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THE EFFECTS OF ETIOLATION AND RINGING ON ROOTING OF
MYROBALAN PLUM (Prunus cerasifera) CUTTINGS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

by

Yousif Hanna Yousif

* * * * * *

The Ohio State University
1972

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LIST OF SYMBOLS

C = Control

D₁ = date one - at which the first measurements were made in the greenhouse

D₂ = date two - at which the second measurements were made in the greenhouse

E = etiolation with the black tape

Ep = etiolation with the black paint

ER = etiolation with the black tape plus ringing

EpR = etiolation with the black paint plus ringing

FDT = freeze dried tissues

IAA = Idoleacetic acid

PPM = parts per million

R₂ = Ohio 2 rootstock

R₂₉ = Myrobalan 29c rootstock
INTRODUCTION

Generally a fruit tree is not a single individual but is composed of two individuals growing together as one. These are the scion of the selected cultivar and the rootstock. However, the selection of the best combination of the scion and the rootstock, which may vary with different localities, is of utmost importance for a successful fruit planting. Therefore, the selected rootstocks ought to be propagated vegetatively in order to maintain the desired characteristic(s) of the rootstocks.

Various methods are used by nurserymen in the vegetative propagation of rootstocks. These include cuttings, layering, suckers, off shoots and apomictic embryos.

The propagation of fruit tree rootstocks by cutting is commercially the most desirable method if a clone reproduces easily by cuttings because: 1) the new plants are genetically identical to the selected parent plant, which usually can't be obtained by seed propagation, 2) usually the growth of plants is faster and more uniform than that of seedlings, 3) more plants can be obtained from a limited source of plant material in comparison with layering or stooling, 4) less space is required for growing equal numbers of plants by cuttings than by stooling or layering, 5) dormant hard-
wood cuttings can be taken anytime during the winter months and stored until planting to suit the propagator's scheduling. In addition, this kind of cutting needs less care and attention than the softwood ones, and 6) sterile cultivars require vegetative propagation; therefore cuttings may often be the best method.

Great differences exist among species and clones in rooting of cuttings taken from them. For example, some grapes, figs, and pomegranates root so readily that the most simple facilities and care give high rooting percentages, while most peaches, apricot, plums and apples root with difficulty, even if special practices are taken into consideration and maintained at the optimum conditions. Softwood cuttings of difficult-to-root woody plants were tried after mist propagation was introduced. Since then, a great advancement in plant propagation by cuttings has been achieved with many plants, but the problem remains unsolved with others.

The Myrobalan plum (Prunus cerasifera L.) is used widely as a rootstock for European and Japanese cultivars of plums because the tree is hardy and vigorous. Two Myrobalan plum rootstocks were used in this study. Myrobalan 29c is an easy to root clone, while Ohio 2 is difficult to root. Therefore, an attempt was made to find a method that could improve rooting of Ohio 2 cuttings.
Etiolation appears to offer considerable promise for successful rooting of fruit trees. Etiolation is defined as growth and development of the plant or plant parts in the absence of light. As early as 1537, the beneficial effect of darkness on rooting of some apple cultivars had been noticed. Recently this has been found true with other plants such as avocado, hibiscus, etc.

Although etiolation enhances remarkably the rooting of cuttings of many plants, its use as a commercial practice is limited, if not completely avoided. That is because the techniques employed in etiolating plants or plant parts are laborious and costly. Therefore, it was thought that the use of black paint and a modification of the tape technique might reduce this cost.

This study was conducted in an attempt to improve the rooting of cuttings of the Ohio 2 clone. This objective was measured by rooting percentages, time required for rooting and the number of roots per cutting. Other objectives of this investigation were to determine whether or not the difference in rooting responses of cuttings of two Myrobalan plum rootstocks was related to the contents of their root promoting substances and growth substances. If so, how are these substances influenced by etiolation and ringing?
LITERATURE REVIEW

The propagation of fruit trees by cuttings has numerous advantages over the other methods of plant multiplication. The new plants are genetically identical to the parent plant; whereas, this usually cannot be obtained from seed propagation. Very often the growth of plants is faster and more uniform than that of the seedlings. Consequently, much research has been conducted in order to induce and/or improve the rooting of cuttings of a variety of plant species. Both environmental influences and plant conditions have been investigated. Recently attempts have been made to elucidate the principles governing the rooting process of cutting. Some of the most important factors that influence rooting of cuttings are:

1. Etiolation

Etiolation has long been known to be very effective in inducing root formation in stem cuttings. According to Frolich (17), as early as 1537, the beneficial effect of etiolation was noticed on rooting of cuttings of some apple cultivars. Reid (50) stated that the Camphor plant which is usually propagated from seed, was propagated by cuttings taken from etiolated shoots. Knight and Witt (41) working with plums, found that etiolation resulted in a marked
increase in rooting of cuttings, and roots were not limited only to the cut base as in the control but they appeared on the whole etiolated area. Blackie et al (8) reported similar results to those of Reid (50) with Camphor plants. The method they employed was to cover the twigs so they would develop for two weeks in darkness, prior to their removal from the parent tree. Smith (54) reported that when a portion of stem of clematis was wrapped with black paper for ten days to three weeks before taking cuttings, root formation resulted at the nodes instead of only internodally, and more rapid rooting in the internodal region occurred. Gardner (20) etiolated apple shoots by using ordinary black insulating tape and black cylinders. A piece 2.5 to 3 inches long was used and wrapped spirally four to five times around the young shoots, starting as near to the growing tip as possible. Cuttings were made from the etiolated shoots with the basal cut made in the etiolated area. He found that cuttings from twelve apple cultivars rooted to different extents with the best responses obtained with McIntosh. When etiolated shoots were girdled early in their growth by means of copper wire, up to 100% rooting of McIntosh cuttings could be obtained. Gardner developed a black paper cylinder method for etiolating individual shoots. Frolich (17) reported that the majority of avocado cultivars cannot be propagated by cuttings, but it has been made possible by etiolation. The method used is by growing
avocado seedlings in small containers and grafting them with the cultivar they wish to root. The plants are cut back to near the original scion and when the buds show signs of pushing, the plants are left in the dark room until shoots are about three inches long, and then they are placed out into the light. A paper cylinder is placed around the shoots and filled with vermiculite or other materials that exclude light. Herman and Hess (25) studied the effect of etiolation on the rooting of cuttings of red kidney beans and three cultivars of Chinese hibiscus. Shoots were etiolated by black tape for three weeks before taking the cuttings. They found that the etiolated red kidney bean cuttings had formed over five times as many roots as non-etiolated ones after four days, and nearly twice as many roots after eight days. Also, the rooting of hibiscus cuttings was improved and the magnitude of the improvement varied with the cultivar. Hackett (21) found that etiolation of the adult forms of Hedra helix stems caused the stems to form aerial roots quite profusely.

Etiolation does not only enhance the rooting of cuttings taken from etiolated shoots or plants, but it also influences the rooting of cuttings taken from non-etiolated shoots. The storage of dormant cuttings in a damp peat moss at a proper temperature for several weeks before planting is a common practice in the propagation of many clonal rootstocks by hardwood cuttings. However, it seems likely that
etiolation is playing a role in the enhancement of rooting of the stored cuttings. Shapiro (52) reported that poplar cuttings kept in darkness for four or more days before being placed under continuous light, showed root emergence in a direct proportion to the length of the dark period. Kawase (40) found that the rooting of mung bean cuttings was increased with increasing duration of etiolation. In addition, when etiolation was made immediately after the cuttings were made, the effect sharply increased up to four days of etiolation. Similar results were reported with willow cuttings by the same author.

When cuttings are made, the cut surfaces are exposed to light. The duration of exposure varies with the circumstances of the operation. However, the question is: to what extent this might influence the rooting of cuttings of a variety of plant species.

Several studies have been made in an attempt to explain the effect of etiolation on rooting of cuttings. Priestly and Ewing (48) working with bean plants suggested that the presence of the endodermis, which develops under etiolation conditions, causes the free development of the adventitious roots on stem cuttings because the distribution of the nutrient sap is changed. Smith (54) considered etiolation to be acting by mechanical softening of the tissues and by restoring the C/N ratio necessary for meristematic activity. Gardner (20) suggested that etiolation brings about a
temporary accumulation of growth substances in the etiolated region due to some anatomical abnormality. Herman and Hess (25) reported that various anatomical modifications in cells and tissues of red kidney beans and hibiscus stems were found as a result of etiolation. In addition, slightly higher endogenous auxins and to somewhat higher rooting cofactors were present in etiolated tissues than in the non-etiolated. They concluded that the effect of etiolation on rooting of cuttings may be attributed to a complex of factors. These factors interact, some synergistically, in a final realization of the rooting response of a plant. Kawase (40) proposed that cuttings under dark conditions retain a higher level of IAA in the cuttings at the time of root initiation and this higher level of IAA results in better rooting than in non-darkened ones.

