SOME ENDOGENOUS FACTORS AFFECTING ROOT FORMATION ON HARDWOOD CUTTINGS OF TWO CLONES OF APPLE (*MALUS SYLVESTRIS* MILL.) ROOTSTOCKS

A Thesis Submitted in Partial Fulfilment of
the Requirements for the Degree of
Doctor of Philosophy
at
Massey University

DONALD STUART TUSTIN

1976

ACKNOWLEDGEMENTS

The author would like to extend his sincere gratitude and appreciation to Mr Murray Richards and Dr David Woolley for their continued encouragement and assistance in their capcity as supervisors of this thesis.

Thanks are also extended to the following people and organisations:

The N.Z. Fruitgrowers Federation Nursery and the Levin Horticultural Research Station for supplying apple rootstocks.

The Asian Vegetable Research and Development Centre for the gift of mung bean seed.

Messrs B. Wilcox, A. Watson and D. Anderson for technical assistance.

Mrs Sara Grant for the typing of the thesis.

The University Grants Committee, whose award of a Postgraduate Scholarship made this study possible.

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

LIST OF PLATES

ABSTRACT

Hardwood cuttings of easy-to-root (MM 106) and difficult-to-root (EM XII) apple (Malus sylvestris M.) rootstocks were sequentially harvested and planted according to the East Malling system, over a period from late autumn (April) until early spring (October). Tissue samples collected on harvest dates and from cuttings undergoing root initiation, were analysed for endogenous, plant growth regulators.

Promotion of root formation by a concentrated, quick-dip treatment of Indole-butyric acid (IBA) was only observed in shoots with a high natural ability to initiate roots, when planted as cuttings. Root initiation potential appeared to be directly related to endogenous levels of an indole-acetic acid (IAA)-like growth promoter. No positive correlation between root initiation and bud dormancy, endogenous Abscisic Acid, Cytokinin or Rooting Cofactor levels was established. Reciprocal donor-grafting experiments showed that a root promoting stimulus was graft transferable from MM 106 into EM XII and that a promoter chromatographically similar to IAA accumulated in EM XII stocks grafted with MM 106 scions. No active rooting inhibitors were located from the seasonal or donor-grafted tissue samples.

A separation of the modes of action of IAA and IBA in promoting root formation was shown. IAA appeared to be the fundamental physiological promoter of adventitious root formation; the number of roots increasing with an increased concentration of IAA. IBA seemed to have a supporting role in promoting root formation, and could not further promote rooting if applied at increasing concentrations above a threshold level. IBA appeared to be active in the root initiation process by protecting the endogenous IAA levels in the cutting base. Studies of the

metabolism of ^{14}C-IAA in the cuttings base indicated that IAA was protected from enzymic degradation and conjugation (both inactivation processes) by the application of IBA prior to planting. IBA was only active in promoting rooting if an optimum level of IAA was present.

The results are discussed in terms of the integrated control of root formation on woody shoots and a hypothetical model of the physiological control of root initiation is proposed on the basis of the present work and evidence available in the literature.

REVIEW OF LITERATURE

A. Introduction

Asexual propagation involves reproduction from vegetative parts of plants which have the capacity for regeneration. The asexual process is of particular importance in horticulture because most valuable fruit and ornamental cultivars are highly heterozygous and sexual reproduction would cause an immediate loss of the unique cultivar characteristics. The characteristics of any single cultivar are perpetuated by establishing a clone, a process entirely dependant on asexual propagating techniques. Apart from the maintenance of specific clones of plants, asexual propagation has some very obvious advantages in terms of production of large quantities of specific cultivars, a factor obviously capitalised upon by the nursery industry.

The simplest method of asexual propagation is by cuttings; leaf, stem, or root. Stem cuttings are most widely used and provide the greatest scope for utilisation of asexual propagating techniques. To establish a small growing plant from a stem cutting, a new root system must develop from the basal tissue of the cutting. This is a complex physiological process under the control of natural plant growth substances, the promotion of which is not well understood. The ability to promote root formation on stem cuttings is clearly critical for the nursery industry and a massive volume of research has been carried out, studying methods of improving the rooting of many species (Thimann and Behnke-Rogers, 1950). Ever since plants were first propagated from stem cuttings, some clones have been considered difficult-to-root. While the number of these clones has decreased with the advancement of research, the problem is still considerable and reflects the level of understanding of the fundamental process of root initiation achieved to date. Tremendous technological advances, in

particular the discovery of the role of IAA by Went (1934), have revolutionised the science of plant propagation. The time is rapidly approaching when any further major advance in plant propagation by stem cuttings will only be achieved with the knowledge of the intimate mechanisms of the root initiation process. By achieving the understanding of such a process, the technical development of methods suitable for commercial use may virtually eliminate the "difficult-to-root" clones of plant species.

In the following sections, the physical, environmental and physiological factors affecting the propagation of hardwood cuttings are reviewed. The influences of cultural treatments and plant growth regulators on adventitious root initiation are examined.

B. Part I. Physical Factors Affecting the Propagation of Apple Rootstocks from Cuttings

1. Physiological Age of Parent Plant

The influence of the physiological age on root initiation of cuttings is a well known phenomenon today and is widely utilised. This juvenility factor is still very difficult to define, but in some cases is involved with changes in form and appearance in certain plants. It can also apply to certain regions of mature plants, e.g. Chadwick (1955) suggested that the basal portion of trunks and lateral roots of woody plants retained juvenile characteristics and this was borne out by the frequency of adventitious shoot production, often observed in many species. These shoots were invariably "juvenile" and nearly always rooted readily. Garner and Hatcher (1955) have found that adventitious shoots produced by shy rooting apple cultivars usually rooted better, particularly with the use of growth regulators, than ordinary vegetative cuttings. Olden (1952) and Smith (1959) also reported that cuttings produced from basal buds on apple cultivars, whether produced naturally or artificially induced, rooted readily. In both cases cuttings derived from mature fruiting wood either failed to

root or rooted very poorly. Smith also noted that cuttings, made from root suckers of a mature ornamental apple tree, rooted well in comparison with cuttings taken from fruiting branches. A general trend found by Danilov (1968) was that cuttings from lower parts of apple tree crowns rooted better than those from the upper parts. Stepanov and Kuzin (1973) classified hybrid apple seedlings on their ability to root from juvenile and mature tissue, the juvenile generally performing better. Later Kuzin (1975) found that cuttings taken from lower, more juvenile shoots rooted earlier and better than those taken from upper, more mature shoots of apple tree crowns.

The physiological age of the whole plant is also important when considering sources of cutting material, because in many cases whole plants are maintained as stock material. Stoutmeyer (1937) recognised two distinct phases of growth in the apple, the juvenile phase rooting readily from cuttings, and the mature phase cuttings rooting very slowly and poorly. Gardner (1929) found rooting decreased with increasing age of the plant from seed with apple hardwood cuttings. 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. Subsequently many workers have studied the juvenility factor with respect to propagation by cuttings and have found that the ease with which roots are formed on cuttings of one year old wood falls off with increasing age of the stock plant; apple rootstocks and cultivars are no exception as found by Knight et al. (1927), Frischenschlager (1938), Zimmerman and Hitchcock (1946), Garner and Hatcher (1947) and Turovskaja (1974).

With the knowledge of the changes in juvenile characteristics as plants age and also the location of juvenile tissue on older plants, more careful selection of propagation material has been carried out, and treatments of stock plants to maintain juvenile characteristics in propagation material

have been utilised. By selecting propagules which might be expected to be juvenile, greater success in propagation by cuttings in apples has been achieved. Garner and Hatcher (1947) noted that young parent plants propagated from roots give more readily rooted stem cuttings than young parents propagated from shoots. After another season's growth the response was reversed; 2 year old parents from shoots were better than 1 year old parents, and 2 year old parents from roots were poorer than 1 year old parents. Stoutmeyer (1937) found cuttings taken from apple shoots derived from root cuttings, rooted easily. Turovskaja (1969a) verified this by using shoots from horizontally planted root cuttings, particularly if taken at the 3-5 leaf stage. Garner and Hatcher (1955a) also found that hedge produced apple hardwood cuttings were more disposed to rooting than those taken from lined out nursery plants. Similar conclusions were obtained by Sinha and Vyvyan (1943) with Myrobalan B plum rootstock. Further work by Garner and Hatcher (1955b) showed that etiolated basal shoots off stools, rooted better than hedge growths but when rooting of non-etiolated cuttings was satisfactory the disparity between hedge derived cuttings and stool derived cuttings diminished. It would be expected that hard pruning of the hedges and stools maintained the production of juvenile shoots. Nelson (1955) and Singh et al. (1957) also noted this phenomenon when comparing stooled stocks and newly lined out plants. The stock source influenced the speed, degree and percentage of rooting of the cuttings. Tarasenko (1964) compared hardwood and root cuttings taken from juvenile and adult plants and plant parts. Propagation of hardwood cuttings was most successful when material was obtained from juvenile plants, juvenile parts of mature plants and plants reverted to a juvenile state by induction. Hatcher (1959) noted that severe top pruning or root pruning promoted rooting of tip cuttings of apple rootstocks. Carlson (1966) reported that the current season's growth was most satisfactory for propagation of fruit trees by cuttings.

It is evident that, although a precise definition of the juvenile state of plants or plant parts is very difficult to express, the influence of this physiological state on the degree of root initiation obtained on cuttings is very important. Thimann's (1942) comment that with shy rooting species of plants, the most important single factor affecting root initiation was the age of the tree from which the cuttings were taken, is significant. By beginning propagation studies with non-juvenile plant material, subsequent treatments will not serve to replace the juvenile factor required for successful root initiation.

2. Choice of Material from Parent Plant

Much of the research into the effect of juvenility on the rooting of cuttings has resulted in relatively detailed mapping of regions of plants which retain juvenile characteristics and also changes in the juvenile state with time. The most significant contribution this has made to propagation is in determining which plant material is best for use in producing rooted cuttings successfully. As early as 1926, Knight suggested that on Pyrus and Prunus species, lateral shoots which were not actively elongating provided a better source of cutting material than terminal shoots or elongating laterals. Lateral cuttings were consistently superior to terminal cuttings of Norway Spruce according to Farrar and Grace (1942). Turovcev (1960) found that, with apples, shoots from adventitious buds of secondary meristems rooted better than shoots from lateral or axillary buds from the primary meristem. These reports confirmed information obtained in juvenility studies that cuttings from the lower parts of the plant, not associated with the primary leader, provided the better source of cutting material.

Perhaps equally important is the region of the shoot to be used. Garner and Hatcher (1947) reported that apple stem cuttings from the base

of yearling shoots were superior to those from upper parts of the shoot, irrespective of the origin of the parent. Further to this they described a decreasing gradient of rooting ability along a yearling shoot from base to terminal. Perhaps one of the more significant advances in propagation from hardwood cuttings was the discovery of the propensity of basal cuttings to root more readily than intermediate or terminal cuttings. Pope (1960) reported that basal cuttings of Ilex, Feijoa, Cocculus and Thryallis all rooted more readily than terminal cuttings. Many reports exist on the success of propagation from basal hardwood cuttings, particularly with reference to fruit tree rootstocks; for example, rooting ability increased with increasing distance from the apex in rootstocks of apple, plum and quince (Garner and Hatcher, 1955; Hartmann, 1955; Peters, 1959). More detailed work by Howard et al. showed that basal hardwood cuttings of apple and plum rootstocks taken as close to the previous season's growth, including the swollen base of the shoot but not a heel of older wood, proved to be the most suitable propagation material. (Howard, 1966; Howard and Nahlawi, 1969b; Howard, 1970a; Howard, 1970b; Howard, 1970c; Howard, 1970d). It was noted however that plums were influenced less by use of basal cutting material than apple rootstocks. Later work by Doud and Carlson (1972) confirmed Howard's work and also showed that a similar situation existed with peaches, cherries and apricots. Contrary to these reports, Danilov (1968) found that the results from apple cuttings taken from different parts of the shoot were contradictory and appeared to be cultivar dependent. It should be noted that when referring to the type of cutting used for propagation, the wood from which it is derived is that which has been produced in the current season of growth immediately prior to the harvesting of the cuttings.

It can now be stated that in general when propagating apple rootstocks from hardwood cuttings, basal hardwood cuttings are likely to result in

higher percentages of rooted cuttings than either intermediate or terminal cuttings.

3. Seasonal Effects and the Time of Taking Cuttings

The time at which cuttings are collected has been found to be critical in terms of the expected successful propagation of apple rootstocks. The timing of the collection is also related to the physiological condition of the stock plant rather than the calendar date. Cuttings of apple, peach, pear, cherry and apricot cultivars collected in autumn rooted in moderately high percentages, but cuttings collected in mid winter rooted very poorly, (Howard, 1966; Doud and Carlson, 1972). A rapid rise in the ability to root occurred in spring. In some cases the autumn peak was not present but the spring flush was always recorded. Upon investigation, Howard (1968a) concluded that the mid winter depression in rooting was not due to the influence of vegetative buds in varying degrees of dormancy. Fadl and Hartmann (1967a) found that root initiation on Old Home pear hardwood cuttings was best in autumn and was correlated with the ability of the buds to shoot immediately. As the buds entered dormancy the ability of the cuttings to root also decreased. Nesterov (1968) noted that the successful propagation of stem cuttings of quince depended on the amount of chilling the mother plant had undergone. Cuttings of Myrobalan B plum rootstock were found to exhibit similar trends observed in apple and pear hardwood cuttings, (Howard and Nahlawi, 1969b). High levels of rooting were recorded in autumn over a period of 4 years, decreasing to virtually no rooting in mid winter and then rapidly improving with the onset of spring. Each of these reports stress that the trend in root initiation was apparent when propagation was carried out with the use of high basal storage temperatures and a synthetic growth promoter (IBA).

Similar work with MM 106 apple rootstock did not show any decline in

the percentage of cuttings rooted on progressive harvest dates from autumn until mid winter but the environmental conditions (mid winter conditions) were considered to be partially responsible for this response (Tustin, 1972). It was noted that the rate of initiation of adventitious roots was slower in the winter months compared with the autumn harvest dates.

In virtually all the reports on the effect of the time of taking fruit tree rootstock hardwood cuttings, autumn or spring were the times when a high degree of success was achieved. Generally much poorer results were obtained during the mid winter months and in some cases the autumn peak was also absent.

Part II. Physical Treatments Prior to Planting

1. Treatment with Synthetic Growth Substances

With the comparison of a root inducing hormone and of auxin (Thimann and Went, 1934) and the subsequent proof that they were one and the same (Thimann and Koepfli, 1935), the breakthrough in hormonal regulation of adventitious root initiation appeared to have occurred. Practical application followed rapidly, e.g. Cooper (1935) applied IAA in lanolin to lemon cuttings and found it promotive to rooting; as well as substitution of IAA by other similar synthetic compounds such as IPA and NAA (Hitchcock, 1935; Zimmerman and Wilcoxon, 1935).

Rapid progress in testing compounds for root forming activity was made. IBA and NAA were found to be better than IAA because of their persistence and the phenoxyacetic acid compounds found to have adverse side effects because threshold levels were also toxic. Once the efficacy of the compounds was established, the methods of application became important also.

Hitchcock and Zimmerman (1939) were the first recorded workers to

use the concentrated dip treatment of growth substances on cuttings. Cooper (1944) found that IBA applied as concentrated dips of varying strengths gave excellent responses on a range of tropical plants, when planted as leafy cuttings. Satisfactory responses were also obtained with lower concentrations when the base of the cutting was immersed for long periods e.g. 24 hour soak treatments.

Hatcher and Garner (1947) established the first positive reaction of hardwood apple cuttings to growth substance treatment, but found that with the concentrated dip method, deleterious results were obtained. In further work, namely trying to establish a standard treatment of fruit tree rootstock cuttings, Hatcher and Garner (1950) found 0.5 mg/ml IBA applied as a quick dip, best for Myrobalan B plum rootstock. With Crab C apple rootstock, while it responded without damage to 5.0 mg/ml IBA, the best response was at 0.5 mg/ml. Subsequently a standard treatment of 500 ppm was adopted to be applied as a concentrated quick dip, which was considered most functional, commercially.

With the development of high temperature storage of cuttings while undergoing root initiation, a re-evaluation of the growth substance treatment occurred. Howard (1966) in an interim report, suggested a quick dip of IBA in 50% methylated spirit; for plums a concentration of 500 ppm and apples, 2,500 ppm. A critical examination of the influence of IBA, (Howard, 1968b) on root initiation in apple hardwood cuttings, showed that rooting in terms of percentage, number of roots and dry weight of roots improved with increasing concentration up to 1,250 ppm. Further improvement in percentage was obtained at 2,500 ppm, but at 5,000 ppm root numbers were higher, though percentage rooted was lower. With plum rootstocks Howard and Nahlawi (1969b) found clonal differences, Myrobalan B and St Julian being optimum at 5,000 ppm, while Brompton and EA.16 show-

ing no improvement above 1,250 ppm. Again the optimum concentration for root number tended to be higher than for rooting percentage. An asessment of new clones of plum rootstocks (Howard and Nahlawi, 1969a) showed that many responded well to increased concentrations of IBA although more variation between clones was apparent than with apple rootstocks.

Critical assessments of the method of application of IBA in solution, resulted in the demonstration that with apple and plum cuttings, highest rooting percentages were obtained when cuttings were dipped as shallowly as possible. This varied between clones and was also influenced by the IBA concentration (Howard and Nahlawi, 1970). Nahlawi and Howard (1971) verified that, on plum cuttings, when only the basal cut end was treated with IBA the best rooting percentages were obtained. The detrimental effect of deeper dipping in higher concentrations of IBA was proved to be caused by IBA application to the cutting epidermis. Epidermal application was only beneficial when insufficient stimulation occurred via the base.

Howard (1970) found that methyl alcohol, methylated sprit and acetone were cheap and suitable alternatives to ethyl alcohol solvents for IBA application. Methylated spirit seemed to increase the level of rooting if propagating conditions were less than optimum. Ensuring adequate ingress of IBA into the cutting was important and the duration of dipping and the moisture content of the cuttings both influenced this. Nahlawi and Howard (1972) noted that a longer duration of dipping was required at lower concentrations of IBA to obtain equivalent rooting responses to quick dips at high concentration. A delay between harvest and dipping causes a water deficit in the cuttings which increases uptake of IBA, improving the response with lower IBA concentrations but reducing the levels of rooting from concentrated dips (Nahlawi and Howard, 1972; Howard, 1974).

Although much of the research into the effect of growth substances on rooting in fruit tree hardwood cuttings has been carried out at East Malling over the last decade, independent workers have verified results obtained by Howard et al. As early as 1940, Hitchcock and Zimmerman noted that a combination of growth substances was more effective in promoting root initiation than either of the components separately. IBA and NAA were most effective in combination where one or the other had a lesser effect on a particular cultivar. Components which were both equally effective on one cultivar did not exhibit the massive promotary effect of the combination. Responses to the combinations which were effective were usually synergistic. Moretti (1954) found IAA and NAA (50 ppm each) in combination gave better promotion to rooting of mulberry cuttings, than either IBA or a commercial preparation applied at 100 ppm separately. Zatyko (1959) found on fruit tree rootstocks and cultivars that NAA was not uniformly effective in promoting root initiation, but by splitting the base of the cutting, rooting was enhanced further. Rooting on EMIX was improved using a 50 ppm soak of IAA and heat, Van Doesburg, (1960), and Pope (1960) found on a range of woody ornamental plants that 1000 ppm IBA quick dip was most effective in promoting root initiation. Hartmann, Hansen and Loreti (1965) found that a 24 hour soak in an aqueous solution of 100 ppm IBA was promotive to rooting of a range of apple rootstock hardwood cuttings, but further treatments such as heat were often required for a high degree of success. Carlson (1966) also reported that the concentration and duration of the IBA treatment had a profound effect on root initiation in fruit tree hardwood cuttings. Fernandez-Sevilla (1969) found various clones of apples responded differently to IBA as a quick dip but in general a concentration within the range of 1000-4000 ppm was optimum. Ashiru and Carlson (1968) concluded the optimum concentration of IBA for obtaining a maximum rooting percentage was 1500 ppm in association with 21°C bottom heat. Ashiru (1968) also found a combination

treatment of 200 ppm IBA and 300 ppm NAA stimulated root initiation in MM 106 cuttings taken in early spring. Further work by Doud and Carlson (1972) with apple, peach, pear, cherry and apricot cultivars confirmed that 1000-1500 ppm IBA as a 10 second quick dip gave the optimal response. A range of clonal apple rootstocks were treated with IBA at varying concentrations (150-300 ppm) for 24 hours or for 5 seconds with 3000 ppm IBA by Guerriero and Loreti (1968). Rooting was lower at 3000 ppm IBA but of the cuttings rooted more survived from this treatment than the soaks. Tkacenko (1970) stimulated rooting in the difficult-to-root paradise apple rootstock by using 24 hour soaks of IAA (.001%), IBA (.0005%) or 2,4-D (.0001%) or by quick dips in IBA (.005%) or (.01%) or IAA (.02%).

It is now accepted that for successful propagation of apple rootstock hardwood cuttings, the use of an effective synthetic growth regulator is essential. The literature confirms the generally better performance produced by quick dip treatments at high concentrations of IBA and also recognises the fact that, although not necessarily related, the use of high temperature storage with the IBA treatment is essential to get optimum results.

2. Temperature Treatments Prior to Planting

With many fruit tree rootstocks, particularly shy rooting clones, temperature treatments prior to planting of the cuttings has shown beneficial effects on the subsequent root initiation.

Hatcher and Garner (1956) studied the effect of overwinter storage of apple and plum hardwood cuttings. By maintaining a minimum heat of 45°F throughout the duration of storage, cuttings which had been treated with IBA prior to storage, generally rooted better when lined out in the spring. The exception was Crab C which, after the IBA treatment, rooted well when planted out directly in autumn. Plum cuttings established well

after winterstorage irrespective of the IBA treatment.

Hartmann et al. (1963) found that hardwood cuttings of the shy-rooting Bartlett Pear which had been planted with basal temperatures of 75°F, responded well to a cold storage treatment for three weeks before lining out, compared with the response of similar cuttings which had received treatments usually successful with Old Home pear cuttings. It was found that with the use of a lower basal temperature treatment, root appearance was not as pronounced as at 75°F. Howard and Garner (1965), when studying the prolonged storage of rooted and unrooted fruit tree hardwood cuttings, noted that with unrooted cuttings treated with IBA after storage and placed on storage beds with a minimum heat of 45°F, the maximum number of roots and root length were often greater than on those rooted before storage. The percentage of the cuttings rooted was considerably increased in most cases and the period of root stimulation was also shorter. It was considered that although rooting was readily achieved, the rapid leafing out of buds on the cuttings was disadvantageous from a commercial aspect. Further work by Garner (1965) found that storage of M 26 apple cuttings with a minimum bottom heat of 45°F for 2-4 months, enhanced rooting and the percentage survival of cuttings lined out in spring. Very little root development occurred during storage.

Fadl and Hartmann (1967a) verified the data of Hartmann et al. (1963) on Bartlett pear cuttings and found that the highest level of root promotion was correlated to the lowest mean temperature the buds had been exposed to. Bartlett cuttings stored artificially at 40°F rather than at high temperatures showed increases in root promoting compounds and decreases in root inhibiting compounds when extracts were assayed. Turovskaja (1969b) subjected apple root cuttings from four year old trees for periods from 30 to 200 days under winter temperatures. Root and

Shoot development on these cuttings when placed in favourable conditions was quickest and best on cuttings which had received the longest storage time.

Cripps (1974), working with apple rootstock hardwood cuttings found a 10 week storage period at $0^{o}C$ was essential to obtain commercially useful percentages of cuttings rooted. M 26 and MM 106 were found to be the best rootstocks for propagation by this method.

It seems apparent that shy rooting hardwood cuttings of fruit rootstocks respond favourably to periods of low temperature storage prior to giving additional, more routine root stimulating treatments. Warm temperature storage of cuttings above the ambient minimums also enhances the subsequent rooting and establishment of cuttings when lined out.

3. Wounding and Bud Removal as an Aid to Root Initiation

Wounding has traditionally been used in propagation by hardwood cuttings, although the reasons for doing so are probably not understood. It has also been proposed that season differences in root initiation may be a result of changing promotory and inhibitory activity of buds on the cuttings. This is in part based on observations that removal of non-dormant buds in autumn and spring is detrimental and chilling and bud removal in mid winter is beneficial to root initiation.

Fadl and Hartmann (1967a) compared easy rooting Old Home and shy rooting Bartlett pear rootstock cuttings. Disbudding of Old Home cuttings reduced the rooting to a low but constant level throughout the season. Bartlett cuttings would not root at all unless disbudded, when a small stimulation in rooting was achieved. Chilling the buds of Bartlett pear cuttings while rooting proceeded, effected improvements in percentage

root formation, except in late winter, when no differences occurred. The greater the number of buds present on the cuttings, the greater was the chilling effect on rooting. Changes in root initiation with bud activity were correlated in terms of inhibitor and promoter levels assayed from treated cuttings.

Howard (1968a) could not substantiate the results of Fadl and Hartmann. Hardwood cuttings of Myrobalan B plum rootstock were collected throughout the season, disbudded and treated in the usual way for rooting. Disbudded cuttings rooted consistently better than normal cuttings but cuttings which were disbudded one month or more prior to harvest quickly lost any stimulus caused by disbudding. Removal of only 30% of the buds was found to stimulate rooting and their position was not important. Cuttings of M 26 apple rootstock were used to investigate the effect of winter chilling on bud activity and rooting and no difference was found between chilled and unchilled cuttings. Also, removal of potentially active buds of plum cuttings did not depress rooting. Therefore none of these results support the view that bud activity fluxes, throughout the season, account for fluctuations in rooting ability.

Because bud removal causes wounding to the cutting, wounding per se was also investigated by Howard (1968a). Wounding was found to give a big stimulus to rooting, similar to disbudding and also to a combination of the two, on both Myrobalan plum and M 26 apple rootstock cuttings. Often the stimulus from wounding was greater than from disbudding. Howard concluded that a wound-induced stimulus and not the state of activity of the buds was responsible for improved root initiation.

A further suggestion on the effect of wounding was that in some plants a tough ring of sclerenchyma in the cortex, external to the site of initiation, prevented root emergence and that wounding provided a

point of exit for developing roots (Beakbane, 1961). The other commonly held view was that increased uptake of growth substances was achieved through a basal wound.

In general, the effects of bud removal and wounding have both been seen to promote root initiation but there is still a divergence of opinion in the interpretation of the data. It still remains to be shown conclusively that one or the other, or both the treatments are responsible for the promotion and exactly what mechanism is involved.

Part III. Physical Treatment During Root Initiation

1. High Temperature Storage of Cuttings

While the effect of growth substances on root initiation of hardwood cuttings was elucidated, another critical treatment was developed in conjunction with it, namely high temperature storage of the bases of cuttings. As success with concentrated dipping techniques was achieved it was also noted that warm or high temperature storage, during the rooting period, greatly improved the rate of root initiation on many fruit tree rootstocks.

Hatcher and Garner (1956) first noted that by maintaining a minimum temperature of 45°F during storage through the winter, after an IBA dip, cuttings rooted much more readily. The effect was most noticeable on material collected in autumn rather than that from later in the winter. Also, with higher storage temperatures (65°F) rooting was accelerated considerably. Reaction to this treatment was obtained whether given immediately after planting, or after an initial period without heat. Hartmann (1955) had also noted that on plum and quince hardwood cuttings, a 30 day storage period in moist peat moss at 50°F, after an IBA treatment, promoted the rooting of cuttings when subsequently lined out.

Howard and Garner (1964) investigated the high temperature storage of M 26 apple hardwood cuttings, as a method of rapid propagation and increasing rooting percentages. A range of high temperatures applied to the base of the cuttings, which had been given the normal IBA concentrated dip (2,500 ppm), were evaluated. Rooting was found to occur most rapidly at 70°-80°F. At higher temperatures the basal tissue was often killed. Rooting was achieved in approximately two weeks at 76°F and 81°F but the effect on root numbers was less clearly defined. In a further experiment, M 26, EM VII and Old Home pear cuttings were sufficiently rooted after four weeks at 65°F to be lined out.

At this time, many workers found the effect of growth substance application could be enhanced greatly by the use of high temperature storage. With a range of clones of apple rootstocks, the most effective treatment in improving rooting was basal storage at 21°C, after an IBA treatment, with the above ground parts of the cuttings at ambient temperatures, (Hartmann et al., 1965; Carlson, 1966; Ashiru and Carlson, 1968; Guerriero and Loreti, 1968; Doud and Carlson, 1972).

Howard (1968b) re-examined the effect of temperature and hormone treatments on root initiation. He found the optimum temperature for rooting percentage was at or above 22°C. As with the IBA concentration, a higher temperature of 27°C favoured root numbers rather than rooting percentage. Auxin treatments and temperature treatments were found to promote rooting independently and it was necessary to use the auxin treatment to obtain satisfactory rooting. It was from this work and that of Howard and Garner (1965) that the standard East Malling treatment of hardwood cuttings was formulated.

Although promotion by growth substances and high temperature treatments

of hardwood cuttings have been found to be independent of each other, the greatest successes in rooting apple hardwood cuttings have combined both treatments. From a commercial point of view, the independent action of each factor offers more scope for variation to achieve the optimum of both treatments.

C. Plant Growth Regulators and Their Effect on Adventitious Root Initiation

1. Endogenous Auxins

Since the original demonstration by 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, research into auxin effects in root initiation has taken two main paths. By far the most common has been the influence of exogenously applied auxins, and synthetic compounds exhibiting auxin activity, on root formation on cuttings and tissue explants. No less important has been investigations into endogenous levels of native plant auxins and their relation to ease of propagation of plants from cuttings. In combination with this work have been studies on ratios of auxins and inhibitors of root initiation, and the correlation of these ratios to the ease of root formation on cuttings and tissue explants.

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 high 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 bases in water, or by treatment with an auxin inhibitor. After 96 hours a distinct polarity existed in the cuttings with a higher distal auxin content. Addition of synthetic

auxins also aided root initiation. In a range of herbaceous species, Odom and Carpenter (1965) related rooting ability to the presence or accumulation of "free auxin" in the cutting bases. Rapidity and vigour of rooting paralleled the level of endogenous auxin. An inverse relationship between the endogenous acidic auxin level and the known beneficial response to added root promoting substances was established. Where auxin levels were low in fresh samples, an accumulation of acidic or neutral auxins occurred just prior to, or during, root initiation, but where high levels of auxins were present initially, the level declined during rooting. Saito and Ogasawara (1960) had noted that in cuttings of Salix gracilistyla, the IAA level was high at harvest time and decreased rapidly during root initiation and suggested that this indicated that IAA was instrumental in the initiation of roots on cuttings of this species. Conversely, Smith and Wareing (1972a) found that endogenous auxin levels at the base of both intact, and disbudded, chilled poplar cuttings were high at the time of root initiation, but were low 15 days later when the roots were several cms long. It must be noted though, that the poplar cuttings had been given several weeks after chilling to allow the "hormonal" changes to occur prior to sampling, so a situation similar to Odom and Carpenter's low initial auxin status cuttings existed with the poplar. Nanda et al. (1968) found that segments of Populus nigra shorter than 3.5 cm in length would only root when exposed to three or more days of darkness and this was related to the auxin status of the segments. The dark requirement could be replaced by IAA or IBA or by using larger segments. The failure of light grown segments was attributed to low auxin status, or more probably, a high rate of destruction. Haissig (1970) used the removal of stem apices, leaves and axillary buds to demonstrate that endogenous auxins were responsible for root initiation in Salix fragilis cuttings. Surgery reduced endogenous auxin levels and caused a reduction in primordial initiation and to a lesser extent primordial growth. As cells lost their meristematic ability, they dif-

ferentiated into mature parenchyma cells. Application of IAA overcame the effects of surgery. More labelled IAA was taken up by primordia in the earliest stage of initiation, indicating that the establishment of a meristematic site was more dependent on IAA, than the subsequent development of the primordia. The auxin also appeared to act directly in the cells which become meristematic. Greenwood and Goldsmith (1970) attributed the regeneration of roots on excised Pinus lambertiana embryos to the transport and accumulation of endogenous IAA. 40% of the cuttings rooted without the addition of exogenous IAA and the first regeneration always occurred at the basal tip of the slanting cut (IAA accumulation point). Regeneration was not affected by the removal of the regenerating region and addition of exogenous IAA could increase the number of roots; application of TIBA totally inhibited rooting. They also concluded that polar auxin transport raised local concentrations of IAA in the cutting base to levels sufficient for promoting root regeneration.

Etiolation of tissue prior to or during planting as a cutting has been found to be beneficial in propagation. Kawase (1965) proposed that etiolated cuttings retained a higher level of endogenous auxin at the site of etiolation during root initiation and that this seemed to account for the better rooting compared to non-etiolated cuttings. IAA was able to replace the etiolation effect in non-etiolated cuttings. 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. Herman and Hess (1963) had noted a slightly higher level of IAA in etiolated tissues compared to non-etiolated tissue of cuttings of Phaseolus vulgaris and several cultivars of Hibiscus rosasinensis. An unidentified auxin at Rf 0.05-0.15 was also present in the same proportions as IAA in etiolated and non-etiolated tissue. Bastin (1966) found that differences in rooting ability of Abies balsemia 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 compounds which inhibit IAA-oxidase activity. A linear increase of diphenol content and number of roots per cutting 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 Co-factor theories.

Seasonal changes in the ability of many species of plants to root from cuttings, has been correlated with levels and fluxes of extractable auxins. In general, periods of dormancy are accompanied by lower free auxin levels and during these phases, lower levels of rooting occur. The optimum time for root initiation is often at the termination of a dormant or bud-rest phase, when a flush of renewed auxin production often occurs. As a result of this observation, a relationship between bud activity and root initiation has been established. Periods of active bud development and growth are associated with better root initiation in some species. Spiegel (1955) investigated rooting of grape cuttings and noted that an auxin peak occurred just prior to bud-break in spring while inhibitor levels were low. Vieitez and Pena (1968) established a loose correlation between seasonal rooting of Salix atrocinerea and IAA content of the stem tissue. Samples from July did not fit the correlation and this was unexplained. No active inhibitors of root initiation were located. Sin and Sung (1968) and Sung (1969) 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 rooting promoter. Nanda and Anand (1970) found that, with the onset of dormancy, and through the winter, low rooting was achieved in Populus nigra, but with the renewed growth in spring, high rooting occurred. 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 initia-

tion, and added auxin thus facilitated root initiation. In spring, added auxin inhibited rooting, presumably because of supra-optimal levels, since cuttings 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. Smith and Wareing (1972b) examined rooting in active and dormant leafy poplar cuttings. Long days were found to promote root initiation and this was related to a high auxin status under these conditions. Rooting over a period from April to August was correlated with auxin levels. Further work by Smith and Wareing (1972a) demonstrated that the buds on the cuttings influenced rooting via the auxin supply. Chilling shortened bud dormancy and increased root initiation. Endogenous auxin levels were higher in chilled than unchilled buds 5 weeks after chilling ended. It has been noted in many cases, that the influence of chilling, and the effects of bud-burst on subsequent root initiation, have been generally promotive. Smith and Wareing (1971) also correlated bud size with root initiation; bud size affecting the quantitative cut-put of IAA. Wareing and Roberts (1956) and Bhella and Roberts (1974) both related long day treatments with improved cambial activity and the possible link of endogenous IAA production as the effector of this process. Seasonal variations in root 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.

With improvements in plant extract assay techniques, it might be more relevant today to examine auxin:inhibitor ratios and ratios between auxins and other plant hormones which are known to antagonise root initiation. Work on tissue explants has defined the importance of auxin:cytokinin ratios in determining whether roots will be initiated. Spiegel (1955)

found in Vitis cuttings that the auxin:inhibitor relationship varied with different cultivars; easy rooting cuttings had a high auxin:low inhibitor ratio and the converse was true for difficult-to-root cuttings. Leachings containing the rocting inhibitor could inhibit rooting of the easy-to-root cultivar. A high auxin:inhibitor ratio was also detected just prior to spring bud break. Ogasawara (1960) found that as Pinus densiflora stock plants increased in age, the rooting capacity of cuttings decreased accordingly. Correlated with this was a change in the auxin:inhibitor status of the stock plants, from a high to a low ratio. He concluded that the auxin:inhibitor balance was important in root initiation. Heide (1967) found that the IAA level in Begonia leaves varied with day length and temperature. High temperatures and long days increased endogenous auxin levels. Short days and low temperatures decreased auxin production and also reduced the propagation ability of Begonia leaves. Day length and temperature influenced the regeneration of Begonia leaves by altering the endogenous auxin:cytokinin ratio (Heide, 1965b, 1968; Heide and Skoog, 1967). Short days decreased auxin synthesis and promoted cytokinin synthesis, thereby reducing the auxin:cytokinin ratio. It was also noted that short days and low temperatures favoured adventitious bud development. In apple, peach, pear and quince cuttings, Sarkisova (1972) found that endogenous auxin:inhibitor ratios were high in easy and difficult-to-root species during the autumn. This time correlated with the best months for rooting cuttings, whether easy or difficult-to-root.

2. Gibberellins

Gibberellins are notable for their inhibition of root initiation in cuttings and tissue culture explants. In his review, Scott (1972) concluded that most reports of gibberellin effects on root initiation showed inhibition, whether in the presence of auxins or not. Early reports on gibberellins and root initiation soon established their inhibitory nature. Brian et al. (1955) and Brian (1959) showed that GA_3 inhibited root ini-

tiation on pea, bean and tomato cuttings. Kato (1958) verified this and also found that GA_3 counteracted the stimulatory effect of auxin on root formation. Gray (1958) found GA_3 inhibited rooting on Saintpaulia ionanthe and Hibiscus rosasinensis cuttings when applied with IBA, at 10 times the concentration of the IBA, i.e. it counteracted the auxin effect on rooting. Schraudolf and Reinert (1959) found that GA inhibited both root and shoot development in isolated Begonia leaf discs, at concentrations of 10^{-6}-10^{-5} gm/ml. The inhibition could be removed by 2,4-D at the same concentration but not by kinetin. Brian et al. (1960) found varietal differences in rootability of pea cultivars but the GA_3 inhibition of rooting was uniformly effective and promoted stem extension. Similar results were obtained with dwarf beans. The degree of rooting was inversely proportional to the promotion of stem extension and the antagonism between GA_3 and auxins was found to be non-competitive. Bachelard and Stowe (1963) noted the inhibition of rooting of Acer rubrum cuttings by GA even in the presence of added auxin. Murashige (1964) showed that with tobacco tissue cultures, the level of GA_3 which stimulated callus growth, inhibited root or shoot differentiation. Cell division per se was not prevented. GA also persisted through two subcultures (dilution 1:400) indicating that it was not easily metabolised. Jansen (1967) reported that root formation on tomato cuttings was always inhibited by GA, never promoted; fewer roots were produced and their emergence delayed. The effect was concentration dependant, the lowest effective concentration being 10^{-3} mg/l and the inhibition increased up to 1000 mg/l. Nanda et al. (1968) found that GA_3 inhibited rooting of Populus nigra segments under favourable dark treatments. Heide (1969) in a further work with Begonia cuttings established that a non-reversible inhibition of rooting and bud formation occurred after treatment with GA_3. Auxin, cytokinin, ABA, CCC or Phosphon were unable to counteract or reverse the GA-induced inhibition of organogenesis. Pierik and Steegmans (1975) noted that the

influence of GA_3 on the rooting of Rhododendron explants was always negative and especially at high concentrations, all parameters of rooting were decreased. Hansen (1975) found that the gibberellin effects were light dependent. High GA_3 concentrations caused inhibition of rooting, regardless of the light regime and cuttings from plants under high light intensities were not affected by low GA_3 levels but higher concentrations gave the usual inhibition. However, cuttings from lowlight treated plants were significantly promoted in rooting by low GA levels (10^{-7}-10^{-8}M) but above 10^{-6}M, inhibition occurred.

Isolated reports have recently reported instances of gibberellin promoted root initiation. Nanda et al. (1972) reported that GA_3 treated Ipomoea fistulosa cuttings rooted with greater numbers of roots and also increased number and length of sprouted buds. They suggested that it was an indirect promotion; GA_3 caused bud burst and increased the endogenous auxin supply from the axillary buds as postulated by Erikson (1971). Another indirect promotion of rooting by gibberellin was found by Varga and Humphries (1974). Pretreatment of the laminae of isolated primary leaves of dwarf bean with GA especially with the addition of tryptophane strikingly increased the rooting of the petioles. TIBA applied to the upper part of the petiole prevented the promotion presumably by blocking IAA translocation. This result demonstrated the effect of GA_3 on increasing IAA synthesis and level in the leaf. Hansen (1975) reported that with plants grown under low light intensities, cuttings supplied with GA_3 (10^{-3}-10^{-8}M) were significantly promoted in root initiation; high concentrations caused the usual inhibitory effect.

Two theories on the mode of action of gibberellin inhibition of root initiation have been proposed. An early theory proposed by Brian (1957) was the nutrient diversion hypothesis. This proposed that gibberellins,

by inducing stem elongation, or bud burst and elongation, set up a new sink for nitrogenous compounds and carbohydrates, which competed more effectively for these food reserves than did meristematic sites of root regeneration. Nanda et al. (1968) subscribed to this theory even though Brian et al. (1960) abandoned the theory after finding that a small apical dose of GA caused extension growth without root regeneration inhibition, and removal of the apical bud prevented extension growth while rooting was inhibited by a small basal dose of GA.

A more recent theory has emerged with substantial support from the literature. This is that gibberellins may inhibit root initiation by acting directly on the site of regeneration, i.e. blockage of the initial organised cell divisions required to form root primordia. The inhibition appears to be non-competitive with auxins, as it is not reversed by auxin treatment. GA_3 does not effect the subsequent development of existing root primordia at the time of treatment. Brian et al. (1960) found that the effectiveness of GA_3 on inhibition of root formation was greatest if applied just prior to removal of the cutting and the effectiveness decreased markedly if applied two days after the cutting was taken. This suggested that GA_3 prevented rooting by direct local inhibition of organised cell divisions preceding the formation of organised root primordia. Jansen (1967) obtained similar results; GA-treated tomato cuttings being most effectively inhibited from rooting by treatment in the first three days. Growth of existing primordia was not affected by GA. These results also indicate that GA inhibits rooting by inhibiting or retarding the induction of root primordia. Heide (1969) found with Begonia leaf cuttings that organogenesis of both roots and buds was inhibited by GA_3 and that in both cases the development of pre-existing primordia was unimpeded. GA-induced petiole elongation could be competitively inhibited by auxin and cytokinin but inhibition of organogenesis was non-competitive with auxin and cytokinin. Heide agreed with Brian et al.(1960), Murashige

(1964), Kaufman (1965) and Jansen (1967) in that organised cell division for root and bud primordial formation were blocked by GA_3. Haissig (1973) found further that in willow, GA_3 blocked the auxin-induced primordial development subsequent to initiation. As Kaufman and Murashige (loc. cit.) concurred, this action of gibberellin was different from that seen in the apical meristem, where gibberellin commonly induced cell division. Heide found that this difference in effects was clearly manifest in Begonia by studying the influence of GA_3 on growth and development applied before and after organised bud primordia are formed.

3. Cytokinins

Cytokinins, like gibberellins, inhibit root initiation but it is doubtful whether the physiological mechanism in the two groups of hormones is similar. Early investigations with kinetin soon demonstrated that it possessed the ability to inhibit root formation and promote callus development. Gregory and Samantarai (1950) found this response using 1 mg/l kinetin, on the petioles of isolated primary leaves of dwarf bean. If the supply of kinetin was removed, rooting from the developed callus on the base of the petiole occurred. Early research soon established that not only was root initiation suppressed by cytokinins, but the influence of added auxins was counteracted also. De Ropp (1956) noted that the IAA-induced rooting of sunflower hypocotyls was suppressed by kinetin, at 1 mg/l and to a lesser extent at 0.1 mg/l. Schraudolf and Reinert (1959) found that kinetin suppressed root initiation on isolated Begonia leaf discs and counteracted their promotion by 2,4-D. Kinetin also abolished the polarity of the leaf discs, so that shoots formed over the whole area of the disc. Humphries (1960) reported the inhibition of root initiation on petioles and hypocotyls of dwarf bean, and the subsequent successful rooting if kinetin was withheld; kinetin also counteracted the effect of NAA in induction of new roots.

Another development was the discovery of the effect of low levels of cytokinin on root initiation in association with a constant level of auxin. Skoog and Miller (1957) found that kinetin appeared to enhance root initiation on tobacco callus cultures, at low concentrations, in the presence of a constant supply of IAA. In contrast a high concentration of kinetin, with the same level of IAA resulted in bud formation. They concluded that the type of organ regenerated was controlled by a delicate interaction of cytokinins and auxins. Mullins (1970) found that the auxin:cytokinin ratio was of importance in the rooting of cuttings as well as of undifferentiated tissue. Similar responses have been reported by Pilet (1960) using carrot callus and Toponi (1963) with endive. Okazawa *et al*. (1967) noted that with potato tuber explants, kinetin appeared essential for root formation in combination with a sufficiently low auxin level. Heide (1965b) also found that at low concentrations both auxins and cytokinins appeared to enhance the processes which both normally opposed if present in high concentration. Thus the interaction of cytokinins and auxins in the root initiation process was established. It has also been noted that cytokinins competitively inhibited the auxin effect on root initiation and that this inhibition could be overcome by increasing the auxin supply. Heide (1971) summarised the evidence for an *in vivo* interaction of auxin and cytokinins in the regeneration of *Begonia* leaves, notably with temperature and day length treatments correlating with endogenous hormonal levels, giving responses which had been induced previously with the use of exogenous auxins and cytokinins.

Further reports provide more information on cytokinin activity on root initiation. Bachelard and Stowe (1963) found that *Acer rubrum* cuttings rooted less well when kinetin was applied to the cutting base; in contrast, if kinetin was applied to the leaves, rooting appeared to be stimulated, but no evidence of changes in the auxin:cytokinin ratio was found. They suggested that an altered nitrogen metabolism in the cuttings

may have occurred causing a stimulation of rooting. Gautheret (1969) noted that on Helianthus tuberosus cultures, kinetin exhibited no rhizogenic activity alone with glucose and light, but it did reduce the rhizogenic action of NAA. Pierek and Steegmans (1975) found that, on Rhododendron explants, the addition of low concentrations of 6-benzylamino-purine (BA) did not affect root initiation, but at 10^{-5} g/ml and above, rooting was strongly inhibited. Heide (1965b) and Heide and Skoog (1967) established a strong reaction to changing auxin:cytokinin ratios in Begonia leaf cuttings. A high cytokinin level stimulated bud formation and inhbition of rooting while a high auxin content had the opposite effect. Short days and low temperatures favoured the production of endogenous cytokinins, a treatment also known to enhance bud formation. Long days and high temperatures favoured root initiation, conditions which were found to produce a high auxin:cytokinin ratio. Varga and Humphries (1974) found that changes in the auxin: cytokinin ratio which occurred after pretreating detached leaves of dwarf bean with cytokinins, were responsible for the failure of the petioles to form roots. They suggested that this ratio was altered partially by reducing protein decomposition, thus making less trypotophane available for auxin synthesis.

The possible mode of action of cytokinins in the inhibition of root initiation has been investigated. Humphries and Maciejewska-Potapczyk (1960) proposed that kinetin inhibited root initiation not by inhibiting cell division, but by influencing the kind of cell produced. They suggested it acted on the meristematic region of the pericycle of the cutting hypocotyl, from which roots originated, rather than on the organised root meristem itself. Chandra et al. (1973) found that BA caused extensive cell division and tracheid differentiation in mung bean hypocotyls, but inhibited root initiation. The cellular organisation usually associated with root initiation was not evident in the BA-treated cuttings. Root primordia already formed prior to treatment with BA, were not affected in any way. They proposed that the production of specific iso-enzymes necessary for root formation was prevented by BA, and that BA was only effective in

the very early stages of root induction. Smith and Thorpe (1975) verified the observations that cytokinins were only effective inhibitors of the pre-initiation phase of root initiation and that once primordia were formed, no inhibition of development occurred. They also concluded that cytokinins were effective by preventing the formation of meristematic root primordia, an opinion shared by Eriksen (1974) who found that kinetin blocked IAA activity in root promotion at a pre-initiation stage on pea cuttings.

4. Abscisic Acid

The effect of Abscisic Acid (ABA) on inhibition and retardation of growth processes in plants is well documented (Addicott and Lyons, 1969). The role of ABA in root initiation processes is relatively unknown and reports are often of conflicting opinions.

Heide (1968) while studying the role of growth regulators on the differentiation of buds and roots on begonia leaf cuttings, noted that bud formation was promoted by short days, suggesting the possible implication of ABA. Application of exogenous ABA promoted bud formation, a process opposed by auxins and GA. Reduction in the number of roots formed occurred at higher ABA concentrations, while bud formation promotion occurred at all concentrations. Heide compared this result with that of Linser (1948) who obtained an inhibitor from Syringia leaves which inhibited root initiation and opposed the action of IAA on Cochlearia officinalis. This inhibitor was considered to be similar to ABA. Eliasson (1969) in a study of growth regulators in Populus, noted the ease with which suckers initiated roots, compared with cuttings taken from the tree crown. He considered the difference was related to the difference in level of inhibitor B (ABA in part) between the two tissues. The bases of the suckers were low in inhibitor content, while there was a high content of inhibitors in the crown derived shoots. Tognoni and Lorenzi (1972) isolated an

acidic root promoting growth inhibitor from Picea glauca and Chamaecyparis lawsoniana. By comparative work it was considered that this inhibitor was more like ABA than any other compound. No synergistic response with IAA on root initiation in the mung bean bioassay was noted and the ease of propagation between the two species could not be related to the growth inhibitor. Pierek and Steegmans (1975) found the addition of ABA always had 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. One cultivar appeared to be tolerant to ABA since the inhibition was much less.

In comparison to reports of ABA-inhibition of root initiation, several reports demonstrate root-promoting activities of ABA. Chin et al. (1969) and Chin and Beevers (1969) found that ABA stimulated rooting in mung bean cuttings. IAA or ABA treatments both promoted rooting, but no evidence for synergistic or additive effects occurred. GA inhibition of rooting could be partially overcome by ABA, but the reduction of rooting by kinetin could not. With Hedera cuttings, the actively growing form rooted readily with no treatment, while the inactive, difficult-to-root, form exhibited rooting comparable with the active form, after treatment with 1 ppm ABA for 48 hours. With 10 ppm ABA both forms rooted equally well. Basu, Gosh and Sen (1968) detected ABA in shoots of mango and from its biological activity, denoted it as a naturally occurring mung bean rooting factor. Basu et al. (1970) found little response of mung bean to ABA up to 1.0 ppm but above this rooting was promoted. Tomato cuttings were significantly promoted in root initiation at 1.0, 10 and 50 ppm. In combination with IAA, ABA gave mainly additive effects. Very little promotion of rooting by ABA in the presence of IBA or NAA occurred on mung bean, french bean or tomato, and only in the 0.1 ppm ABA + IBA on tomato cuttings was any synergistic response evident.

5. Rooting Cofactors

During the initial research into auxins and root initiation, Went (1934) found that budless pea cuttings would not root even in an auxin-rich medium. Also substances in the cotyledons, leaves and buds which stimulated rooting of cuttings were isolated (Bouillenne and Went, 1933). This led to the postulation of the presence of specific factors other than auxin, which were produced in the leaves, and were necessary for root initiation (Went, 1938). This factor was termed 'Rhizocaline'.

Further evidence for the involvement of another factor other than auxin in root initiation was provided by Cooper (1935)(1936). Girdling could prevent root initiation on lemon cuttings treated with IAA. A partial girdle reduced rooting. Removal of the cutting base after the initial auxin treatment caused a large decrease in rooting which could not be replaced by a further IAA treatment. Cooper concluded that auxin plus another factor synthesised slowly in lemon leaves were necessary for root formation. Similar conclusions were reached by Gregory and van Overbeek (1945) and van Overbeek and Gregory (1945). Donor grafting an easy rooting red hibiscus scion to a difficult rooting white hibiscus stock, enhanced the rooting of the stock. Girdling prevented rooting on the compound cuttings even with a basal IBA treatment but a partial girdle did not reduce rooting. A factor other than auxin had been produced in the red hibiscus and translocated through the graft union. Further work by van Overbeek *et al.* (1946) concluded that the main function of the leaf was a nutritional one; sugars and nitrogenous compounds replacing the leaf function and these compounds being actually contributed by the hibiscus leaves. No evidence for a specific 'Rhizocaline' compound could be found.

Hess (1959, 1960, 1962a, 1964) reported the presence of several root promoting compounds in *Hedera helix* and *Hibiscus rosasinensis.* No dif-

ferences in auxins or inhibitors could account for the differences in root initiation ability of mature and juvenile Hedera helix cuttings, or in the red or white hibiscus varieties. Four "rooting cofactors" were isolated from Hedera as determined by activity in the mung bean rooting bioassay; the levels in the easily rooted juvenile stage being much higher than in the mature stage. The promotion of rooting was transferable across a graft union from a juvenile to a mature cutting. Hess found that the white hibiscus only contained one rooting cofactor, an intermediate rooting white variety contained three cofactors and the readily rooted red hibiscus contained all four, similar to those found in Hedera helix. Hess postulated that seasonal changes in root initiation may be due to changes in content of cofactors, especially when leaves were absent. Hess found with easy and difficult rooting Chrysanthemums, cofactor levels correlated with differences in rooting and that the cofactors corresponded to both Hedera and Hibiscus cofactors. With the development of the mung bean root initiation bioassay by Hess, many subsequent reports of rooting cofactors have appeared.

Richards (1964) equated the ease of rooting of Camellia species with the presence of a cofactor promoter, which was graft transmissible and promoted rooting in a difficult-to-root cultivar lacking the natural accumulation of the promoter. Lee et al. (1969) relate ease of rooting in Rhododendron cultivars to levels of cofactors detected. Promotion from easy to difficult rooting cultivars by donor grafts was achieved. Also seasonal changes in rooting were explained by fluxes in cofactor contents. Stoltz (1968) found that in a hard-to-root Chrysanthemum cultivar, the leaves had a high cofactor four content but the stem had a very low level when compared with an easy to root cultivar. Challenger et al. (1965) established an increase in the activity of a rooting promoter when Malus and Prunus hardwood cuttings were given high bottom heat while rooting, a treatment known to enhance root initiation. This pro-

moter of rooting inhibited coleoptile straight growth. Ashiru and Carlson (1968) also found cofactors in Malus rootstock cuttings. The differences in root initiation seemed to be due to an active inhibitor rather than differing levels of promoters as shown by reciprocal grafting experiments. By centrifuging cuttings, Kawase (1964a and b, 1965) demonstrated that a rooting promoter was present in Salix alba which synergistically promoted root initiation of mung beans with IAA. Rooting of Salix alba was reduced if the cutting base was removed after centrifugation. The diffusate of centrifugation was heat stable and most of the activity resided in the aqueous phase after basic and acidic ether partitions. Kawase proposed that this compound might be Rhizocaline and that it has been found in various other species using similar techniques. Fadl and Hartmann (1967a, b, c) correlated the ease of rooting with different levels of rooting cofactors in Pyrus hardwood cuttings. Treatments which improved rooting responses gave corresponding increases in the levels of cofactor promoters. Donor grafting and centrifugation both increased the activity of basal samples from cuttings. A potent promoter was isolated from cuttings which had received root promoting conditions for 10 days. It was considered to be a condensation product between exogenous auxin and a phenolic compound from the buds of Old Home pear. Quamme and Nelson (1965) isolated an unidentified auxin synergist from juvenile and adult tissue of Malus robusta 5 apple rootstock. This factor, more than the auxin levels, was related to the difference in propagation success of the two phases of the rootstock. Shah (1969) working on Citrus propagation noted that differences in propagation of cultivars, was directly related to the levels of cofactors or rooting inhibitors.

Rooting cofactors have also been located in coniferous plants. Zimmerman (1963) found active cofactors in several Pinus species and these appeared to be similar to those described by Hess. Similar contents were obtained from 22 year old flowering trees and one year old seedlings. It

was not established if they promoted rooting in pine cuttings. Lanphear and Meahl (1963) found four cofactors present in extracts taken from Taxus and Juniperus plant tissue. The level of these remained constant over the whole year even though the rooting ability of cuttings fluctuated. They suggest that the cofactor levels may reflect the rooting potential of the cuttings but other factors, particularly dormancy, also have a mediating effect on the formation of roots. Tognoni and Lorenzi (1972) isolated a coleoptile straight growth inhibitor from Picea and Chamaecyparis which gave root promotion activity in the mung bean bio-assay. Levels in both species were similar although rooting capacities varied widely.

Treatments known to enhance root initiation, such as girdling and etiolation have been examined for changes in rooting cofactor contents as an explanation of their efficacy. Stoltz and Hess (1966) studied levels of the four cofactors above and below and girdled stem of easy and difficult-to-root hibiscus cultivars. Changes in the level of cofactor four were most dramatic, with large increases in level occurring above the girdle in the easy rooting red variety. The difficult rooting white variety showed no such accumulation. This was in accordance with earlier work by Hess which related the difference in rooting ability to differing levels of cofactor four in Hibiscus. Etiolation is also a treatment known to improve rooting responses of cuttings. Herman and Hess (1963, 1966) examined the physiology of etiolation to find how rooting was improved. Only slight differences in auxin content occurred in etiolated and non-etiolated tissue. An indication of a higher 'cofactor' content in some etiolated tissue compared with non-etiolated tissue was obtained. Although bioassay of these cofactors was not entirely successful, a great response to added auxin in etiolated cuttings suggested the presence of an auxin synergist. The use of mist for propagation has been shown to have many beneficial effects. Lee and Tukey (1971) found on Euonymus alatus that

misting stock plants prevented the onset of dormancy and natural root inducing phenolics and flavanoids accumulated in the leaves. Consequently stem cuttings taken and grown under mist, rooted easily while cuttings from non-misted stocks rooted less well. Rutin, a flavenol similar to the naturally occurring ones, replaced the misting treatment of stock plants, resulting in equally well rooted cuttings as those from misted stock.

Characterisation of the four rooting cofactors isolated by Hess has been attempted, as well as identifying their mode of action. Most work has involved cofactor four which appears to be the most important one over a range of plants. All the cofactors are auxin synergists by definition. Hess (1961, 1965) identified cofactor four as a phenolic complex, more specifically a group of oxygenated terpenoids. Structural requirements for activity in the mung bean bioassay, seem to be an ortho-dihydroxy configuration with an open para position. Cofactor three has been shown to consist of at least partially, of isochlorogenic acid, another phenolic compound possessing an ortho-dihydroxy structure. Hess suggested that the first step in root initiation may be oxidative, under the control of these phenolic compounds, but experiments with phenolase inhibitors and reducing agents did not substantiate this (Hess, 1961). Bouillenne and Bouillenne-Walrand (1955) came to the same conclusions as Leopold and Plummer (1961), which suggested that IAA complexes with the quinones formed from the oxidation process, thus affording protection of IAA from enzymic oxidation. Using gradient elution techniques Girouard (1969) found five main peaks of promotion from the cofactor four complex in Hedera helix, all being identified as possessing oxygenated terpenoid characteristics. Hess had previously found that cofactor four from Hedera had three highly volatile peaks in the juvenile extract but these were absent in the mature extract, when analysed by gas chromatography.

Heuser and Hess (1972) have also isolated three lipid-like root promoting compounds from juvenile Hedera helix. These lipids were derived from the cofactor four complex and are unstable compounds.

During characterisation of cofactor four, Hess (1961) found the structural requirements of phenolic compounds which were synergistic with IAA in root initiation. Other unrelated reports also record the synergism of IAA by phenols in the rooting process. Bastin (1966) related auxin-induced rooting to an increase in the synthesis of phenolic compounds which are inhibitors of the IAA-oxidizing enzyme. Changes in the diphenol-like substances increased the level of endogenous IAA by inhibiting the IAA oxidation. Basu et al. (1969) treated Eranthemum cuttings with a range of phenolics prior to auxin treatments. None of the phenolics showed any activity singly. Tannic acid and gallic acid were synergistic with IBA and NAA but not with IAA. Salicylic acid promoted IBA, NAA and IAA-induced rooting and p-hydroxybenzoic acid was synergistic with IAA or NAA. These results agreed with those of Gorter (1962) in that no close structural resemblance between auxins and synergists occurred, and did not agree with Hess's structural requirements for synergism. If it is assumed that synergism is via IAA oxidase inhibition, then the enzyme system must be non-specific since IBA and NAA showed synergistic responses, also agreeing with the results of Gorter (1962). Poapst and Durkee (1967) found the oxidation products of a range of simple aromatic compounds from apple fruit greatly improved the rooting of dwarf bean cuttings. Effective molecular arrangements included vicinal di and trihydroxylation and para dihydroxylation. Methoxylation which blocked oxidation also nullified root promotion. Oxidation productions of phenols such as catechol and pyrogallol were nearer the active molecule than the phenolic itself.

6. Endogenous Rooting Inhibitors

Certain difficult-to-root plants may fail to form roots because of the presence of naturally occurring chemical inhibitors. These may act in the presence or absence of rooting promoters by changing the promoter: inhibitor balance, this balance probably being more important than the presence of any one promoter or inhibitor.

Spiegel (1955) found that rooting in Vitis cuttings was controlled by an auxin:rooting inhibitor balance. The difficult-to-root cultivar had a high inhibitor level which could be reduced by water steeping and cold treatment. These treatments resulted in improved rooting while an aqueous extract of the inhibitor could reduce the rooting response of the easy-to-root cultivar. Odom and Carpenter (1965) studying root initiation in several herbaceous species, located inhibitors but no correlation between their presence and the rooting of the cuttings could be established.

Fadl and Hartmann (1967a, b, c), working with pear hardwood cuttings, found that treatments which promoted rooting in Old Home cuttings, were not successful with Bartlett cuttings. Extracts from the bases of the two rootstocks, three weeks after planting showed that Old Home cuttings had a high level of strong rooting promoters while Bartlett had produced a considerable amount of inhibitors. These inhibitors could be reduced and rooting improved by simultaneous chilling of the buds while the cuttings were rooting. They proposed that the inhibitors were produced by the buds of Bartlett and that they interfered with the metabolic processes leading to adventitious root initiation. Ashiru and Carlson (1968) found that with apple rootstock hardwood cuttings, the difference between easy and difficult rooting clones was due to both cofactor and inhibitor levels. Reciprocal grafts resulted in the easy-rooting clone being inhibited significantly by a scion of the difficult-to-root clone. No significant

promotion of rooting was achieved with the opposite combination. Shah (1969) found that decreasing levels of rooting promoters and increasing levels of inhibitors accounted for the increasing difficulty of some clones of Citrus species to root from cuttings.

The rooting of offshoots of Phoenix dactylifera was positively correlated to carbohydrate levels and negatively correlated to rooting inhibitor levels (Reuveni and Adato, 1974). In all difficult-to-root offshoots, high concentrations of a rooting inhibitor (Rf 0.5-0.8) was found. Easy-rooting offshoots, whether by cultivar or position on the mother stock, invariably contained a lower level of rooting inhibitor.

Three closely related inhibitors from adult tissue of Eucalyptus grandis were isolated, one of them being determined by x-ray diffraction (Nicholls, Crow and Paton, 1970). The most closely related compounds appeared to be B-triketones. Each compound inhibited rooting of stem cuttings of E. grandis seedlings, E. deglupta seedlings and mung bean seedlings at 10^{-4}M concentration. The natural concentration in the plant (10^{-3}M) appeared sufficient to cause inhibition of root initiation on E. grandis. It was considered that the three inhibitors were responsible for the increasing rooting inhibitor level in successively higher leaf pairs and the related decrease in rooting ability as seedlings grew older.

The occurrence of root initiation inhibitors appears to be definitely established today. What is still to be determined is whether inhibition of root initiation is a function of a more general plant growth inhibitor such as ABA, or whether inhibitors of root initiation are species or genera dependent such as might be expected from Eucalyptus grandis. It is notable that reports on inhibitors of root initiation often demonstrate chromatographic zones which are similar to known plant inhibitors

(Challenger et al., 1965; Ashiru and Carlson, 1968; Reuveni and Adato, 1974).

7. Ethylene

With reports of IAA-induced ethylene production in plants, the effects of ethylene on adventitious root formation have been studied in several instances.

Initial work by Zimmerman and Hitchcock (1933) established the promotory activity of unsaturated hydrocarbon gases on adventitious root initiation. Ethylene, acetylene and propylene stimulated rooting in 15 plant species. Latent root primordia in hardwood cuttings were also stimulated to develop by the gases. Root hairs and secondary roots could be induced by intermittent exposure to the gases.

Kawase (1971) investigated the cause of centrifugal and water-steeping promotion of root initiation in cuttings of Salix fragilis. It was found that these treatments increased the ethylene concentration within the cuttings. With the demonstration of the root-promoting properties of ethylene and ethrel, it was proposed that centrifugation and the submerging of cuttings in water, caused an injection of water into the cutting, triggering a peak of ethylene production which in turn stimulated root initiation. In contrast, Mullins (1970) found that ethylene inhibited formation of adventitious roots on mung bean hypocotyl cuttings, but enhanced the emergence of pre-formed root initials. He proposed that the promotive effects of auxin on rooting were opposed by the inhibitory effects of auxin-induced ethylene, i.e. a feedback mechanism. Adventitious root promotion occurred when ethylene production was low compared to auxin concentration. Further work by Batten and Mullins (1975) concluded that ethylene was not directly involved in auxin-induced root promotion. No

relationship between various auxins, their rhizogenic ability or their capacity to induce ethylene biosynthesis was established. Application of ethylene at physiological levels had no effect on root initiation. The ineffectiveness of IAA in the mung bean bioassay was found not to be due to the stimulation of the biosynthesis of ethylene. Also ethylene had no effect on the IBA stimulation of rooting.

With only sparse and conflicting evidence on the role of ethylene in adventitious root formation, it is very difficult to draw any conclusions on the involvement of ethylene in this process.

D. Metabolism of Indoleacetic Acid during Adventitious Root Initiation

The definition of physical requirements for successful root initiation on cuttings and the related effects and correlation with exogenous and endogenous plant growth regulators has been well documented (Sections B and C). One clearly apparent factor was common throughout most of this review; the necessity and central importance of indole-3-acetic acid and/or its analogs in root initiation. Despite the many theories on root initiation, IAA is always considered an essential factor; the mechanism of its action being the controversial point in the current theories. While the metabolism of IAA in other plant processes has been extensively studied and reviewed (Schneider and Wightman, 1974), it is surprising that techniques for studying the metabolism of IAA during root initiation have been used only occasionally.

The first reported use of ^{14}C-IAA for studying root initiation was by Strydom and Hartmann (1960) on plum cuttings. They observed rapid absorption and movement of IAA-2^{14}C into the cuttings, the bulk of the IAA remaining in the basal portion of the cutting. After 12 hours, activity was detectable in $^{14}CO_2$, the level peaking after three days and then

decreasing to a lower level for the remaining 25 days. After 28 days, adventitious roots showed appreciable levels of activity, but this was probably not due to IAA but rather to some compound formed from $^{14}CO_2$ derived from the ^{14}C-IAA. Two possible mechanisms of destruction of IAA were apparent. Enzymic destruction resulting in $^{14}CO_2$ accounted for most of the loss of activity, but degradation by binding with phenolic compounds was also evident. Two methods of enzymic oxidation of IAA were proposed, both requiring the initial cleavage of the indole ring. Geronimo et al. (1964) virtually repeated the work of Strydom and Hartmann and also found the appearance of $^{14}CO_2$. The $^{14}CO_2$ peaked at three days, but did not reflect an increase in respiration, or coincide with the peak of auxin destruction. Considerable metabolism of the non-IAA-2^{14}C had occurred prior to the appearance of $^{14}CO_2$. 70% of the non-indolic activity was in aromatic compounds especially quinic and shikimic acid, suggesting that the shikimic acid pathway may be important in IAA destruction. No evidence of indoleacetyl aspartate formation was detected although an unidentified indole metabolite was located. They concluded that ring cleavage was an early reaction in the metabolism of IAA, but extensive metabolism occurred before $^{14}CO_2$ appeared. This suggested that $^{14}CO_2$ was produced by decarboxylation processes of a metabolite rather than by α or B oxidation as postulated by Strydom and Hartmann. It was interesting that, from both of these reports, no postulation of IAA mechanism of action in root initiation was discussed.

Catechol, the phenolic auxin rooting cofactor appeared to act partially by protecting and enhancing the IAA-aspartate conjugation (Hess, 1969). It was also shown that IAA-aspartate was very active in the mung bean rooting bioassay, but secondary metabolites were not. This tended to support Hess's cofactor theory for the mechanism of root initiation, since a conjugation product showed activity in the mung bean rooting test.

Ryugo and Breen (1974) agreed with Hess, that IAA-aspartate may be the first step to promoting root initiation by IAA. They found IBA enhanced the production of ^{14}C-IAA-aspartate in plum cuttings and ^{14}C-IAA-aspartate and ^{14}C-IAA could be obtained after a peptidase treatment of the alcohol-insoluble residue. 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 promotions (Galston, 1967; Kobayashi and Yamaki, 1972; Venis, 1972; Morris et al., 1969, Masuda, 1965; Masuda et al., 1967; Pilet and Braun, 1967; Tautvydas and Galston, 1970).

Lesham and Schwartz (1968) considered that the free IAA level was responsible for short day-induced root initiation of chrysanthemums. Dichlorophenol and/or tyrosine, both promoters of IAA-oxidase activity, reduced the rooting of the cuttings in inductive day lengths. Inhibition was also achieved with TIBA via IAA transport inhibition. Greenwood et al. (1974) also concluded that the free auxin level and not the conversion products were essential for root initiation. This was because 10^{-7}M NAA was more effective at promoting root formation than 10^{-6}M IAA, yet it formed conversion products much less rapidly. Continuous exposure to IAA was necessary for optimum root formation. Muir (1970) considered that the free-IAA level was important, not the conjugation products of IAA metabolism, and that increased growth was always related to increases in free IAA level. He illustrated this with growth in Avena coleoptiles, dwarfism in peas and GA-induced growth of savoy cabbage. This theory supported both Lesham and Schwartz and Greenwood et al. Various other reports indicated that conjugation of IAA, especially to IAA-aspartate was a detoxification process of excess IAA, converting it into a stable storage form which was inactive physiologically. (Andreae, 1967; Lantican and Muir, 1969; Morris et al., 1969).

Many workers recognise that one of the main methods by which IAA influences growth and development is by promoting the synthesis of new RNA, (Masuda, 1965; Masuda et al., 1967; Galston, 1967; Kobayashi and Yamaki, 1972; Morris et al., 1969) which in turn affects IAA oxidation and conjugation. (Sudi, 1964; 1966; Galston et al., 1968; Pilet and Braun, 1967; Lesham et al., 1970; Lee, 1971; Venis, 1972). By this feedback mechanism auxin-induced growth promotions may be regulated in vivo. The mechanism by which IAA induces the synthesis of new RNA, particularly in root initiation, is where current theories diverge, as seen in the literature just reported.

Rationale of the Present Work

Adventitious root initiation on cuttings is one of the most complex plant physiological phenomenon known. The theories on the mechanism of root initiation are many and varied, all substantially supported by the literature. A complex set of physical requirements has been shown to aid and improve the rooting response of many species of cuttings. Perhaps the largest field of study is the hormonal and biochemical regulation of root initiation which is still far from being clearly understood.

As with most physiological processes, certain physical treatments, known or observed to cause a response, can be explained and more closely understood by observing the concurrent biochemical changes induced by the treatments. Root initiation on cuttings is no exception, although the many and varied chemical processes observed, have contributed to demonstrating that a number of possible control mechanisms might be involved.

While the definition of physical requirements for successful root formation has been achieved in many plant species, the resistance to such treatments by difficult-to-root species only indicates that the processes involved are not fully understood. The objective of the present work was to

attempt to gain a further understanding in the control of the process of root formation on cuttings of woody shoots of apple rootstocks. This was achieved in the following manner:-

Section I:- Most complex physiological responses are probably the result of a delicate balance between hormones (Galston and Davies, 1969). Therefore by studying seasonal changes in endogenous growth regulators and relating these changes to seasonal variations in root formation of apple rootstock hardwood cuttings, a clearer understanding of the endogenous promotion and control of root formation might be achieved. This was done using easy and difficult-to-root clones of apple rootstocks. An attempt to locate the fundamental promoter of root formation was undertaken. The effects of root-promoting treatments of cuttings on endogenous levels of plant growth regulators and rooting processes were also investigated.

Section II:- Any promoter or inhibitor of root formation might be expected to be transferable across a graft union and influence a rootstock with the opposite rooting potential. To further identify rooting promoters or inhibitors, reciprocal cutting-graft transfers were carried out. Analyses of endogenous growth regulators and rooting trials were used to identify endogenous root-promoting substances.

Section III:- A study of the physiology of root formation and the influence of IAA upon this process was attempted. In relating the effect of an endogenous rooting promoter located in Sections I and II to the role of IAA in the rooting process, attempts to gain further information on the endogenous promotion of root formation in apple rootstock hardwood cuttings, were carried out. An investigation of the mode of action of root-promoting IBA hormone treatments was also attempted.

MATERIALS AND METHODS

A. Plant Materials

Mature stoolbeds of Merton-Malling 106 and East Malling XII apple rootstocks provided the source of plant material for experimental use. A widely different ability to produce adventitious roots when planted as cuttings made these rootstocks very suitable for studies on adventitious root initiation. Stoolbeds were maintained according to normal nursery practice with regular pest and disease control sprays being applied.

B. Collection and Preparation of Cutting Materials

Vigorous adventitious shoots approximately 1 cm in diameter, of current season's growth, were selected for use as cutting material. These were harvested 15 cm above ground level. Leaves were usually about to absciss, or had fallen but if necessary the leaves were removed immediately. Collection was always carried out in the early morning. Hardwood cuttings, 25 cm long, were made by trimming the basal end of the shoots to just below an axillary bud and trimming the rod to the required length.

C. Treatment and Planting of Cuttings

Considerable studies have been made on the physical requirements needed to induce root formation on apple rootstock hard wood cuttings. Most notable in this field has been research conducted at East Malling Research Station (Howard, 1966, 1968a, b, 1970, 1971, 1974; Howard and Garner, 1964, 1965). From this research and additional work on plum hardwood cuttings (Howard and Nahlawi, 1969a, b, 1970), a set of standard recommendations for improving the propagation of hardwood cuttings was developed. The East Malling technique for hardwood cutting propagation was used in this study.

Cuttings were wounded opposite the basal bud and then given a 10 second quick dip, 1 cm deep, in a 50% ethanol solution of 2,500 ppm Indolebutyric acid (IBA). They were then air-dried and planted 10 cm deep, in coarse river-washed sand. A temperature of $23^{o} \pm 2^{o}C$ was maintained by means of a thermostatically controlled electric cable, layed at 135 watts/ square metre and cuttings were planted to a depth 2.5 cm above the cable.

After six weeks on the heat bed, cuttings were lifted. The number of cuttings rooted and the number of roots per cutting was recorded. Cuttings were then discarded and the rooting trials terminated.

D. Production of Maiden Trees

The use of apple rootstocks grown under controlled conditions for manipulations such as reciprocal grafting, necessitated the production of maiden trees of each rootstock under glasshouse conditions.

Rooted stool stocks of MM 106 and EM XII, cut back to a length of 15 cm, were planted in 2 litre black polythene containers in a medium of coarse sand and peat, (1:1 v/v) in early spring (August). Nutrition was provided by the following fertiliser programme:-

Osmocote	18:2.6:10 = 2.5 kg/m^3
	14:6:11.6 = 1 kg/m^3
Superphosphate	1.5 kg/m^3
Lime	1.5 kg/m^3
Dolomite Lime	1.5 kg/m^3

This was estimated to be adequate for a growth period of 10 months. The potted stocks were lined out on foam plastic pads in a 6 m x 6 m glasshouse. Watering was achieved by capillary action from the foam plastic pads, these being fed from a header pipe via four microtubes per

pad. Watering time was controlled by a solenoid valve regulated by a time clock and was adjusted to suit the climatic and growing conditions as necessary. Once a week, the media was leached by overhead watering to remove any excess soluble salts. Two shoots on each tree were trained up during the growing season and in mid-summer were grafted.

Regular pest and disease control measures were provided for protection against powdery mildew and aphid infestations.

E. Reciprocal Donor Grafting

Reciprocal donor grafting has been a useful method of studying the presence and translocation of endogenous root-promoting regulators in comparative root initiation studies. The method has been employed by several workers in this field of study (Gregory and van Overbeek, 1945; Richard, 1964; Fadl and Hartmann, 1967; Ashiru and Carlson, 1968; Lee et al., 1969; Shah, 1969). The effects on root initiation of endogenous promoting and inhibiting growth substances can be identified in difficult or easy-to-root cuttings using this method.

Stocks to be grafted were sprayed with a commercial anti-transpirant ("Wilt-pruf") and cleft grafts of MM 106 scions onto EM XII rootstocks and vice versa were made. At this stage, the stock material was divided into four equal parts; MM 106 (ungrafted), MM 106 with EM XII scions, EM XII (ungrafted) and EM XII with MM 106 scions. Grafts were wrapped tightly with plastic tape and the scion and graft union completely covered with plastic bags.

One month after grafting, the unions were well callused and the plastic bags and tape removed. The scions commenced extension growth almost immediately and by autumn (April), had made approximately 30 cm of growth.

Just prior to dormancy, 1 cm wide girdles were made 15 cm below the graft unions, or in comparable positions on ungrafted stocks. This was to halt any downward transport of growth substances being exported from the scions or upper parts of the control plants. The distance below the graft union was 15 cm to ensure no rooting of the scions occurred when planted subsequently.

F. 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 sliced up and immediately frozen in dry ice and then freeze dried for 48 hours. Once dried, the tissue samples were stored i in sealed jars at $-15^{\circ}C$.

The stem tissue used for hormone extraction was taken from plant material considered suitable for use as cuttings.

G. Extraction of Plant Material for Hormone Analysis

(1) Initial Extraction

Determinations of endogenous levels of auxins, cytokinins, abscisic acid and rooting cofactors were made in conjunction with field experiments.

By comparisons with freeze dried and oven dried stem tissue, it was found that 5 grams of dried tissue were equivalent to 10 grams fresh weight. The procedure employed for initial extraction and purification was basically that described by Yadava and Dayton (1972).

A 5 gram sample of finely ground, freeze dried stem tissue was suspended in 100 ml of chilled 80% aqueous, redistilled methanol, giving a

10:1 v/w ratio of solvent to plant fresh weight. This was placed under refrigeration at 1°C for 18 hours in the dark, with intermittent shaking. After 18 hours, the extract was filtered and the residue extracted twice more in 5 volumes of 80% methanol for four 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) and dry ice trapping system, 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 x g for 40 minutes at 0°C.

(2) Procedure for Separation of Auxins, Inhibitors and Cytokinins

After centrifugation, the clear aqueous phase was adjusted to pH 2.5 with 50% HCl. This was then shaken vigorously with three separate, equal volumes of diethyl ether for five minutes each. The bulked ether fraction was backwashed for five minutes with 15 ml distilled water (pH 2.5) to remove any remaining cytokinins from the ether fraction (Park, pers. comm.). The aqueous phase of the backwash was combined with the initial aqueous extract and retained for further separation of cytokinins.

The ether fraction was then extracted twice with 50 ml volumes of 5% sodium bicarbonate (pH 8.5) by shaking for five 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 three 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 Abscisic Acid. The remaining aqueous fraction was discarded.

The Neutral and Acidic Ether fractions were dried over anhydrous sodium sulphate at 1°C for two hours, then filtered and evaporated to dryness in the rotary evaporator. The residue was redissolved 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.

The combined aqueous fraction and backwash from the first acidic ether extraction, was adjusted to pH 8.0 with 50% NH_4OH and extracted three times by shaking with equal volumes of water saturated n-butanol. The butanol fraction was reduced to dryness on the rotary evaporator and retained for assaying for the free bases and ribosides of cytokinins and the aqueous fraction was also retained for ribotides of cytokinins. These were both transferred to sample tubes by dissolving the residue in 100% redistilled methanol, dried under vacuum and stored at -15°C until required.

A summary of the extraction procedure is presented in figure 1.

(3) Extraction and Separation of Rooting Cofactors

Extraction and separation of samples for bioassay of rooting cofactors was initally achieved using the same procedure as for auxins and inhibitors. Inhibitory activity located in oat and wheat coleoptile bioassay of acidic ether fractions could be restricted to one zone of Rf 0.2-0.3 by using only 1 gram dry weight tissue samples. This was necessary, since no further purification of the extracts was undertaken, as rooting cofactors have been described as being phenolic in nature. Purification procedures used for other growth substances removed most of the phenolic content of the extracts.

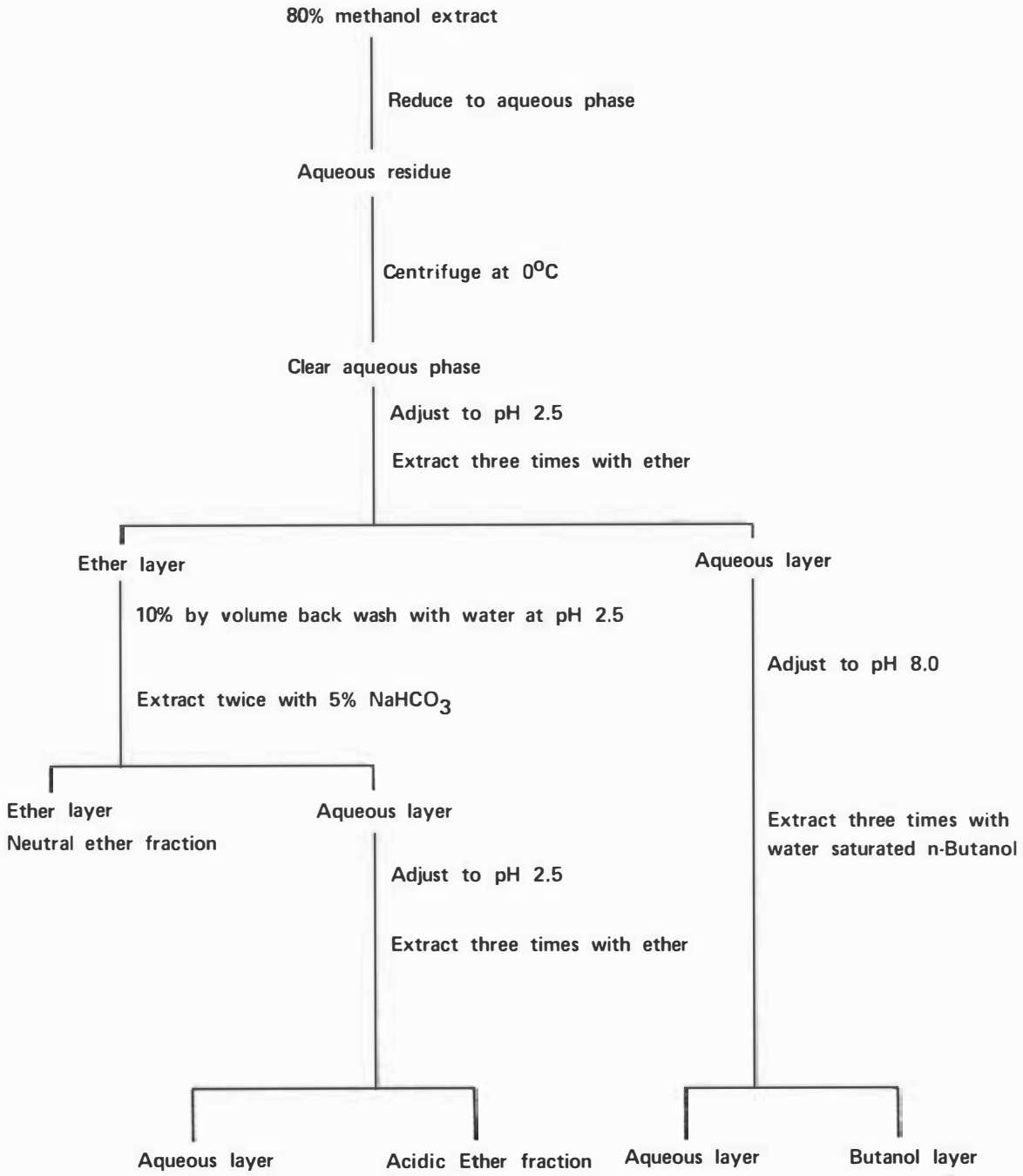

FIGURE 1

Summary of Extraction procedure.

A revised scheme for the extraction and separation of rooting cofactors was devised, to allow the examination of the aqueous fraction remaining after neutral and acidic ether extractions. A summary of this procedure is presented in figure 2.

H. Chromatography of Extracts for Hormone Analysis

Preliminary experiments with the purification and bioassay procedures to be employed, resulted in large areas of inhibitory activity being located from Rf's 0.1-0.5, in acidic ether extracts, tested in the wheat and oat coleoptile bioassays. It was assumed a large amount of the inhibitory activity was caused by the glycoside Phloridzin, since its chromatographic behaviour and effects on the bioassay were similar to those described by Hancock et al. (1961); Challenger et al. (1964); Grochowska, (1966); and Robitaille, (1970). Phloridzin is also known to be present in apple tissue in high levels. Because of the masking effect of the inhibitor further purification of the extracts was needed.

Polyclar A.T., an insoluble form of the polymer n-polyvinylpyrrolidone (P.V.P.) has been used by various methods to remove phenolic compounds from plant extracts (Simpson and Saunders, 1972; Lenton et al., 1971; Glenn et al., 1972 ; Hewett and Wareing, 1973). Hewett and Wareing (1973) slurried the aqueous alcohol extract with P.V.P. for 30 minutes. This was examined and found to be ineffective in removing the zone of inhibition, although added synthetic IAA and ABA were not bound to the P.V.P. Slurrying the aqueous phase prior to partitioning removed the zone of inhibitory activity from the extract, but when the technique was examined using synthetic IAA and ABA, it was found that 25% of the added IAA had been retained by the P.V.P. Because neither of these methods were entirely satisfactory, it was decided to examine the use of P.V.P. as a column.

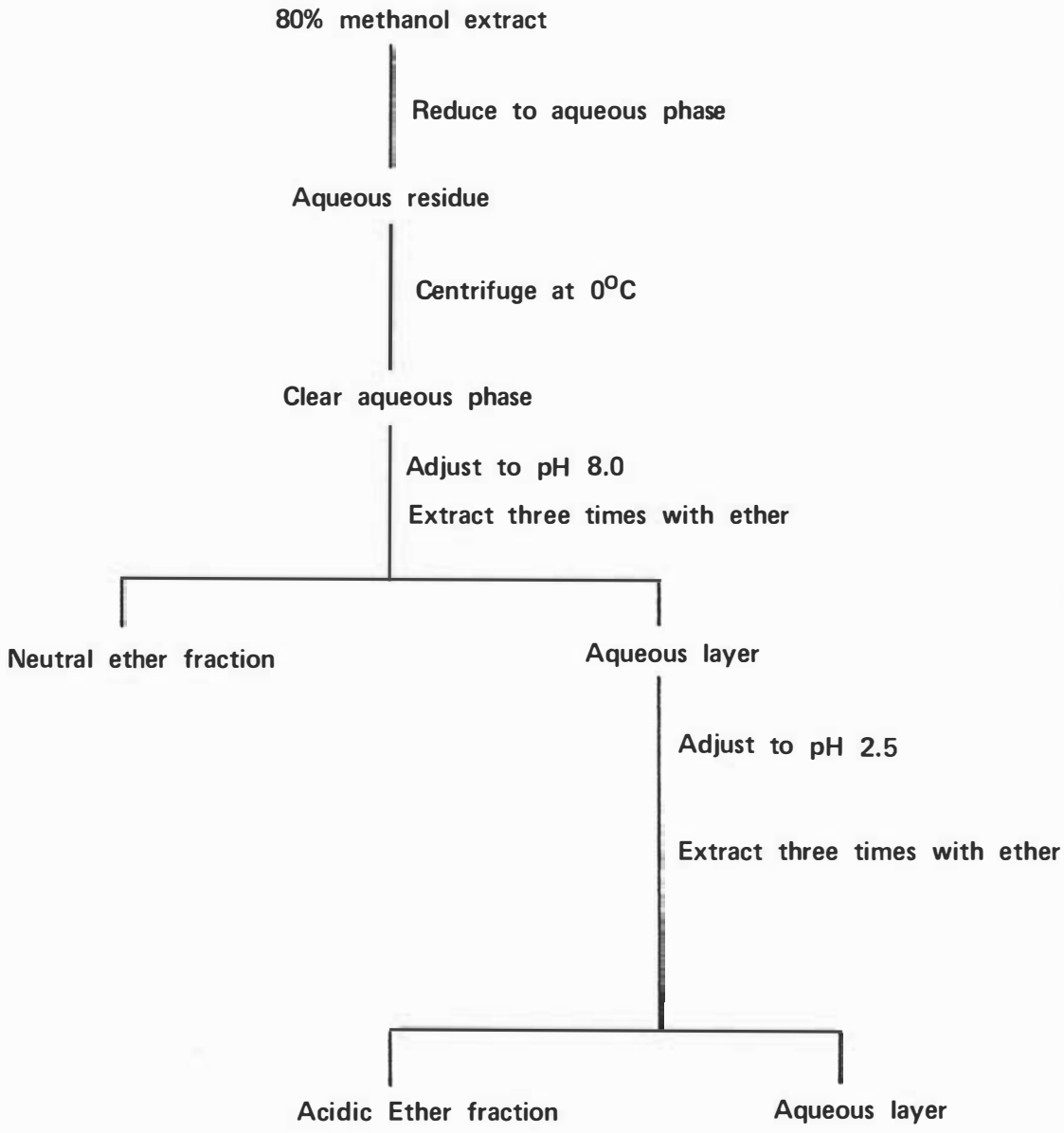

FIGURE 2

Summary of Revised Mung Bean Extraction procedure.

(1) Column Chromatography of the Acidic Ether Fraction

The P.V.P. was prepared in the manner described by Glenn et al. (1972). Dry P.V.P. powder, sieved to a particle size of 125-250μ diameter, was slurried in five times its own volume of distilled water and left to settle for 15 minutes. The fines were then decanted off and the process repeated for a further five times. To pack the column, the P.V.P. was suspended in an equal volume of distilled water and pured into a glass column (1.8 cm, internal diameter) and the excess water run off. The suspension was added until the final length of the packed column was 10 cm. A filter paper disc was placed on top of the P.V.P. packing, which was then washed exhaustively with 0.1M phosphate buffer (see Appendix I) at pH 8.0. The flow rate of such columns was approximately 100 mls per hour.

Characterisation of the column was achieved in the following manner. Approximately 1 mg of ABA or IAA was dissolved in 2 mls of ammonia solution (S.G. 0.91) and the ammonia was removed under vacuum on a rotary evaporator. The aqueous solution was then loaded onto the top of the column and subsequently eluted with buffer at pH 8.0. 10 ml fractions were collected and analysed on a Unicam S.P. 800 U.V. Spectrophotometer. Approximate elution volumes had been calculated from data of Glenn et al. (1972). First detection of ABA was in fraction 3, the bulk appearing in fraction 4. There was no detection in fraction 6. Recovery of ABA was calculated to be greater than 95%. First detection of IAA occurred in fraction 7 which also contained the bulk of the compound. No detection was evident in fraction 10. Recovery of IAA was calculated to be approximately 97%.