It is evident from this review that etiolation does enhance the rooting of stem cuttings of many plants, but the actual mechanism(s) by which the rooting of cuttings is promoted is not completely understood. In addition, the methods employed in etiolating plants or plant parts are laborious and costly. These techniques can be classified into two main categories: First, etiolating the entire plant either by covering it with a black material for a certain period of time to exclude light completely or by putting plants in dark rooms. Second, etiolating parts of the plant by wrapping or covering the individual shoots with a black
material for a limited period of time prior to taking of cuttings.

The problems associated with the first technique are: (1) the stock plants will be weakened as a result of covering with the black cover, (2) if cuttings are made immediately after the cover is removed, the etiolated cuttings do not withstand the high temperatures and light intensities in the greenhouse. This might result in the death of a considerable number of cuttings, (3) the potential of disease infection is increased due to the high relative humidity and temperatures inside the cover, and (4) wrapping the base of the etiolated shoots is required after the removal of the black cover and before taking the cuttings.

The problems associated with the second technique are: (1) wrapping the individual shoots with the black tape spirally several times around the shoots is a time consuming process, (2) if specially prepared black bags are used, the bags are troublesome to fasten over the shoot terminals, (3) in addition, the basal portion of the shoot must be wrapped with a black tape after the removal of the bags, and (4) if black cylinders are used for etiolating individual shoots there is a chance of light penetration through them which might reduce the effectiveness of etiolation on rooting of cuttings.
2. Ringing

Several workers have reported that constricting the base of new shoots by wiring or ringing increased rooting of cuttings taken from such treated shoots. Bobilioff (9) obtained increased rooting in *Hevea brasiliensis* by binding shoots with wire for three weeks before taking cuttings with the base immediately below the placed wire. Stoltz and Hess (59) reported that girdling greatly increased the rooting of red hibiscus cuttings, but the root emergence began at about the same time in both the girdled and controlled cuttings. Higdon and Westwood (33) found girdled Old Home pear cuttings taken in August rooted better than those taken from ungirdled ones. On the other hand, there are reports which indicate that girdling does not affect rooting of cuttings considerably. Luckan (45) failed to obtain a successful rooting of walnut cuttings after being girdled. Vierheller (64) found that girdling of apple shoots during the summer resulted in no superior rooting of cuttings over the control. This might be explained with the findings and conclusions of Hunter (35) when he found that Rangpur lime cuttings gave optimum percentage of rooting when shoots were ringed for 15 days prior to the taking of cuttings, in comparison with 5, 10, 20, 25, and 30 days of ringing. He concluded that it was necessary to ring neither too short nor too long before taking the cuttings.
The physiological basis for the enhancement of rooting of cuttings by girdling is not clarified yet. The accumulation of carbohydrates above the girdle is thought to be an important factor in promotion of rooting of cuttings. Bobilioff (9) noticed that swelling occurred immediately above the constriction and suggested that the extra stored food in cuttings assists regeneration of roots. Weaver (65) found that total sugars plus starch in the basal portions of girdled shoots of Red Malaya grapes were increased in comparison to non-girdled ones. Stoltz and Hess (59) stated that carbohydrate represent the major component to increase in the tissue above the girdle of red hibiscus plants, in which much higher sugars and starch were present in comparison with the white cultivar. They suggested that the latter may account for the difference in their rooting ability. Reid (49) suggested that any conditions favoring high C/N ratio favored rooting of cuttings.

Root promoting substances have been shown to build up above the girdle. Van Overbeek and Gregory (63) worked with red hibiscus, which is propagated easily by cuttings, and a white cultivar, which is very difficult to root. When the red cultivar was grafted onto the white one, the rooting of the latter was promoted when auxin was applied, but, when a complete girdling was made on the scion just above the graft union, no rooting of cuttings of the white form was
obtained, even when auxin was applied.

Stoltz and Hess (60) found that rooting co-factors 1, 2, and 4 were present in the tissues above and below the girdle. The concentration of co-factors 1 and 2 increased during the first 10 days, but decreased at 20-30 days; while co-factor 4 increased substantially in tissues above the girdle of the easy-to-root cultivar, but not with the difficult-to-root one, or in the tissues below the girdle of either cultivar. They suggested that the increases in the rooting ability of girdled hibiscus cuttings may be due to the accumulation of root promoting substances or their precursor.

The effect of girdling on auxin (IAA) levels above the girdle has been reported to decrease. Kato and Ito (38) found that girdling of apple shoots decreased the endogenous auxin content in the apical buds of girdled McIntosh apple shoots. Stoltz and Hess (60) reported that one auxin (IAA) was found in the tissues of both white and red hibiscus cultivars in the tissues above the girdle, and that the concentration decreased below the original content 10 days after girdling in most tissues, above and below the girdle of both hibiscus cultivars.

It is evident from this review that ringing or constricting of shoots prior to taking of cuttings enhances the root formation of cuttings. The physiological role which ringing plays in the rooting process of cuttings is not completely understood. It could be due to the accumulation of
carbohydrates, root-promoting substances, or, a combination of two or more of these factors.

3. **Endogenous rooting substances**

   a. Root promoting substances (auxin, cofactors, water soluble substances)

According to Audus (4) our present understanding of the subject of root promoting substances originated from the work of Van Der Lek who published the first extensive investigation of the role of these internal factors on rooting of cuttings. He demonstrated that in currant, willow, poplar and grape, the presence of developing buds was a prerequisite for rooting and that the intensity of root production was directly correlated with the rate of bud development. Cuttings with dormant buds failed to root even under the most favorable conditions, but in the spring when the buds renewed their activity, rooting occurred. The removal of a ring of bark from a short section of stem below the buds also prevented rooting. He suggested the formation of a hormone(s) in the developing buds and its conduction in the bark to the base of the cuttings where it initiated rooting. Went (66) stated that a substance diffused from petioles of Acalypha and *Caria papaya* leaves in water which promoted rooting of Acalypha cuttings.

Root promoting substances have been shown to be produced by leaves, buds and cotyledons. They are translocated downward and accumulate at the base of the cuttings where
root formation is stimulated. Went (68) reported that the presence of at least one bud on pea cuttings was essential for rooting even when auxin-rich preparation was applied to the cuttings. Cooper (12) reported that lemon and trade-cantia cuttings without leaves failed to root, but when they were treated with IAA, the rooting occurred. Went (68) working with pea cuttings, found that auxin is not the only substance responsible for root formation, but other substances distributed over the whole length of the stem which are manufactured in the leaves are necessary for root formation. He suggested that when auxin is applied to the cuttings it causes the rapid downward movement of the rooting substances which accumulate at the base of cuttings. Cooper (14) found that there was little difference in the amount of auxin recovered from treated apple and lemon cuttings, yet the apple cuttings did not form roots. He assumed that apple cuttings are lacking in certain internal substances necessary for root formation. In addition, he found that lemon cuttings treated at the base with 0.2% IAA solution for 20 hours produced 17 roots per cutting more than the control. Cutting off the treated base destroyed the effect of the treatment, and treating the new base for 20 hours with the same treatment caused only 5.8 more roots than the control. Excising the treated base likewise destroyed the effect of a 40 hours treatment with 0.2% IAA solution, but treating the new base for 20 hours gave no more roots than when not treated. He concluded that
IAA treatment causes the downward movement of a substance(s) called rhizocaline which is necessary for root formation. Cooper (14) presented evidence which indicates that leaves of lemon cuttings supply a substance necessary for the differentiation of the root primordia and another for the outgrowth of these primordia.

There are other means for further demonstration of the presence of rooting substances in cuttings and in intact plants. Cooper (13) reported that when a complete girdle was removed from the stem of the lemon cuttings about 4 cm. from the base, no rooting occurred even though cuttings were treated with auxin. Van Overbeck and Gregory (63) working with hibiscus plants found that when a complete girdle was made just above the graft union of the red form onto the white form, the root promoting substances movement from the red form was blocked. This was indicated by the inability of the cuttings taken from the rootstock to form roots.

Kawase (39) has shown that centrifugation of willow cuttings in a basipetal direction promotes root initiation. The number of roots per cutting increased linearly with increased centrifugal force from 0 to 2540 g. and with increased centrifugal duration from 0 to 90 minutes. When cuttings were centrifuged basipetally in small amount of distilled water, it was possible to collect a substance(s) which promotes root initiation in willow and mung bean cuttings. The amount of the root promoting substance collected was propor-
tional to the gravitational force.

Attempts have been made for the isolation, characterization and elucidation of the mode of action of rooting substances. Bouillenne and Bouillenne-Walrand (10) proposed that rhizocaline can be considered as a complex of three components: (1) a specific factor translocated from the leaves and characterized chemically as an ortho-dihydroxy phenol, (2) a nonspecific factor (auxin) which is translocated and found in biologically low concentration and (3) a specific enzyme located in cells of certain tissues which is probably of the polyphenol-oxidase type.

Hess (29) reported that methanol extract from juvenile cuttings of Hedra helix, when tested on mung bean cuttings did not increase the number of roots per cutting, but when auxin was added to it, the number of roots increased greatly. When the extract was fractionated by paper chromatography, he found four regions which promote rooting on mung bean cuttings when used with IAA solutions. Hess (30) working with Hedra helix and hibiscus, reported that rooting substances are soluble in water and methyl and ethyl alcohol. They are insoluble as a group in chloroform and ether, they are thermostable and nondialytic. Hess (30, 31) found that rooting substances react with IAA synergestically in the mung bean rooting test. Because of this characteristic, Hess called them rooting cofactors. According to Hess (28), there are four cofactors (1, 2, 3, 4) present in the extract of easy to
root plants. They are named according to their locations on the chromatograph after being developed. The first one is the closest to the spotting line and the fourth one is the farthest.