Because of the efficiency of recovery of ABA and IAA off P.V.P. columns, it was considered to be a suitable method for further purification of the acidic ether fractions. The dried acidic ether extracts were redissolved in 2 ml of ammonia solution (S.G. 0.91) and the ammonia was removed under vacuum. The sample was then loaded onto the top of the

column and eluted off with buffer at pH 8.0. Samples for ABA determinations were collected as a bulk fraction of 60 ml, while samples for IAA determinations were collected as a bulk fraction of 100 ml. The bulk samples were acidified to pH 2.5 and partitioned three times with equal volumes of diethyl ether and the combined ether fractions were reduced to dryness on the rotary evaporator. Samples were then transferred to test tubes, dried under vacuum and stored at -15°C until required for further purification.

Almost all the coloured pigments associated with the zone of inhibition were retained on the top 1 cm of the column and the purified plant extracts had only a faint pink colouration.

(2) Column Chromatography of the Neutral Ether Fraction

Column chromatography of neutral ether extracts was unsuccessful on P.V.P. Synthetic indoleacetonitrile (IAN) could not be recovered from a 10 cm column over a range of pH's from 8.0 to 4.0. Elution volumes of 500 ml were taken at each pH.

Silica gel columns were prepared by slurrying silica gel powder (100-200 mesh) in five times the volume of distilled water and left for 15 minutes. The fines were decanted and the process repeated a further five times. To pack the column, the silica gel was suspended in an equal volume of water and poured into a glass column (1.8 cm internal diameter) to a height of 10 cm. A filter paper disc was placed on top of the packing and the column was washed exhaustively with 0.1 M phosphate buffer, pH 5.0 (see appendix I). 1 mg of IAN was dissolved in 1 Ml of absolute methanol and 2 ml distilled water were added and the methanol was evaporated off on a rotary evaporator. The IAN solution was loaded onto the top of the column and 10 ml fractions of eluant were collected. IAN was first detected in fraction 5, the bulk of the sample eluting off in the 6th fraction. No IAN was detected in fraction 9. Recovery was calculated to

be greater than 90%.

Neutral ether samples were dissolved in 1 ml of absolute methanol and 2 ml of distilled water were added and the methanol was removed on the rotary evaporator. The samples were loaded onto the top of the column and eluted with 0.1 M phosphate buffer at pH 5.0. All the chlorophyll pigments eluted through the column in the first 30 mls. This was discarded and a bulk fraction of 70 ml was collected. The bulk samples were adjusted to pH 8.0 and partitioned three times with equal volumes of diethyl ether. The combined ether fractions were reduced to dryness and transferred into sample tubes for storage until required.

(3) Column Chromatography of Butanol-soluble Cytokinins

Several workers have reported the separation of cytokinins on insoluble polyvinylpyrrolidone (Polyclar A.T.) (Glenn et al., 1972; Thomas et al., 1975). Reported elution volumes are relatively similar and it appears that the elution volume for each compound is pH dependant.

Preliminary trials using preparations of synthetic cytokinins were conducted, using a 10 x 1.8 cm P.V.P. column connected with an ISCO, Type 6 optical unit and U.A.5 U.V. absorbance monitor.

Samples of single cytokinins and also mixtures of several cytokinins were loaded onto the column and eluted with distilled water or 0.01 M phosphate buffer at pH 6.0 and pH 8.0.

The results obtained suggested that at a slightly acid pH, elution volumes were nearer to those reported by Glenn et al. (1972). At pH 8.0 elution volumes were nearer to those reported by Thomas et al. (1975) at pH 6.4.

Good recovery of zeatin and zeatin riboside was obtained with the total sample of each cytokinin eluting in 3, 9 ml fractions or less. Zeatin riboside was eluted in the 5th and 6th fractions (36-50 ml) and zeatin, in the 6th, 7th, and 8th fractions (50-65 ml), at pH 8.0.

Butanol-soluble extracts were dissolved in 1 ml of ammonia solution (S.G. 0.91) and the ammonia was evaporated off under vacuum. The samples were loaded onto the top of the column and eluted off with 0.01 M phosphate buffer at pH 8.0. A bulk fraction of 100 ml was collected for each sample. The bulked eluate of each sample was partitioned three times with equal volumes of water saturated n-butanol at pH 8.0. The combined butanol fractions were reduced to dryness and transferred into sample tubes and stored until required.

(4) Paper Chromatography of Auxins and Inhibitors

Further purification, immediately prior to bioassaying, was achieved by use of paper chromatography 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). A 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, onto pre-washed Whatmann no. 1 chromatography paper. All paper used for chromatography was 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 (Wright and Hiron, 1969). 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 removed and the solvent front marked immediately. The chromatograms were air-dried for one hour and then dried under vacuum for a further 12 hours. The samples were now ready for measurement of

activity in the respective bioassays.

(5) Paper Chromatography of Rooting Cofactors

Acidic and neutral ether 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 streak-loaded onto 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.

(6) Paper Chromatography of Butanol-soluble Cytokinins

Chromatography of butanol-soluble cytokinins was carried out using the same technique as for auxins and inhibitors. The solvent system used was n-butanol: ammonia (4:1 v/v).

I. Bioassay Procedures

(1) Triticum Coleoptile Bioassay

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

Seed of the wheat variety "Aotea" was soaked in tap water for two 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 third day after sowing, the coleoptiles were 2 to 3 cm long and ready for use. 10 mm 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 25 mm glass vial, so that the paper was in contact with the wall of the vial. Ten coleoptiles were introduced into each vial, under the green safe light and each vial was capped with a plastic top with a needle hole in the centre. The vials were then placed in 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 3 x magnification.

With each bioassay, a standard series of ABA solutions from 0.001 μg/ml to 10 μg/ml in a log dilution series, was run concurrently. Strips of washed chromatography paper were 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.

(2) Avena Coleoptile Bioassay

Attempts to use wheat coleoptiles, employing the method of Nitsch and Nitsch (1956) for measuring levels of auxins were unsuccessful. Standard solutions in logrithmic concentration from 10 μg/ml to 0.001 μg/ml gave consistently poor responses.

By combining the technique of Sirois (1967) and Burström (1973), a very sensitive bioassay for auxins was developed. Initial experimentation demonstrated that the oat coleoptile was more sensitive than either the wheat coleoptile, which Burström used, or the oat mesocotyl.

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

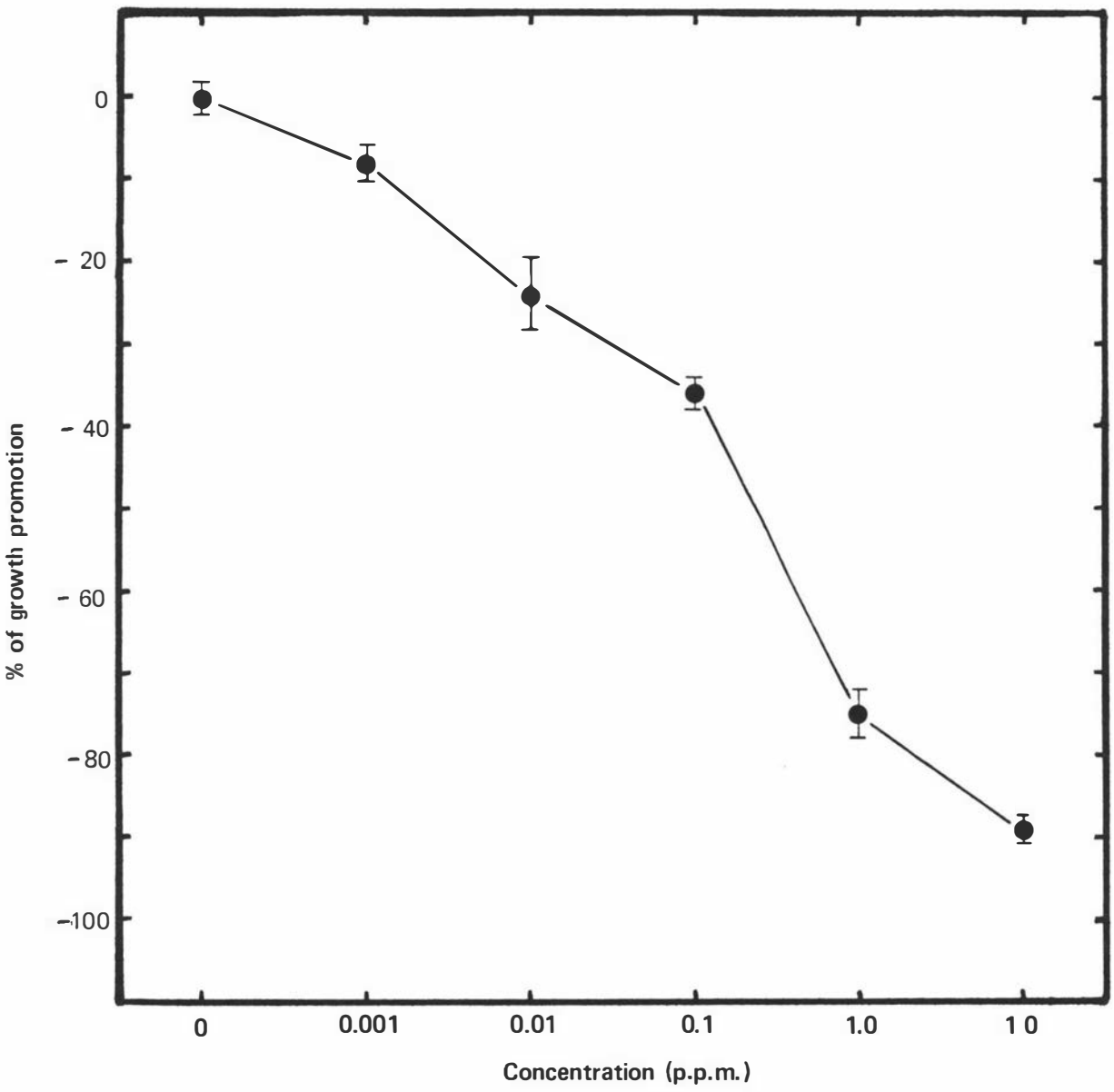

FIGURE 3

Standard curve for wheat coleoptile response to abscisic acid. Standard errors shown.

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 two 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 Burström's basal solution (see appendix I) for one hour prior to use.

Chromatograms were cut into 10 equal transverse strips, plus a control strip taken from above the base line and placed in 40 x 25 mm glass vials, as described in the Triticum coleoptile bioassay. 2 ml of Burström's basal solution was then added to each vial. All manipulations of the coleoptiles were carried out in the dark, under a green safe light. The vials were capped after loading and placed on the turntable and run at approximately 1 r.p.m. for six hours. The vials were removed and 2 ml of 10% methanol added to kill the coleoptiles. Measurements were done as previously described in the Triticum coleoptile bioassay.

Standards of IAA or IAN from 10 μg/ml to 0.001 μg/ml, were run concurrently with each bioassay. A strip of washed chromatography paper was added to each vial, to standardise the procedure. Standard curves for IAA and IAN responses are shown in figures 4 and 5. Standard errors were so small, they fell within the boundaries of the symbols denoting responses.

(3) Mung Bean Root Initiation Bioassay

Preliminary experiments, using a locally available, un-named source of mung bean (Phaseolus aureus) seed, indicated that variation was so great that an improved bioassay method was required.

Incorporation of Boron (Jackson and Harney, 1970), Nicotinic acid (Peters et al., 1974) and a dilute phosphate buffer, either separately or in combination, failed to give any improvement in the response, when used

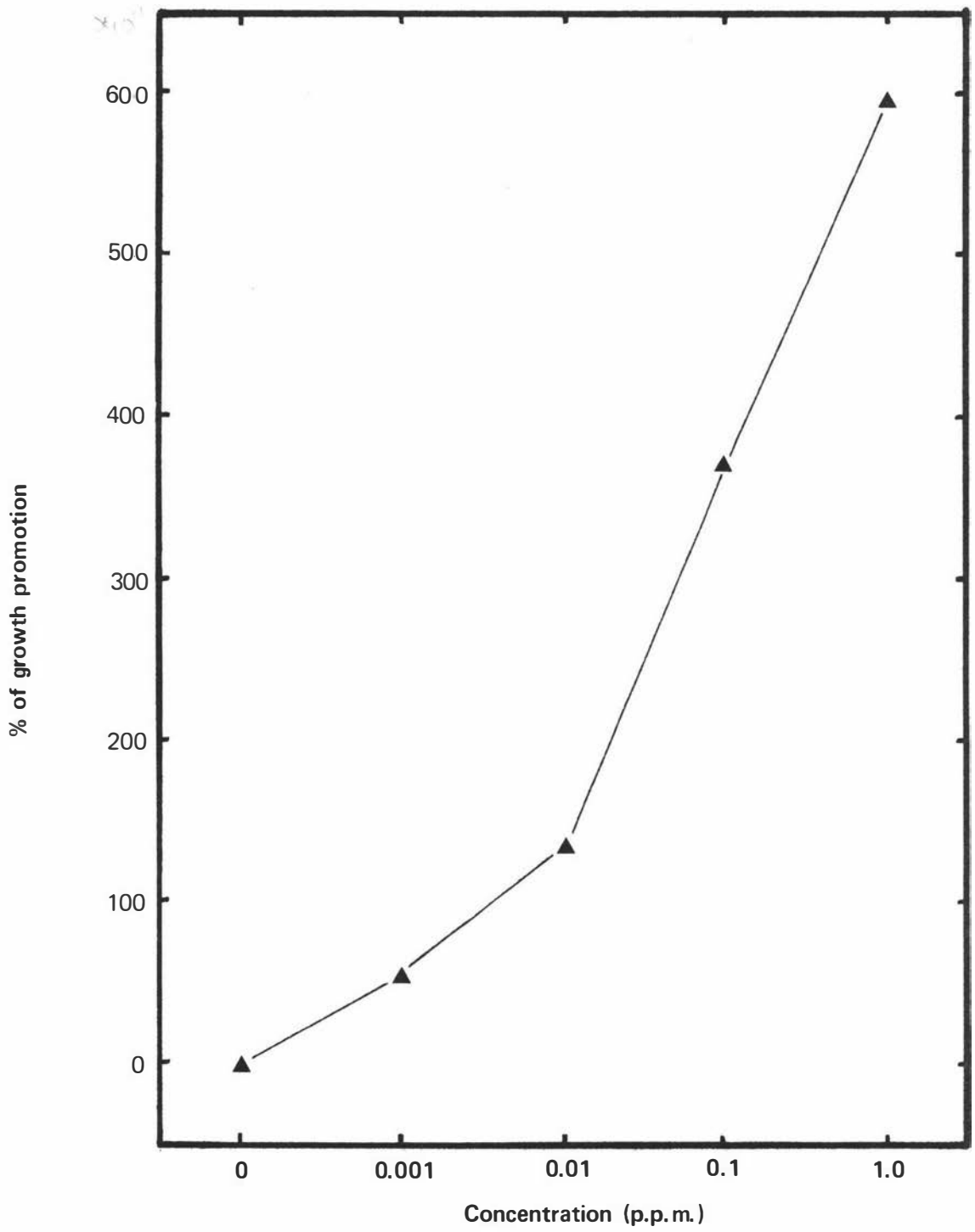

FIGURE 4

Standard curve for oat coleoptile response to indole-acetic acid.

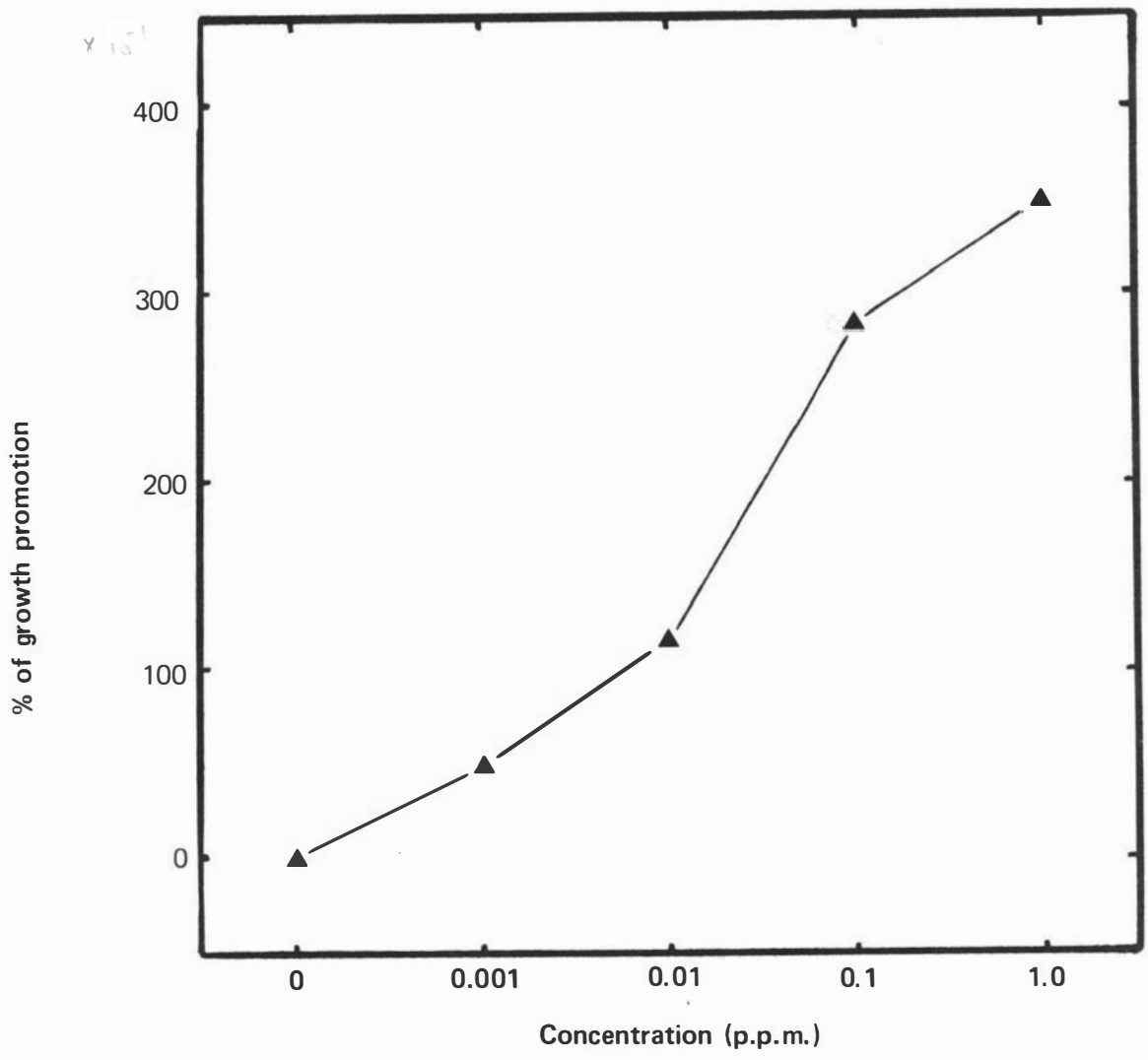

FIGURE 5

Standard curve for oat coleoptile response to indole-acetonitrile.

with 1 μg/ml IAA in the bioassay.

A selection of improved mung bean varieties was obtained from the Asian Vegetable Research and Development Centre, Taiwan, and screened for their response to added IAA (1 μg/ml) and Catechol (10 μg/ml), both separately and in combination (Challenger et al., 1964). It was noted that although the responses to added growth promoters varied widely, the variation between replicates in any one treatment, with all the cultivars, was reduced markedly. From these trials, one cultivar was selected and then tested with the addition of boron, nicotinic acid and phosphate buffer, either separately or in combination. No further improvement in response was achieved so none of these additional treatments were utilised in the bioassay.

Seed of mung bean, cultivar Tainan no. 1, were soaked in a chlorox: water solution (1:16 v/v) for five minutes and then washed in running tap water for 24 hours. The seed was then sown in moist, fine, pumice to a depth of 1 cm and placed in a high humidity cabinet at 27^{o}C under continuous light. Lighting was provided by seven double banks of 40 watt, cool, white fluorescent lamps, arranged above and around the cabinet. It was found that high humidity conditions were needed to maintain a low degree of variation of root initiation within replicates and that even small degrees of water stress had an adverse effect on both response and variation of the bioassay.

After four days the plants were ready for use. Cuttings were harvested at ground level and leached in distilled water for six hours (Zabkiewicz and Steele, 1974) to remove any exudate that was emitted from the cut bases of the cuttings.

Chromatograms of acidic and neutral fractions of stem extracts were

cut into 10 equal transverse strips plus a control strip taken from above the base line. Each strip was rolled and placed in a dark brown 40 x 20 mm glass vial and 6 ml distilled water with 1 μg/ml IAA was added to each vial. These were eluted for three hours in the dark and then used in the bioassay.

Cuttings of similar size were selected and cut 3 cm below the cotyledonary node. Any remaining 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 42 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 roots on the cuttings had developed sufficiently to allow counting. This took four days from the time the cuttings were placed in the test solutions.

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

(4) Radish Cotyledon Expansion Bioassay

The procedure of Letham (1971) was adopted for the bioassay of cytokinin activity in tissue extracts.

Radish seed (Raphanus sativus L.), cultivar Long Scarlet, Market Strain, retained by a 2 mm sieve, were soaked for five minutes in a 1% chlorox solution and then thoroughly rinsed. Seed was then sown on moist filter paper in plastic trays, covered with a sheet of glass and then grown in darkness at 25°C for 36 hours.

The smaller cotyledon of each seed was excised from the rest of the

seed making sure no part of the hypocotyl was retained. Chromatogram segments were cut up and placed in small petri dishes containing 2 ml distiled water (pH 6.5). Eight cotyledons of uniform size were placed in each petri dish, with the upper surface in contact with the chromatogram segment. The petris dishes were placed in a plastic tray on moist filter paper and covered with a sheet of glass. The trays were placed under continuous, low intensity, fluorescent light at 24°C and left for three days.

After 72 hours, the cotyledons were blotted dry with filter paper and weighed.

With each bioassay, kinetin standards from 3.0 μg/ml to 0.003 μg/ml were run and relative activity was estimated from these standard curves. A standard curve for the radish cotyledon response to kinetin standards is shown in figure 6.

J. Lanolin Pastes

The application of lanolin impregnated with plant hormones is a classical method of studying responses of plants or plant parts to the hormones applied. Woolley (1971) and Patrick and Woolley (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% or 1.0% IAA. The required amount of IAA was dissolved in a minimum quantity of ethanol and warm, semi-molten lanolin (35-40°C) was added until the correct proportion was obtained. The liquid mixture was stirred vigorously for some time to ensure complete homogeneity and then smeared thinly around the wall of a beaker and left

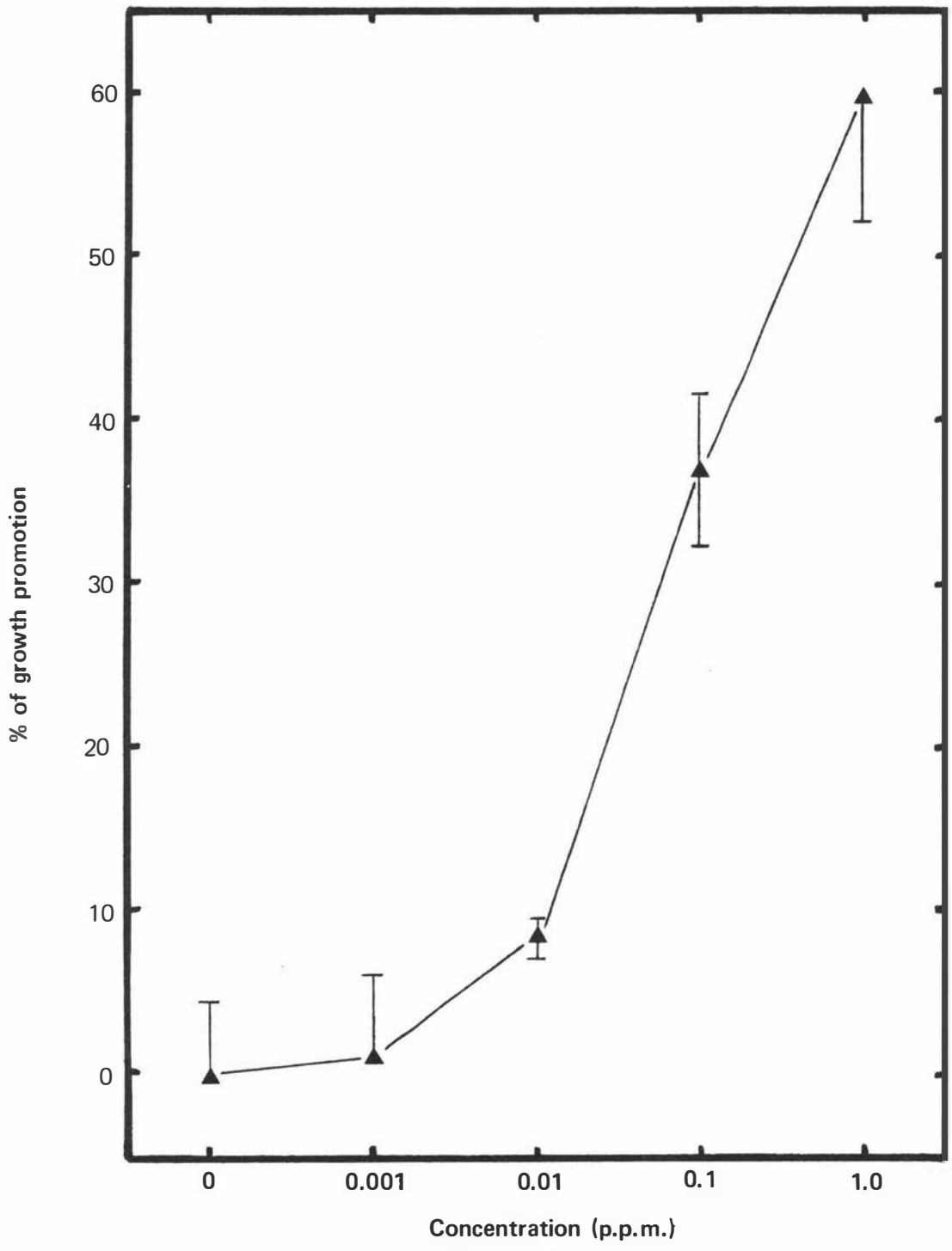

FIGURE 6

Standard curve for radish cotyledon response to kinetin. Standard errors shown.

in the dark at room temperature overnight for the ethanol to evaporate. The paste was then dispensed via a disposable plastic syringe into small gelatine capsules. The capsules were applied firmly to the cutting apices, and covered with tin foil caps to prevent photodegradation of the IAA.

K. Radioactive Chemicals

1-^{14}C-IAA (sp. act. 46 mc/mM) and 2-^{14}C-IAA (sp. act. 55 mc/mM) were obtained from the Radiochemical Centre, Amersham, Bucks.

All ^{14}C-hormones were in the ammonium salt form which allowed easy dissolution in water.

L. Radioassay of ^{14}C

The level of ^{14}C present in extracts and solutions was determined by counting on a Beckman LS-350, ambient temperature, beta-counting spectrometer.

(1) Scintillation Cocktails

For trapped $^{14}CO_2$

Toluene	1 litre
Ethylene glycol monomethyl ether	700 mls
Ethanolamine	100 mls
2,5 diphenyloxazole (PPO)	5.5. g/l

For aqueous methanolic extracts

Birks (1964) indicated the while 8 g PPO/l gave the greatest counting efficiency, reduction to 3-4 g gave considerable economy with only a small decrease in efficiency. The cocktail described by Patterson and Greene (1965) was used:

Toluene	1 litre
PPO	4 g
Triton x-100	500 mls

Scintillation vials were allowed to stand for 24 hours, in the dark prior to counting to allow chemiluminescence to fade.

(2) $^{14}CO_2$ trapping system

One problem with trapping $^{14}CO_2$ was doing so in a form which was suitable for counting. Methods of trapping which relied on precipitation did not lend themselves to liquid scintillation counting. The method of Jeffay and Alvarez (1961) for trapping CO_2 in a soluble form was used.

A solution of ethanolamine in ethylene glycol monmethyl ether (1:2 v/v) was made up and 9 ml placed in a scintillation vial. This was connected into a closed system using a Watson/Marlow peristaltic pump with a gas bubbler immersed to the bottom of the vial (Ryugo and Breen, 1974). Every 12 hours, the vials were replaced with new ones containing fresh trapping solution.

Two aliquots of 3 ml each of the solution were placed in scintillation vials and 15 mls of scintillation medium were added.

Scintillation medium: Toluene:- ethylene glycol monomethyl ether 2:1 v/v, PPO 5.5 g/l. This gives a final scintillation cocktail of:

Toluene: ethylene glycol monomethyl ether: ethanolamine 10:7:1 v/v.

(3) Preparation of External Medium for Counting

After 72 hours exposure to an ^{14}C-IAA solution, cuttings were rinsed thoroughly and the remaining external ^{14}C-IAA solution was made up to volume with 80% methanol. One aliquot was pipetted into a scintillation vial and counted while another was streaked onto Whatmann no. 3 MM chromatography paper and developed in isopropanol:ammonia:water (8:1:1.5 v/v). The radiochromatograms were cut into 10 equal transverse strips,

diced into small pieces and eluted in scintillation vials with 2 ml 80% ethanol for 24 hours at 0°C. 11 mls of scintillant were dispensed into each vial which was then capped, shaken and left in the dark to allow chemiluminescence to fade.

(4) Preparation of Samples for Counting

Cuttings were cut into small pieces and homogenised in a blender in chilled aqueous 80% methanol at 0°C and extracted for 48 hours. The extract was filtered and the filtrate reduced to 1 ml on a rotary evaporator and then made up to volume with 80% methanol.

One aliquot was counted immediately, while another was chromatographed and eluted in the same manner as external medium samples and prepared for counting.

(5) Treatment of Tissue Residue

The remaining woody tissue, after alcohol extractions, was immersed in 25 mls of 0.3N KOH and heated for one hour to hydrolyse any bound IAA, (Galston, 1967). After cooling and filtering, the filtrate was acidified to pH 2.5 and extracted three times with diethyl ether. The organic phase was reduced to dryness on the rotary evaporator and then dissolved and made up to volume with 80% methanol. One aliquot was counted and another was chromatographed, eluted and then counted.

(6) Counting of Radioactive Samples

The Beckman L-S 350 spectrometer has three channels into which iso-sets can be inserted for counting ^{3}H, ^{14}C and ^{32}P, either alone or in combination, and an external caesium-137 standard source from which quenching errors can be estimated. The master gain setting was set at 4.15 with the window width for counting set at the full ^{3}H and ^{14}C spectrum. This was achieved using Beckmann prepared standard ^{3}H and ^{14}C iso-sets and counting the lowest quenched sample at varying gain settings until most of the counts

fell in the ^{14}C channel.

Background counts were established by counting three non-active vials, containing the appropriate cocktail, prior to each counting run, for 10 minutes each. These were found to be 38-40 cpm for all runs and the average was subtracted from sample values.

Counting of active samples was achieved using a double check system. A pre-set counting error (20) programmed to give a 1.5% counting error for all samples over 1000 cpm was used. As soon as the 1.5% error was achieved, the teletype printed out the counts per minute. Samples of low activity were counted for 10 minutes if a 1.5% pre-set error was not achieved. All samples counted achieved a maximum counting error of 10%. Zones of radioactive metabolites of ^{14}C-IAA all achieved counting errors of 1.5%

(7) Quench Correction

Various factors in liquid scintillation counting cause quenching of prepared samples, resulting in a lower number of counts being recorded than are actually emitted. The main source of quenching was due to plant pigments discolouring scintillation mediums. A colour quench correction curve, based on an external standard ratio was prepared (figure 7) to allow accurate calculation of activity.

A sample of apple stem tissue equivalent to the volume of tissue to be extracted experimentally was homogenised and extracted for 48 hours in 80% methanol. The alcohol filtrate was reduced to 1 ml and made up to volume with 80% methanol. A linear dilution series was made up so that each vial contained 2 ml, 80% methanol with varying degrees of colour. 11 ml of Toluene-Triton scintillation cocktail with a known amount of radioactivity added, was dispensed into each vial.

Counted samples were usually within 5% efficiency of the least quenched samples; larger differences in quenching were corrected by reference to the colour quench correction curve.

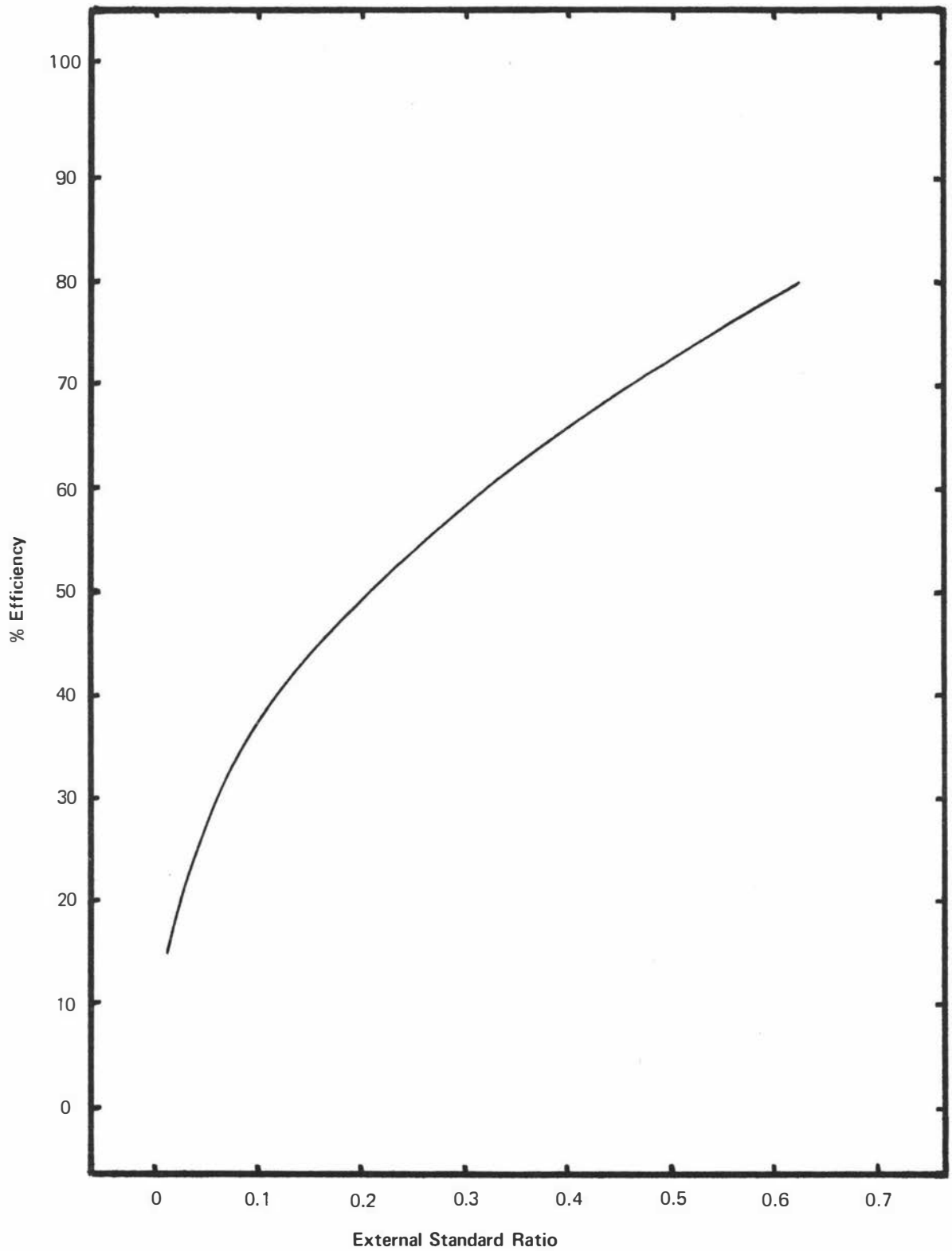

FIGURE 7

Colour Quench Correction Curve.

Least quenched samples of both cocktails used gave counting efficiencies of 80%.

EXPERIMENTAL

Section I

Seasonal Changes in Root Initiation, Bud Dormancy and Hormonal Status in MM 106 and EM XII Apple Rootstock Hardwood Cuttings

A. Seasonal Changes in Root Initiation of Hardwood Cuttings

Introduction and Methods

The need to develop a satisfactory alternative to traditional methods of fruit tree rootstock propagation has been emphasised over the last decade. Extensive study in the UK and USA has demonstrated the potential of using hardwood cuttings subjected to certain root-inducing hormone and high temperature treatments, particularly with apple rootstocks. While the physical treatments which promote root formation on cuttings have been well defined, very little study of the hormonal physiology associated with rooting promotion has been reported. Another facet relatively unexplored is the factors associated with the widely varying responses to root formation promoting treatments.

Many physiological factors can affect the ability of a hardwood cuttings to initiate roots (Section B, Literature Review). To enable the study of seasonal changes in the ability of apple rootstock hardwood cuttings to form adventitious roots, juvenile plant material from established stool beds was used to minimise complicating factors such as the age and condition of the parent plant. Details of specific treatments of plant material are presented in the Materials and Methods. The use of juvenile plant material also allowed the study of inherent factors affecting the process of adventitious root formation in apple rootstocks, particularly the endogenous hormonal physiology.

Cuttings of MM 106 and EM XII apple rootstocks were harvested from stools each month, from a period in late autumn (April) until the spring bud burst (October). Cuttings 25 cm in length were treated according to the East Malling hardwood cuttings technique (Howard, 1966, 1968b, 1971; Howard and Garner, 1964) and four blocks of 10 cuttings each, of each rootstock were planted in the heat bed. Another four blocks of 10 cuttings of MM 106 and EM XII rootstocks were collected at the same time and treated in a similar fashion, except that they were dipped in 50% ethanol only, i.e. no exogenous auxin treatment was applied.

Cuttings were stored in the heat bed for six weeks and then lifted carefully and the number of rooted cuttings recorded. Each trial was terminated at this point and unrooted cuttings were discarded.

The last harvest of MM 106 cuttings was taken in September at a time when bud movement had just begun. Bud movement in EM XII occurred one month later so an extra harvest of cuttings was taken in October. Axillary bud development on MM 106 rootstocks in October was so advanced, that the shoots were considered unsuitable for use as cutting material for propagation purposes.

Results

Preliminary experiments indicated a large difference in the ability of MM 106 and EM XII apple rootstocks to initiate roots when planted as hardwood cuttings. This difference in root formation was maintained throughout the harvesting time of April to October, in cuttings both treated and untreated with IBA (figure 8).

A very high level of rooting was achieved throughout the whole season, with MM 106 treated with IBA. There was no significant difference between

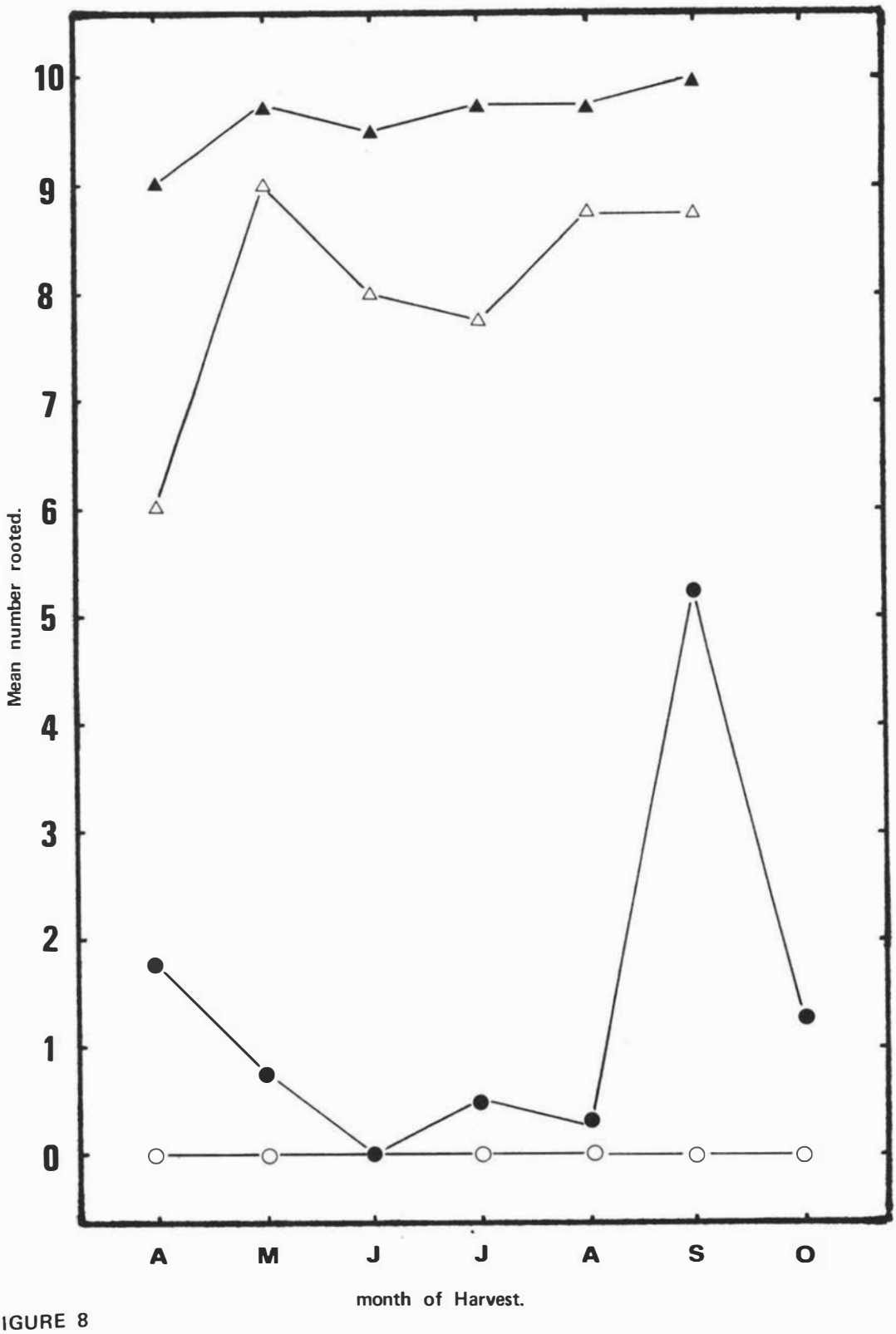

FIGURE 8

Seasonal Changes in Root Initiation of Root Initiation of hardwood cuttings of apple rootstocks. ▲—▲ MM 106 + IBA. △—△ MM 106 – IBA. ●—● EM XII + IBA. ○—○ EM XII – IBA.

any harvest dates and at all harvests, 90% or more cuttings rooted. MM 106 hardwood cuttings not treated with IBA tended to vary more in their response, with the April harvest being significantly lower than the other harvest dates (see Table 1.)

Table 1. Mean Number of Cuttings Rooted on Successive Harvest Dates

	Month of Harvest						
	April	May	June	July	August	Sept.	Oct.
MM 106 + IBA	9.0 a	9.75 a	9.5 a	9.75 a	9.75 a	10 a	- N.S.
MM 106 - IBA	6.0 b	9.0ab	8.0 b	7.75 b	8.75ab	8.75 b	- 5.0%
EM XII + IBA	1.75 c	0.75 c	0.0 c	0.5 c	0.25 c	5.25 c	1.25 c 1.0%
EM XII - IBA	0.0cd	0.0 d	0.0cd	0.0 d	0.0cd	0.0 d	0.0 d N.S.

Treatments joined by the same line were not significantly different when analysed by Duncan's Multiple Range Test.

EM XII hardwood cuttings treated with IBA showed very low numbers of cuttings rooted on successive harvest dates until September, when a large increase in the mean number of cuttings rooted occurred. This time coincided with axillary bud burst on the cuttings, shortly after being placed on the heat bed. Successive harvests of EM XII hardwood cuttings not treated with IBA, failed to root. A comparison between each treatment on

each harvest date also yielded some interesting data (see Table 1). Treatments sharing the same letter (looking vertically) are not significantly different when analysed by T-tests.

Treating MM 106 cuttings with IBA gave significantly higher numbers of cuttings forming roots with the exception of May and August where there was no significant difference between treated and untreated cuttings. Untreated cuttings of MM 106 rooted more readily than any of the EM XII cuttings, irrespective of their treatment ($P \geq 0.05$ minimum).

EM XII hardwood cuttings treated with IBA, fluctuated quite widely in response, with significant differences from untreated EM XII cuttings in May, July, September and October. Nevertheless, only low numbers of cuttings formed roots, with the exception of the September harvest, when a large increase occurred in the number of cuttings rooted.

B. The Relationship between Bud Dormancy and Root Initiation

Introduction and Methods

The role of bud dormancy in the control of adventitious root initiation is both obscure and complex. Conflicting reports have been made, pertaining to the influence of dormant buds on inhibition of root initiation of cuttings (Fadl and Hartmann, 1967a; Howard, 1968a). An investigation to find if any correlation existed between the ability of cuttings to initiate roots and the ability of the buds on the cuttings to shoot, was undertaken.

At each harvest date, 10 cuttings of MM 106 and EM XII rootstocks 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. When 50% of the cuttings had exhibited bud movement (i.e. sprouting had begun) the number of days from harvest was recorded.

Results

The results are presented in Table 2.

Table 2. Time in days, to 50% bud burst of cuttings of MM 106 and EM XII apple rootstocks

	Month of Harvest						
	April	May	June	July	August	Sept.	Oct.
MM 106	7	12	12	11	9	0	-
EM XII	7	12	14	failed to shoot	11	8	5

Both MM 106 and EM XII hardwood cuttings tended to take similar times for buds to renew activity while going into winter rest. In later winter, it was noticeable that the buds of MM 106 cuttings resumed activity more quickly than EM XII buds. Buds on the established stools in the field, burst one month earlier on MM 106 than on EM XII

C. Seasonal Changes of Endogenous Growth Regulators of MM 106 and EM XII Apple Rootstocks

Methods

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

Samples were 80% methanol extracted and purified initially by basic and acidic ether extractions (Section G, materials and methods). Further purification was required and was achieved by column chromatography and paper chromatography (section H, materials and methods).

Additional IBA-treated cuttings of both rootstocks were planted in

the heat bed at each harvest date and these were lifted after being on the heat bed for three weeks. The basal 5 cm was cut off and freeze-dried for examination of endogenous growth regulators. These samples should reflect the hormonal status of the cutting while undergoing adventitious root initiation.