Some of these cofactors have been isolated and characterized. Hess (30) isolated cofactor 3 from the easy to root juvenile form of *H. helix*. He mentioned that cofactor 3 is isochlorogenic acid, which is a phenolic compound and provides protection for IAA from destruction. Cofactor 4 is a group of oxygenated terpenoid compounds which have been suggested to be the most active of all naturally occurring root promoting substances (25). It has 4 major areas of activity and it has a bluish fluorescence in u.v. light. Hess (29) considered cofactor 1 as having the least significance among the four cofactors in promotion of rooting of mung bean cuttings. Fadl and Hartmann (15) isolated and purified a rooting cofactor from Old Home pear cuttings during the maximum rooting activity of cuttings which they termed IAA-cofactor complex. They concluded that it is synthesized in the bud or it is a product of a reaction(s) which take place in the base of the cuttings in the presence of relatively high concentration of reactants, which are: a) materials produced by the buds, and translocated to the base of cuttings during the 10 day period, or, b) the applied auxin.

After the discovery of the rooting cofactors by Hess many investigators have reported their presence in extracts
of many plant species. But the quantity and the numbers of these cofactors which are present in the extract varies with the plant species or variety and the time of the year at which the extracts are made. Zimmerman (70) reported the presence of four rooting cofactors in Loblolly pine, but cofactor 1 had a slightly active substance. In addition, he reported that in one eight year old tree had only cofactors 3 and 4, while in another tree similar to the first one, had only cofactors 1, 3, and 4. He also mentioned that the amount of rooting substances do not depend on the age of the tree. Herman and Hess (25) found that both red kidney beans and Wilson hibiscus contained higher levels of rooting cofactors when etiolated than non-etiolated. On the other hand, the results were non consistent with Cornell Red and Ruth Wilcox hibiscus varieties. Ashire and Carlson (3) working with clonal apple rootstocks found that their extracts contained cofactor 1 and 2, in addition, cofactor 3 was present in MM106 extract only. Challenger et. al (11) stated that rooting cofactor activity was obtained in extract of hardwood cuttings of Crab-C and EM 26 apple rootstocks. This activity was increased by high temperature during winter storage of cuttings. Richard (51) found that extract of difficult to root Camellia variety cuttings lacked some of root promoting zones. Fadl and Hartmann (16) found that high levels of root promoting activity occurred in the extract of Old Home buds and basal segments during the period of maximum rooting,
while Bartlett extract showed considerably less promotion activity.

It is evident from this review that rooting substances are present in plants. They are synthesized in leaves, buds, and cotyledons, and they are translocated downward in the phloem which accumulates at the base of the cuttings. Rooting substances have been isolated and some of them are characterized, but their mode of action is not fully known yet.

The present hypothesis about the rooting of cuttings is that in easy to root ones all the four cofactors and auxin are present in adequate amounts. On the other hand, cuttings which are difficult to root, either auxin or one or more of the cofactors are limiting. If auxin is limiting, an exogenously applied auxin enhances the rooting of cuttings. But if cofactor(s) are limiting, an auxin treatment would not enhance rooting, of course depending on the plant in question (23).

According to this hypothesis, a cutting must root when all cofactors and auxin are present in proper amounts provided that environmental conditions are maintained at optimal levels. But this is not the case, because a large number of plants fail to root even when the previous requirements and conditions are met.

b. Root inhibiting substances

The naturally occurring inhibitors in many plants and plant parts have been demonstrated beyond any doubt. Their
role in many physiological processes has been well established such as in dormancy of seeds, tubers, and bulbs, the rest period of deciduous woody plants, leaves and fruit abscission. Unfortunately, there has been little work in connection with rooting of cuttings.

Inhibitors in plant extracts have been classified into two main groups by Barlow et al. (7) and Luckwill (46). First, substances which are of a phenolic nature and are merely toxic. Second, substances which are readily reversible while they act by blocking some essential reactions in the chain of processes involved in growth.

Some of these inhibitors have been isolated and characterized from various plants. The typical procedure for their studies is the extraction of the plant materials with alcohol or other organic solvents using paper chromatography techniques for the separation of the extracts. Many bioassays are used for testing the activity of the inhibitors such as wheat coleoptile, and seed germination tests. Barlow (6) working with Myrobalan B plum and Crab C apple rootstocks, reported that inhibitors were obtained at Rf 0.5 to 0.6, which retained their activity over a long period in comparison with growth promoting substances. They were heat stable and the inhibiting effect was proportional to the logarithmic concentration, either alone or in the presence of IAA. Hemberg and Larson (24) reported that inhibitor B-complex from resting potato tuber was isolated which inhibited
\( \alpha \)-amylase activity. Lane and Bailey (43) found that growth inhibitor occurring in dormant maple buds is most evident in the Rf 0.5 to 0.8 zone in isopropanol:ammonium hydroxide:water (10:1:1 v/v) solvent system especially at Rf 0.6 to 0.7, which was acidic in nature and soluble in water, methanol, ethanol and acetone, and it is a phenolic compound.

Perhaps Spiegle (55, 56) was the first one to demonstrate that inhibitors were important factors in rooting of cuttings. He reported that when dormant cuttings taken from many grape species and hybrids were leached with water for periods up to 96 hours their rooting was most favorably influenced. Furthermore, when the water extract of the difficult to root grape cuttings was applied to the easily rooting ones, their rooting was retarded. Kahn and Hall (37) stated that immersion of sugar cane cuttings in water for 24 hours prior to planting was found to be definitely superior to the control in the rooting percentage, number, weight and volume of roots produced. In addition, further enhancement was obtained when soaked cuttings were treated with auxin in comparison to the soaked cuttings but not treated with auxin. They suggested that the improvement could be due to leaching of inhibitors from cuttings. Tyce (62) reported that extracts from willow cuttings which had been left in water to root showed a striking decrease in their inhibitory action on IAA. On the other hand, Hess (27)
mentioned that cuttings made from the mature form of *Hedra helix* and soaked in water remained difficult to root.

The presence of buds on cuttings has been shown to exert a major influence on their rooting. It could be a promotive or inhibitory effect depending on the physiological status of the buds. Van Der Leek (44) stated that cuttings of many woody plants failed to root even under the most favorable conditions, but in the spring, when buds renewed their activity, rooting occurred. Howard (34) working with plum and apple rootstocks found that at each collection date at monthly intervals from November to March, rooting of disbudded cuttings was consistently higher than the control. Fadle and Hartmann (15) reported that a seasonal fluctuation in rooting pattern similar to that of potential bud activity was found in Old Home pear hardwood cuttings. When buds and basal segments of cuttings collected at intervals from July to March, were extracted and fractionated by chromatography using mung bean bioassay, they found that high levels of root promoting activity occurred in extracts of Old Home buds and basal segments during the period of maximum rooting. In contrast, Bartlett pear cuttings and extracts not only showed considerably less promotion but high inhibition. High levels of inhibitors occurred in Old Home buds only during their rest period while Bartlett buds extracts had large amounts of inhibitors most of the year.
In general, extracts from difficult to root plants have been shown to contain higher levels of inhibitory substances, and less amounts of promoting ones compared with the easy to root cuttings. Ashire and Carlson (3) working with EM 11 and MM 106 clonal apple rootstocks, found that an endogenous inhibitor of rooting in EM 11 extract at Rf 0.6 - 0.9 in isopropanol:water (8:2 v/v) solvent system was present, while it was absent in easy to root MM 106 extract. Spiegle (55) reported that a larger amount of inhibitors were present in *Vitis berlandieri* grape cross which roots with difficulty in contrast with the easy rooting *V. rupesteris* cross. Two rooting inhibitors were found occurring at Rf .65 to 0.75 and at Rf .95 to 1.0 in isopropanol:ammonia (10:1 v/v) solvent system. Challenger et al. (11) and Barlow et al. (6) have isolated and characterized inhibitors from Myrobalan plum and Crab C apple rootstocks. They occurred at Rf 0.5 to 0.6.

Richard (51) using mung bean bioassay, found that extracts from leaves of Camellia reticulata, which is very difficult to root contains a number of inhibitors of root formation which did not occur in *C. Japonica* which roots easily. Hess (30) reported the presences of inhibitors and promoters in extracts of mature and juvenile forms of *H. Helix*. He concluded that the difference in rooting ability of the two forms cannot be attributed to a lack of extractable auxin or to the presence of inhibitors. But when
the extracts were tested with mung bean test, he found that
the extract of the juvenile form contains high amounts of
rooting cofactor 4 which is absent in the mature form (32).
MATERIALS AND METHODS

The field experiments were conducted at the Horticultural Farm of The Ohio State University in Columbus, Ohio.

Two Myrobalan plum (Prunus cerasifera) rootstocks were used in this study. They were Myrobalan 29c which is an easy to root clone and Ohio 2 which is difficult to root. The plants were growing in hedge rows about 6 feet apart with 2 feet between the plants in the row. They were 3-4 years of age and bearing fruits.

A randomized complete block design was used in the laying out of the field experiments. Three treatments with three replications per treatment and ten cuttings per replicate were used. All the data were subjected to analysis of variances and Duncan's Multiple Test Range was used to compare treatment means (57).

A. Field Experiment 1

1. Black tape experiment

On May 15, 1971 the following treatments were applied to both rootstocks. Each tree was divided perpendicularly into approximately three equal portions. A replicate consisted of similar portions of the trees. The treatments within the replicate were applied randomly to the shoots. In all cases shoots of similar
length and vigor were used.

a. Etiolation with the black tape

Shoots of 3-4 inches long having approximately equal vigor were used. The expanded leaves below the shoot tip and down to a distance of 1\frac{1}{2} to 2 inches were removed. Black plastic electric tape of 3/4 in. width was used. A piece 1.5 to 2 inches in length was folded lengthwise around the shoot starting as close as possible to the shoot tip.

b. Etiolation with the black tape plus ringing

Ringing of shoots was made with a small piece of thin copper wire about 2 inches long. The wire was wrapped around the shoot twice at the end of the etiolated area and the ends of the wire were twisted together several times.

2. Black paint etiolation

A black paint material was obtained from AMCHEM Company which consisted of 100% asphalt base.

The procedures followed in both paint and paint plus ringing applications were the same as those described earlier for the black tape except that paint was applied instead of tape. The paint was applied with a small paint brush which provided a thin layer around the etiolated portions of the shoots.
B. Greenhouse Experiment

The rooting studies were conducted at the greenhouses of the Horticulture Department of the Ohio State University in Columbus, Ohio.

The rooting medium consisted of a 2:1 (v/v) ratio of perlite (horticulture coarse type) and Indiana peat moss, respectively. The medium was steam sterilized for 30 minutes. The temperatures of the medium at 2-3 inch depth ranged from 75 to 80°F during the warmest hours of the day, while the air temperature and the relative humidity were automatically controlled and ranged from 75 to 80°F and 50-60% respectively.

On June 15, 1971, cuttings were taken in the late afternoon hours. The base of the cuttings was stuck into moist peat moss until they were transferred to the greenhouse and placed under intermittent mist (5 sec/6 minutes). The basal cut was made directly at the end of the etiolated area. The control cuttings were prepared by making the cut at a similar distance from the base of the shoots to that of the treated ones. The shoot tips were removed and the length of cuttings was from 5-6 inches. The leaves from the basal portion of the cuttings were removed and only 4-5 leaves per cutting were left. The black tape was removed by making a cut through the united edges with a razor blade and the whole tape was peeled off and the cutting was stuck into the media. The black paint was left on the cuttings. Cuttings from ringing treatments were treated similarly after the wire was
Cuttings were set at 3.0 x 3.5 inches in the rows and between the rows, respectively. The replicates were assigned randomly to the propagation bed being 10 inches apart.

The cuttings were covered with a shade cloth for 2 weeks after the time of planting. No wilting occurred during the entire period of the experiment. The cuttings were sprayed occasionally with water to maintain adequate moisture in the medium.

Two counts were made during the course of the experiment. These are referred to as date one (D₁) and date two (D₂). Each time the number of rooted cuttings and the number of roots per cutting were recorded. The readings were made at 4-week intervals after the time of planting.

Taking samples for the laboratory studies

Samples for the laboratory studies were taken the day following the taking of cuttings. Eighteen samples were collected. Each sample consisted of 5 to 10 cuttings. Only etiolated portions were used. For the control, similar portions of the shoot to that of the etiolated ones were collected.

When each sample was prepared it was placed in a small polyethylene bag, labelled and placed on dry ice and kept in an insulated ice box. After that, the samples were stored at -20°C until they were freeze-dried and ground to pass through a 40 mesh screen. They were stored in small glass jars.
having a screw-type cap and placed inside a desiccator which was partially evacuated and stored until the time of the chemical analysis in the winter of 1972.

No samples for laboratory studies were collected for the black paint experiment.

C. Field Experiment II

Materials and methods similar to those in Field Experiment I were used. The plants were pruned severely on June 10, 1971, and the trees had made sufficient growth by July 10, at which time the treatments were applied.

D. Greenhouse Experiment II

This study was conducted at the greenhouses of the Horticultural Department of the Ohio Agriculture Research and Development Center at Wooster, Ohio. The greenhouse was shaded with white shade material and the propagation bed was covered with the green saran screen. The mist system was controlled by a solatrol and the amount of mist provided varied with light intensity conditions. It averaged about a 10 sec. mist every 20 minutes.

Cuttings were taken on August 8, 1971. They were similar to those of the first experiment and were treated alike. The cuttings were put in carton boxes which contained moistened peat moss and the entire box was put inside a plastic bag to reduce transpiration during transportation to Wooster, Ohio. The cuttings were in excellent shape at the time of arrival in Wooster.
Only two replications per treatment were obtainable because of a windstorm which resulted in the breakage of a considerable number of shoots having the ringing treatment. In addition the paint treatment retarded the growth of many shoots of the Ohio 2 rootstock.

Two readings were made at 4 week intervals as in Experiment I. They are referred to as date one \( (D_1) \) and date two \( (D_2) \). At both dates the number of rooted cuttings and number of roots per cutting were recorded.

Samples for the laboratory studies were collected and treated similarly to those of the first experiment.

The Laboratory Studies

1. Auxin \( (IAA) \) determination

Three samples were drawn randomly from the sample population and were run together at each test.

a. Extraction:

A fifty mg. sample of lyophilized tissue was extracted three times with 3 volumes 50 ml. of absolute methanol at 0°C. for 30 minutes at each time. The three extracts were combined and reduced almost to dryness with a flash evaporator at 36°C. The residue was taken up three times in 0.5 ml. methanol and spotted on a paper chromatograph strip.

b. Chromatography:

A 2.5 cm. wide strip of Whatman No. 3 MM chromatography paper was used. The residue was spotted as a
line on both sides of the paper with capillary tubes of 0.9 mm. (I.D.). A hair dryer was used for drying the chromatographs during the spotting process. They were air dried for at least 30 minutes before they were put for equilibration.

Ascending paper chromatography was adopted in a glass cylinder in a dark room at 24°C. The developer consisted of isopropanol, ammonium hydroxide (28%) and water in a ratio of 8:1:1 (v/v) respectively. Chromatographs were left overnight to equilibrate. When the solvent front reached 20 cm. from the starting line, the chromatographs were removed and dried for at least half an hour before incubation. In most cases they were wrapped with aluminum foil and stored at -20°C. until they were used.

Each chromatograph ranging from Rf. -0.05 to 1.0 was sectioned into 21 equal pieces of 1 cm. long each. The auxin activity of the sections was assayed by the wheat coleoptile test method.

c. Bioassay

Wheat Coleoptile test as described by Nitsch and Nitsch (47) was adopted with slight modifications. Wheat seed var. "Knox" was soaked in water for two hours at room temperature. Kitchen dish washing pans of 14 x 6 x 5 inches were used for growing the coleoptiles. One liter of water was added to two liters of
vermiculite in the pan. The seeds were sown on the leveled surface of the moistened vermiculite and covered evenly with half a liter of vermiculite. The pan was put inside a polyethylene bag which was sealed tightly and placed in a dark room at 24°C. After 69 to 72 hours the coleoptiles were ready for sectioning. Only straight ones of approximately 2.0 cm. long were used in all tests reported in this study.

A Barlow cutter as described by Barlow (5) was constructed from plexiglass material. Sections of 4.5 mm. long were cut 3 mm. below the tip of the coleoptile. The primary leaf was left inside the sections. Ten sections were cut at a time and soaked in glass distilled water for three hours before they were incubated in test solutions. Five sections were used per chromatogram segment in all experiments reported here.

Each chromatogram segment was cut into two pieces and placed into a shell vial of 1.9 x 10.6 cm. to which 1 ml. of a buffer solution at pH 5.2 (K2HPO4 1.794 gr./l. plus citric acid monohydrate 1.091 gr./l.), plus 2% sucrose and 0.1% Tween 80 was added.

The coleoptile sections were removed from the distilled water and blotted dry on a clean piece of chromatography paper before they were added to the test solutions. The incubation period ranged from 22-24 hours in darkness at 24°C. The whole process of sectioning,
soaking and incubation was conducted in a dark room under a dim green light.

The increase in section lengths was measured by means of calipers supplied with a thumbwheel and with micrometer dial which gives a direct reading up to 0.05 mm. The sections were blotted dry on a filter paper and placed on a black scotch tape fixed on the table surface in such a way that the sticky surface was maintained upward to which the sections were stuck and measured. The mean elongation of the sections in different solutions was compared with that of the control. The control treatment consisted of a piece of blank chromatography paper which was taken from the lower end of the chromatograph and incubated with 1 ml. of the incubation solution.