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 oat coleoptile bioassay, Abscisic Acid, using the wheat coleoptile bioassay, Cytokinins using the radish cotyledon expansion bioassay and rooting cofactors were located using the mung bean bioassay. Details of these procedures are presented in section I of the Materials and Methods.

Results

(1) Auxins

An acidic growth promoter, the same or similar to IAA was isolated chromatographically from both MM 106 and EM XII apple rootstocks. 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:ammonia: water (10:1:1 v/v).

Values of promoter concentration are expressed as μg equivalents per 5 grams dry weight of plant stem tissue and are plotted in figure 9.

Confidence limits of P = 0.01 were calculated by the Link and Wallace method (Tukey, 1953) for histograms derived from the oat coleoptile bioassay. Values below 0.001 μg/ml IAA (from standards run concurrently) were not significantly different from controls but are plotted in figure 9 for comparative purposes. These are the EM XII values for three week harvest dates of April, May, June and July.

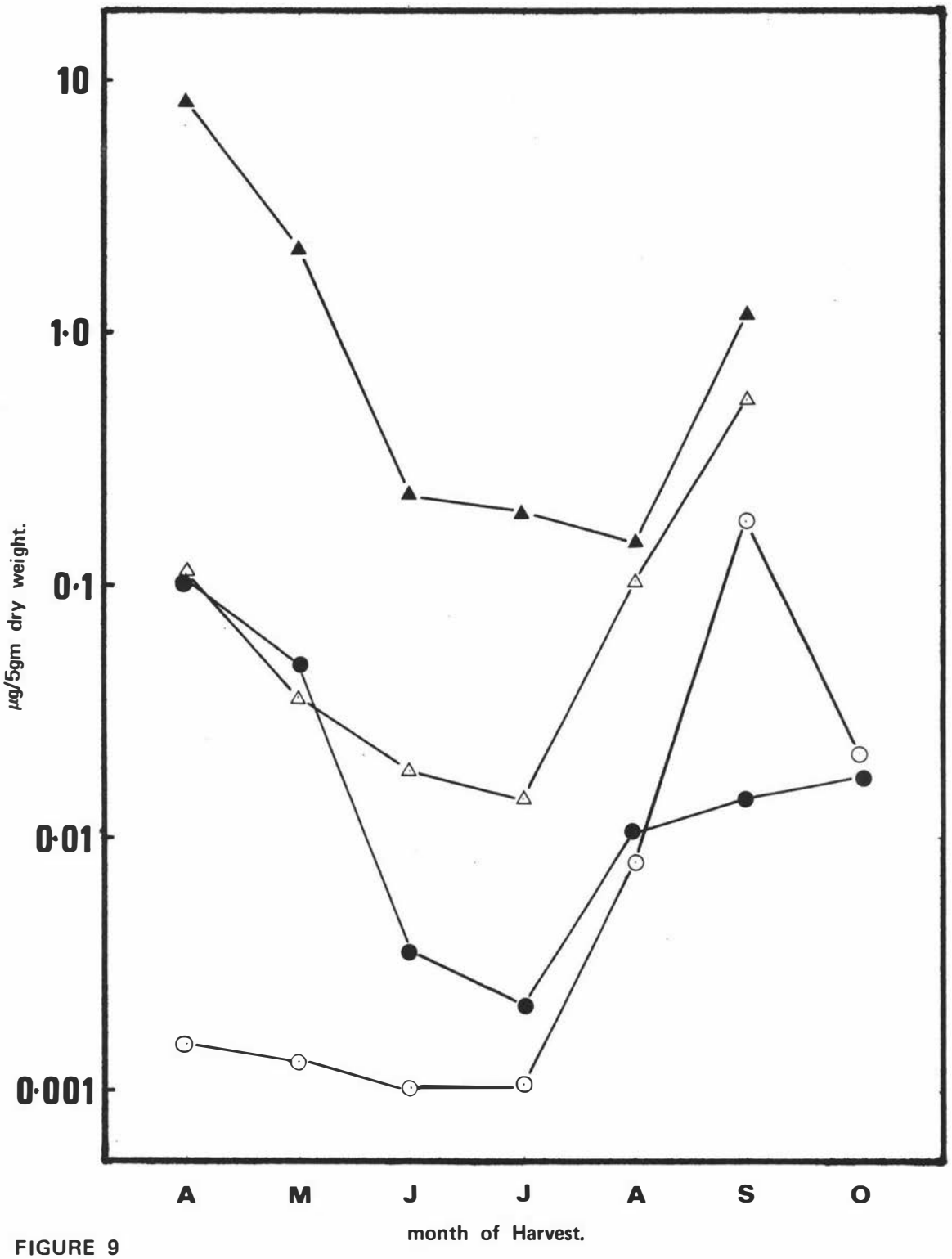

FIGURE 9

Seasonal Changes of an acidic growth promoter, the same or similar to IAA, determined by the oat coleoptile bioassay of the acidic ether fractions of stem tissue samples of apple rootstocks.

▲—▲ MM 106 △—△ MM 106 3 week ●—● EM $\overline{\text{XII}}$. ○—○ EM $\overline{\text{XII}}$ 3 week

The notations 'MM 106 three week' and 'EM XII three week' refer to samples taken from cutting bases which had been treated with IBA and planted on the heat bed for three weeks and then lifted and sampled.

A very significant difference in the IAA content of the two rootstocks existed at all harvest dates throughout the season; MM 106 had a level more than 10 times that of EM XII at any single harvest date, with the exception of the September three week sample.

Both MM 106 and EM XII show similar trends from autumn until spring, with higher levels in autumn, decreasing to a minimum in mid winter and subsequently increasing in early spring when bud burst occurred on cuttings on the heat bed soon after being planted. A quite dramatic decrease in IAA content occurs within the cutting bases of two rootstocks while on the heat bed. MM 106 three week and EM XII three week samples show large decreases in IAA content after three weeks on the heat bed. This sampling time coincides with the emergence of roots from cutting bases. It is notable that this decrease is virtually eliminated in early spring when buds are beginning to shoot while on the heat bed (August, September) and that a peak of IAA production in EM XII occurs in the September three week sample. This peak coincided with the significant promotion of root initiation in EM XII cuttings shown in figure 8.

A neutral growth promoter chromatographically similar to indoleacetonitrile (IAN) was also isolated from both MM 106 and EM XII apple rootstocks. Considerable promotive activity in the oat coleoptile bioassay was observed in Rfs 0.7-0.9 from paper chromatograms developed in isopropanol:ammonia:water (10:1:1 v/v).

Values of promoter concentration are expressed in a similar way as the IAA levels and are represented in figure 10. May, June, July, April

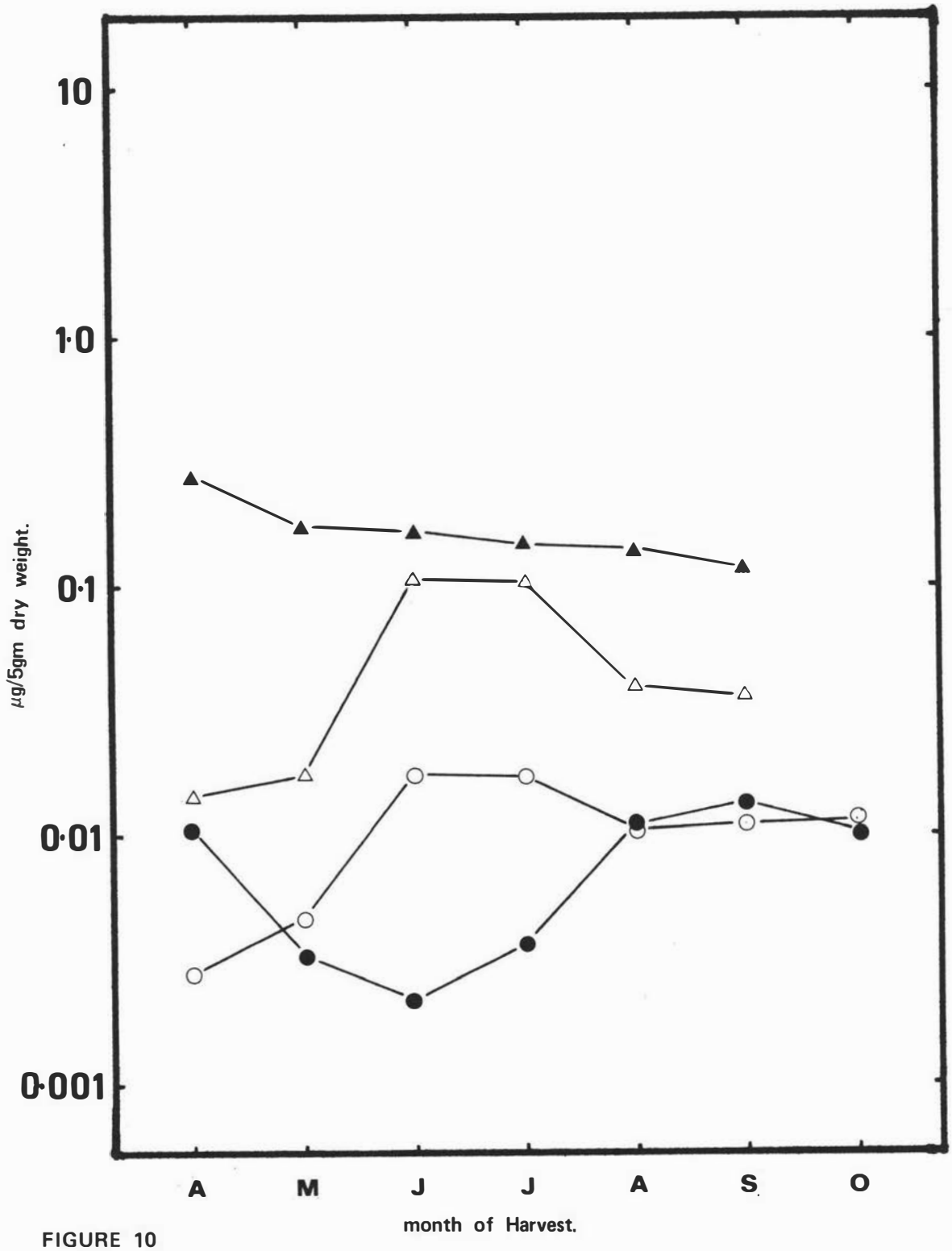

FIGURE 10

Seasonal Changes of a neutral growth promoter, the same or similar to IAN, determined by the oat coleoptile bioassay of neutral ether fractions of stem tissue samples of apple rootstocks.

▲—▲ MM 106 △—△ MM 106 3 week ●—● EM XII. ○—○ EM XII 3 week

three week and May three week samples from EM XII were not significantly different from controls but are plotted for comparative purposes.

Although the absolute levels of IAN in MM 106 and EM XII were lower than for IAA, the same magnitude of difference in seasonal levels between the two rootstocks existed. Levels of endogenous IAN in MM 106 did not change much through the winter, a slight decrease being evident. IAN levels from EM XII followed a similar trend as IAA from EM XII, with higher levels in autumn and spring than in mid winter.

Samples taken from cuttings after being on the heat bed for three weeks initially showed the same trend as IAA, with large decreases in level occurring. In the winter months, the recorded decrease in MM 106 was much smaller and in EM XII, higher levels of IAN were recorded than from the initial samples. In early spring the level in MM 106 three week samples decreased again and the EM XII three week samples remained at approximately the same concentration as the initial samples indicated.

(2) Abscisic Acid

Marked inhibitory activity was observed in acidic ether extracts at Rf 0.5-0.7, the same zone described by Bennet-Clarke and Kefford (1952). ABA marker spots run concurrently with extracts in isopropanol:ammonia: water (10:1:1 v/v) corresponded to the inhibitory zone. A Polyclar A.T. separation, similar to that used by Lenton et al., (1971) prior to GLC of endogenous ABA, removed virtually all the phenolic contamination from the apple tissue extracts. Since phenolic compounds constitute most of the inhibitory activity other than ABA, the inhibitory activity located was probably mainly due to endogenous ABA.

ABA was isolated chromatographically in both MM 106 and EM XII stem tissue samples. Values of inhibitory activity are expressed as μg ABA

equivalents per 5 grams dry weight of stem tissue sample and are shown in figure 11.

In tissue samples taken at harvest dates, similar levels of ABA were found to be present in both MM 106 and EM XII rootstocks. The seasonal trend in ABA levels closely followed the trend of the buds' ability to shoot and renew growth, over the same time period. Samples taken from cuttings which had been on the heat bed for three weeks showed quite wide variation, but in general, the levels of ABA in both rootstocks showed a considerable decrease, except in the early spring months. In August, the levels were approximately the same as the initial harvest, while in September they rose considerably higher than the initial levels recorded. The increased levels of ABA in the three week samples coincided with the bursting of buds and renewed growth while the cuttings were still undergoing root initiation.

(3) Rooting Cofactors

An acidic rooting promoter was isolated in the acidic ether fractions of MM 106 and EM XII extracts at Rf 0.7-0.9 on chromatograms developed in isopropanol:ammonia:water (10:1:1 v/v). Levels were high in April but relatively low in subsequent months in both rootstocks, (figure 12), when assayed in the mung bean bioassay. Bioassays of samples taken from the heat bed after three weeks showed that the promoter had disappeared or the activity was not significant.

Using a revised separation system (figure 2), the aqueous fraction was bioassayed after neutral and acidic extractions with ether. Since a full seasonal analysis was not possible, June and June three week samples of MM 106 and EM XII rootstocks were assayed. A very active zone of promotion was detected at Rf 0.2-0.5 in both rootstocks, using the solvent

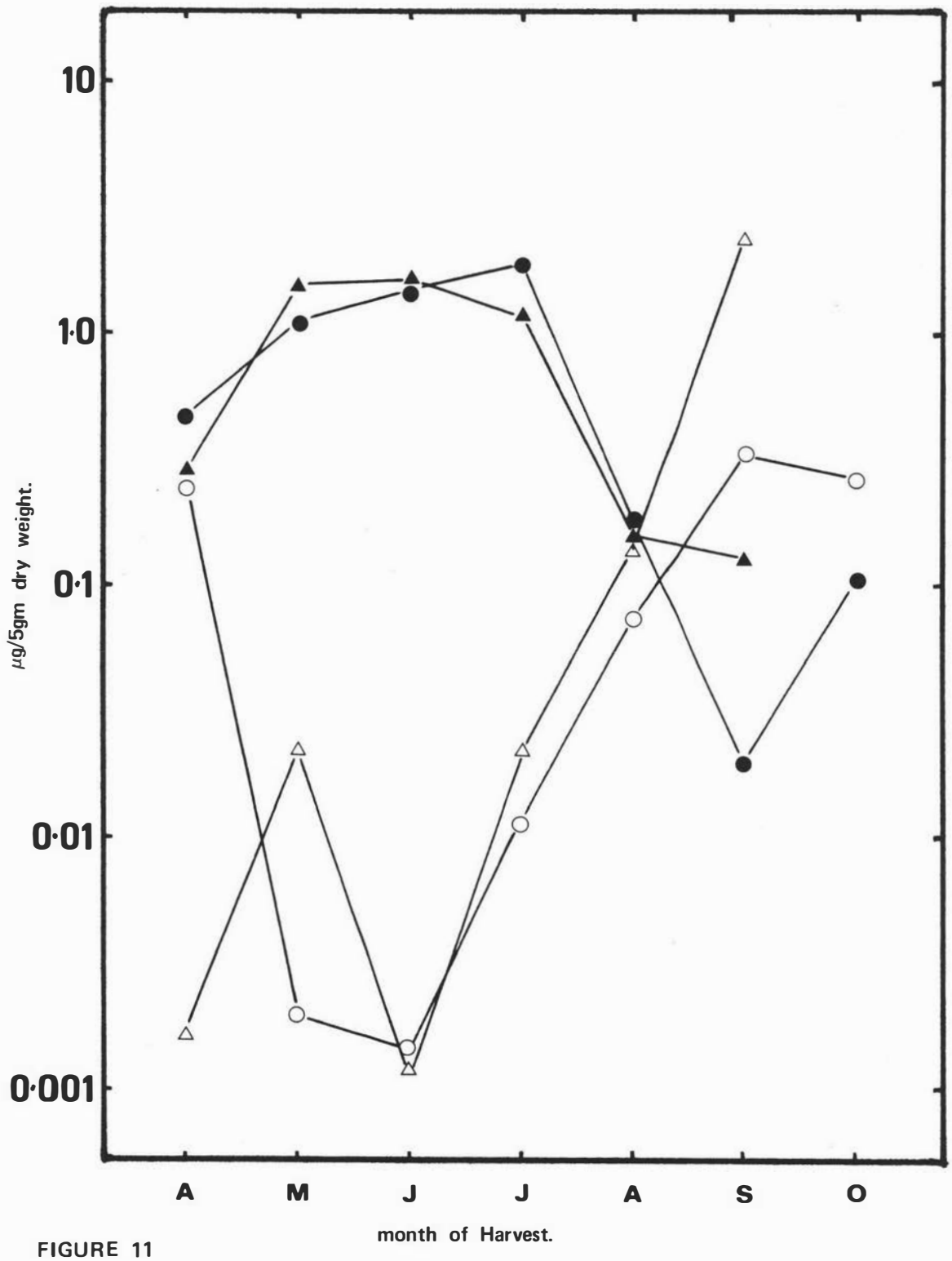

FIGURE 11

Seasonal Changes of an acidic growth inhibitor, the same or similar to ABA, determined by the wheat coleoptile bioassay of acidic ether fractions of stem tissue samples of apple rootstocks.

▲——▲ MM 106 △——△ MM 106 3 week ●——● EM $\overline{\text{XII}}$. ○——○ EM $\overline{\text{XII}}$ 3 week

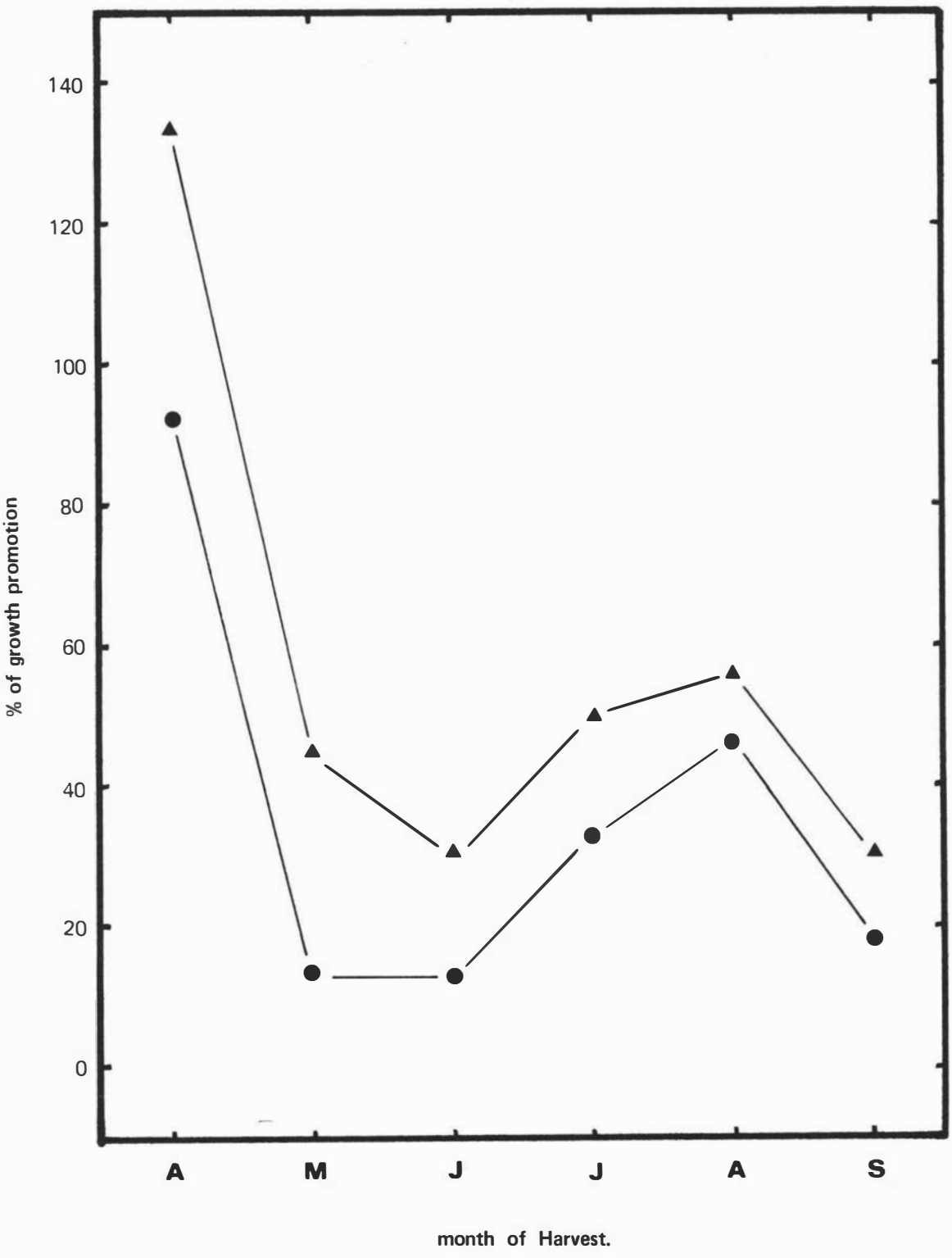

FIGURE 12

Seasonal changes in an acidic root initiation promoter from MM106 and EMXII apple rootstocks, determined by the mung bean bioassay.
▲—▲ MM 106 ●—● EM XII.

system isopropanol:ammonia:water (10:1:1 v/v) (figure 13). This water-soluble promoter appeared to be chromatographically similar to Kawase's "Rhizocaline-like" compound (Kawase, 1964). Promotory activity was very similar for both MM 106 and EM XII rootstocks and levels from three week samples were similar to the original extracts. Further bioassays were conducted for the promoter, with the exclusion of 1 μg/ml IAA from the mung bean bioassay. The promotive activity of both MM 106 and EM XII aqueous extracts was similar, and of the same magnitude and Rf, as the zones identified by the standard mung bean bioassay which included 1 μg/ml IAA in the test solution (figure 14).

It was noted that while root initiation on mung bean cuttings was dramatically increased b the aqueous promoter, root elongation was retarded, resulting in an increase of one or two days more being required for satisfactory recording of root numbers. In some bioassays of the aqueous extract it was noticed that Rf 0.3-0.4, promoter levels were so high that inhibition of rooting occurred. The hypocotyls became very flaccid and glassy in appearance and the leaves wilted and died. A ten-fold dilution of the eluant of the chromatogram segment resulted in a promotion of approximately 30% of the normal promotion recorded.

No significant promotion or inhibition of rooting from neutral ether extracts of either rootstock was recorded in the mung bean bioassay.

(4) Butanol-soluble Cytokinins

A peak of rowth promotion of radish cotyledons was located at Rf 0.4-0.7 in butanol-soluble extracts of both apple rootstocks, when chromatograms were developed in n-Butanol:ammonia (4:1 v/v). This peak coincides with the Rf's of zeatin and zeatin riboside, as found using marker spots run concurrently with chromatograms.

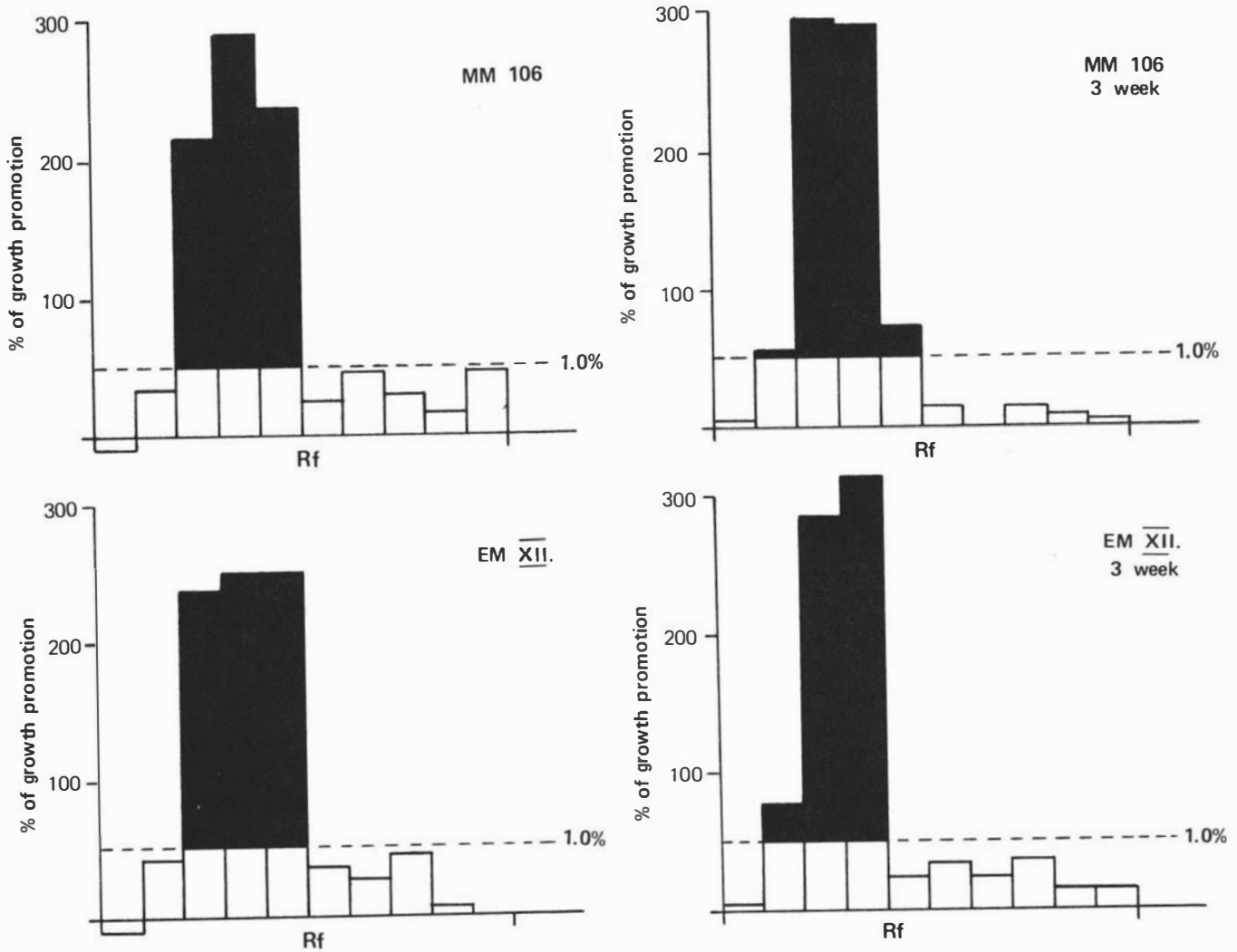

FIGURE 13

Histograms of an aqueous promoter from stem tissue samples of MM 106 and EM XII apple rootstocks, determined by the mung bean bioassay.

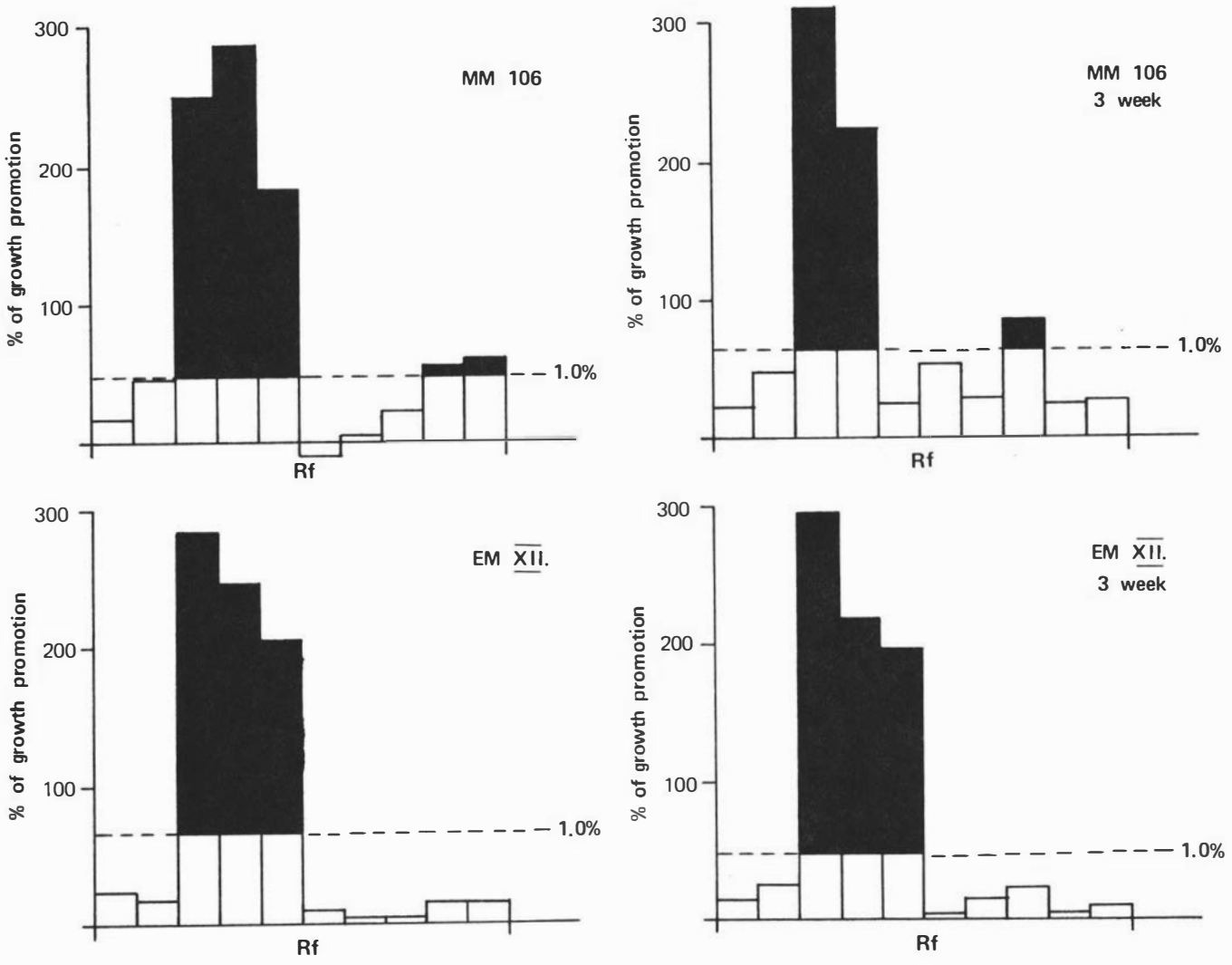

FIGURE 14

Histrograms of an aqueous promoter from stem tissue samples of MM106 and EMXII apple rootstocks, determined by the mung bean bioassay, minus 1ppm IAA.

Values of the total promotion are expressed as g equivalents of kinetin per 5 grams dry weight of plant tissue and are plotted in figure 15.

Confidence limits of P = 0.01 were calculated for the histograms derived from the radish cotyledon bioassay, by the Link and Wallace analysis. Values plotted as 0.001 µg/5 gm dry weight represent samples where no cytokinin activity was detected or was not significantly different from the controls. These include the MM 106 June and July samples, the EM XII May, June, July and August samples, the MM 106 three week and EM XII three week June samples.

Similar trends in seasonal changes occurred with both MM 106 and EM XII rootstocks. There was no large difference between the two rootstocks in cytokinin content, except in the early spring samples off the stools, when MM 106 exhibited bud movement one month earlier than EM XII.

Cytokinin levels from samples taken on harvest dates decreased rapidly in autumn in both rootstocks, with the EM XII status decreasing more rapidly. Through the mid winter months no cytokinin activity was detectable in either rootstocks but in early spring a rapid increase in activity occurred. The increase was coincident with bud break on both rootstocks, the rise in activity, slightly preceding bud break, but with a peak at the time of bud break in the field rootstocks. Because of this, MM 106 rootstock showed a rise in activity one month earlier than EM XII.

Samples taken after the cuttings had been exposed to three weeks on the heat bed showed slightly different trends. At tim s when the buds have the ability to rapidly renew growth if placed in conducive conditions (autumn and spring), higher levels of cytokinin activity were detected

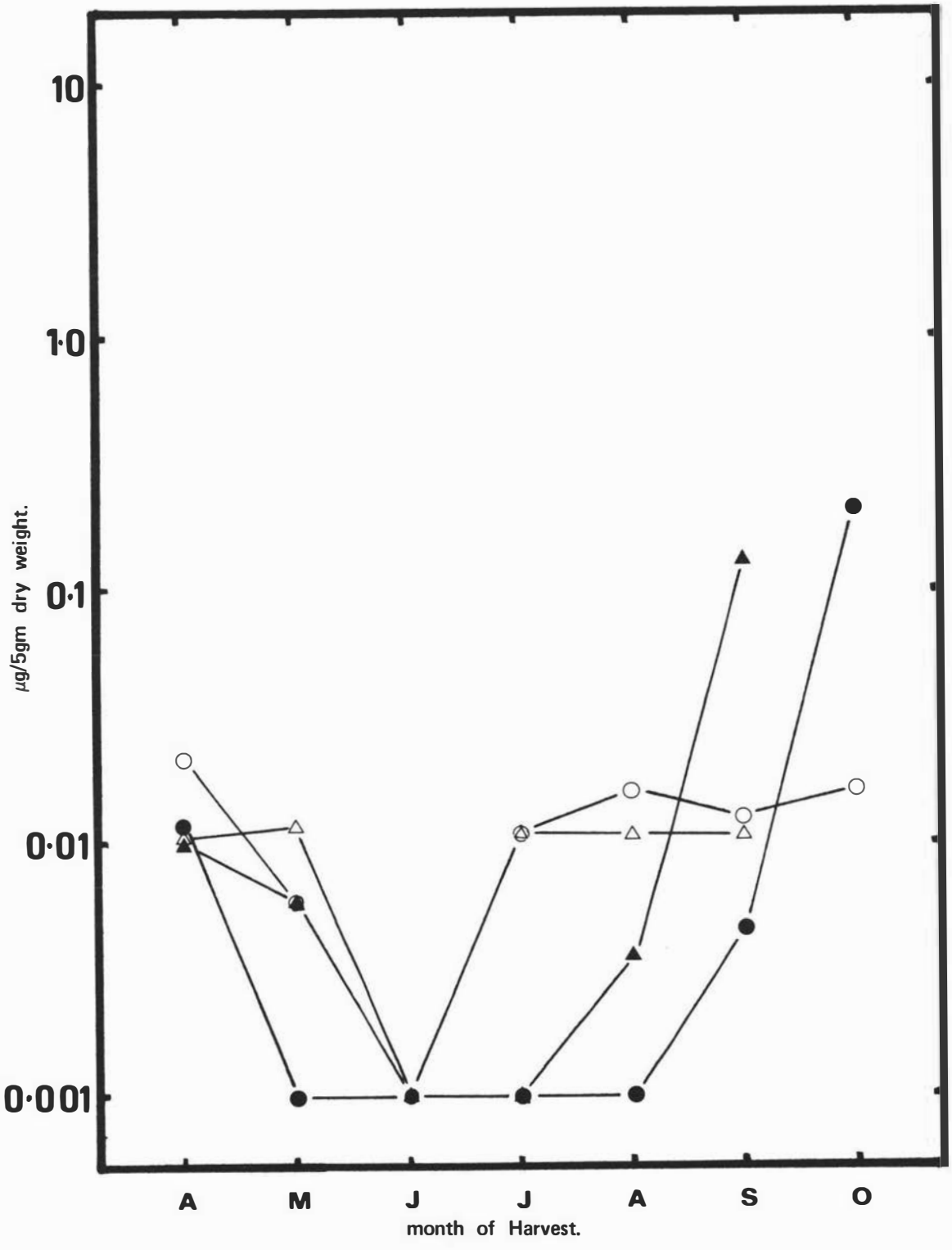

FIGURE 15

Seasonal changes in butanol soluble cytokinins from MM106 and EMXII apple rootstocks.
▲—▲ MM 106 △—△ MM 106 3 week ●—● EM XII. ○—○ EM XII. 3 week

in the three week samples, compared with the initial samples. When leafing cut was further advanced (spring) the levels of three week samples were lower than the original sample levels, e.g. MM 106 September and EM XII October.

DISCUSSION

Preliminary rooting trials with hardwood cuttings of MM 106 and EM XII apple rootstocks demonstrated a large difference in their ability to initiate roots, when treated by the East Malling technique. The difference was evident throughout the whole season of planting. According to Howard (1966, 1968b, 1971), treatments of high bottom heat and concentrated IBA dips promoted root initiation in some clones of apple and plum rootstocks. Results presented in Table 1 confirmed that a promotion of rooting can be achieved by the application of IBA and bottom heat, but the degree of promotion by IBA application was limited by some other factor. Cuttings possessing a high natural ability to initiate roots (MM 106) responded more readily than did difficult-to-root cuttings (EM XII). In early spring, EM XII showed a much improved response to IBA application and since no treatment factor was varied some other factor which interacts with IBA and varies with the season, must have altered.

Howard (1966, 1971) and Howard and Nahlawi (1969b) found that under standard East Malling propagating conditions, rooting decreased from autumn to mid winter and increased to a second peak in spring. The autumn peak may have been absent, but the high rooting ability in spring was always present with apple rootstocks. In the easily rooted MM 106 cuttings, no apparent decrease in rooting ability occurred through the winter months. Since the degree of rooting was so low in EM XII cuttings, no significant decrease occurred in mid winter.

In both rootstocks treated with IBA an increase to a seasonal maximum number of rooted cuttings occurred in spring. The increase was also evident in MM 106 untreated with IBA, but not in untreated EM XII cuttings. Since an increase in root formation occurred on IBA-treated MM 106 and

EM XII and untreated MM 106 cuttings in spring, a change in an endogenous factor necessary for rooting may have occurred because the only variable experimental factor was the season of harvest. The ability of IBA to promote root formation was also enchanced at this time which suggested that IBA alone could not promote root initiation. Some decrease in the level of rooting through the winter months was apparent in untreated MM 106 cuttings but the decline was much less than that described by Howard and Nahlawi (loc. cit.), and was statistically insignificant. Untreated MM 106 and IBA-treated EM XII cuttings exhibited seasonal changes in rooting ability most like those reported by Howard, with both autumn and spring peaks being evident. Since only small decreases in rooting ability of easy-to-root cuttings were recorded, it is possible that endogenous factors necessary for root formation were maintained at high levels throughout the harvest season. Decreases in the ability of cuttings to root on successive harvest dates from autumn through mid winter have also been reported by Fadl and Hartmann (1967a), Nesterov (1968) and Doud and Carlson (1972) for other fruit tree species. Results in Table 1 confirmed earlier results obtained by the author, in which no decline in the rooting of IBA-treated MM 106 cuttings was recorded from autumn until mid winter (Tustin, 1972).

The idea that bud dormancy may influence root initiation has been suggested by several workers (Wareing and Smith, 1963; Fadl and Hartmann, 1967a; Roberts and Fuchigami, 1973; Roberts et al., 1974). In the present work it would seem that seasonal changes in root formation of both MM 106 and EM XII cuttings were not related to the degree of bud dormancy of the cuttings, an opinion shared by Howard (1968a) from studies on M. 26 apple rootstock and Myrobalan B plum rootstock. While bud dormancy followed an expected trend of increasing from autumn until mid winter and then declining until bud break in spring, no positive or negative relationship to rooting ability of the two rootstocks was evident. However

a good correlation was found between the termination of bud rest (bud break) in early spring and promotion of root initiation in both MM 106 and EM XII. When axillary bud burst occurred on cuttings, shortly after being placed in the heat bed, strong promotion of root initiation was achieved, particularly when combined with an IBA treatment as emphasised previously. This correlation of bud burst with root initiation is well documented (Speigel, 1955; Lanphear and Meahl, 1963; Howard, 1965; Fadl and Hartmann, 1967; Nanda and Anand, 1970; Roberts et al., 1974) and treatments which induce bud burst (e.g. chilling and day length manipulations) have been known to enhance root initiation (Fadl and Hartmann, 1967; Smith and Wareing, 1972, a, b; Roberts and Fuchigami, 1973; Roberts et al., 1974; Bhella and Roberts, 1974; Whitehill and Schwabe, 1975). It would appear however, that the exact time at which bud burst occurs in the heat bed is important. For example, the October planted EM XII cuttings came into growth rapidly when planted in the propagating bed but the cuttings rooted poorly. This failure to root may have been due to dehydration of the cuttings, the formation of a strong alternative sink being set up by the rapidly developing buds which thus competed with the rooting process for a limited nutrient supply, or some other effect on the apparently delicate balance of factors influencing the complex sequence of events leading to root formation.

A considerable difference in the IAA status of the two rootstocks was evident throughout the harvest season (figure 9). The easily-rooted MM 106 rootstock contained a substantially higher level of endogenous free IAA in the tissue than EM XII, on each harvest date. The promotive activity of IAA on root initiation has been well documented (Went, 1934; Thimann and Went, 1934; Warmke and Warmke, 1950), and is now considered to be an essential factor for successful induction of rooting of cuttings. The seasonal changes in root formation of cuttings of both rootstocks closely followed the seasonal changes in endogenous IAA levels for each

harvest date. An exception to this was on the September EM XII harvest, when a very significant rise in root initiation was related to a peak of IAA production while on the heat bed. Seasonal changes in root initiation have also been related to patterns of endogenous auxin production in Salix (Vieitez and Pena, 1968), and Populus species (Nanda and Anand, 1970; Smith and Wareing, 1972b).

Cuttings of MM 106 rootstock, rooted much more readily than EM XII cuttings throughout the season of harvest, possibly because the motherstock retained a high IAA status during the dormant period compared to EM XII. The mechanism by which MM 106 retained the high IAA status is unknown. Hatcher (1959) found that mature internode tissue could either produce considerable diffusable auxin after periods of temporary rest, or auxin which had been converted to a bound form could be released again. Earlier work by Zimmerman (1936) and Soding (1937) had shown that diffusable auxin levels in the lower stem could be higher than present in the apex. Mirov (1941) and Alden (1971) both found that in Pinus species the greatest amount of diffusable auxin was present in the basal parts of the shoot, and declined towards the apex. Gunkel and Thimann (1949) found in Gingko that in the later stages of stem growth, an increasing part of the auxin supply was derived from the basal internodes. Basal internodes of MM 106 contained levels of IAA comparable with the basal internodes of pine shoots studied by Alden (1971). MM 106 may possess a mechanism, lacking in EM XII, by which mature internodes can produce IAA, or store and remobilise IAA in the late summer and winter months. Alternatively, destruction of IAA in MM 106 may be limited and the higher endogenous IAA levels may, in part, reflect the lower degree of dormancy exhibited by the rootstock when compared to EM XII.

Maximum root initiation potential was realised in early spring at a time which coincided with bud burst on the cuttings shortly after being placed on the heat bed. A peak of IAA production occurred in both root-

stocks just prior to and during bud burst, suggesting that improved rooting of cuttings, especially in the EM XII clone, was brought about by a flush of auxin produced in the rapidly developing buds. A lower endogenous IAA level detected in the October EM XII 3 week sample might indicate that the auxin flush actually preceded bud burst rather than occurred as a result of it. The lower degree of rooting of October EM XII cuttings tended to support this hypothesis. A distinct and transient flush of auxin production in buds of trees resuming growth after winter rest, has been reported by many workers, (Czaja, 1934; Zimmermann, 1936; Söding, 1937; Speigel, 1955; Nanda and Anand, 1970; Smith and Wareing, 1971, 1972a), some of whom have related the peak to times of maximum rooting potential of cuttings. Artificial termination of bud rest has also induced the flush of auxin production (Smith and Wareing, 1972a; Tomsett and Schwabe, 1974).

A large decrease in the IAA content in the cutting bases of both rootstocks was evident after being on the heat bed for 3 weeks. This decrease was only apparent when buds remained dormant on cuttings undergoing root initiation; a renewed auxin supply from developing buds in the early spring maintained or increased the levels present at harvest date. Metabolism or inactivation of IAA appeared to have occurred in the cutting bases, caused by either the bottom heat treatment or by the presence of newly formed roots, since roots themselves are known to retain high IAA-oxidase levels. It is difficult to propose any mechanism by which the decrease in IAA level was achieved, since it occurred irrespective of whether root initiation took place or not. Odom and Carpenter (1965) found that when higher levels of endogenous acidic auxins were initially present in cuttings, the level declined during rooting. Saito and Ogasawara (1960) noted that IAA levels in Salix were high at harvest time and decreased rapidly during root initiation. It was suggested that the decline indicated IAA was instrumental in the initiation of roots on Salix. Bonner and Bonner (1948) and Galston (1955) both believed that

tissues responding to IAA, exhibited a reduction in IAA levels as the response was manifest. Smith and Wareing (1972a) found endogenous levels of IAA at the cutting base were high at the time of root initiation and low once root emergence had occurred. These reports indicated that once root initiation had occurred, the level of endogenous IAA decreased; the apparent mechanism which operated in MM 106 cuttings. It is conceivable that the same metabolic reduction of endogenous IAA levels occurred in EM XII cuttings, but the initial level was below that required to facilitate promotion of root initiation. Possibly in early spring, a flush of endogenous IAA production from the bursting buds of EM XII cuttings enabled an optimum level of IAA to be reached, thus resulting in a considerable increase in the number of cuttings initiating roots.

Auxin activity, chromatographically similar to IAN was observed in both rootstocks. Absolute levels of IAN in both rootstocks were considerably lower than IAA levels, but the magnitude of difference between the two rootstocks was similar to that found for IAA. The biological significance of IAN in root initiation is relatively unknown. 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. It is well known that grain coleoptiles are capable of converting IAN to IAA readily, and that to be biologically active, the conversion of IAN to IAA is necessary. Gur and Samish (1968) reported the ability of apple rootstock roots to convert IAN to IAA and suggested that upsetting the IAN-IAA equilibrium by IAA-oxidase, might result in IAN being converted to IAA, to maintain a stable IAA level. Odom and Carpenter (1965) suggested that neutral auxins were active in root initiation through conversion to IAA in Coleus sp. and Chrysanthemum sp.