The results are expressed as an increase or decrease in mm. in length of coleoptile sections in comparison with the control.

**Synthetic IAA Standard Curve**

A stock solution of 10 ppm was prepared by dissolving 5 mg. of synthetic IAA in 500 ml. of phosphate-citrate buffer (pH 5.2). A series of IAA dilutions (10^{-0} through 10^{-4} ppm) were prepared and contained 2% sucrose and 0.1% Tween 80. The growth of coleoptile sections in different IAA concentrations is shown in Figure 13. The results are expressed as a percent increase in coleoptile section lengths of the control.
When a synthetic IAA solution was spotted, developed and assayed similarly to that of the experimental material, the auxin activity appeared at segments 8, 9, and 10 or at Rf. ranged between 0.35 and 0.5. Therefore, the promotive activity of the segments 8, 9, and 10 of each chromatograph was considered to be due to IAA present in these segments. Concentrations of IAA in 8, 9, and 10 sections were estimated from Figure 13. The three values were added together, thus giving an IAA equivalent present in that sample. Similar procedure was used for all samples of the experiment. The results were subjected to least square analysis of variances, and Duncan's Multiple Test Range (57) was used to compare treatment means.

Rooting Cofactors Study

The mung bean (Phaseolus aureus Robx.) rooting test originally developed by Hess (27) was used with modifications.

Extraction and chromatography procedures were essentially the same as those previously described for the auxin study. Also, three samples were run together at each test.

Growing the seedlings

Mung bean seeds (30 cm³) were soaked in a mixture of methanol: chlorox (1:1 v/v) ratio for 3.5 minutes. Kitchen dish washing pans of 14 x 6 x 5 inches were used. Two liters of horticultural perlite were placed in the pan, to which 1½ liters of water were added. The seeds were set at about 1 x
1 inches apart or approximately 100 seeds per dish from which about 75 cuttings were obtained. The seeds were covered evenly with 1 liter of a mixture of perlite-vermiculite at 1:1 v/v ratio. The pans were put inside polyethylene bags which were sealed tightly and covered with two layers of cheesecloth and one layer of regular brown paper. This cover provided about 20 ft. candle light intensity inside the pans under which the seedlings were left for 5 days before they were removed and placed under light intensity of 1800 ft. candle for another 4-5 days at which time the seedlings were ready for use. The temperatures in the room ranged from 23°C for the initial growing of seedlings to 27°C when they were placed under light. The growth room was controlled to give a daily 18 hr. light period with an intensity of 1800 ft. candle at the cuttings level from a mixed light source of cool white fluorescent tubes and tungsten bulb.

The original weight (5.5 lb.) of the pans was maintained twice a day after being placed under the light.

**Bioassay**

The seedlings were ready for use 9-10 days after planting. Cuttings were made by excising the root system 4 cm. of hypocotyl plus epicotyl including a pair of primary leaves. Cotyledons were removed if they were still intact. Uniform seedlings were used per test. Five cuttings per chromatogram section were employed. They were placed in a 6.8 x 2.0 cm. vial which contained a chromatogram section plus 4 ml. of
$5 \times 10^{-6}$ M IAA solution. Twenty-two vials were needed per chromatograph. Each vial contained a section of paper chromatograph and the control contained a piece of blank paper. Also, the vials were marked at a height of $3.5$ cm. so that the cotyledonary node was maintained above the solution level during the course of the experiment.

After 24 hours from incubation, the cuttings had absorbed about $2/3$ of the solution. Glass distilled water (GDW) was added to a point where the cotyledonary nodes remained above the solution level. Later on GDW was added twice a day to maintain the original level of the solution in the vial. After 6-7 days, the experiment was terminated, and the number of roots per chromatogram section was compared with the average number of roots for the control.
RESULTS

A. Experiment I

1. Effects of tape etiolation

Tables 1 and 2 and Figure 1 show that the Ohio 2 rooting percentages and number of roots per cutting were significantly increased over the control. However, Myrobalan 29c was very slightly increased by the treatment.

Etiolation resulted in no significant difference in number of rooted cuttings between dates (Fig. 2 and Table 1). However, the number of roots per cutting was significantly greater at $D_2$ than at $D_1$ (Fig. 2 and Table 2).

2. Effects of tape etiolation plus ringing

The Ohio 2 rooting percentage was significantly increased by ER over that of etiolation (Fig. 1 and Table 1). However, the number of roots was increased but not significantly (Fig. 1 and Table 2). The treatment (ER) increased somewhat the rooting percentages of Myrobalan 29c but not the number of roots (Fig. 1 and Tables 1 and 2).

Etiolation plus ringing did not result in significant differences in rooting percentages between dates.
Fig. 1 The effects of black tape etiolation "E", tape etiolation plus ringing "ER" (and control "C") interactions with rootstock on rooting percentages and number of roots per cutting of plum rootstocks (Exp. 1). R<sub>2</sub> and R<sub>29</sub> refer to rootstocks Ohio 2 and Myrobalan 29c, respectively.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 2 The effects of black tape etiolation "E", tape etiolation plus ringing "ER" (and control "C") interactions with time of measurement on rooting percentage and number of roots per cutting of plum rootstocks (Exp. 1). D₁ and D₂ refer to date one and date two of measurements.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 3 The rooting percentages and number of roots per cutting of Myrobalan plum rootstocks (Exp. 1 tape). The symbols are as in Figure 1.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 4 The effects of black tape etiolation "E", tape etiolation plus ringing "ER" (and control "C") interactions with rootstock on rooting percentages and number of roots per cutting of plum rootstocks (Exp. 2). The symbols are as in Figure 1.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 5 The effects of black tape etiolation "E", tape etiolation plus ringing "ER" (and control "C") interactions with time of measurements on rooting percentages and number of roots per cutting of plum rootstocks (Exp. 2). The symbols are as in Figure 2.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 6 The rooting percentages and number of roots per cutting of Myrobalan plum rootstocks (Exp. 2 tape). The symbols are as in Figure 1.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
(Fig. 2 and Table 1). However, the number of roots was significantly greater at D₂ than D₁ (Fig. 2 and Table 2).

3. Effects of paint etiolation

The rooting percentage and number of roots of Ohio 2 cuttings were significantly increased by etiolation compared to control (Fig. 4 and Tables 5 and 6). Myrobalan 29c however was not significantly improved by the treatment. Paint etiolation at D₂ was significantly greater for both rooting percentage and number of roots than at D₁ (Fig. 5 and Tables 5 and 6).

4. Effects of paint etiolation plus ringing

The treatment resulted in no significant differences over etiolation alone for both rootstocks in regard to rooting percentages and number of roots (Fig. 4 and Tables 5 and 6).

No significant difference occurred in rooting percentages between dates (Fig. 5 and Table 5). However, the number of roots was significantly greater at D₂ than at D₁ (Fig. 5 and Table 6).

5. Rootstock differences

The rooting percentages and number of roots per cutting were significantly higher for Myrobalan 29c compared to Ohio 2 rootstock (Figs. 3 and 6).
E. Experiment II

1. Effects of tape etiolation

Tables 3 and 4 and Figure 7 show that for both rootstocks rooting percentages and number of roots per cutting were not significantly increased over the control. However, higher mean values occurred with etiolation than the control.

Etiolation increased significantly both rooting percentages and number of roots at D2 than D1 (Fig. 8 and Tables 3 and 4).

2. Effects of tape etiolation plus ringing

The treatment resulted in no significant differences over etiolation for both rootstocks (Fig. 7 and Tables 3 and 4). In most cases the mean values of ER treatment were less than that of etiolation alone.

Etiolation plus ringing resulted in significantly higher rooting percentages and number of roots at D2 than D1 (Fig. 8 and Tables 3 and 4).

3. Effects of paint etiolation

Paint etiolation did not result in significant increase in rooting percentages of either rootstock over the control (Fig. 10 and Table 7). However, the number of roots per cutting was significantly greater for Myrobalan 29c only at D2 than the other interactions (Fig. 10 and Table 8).
Fig. 7  The effects of black paint etiolation "Ep", paint etiolation plus ringing "ER" (and Control "C") interactions with rootstock on rooting percentages and number of roots per cutting of plum rootstocks (Exp. 1). The symbols are as in Figure 1.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 8 The effects of black paint etiolation "E_p", paint etiolation plus ringing "E_pR" (and control "C") interactions with time of measurement on rooting percentage and number of roots per cutting of plum rootstocks (Exp. 2). The symbols are as in Figure 2.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 9 The rooting percentages and number of roots per cutting of Myrobalan plum rootstocks (Exp. 1 paint). The symbols are as in Figure 1.
Fig. 10 The effect of black paint etiolation "Ep", paint etiolation plus ringing "EpR" (and control "C") interactions with rootstock on rooting percentages and number of roots per cutting (Exp. 2). The symbols are as in Figure 1.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 11 The effect of black paint etiolation "Ep", paint etiolation plus ringing "EpR" (and control "C") interactions with time of measurement on rooting percentage and number of roots per cutting of plum rootstocks (Exp. 2). The symbols are as in Figure 2.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
Fig. 12 The rooting percentages and number of roots per cutting of Myrobalan plum rootstocks (Exp. 2 paint). The symbols are as in Figure 3.

1- Bars within each graph not having a common letter are significantly different at 5 percent level.
4. Effects of paint etiolation plus ringing

EpR did not improve the rooting percentages and number of roots per cutting of both rootstocks over etiolation alone (Fig. 10 and Tables 7 and 8).

The treatment resulted in a significantly higher rooting percentage and number of roots at D₂ than at D₁ (Fig. 11 and Tables 7 and 8).

5. Rootstock differences

In both instances the rooting percentages and number of roots per cutting of Myrobalan 29c were significantly higher than Ohio 2 (Figs. 9 and 12).

Results of Auxin Study

1. Synthetic IAA standard curve

The growth of wheat coleoptile sections in 1 ml. of synthetic 3-indoleacetic acid solutions of various concentrations are shown in Figure 13. The growth shown on the vertical scale is a percent increase in the length of coleoptile sections of the control. It is evident that the maximum elongation occurred at 1 p.p.m. concentration.

2. Auxin activity in plant extracts

a. Myrobalan 29c rootstock

Figure 14 shows the auxin activity in the methanol extracts of the first experiment. Extracts from the control tissues had three promotive areas (Fig. 14, C). They occurred at Rf. values 0.05-0.1, 0.15-0.25 and
Fig. 13 Wheat coleoptile responses to synthetic IAA solutions
Fig. 14 Histograms indicating the auxin activity in methanol extracts of Myrobalan 29c plum tissues of the first experiment. The black and white bars represent elongation above and below the control (mm). Abscissa: Rf values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are: C = Control, E = etiolation and ER = etiolation plus ringing.
Fig. 15 Histograms indicating the auxin activity in methanol extracts of Myrobalan 29c plum tissues of the second experiment. The black and white bars represent elongation above and below the control (in mm.) respectively. Abscissa : Rf values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are as in Figure 14.
0.4-0.45. It is apparent that the largest promotive activity occurred at Rf. 0.4-0.45 which corresponded to that of the synthetic IAA (Rf. 0.4-0.5) which was spotted, developed and assayed similarly to that of the plant extracts. The inhibitory activity occurred at Rf. values 0.5-1.0 with the highest inhibitory peak occurring at Rf. 0.65-0.7.

The control tissue extracts activity of the second experiment is shown in Figure 15(C). Three peaks of growth promotion occurred at Rf. values 0.05-0.15, 0.2-0.35 and 0.75-0.85. The inhibitory activities appeared at Rf. values 0.35-0.75 and 0.85-1.0 with the largest inhibitory peak at 0.6-0.7.

The auxin activity in the extracts of etiolated tissues of the first experiment is shown in Figure 14(E). In general, a high increase in the promotive activity and a decrease in the inhibitory one resulted from etiolation. There was a considerable increase at Rf. 0.4-0.5 which corresponded to that of the synthetic IAA. Other peaks are also evident at Rf. values 0.0-0.05 which was an inhibitory one in the control extracts, 0.05-0.15 which did not change noticeably, 0.15 - 0.2 and 0.25-0.35. The inhibitory activities were markedly reduced by etiolation (Fig. 14,E) as opposed to the control.

The growth promotive activity of etiolated tissue
extracts of the second experiment is shown in Figure 15(E). It is clear that a significant increase in auxin activity resulted from etiolation as compared to control. The most prominent increase occurred at \( \text{Rf.} \ 0.05-0.15, 0.15-0.25, \) and \( 0.35-0.45 \). The inhibitory activities were noticeably reduced by etiolation at \( \text{Rf.} \) values \( 0.45-0.55 \) and were increased at \( 0.55-0.6 \).

The effect of etiolation plus ringing on auxin activity in the extracts of the first experiment is shown in Figure 14(ER). It is evident that a sharp decrease in growth of wheat coleoptile sections resulted compared to that of etiolation and to a lesser degree in comparison with the control. The number of the inhibitory zones was increased by ER treatment. For example, \( \text{Rf.} \) values \( 0.0-0.05, 0.2-0.25, 0.5-0.55 \) had been changed to inhibitory areas while they were promotive with etiolation. However, the \( \text{Rf.} \) \( 0.65-0.7 \) which exhibited the peak of the inhibitory activity with E and C was greatly reduced by ringing.

The auxin activity in the extracts of ER treated tissues of the second experiment is shown in Fig. 15(ER). The growth promoting activity was reduced compared to etiolation (Fig. 15,E). The highest promoting activities occurred at \( \text{Rf.} \) values \( 0.05-0.15 \) and \( 0.35-0.45 \). The inhibitory activities were increased at \( \text{Rf.} \) \( 0.8-1.0 \) and decreased at \( 0.55-0.8 \).
b. The Ohio 2 Rootstock

Figure 16 shows the auxin activity in the methanol extracts of the tissues of the first experiment. Extracts from the control tissues had three growth promoting areas at Rf. values of 0.05-0.15, 0.15-0.3 and 0.35-0.5 (Fig. 16, C). The largest promotive peak occurred at Rf. 0.35-0.45 which corresponded to that of the synthetic IAA. On the other hand, the inhibitory activities started from Rf. 0.5-1.0 with a highest peak at 0.65-0.7.

The control extracts activity of the second experiment is shown in Figure 17(C). Two large growth promoting zones occurred at Rf. values 0.1-0.2 and 0.35-0.4. The inhibitory activities started from Rf. 0.45-1.0.

The auxin activity in the extracts of etiolated tissues of the first experiment showed a marked increase as opposed to the control (Fig. 16, E). The highest promotive activities occurred at Rf. values 0.0-0.2 and 0.3-0.45. In addition, three new promotive zones emerged at Rfs. 0.0-0.05, 0.5-0.55 and 0.75-0.8. The inhibitory activities were lower than the control and the highest peak occurred at 0.6-0.7.

The growth promotive activity of etiolated tissue extracts of the second experiment is shown in Figure 17(E). It is apparent that a significant increase in
Growth responses of coleoptile sections, mm

Fig. 16 Histograms indicating the auxin activity in methanol extracts of Ohio 2 plum tissues of the first experiment. The black and white bars represent elongation above and below the control (in mm.) respectively. Abscissa: $R_f$ values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are as in Figure 14.
Fig. 17  Histograms indicating the auxin activity in methanol extracts of Ohio 2 plum tissues of the second experiment. The black and white bars represent elongation above and below the control (in mm.) respectively. Abscissa : Rf values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are as in Figure 14.
auxin activity resulted from etiolation. The highest increase occurred at Rf. 0.05-0.2, 0.35-0.45 which corresponded to that of the synthetic IAA and 0.75-0.85. The inhibitory activities were higher at Rf. 0.85-1.0 and lower at 0.55-0.75 as opposed to the control (Fig. 17,C).

The auxin activity in the extracts of ER treated tissues of the first experiment is shown in Fig. 16(ER). It is evident that the growth of coleoptile sections was not reduced noticeably in comparison with etiolation, at the same time, it was much greater than that of the control. The highest growth promotive peaks occurred at Rf. 0.05-0.25 and 0.3-0.5. The inhibitory activities were increased at Rf. 0.6-0.7 which represented the peak of inhibition.

Figure 17(ER) shows the growth promoting activity of tissue extracts of the second experiment. In general, a slight reduction occurred in these activities as compared to etiolation but it was higher than that of the control. The inhibitory activities were reduced remarkably by the treatment as compared to both etiolation and control.

3 Endogenous IAA estimation in plant tissues

The results of the first experiment indicate that Myrobalan 29c content of IAA equivalent was slightly higher than that of Ohio 2 clone (Fig. 18 and Table 9). However, the
Fig. 18  The effects of etiolation "E", etiolation plus ringing "ER" (and control "C") interactions with rootstock on the endogenous IAA levels (Exp. 1).

Bars not having a common letter are significantly different at 5 percent level.
The effects of etiolation "E", etiolation plus ringing "ER" (and control "C") interactions with rootstock on the endogenous IAA levels (Exp. 2).

Bars not having a common letter are significantly different at 5 percent level.
reverse was the case in the second experiment (Fig. 19 and Table 10).

Etiolation resulted in a significant increase in the endogenous IAA levels of both rootstocks as opposed to the control in the first experiment (Fig. 18). On the other hand, the increase was only significant with Ohio 2 in the second experiment (Fig. 19).

Etiolation plus ringing reduced the endogenous IAA levels as compared to etiolation. The reduction was significant only with Myrobalan 29c, and considerably high with Ohio 2 in the first experiment (Fig. 18). In the second experiment, the reduction was significant only with Ohio 2 and very slightly with Myrobalan 29c (Fig. 19).

Results of Rooting Cofactors Study

1. Myrobalan 29c clone

Figure 20 shows the root promoting substances activity in the methanol extracts of Myrobalan 29c rootstock tissues of the first experiment. Extracts from the control tissues contained five or possibly six promoting zones at Rf. values -0.05 - 0.05, 0.2-0.3, 0.55-0.65, 0.7-0.75, 0.8-0.9 and 0.95-1.0 (Fig. 20,C).