Seasonal changes in IAN levels in the two rootstocks did not closely resemble the seasonal pattern in root initiation. MM 106 retained a quite

stable IAN content throughout the whole harvesting season. EM XII demonstrated a change in IAN content similar to the seasonal change in IAA levels with the exception of the high spring peak, which was not evident for IAN. The bases of cuttings from the heat bed showed a decrease in level of IAN in the autumn months; through the mid winter, the decrease in IAN levels was not apparent, and, in EM XII, an increased level of IAN was observed. With the onset of spring, MM 106 again showed the decrease in IAN levels after three weeks on the heat bed, but EM XII retained a level similar to the initial harvest dates (figure 10). Conversion of IAN to IAA may have occurred during the more active growth periods (autumn and spring) in cuttings undergoing root initiation, to maintain an IAA pool, as suggested by Gur and Samish (1968). This was more evident in MM 106 but did not appear to occur in mid winter. EM XII showed an increased level of IAN after 3 weeks which suggested that a reversal of the IAA-IAN equilibrium (Gur and Samish, 1968), or release from a further storage form, such as glucobrassicin (Gmellin and Virtanen, 1961), had possibly occurred. The role of IAN in root initiation of apple cuttings remains obscure. The detection of IAN levels which were proportionally similar to IAA levels, indicated that biosynthesis of both auxins in each rootstock was regulated by a common factor or factors. It is conceivable that the biosynthesis of IAA in the apple rootstocks involves an intermediate step resulting in the formation of IAN. A reduction in the conversion of IAN to IAA during the mid winter dormant period may account for the absence of the decrease in IAN levels in MM 106 (3 week) and for the increase in level in EM XII (3 week) extracts, particularly if a stored form of auxin was being remobilised.

A potent growth-inhibiting compound, probably abscisic acid (ABA), was located in both rootstocks (figure 11). In tissue samples from the same harvest dates, similar ABA levels were located in both rootstocks. The seasonal changes of ABA content in both rootstocks were closely related to the dormancy of buds on the cuttings. The correlation between endogenous

ABA and the degree of dormancy, as well as the importance of inhibitor: promoter ratios in regulating bud dormancy have been well researched and reviewed (Milborrow, 1967; Wareing and Saunders, 1971; Milborrow, 1974). An increase in ABA level in both rootstocks, progressed into the winter months and then declined; bud burst on cuttings on the heat bed occurred at a time when a minimum level of ABA was present. Bud burst on stool stocks was also related to ABA levels, with MM 106 demonstrating bud movement one month earlier than EM XII.

ABA has been found to promote root initiation on cuttings of some plant species (Chin et al., 1969; Basu et al., 1970), but as many reports noted the inhibition of root initiation by ABA (Heide, 1968; Eliasson, 1969; Pierek and Steegmans, 1975). No evidence of root-promoting activity of compounds in the inhibitor-B complex was noted in the mung bean rooting bioassay, and differences between ABA levels of MM 106 and EM XII could not account for the differences in ability to initiate roots.

As was found with IAA levels, samples taken from the heat bed after 3 weeks, showed a dramatic decrease in ABA levels, although considerable fluctuations occurred in these samples. In early spring (August, September) ABA levels increased substantially when cuttings were on the heat bed. The increased levels of ABA coincided with early leafing out of buds while cuttings were still undergoing root initiation. Wright and Hiron (1969); Zeevart, (1971); Most, (1971); and Ivey (1974) all reported that ABA levels increased with water stress in wheat, spinach, sugar cane and peas respectively. It was considered probable that the presence of young leaflets on apple cuttings induced a degree of water stress on unrooted cuttings, resulting in increased ABA levels in cuttings on the heat bed. Increases in ABA levels were recorded in cuttings on the heat bed, irrespective of whether root formation took place.

Since the initial discovery of the action of IAA on root initiation processes, the postulation of the involvement of compounds ("Rhizocaline, auxin synergists, rooting cofactors") other than auxins in the "triggering" of root initiation has been prominent. Perhaps the most notable recent advance was by Hess (1957, 1959, 1960, 1961, 1962, 1964, 1966), who developed the rooting cofactor theory. In the present work a zone of root-promoting activity from the acidic ether fractions of both rootstocks was found using the mung bean bioassay (figure 12). Levels of the acidic rooting promoter were high during the autumn but fell to low level during the mid winter and spring months in both rootstocks and disappeared from extracts taken from cuttings which had been in the propagating bed for 3 weeks. Levels of the promoter were similar in the two rootstocks which was not unexpected since both sources of cutting material were in a very juvenile state. Differences in the level of the acidic rooting promoter could not account for the differences in root formation between MM 106 and EM XII cuttings. Although numerous reports exist describing relationships between "rooting cofactors" and root initiation (see Part B. 6 of Literature Review), no root promoting cofactors or inhibiting compounds were located in the neutral ether extracts.

A very active promoter of mung bean root initiation was chromatographically isolated from the aqueous residue of both MM 106 and EM XII. Activity was very similar in both rootstocks irrespective of whether sampled at harvest date, or after 3 weeks on the heat bed. The inclusion of IAA in the bioassay had no influence on the activity of the promoter, Kawase (1964) described a compound extracted from Salix, thought to be "Rhizocaline", which appeared to be chromatographically similar to the aqueous promoter from apple. Kawase noted a synergism of IAA with the "rhizocaline-like" promoter. The promoter was so active that the number of roots initiated on the mung bean cuttings appeared only to be restricted by a physical limita-

tion of space for root initials to develop. Since levels of the aqueous promoter were very similar in both MM 106 and EM XII rootstocks, and the biological activity seemed independent of IAA, the physiological role of the promoter in adventitious root initiation remained obscure. As both sources of cuttings were considered to be in a juvenile state, it would seem likely that the aqueous rooting promoter was present in levels above a required threshold and therefore did not limit and promotion of root formation. 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.

Cytokinin activity in the butanol-soluble extracts from both rootstocks followed similar patterns to, and was positively related to, the buds ability to shoot rapidly when placed in inductive conditions. Hewett and Wareing (1973) reported similar changes from mid winter until spring in dormant shoots of poplar, with a peak of cytokinin activity at bud break. An increase in cytokinin production may well be a critical factor for bud burst to occur, since in MM 106, both increased cytokinin activity and bud burst occurred one month earlier than in EM XII and artificially induced bud burst by the high temperature storage conditions of the cuttings' bases, was also preceded by increases in endogenous cytokinin levels. These induced levels reached a lower maximum concentration than those recorded at natural bud burst, but the levels appeared to increase earlier (see figure 15; 3 week determinations). Similar observations have been noted by Hewett and Wareing (loc. cit.) also. Thus it seems likely that buds appear to depend on an increase in cytokinin levels in the spring before resuming growth, an opinion shared by Pieniazek et al., (1972) who suggested that cytokinins may be primarily responsible for inducing bud break in apple, as have Chvojka et al., (1962) and Williams and Stahly, (1968).

The importance of auxin:cytokinin ratios in the control of growth and development of plants has become increasingly well documented. In particular, control of organogenesis has been found largely to depend on the auxin: cytokinin ratios (Skoog and Miller, 1957). Heide, 1965b; Mullins, 1970; and Heide, 1971 produced evidence for the existence of both *in vitro* and *in vivo* interactions of auxins and cytokinins in the regeneration of roots on cuttings. Along with many other reports (Part B. 3 Literature Review) the inhibitory effect of high cytokinin levels on root initiation and more important, the competitive inhibition of auxin-induced root initiation has been clearly demonstrated. No correlation was established between differences in root initiation and cytokinin levels obtained throughout the harvest season on either apple rootstock. Cytokinins could not be identified as rooting promoters and lack of detection throughout the winter months precluded their being involved in promoting root initiation. In MM 106, a high auxin:cytokinin ratio (minimum = 10:1) existed throughout the whole harvesting season, while in MM XII the ratio was much lower (minimum = 1:1). In the early spring when bud burst was occurring naturally or was induced by root initiation treatments, the root initiation promoting auxin:cytokinin ratios were not so obvious. This was likely to be due to the physical separation in time of the auxin and cytokinin peaks at bud burst. Cytokinins can induce the breaking of dormancy of apple buds (Chvojka *et al.*, 1962; Weinberger, 1969; Pieniazek *et al.*, 1970) and the developing buds subsequently produce a transient flush of auxin production as they initially develop (Smith and Wareing, 1971, 1972a; Tomsett and Schwabe, 1974; Pieniazek *et al.*, 1970). This could account for the realisation of maximum rooting potential of both rootstocks at a time of an apparently unfavourable auxin:cytokinin balance.

Seasonal changes in root initiation and the differences in capacity to form roots on hardwood cuttings of the 2 rootstocks, could only be related to changes in endogenous IAA levels. Endogenous levels of IAN were

present in the same differential magnitude as IAA levels and could account for the differences in root initiation ability between MM 106 and EM XII cuttings, but the pattern of seasonal change was inconsistent with the observed seasonal changes in root initiation potential. No auxin synergists were located, but commonly present acidic and aqueous rooting promoters were found; the physiological significance of these promoters being somewhat obscure. Seasonal changes of ABA and butanol-soluble cytokinins correlated well with commonly observed physiological phenomena such as the induction and termination of winter dormancy and bud burst. Levels of both ABA and butanol-soluble cytokinins were similar for both rootstocks and bore no direct relationship to promotion of root initiation on hardwood apple cuttings. Bud dormancy *per se* had no influence on root initiation, but changes in endogenous growth promoters and inhibitors associated with termination of bud dormancy appeared to result in a physiological balance most suitable for induction of roots on apple hardwood cuttings.

In many studies on rooting promoters and inhibitors, the transmission of a rooting stimulus or inhibition across a graft union has been used to help elucidate the physiological basis of adventitious root initiation. Promotion of root initiation in apple rootstock hardwood cuttings does not seem to be a simple auxin effect since IBA did not promote rooting in EM XII. It is conceivable that IBA and IAA do not act in the same way within apple cuttings and that promotion of rooting is dependent on a specific auxin(s). MM 106 apple rootstock has been shown to retain a considerably higher concentration of IAA than EM XII, which appeared to account for the higher degree of rooting. By use of reciprocal grafting techniques, further studies on endogenous promotion of root initiation in apple rootstock hardwood cuttings were undertaken in Section II. Using this technique, the promotional activity by IAA could be more clearly demonstrated by transfer of rooting ability into EM XII rootstocks from cleft grafts of MM 106.

SECTION II

Demonstration and Location of a Transmissable Root Promoter in Apple Rootstock Hardwood Cuttings

A. Root Initiation Trials of Reciprocal Donor Grafted Rootstocks

Introduction and Methods

Maiden trees of MM 106 and EM XII were grafted with scions of one another, grown on and prepared for planting as cuttings in early winter. Detailed procedure is outlined in Materials and Methods, Sections C, D and E.

By using two rootstocks with such a large difference in capacity to initiate roots, it could be expected that any activity of a root promoting or inhibiting growth regulator would be demonstrated by transfer across a graft union. This would subsequently affect the ability of cuttings to initiate roots. A notation has been adopted for explanatory purposes when discussing stock-scion combinations. Control plots of each rootstock are referred to by their clonal names (MM 106 and EM XII). Compound cuttings with a scion of EM XII and a stock of MM 106 are referred to as $\frac{\text{EM XII}}{\text{MM 106}}$ and the opposite combination is called $\frac{\text{MM 106}}{\text{EM XII}}$.

Root initiation trials were conducted using standard techniques previously discussed.

Results

Results are presented in Table 3.

Table 3. Results of Root Initiation Trials of Stock-scion Combinations of MM 106 and EM XII Apple Rootstocks.

	Treatment			
	MM 106	EM XII / MM 106	MM 106 / EM XII	EM XII
Replicate	9	9	6	4
(Number	9	9	5	3
Rooted)	9	9	6	3
	10	9	5	3
Mean number rooted	9.25	9.0	5.5	3.25

1%

Numbers joined by the same line are not significantly different when analysed by Duncan's Multiple Range Test.

Both MM 106 and EM XII treatments verified earlier results obtained in Section I, A. MM 106 demonstrated a high degree of root initiation and EM XII rooted slightly better than would normally be expected, but this was probably a result of the girdling treatment imposed three weeks prior to cutting harvest.

The compound cuttings EM XII / MM 106, showed no significant difference in rooting ability, from the MM 106 control treatment, the mean number of cuttings rooted being almost identical. The opposite combination, MM 106 / EM XII, demonstrated a degree of root initiation which was intermediate between the MM 106 and EM XII control treatments, but significantly higher than the EM XII treatment. These results indicated strongly that an active promoter or promoters, were responsible for the differences in root initiation capacities between the two rootstocks, rather than the presence

of an active root initiation inhibitor.

B. Examination of Endogenous Growth Regulators from Reciprocal Donor-grafted MM 106 and EM XII Apple Rootstocks

Methods

Stem tissue samples, taken from the basal 5 cm of cuttings from each treatment at the harvest date, were examined for the presence of endogenous growth regulators using standard techniques described in the Materials and Methods, Sections F, G, H and I.

Results

(1) Auxins

Promotive activity was found in the acidic ether fractions of stock-scion combination extracts at the Rf of IAA. The large difference in auxin level between MM 106 and EM XII was verified in this analysis. The stock-scion combination $\frac{\text{EM XII}}{\text{MM 106}}$ contained a concentration of auxin very similar to that of the MM 106 sample, while the combination $\frac{\text{MM 106}}{\text{EM XII}}$, was found to have a level intermediate to MM 106 and EM XII; a considerable increase over ungrafted EM XII cutting samples. It would appear that a transfer or partial transfer of endogenous IAA from MM 106 scions into EM XII stocks had occurred, being limited from further transport by the girdle below the graft union. A graphical representation is shown in figure 16. Horizontal bars denote IAA standards run concurrently with the bioassay.

Bioassays of the Neutral Ether Extracts resulted in promotive activity being isolated in Rf 0.7-0.9, the same Rf as IAN. As was found in the IAA extracts, a large difference in levels of IAN occurred between MM 106 and EM XII. Both $\frac{\text{EM XII}}{\text{MM 106}}$ and $\frac{\text{MM 106}}{\text{EM XII}}$ showed a level between MM 106 and EM XII, but both had concentrations considerably higher than EM XII alone, It

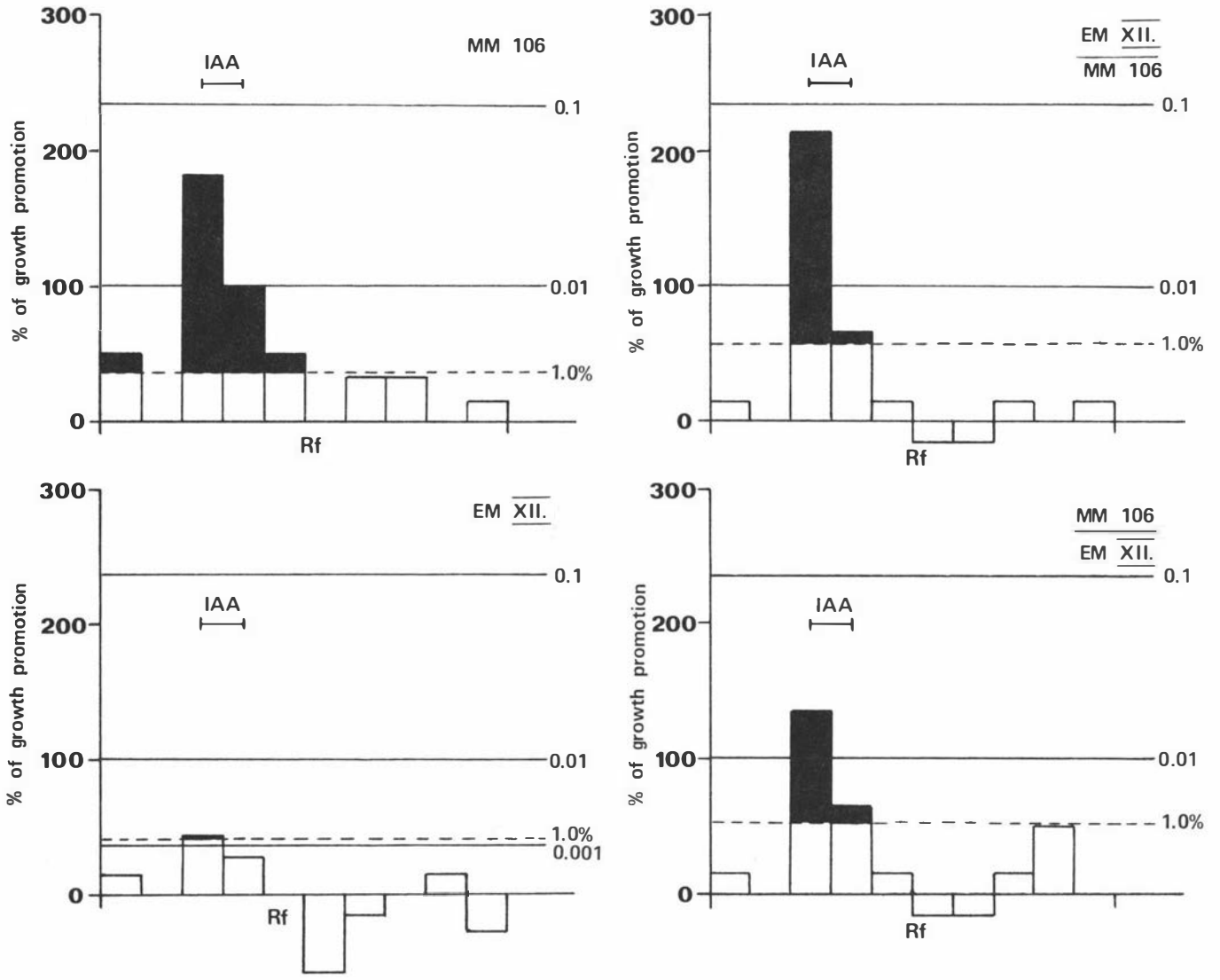

FIGURE 16

Histograms of IAA-like acidic growth promoters from stem tissue samples of reciprocal donor-grafted apple rootstocks, determined by the oat coleoptile bioassay.

would appear that a transfer of IAN had occurred from MM 106 scions into EM XII stocks along with IAA, or that some of the transferred IAA had been converted to IAN. It also appears that the level of IAN in $\frac{\text{EM XII}}{\text{MM 106}}$ had decreased relative to MM 106, possibly by conversion to IAA or by the transfer of a destructor from EM XII. No such effect was apparent with IAA.

A graphical representation of IAN levels, and IAN standards is shown in figure 17.

(2) Abscisic acid

An acidic growth inhibitor, coincident with ABA marker spots was isolated from all four stock-scion combinations. Was was found with the seasonal changes in inhibitor level of the two rootstocks, virtually no difference in levels of ABA were detected between any of the stock-scion combinations. The absolute levels of all samples were very similar to those determined from the same time period during the seasonal ABA determinations. Figure 18 shows ABA levels in all stock-scion combinations and ABA standards.

(3) Rooting cofactors

Examination of the aqueous phase of the revised extraction procedure (figure 2), resulted in the location of the potent root initiation promoter at Rf 0.2-0.5, in all cutting-graft combinations. Histograms of promoter levels are shown in figure 19. Levels of the promoter were approximately similar although it could be argued that an increase in $\frac{\text{MM 106}}{\text{EM XII}}$, compared to EM XII, had occurred. Nevertheless, the fact that such high levels of the promoter are present in all stock-scion combinations places the significance of the small increase in some doubt.

(4) Butanol-soluble cytokinins

Examination of the butanol extract of all four stock-scion combinations

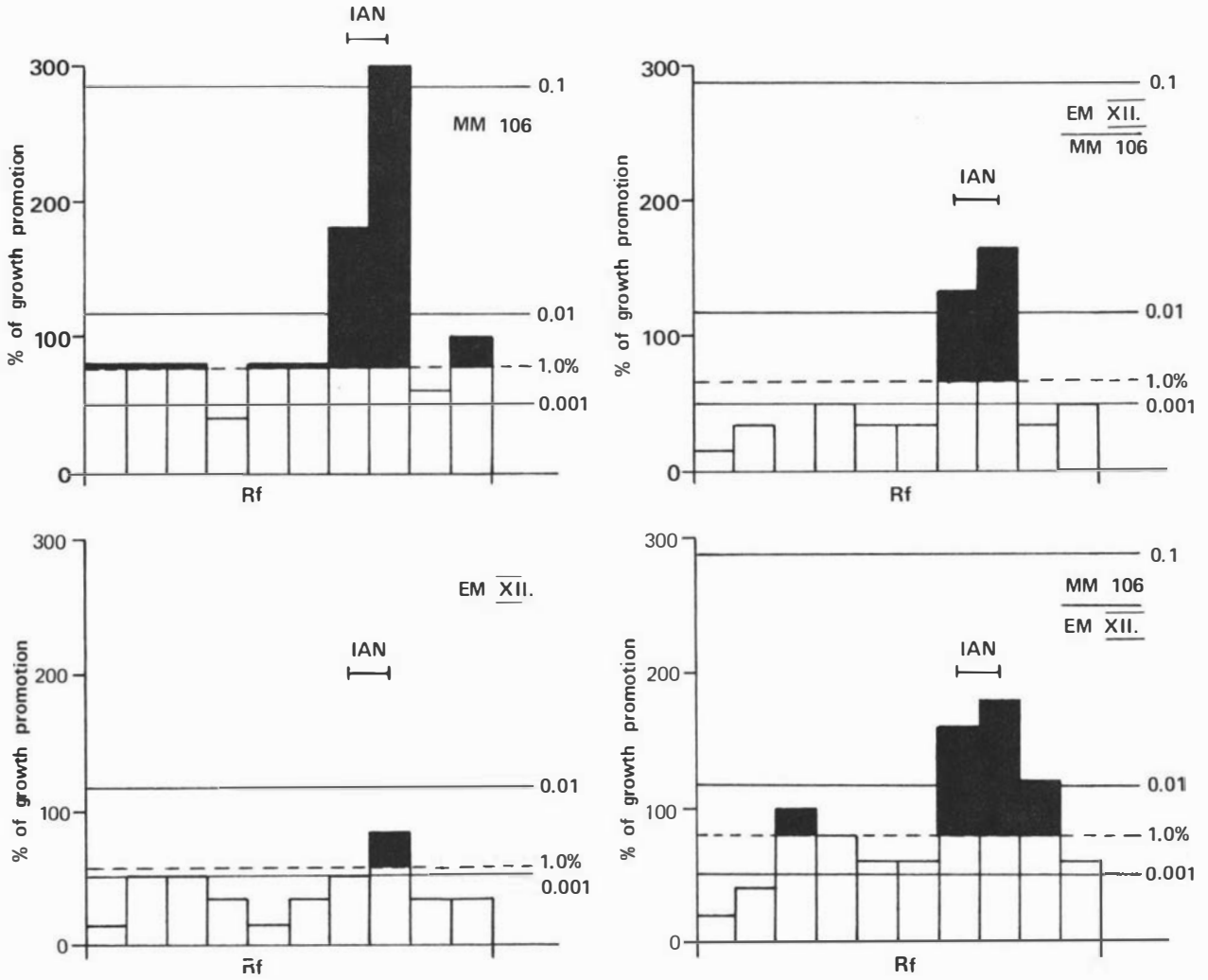

FIGURE 17

Histograms of IAN-like neutral growth promoters from stem tissue samples of reciprocal donor-grafted apple rootstocks, determined by the oat coleoptile bioassay.

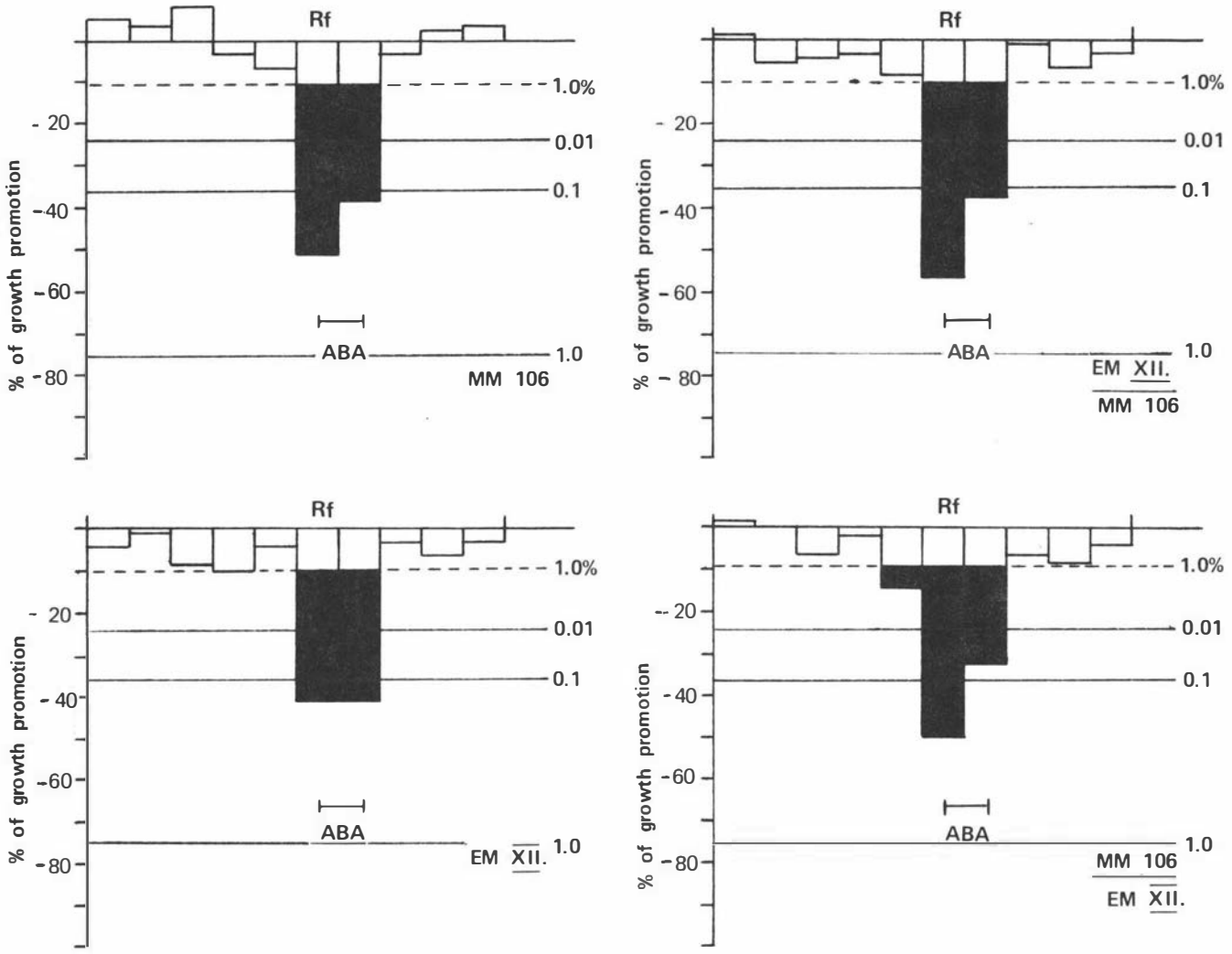

FIGURE 18

Histograms of ABA-like acidic growth inhibitors from stem tissue samples of reciprocal donor-grafted apple rootstocks, determined by the wheat coleoptile bioassay.

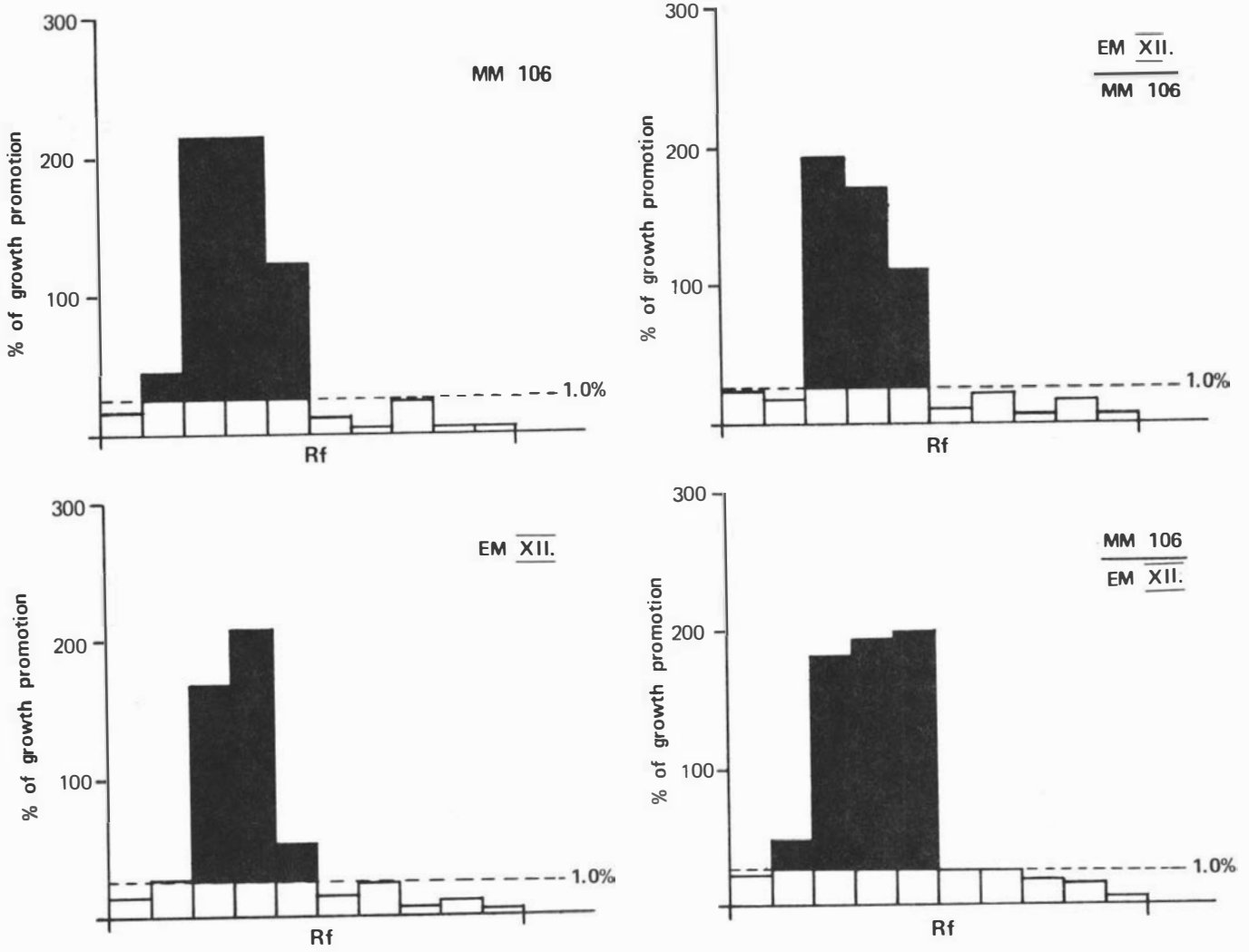

FIGURE 19

Histograms of an aqueous promoter from stem tissue samples of reciprocal donor-grafted apple rootstocks, determined by the mung bean bioassay.

was carried out. No significant cytokinin activity was detected in any of the extracts, which is in accordance with the results obtained in the seasonal changes study.

DISCUSSION

The transferance of a rooting stimulus or inhibition has been demonstrated frequently in studies on adventitious root initiation. Fadl and Hartmann (1967c), noted on pear hardwood cuttings that a girdle below the graft union was necessary to ensure accumulation of rooting promoters and inhibitors. This treatment was applied to the apple shoots to enable a clear demonstration of endogenous promotion or inhibition of rooting.

The high rooting ability of MM 106 was retained on control cuttings and those grafted with EM XII scions. This indicated that the situation in the present study was clearly different from that found with MM 106 and EM II apple rootstock cuttings (Ashiru and Carlson, 1968) and Old Home and Bartlett pear rootstock cuttings (Fadl and Hartmann, 1967c). Several alternative possibilities could account for the different response. There may not be an active rooting inhibitor present in EM XII rootstock and studies in Section I have shown this to be a possible explanation. Alternatively an inhibitor in EM XII may not be graft-transferable or may not be active in the MM 106 rootstocks. EM XII cuttings showed a low ability to initiate roots, while the compound $\frac{\text{MM 106}}{\text{EM XII}}$ cuttings exhibited a degree of rooting less than that of MM 106 but significantly higher than EM XII (Table 3). A promotory compound appeared to have been accumulated in EM XII which was translocated from the MM 106 scion. Indications from the studies on seasonal changes of endogenous growth regulators in MM 106 and EM XII apple rootstocks suggested that promotion of root initiation of cuttings was in part related to endogenous IAA levels. Other growth regulators were not directly involved in promoting root initiation but their influence on the physiological state of cutting tissue indirectly affected the degree of rooting. Studies of auxin, ABA, cytokinin and rooting promoter levels from donor-grafted stocks, demonstrated that the growth regulator status in

control cuttings corresponded to levels obtained for the same time period in the seasonal studies (figures 16-19).

Root initiation on reciprocal grafted cuttings showed a positive correlation with both IAA and IAN levels. A high level of both auxins was retained in cuttings which utilised MM 106 as the rootstock. When used as a scion some factor allowed the accumulation of IAA and IAN to occur in the EM XII rootstock. Several possible alternatives exist which could explain this phenomenon. Endogenous IAA and/or IAN could have been translocated across the graft union and accumulated in the basal tissue of the EM XII rootstock. Endogenous IAA may have been translocated, some of which was converted to IAN in the EM XII tissue. Alternatively, some factor which slows the destruction of auxin may have been translocated from MM 106 into EM XII, thus allowing local IAA levels to increase within the EM XII rootstock. Improved rooting could be related to increases in both IAA and IAN in EM XII with a MM 106 scion. A number of reports document the transference of a root promoting stimulus across a graft union, (Muzik and Cruzado, 1958; Stoutmeyer et al., 1961; Richards, 1964; Shah, 1969; Lee et al., 1969), but reference is only made to rooting cofactors or a "juvenile factor".

ABA inhibitor levels were similar in both rootstocks, as found in the seasonal studies in Section I. A level consistent with the corresponding seasonal determination, was located in all extracts. No cytokinin activity was detected in any extract which indicated that promotion of rooting depended only on an optimum level of endogenous IAA being reached, rather than additionally overcoming an adverse auxin:cytokinin interaction. The potent aqueous rooting promoter located in the seasonal studies was found to be present in approximately equal quantities in all stock-scion combinations. As was concluded in the seasonal studies, the physiological

significance of the promoter was not clear as difficult-to-root cuttings had a high level of the promoter present, although this might be expected since both sources of cuttings were physiologically juvenile.

Promotion of root initiation in apple rootstock donor-grafted cuttings could only be related to changes in endogenous auxin levels (IAA and IAN). While IAA is known to promote root initiation in cuttings, the promotional activity of IAN in rooting is virtually unknown. IAA has been shown to be the main physiologically active auxin in plants, and IAN probably has to be converted to IAA to be active, a reaction which has been found to occur in the roots of apple rootstocks (Gur and Samish, 1968). Studies of seasonal changes and donor-grafting trials both suggested that root initiation in apple rootstock hardwood cuttings was controlled primarily by endogenous levels of IAA. No indication of promotion of root initiation by ABA, rooting cofactors or cytokinins was obtained in either seasonal or donor-graft studies. Root initiation appeared to be a predominantly auxin specific process in apple rootstocks, i.e. there was no indication of the necessity of an auxin:cofactor complex which was essential for the promotion of root initiation, although IAA alone could not entirely promote rooting on EM XII. Some factor other than IAA but not a complex-forming cofactor seemed to be essential for promoting root formation in apple rootstock hardwood cuttings. It is conceivable that the aqueous rooting promoter may be an essential cofactor which is present in both rootstocks, but requires an optimum level of IAA with which to interact. This seemed unlikely, since no synergism was recorded between IAA and the promoter and activity was unaffected by the addition of IAA to the mung bean rooting test. Hess (1965) reported that phenolic rooting cofactors could stimulate root initiation on mung beans when applied alone, but reacted synergistically with IAA.

The two most notable promotions of rooting of EM XII, irrespective of

the method, were related to considerable increases in the endogenous IAA levels. The easily-rooted MM 106 cuttings retained high concentrations of IAA compared with EM XII on all harvest dates during the seasonal studies as well as in the donor-grafting experiment. The donor-graft experiment indicated that IAA, although an essential requirement for adventitious root formation, was not the only necessary factor since the rooting percentage of $\frac{\text{MM 106}}{\text{EM XII}}$ compound cuttings was not as high as MM 106 or $\frac{\text{EM XII}}{\text{MM 106}}$. It is possible that any other factor necessary for root formation acts by maintaining a high IAA level, i.e. restricting IAA metabolism. If IAA alone is the fundamental promoter of root formation application of high levels of exogenous IAA should reflect a promotion of rooting to levels similar to that seen in MM 106 cuttings. A major problem associated with exogenous application of IAA to cuttings, has been the rapid destruction of IAA by IAA-oxidase. By providing a continuous supply, the problem should be minimised.

Studies of seasonal root initiation and donor-grafted promotion of rooting showed that any major promotion of root initiation by IBA application, only occurred in cuttings with a high natural ability to initiate roots. Lanphear and Meahl (1963) noted the same relationship when studying the propagation of Taxus and Juniperus cuttings. This indicated that for IBA to be an effective rooting promoter, optimum levels of endogenous IAA were required. This further suggested that an IBA-IAA interaction existed which improved root initiation responses and that IBA did not act in the role that it had traditionally been thought to do, i.e. as an 'auxin' increasing the total auxin content to overcome a threshold level required for rooting, as suggested by Nanda and Anand (1970). In Section III, a study is made of the interactions between IAA and IBA including an investigation of the effect of IBA and rootstock tissue on the metabolism of ^{14}C-IAA.

SECTION III

Examination of the Roles of IAA and IBA in Root Formation of Difficult and Easy-to-Root Cuttings of Apple Rootstocks

INTRODUCTION

Since the use of IBA is so important and widespread in propagation, elucidation of the mode of action of IBA would enable improvement of existing treatments using exogenous rooting promoters. Section III consists of a series of experiments which evaluate the action of IAA and IBA in this process. These studies led to the investigation of the metabolism of IAA within the two rootstocks using radio-isotope tracing techniques. In the same experiment, the influence of IBA on IAA metabolism was studied. The hypothesis that IAA alone directly promotes root initiation in apple rootstock hardwood cuttings and that IBA can only promote rooting in the presence of a threshold level of IAA, was evaluated.

A. The Effect of IAA and IBA on Root Formation when Applied Alone or in Combination, I.

Methods

By applying IBA basally as a concentrated dip and providing a continuous supply of IAA, by apical application of IAA-lanolin (0.1%), it was intended to evaluate the roles of IAA and IBA in the root initiation process.

15 cm long hardwood cuttings of MM 106 and EM XII were harvested and the buds removed by excision. Buds were removed to avoid interactions with endogenous plant growth regulators from the buds which might obscure the effects of the treatments. Cuttings were planted 10 cm deep in the heat bed and were treated by one of the four following methods:

Treatment 1	No IAA	No IBA
Treatment 2	+ IAA	No IBA
Treatment 3	No IAA	+ IBA
Treatment 4	+ IAA	+ IBA

IBA was applied basally as a 2,500 ppm quick dip in 50% ethanol. IAA was applied apically as a 0.1% lanolin-IAA mixture. Cuttings not treated with IBA, received a quick dip in 50% ethanol and pure lanolin was applied to cuttings untreated with IAA. The IAA-lanolin and pure lanolin treatments were replaced every second day and a 1 mm slice removed from the top of each cutting each week, to ensure a continuing supply of IAA to the cutting tissue.

After six weeks, the cuttings were lifted and the number of cuttings rooted and the number of roots per cutting was recorded.

Results

The results are presented in Tables 4 and 5.

Table 4. The number of MM 106 cuttings rooted, and the average number of roots per cutting when treated either singly or in combination with IAA or IBA

	Treatment			
	- IBA - IAA	- IBA + IAA	+ IBA - IAA	+ IAA + IBA
	6	9	20	6
	8	1	15	32
	0	6	3	28
Replicate	3	4	14	25
	1	12	10	19
	0	16	22	18
	5	8	9	22
	1	2	6	30
	4	5	20	27
	2	13	10	12
Total Rooted	8	10	10	10
Mean No. roots/cutting	3.0	7.6	12.9	21.9

1%

Treatments linked by the same line are not significantly different when analysed by Duncan's Multiple Range Test. Figures in the columns of replicates indicate the number of roots per cutting, each treatment being made up of 10 cuttings.

Table 5. The number of EM XII cuttings rooted, and the average number of roots per cutting, when treated either singly or in combination with IAA and IBA

	Treatment			
	- IBA - IAA	- IBA + IAA	+ IBA - IAA	+ IBA + IAA
	0	0	0	2
	0	0	0	0*
	0	0	0	0
	0	0	0	1
Replicate	0	0	0	3
	0	0	0	1
	0	0	0	0*
	0	0	0	0
	0	0	0	0
	0	0	0	0*
Total rooted	0	0	0	4
Mean No. roots/cutting	0	0	0	0.7
	5%			

* Base decayed

Non-rooted cuttings were included in the calculations of the average number of roots per cutting for purposes of statistical analysis, which was by Duncan's Multiple Range Test. A photographic record of these results can be seen in Plate 1. This is a representative sample of cuttings

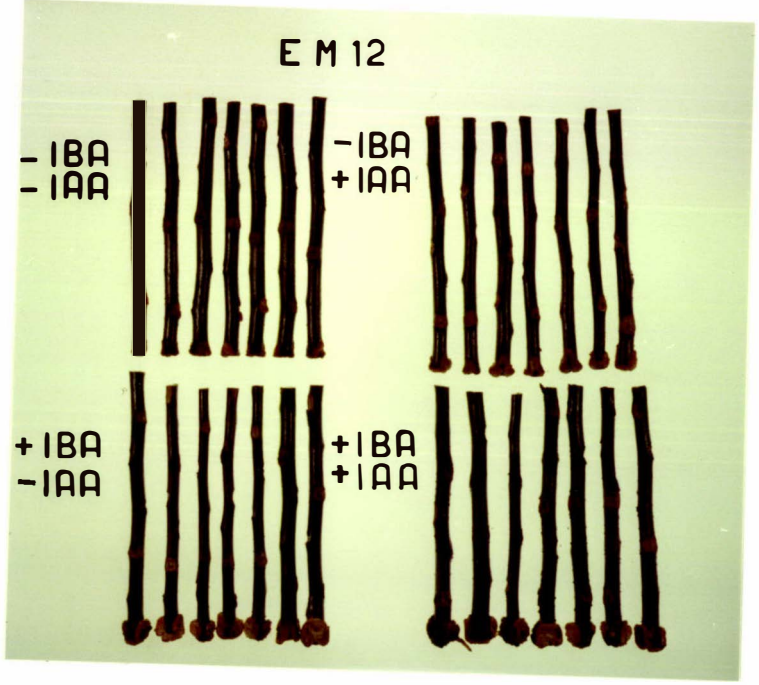

PLATE 1.

A photographic representation of the effects of IBA and 0.1% IAA in lanolin, applied either separately or in combination, on root formation of apple root-stock hardwood cuttings.

from the various treatments.

Root development was less advanced on EM XII than on MM 106 hence the poor definition achieved photographically. Three of the 10 cuttings of EM XII from the IAA/IBA treatment had rotted at the base, thus detracting from the recorded results.

In MM 106 rootstock, there was little difference in the number of cuttings forming roots, indicating the ease with which these can be propagated. All treatments with IBA or IAA resulted in all 10 cuttings initiating roots. Untreated controls produced 80% of the cuttings rooted. More important is the number of roots per cuttings in each treatment. There was no significant difference (5%) between the number of roots per cutting produced by the IAA-treated cuttings or the control, although this was only marginal. Even so the IAA-treated cuttings produced 4.6 roots per cutting more than the control. The IBA-treated cuttings produced significantly (1%) more roots per cutting than the control, but not from the IAA-treated cuttings, although the IBA-treated cuttings produced 5.3 roots per cutting more than the IAA treatment. Cuttings treated with both IAA and IBA produced significantly (1%) more roots per cutting than any other treatment, the average number of roots being the sum of the IBA and IAA treatment means, i.e. an additive promotion was evident.

With EM XII cuttings, only the combined IAA/IBA treatment resulted in any root initiation. This indicated that neither IAA or IBA alone was sufficiently promotive to induce root initiation, but in combination, a threshold was reached which induced 40% of the cuttings to initiate roots.

B. The Effect of IAA and IBA on Root Formation when Applied Alone or in Combination, II.

Introduction

From the indications of Part A of this experiment, several complicating factors were recognised and further examination was considered necessary. With the knowledge of the endogenous levels of IAA in the two rootstocks obtained from Section I c, EM XII and MM 106 rootstocks were treated differently.

Methods

(1) EM XII Hardwood Cuttings

It was found in Section I c than EM XII rootstock had a very low level of endogenous IAA. To ensure that a threshold level of IAA was obtained, a similar experiment to part A, but with a ten-fold increase in IAA concentration, was set up.

Cuttings of EM XII were made and treated apically with a 1.0% IAA-lanolin mixture. All other procedures were done in a similar manner to part A.

Results

Results are presented in Table 6. Non-rooted cuttings were included in the estimation of average numbers of roots per cuttings for statistical purposes. Statistical significance was established by Duncan's Multiple Range Test.

Table 6. The number of EM XII cuttings rooted and the average number of roots per cutting, when treated either singly or in combination with IAA (1.0%) and IBA.