Extracts of the control of Myrobalan 29c of the second experiment are shown in Figure 21(C). It can be seen clearly that more root promoting substances activity occurred here than that of the first experiment (Fig. 20,C). The peaks of promotion occurred at Rf. -0.05-0.05, 0.05-0.2, 0.2-0.35,
Fig. 20 Histograms showing root-promoting and inhibiting activities in methanol extracts of Myrobalan 29c plum tissues on mung bean cuttings of the first experiment. The black and white bars represent promotion and inhibition of root formation as opposed to the controls respectively. Rf. values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are as in Figure 14.
Fig. 21  Histograms showing root-promoting and inhibiting activities in methanol extracts of Myrobalan 29c plum tissues on mung bean cuttings of the second experiment. The black and white bars represent promotion and inhibition of root formation as opposed to the controls respectively. Rf values in isopropanol: ammonia: water (8:1:1 v/v). The symbols are as in Figure 14.
The root promoting substances in the extracts of etiolated tissues of Myrobalan 29c of the first experiment are shown also in Figure 20(E). It is apparent that the activity of rooting substances have slightly increased in comparison with the control. The zones of rooting promotion corresponded very closely to that of the control. These areas occurred at Rf. values -0.05-0.05, 0.1-0.2, 0.25-0.35, 0.5-0.55, 0.6-0.65, 0.7-0.8 and 0.95-1.0. An inhibitory zone appeared at Rf. 0.45-0.5 and a reduction in Rf. 0.8-0.95 activity happened.

Etiolation caused a remarkable increase in root promoting activity of the extracts of Myrobalan 29c of the second experiment (Fig. 21, E), except for the Rf values 0.15-0.2 and a slight reduction at Rf. 0.85-1.0.

In the first experiment, ER decreased the root promoting activity of the extracts of Myrobalan 29c (Fig 20, ER) in comparison with etiolation (Fig. 20, E). As a matter of fact, these activities were reduced in all Rf. values. But still some weak peaks existed at the following Rfs.: -0.05-0.0, 0.05-0.15, 0.2-0.35, 0.6-0.7 and 0.85-0.9.

Etiolation plus ringing results are shown in Figure 21 (ER) for rootstock Myrobalan 29c of the second experiment. In general, there was a reduction in the activity of root promoting substances in all Rfs. as compared to etiolation (Fig. 21, E). Although the reduction occurred but some peaks of promotion existed. These peaks were at Rf values -0.05-
0.05, 0.1-0.2, 0.3-0.35, 0.55-0.65, and 0.7-0.75 and 0.95-1.0.

2. Ohio 2 Rootstock

The activity of root promoting substances in methanol extracts of Ohio 2 clones tissues of the first experiment are shown in Figure 22. The control had five promoting zones at Rf. values of -0.05-0.05, 0.25-0.4, 0.45-0.6, 0.65-0.7 and 0.95-1.0 (Fig. 22,C).

The activity of rooting substances in the extracts of the second experiment tissues of Ohio 2 are shown in Figure 23. The control contained higher peaks of promotion in many Rfs than that of the first experiment (Fig. 22,C). These peaks occurred at Rf. values -0.05-0.05, 0.1-0.2, 0.25-0.3, 0.45-0.5, 0.6-0.7 and 0.7-0.8. There was a rooting inhibition at Rf. 0.8-1.0 of the second experiment extracts.

Extracts of the etiolated tissues of the first experiment, in general showed a higher root promoting activity (Fig. 22,E). It is evident that five promoting zones appeared in the histogram at Rf. values -0.05-0.05, 0.2-0.35, 0.4-0.6, 0.65-0.75 and 0.8-0.95. The Rf's which were changed by etiolation are a slight reduction at -0.05-0.05, 0.95-1.0, and an increase at 0.45-0.6, 0.65-0.75 and 0.75-0.95.

In the second experiment, the etiolated tissues extracts of Ohio 2 clone showed an increase in root promoting substances activity in some Rf. values and a decrease in others as compared with the control (Fig. 23,E). The increase
Fig. 22 Histograms showing root-promoting and inhibiting activities in methanol extracts of Ohio 2 plum tissues on mung bean cuttings of the first experiment. The black and white bars represent promotion and inhibition of root formation as opposed to the controls respectively. \( R_f \) values in isopropanol: ammonia: water (8:1:1 v/v). The symbols are as in Figure 14.
Fig. 23  Histograms showing root-promoting and inhibiting activities in methanol extracts of Ohio 2 plum tissues on mung bean cuttings of the second experiment. The black and white bars represent promotion and inhibition of root formation as opposed to the controls respectively. Rf values in isopropanol:ammonia:water (8:1:1 v/v). The symbols are as in Figure 14.
occurred at Rf values -0.05-0.0, 0.3-0.45, 0.45-0.65 and 0.8-1.0.

Etiolation plus ringing resulted in a higher root promoting activity than the control and somehow similar to that of etiolated tissues of Ohio 2 rootstock of the first experiment (Fig. 22, ER). Seven peaks occurred at Rf values -0.05-0.05, 0.1-0.2, 0.25-0.5, 0.55-0.65, 0.7-0.75, 0.8-0.85 and 0.9-1.0.

The Rf's which were changed by ER in comparison with etiolation are an increase at Rf. -0.05-0.05, 0.1-0.2, 0.55-0.65, and 0.7-0.75 (Fig. 22, ER).

In the second experiment, Ohio 2 extracts showed a similar trend to that of the first experiment in regard to the effect of ER on root promotive activity. But in general, they were slightly higher in many instances than that of etiolation (Fig. 23, E), for example, Rf's 0.05-0.1, 0.25-0.3 and 0.65-0.7.
DISCUSSION

It should be made clear that the conditions under which the second experiment was conducted were different in many aspects from that of the first experiment. For example, the trees were pruned severely on June 10, and after one month the treatments were applied to the new shoots. However, in the first experiment the trees were pruned moderately in the winter and the treatments were applied to shoots that developed in the spring. The time of the treatment applications and the rooting time were later in the season compared to the first experiment. Finally, the greenhouse conditions were also different in regard to light intensity and the mist system under which the cuttings were set.

1. Rootstock differences

The higher rooting percentages that occurred with Myrobalan 29c probably can not be related to the endogenous IAA content. This is because there is no significant differences in IAA content between Myrobalan 29c and Ohio 2 controls (Figs. 18, 19).

The root promoting substances activity in Myrobalan 29c controls tissue extracts had more root promoting zones than Ohio 2 clone. When these zones were compared with the rooting cofactors described by Hess (31), it could be seen that
all cofactors were present in Myrobalan 29c. However, the Ohio 2 controls extracts showed that cofactor 3 (Rf 0.6-0.73) and cofactor 4 (Rf 0.8-0.93) were present in a lower concentration in the first experiment compared to Myrobalan 29c (Figs. 20 and 22,C). In the second experiment cofactor 4 was absent from Ohio 2 extracts (Fig. 23,C). Hess (30,31) suggested that the presence of all cofactors in adequate amounts in cuttings is necessary for good rooting when other influences are not limiting. Consequently, it seems likely that the rooting of Myrobalan plum cuttings might be related in part to their content of rooting cofactors. This conclusion is further supported by the fact that the significantly improved rooting of Ohio 2 cuttings in the first experiment was associated with the increased concentration of cofactors (Fig. 20,E). However, in the second experiment, the rooting of Ohio 2 cuttings was not significantly increased when the rooting cofactor 4 concentration was increased (Fig. 21,E). Cofactor 3 (Rf 0.6-0.73) content was much higher in experiment 2 than in experiment 1 although there was no improved rooting of Ohio 2 cuttings in experiment 2 (Figs. 22, 23 and Tables 1, 3).

The reason for the absence of cofactor 4 in the tissue extracts of Ohio 2 in the second experiment is probably due to the effect of the season in which the cuttings were taken. Fadl and Hartmann (16) found that changes in root promoting substances occurred in tissue extracts of both Old Home and
Bartlett pear cuttings collected at different months of the growing season.

The difference in rooting ability of cuttings of these clones can not be explained on the basis of their growth inhibitors content. This is because their extracts showed in general similar inhibitory activities in regard to their magnitudes and the Rf values. This was consistent in both experiments (Figs. 14, 17).

The rooting inhibitors did not exist in either clone tissue extracts of the controls in the first experiment (Figs. 20, 22,C). However, in the second experiment a slight rooting inhibition occurred with Ohio 2 only at Rf. 0.8-1.0 (Fig. 23,C). Rooting inhibitors have been reported by several workers (51, 55, 62) to be present in tissue extracts or in cuttings of many plant species. Since the presence or absence of rooting inhibitors from tissue extracts of Ohio 2 clone did result in similar rooting percentages of cuttings in both experiments (Figs. 1, 7,C). Therefore, it seems likely that the rooting inhibitors probably are not involved in determining the difference in rooting ability of cuttings of these clones.