	Treatment			
	- IBA - IAA	- IBA + IAA	+ IBA - IAA	+ IBA + IAA
Replicate	0	0	1	0
	0	2	0	0
	0	0	0	5
	0	0	0	3
	0	0	0	3
	0	0	0	0
	0	0	0	1
	0	0	0	2
	0	0	0	0
	0	0	0	6
Total rooted	0	1	1	6
Mean No. roots/cutting	0	0.2	0.1	2.0
			1%	

The trend observed on EM XII cuttings treated with 1% lanolin was similar to cuttings treated with 0.1% lanolin. No cuttings initiated roots in the untreated controls and only one cutting in 10 formed roots in the IAA or IBA treatments. The combined IAA/IBA treatment showed 60% of the cuttings initiated roots, an increase of 20% over the 0.1% IAA-lanolin. It was also evident that on rooted cuttings, more roots per cutting were initiated. Therefore by increasing the IAA level, relative to the IBA concentration, a further promotion in root initiation was

achieved, and a synergistic interaction between IAA and IBA was clearly demonstrated.

Most of the EM XII cuttings which received the combined IAA/IBA treatment, had developed a basal rot which rendered them useless for photographic purposes. Those which had rooted and then rotted, had distinct root skeletons of up to 2 cm long which facilitated recording of root numbers and also the number of cuttings rooted. Howard (1968b, 1974) reported that some clones of apple rootstocks were more predisposed to rotting when planted as cuttings on the heat bed and that the rotting is correlated with the high concentration auxin treatment. It had been noticed that in previous experiments, varying degrees of rotting of EM XII had occurred (normally 5-10%), but an increased incidence stemmed from the combined IAA/IBA treatments, particularly at 1.0% IAA concentrations. MM 106 did not exhibit the rotting induced by high auxin concentration. It was considered that the rot was not due to an active pathogen in the heat bed medium, since cuttings of MM 106 immediately adjacent to affected EM XII cuttings were not damaged.

(2) MM 106 Hardwood Cuttings

Introduction

It is known from Section I c that MM 106 apple rootstock contains a high concentration of endogenous IAA in the stem tissue. It is considered that this factor may have reduced the effective demonstration of a synergistic promotion of rooting by IAA and IBA in part A, because supraoptimal levels of IAA may have been reached in the base of the cuttings. This opinion is reinforced by results presented in Section III c where it is shown that increasing IBA concentrations above 2,500 ppm do not promote root formation. As a result a different treatment of MM 106 cuttings was formulated.

Methods

15 cm long hardwood cuttings of MM 106 rootstock were disbudded and centrifuged at 2000 x g for three hours, base down in 20 ml water. The basal 2.5 cm of each cutting was removed and the following treatments applied:

Treatment 1	No IAA	No IBA
Treatment 2	+ IAA	No IBA
Treatment 3	No IAA	+ IBA
Treatment 4	+ IAA	+ IBA

IAA was applied as a 1.0% lanolin mixture, a ten-fold increase in concentration over Part A. All other procedures were conducted in the same manner as in Part A.

The centrifugate of the cuttings was retained and acidified and partitioned three times 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 homogenised and extracted in chilled 80% methanol. Purification of the extracts was done according to the revised mung bean separation procedure and column chromatography.

The centrifugation procedure adopted was designed to reduce the endogenous levels of rooting promoters within the cuttings, by extending treatments used by Kawase (1964) and Fadl and Hartmann (1967) over a greater time period. Removal of the cutting base was an added treatment to ensure reduced growth factor levels.

Results

Table 7 shows the results of root initiation trials on MM 106 cuttings after the centrifugation treatment. Statistical significance was established by Duncan's Multiple Range Test.

Table 7. The number of centrifuged MM 106 cuttings rooted and the average number of roots per cutting, when treated either singly or in combination with IAA (1.0%) and IBA

	Treatment			
	- IBA - IAA	- IBA + IAA	+ IBA - IAA	+ IBA + IAA
Replicate	0	1	17	15
	4	13	11	16
	0	13	7	18
	0	9	10	15
	0	13	6	21
	0	5	8	4
	0	8	17	21
	0	7	6	29
	0	10	7	24
	0	5	6	20
Total rooted	1	10	10	10
Mean no. roots/cutting	0.4	8.4	9.5	18.3
		1%		

The most notable effect after centrifugation of the cuttings was that a dramatic decrease occurred, in the number of cuttings rooted in the untreated control. Only one of the 10 cuttings formed roots. Application of IAA or IBA singly, restored the ability of the cuttings to form roots, but the average number of roots per cutting for each treatment was not significantly different (5%). Cuttings treated with the IAA/IBA combination all rooted and the average number of roots per cutting was equivalent to the sum of the IAA and IBA treated cuttings (an additive effect). Since IBA

promoted rooting equally as well as IAA and an additive promotion of rooting was obtained with the IAA/IBA treatment, it is possible that the IBA treatment lowered the optimum IAA level required for root formation. Application of IAA with IBA may have resulted in superoptimal IAA levels in the cutting base. The combined IAA/IBA treatment was significantly different from all other treatments (1%). A photographic record of the results is presented in plate 2.

Since centrifugation caused a loss of rooting ability which could be replaced by IAA or IBA or more effectively by a combination of both, some endogenous root promoting factor had been reduced in the cuttings. An examination of the centrifugate, excised bases and remaining cuttings indicated the nature of the promoter.

An examination of the aqueous root initiation promoter in the three samples is presented graphically in figure 20. It was evident that centrifugation had only a small effect on the mobilisation of the aqueous root initiation promoter, as seen in the concentration present in the centrifugate. Although a considerable level is present in the bases which were excised, the cuttings still retained a high level of the promoter. From the concentration present in the cuttings, it would not appear to be a limiting factor in the control of root initiation in the centrifuged MM 106 cuttings.

The other root initiation promoter that was studied was endogenous IAA. Figure 21 snows the extractable levels of an IAA-like growth promoter from the centrifugate, excised bases and cuttings of MM 106 rootstock after centrifugation. Horizontal bars denote IAA standards run concurrently with the bioassay. A considerable reduction in endogenous IAA concentration was achieved by centrifugation. The centrifugate contained the highest level of IAA, of the three extracts. The basal extract contained the next highest concentration, a supply which was removed from the cutting. The cuttings

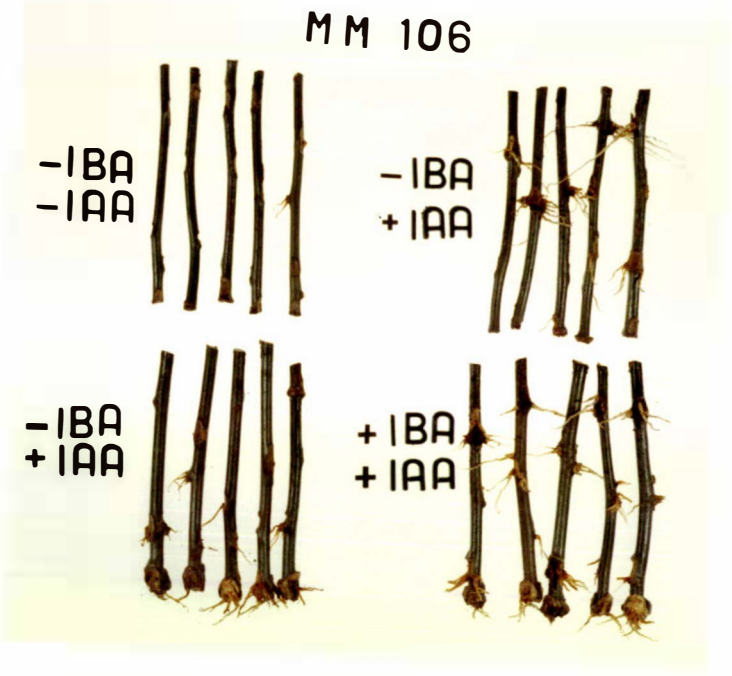

PLATE 2.

A photographic representation of the effects of IBA and 1.0% IAA in lanolin, applied either separately or in combination, on root formation of MM 106 apple rootstock hardwood cuttings after centrifugation and base removal. The lower left hand group should read + IBA, - IAA.

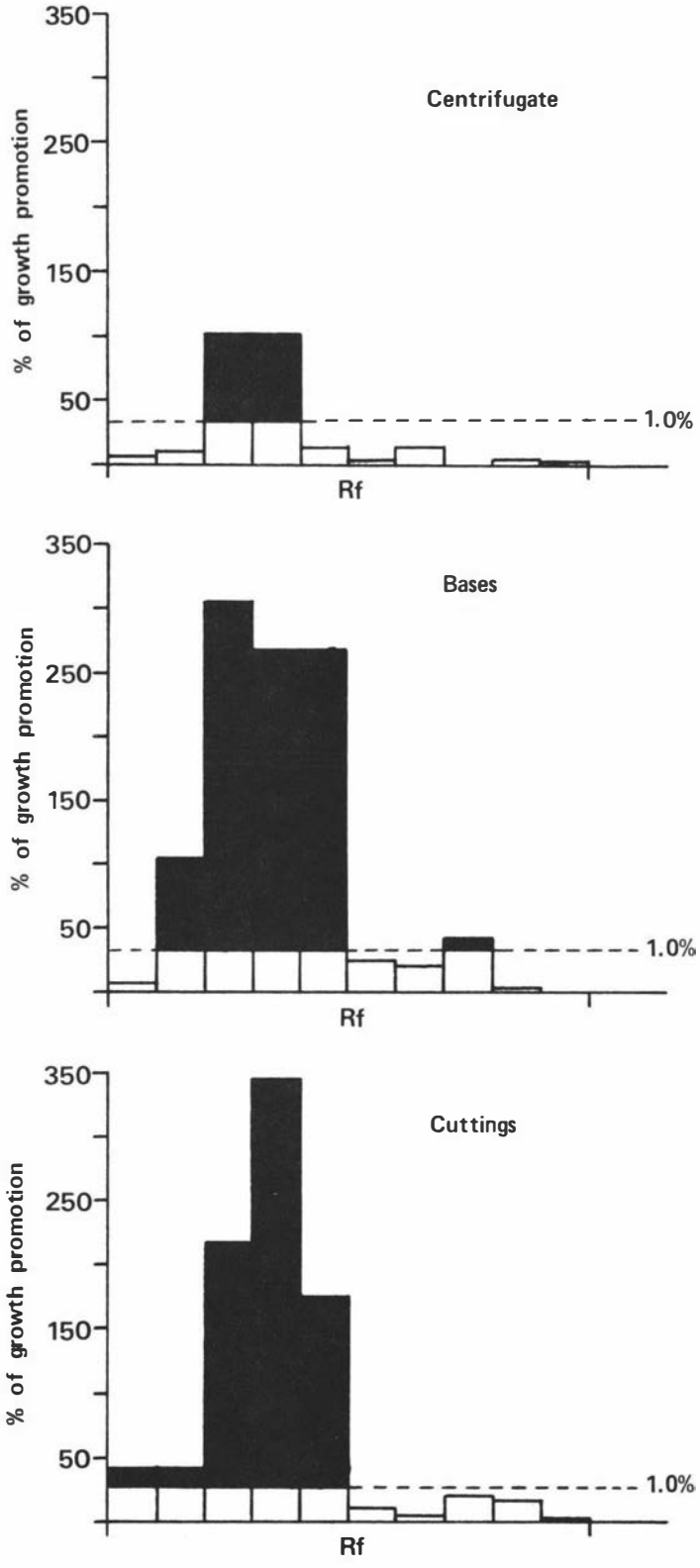

FIGURE 20

Histograms of an aqueous promoter from stem tissue and centrifugate of MM 106 apple rootstock.

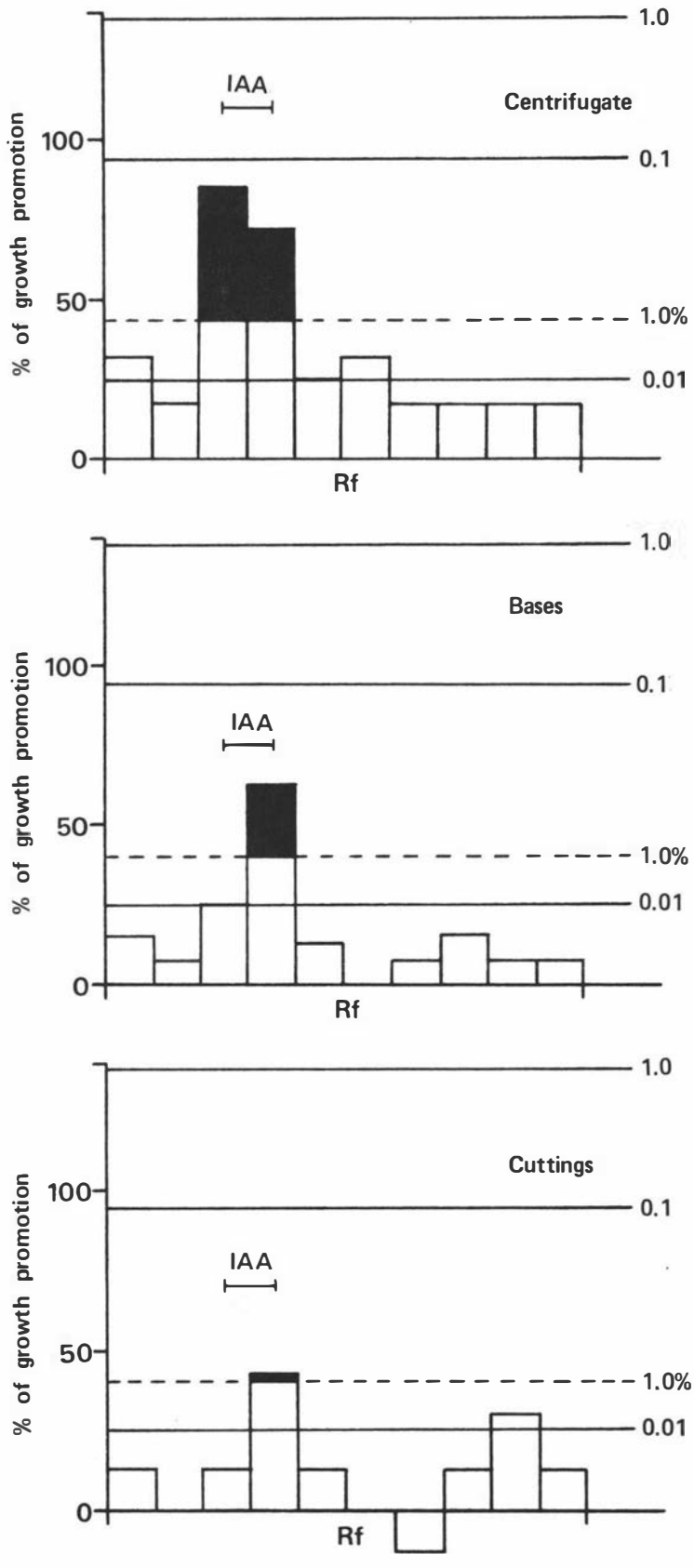

FIGURE 21

Histograms of IAA-like growth promoters from stem tissue and centrifugate of MM 106 apple rootstock.

retained the lowest concentration of IAA. The reduction of root initiation by centrifugation seems likely to be a result of depleted endogenous IAA, since restoration of the rooting ability was possible by the addition of exogenously supplied auxins.

C. The Effect of Varying Concentrations of IBA on Root Formation of MM 106 and EM XII Hardwood Cuttings

Although much work has defined the optimum levels of IBA in terms of commercial propagation of hardwood cuttings, it is still necessary to critically evaluate the effects of increasing concentrations of IBA on root initiation. Using the assumption that 2,500 ppm IBA is at or above the optimum level required to promote root initiation on apple rootstock hardwood cuttings, the influence of higher concentrations of IBA was investigated.

Cuttings 15 cm long were prepared and disbudded. Solutions of IBA at concentrations of 2,500, 5,000, 10,000 and 20,000 ppm were prepared in 50% ethanol solutions. Ten cuttings were treated accordingly to standard techniques, in each concentration and planted on the heat bed. Lanolin capsules were placed on the cutting apices and the cuttings were left for 6 weeks.

Tables 8 and 9 show the effect of IBA concentration on root initiation of MM 106 and EM XII hardwood cuttings. Statistical analysis was by Duncan's Multiple Range Test.

Table 8. The effect of IBA concentration on root initiation of EM XII apple rootstock hardwood cuttings

	IBA Concentration (ppm)			
	2,500	5,000	10,000	20,000
Total rooted	1	0	0	0
Mean No. roots/cutting	0.1	0	0	0
				N.S.

Table 9. The effect of IBA concentration on root initiation of MM 106 apple rootstock hardwood cuttings

	IBA Concentration (ppm)			
	2,500	5,000	10,000	20,000
	8	14	11	1
	9	4	8	3
	14	13	12	1
	9	17	10	10
Replicate	2	1	18	5
	13	12	6	13
	7	12	8	1
	1	13	14	18
	18	9	8	7
	0	0	0	0
Total rooted	9	9	9	9
Mean No. roots/cutting	8.1	9.5	9.5	5.9 N.S.

No significant increase or decrease in the number of cuttings initiating roots, or in the average number of roots per cutting was evident as a result of increasing concentration of IBA. Some toxic effects on rooting were noted in MM 106 cuttings at 10,000 and 20,000 ppm IBA. In both cases, the damage was in the form of stunted and twisted roots. Normal elongation of the roots had been inhibited particularly at 20,000 ppm. Although not significant, reduction of root number was apparent in MM 106, 20,000 ppm IBA. In the EM XII plots, increased damage in the form of basal rot or failure to form callus tissue was correlated with the rise in IBA concentration.

Indications from this experiment suggest that once the optimum level of activity of IBA in root initiation processes has been reached, no further promotion of rooting can be obtained by increasing the dosage of IBA. These results suggest that the additive interaction between IAA and IBA in MM 106 is not simply due to an increase in the total auxin-promotion of root initiation.

D. The Metabolism of ^{14}C-IAA in Hardwood Cuttings of MM 106 and EM XII Apple Rootstocks

Further examination of the roles of IAA and IBA in the physiology of root initiation was achieved by studying the metabolism of IAA in the cuttings, using 1-^{14}C-IAA and 2-^{14}C-IAA radio-isotopes. Several aspects of the metabolism of IAA were studied simultaneously. Comparison of the metabolism between the two rootstocks by studying the formation of metabolites and the destruction of IAA by measurement of $^{14}CO_2$ evolution, were made to evaluate differences in ability to initiate roots. The effect of a basal IBA treatment on the metabolism of IAA was compared in both rootstocks to attempt to elucidate the mechanism of action of IBA on root formation processes, in relation to IAA.

Methods

Hardwood cuttings of MM 106 and EM XII apple rootstocks approximately 5 mm in diameter were collected in mid-winter, immersed in a 1% chlorox solution for five minutes, and then rinsed twice in sterile water. The cuttings were then trimmed to a length of 4 cm including a basal bud. All treatments were conducted under sterile conditions in a transfer cabinet. The following treatments were applied to both rootstocks.

Treatment 1:- a basal 10 second quick dip in IBA (2,500 ppm) in 50% ethanol, and then transferred to a sterile scintillation vial containing

1 ml of 1-^{14}C-IAA aqueous solution, concentration 1 ppm (580,000 cpm Sp. act. 46 mci/mM).

Treatment 2:- a similar treatment, dipping in 50% ethanol minus the IBA.

After loading, the vials were capped with sterile screw caps which had had inlet and outlet tubes packed with cotton wool prior to autoclaving. This was to maintain sterile conditions for as long as possible. The vials were connected into a closed system between an air humidifier and a CO_2 trapping system. The system used was a modification of the apparatus used by Ryugo and Breen (1974). A peristaltic pump was connected in series to an air humidifier, a vial containing cuttings and ^{14}C-IAA, a $^{14}CO_2$ trapping system and then back to the pump forming a closed air system.

The same methods were repeated using 2-^{14}C-IAA (690,000 cpm sp. act. 55 mci/mM) and each treatment was duplicated. Each set of treatments were run for 72 hours in darkness at 25^{o}C.

Results

(1) $^{14}CO_2$ Evolution

The rate curves of $^{14}CO_2$ evolution from 1-^{14}C-IAA treated cuttings are presented in figure 22, each point representing the total cpms of the 12 hour period prior to the recorded time.

In MM 106 and EM XII cuttings without IBA, a relatively steady evolution of 1-$^{14}CO_2$ occurred for the first 36 hours, but then a considerable increase occurred in the level of labelled $^{14}CO_2$ evolved, continuing to increase until the termination of the treatment. Although a similar trend occurred in both rootstocks, the effect was most pronounced in EM XII. In cuttings which had been treated with IBA prior to exposure to 1-^{14}C-IAA

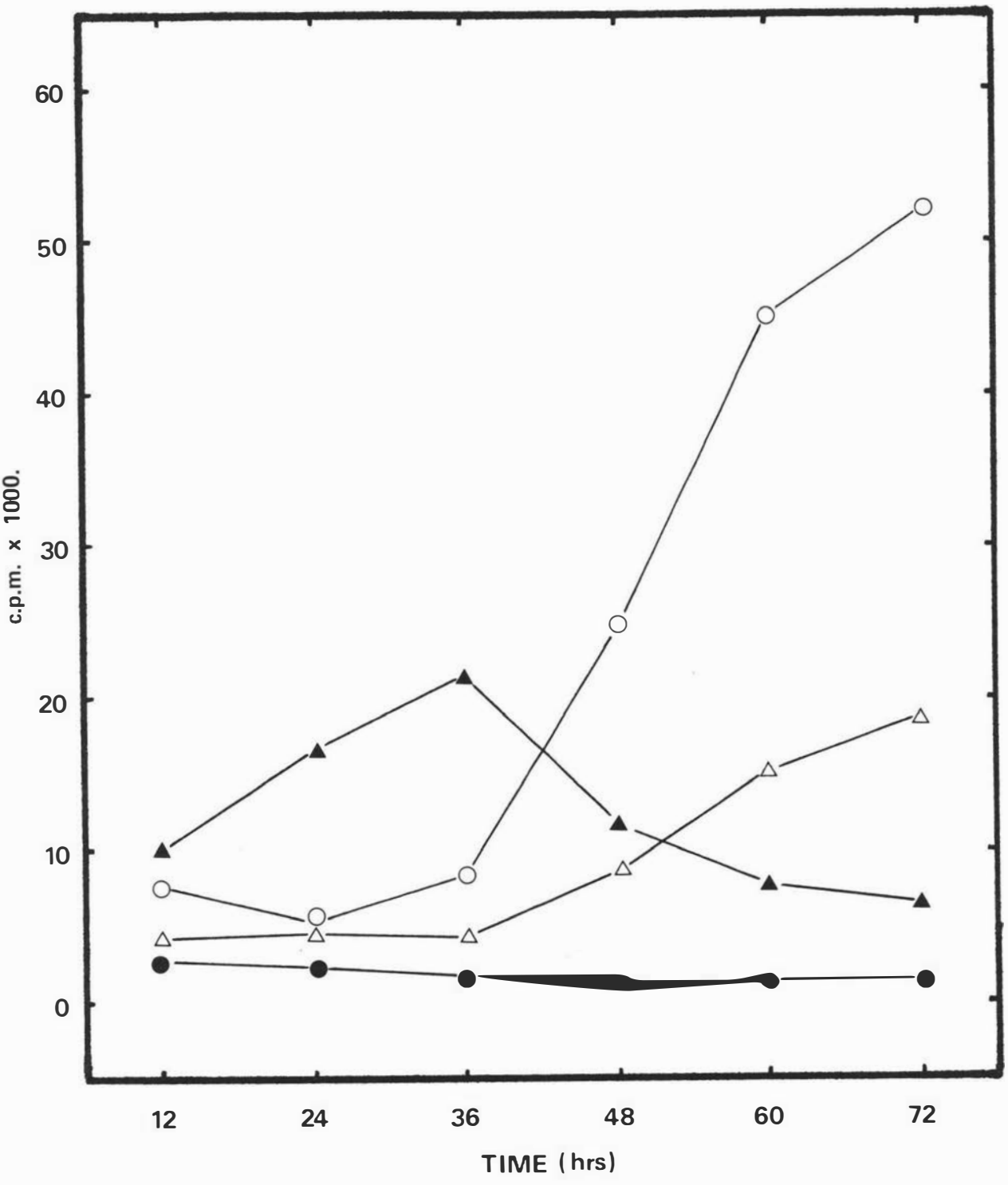

FIGURE 22

Rate curves of $1^{14}CO_2$ evolution by M.M. 106 and EM XII hardwood cuttings. ▲—▲ MM 106 + IBA. △—△ MM 106 – IBA. ●—● EM $\overline{\text{XII}}$. + IBA. ○—○ EM $\overline{\text{XII}}$ – IBA.

a different pattern was evident. In EM XII, a rate of $^{14}CO_2$ evolution, half or less than the initial rate of undipped cutting was observed and was maintained at the low level for the duration of the experiment. With MM 106 initial $^{14}CO_2$ evolution increased quite rapidly until 36 hours, but then rapidly decreased to a level similar to the initial rate of evolution of undipped cuttings.

The same treatments were repeated using 2-^{14}C-IAA, and high levels of $^{14}CO_2$ were detected. The rate curves of $^{14}CO_2$ evolution from 2-^{14}C-IAA treated cuttings are presented in figure 23. In both rootstocks pre-treated with IBA, a low level of $^{14}CO_2$ evolution occurred, increasing only slowly with time. In untreated cuttings, with both rootstocks, the rate of $^{14}CO_2$ evolution increased rapidly to a peak 48-60 hours after the treatment began and then declined slowly. In both experiments, the production of labelled CO_2 from untreated cuttings is greater and more rapid from EM XII, than MM 106. In general, the degradation of IAA which results in $^{14}CO_2$ evolution in both experiments, is retarded by the IBA treatment administered prior to the addition of the radio-isotope.

(2) External Solutions

The remaining solutions of ^{14}C-IAA at the cuttings bases of each treatment were made up to a known volume with 80% methanol and called the external solutions. These were tainted a brown colour from pigments leached from the cutting bases. The radiochromatograms of external solutions of 1-^{14}C-IAA and 2-^{14}C-IAA are presented in figure 24 and 25 respectively.

The only prominent area of activity was in the zone corresponding to IAA. A low level of activity was always present in the first chromatogram segment, the same zone in which the brown pigments in the solutions were limited. This is probably due to labelled IAA complexing with phenolic

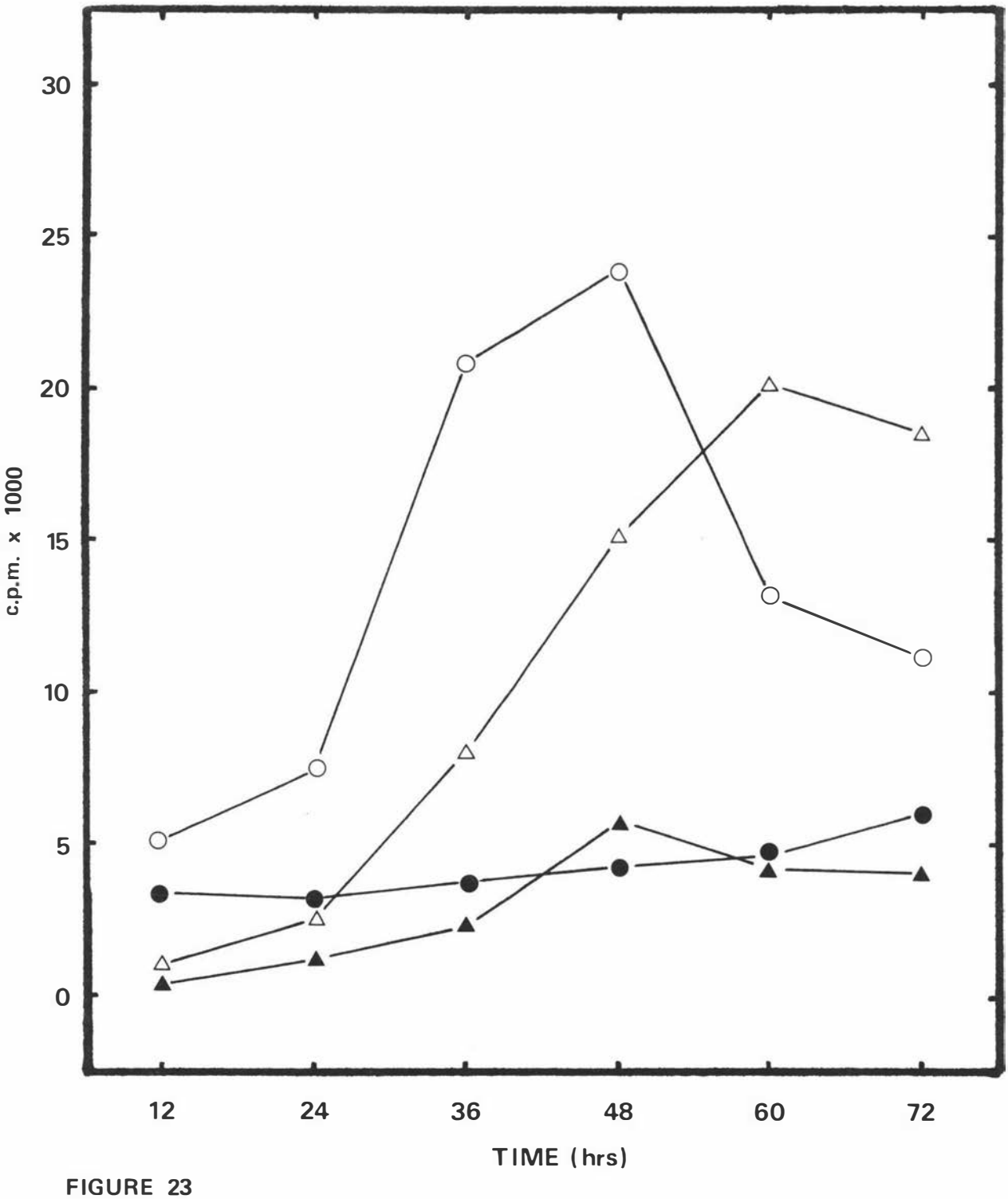

FIGURE 23

Rate Curves of $2^{14}Co_2$ evolution by MM106 and EMXII hardwood cuttings.
▲—▲ MM 106 + IBA. △—△ MM 106 – IBA. ●—● EM XII + IBA.
○—○ EM XII – IBA.

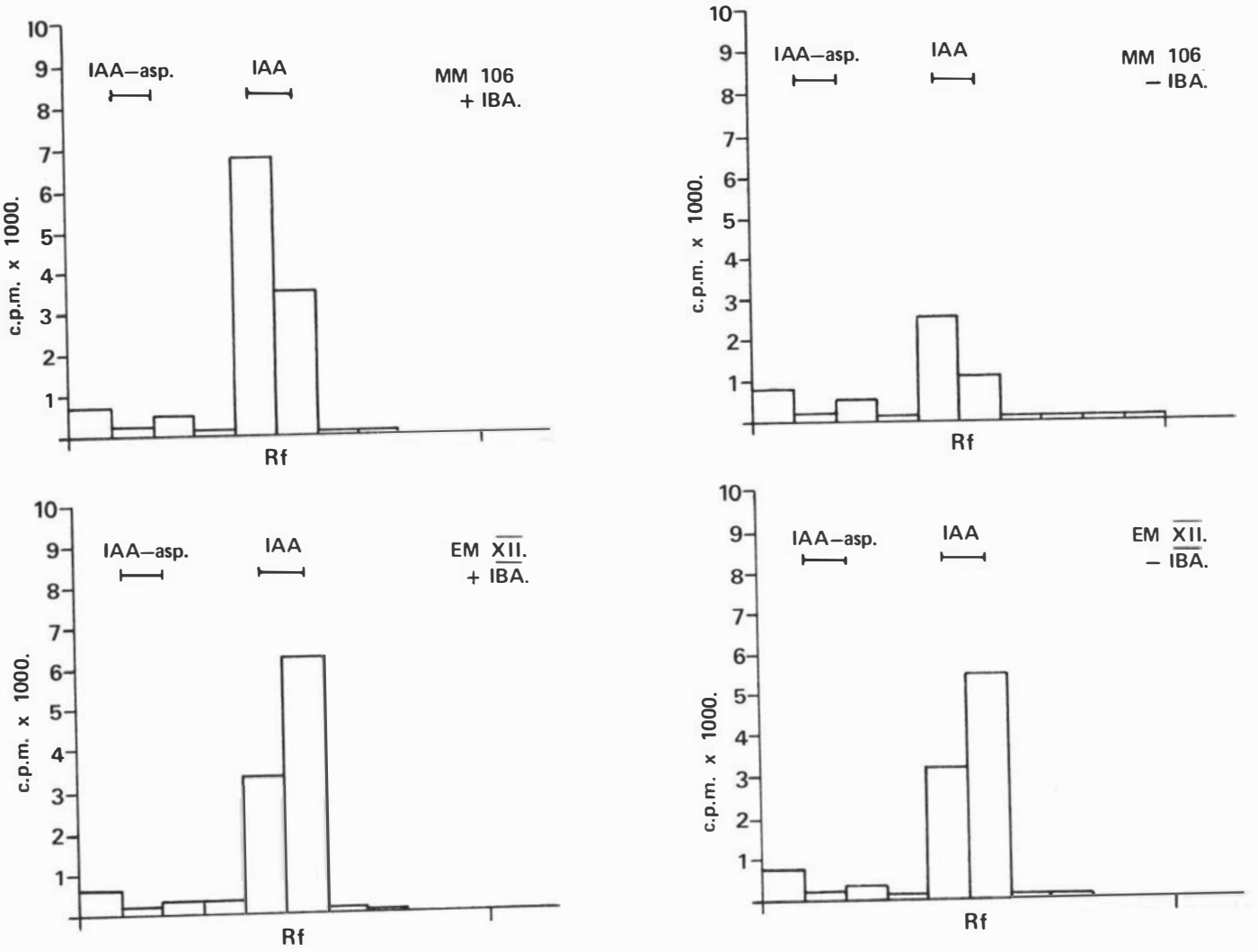

FIGURE 24

Histograms of chromatogram segments of the $1-{}^{14}C$ – IAA external solution. The solvent system was isopropanol: NH_4OH : water (8 : 1 : 1.5 v/v).

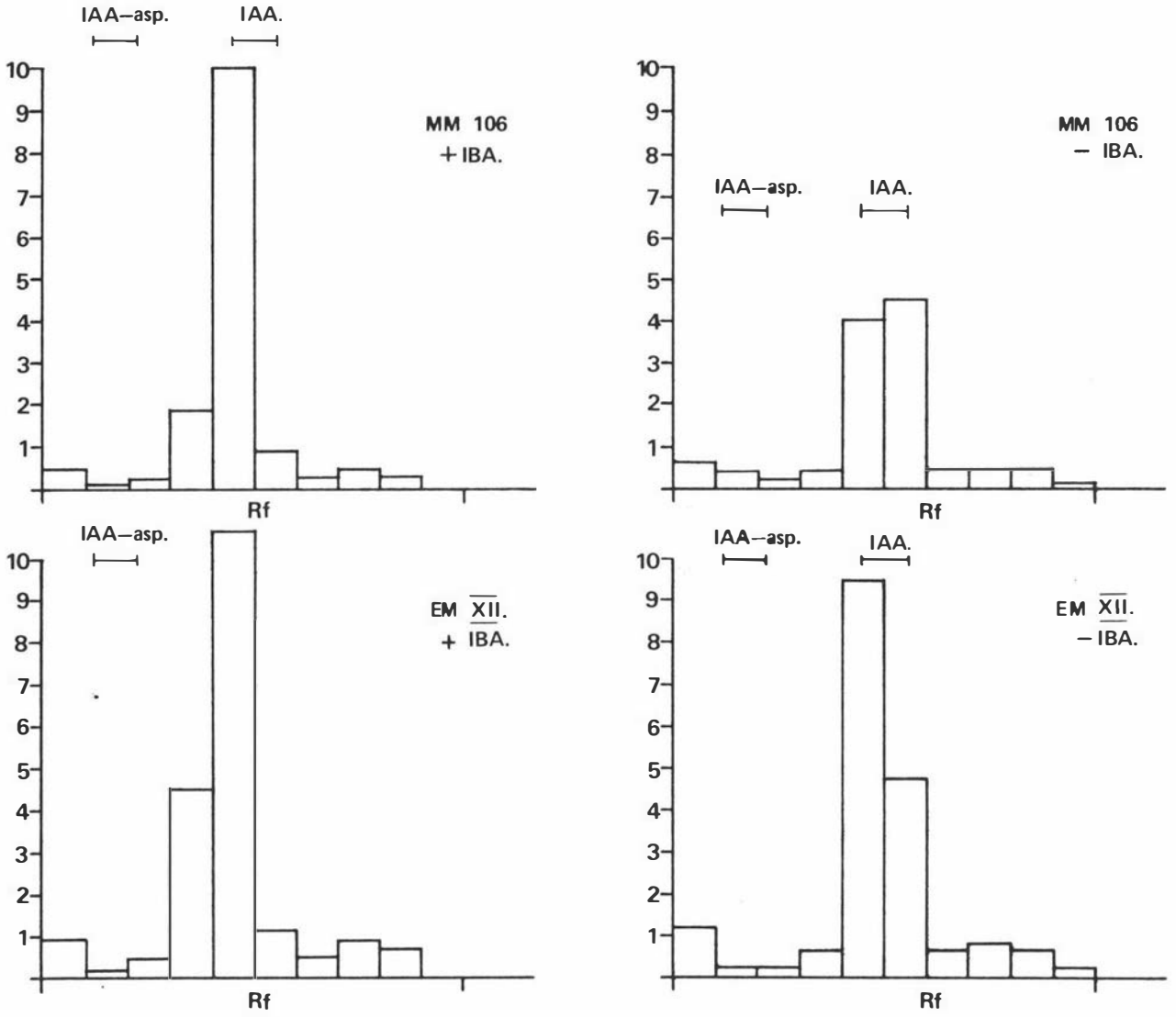

FIGURE 25

Histograms of chromatogram segments of the 2 – ^{14}C – IAA external solution. The solvent system was isopropanol : NH_4OH : water (8 : 1 : 1.5 v/v).

compounds described by Leopold and Plummer (1961), released from the cut ends of the cuttings and leached from the cutting bases. The activity in this zone was not considered to be of any real significance in the metabolism of ^{14}C-IAA. No major metabolite was located in the external media, suggesting that they are not freely mobile once formed.

In external solutions of both 1-^{14}C-IAA and 2-^{14}C-IAA experiments, the level of remaining ^{14}C-IAA in the external solution was always higher in treatments which had included an IBA pre-treatment of the cuttings. This was much more evident in MM 106 than in EM XII.

(3) Alcohol Extracts of Cuttings

Two major zones of activity were located on chromatograms of the alcohol extracts, one corresponding to IAA and one corresponding to indoleacetyl aspartic acid (IAA-asp.). Figure 26 presents radiochromatograms of alcoholic extracts of the two rootstocks treated with 1-^{14}C-IAA. Cuttings of both MM 106 and EM XII pretreated with IBA retained a higher level of activity in the IAA zone than untreated cuttings. In both cases the ratio of IAA level between IBA-treated cuttings and untreated cuttings was approximately 2:1. In MM 106 cuttings the degree of IAA-asp. conjugate formation is very different between IBA-treated and untreated cuttings. The level of IAA-asp. in IBA-treated cuttings is only one quarter of the free IAA level, whereas in untreated cuttings, conjugation has proceeded to a much greater degree, so that twice as much activity resides in the IAA-asp. zone compared to free IAA.

In EM XII cuttings, those treated with IBA show no development of IAA-asp. conjugation, whereas untreated cuttings show only a low development of IAA-asp. In general the conjugation reaction seems to be much slower in EM XII than in MM 106.

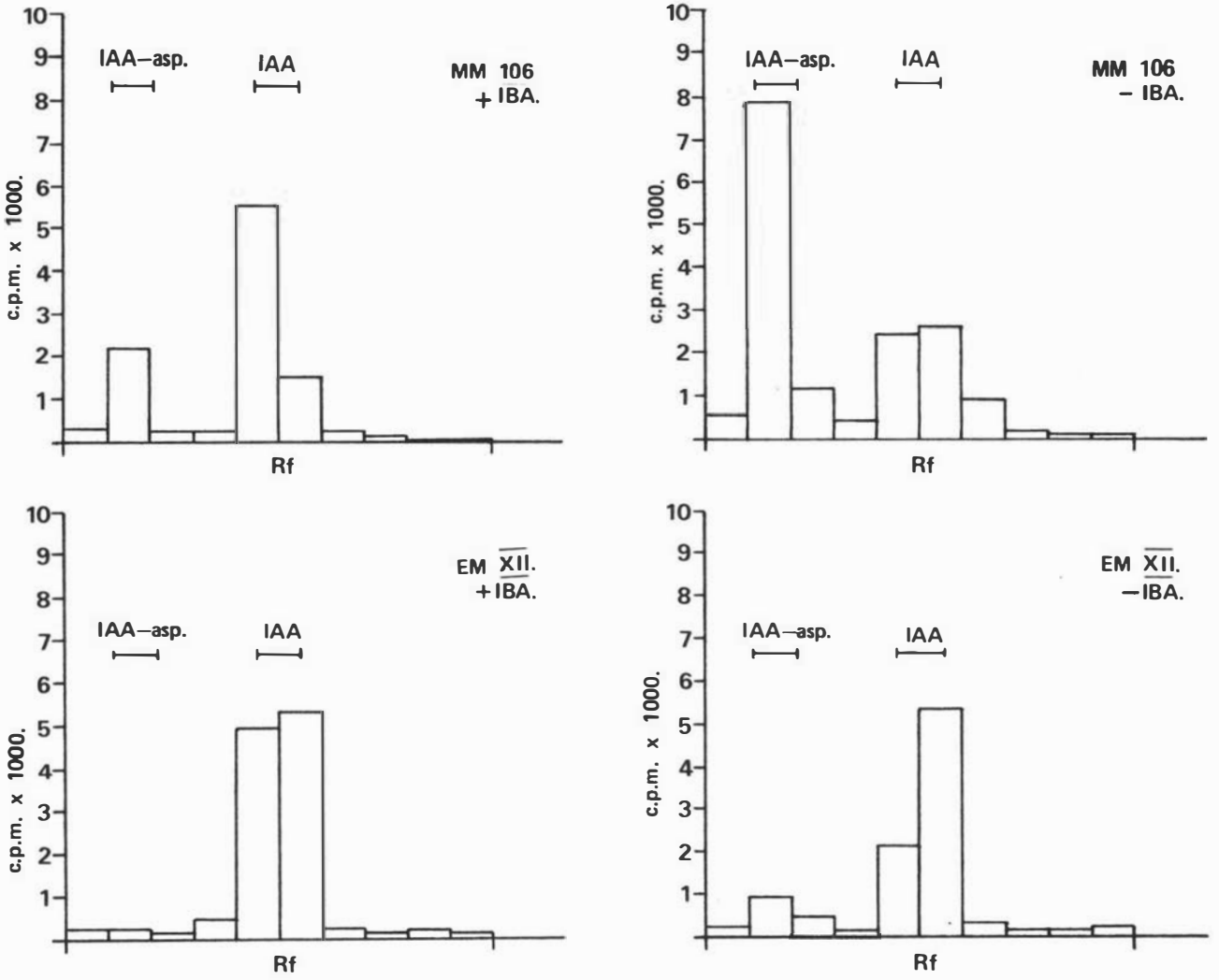

FIGURE 26

Histograms of chromatogram segments of the alcoholic extracts of MM 106 and EM XII hardwood cuttings treated with $1 - {}^{14}C - IAA$. The solvent system was isopropanol : NH_4OH : water (8 : 1 : 1.5 v/v).

Figure 27 shows radiochromatograms of alcoholic extracts of EM XII and MM 106 cuttings after exposure to 2-^{14}C-IAA. A similar pattern emerges with 2-^{14}C-IAA as was evident in cuttings treated with 1-^{14}C-IAA. In IBA-treated MM 106 cuttings, the IAA-asp. activity is restricted to approximately one third of the activity of IAA, but in untreated cuttings, 2.5 times the activity of IAA was located in the IAA-asp. zone. More than twice as much activity in the free IAA zone was present in the IBA-treated cuttings compared with untreated MM 106 cuttings.

EM XII cuttings pre-treated with IBA show only minimal IAA-asp. conjugation, but untreated cuttings have an IAA-ASP. activity approaching one half the activity of free IAA. As was found in MM 106, the activity found in the free IAA zone was twice as high for IBA-treated cuttings than untreated EM XII cuttings.

(4) Alcohol Insoluble Compounds

Tissue remaining after alcohol extraction underwent alkaline hydrolysis and ether extraction. In both MM 106 and EM XII tissue treated with both carboxyl and methylene labelled IAA, very little activity was obtained after hydrolysis. Chromatograms showed that any activity present was located at Rfs 0.0-0.1 and Rf 0.5-0.6 (IAA). The activity obtained was only minimally above background levels and was not considered to be significant in the metabolism of ^{14}C-IAA. This was verified by analysis of activity budgets which showed that almost all the activity was accounted for in the $^{14}CO_2$, external solutions and alcohol extracts of the various treatments.

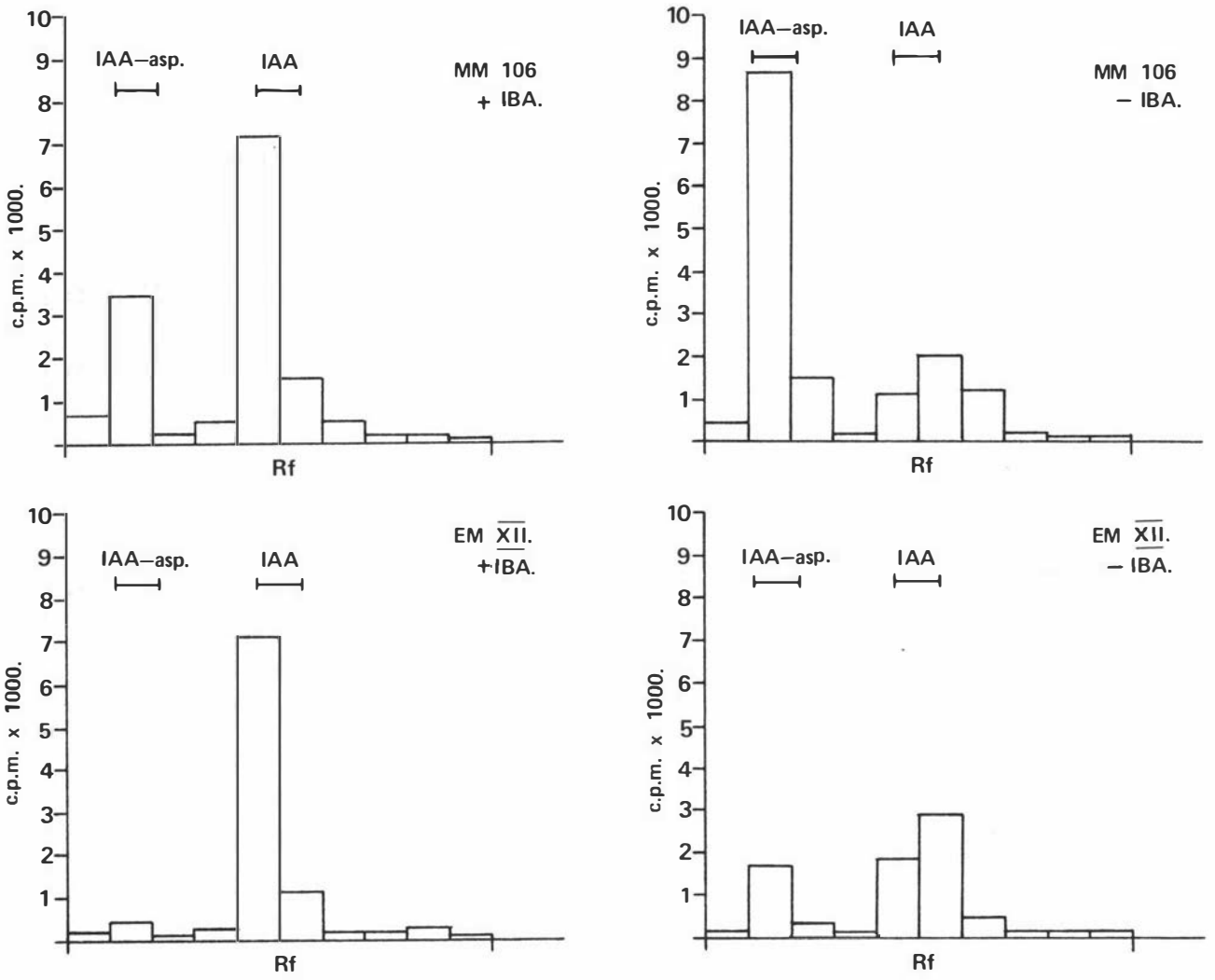

FIGURE 27

Histograms of chromatogram segments of the alcoholic extract of MM 106 and EM XII hardwood cuttings treated with $2 - {}^{14}C - IAA$. The solvent system was isopropanol : NH_4OH : water (8 : 1 : 1.5 v/v).

DISCUSSION

The concept of promotion of root initiation by IAA is now generally accepted. Exogenous applications of IAA or synthetic auxins (IBA, NAA, 2,4-D) have been shown to promote root initiation on cuttings of fruit tree rootstocks (Cooper, 1935; Hatcher and Garner, 1947; Hartmann et al., 1965; Howard, 1966, 1968a; Carlson, 1966; Nahlawi and Howard, 1971; Doud and Carlson, 1972). A traditional interpretation has been that exogenous auxin applications increase the total auxin pool to a level which will promote root initiation. Results from Sections I and II demonstrated 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; a similar situation was observed by Lanphear and Meahl (1963).

Treatments of IAA, IBA or both promoted rooting in MM 106 rootstock (see Table 4), when compared with the control in terms of the number of cuttings rooted. When compared on the basis of average number of roots per cutting, the IAA treatment was not significantly different from the control but the lack of statistical significance was only marginal. Similarly the IBA treatment was not significantly different from the IAA treatment but this lack of significance was also marginal. A combined treatment was significantly better than all other treatments. With EM XII rootstock, the only treatment to induce root initiation was the IAA/IBA combined treatment (Table 5). Root initiation on MM 106 appeared to be promoted more readily by IBA than IAA, the combination producing an additive promotion. This suggested that both IAA and IBA have a role in the same physiological process but that they do not both act on the same process. In EM XII rootstock, neither exogenous auxin stimulated root initiation when applied alone, but the combined treatment induced rooting on 40% of the cuttings.

This indicated that neither IAA nor IBA alone could reach an optimum level for promoting rooting, but in combination, either the total auxin level or the combined reactions of IAA and IBA, served to promote root initiation.

Knowledge of the endogenous levels of IAA in the two rootstocks led to the conclusion that confounding of the results had occurred. Since MM 106 had a level of endogenous IAA which facilitated the rooting of a high percentage of the cuttings throughout the season, marked promotion by the addition of further IAA was not likely to be forthcoming. Furthermore, the addition of IBA would be expected to give a considerable degree of rooting promotion. EM XII was found to retain a very low level of endogenous IAA through the season, so the concentration applied (0.1%) did not necessarily raise IAA levels to an optimum level. The failure of IBA alone, to promote rooting in EM XII noted in Sections I and II, was again evident.

Treatments designed to overcome the respective complicating factors were used to modify the experiments. A spectacular removal of the rooting ability of MM 106 was achieved using centrifugation and cutting base removal. Examination of centrifugates and tissue 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 MM 106 in terms of numbers rooted and the average number of roots per cutting was achieved by both IAA and IBA application; the combined treatment again showed an additive promotion effect (Table 7). A ten-fold increase in the IAA concentration applied to EM XII cuttings did not promote rooting in the IAA treatment. An effective promotion of rooting of 50% (i.e. from 40% to 60% of the cuttings rooted) was observed in the combined treatment, i.e. by increasing the IAA level, rooting was promoted in the IAA/IBA treatment (Table 6).

Removal and the subsequent restoration of a root-promoting stimulus

and the increased stimulation of rooting in a difficult-to-root species, were related to the changes in level of endogenous and exogenously supplied IAA. Since IBA also restored the ability of MM 106 cuttings to root, the effect of changing IBA concentrations on root initiation was evaluated.

Changes in IBA concentration from 2,500 ppm to 20,000 ppm had no significant effect on the number of cuttings rooted or the average number of roots per cutting in MM 106 and EM XII (Tables 8 and 9). This indicated that once an optimum level was achieved in the cutting base, no further promotion was obtained by increasing the IBA concentration. Because the IBA-promoted rooting stimulus could easily be saturated, the postulation 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 to be the most likely role of IBA in promoting root formation.

IAA appeared to be the fundamental promoter of root initiation in apple rootstock hardwood cuttings. The most recent research reports on the physiology of root initiation indicated that root initiation was an IAA-specific mechanism and that IAA influenced the first "initiation of root meristems" phase of adventitious root regeneration (Greenwood and Goldsmith, 1970; Haissig, 1970, 1973; Smith and Wareing, 1971, 1972a, b; Eriksen and Mohammed, 1974, Mohammed and Eriksen, 1974; Greenwood et al., 1974; Ryugo and Breen, 1974; Mohammed, 1975). Furthermore, a continuous IAA supply during the initial primordial development was essential for root initiation (Haissig, 1970; Greenwood et al., 1974, Mohammed and Eriksen, 1974). Results in Section III suggested that root initiation in MM 106 and EM XII was controlled primarily by endogenous IAA levels. No evidence that IAA acted by forming complexes with cofactor-like compounds as suggested by Hess (1965) and Fadl and Hartmann (1967a, b), was found. Total removal and restoration of the rooting stimulus in MM 106 were directly related to

levels of free IAA within the cuttings. While root initiation in EM XII could not be promoted by IAA alone, different levels of applied IAA, in combination with a standard IBA treatment, increased the promotion of rooting considerably. Because EM XII had a very low free IAA level *in vivo*, a rapid and vigorous IAA-inactivation system may operate which inactivates exogenously-applied IAA when administered alone, thus preventing the induction of root primordia. This could also explain why a spring peak of rooting of EM XII seen in Section I was only evident after an IBA pre-treatment. MM 106 could have a less vigorous IAA-inactivation system *in vivo*, enabling high levels of endogenous IAA to be retained by the root-stock.

There was no evidence that IBA acted on the same root-promoting processes as IAA in either rootstock. This casts doubt on the interpretation of results by equating exogenously-applied synthetic auxins with endogenous IAA; in terms of a total auxin pool reaching optimum requirements. IBA could only promote rooting in cuttings containing near-optimum levels of IAA. Therefore IBA must have promoted root initiation by some supporting reaction to that of IAA. Ryugo and Breen (1974) found that IBA inhibited IAA-oxidase activity as measured by the rate of $^{14}CO_2$ evolution, in plum cuttings. Inhibition of the IAA-inactivation systems in apple rootstocks could account for the observed promotions of root initiation in MM 106 and EM XII. In rootstocks with a high endogenous IAA level (MM 106), any inhibition of IAA inactivation would allow optimum levels of IAA to accumulate more rapidly in the cutting base, 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, both in numbers rooted and average number of roots per cutting. In rootstocks with a low IAA status (EM XII) 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 (e.g. by spring bud-burst or by exogenous IAA-lanolin application) simultaneously, the IAA would be protected from inactivation and could then induce root initiation. It is proposed that this mode of action could occur in EM XII cuttings since:-

(i) an increase in endogenous IAA resulting in improved rooting in early spring was either protected by or the result of an IBA pretreatment prior to planting the cuttings.

(ii) the only treatment to initiate roots on EM XII cuttings was the IAA/IBA combination.

(iii) by increasing the IAA level to 1% IAA in lanolin, a 50% promotion in root initiation was achieved on EM XII cuttings receiving the combined IAA/IBA treatment, compared with a 0.1% IAA-lanolin application.

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 the centrifuged MM 106 cuttings treated with IBA and the EM XII cuttings receiving the IAA/IBA treatment. Odom and Carpenter (1965) found that the greatest response to synthetic root-promoting substances was from cuttings which exhibited a slowly accumulated acidic auxin content.

In order to study the exact mode of action of IBA in root initiation on apple rootstocks and to understand any differences in IAA metabolism between MM 106 and EM XII, further investigations of their respective roles were achieved using 1-^{14}C-IAA and 2-^{14}C-IAA radio-isotopes. High activity, low concentration solutions of both radioactive compounds were used to facilitate clear tracing of metabolites. Radioactive IAA solutions

were made up to 1µug/ml concentration, the level observed to be near the optimum for root initiation in MM 106, in the seasonal studies (1 µg/5 gm dry tissue).

IAA-decarboxylation and inactivation activity was measured by monitoring the production of $^{14}CO_2$. The evolution of $^{14}CO_2$ from 1-^{14}C-IAA was observed to increase rapidly after 36 hours exposure to the labelled IAA, in the cuttings untreated with IBA. A constant low level of evolution was seen in IBA-treated EM XII cuttings and in IBA-treated MM 106 cuttings, a rapid initial increase in $^{14}CO_2$ was reduced to low levels after 48 hours.

IAA-decarboxylation activity appeared to be more vigorous in EM XII than in MM 106, but was more effectively blocked by IBA treatment. In both rootstocks, IBA exhibited IAA-decarboxylation-inhibiting activity as was found by Ryugo and Breen (1974) in plum cuttings.

Cuttings exposed to 2-^{14}C-IAA ± IBA produced considerable levels of $^{14}CO_2$, with a peak of $^{14}CO_2$ occurring in both untreated rootstocks after 48-60 hours. Evolution of $^{14}CO_2$ from 2-^{14}C-IAA treated cuttings suggested IAA inactivation could be caused by either α-α decarboxylation or ring cleavage resulting in fragmentation of the IAA molecule. Both inactivation processes have been found to occur in plum cuttings by Strydom and Hartmann (1960) and Geronimo *et al*. (1964) but not by Ryugo and Breen (1974). Cuttings treated with IBA maintained a low steady level of $^{14}CO_2$ evolution.

Enzymic degradation of free IAA by IAA-oxidase (Ryugo and Breen, 1974) and by α-α decarboxylation (Strydom and Hartmann, 1960) and/or ring cleavage (Geronimo *et al*., 1964), has been shown to be significant inactivation processes in fruit tree rootstocks. IBA may possibly inhibit the rapid destruction of IAA by all the inactivation processes; the mode of action

which was proposed to operate in MM 106 and EM XII. As was postulated, a more vigorous IAA-decarboxylation system was apparent in EM XII, possibly accounting for the lower endogenous levels of free IAA present, over the harvest season (Section I). Nevertheless, IBA appeared to be most effective in inhibiting the EM XII IAA-inactivation system.

Solutions of ^{14}C-IAA remaining after the treatment of cuttings, showed that treatments which included an IBA dip on the cuttings, took up less IAA from the external solutions. This correlated with reduced inactivation by IAA-decarboxylation, a process already found to be inhibited by IBA treatment.

Examination of the alcohol extracts of the cuttings showed two major zones of activity corresponding to IAA and IAA-asp. (figures 26 and 27). In both 1-^{14}C-IAA and 2-^{14}C-IAA extracts, cuttings which had received a basal IBA treatment, retained twice the level of activity in the IAA zone, than untreated cuttings. This was apparent in both EM XII and MM 106 and suggested that IBA did not act solely by inhibiting decarboxylation. IAA-asp. conjugation was very different between the rootstocks, but appeared to be inhibited by IBA application in both EM XII and MM 106, contrary to the report of Ryugo and Breen (1974). MM 106 showed a high propensity to form the IAA-asp. conjugate whereas EM XII demonstrated a very low ability to do so.

Since IBA promoted rooting if IAA was present; but prevented the conjugation of IAA with aspartic acid, it is unlikely that the conjugation reaction is the first step in root initiation as suggested by Hess (1969) and Ryugo and Breen (1974). Several workers considered that IAA-asp. formation to be an inactivation, detoxification or storage process of excess endogenous IAA (Andreae and Good, 1957; Andreae and van Ysselstein,

1956, 1960; Andreae, 1967; Lantican and Muir, 1969; Morris et al., 1969; Muir, 1970) in which the conjugation product has no physiological auxin activity. The significance of the widely differing levels of IAA-asp. conjugation between MM 106 and EM XII was most likely related to the differences in endogenous IAA concentration. Natural formation of IAA-asp. in tomato indicated that formation of the conjugate could occur under physiological conditions (Row et al., 1961) and that high endogenous IAA levels induced the formation of IAA-asp. synthetase (Zenk, 1962; Andreae, 1967). Morris et al. (1969) also found that conditions that reduced destruction of IAA favoured IAA-asp. conjugation and also promoted the conjugation reaction. MM 106 rootstock has both a lower rate of IAA inactivation and a high endogenous IAA level, both of which would enccurage high IAA-asp. synthetase activity. Because EM XII had a low endogenous IAA level, stimulation of the synthesis of the conjugation enzyme would not occur to the same extent as in MM 106. Therefore the large difference in degree of IAA-asp. conjugation appeared to be related to the respective endogenous IAA levels of the two rootstocks. The formation of IAA-asp. was considered to be a storage or inactivation process of excess free IAA by the plant tissue, which was independent of the root initiation process.

An alternative explanation of the action of IBA on IAA-asp. formation could be that IAA-asp. is rapidly metabolised by EM XII resulting in low detectable levels of the conjugate. IBA may protect IAA-asp. from further metabolism in both rootstocks, thus facilitating the deconjugation of IAA-asp. to release free IAA over a longer period of time. There is little evidence to support this hypothesis in the literature and considerable evidence that suggests that IAA-asp. synthetase is only induced by high endogenous IAA levels (Zenk, 1962; Andreae, 1967; Morris et al., 1969). It would seem more likely that the difference in level of IAA-asp. formation was related to the differing IAA status of MM 106 and EM XII particularly

when IAA-asp. appears to be a stable conjugate whose most likely fate is incorporation into proteins as suggested by Ryugo and Breen (1974).

The action of IBA, in promoting root initiation on apple rootstock cuttings, may well be by a mechanism of protection of IAA from inactivation or oxidation; the basic method suggested by Hess (1969) and Ryugo and Breen (1974), although the mechanism was apparently different from that proposed by Hess. Induction of root formation appeared to be an IAA-specific reaction which did not require the formation of a conjugation product, contrary to reports from Fadl and Hartmann (1967), Hess (1969) and Ryugo and Breen (1974). It would appear to be more logical that IBA inhibited all IAA-inactivation processes, as suggested in this study, rather than inhibiting IAA-oxidase and promoting IAA-asp. conjugation (both inactivation processes) as Ryugo and Breen (1974) suggested. It is postulated that the role of IBA in promoting root initiation is by inhibiting the IAA-inactivation and conjugation mechanisms which regulate and reduce free IAA levels in the woody shoot. Since IBA is a structural analog of IAA, the most likely mechanism would be by occupying the active sites on the respective enzymes, thus blocking IAA destruction and allowing local IAA levels in the cutting base to increase. The aspartate conjugation enzyme in pea tissue has been shown to be capable of forming conjugates with IAA, NAA and benzoic acid (Venis, 1972). The induction of this enzyme was absolutely dependant on physiologically active auxins (Sudi, 1964, 1966; Venis, 1972). It would therefore be reasonable to assume that IBA could form conjugates with aspartic acid and thus block the inactivation of IAA by competitive inhibition of IAA-inactivation systems by irreversible binding, the apparent mechanism operating in MM 106.

High levels of physiologically active auxins induce the synthesis of IAA-conjugation and oxidation enzymes thus providing an IAA-regulating

system in plants. Blocking the IAA regulatory system by IBA application in the cutting base may increase IAA levels to an optimum concentration required to trigger root meristem formation.

SUMMARY AND GENERAL DISCUSSION

A comparison between easily-rooted (MM 106) and difficult-to-root (EM XII) apple rootstocks showed that the difference in ability to form roots on hardwood cuttings occurred throughout the whole harvesting season when planted according to the East Malling system. Only with the spring harvest did the shy-rooting EM XII rootstock show any significant improvement in root formation, following an IBA quick dip treatment. Reports on the influence of the season of harvest on adventitious root formation of fruit tree rootstock cuttings indicated that autumn or spring-planted cuttings rooted most readily with a definite falling-off of rooting ability occurring during the mid winter (Howard, 1966; Fadl and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi, 1969b; Doud and Carlson, 1972). No strong seasonal trend in root formation was evident in either rootstock in the present study, although untreated MM 106 and IBA-treated EM XII cuttings exhibited seasonal changes most like those reported by Howard (loc. cit.). Nevertheless an increase to a seasonal maximum percentage of cuttings rooted was demonstrated in both IBA-treated rootstocks and in MM 106 untreated with IBA. It also became apparent that the seasonal promotion of rooting by IBA was only possible on cuttings exhibiting a high potential ability to root.

The effect of bud dormancy on root formation has been demonstrated by several workers (Wareing and Smith, 1963; Fadl and Hartmann, 1967a; Roberts and Fuchigami, 1973; Roberts *et al.*, 1974), but could not be substantiated on cuttings of apple rootstocks (Howard, 1968a). Seasonal changes in root formation of both MM 106 and EM XII could not be related to the degree of dormancy of the buds on the cuttings although a correlation was found between the termination of winter rest and promotion of rooting. The physiological state of the cutting resulting in axillary bud

burst may also be very conducive to promoting root formation. Spiegel (1955), Lanphear and Meahl (1963), Howard (1965), Nanda and Anand (1970) and Roberts et al. (1974) have reported similar promotions of root formation coinciding with the resumption of growth in the spring with a range of cutting species.

The importance of IAA in promoting adventitious root formation is now universally accepted and changes in the patterns of root formation have often been related to changes in endogenous auxin levels (Warmke and Warmke, 1950; Spiegel, 1955; Odom and Carpenter, 1965; Haissig, 1970; Greenwood and Goldsmith, 1970; Smith and Wareing, 1972a, b). In the present study, seasonal changes in root formation on both rootstocks could be related to changes in an IAA-like growth promoter. The difference in rooting ability of EM XII and MM 106 cuttings was also directly correlated with levels of IAA and the increased rooting in early spring was correlated with an increase in extractable IAA.

A measure of the critical minimum level of free endogenous IAA required to promote rooting might be broadly defined from the data presented in the previous three sections. The critical level of IAA which appeared necessary to promote rooting was approximately 0.1 ug/5 gm dry weight of stem tissue. Cuttings retaining auxin levels at or above this concentration of auxin exhibited a high potential to root when treated with IBA. Promotion of rooting with IBA was most spectacular on cuttings with a higher IAA level and seemed dependant on an optimum level of endogenous IAA within the cutting base. Cuttings which had very low endogenous auxin levels failed to root after IBA application, but promotion of rooting (significant at the 1% level) on EM XII was achieved with IBA when endogenous levels of IAA rose above a critical minimum in early spring.

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). Differences in levels of IAN in MM 106 and EM XII were of a similar magnitude to IAA but seasonal changes of the IAN content of either rootstock could not be correlated with the seasonal changes in root formation. No increase in IAN level was evident in the early spring when adventitious root formation increased. It is conceivable that IAN may be an inactive storage form of auxin which can be converted to IAA at times of increased physiological activity such as during spring bud break. This could explain why reduced levels of IAN were only located in samples taken from the propagating bin in autumn and spring and why no peak of IAN production coincided with bud burst as was seen with endogenous IAA levels.

Abscisic acid has been found to both promote root initiation (Chin and Beevers, 1969; Basu et al., 1970) or alternatively inhibit root formation (Heide, 1968; Eliasson, 1969; Pierek and Steegmans, 1975). In the present study, no root-promoting activity was found in the inhibitor-B complex and therefore ABA could not be shown to be an active rooting promoter. Heide (loc. cit.) suggested that ABA antagonised rooting on Begonia cuttings, since ABA enchanced processes which had been shown to reduce auxin synthesis and root formation. Therefore IAA:ABA interactions may be important in the correlative regulation of root formation. Both apple rootstocks were found to have similar ABA levels throughout the season, therefore the easily-rooted MM 106 retained a higher IAA:ABA ratio than EM XII. Increasing ABA and decreasing IAA levels through the winter months reduced the IAA:ABA ratios in both rootstocks, a time when difficult and poor rooting may occur (Howard, 1966, 1971; Fald and Hartmann, 1967a; Nesterov, 1968; Howard and Nahlawi, 1969; Doud and Carlson, 1972). In both rootstocks, an IAA:ABA ratio of

1:1 favoured root formation suggesting that ABA was not a potent competitive rooting inhibitor.

Most prominent of the growth regulator interactions affecting root formation on cuttings has been shown to be the auxin:cytokinin ratio (Skoog and Miller, 1957; Heide, 1965b, 1971; Mullins, 1970). As has been seen with ABA, MM 106 retained a high IAA:cytokinin ratio through the winter months compared with EM XII. In the early spring, when the auxin:cytokinin ratio in EM XII was increased by a high auxin flush, a significant promotion (1.0%) in rooting occurred. Although no intimate relationship between IAA, cytokinins and root initiation was established, the importance of the interaction has been reported (Heide, loc. cit.). Whenever the IAA:cytokinin ratio was high, a high degree of root formation was achieved in both MM 106 and EM XII. Further study of the effects of exogenously applied IAA and cytokinins is required to ascertain whether an antagonistic IAA:cytokinin ratio is instrumental in controlling root formation in apple rootstock cuttings.

Numerous workers have reported the involvement of rooting cofactors or auxin synergists in root initiation of cuttings including fruit tree rootstocks (Challenger et al., 1965; Fadl and Hartmann, 1967; Ashiru and Carlson, 1968). In Section I two promoters of mung bean root initiation were located, one being present in high levels only in the autumn and the other being a potent aqueous promoter. Both rooting promoters were present in approximately similar concentrations in MM.106 and EM XII rootstock cuttings and differences in root initiation between the two rootstocks could not be related to differential levels of the promoters. Hess (1959, 1960, 1961, 1962, 1964) located high levels of rooting cofactors in juvenile plant tissue of Hedera helix, Chrysanthemum and Hibiscus rosa-sinensis and suggested that differences in ease of rooting

were related to the presence and level of such cofactors. Since both EM XII and MM 106 stock sources were in a juvenile condition it is not unexpected that high levels of promotive compounds were located with the mung bean bioassay. With both rootstocks exhibiting high root promoting activity but possessing widely varying ability to form roots, neither the acidic nor the aqueous promoter appeared to be a factor limiting root formation. No evidence from the ^{14}C-IAA metabolism studies (Section III) was found to suggest that the promoters complexed with an auxin as proposed by Bouillenne and Bouillenne-Walrand (1955), Hess (1965) and Fadl and Hartmann (1967). Nevertheless it does not preclude the possibility that the promoters may be active in some supporting mechanism of root formation, such as protecting IAA from oxidation by IAA-oxidase as found by Bastin (1966). Further identification and study of the physiological properties of the promoters should indicate more clearly their role and significance on adventitious root formation.

A number of reports document the transferance of a root promoting stimulus across a graft union (Muzik and Cruzado, 1958; Stoutmeyer et al., 1961; Richards, 1964; Shah, 1969). Rooting of EM XII stocks bearing MM 106 scions was promoted while rooting of MM 106 and MM 106 stocks grafted with EM XII scions remained both similar and at a very high level. The increased rooting of $\frac{\text{MM 106}}{\text{EM XII}}$ cuttings was directly related to increased levels of IAA and IAN in the cutting base prior to planting, compared to EM XII controls. No evidence of inhibition of rooting, as suggested by Ashiru and Carlson (1968) and Fadl and Hartmann (1967) was found with $\frac{\text{EM XII}}{\text{MM 106}}$ cuttings. Some factor, either auxin or a factor inhibiting auxin metabolism in EM XII cuttings was apparently transferred from MM 106 across the graft union into EM XII. Endogenous levels of ABA, cytokinin and rooting cofactors correlated with the seasonal trends seen in Section I for the season of harvest and did not account for rooting promotion of $\frac{\text{MM 106}}{\text{EM XII}}$ cuttings. In both the seasonal studies and

the donor grafting experiments, improved rooting of EM XII cuttings was associated with considerable increases in endogenous IAA levels combined with an IBA dipping treatment. The degree of root formation in $\frac{\text{MM 106}}{\text{EM XII}}$ was considerably less than for both MM 106 and $\frac{\text{EM XII}}{\text{MM 106}}$, suggesting that root initiation may not be entirely dependant on auxin. This was also evident when IBA failed to promote root formation on EM XII cuttings throughout most of the season of harvest and in the donor grafting experiments.

Improved rooting on apple rootstock hardwood cuttings in Sections I and II appeared to be directly related to high levels or increases in endogenous IAA levels and promotion of rooting by IBA also seemed dependant on high endogenous auxin levels. Studies in Section III showed that, with MM 106 cuttings, combined application of IAA and IBA produced an additive promotion of rooting when compared with either auxin applied singly. EM XII cuttings showed a strongly synergistic response to a combined IAA/IBA treatment, when compared with IAA or IBA applied alone. A dramatic removal of rooting ability of MM 106 by centrifugation and base removal could be restored with either IAA or IBA applied alone, a combined treatment producing an additive promotion. Increasing concentrations of IBA could not promote rooting further than was achieved with 2,500 ppm when applied alone. These observations indicated that the additive promotion of rooting in MM 106 by IAA/IBA was not due simply to an increase in total auxin concentration promoting root formation, and that promotion of rooting by IBA was possibly not through the same metabolic functions as by IAA. A strong synergistic promotion of rooting by combined IBA/IAA treatments with EM XII cuttings supported this hypothesis.

A more detailed examination of the effects of IBA on the metabolism of IAA in the cutting base showed that the possible mode of action of IBA was by prevention of inactivation of IAA by decarboxylation or conju-

gation with aspartic acid. Cuttings of both rootstocks retained higher levels of labelled IAA and exhibited lower $^{14}CO_2$ evolution and IAA-asp. conjugation if pretreated with IBA. External solutions from IBA-treated cuttings also exhibited higher levels of IAA-^{14}C, suggesting reduced inactivation of IAA within the cuttings. While IBA appeared to act as an IAA-sparing device, no evidence of an IAA-cofactor complex was found, which could be regarded as an IAA protective process. From these studies, the possibility that IBA acts in an IAA-sparing role rather than boosting the total auxin pool appears more tenable. In support of this hypothesis, it should be noted that improved rooting of EM XII cuttings only occurred when high levels of IAA were present in the cutting base accompanied by an IBA treatment. The interpretation that IBA promotes root formation by increasing the active auxin pool (Nanda and Anand, 1970) seems unlikely when it is considered that IBA has only weak auxin activity (Weaver, 1972). Auxin-promoted growth by IBA in the oat coleoptile bioassay was only 1% of a similar concentration of IAA, as found by the author. A suggestion that IBA acts by undergoing B-oxidation to form IAA as found 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, loc. cit.).

IAA inactivation processes appeared to be more vigorous in EM XII than in MM 106 which may account for the lower level of endogenous IAA found in the seasonal studies and donor-graft experiments. It might also indicate why root formation only occurred on EM XII when a high IAA plus a high IBA level were present. If the rapid metabolism of IAA by EM XII was arrested by IBA application, optimum levels of IAA required for root formation could be achieved as seen in the three sections, provided that a continuing supply of IAA was available (e.g. at spring bud burst; with the presence of a MM 106 scion, or with a continuous IAA supply from lanolin caps). Presumably IAA is rapidly metabolised by EM XII tissue

when IBA is not present. The large difference in IAA-asp. conjugation between the two rootstocks seemed to be more related to their differential endogenous IAA levels, since IAA-asp. synthetase depends on the presence of a physiologically active auxin in high levels (Sudi, 1964, 1966; Venis, 1972), rather than accounting for differences in root formation between the two rootstocks. It would also seem more likely that IBA would inhibit all IAA inactivation processes as found in the present study, rather than inhibiting one inactivation process (IAA-destruction) and promoting another (IAA-asp. conjugation) as suggested by Ryugo and Breen (1974).

With the knowledge that IBA may protect and ensure the persistence of free IAA in the basal tissue, a reassessment of current commercial treatments of cuttings could improve propagating success. Examination of the endogenous IAA content of shy-rooting cultivars might show that IAA is often the limiting factor restricting root initiation, as has been shown in apple rootstocks in this study. A major problem to overcome will be the administering of IAA to the cuttings without losing the 'auxin' activity. While this was achieved experimentally by using apically-applied lanolin-IAA capsules, the commercial application of an IAA treatment will be more difficult and requires further study. Meanwhile by manipulation of the parent plant and environment to enable the harvest of propagating material to coincide with a time when high endogenous IAA levels are present, improved rooting of shy-rooting cultivars may be achieved. An example of this was seen with EM XII cuttings taken in early spring.

A Proposed Model of the Initiation Phase of Adventitious Root Formation

The process of root formation on cuttings has been separated into two phases; the initiation phase and the root growth and elongation phase (Lovell et al., 1971; Eriksen, 1973). Further work by Eriksen and

Mohammed (1974) and Mohammed and Eriksen (1974) showed that the initiation phase may have two stages, an auxin active and an auxin-inactive phase and that auxin (IAA) was more important in the earlier part of the initiation phase, a factor already stressed by Haissig (1970, 1972). Considerable evidence exists indicating that IAA influences growth processes by triggering the synthesis of specific new RNA's and enzyme proteins (Masuda, 1965; Galston, 1967; Morris et al., 1969; Tautvydas and Galston, 1970; Kobayashi and Yamaki, 1972). Anzai (1975) showed that root formation on mung bean cuttings was a two-phase process, the initial phase being sensitive to RNA synthesis inhibitors and the latter phase, to DNA synthesis inhibitors.

The initiation phase of root formation is dependant upon IAA as a continuous supply over the period of initiation, rather than its availability at one time in higher concentrations (Greenwood and Goldsmith, 1970; Haissig, 1970, 1972; Eriksen, 1973; Eriksen and Mohammed, 1974; Mohammed and Eriksen, 1974; Ryugo and Breen, 1974; Greenwood et al., 1974; Mohammed, 1975). Evidence suggests that the role of IAA in root initiation may be by inducing the synthesis of specific enzymes and proteins (Ryugo and Breen, loc. cit.) which induces the cell to become a rudimentary root meristematic zone. Figure 28 is a scheme postulating the integrated control of root initiation by plant growth regulators, incorporating evidence from this study and the relevant literature.

While the induction of root initiation is possibly an IAA-specific reaction, the interactions of plant growth regulators mediate the manifestation of the process. Organogenesis of roots on cuttings is partly controlled by the delicate balance between IAA and cytokinins (Heide, 1965b, 1971; Mullins, 1970) and possibly by interactions with ABA and gibberellins also. The auxin:cytokinin ratio appears to be critical in

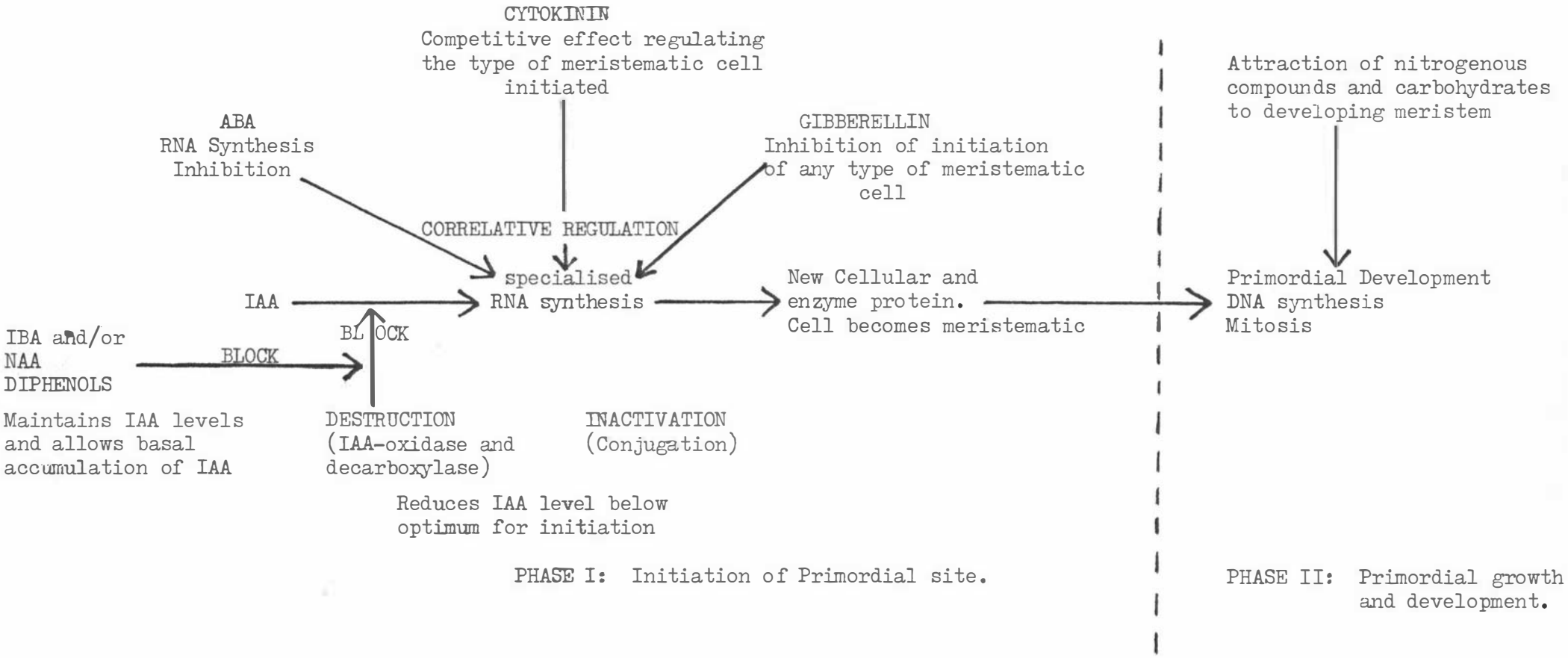

FIGURE 28: A hypothetical model of the integrated control of the initiation phase of root formation on cuttings.

determining whether adventitious roots will initiate on cuttings of some species or whether roots or buds will initiate on various tissue explants, (Skoog and Miller, 1957; Heide, loc. cit.; Heide and Skoog, 1967; Gautheret, 1969; Mullins, loc. cit.; Pierek and Steegmans, 1975). A competition effect regulated by the ratio seems to determine the type of meristematic cell initiated (Humphries and Maciejewska-Potapczyk, 1960; Chandra et al., 1973). This is conceivably regulated by the synthesis of specific RNA's within the dedifferentiating cell. ABA has been found to influence growth processes by inhibiting RNA synthesis (Villiers, 1968; Chen and Osborne, 1970; Bex, 1972; Walbot et al., 1975). A competitive balance with IAA could operate, the ratio determining the rate of RNA synthesis at the site of action of root initiation. Recent reports indicated that a continuous supply of IAA during the initiation phase was critical. This may be important in maintaining a high IAA:ABA ratio to facilitate the necessary RNA synthesis required for initiation. Several reports have shown that root initiation could be promoted by exogenously applied ABA (Chin et al., 1969; Chin and Beevers, 1969; Basu et al., 1968, 1970). Anzai (1975) found that several RNA synthesis inhibitors inhibited root initiation as measured by the number of roots appearing with time, but increased the number of roots per cutting when the cuttings ultimately rooted. The observed promotion of root initiation by ABA may have been induced by a similar process. Gibberellins have been shown to non-competitively inhibit natural or IAA-induced root initiation (Kato, 1958; Brian et al., 1960; Bachelard and Stowe, 1963; Jansen, 1967; Pierek and Steegmans, 1975) and totally inhibit root and bud organogenesis on Begonia leaf cuttings (Schraudolf and Reinert, 1959; Heide, 1969). No interaction with IAA was evident in any report and the mode of action appeared to be via an inhibition of the production of specialised meristematic cells. Cell division per se was not prevented.

The interactions between IAA and cytokinins and ABA, and the inhibi-

tion of root initiation by gibberellin are all effective on the first phase of root formation; the initiation of a meristematic root primordial site. Competitive and non-competitive inhibition of root initiation by growth regulators other than IAA, appear to act on the initiating phase, rather than the retardation of the subsequent development of root primordia, since primordia already initiated prior to treatment remain unaffected and develop normally.

It has been shown that the initiation phase of root formation is dependant on a continuous supply of free IAA above a threshold concentration. The present study supports the hypothesis, that free IAA alone, rather than the conjugation production of IAA plus another compound, may be the active primary effector of the initiation phase. The ability to maintain a promotive level of IAA in the cutting base is counteracted not only by interactions with other growth regulators, but also by the endogenous IAA destruction and inactivation processes, some of which have been reported to be very active just after the cutting is made (Chandra et al., 1971; Nanda et al., 1973). The inhibition of the enzymic destruction of IAA by synthetic or non-auxin compoun s has been shown (Bastin, 1966; Ryugo and Breen, 1974). The present study provides evidence that IBA, applied to the base of apple rootstock cuttin s (a commercial nursery practice), not only inhibits the enzymic degradation of IAA but also inhibits the inactivation by the IAA-aspartate synthetase enzyme system. The protection mechanism ensures the maintenance of a maximal level of IAA in the cutting base. IBA itself, did not seem to promote root initiation directly. The apparent promotion of rooting by synthetic auxins may be by the method of protecting the endogenous IAA from metabolism rather than by acting on the same metabolic process as IAA. No evidence in the present study or in the most recent research reports was found to indicate that an auxin-cofactor complex was a necessary pre-requisite for root initiation to occur, although it is possible that auxin-synergists are active in protect-

ing IAA from metabolism by inhibiting IAA-inactivation processes as shown by Bastin (1966).

While the separation of the roles of IAA and IBA offers one explanation of why basal 'hormone' treatments with synthetic auxins are not universally successful, until the mechanism of root initiation and auxin activity within this process is more clearly defined, further improvement of propagation techniques will be limited. The ability to achieve the initiation of roots on cuttings of difficult-to-root species parallels the physiological advances made in the understanding of the process of adventitious root initiation. The role of rooting cofactors and auxin-synergists is still unresolved although recent reports seem to indicate that IAA is the specific effector of root initiation. The concept of auxin-protectors has been suggested and shown to operate within the initiating phase of adventitious root formation. A major field of study yet to be undertaken, is in establishing the extent to which the hormonal interactions suggested in the present study influence root formation on woody shoots, and to elucidate the mechanisms involved in these processes. Only by understanding the correlative physiology of the control of root initiation will major advances be achieved in further developing the science of asexual plant propagation by rooting cuttings of shoots.

APPENDIX I

Buffer Solutions

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

K_2HPO_4	4.485 gm
Citric acid monohydrate	2.547 gm

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

2. Burström Basal Solution.

To obtain maximum response to auxins in the Avena coleoptile bioassay, the Burström basal solution (Burström, 1973) was found to be most satisfactory.

K_2HPO_4	10^{-3} mol/litre
$Ca(NO_3)_2$	10^{-4} mol/litre
$MgSO_4$	10^{-5} mol/litre
Glucose	16 gm/litre

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

3. Phosphate Buffers for Column Chromatography.

Buffer pH 8.0. K_2HPO_4 solution (10^{-1} mol/litre) adjusted to pH 8.0 with KH_2PO_4 (10^{-1} mol/litre).

Buffer pH 5.0. KH_2PO_4 solution (10^{-1} mol/litre) adjusted to pH 5.0 with K_2HPO_4 (10^{-1} mol/litre).

Buffer pH 8.0 for cytokinin extracts:- 0.1 M phosphate buffer (pH 8.0) was diluted 1 in 10.

APPENDIX II

Summary of Statistical Analyses

1. Seasonal Changes in Root Initiation of MM 106 and EM XII apple rootstocks.

Analysis of Variance

(a) MM 106 treated with IBA.

Source of variation	d.f.	s.s	m.s.	Observed F.	5%	1%
Total	23	9.6				
Block	3	0.43	0.14	0.31 N.S.	2.90	4.56
Treatment	5	2.35	0.47	1.04 N.S.		
Error	15	6.82	0.45			

S.E. = 0.112

(b) EM XII treated with IBA.

Source of variation	d.f.	s.s.	m.s.	Observed F.	5%	1%
Total	23	96.625				
Block	3	3.458	1.153	0.742 N.S.	2.90	4.56
Treatment	5	69.875	11.645	7.498 **		
Error	15	23.292	1.553			

S.E. = 0.623

(c) MM 106 untreated with IBA.

Source of variation	d.f.	s.s.	m.s.	Observed F.	5%	1%
Total	23	43				
Block	3	1.46	0.486	0.433	2.90	4.56
Treatment	5	24.71	4.942	4.412 *		
Error	15	16.83	1.12			

2. Root Initiation of Donor-grafted rootstocks.

Source of variation	d.f.	s.s.	m.s.	Observed F.		5%	1%
Total	15	103					
Block	3	0.5	0.166	0.747	N.S.		
Treatment	3	100.5	33.5	150.9	**		6.99
Error	9	2.0	0.222				

S.E. = 0.234

3. 0.1% IAA-lanolin Experiment.

(a) MM 106.

Source of variation	d.f.	s.s.	m.s.	Observed F.		5%	1%
Total	39	3112					
Block	9	138.1	15.344	0.414	N.S.	2.96	4.6
Treatment	3	1974.9	658.3	17.79	**		
Error	27	999	37.0				

S.E. = 1.923

(b) EM XII.

Source of variation	d.f.	s.s.	m.s.	Observed F.		5%	1%
Total	39	13.775					
Block	9	2.525	0.28	1.0	N.S.	2.96	4.6
Treatment	3	3.675	1.225	4.375	*		
Error	27	7.575	0.28				

S.E. = 0.167

4. 1.0% IAA-lanolin and Centrifugation of MM 106.

Source of variation	d.f.	s.s.	m.s.	Observed F.		5%	1%
Total	39	2333.1					
Block	9	146.1	16.23	0.759	N.S.		
Treatment	3	1609.7	536.56	25.09	**	2.96	4.6
Error	27	577.3	21.38				

S.E. = 1.462

5. 1.0% IAA-lanolin on EM XII.

Source of variation	d.f.	s.s.	m.s.	Observed F.		5%	1%
Total	39	75.78					
Block	9	9.03	1.0	0.68	N.S.		
Treatment	3	27.28	9.09	6.22	**	2.96	4.6
Error	27	39.47	1.46				

S.E. = 0.382

BIBLIOGRAPHY

Addicott, F.T., and J.L. Lyon., 1969: Physiology of abscisic acid and related inhibitors. Ann. Rev. Plant Physiol., 20: 139-164.

Alden, T., 1971: Seasonal variations in the occurrence of Indole-3-acetic acid in buds of Pinus sylvestris. Physiol. Plant., 25: 54-57.

Andreae, W.A., 1967: Uptake and metabolism of indoleacetic acid, napthaleneacetic acid and 2,4, dichlorophenoxyacetic acid by pea root segments in relation to growth inhibition during and after auxin application. Can. J. Bot., 45: 737-753.

_____., and N.E. Good, 1957: Studies on 3-indoleacetic acid metabolism IV. Conjugation with aspartic acid and ammonia as processes in the metabolism of carboxylic acids. Plant Physiol., 32: 566-572.

_____., and M.W.H. van Ysselstein, 1956: Studies on 3-indoleacetic acid metabolism III. The uptake of 3-indoleacetic acid by pea epicotyls and its conversion to indoleacetylaspartic acid. Plant Physiol., 31: 235-240.

_____., _____ ., 1960: Studies on 3-indoleacetic acid metabolism VI. 3-indoleacetic acid uptake and metabolism by pea epicotyls and roots. Plant Physiol., 35: 225-232.

Anzai, T., 1975: Two phases in adventitious root formation in Phaseoulus mungo hypocotyl cuttings, a phase sensitive to an inhibitor of RNA or protein synthesis and a phase sensitive to an inhibitor of DNA synthesis. J. exp. Bot., 26: 580.588.

Ashiru, G.A., 1968: Physiological studies of rooting cuttings of Malling-Merton 106 and East Malling II apple (Malus sylvestris M.) clones. Diss. Abst., 28: 3550.

_____., and R.F. Carlson, 1968: Some endogenous factors associated with rooting of East Malling II and Malling-Merton 106 apple clones. Proc. Am. Soc. hort. Sc., 92: 106.112.

Bachelard, E.P. and B.B. Stowe, 1963: Rooting of cuttings of Acer rubrum and Eucalyptus camaldulensis D. Aust. J. biol. Sc., 16: 751-767.

Barlow, H.W.B., C.R. Hancock and H.J. Lacey, 1961: Some biological characteristics of an inhibitor extracted from woody shoots. In: "Plant Growth Regulation" Iowa State Univ. Press, 127-140.

Bastin, M., 1966: Root initiation, auxin level and biosynthesis of phenolic compounds. Photochem. and Photobiol., 5: 423-429.

Basu, R.N., T.K. Bose, B.N. Roy and A. Mukhopadhyay, 1969: Auxin synergists in rooting of cuttings. Physiol. Plant., 22: 649-652.

_____., _____, _____, 1970: Interaction of abscisic acid and auxins in rooting of cuttings. Plant and Cell Physiol., 11: 681-684.

Batten, D.J. and M.G. Mullins, 1975: Auxin and ethylene in adventitious root formation in mung bean. Res. Rep. 3: 24. Dept. Agron. and Hort. Univ. Sydney.

Beakbane, A.B., 1961: Structure of the plant stem in relation to adventitious rooting. Nature, Lond., 192: 954-955.

Bennet-Clark, T.A. and N.P. Kefford, 1953: Chromatography of the growth substances in plant extracts. Nature, Lond., 171: 645-647.

Bex, J.H.M., 1972: Effects of abscisic acid on nucleic acid metabolism in maize hypocotyls. Planta, 103: 1-10.

Bhella, H.S. and A.N. Roberts, 1974: The influence of photoperiod and rooting temperature on rooting of Douglas Fir (Pseudotsuga menziesii M.). J. Am. Soc. hort. Sc., 99: 551-555.

Birks, J.B., 1964: The theory and practice of scintillation counting. Pergamon Press, Oxford.

Bonner, J., and H. Bonner, 1948: The B vitamins as plant hormones. Vit. and Hormones., 6: 225-275.

Bouillenne, R. and F.W. Went, 1933: Recherches experimentales sur la neoformation des racines dans les plantules et les boutures des plantes superieures. Ann. J. Bot. Buitenzorg., 43: 25-202.

_____., and M. Bouillenne-Walrand, 1955: Auxines et Bouturage. Proc. Proc. XIV Int. Hort. Congress, 1: 231-238.

Brian, P.W., 1957: The effects of some microbial metabolic products on plant growth. Symp. Soc. exp. Biol., 9: 166-182.

_____., 1959: Effects of Gibberellins on plant growth and development. Biol. Rev., 34: 37-84.

_____., H.G. Hemming and M. Radley, 1955: A physiological comparison of gibberellic acid and some auxins. Physiol. Plant., 8: 899-912.

_____., _____ and D. Lowe, 1960: Inhibition of rooting of cuttings by gibberellic acid. Ann. Bot., 24: 407-419.

Burström, H.G., 1973: Mineral nutrition and auxin induced growth of Triticum coleoptile sections. Plant and Cell Physiol., 14: 941-951.

Carlson, R.F., 1966: Factors influencing root formation in hardwood cuttings of fruit trees. Quart. Bull. Mich. ag. Exp. Stn., 48: 449-454.

Chadwick, L.C., 1955: The choice of shoot: a consideration of the influence of source factors on regeneration. Proc. XIV Int. Hort. Congress, 1: 215-222.

Challenger, S., H.J. Lacey and B.H. Howard, 1964: The demonstration of root promoting substances in apple and plum rootstocks. Ann. Rep. E. Mall. Res. Stn., A48: 124-128.

Chandra, G.R., L.E. Gregory and J.F. Worley, 1971: Studies on the initiation of adventitious roots on mung bean hypocotyls. Plant and Cell Physiol., 12: 317-324.

_____., _____, _____ and H.D. Clark, 1973: Effect of 6-benzyladenine on the initiation of adventitious roots on mung bean hypocotyls. Plant and Cell Physiol., 14: 1209-1212.

Chen, D. and D.J. Osborne, 1970: Hormones in the translational control of early germination in wheat embryos. Nature, 266: 1157-1160.

Chin, T.Y., and L. Beevers, 1969: The stimulation of rooting by abscisic acid. Plant Physiol., 44: XXXIII suppl.

_____., M.M. Meyer, and L. Beevers, 1969: Abscisic acid-stimulating rooting of stem cuttings. Planta, 88: 192-196.

Chvojka, L., M. Travnicek and M. Zakourilova, 1962: The influence of stimulating doses of 6-benzylaminopurine on awakening apple buds and on their consumption of oxygen. Biol. Plant., 4: 203-206.

Cooper, W.C., 1935: Hormones in relation to root formation on stem cuttings. Plant Physiol., 10: 789-794.

_____., 1936: Transport of root-forming hormones in woody cuttings. Plant Physiol., 11: 779-793.

_____., 1944: The concentrated-solution-dip method of treating cuttings with growth substances. Proc. Am. Soc. hort. Sc., 44: 533-541.

Cripps, J., 1974: Rooting of hardwood cuttings. Aust. hort. Res. Newsl., 38: 12.

Czaja, A.T., 1934: Der nachweiss des Wuchsstoffes bei holzpflanzen. Ber. Deutsch. Bot. Ges., 52: 267-271.

Danilov, A.I., 1968: Root forming capacity of apple cuttings taken from different parts of the crown and different regions of the shoot. Hort. Abst., 38: 7005.

Doud, S.L. and R.F. Carlson, 1972: Propagation methods of fruit tree cultivars from hardwood cuttings. Fruit vars. and Hort. Dig., 26: 80-83.

Eliasson, L., 1969: Growth regulators in Populus tremula - distribution of auxin and growth inhibitors. Physiol. Plant., 22: 1288-1301.

Eriksen, E.N., 1971: Promotion of root initiation by gibberellin. Roy. Vet. and Ag. Yearbook, Copenhagen. Pp. 50-59.

_____., 1973: Root formation in pea cuttings I. Effects of decapitation and disbudding at different developmental stages. Physiol. Plant., 28: 503-506.

_____., 1974: Root formation in pea cuttings III. The influence of cytokinin at different developmental stages. Physiol. Plant., 30: 163-167.

_____., and S. Mohammed, 1974: Root formation in pea cuttings II. The influence of indole-3-acetic acid at different developmental stages. Physiol. Plant., 30: 158-162.

Fadl, M.S. and H.T. Hartmann, 1967a: Relationship between seasonal changes in endogenous promoters and inhibitors in pear buds and cutting bases and the rooting of pear hardwood cuttings. Proc. Am. Soc. hort. Sc., 91: 96-112.

Fadl, M.S. and H.T. Hartmann, 1967b: Isolation, purification and characterisation of an endogenous root promoting factor obtained from basal sections of pear hardwood cuttings. Plant. Physiol., 42: 541-549.

_____., _____, 1967c: Effect of reciprocal bud-graft transfer between Old Home and Bartlett pears and centrifugation on translocation of endogenous growth substances in hardwood cuttings. Physiol. Plant., 20: 802-813.

Farrar, J.L. and N.H. Grace, 1942: Vegetative propagation of conifers XI. Effects of type of cutting on the rooting of Norway Spruce cuttings. Can. J. Res., 20: 116-121.

Fawcett, C.H., R.L. Wain and R. Wightman, 1958: Beta-oxidation of omega (3-indolyl) alkanecarboxylic acids in plant tissue. Nature, Lond., 181: 1387-1389.

Fernandez-Sevilla, J.L., 1969: Apple propagation by hardwood cuttings. Hort. Abst., 40: 2872.

Frischenschlager, B., 1938: Versche uber die heranzucht von unterlagen aux wurzeln die weitere vermehrung derselben. Gartenbauwiss., 12: 77-88.

Galston, A.W., 1955: In chemistry and mode of action of plant growth substances. Ed. Wain, R.L. and F. Wightmann, Butterworths, Lond.

_____., 1967: Regulatory systems in higher plants. Am. Sci., 55: 144-160.

_____., and P.J. Davies, 1969: Hormonal regulation in higher plants. Sci., 163: 1288-1297.

_____., S. Lavee and B.Z. Seigel, 1968: The induction and repression of perioxidase isoenzymes by 3-indole-acetic acid. In "Biochem. and Physiol. of Plant Growth Subst." ed. G. Setterfield and F. Wightmann. Pp. 455-472.

Gardner, F.E., 1929: The relationship between tree age and the rooting of cuttings. Proc. Am. Soc. hort. Sc., 26: 101-104.

Garner, R.J., 1965: Propagation of M.26 apple rootstocks by hardwood cuttings for direct lining out. Ann. Rep. E. Mall. Res. Stn., A49: 77-79.

_____., and E.S.J. Hatcher, 1947: The selection of hardwood stem cuttings of an apple rootstock. Ann. Rep. E. Mall. Res. Stn., A31: 71-75.

_____., _____, 1955a: Influence of source and growth substances on behaviour of apple and plum cuttings. J. Hort. Sc., 30: 116-128.

_____., _____, 1955b: The interplay of factors influencing rooting behaviour of shoot cuttin s. XIVth Int. Hort. Congress, 1: 204-214.

Gautheret, R.J., 1969: Investigations on the root formation in the tissues of Helianthus tuberosus, cultured in vitro. Am. J. Bot., 56: 702-717.

Geronimo, J., P.B. Catlin and E.C. Maxie, 1964: Metabolism of indoleacetic acid - 2 ^{14}C in plum stem cuttings. Physiol. Plant., 17: 644-653.

Girouard, R.M., 1969: Physiological and biochemical studies of adventitious root formation. Extractable rooting cofactors from Hedera helix. Can. J. Bot., 47: 687-699.

_____., and C.E. Hess, 1964: The diffusion of root promoting substances from stems of Hedera helix. Proc. Int. Plant Prop. Soc., 14: 162-166.

_____., _____, 1966: Distribution and mobility of rooting cofactors in juvenile and adult growth phases of Hedera helix. Proc. XVII Int. Hort. Cong., 1: 370.

Glenn, J.L., C.C. Kuo, R.C. Durley, and R.P. Pharis, 1972: Use of insoluble polyvinylpyrrolidone for purification of plant extracts and chromatography of plant hormones. Phytochem., 11: 345-351.

Gmelin, R. and A.I. Virtanen, 1961: Glucobrassicin: the precursor of indolyl acetglnitrile, ascorbigen and SCN in Brassica oleracea. Suomen. Kemistil., 34: 15-18.

Gorter, C.J., 1958: Synergism of indole and indole-3-acetic acid in the root production of Phaseoulus cuttings. Physiol. Plant., 11: 1-9.

_____., 1962. Further experiments on auxin-synergists. Physiol. Plant., 15: 88-95.

_____., 1969: Auxin-synergists in the rooting of cuttings. Physiol. Plant., 22: 497-502.

Gray, R.A., 1958: The antiauxin effect of Gibberellic acid in the rooting of cuttings. Plant Physiol., 33: XL Suppl.

Greenwood, M.S. and M.H.M. Goldsmith, 1970: Polar transport and accumulation of indole-3-acetic acid during root regeneration by Pinus lambertiana embryos. Planta, 95: 297-313.

_____., A.C. Harlow and H.D. Hodgeson, 1974: The role of auxin metabolism in root meristem regeneration by Pinus lambertiana embryo cuttings. Physiol. Plant., 32: 198-202.

Gregory, L.E. and J. van Overbeek, 1945: An analysis of the process of root formation on cuttings of difficult to root hibiscus variety. Proc. Am. Soc. hort.Sc., 46: 427-433.

Gregory, F.G. and B. Samantarai, 1950: Factors concerning the rooting responses of isolated leaves. J. exp. Bot., 1: 159-193.

Grochowska, M.J., 1966: Chromatographic degradation of phloridzin. Plant Physiol., 41: 432-436.

Guerriero, R. and F. Loreti, 1968: Studies on the propagation by cuttings of clonal apple rootstocks with bottom heat. Riv. Orto. ital., 52: 757-778.

Gunkel, J.E. and K.V. Thimann, 1949: Studies of development of long shoots and short shoots of Ginko bilboa L. III. Auxin production in shoot growth. Am. J. Bot., 36: 145-151.

Gur, A. and R.M. Samish, 1968: The role of auxins and auxin destruction

in the vigor effect induced by various apple rootstocks. Bietr. biol. Pflanzen., 45: 91-111.

Haissig, B.E., 1970: Influence of indole-3-acetic acid on adventitious root primordia of Brittle Willow. Planta, 95: 27-35.

_____., 1972: Meristematic activity during adventitious root primordium development. Plant Physiol., 49: 886-892.

Hancock, C.R., H.W.B. Barlow and H.J. Lacey, 1961: The behaviour of phloridzin in the coleoptile straight growth test. J. exp. Bot., 12: 401-408.

Hansen, J., 1975: Light dependant promotion and inhibition of adventitious root formation by gibberellic acid. Planta, 123: 203-205.

Hartmann, H.T., 1955: Auxins for hardwood cuttings. Calif. Ag., 9: 12-13.

_____., W.H. Grigg and C.J. Hansen, 1963: Propagation of own-rooted Old Home and Bartlett pears to produce trees resistant to pear decline. Proc. Am. Soc. hort. Sc., 82: 92-102.

_____., C.J. Hansen and F. Loreti, 1965: Propgation of apple rootstocks by hardwood cuttings. Calif. Ag., 19: 4-5.

_____., and D.E. Kester, 1968: Plant propgation: principles and practices. 2nd. ed. Prentice-Hall.

Hatcher, E.S.J., 1959: The propagation of rootstocks from stem cuttings. Ann. appl. Biol., 47: 635-639.

_____., 1959b: Auxin relations of the woody shoot. Ann. Bot., 23: 409-425.

_____., and R.J. Garner, 1947: Growth substances in relation to hardwood stem cuttings of fruit tree rootstocks. Ann. Rep. E. Mall. Res. Stn., A31: 117-120.

_____., _____, 1950: Aspects of rootstock propagation II. The development of the concentrated dip method of treating hardwood cuttings with gorwth substances. Ann. Rep. E. Mall. Res. Stn., A34: 116-121.

_____., _____, 1956: Aspects of rootstock propagation IV. The winter storage of cuttings. Ann. Rep. E. Mall. Res. Stn., A40: 101-106.

Heide, O.M., 1965b: Interaction of temperature, auxins and kinins in the regeneration ability of Begonia leaf cuttings. Physiol. Plant., 18: 891-920.

_____., 1967: The auxin level of Begonia leaves in relation to their regeneration ability. Physiol. Plant., 20: 886-902.

_____., 1968a: Auxin level and regeneration of Begonia leaves. Planta, 81: 153-159.

_____., 1968b: Stimulation of adventitious bud formation in Begonia leaves by Abscisic Acid. Nature, Lond., 219: 960-961.

_____., 1969: Non-reversibility of gibberellin-induced inhibition

of regeneration of Begonia leaves. Physiol. Plant., 22: 671-679.

Heide, O.M., 1971: The role of cytokinins in the regeneration process. In "Hormonal regulation in plant growth and development". Proc. Adv. Study Inst. Izmir. Pp. 207-219.

_____., and F. Skoog, 1967: Cytokinin activity in Begonia and Bryophyllum. Physiol. Plant., 20: 771-780.

Herman, D.E. and C.E. Hess, 1963: The effect of etiolation upon the rooting of cuttings. Proc. Int. Plant. Prop. Soc., 13: 42-62.

_____., _____, 1966: The physiology of etiolation in relation to the rooting of cuttings. Proc. XVII Int. Hort. Cong., 1: 368.

Hess, C.E., 1957: A physiological analysis of rooting in cuttings of juvenile and mature Hedera helix L. Ph.D. Cornell Univ. Ithaca, New York.

_____., 1959: A study of plant growth substances in easy and difficult-to-root cuttings. Proc. Int. Plant. Prop. Soc., 9: 39-45.

_____., 1960: Research into root initiation; a progress report. Proc. Int. Plant Prop. Soc., 10: 118-123.

_____., 1961: Characterisation of the rooting cofactors extracted from Hedera helix L. and Hibiscus rosa-sinensis L. Proc. Int. Plant. Prop. Soc., 11: 51-56.

_____., 1962: A physiological comparison of rooting in easy and difficult-to-root cuttings. Proc. Int. Plant. Prop. Soc., 12: 265-269.

_____., 1962b: A physiological analysis of root initiation in easy and difficult-to-root cuttings. XVI Int. Hort. Cong., 4: 375-381.

_____., 1964: Naturally occurring substances which stimulate root initiation. "Reg. Nat. de la Croiss. Vege". 5th Int. Con. Plant Growth Subst. Ed. J.P. Nitsch. Pp. 517-527.

_____., 1965: Rooting cofactors - identification and function. Proc. Int. Plant Prop. Soc., 15: 181-186.

_____., 1965b: Phenolic compounds as stimulators of root initiation. Plant Physiol., 40: XLV Suppl.

_____., 1966: Root Initiation and juvenility - a possible implication of terpene compounds. Proc. XVII INT. Hort. Cong., 3: 443-451.

_____., 1969: In "Root Growth". Ed. W.J. Whittington. Plenum Press, New York.

Heuser, C.W. and C.E. Hess, 1966: The role of oxygenated terpenes in the rooting of chrysanthemum cuttings. Proc. XVII Int. Hort. Cong., 1: 396.

_____., _____, 1972: Endogenous regulators of root initiation in mung bean hypocotyls. J. Am. Soc. hort. Sc., 97: 392-397.

Heuser, C.W. and C.E. Hess, 1972b: Isolation of three lipid root initiating substances from juvenile Hedera helix shoot tissue. J. Am. Soc. hort. Sc., 97: 571-574.

Hewett, E.W. and P.F. Wareing, 1973: Cytokinins in Populus x robusta: Changes during chilling and bud burst. Physiol. Plant., 28: 393-399.

Hitchcock, A.E., 1935: Indole-3-propionic acid as a growth hormone and the quantitative measurement. Contr. Boyce Thomp. Inst., 7: 87-95.

_____., and P.W. Zimmerman, 1939: Comparative activity of root-inducing substances and methods for treating cuttings. Contr. Boyce Thomp. Inst., 10: 461-480.

Howard, B.H., 1965: Increase during winter in capacity for root regeneration in detached shoots of fruit tree rootstocks. Nature, Lond., 208: 912-913.

_____., 1966: Rootstock propagation by hardwood cuttings: a progress report for nurserymen. Ann. Rep. E. Mall. Res. Stn., A50: 202-204.

_____., 1968a: Effects of bud removal and wounding on rooting in hardwood cuttings. Nature, Lond., 220: 262-264.

_____., 1968b: The influence of 4(indolyl-3)-butyric acid and basal temperature on the rooting of apple rootstock hardwood cuttings. J. hort. Sc., 43: 23-31.

_____., 1970: Solvent for application of Indolylbutyric acid to hardwood cuttings. Ann. Rep. E. Mall. Res. Stn., A53: 95-97.

_____., 1970a: Propagating with hardwood cuttings. Grower, p. 540.

_____., 1970b: Hardwood cutting technique is practical success. Grower, pp. 613-614.

_____., 1970c: Hardwood cutting technique is perfected. Comm. Gr., pp. 191-193.

_____., 1970d: Rapid propagation in the nursery. Decid. Fruit., pp. 54-56.

_____., 1971: Propagation techniques. Sci. Hort., 23: 116-126.

_____., 1974: Factors which affect the response of cuttings to hormone treatments. Proc. Int. Plant Prop. Soc., 24: 142-143.

_____., and R.J. Garner, 1964: High temperature storage of hardwood cuttings as an aid to improved establishment in the nursery. Ann. Rep. E. Mall. Res. Stn., A48: 83-87.

_____., _____, 1965: Prolonged cold storage of rooted and unrooted hardwood cuttings of apple, plum and quince rootstocks. Ann. Rep. E. Mall. Res. Stn., A49: 80-82.

Howard, B.H. and N. Nahlawi, 1969a: A progress report on the propagation of some new plum rootstocks from hardwood cuttings. Ann. Rep. E. Mall. Res. Stn., A52: 71-73.

_____., _____, 1969b: Factors affecting the rooting of plum hardwood cuttings. J. hort. Sc., 44: 303-310.

_____., _____, 1970: Dipping depth as a factor in the treatment of hardwood cuttings with indolylbutyric acid. Ann. Rep. E. Mall. Res. Stn., A53: 91-94.

Humphries, E.C., 1960: Inhibition of root development on petioles and hypocotyls of dwarf bean (Phaseolus vulgaris) by Kinetin. Physiol. Plant., 13: 659-663.

_____., and W. Maciejewska-Potapczyk, 1960: Effect of indole-acetic acid, napthaleneacetic acid and kinetin on phosphorus fraction in hypocotyls of dwarf bean (Phaseolus vulgaris). Ann. Bot., 24: 311-316.

Ivey, I.D., 1974: Changes in inhibitor levels, stomatal aperture and growth of Pisum sativum L. subjected to wilting stress cycles during different developmental stages. Thesis, M.Hort.Sc., Massey Univ. New Zealand.

Jackson, M.B. and P.M. Harney, 1970: Rooting cofactors, indoleacetic acid and adventitious root initiation in mung bean cuttings (Phaseoulus aureus). Can. J. Bot., 48: 943-946.

Jansen, H., 1967: The effect of gibberellic acid and indoleacetic acid on the root formation of tomato cuttings. Planta, 74: 371-378.

Jeffay, H. and J. Alvarez, 1961: Liquid scintillation counting of ^{14}C. Use of ethanolamine-ethylene glycol monomethyl ether-toluene. Anal. Chem., 33: 612-615.

Kato, J., 1958: Studies on the physiological effect of gibberellin II. On the interaction of gibberellin with auxin and growth inhibitors. Physiol. Plant., 11: 10-15.

Kaufman, P.B., 1965: The effects of growth substances on intercalary growth and cellular differentiation in developing internodes of avena sativa II. Effects of gibberellic acid. Physiol. Plant., 18: 703-724.

Kawase, M., 1964: Centrifugation, rhizocaline and rooting in Salix alba L. Physiol. Plant., 17: 855-865.

_____., 1965a: Centrifugation promotes rooting in softwood cuttings. Proc. Int. Plant Prop. Soc., 15: 191-199.

_____., 1965b: Etiolation and the rooting of cuttings. Physiol. Plant., 18: 1066-1076.

_____., 1971: Cause of centrifugal root promotion. Physiol. Plant., 25: 64-70.

Knight, R.C., 1926: The propagation of fruit tree stocks by stem cuttings. J. Pomol., 5: 248-266.

_____., R.G. Hatton, J. Amos and A.W. Witt, 1927: The vegetative propagation of fruit tree rootstocks. Ann. Rep. E. Mall. Res. Stn., A10: 11-30.

Kobayashi, K., and T. Yamaki, 1972: Studies on scluble RNA binding indoleacetic acid in etiolated mung bean hypocotyl sections. Plant and Cell Physiol., 13: 49-65.

Kogl, F., A.J. Haagen Smit and H. Erxleben, 1934: Uber ein neues auxin (heteroauxin) aus harn. Z. Physiol. Chem., 228: 90-103.

Kuzin, E.N., 1975: The rooting and growth of softwood apple cuttings in relation to maturity and position on the tree. Sadovodstva, U.S.S.R.

Lanphear, F.O. and R.P. Meahl, 1963: Influence of endogenous rooting cofactors and environment on the seasonal fluctuation in root initiation of selected evergreen cuttings. Proc. Am. Soc. hort. Sc., 83: 811-818.

_____., _____, 1966: Influence of the stock plant environment on the rooting of Juniperus horizontalis "Plumosa". Proc. Am. Soc. hort. Sc., 89: 666-671.

Lantican, B.P. and R.M. Muir, 1969: Auxin physiology of dwarfism in Pisum sativum. Physiol. Plant., 22: 412-423.

Lee, C.I., J.J. McGuire and J.T. Kitchin, 1969: The relationship between rooting cofactors of easy and difficult-to-root cuttings of three clones of Rhododendron. J. Am. Soc. hort.Sc., 94: 45-48.

_____., and H.B. Tukey, 1971: Induction of root-promoting substances in Euonymus alatus "Compactus" by intermittent mist. J. Am. Soc. hort. Sc., 96: 731-736.

Lee, T.T., 1971: Promotion of indoleacetic acid oxidase isoenzymes in tobacco callus cultures by indoleacetic acid. Plant Physiol., 48: 56-59.

Lenton, J.R., V.M. Perry and P.F. Saunders, 1971: The identification and quantitative analysis of Abscisic Acid in plant extracts by gas-liquid chromatography. Planta, 96: 271-280.

Leopold, A.C. and T.H. Plummer, 1961: Auxin-phenol complexes. Plant Physiol., 36: 589-592.

Lesham, Y. and M. Schwartz, 1968: Interaction of photoperiod and auxin metabolism in rooting of Chrysanthemum morifolium cuttings. Proc. Am. Soc. hort. Sc., 93: 589-594.

Letham, D.S., 1971: Regulators of cell division in plant tissue XII. A cytokinin bioassay using excised radish cotyledons. Physiol. Plant., 25: 391-396.

Lipecki, J. and F.G. Dennis, 1972: Growth inhibitors and rooting co-factors in relation to rooting response of softwood apple cuttings. Hort. Sc., 7: 136-138.

Lovell, P.H., A. Cobb and K.G. Moore, 1971: The control of root initiation in detached cotyledons of Sinapsis alba and Raphanus sativus. Ann. Bot., 35: 501-509.

Masuda, Y., 1965: RNA in relation to the effect of auxin, kinetin and gibberellin on the tuber tissue of Jerusalem artichoke. Physiol. Plant., 18: 15-23.

Masuda, Y., E. Tanimoto and S. Wada, 1967: Auxin-stimulated RNA synthesis in oat coleoptile cells. Physiol. Plant., 20: 713-719.

Milborrow, B.V., 1967: The identification of (+)-Abscissin II ((+)-Dormin) in plants and measurement of its concentrations. Planta, 76: 93-113.

_____., 1974: The chemistry and physiology of abscisic acid. Ann. Rev. Plant Physiol., 25: 259-307.

Mirov, N.T., 1941: Distribution of growth hormones in shoots of two species of pine. J. For., 39: 457-464.

Mohammed, S., 1975: Further investigations on the effect of decapitation and disbudding at different developmental stages, on the rooting of pea cuttings. J. hort. Sc., 50: 272-272.

_____., and E.N. Eriksen, 1974: Root formation in pea cuttings IV. Further studies on the influence of indole-3-acetic acid at different developmental stages. Physiol. Plant., 32: 94-96.

Moretti, A., 1954: Growth substances in the propagation of mulberry. Riv. Fruttic., 16: 1-17.

Morris, D.A., R.E. Briant and P.G. Thompson, 1969: The transport and metabolism of ^{14}C-labelled indoleacetic acid in intact pea seedlings. Planta, 89: 178-197.

Most, B.H., 1971: Abscisic acid in immature apical tissue of sugar cane and leaves of plants subjected to drought. Planta, 100: 67-75.

Muir, R.M., 1970: The control of growth by the synthesis of IAA and its conjugation. In "Plant Growth Subst., 1970" Ed. P.J. Carr, pp. 96-101. Springer-Verlag.

Mullins, M.G., 1970: Auxin and ethylene in adventitious root formation in Phaseolus aureus (Roxb). In "Plant Growth Subst., 1970". Ed. P.J. Carr, pp. 525-533. Springer-Verlag.

Murashige, T., 1964: Analysis of the inhibition of organ formation in tobacco tissue culture by gibberellin. Physiol. Plant., 17: 636-643.

Muzik, T.J. and H.J. Cruzado, 1958: Transmission of juvenile rooting ability from seedlings to adults of Hevea brasiliensis. Nature, Lond., 181: 1288.

Nahlawi, N. and B.H. Howard, 1971: Effect of position of IBA application on the rooting of plum hardwood cuttings. H. hort.Sc., 46: 535-543.

_____., _____, 1972: Rooting response of plum hardwood cuttings to IBA in relation to treatment duration and cutting moisture stress. J. hort. Sc., 47: 301-307.

Nanda, K.K., A.N. Purohit and K. Mehrotra, 1968: Effect of sucrose, auxins and gibberellins on rooting of stem segments of Populus nigra under varying light conditions. Plant and Cell Physiol., 9: 735-743.

_____., and V.K. Anand, 1970: Seasonal changes in auxin effects on rooting of stem cuttings of Populus nigra and its relationship to the mobilization of starch. Physiol. Plant., 23: 99-107.

_____., _____, and R.N. Chibbar, 1972: The promotive effect of gibberellic acid on the production of adventitious roots on stem cuttings of Ipomoea fistulosa. Planta, 105: 360-363.

Nanda, K.K., N.C. Bhattacharya and N.P. Kaur, 1973: Disc electrophoresis studies of IAA-osidases and their relationship with rooting of etiolated stem segments of Populus nigra. Physiol. Plant., 29: 442-444.

Nelson, S.H., 1955: Propagation by leaf-bud cuttings. Am. Nurs., 102: 11 and 41.

Nesterov, I.S., 1968: Quince cuttings root formation depends on date of preparation of cutting. Hort. Abs. 38: 2576.

Nichools, W., W.D. Crow and D.M. Paton, 1970: Chemistry and physiology of rooting inhibitors in adult tissue of Eucalyptus grandis. In "Plant Growth Subst., 1970". Ed. P.J. Carr, pp. 324-329. Springer-Verlag.

Nitsch, J.P. and C. Nitsch, 1956: Studies on the growth of coleoptile and first internode sections. A new sensitive straight-growth test for auxins. Plant Physiol., 31: 94-111.

Odom, R.E. and W.J. Carpenter, 1965: The relationship between endogenous indole auxins and the rooting of herbaceous cuttings. Proc. Am. Soc. hort. Sc., 87: 494-501.

Ogasawara, R., 1960: Physiological studies on the formation of adventitious roots in Pinus densiflora I. Relationship between growth substances and age. J. Jap. For. Soc., 42: 356-358.

Okazawa, Y., N. Katsura and T. Tagawa., 1967: Effects of auxin and kinetin on the development and differentiation of potato tissue culture in vitro. Physiol. Plant., 20: 862-869.

Olden, E.J., 1952: Developmental stages in apple seedlings. Hort. Abst., 23: 3834.

Overbeek, J. van and L.E. Gregory, 1945: A physiological separation of two factors necessary for the formation of roots on cuttings. Am. J. Bot., 32: 336-341.

_____, S.A. Gordon and L.E. Gregory, 1946: An analysis of the function of the leaf in the process of root formation. Am. J. Bot., 33: 100-107.

Park, H.S. Personal communication.

Patrick, J.W. and D.J. Woolley, 1973: Auxin physiology of decapitated stems of Phaseoulus vulgaris L. treated with Indol-3yl-acetic acid. J. exp. Bot., 24: 949-957.

Patterson, M.S. and R.C. Greene, 1965: Measurement of low energy Beta-emitters in aqueous solution by liquid scintillation counting of emulsions. Anal. Chem., 37: 854.

Peters, A., 1959: Trials in the experimental garden "Boom en Vrucht". Jaarb. Proefst. Boomkw. Boskoop., 55-56.

Peters, J.E., P.H.L. Wu, W.R. Sharp and E.F. Paddock, 1974: Rooting and metabolism of nicotine in tobacco callus cultures. Physiol. Plant., 31: 97-100.

Pierik, R.L.M. and H.H.M. Steegmans, 1975: Analysis of adventitious root formation in isolated stem explants of Rhododendron. Scien. Hort., 3: 1-20.

Pilet, P.E., 1961: Culture in vitor de tissus de carotte et organogense. Bull. Soc. Bol. Suisse, 71: 189-208.

_____., 1964: Effect of chlorogenic acid on the auxin catabolism and the auxin content of root tissue. Phytochem., 2: 617-621.

_____., and R. Braun, 1967: The interrelationship of RNA, auxin and auxin oxidases in lentil roots. Physiol. Plant., 20: 870-878.

Poapst, P.A. and A.B. Durkee, 1967: Root differentiating properties of some simple aromatic substances of apple and pear fruit. J. hort. Sc., 42: 429-438.

Pope, T.E., 1960: Some factors affecting propagation of woody ornamentals. Fla. Ag. Exp. Stn. Ann. Rev., 128-129.

Price, L.W., 1973: Practical course in liquid scintillation counting. Lab. Prac., 22: series of articles.

Quamme, H.A. and S.H. Nelson, 1965: Root promoting substances in the juvenile phase of Malus robusta S. Can. J. PlantSc., 45: 509-511.

Reuveni, O. and I. Adato, 1974: Endogenous carbohydrates, root promoters and root inhibitors in easy and difficult-to-root date palm (Phoenix dactylifera) offshoots. J. Am. Soc. hort. Sc., 99: 361-363.

Richards, M., 1964: Root formation on Camellia reticulata variety, Captain Rawes. Nature, Lond., 204: 601-602.

Roberts, A.N. and L.H. Fuchigami, 1973: Seasonal changes in auxin effect on rooting of Douglas Fir stem cuttings as related to bud activity. Physiol. Plant., 28: 215-221.

_____., B.J. Tomasovic and L.H. Fuchigami, 1974: Intensity of bud dormancy in Douglas Fir and its relationship to scale removal and rooting ability. Physiol. Plant., 31: 211-216.

Robitaille, R.A., 1970: The relationship of endogenous hormones to growth characteristics and dwarfing in Malus L. Ph.D. Thesis. Michigan State Univ. U.S.A.

Ropp, R.S. De, 1956: Kinetin and auxin activity. Plant Physiol., 31: 253-254.

Row, V.V., W.W. Sanford and A.E. Hitchcock, 1961: Indole-3-acetyl-D.L.-aspartic acid as a naturally occurring compound in tomato seedlings. Cont. Boyce Thomp. Inst., 21: 1-9.

Ryugo, K. and P.J. Breen, 1974: Indoleacetic acid metabolism in cuttings of plum (Prunus ceracifera x Prunus munsoniana cv. Marianna 2624). J. Am. Soc. hort. Sc., 99: 247-251.

Saito, Y. and R. Ogasawara, 1960: Studies on rooting of cuttings and changes in growth substances in Salix gracilistyla. J.Jap. For. Soc., 42: 331-334.

Sarkisova, M.M., 1972: Formation and changes in endogenous growth regulators in shoots of fruit crops during the annual growth cycle. Hort. Abst., 42: 345.

Schneider, E.A. and F. Wightman, 1974: Metabolism of auxins in higher plants. Ann. Rev. Plant Physiol., 25: 487-513.

Schraudolf, H. and J. Reinert, 1959: Interaction of plant growth regulators in regeneration process. Nature, Lond., 184: 465-466.

Scott, T.K., 1972: Auxins and roots. Ann. Rev. Plant Physiol., 23: 235-258.

Shah, R.B., 1969: Some physiological factors affecting root formation in cuttings of Citrus. M.Hort.Sc. Thesis, Massey Univ. New Zealand.

Simpson, G.M. and P.F. Saunders, 1972: Abscisic acid associated with wilting in dwarf and tall Pisum sativum. Planta, 102: 272-276.

Sin, K.H. and Sung, O.H., 1968: Fundamental mechanism of root formation in the cuttings of forest trees. Res. Rep. Inst. For. Gen., 6: 1-52.

Singh, S., R.J. Garner and E.S.J. Hatcher, 1957: Influence of source and environment on the performance of apple leaf-bud cuttings. J. hort. Sc., 32: 248-253.

Sinha, A.C. and M.C. Vyvyan, 1943: Studies on vegetative propagation of fruit trees II. By hardwood cuttings. J. Pomol., 20: 127-135.

Sirois, J.C., 1967: A quantitative coleoptile elongation test for growth regulators. In "Biochem. and Physiol. of Plant Growth Subst." Ed. F. Wightmann and G. Setterfield, pp. 1611-1618. Runge Press, Ottawa.

Skoog, F. and C.O. Miller, 1957: Chemical regulation of growth and organ formation in plant tissue cultured in vitro. Symp. Soc. Exp. Biol., 11: 118-131.

Smith, D.L., 1959: The effect of juvenility on rooting of cuttings from apple seedlings. J. Arnold Arbor., 40: 172-175.

Smith, D.R. and T.A. Thorpe, 1975: Root initiation in cuttings of Pinus radiata seedlings II. Growth regulator interactions. J. exp. Bot., 26: 193-202.

Smith, N.G. and P.F. Wareing, 1971: The effect of gravity on root emergence from cuttings of some tree species. Forestry, XLIV: 177-187.

_____., _____, 1972a: Rooting of hardwood cuttings in relation to bud dormancy and the auxin content of the excised stem. New Phytol., 71: 63-80.

_____., _____, 1972b: The rooting of actively growing and dormant leafy cuttings in relation to endogenous hormone levels and photoperiod. New Phytol., 71: 483-500.

Söding, H., 1937: Wuchsstoff und Kambiemtatigkeit der Baume. Jaarb. wiss. Bot., 84: 639-670.

Spiegel, P., 1955: Some internal factors affecting rooting of cuttings. Rep. 14th Int. Hort. Cong., 1: 239-246.

Stepanov, S.N. and E.N. Kuzin, 1973: The rooting capacity of apple layers in relation to their ontogenic state. Hort. Abst., 44: 5274.

Stoltz, L.P., 1968: Factors influencing root initiation in an easy and difficult-to-root Chrysanthemum. Proc. Am. Soc. hort. Sc., 92: 622-626.

_____., and C.E. Hess, 1966: The effect of girdling upon root initiation: Auxin and rooting cofactors. Proc. Am. Soc. hort. Sc., 89: 744-751.

Stoutmeyer, V.T., 1937a: Regeneration in various types of apple wood. Res. Bull. Ia. ag. Exp. Stn., 220: 308-352.

_____., 1937b: The vegetative growth phase of apple shoots and their relation to root formation. Proc. Iowa Acad. Sci., 44: 104-105.

_____., O.K. Britt and J.R. Goodwin, 1961: The influence of chemical treatments, understocks and environment on growth phase changes and propagation of Hedera canariensis. Proc. Am. Soc. hort. Sc., 77: 552-557.

Strydom, D.K. and H.T. Hartmann, 1960: Absorption, distribution and destruction of indoleacetic acid in plum cuttings. Plant Physiol., 35: 435-442.

Sudi, J., 1964: Induction of the formation of complexes between aspartic acid and indolyl-3-acetic acid or 1-naphthalene acetic acid by other carboxylic acids. Nature, Lond., 201: 1009-1010.

_____., 1966: Increases in capacity of pea tissue to form acyl-aspartic acids specifically induced by auxins. New Phytol., 65: 9-21.

Sung, O.K., 1969: Endogenous growth substances affecting rooting of cuttings of pine. Res. Rep. Inst. For. Gen., 7: 1-33.

Tarasenko, M.T., 1964: Juvenile stage and its importance in vegetative propagation. Hort. Abst., 35: 2686.

Tautvydas, K.J. and A.W. Galston, 1970: Binding of indoleacetic acid to isolated pea nuclei. In "Plant Growth Subst. 1970" Ed. P.J. Carr, pp. 256-264. Springer-Verlag.

Thimann, K.V., 1942: Notes on the rooting of some conifers from cuttings. J. Arnold Arbor., 20: 103-109.

_____., and F.W. Went, 1934: On the chemical nature of the root-forming hormone. Proc. Kon. Ned. Akad. Wetensch. Amsterdam, 37: 456-459.

_____., and Koepfli, 1935: Identity of the growth-promoting and root-forming substances in plants. Nature, Lond., 135: 101.

_____., and J. Behnke-Rogers, 1950: The use of auxins in the rooting of woody cuttings. Harvard For. Cabot Foundation, Petersham, Mass.

Thomas, T.H., J.E. Carroll, F.M.R. Issenberg, A. Pendergrass and L. Howell, 1975: Separation of cytokinins from Danish cabbage by column chromatography on insoluble polyvinylpyrrolidone. Physiol. Plant., 33: 83-86.

Tizio, R., J.S. Moyano and H. Morales, 1968: Inhibitor-like substances in vine cuttings and their possible relationship to the rooting process. Phyton., 25: 123-128

Tkacenko, T., 1970: The effect of growth stimulators on the rooting of clonal rootstock cuttings and the production of standard apple transplants. Hort. Abst., 41: 8124.

Tognoni, F. and R. Lorenzi, 1972: Acidic root-promoting growth inhibitors found in Picea and Chamaecyparis. J. Am. Soc. hort. Sc., 97: 574-578.

Toponi, M., 1963: Action combinee de lakinetine et de l'acide indolylacetique sur la neoformation d'organes par de fragments de faulles d'endive cultive in vitro. C.R. Acad. Sc. Paris, 257: 3030-3033.

Turovcev, N.I., 1960: Concerning the propagation of apples and pears by stem cuttings. Sadovodstvo, 10: 36-37.

Turovskaja, N.I., 1969a: Towards a method of quicker propagation of genetically identical plants. Hort. Abst., 41: 259.

_____., 1969b: The effect of the length of low temperature period on growth of root cuttings. Hort. Abst., 41: 260.

_____., 1974: Regeneration of plants from root cuttings in relation to age and morphological characteristics of the root systems of the seedlings. Hort. Abst., 44: 8206.

Tustin, D.S., 1972: The propagation of apple rootstocks by the hardwood cutting technique. B.Hort.Sc. Hons Thesis, Massey Univ. New Zealand.

Van Doesburg, J., 1960: Experiments with winter cuttings. Hort. Abst., 32: 1302.

Varga, M. and E.C. Humphries, 1974: Root formation on petioles of detached primary leaves of dwarf bean (Phaseolus vulgaris) pretreated with Gibberellic acid, Triiodobenzoic acid and cytokinins. Ann. Bot., 38: 803-808.

Vekhov, N.K. and M.P. Iljin, 1934: Vegetative propagation of trees and shrubs by means of summer cuttings. Suppl. 61, Bull. appl. Bot. Gen. Plt. Breed. P. 284.

Venis, M.A., 1970: Auxin reactive proteins. In "Plant Growth Subst. 1970". Ed. P.J. Carr, pp. 240-247. Springer-Verlag.

_____., 1972: Auxin-induced conjugation systems in peas. Plant Physiol., 49: 24-27.

Vieitez, E. and J. Pena, 1968: Seasonal rhythm of rooting in Salix atrocinerea cuttings. Physiol. Plant., 21: 544-555.

Villiers, T.A., 1968: An autoradiographic study of the effect of the plant hormone abscisic acid on nucleic acid and protein metabolism. Planta, 82: 342-354.

Walbot, V., M. Clutter and I. Sussex, 1975: Effects of Abscisic acid on growth, RNA metabolism and respiration in germinating bean axes. Plant Physiol., 56: 570-574.

Wareing, P.F. and D.L. Roberts, 1956: Photoperiodic control of cambial activity in Robinia pseudoacacia L. New Phytol., 55: 356-366.

_____. and P.F. Saunders, 1971: Hormones and dormancy. Ann. Rev. Plant Physiol., 22: 261-364.

Wareing, P.F., and N.G. Smith, 1963: Physiological studies on the rooting of cuttings. Rep. For. Res. Lond., 120.

Warmke, H.E. and G.L. Warmke, 1950: The role of auxin in the differentiation of root and shoot primordia from root cuttings ofTaraxicum and Cichorium. Am. J. Bot., 37: 272-280.

Weaver, R.J., 1972: Plant growth substances in agriculture. W.H. Freeman and Co., San Francisco.

Went, F.W., 1934a: A test method for rhizocaline, the root-forming substance. Proc. K. Ned. Akad. Wet., 37: 445-455.

_____., 1934b: On the pea test method for auxin, the plant growth hormone. Proc. K. Ned. Akad. Wet., 37: 547-555.

_____., 1938: Specific factors other than auxin affecting growth and root formation. Plant Physiol., 13: 55-80.

Whitehill, S.J. and W.W. Schwabe, 1975: Vegetative propagation of Pinus silvestris. Physiol. Plant., 35: 66-71.

Williams, M.W. and E.A. Stahly, 1968: Effect of cytokinin on apple shoot development from axillary buds. Hort.Sci., 3: 68-69.

Woolley, D.J., 1971: Hormonal interaction, metabolism and movement of cytokinins. Ph.D. Thesis Univ. College of Wales, U.K.

Wright, S.T.C., and R.W.P. Hiron, 1969: (+) Abscisic acid, the growth inhibitor induced in detached wheat leaves by a period of wilting. Nature, Lond., 224: 719-720.

Yadava, U.L. and D.F. Dayton, 1972: The relation of endogenous abscisic acid to the dwarfing capability of East Malling apple rootstocks. J. Am. Soc. hort. Sc., 97: 701-705.

Zabkiewitz, J.A. and K.D. Steele, 1974: Root promoting activity of Pinus radiata bud extracts. In "Mechanisms of regulation of plant growth", Bull. 12, Royal Soc. N.Z., Eds, R. Bieleski, A. Ferguson and M. Cresswell. Pp. 687-692.

Zatkyo, J., 1959: The rooting of cuttings of fruit tree rootstocks and scion varieties with the aid of synthetic growth substances and tissue transplants. Hort. Abst., 31: 222.

Zeevart, J.A.D., 1971 (+) Abscisic acid content of spinach in relation to photoperiod and water stress. Plant Physiol., 48: 86-90.

Zenk, M.H., 1962: Aufnahme und Stoffwechsel von -napthylessigsäure durche erbsenepikotyle. Planta, 58: 75-94.

Zimmerman, P.W. and A.E. Hitchcock, 1933: Initiation and stimulation of adventitious roots caused by unsaturated hydrocarbon gases. Contr. Boyce Thomp. Inst., 5: 351-369.

_____., _____, 1946: The relation between age of stem tissue and the capacity to form roots. J. Geront., 1: 27-32.

_____., and F. Wilcoxon, 1935: Several chemical growth substances which cause initiation of roots and other responses in plants. Contr. Boyce Thomp. Inst., 7: 209-229.

Zimmerman, R., 1963: Rooting cofactors in some southern pines. Proc. Int. Plant Prop. Soc., 13: 71-74.

Zimmerman, W.A., 1936: Untersuchungen uber die raumliche verteilung des wuchstoffes bei baumen. Z. Bot., 30: 209-232.