2. The effect of etiolation on rooting of cuttings

The beneficial effect of etiolation on rooting of cuttings of Myrobalan plum rootstocks is apparent from this study (Tables 1, 3). In the first experiment, the effect of
etiolation was more pronounced with the difficult to root Ohio 2 clone than with the easy to root Myrobalan 29c. These results are in close agreement with those of Herman and Hess (26) who found that etiolation enhanced rooting of cuttings of several hibiscus cultivars and red kidney bean cuttings when taken from shoots etiolated for three weeks prior to taking of cuttings. In addition, the effect of etiolation was more pronounced with Wilson White, a relatively difficult to root cultivar than with Cornell Red, an easy to root clone. The results are also in agreement with those of Gardner (20) with apples, Frolich (17) with avocado, Knight and Witt (41) with plums, Reid (50) and Blackie et al. (8) with camphor plants and Smith (54) with clematis in regard to the role of etiolation in enhancing the rooting of cuttings although different methods of etiolating shoots were employed in these various studies.

In the second experiment, no significant interactions occurred between either treatment and rootstock or treatment and date (Table 3). The overall effect of etiolation on rooting percentages was significantly greater than the control (Table 3). These results also indicate the beneficial effect of etiolation on rooting of plum cuttings.

The endogenous IAA determinations in this study show that etiolation increased significantly the endogenous IAA level in cuttings of both rootstocks compared to control (Figs. 18, 19). The only nonsignificant increase in IAA
levels due to etiolation occurred with Myrobalan 29c in the second experiment (Fig. 19). There has been a report which indicated that etiolation increased the IAA content in plants. Thus, these results are in agreement with those of Herman and Hess (25).

The adventitious root formation in cuttings is dependent upon the initiation of cell division which is the first step in root production. Auxin (IAA) has been shown to initiate cell division and enlargement processes (12, 59, 67, 69). However, the increased levels of endogenous IAA due to etiolation did not necessarily correlate with the improved rooting of cuttings (Figs. 1, 4 and Tables 9, 10).

The high IAA levels that occurred in etiolated tissues as compared with the nonetiolated may be due to the absence of light. Light has been reported by Galston and Baker (18) to increase the activity of IAA-oxidase enzyme system of etiolated pea seedling which destroy IAA. The same authors (19) found that the red light grown pea epicotyl initiated fewer roots in the absence of IAA than the dark grown cuttings, but when IAA was present they initiated as many roots as the dark control. Kawase (40) also found that IAA decreased from mung bean hypocotyls much faster in the light than under etiolation.

The growth inhibitory activities were reduced by etiolation in both rootstocks compared to the controls (Figs. 14, 17) in the first experiment. However, in the second experi-
ment, the reduction occurred with Ohio 2 only (Figs. 15, 17). Whether this reduction in the inhibitory activities has influenced favorably the rooting of cuttings or not is unknown. No specific determination of the role of growth inhibitors in rooting of cuttings was conducted. Also, no rooting inhibition for mung bean cuttings was observed with Ohio 2 extracts of the etiolated tissues in both experiments (Figs. 22, 23,E), however, it did occur with Myrobalan 29c of the first experiment only at Rf. 0.45-0.5 which was a growth promoting zone (Fig. 20,E). The results of growth inhibitors in this study are not in full agreement with those of Challenger et al (11) who stated that a growth inhibitor was present in extracts of Myrobalan B plum and apple rootstocks which was a strong root promoting cofactor in mung bean cuttings. The strongest growth inhibitory zone which occurred consistently in both experiments at Rf. value 0.6-0.7 in the extracts of both rootstocks did not show any strong root promoting activity on mung bean cuttings. The only case in which this root promotion occurred was with Myrobalan 29c of the second experiment (Fig. 21,E). This could be due to the fact that Challenger et al used dormant cuttings, different extracting solvent and a longer extracting time.

a. Ohio 2 clone

The significantly improved rooting of Ohio 2 cut-
tings by etiolation over the control in the first experiment (Table 1) could be due to the increased root promoting substances activities at Rf. 0.45-0.6, 0.65-0.75 and 0.75-0.95. The last two Rfs. correspond closely to cofactor 3 (Rf. 0.6-0.73) and cofactor 4 (Rf. 0.8-0.93) which were described by Hess (30). He stated that their presence in adequate amounts with other factors is necessary for good rooting of cuttings. Also, the significantly higher endogenous IAA that occurred with etiolated tissue extracts might have played a role in the improved rooting of cuttings, or it could be due to both IAA and root promoting substances.

In the second experiment, the rooting of Ohio 2 cuttings was not significantly increased by etiolation over the control (Table 3). The endogenous IAA levels were increased significantly by etiolation over the control (Fig. 19). Also, the rooting cofactor 4 (Rf. 0.8-0.93) which was absent in the control tissue extracts (Fig. 23,C) was increased considerably by etiolation. Cofactor 2 (Rf. 0.33-0.56) was increased also by etiolation. Cofactor 3 (Rf. 0.6-0.73) content was much higher in experiment 2 than in experiment 1 although there was no improved rooting of Ohio 2 cuttings in experiment 2 (Figs. 22, 23 and Tables 1, 3). In other words, all the rooting cofactors were present in tissue extracts of Ohio 2 in the second experiment.
However, the rooting of cuttings was insignificantly improved by etiolation. Therefore, it is concluded that the rooting of Ohio 2 cuttings probably is not solely governed by the endogenous IAA levels and root promoting substances content.

b. Myrobalan 29c clone

The effect of etiolation on rooting percentages of Myrobalan 29c cuttings was insignificant in both experiments compared to controls (Tables 1,3). Etiolation generally increased the endogenous IAA levels in etiolated tissue extracts in both experiments (Figs. 18,19). The root promoting substances activity was reduced by etiolation compared to control in the first experiment (Fig. 20,E). The reduction was more noticeable at Rf's 0.55-0.6 which became a root inhibiting zone and Rf. 0.8-0.93 which correspond to rooting cofactor 4 (Rf. 0.8-0.93). In the second experiment, the root promoting substances were increased remarkably by etiolation (Fig. 21,E) and no rooting inhibition occurred. However, the endogenous IAA levels were about one-fifth of that of the first experiment (Tables 9,10). Therefore, it seems likely that the rooting of Myrobalan 29c cuttings might not be related to its endogenous IAA and rooting cofactors contents.

A question might be asked as to why etiolation inconsistently increased the root promoting substances
activity in Myrobalan 29c in both experiment? Probably, the reason is that the type of shoots that were used in the second experiment were juvenile shoots (vegetative stage). Hess (29) stated that tissue extracts of the juvenile shoots of Hedra helix contain higher levels of rooting cofactors than those from the adult form. This phenomena was well illustrated by the control tissue extracts of Myrobalan 29c of the second experiment (Figs. 20, 21).

c. Time of measurement

The results of time of measurement of the first experiment indicated the possibility of shortening the rooting time by 50% in comparison with the control (Table 1). This is because no significant differences occurred in rooting percentages of etiolation treated cuttings at either date (Fig. 2). However, the number of roots per cutting was significantly greater at $D_2$ than $D_1$ (Table 2). Therefore, the determining factor in reducing the rooting time will be the percentage of the survival of the rooted cuttings after transplanting.

The enhancement of rooting of cuttings in regard to time of rooting agree with the results of Herman and Hess (25) who found that the rooting of Wilson White hibiscus cuttings taken from etiolated shoots was increased about twice over the nonetiolated cuttings in 26 days rooting. Also the results agree with those of
Smith (54) who stated that a more rapid rooting occurred in the internodal region of clematis cuttings taken from etiolated shoots compared to nonetiolated.

In the second experiment the rooting percentages and number of roots per cutting were significantly greater at D₂ than D₁ (Table 3 and Fig. 5). A possible explanation for these results is that the light intensity conditions under which the second experiment was conducted. The light intensities at the tip of the cuttings were about ¼ that of the outdoors conditions as measured with a photocell. Cuttings of the first experiment were exposed to full sunlight in the greenhouse. This reduction in light intensity seems to have influenced unfavorably the time required for rooting of cuttings. This is because the higher these intensities are (within certain limits) the higher the photosynthetic rates when other factors are not changed. Consequently, more food and other necessary substances for root formation are formed (26). Went and Thiman (69) reported that light is required by softwood cuttings for auxin formation and therefore rooting.

It is evident from this discussion that the effect of etiolation on the rooting time required by cuttings is probably dependant on the light conditions under which the cuttings are set.
3. The effects of etiolation plus ringing on rooting

Etiolation plus ringing in the first experiment improved significantly the rooting of Ohio 2 cuttings over etiolation (Table 1 and Fig. 1). Thus, the results are in agreement with those of Gardner (20) with apples who found that the effect of etiolation on rooting of cuttings was further enhanced by wiring the base of the shoots at the end of the etiolated area with a piece of a copper wire. Also the result agree with those of Johnson and Frolich (17) with avocado who found that rooting of intact etiolated shoots was enhanced by wiring the base of the shoot to induce roots in situ. In addition, the results are in agreement with those of Stoltz and Hess (59) with red hibiscus, a relatively difficult to root cultivar, Higdon and Westwood (33) with pears, Hunter (35) with Rangpur lime and Bobilioff (9) with *Hypea brasiliensis*.

The rooting of Myrobalan 29c cuttings in the first experiment was only slightly improved by ER treatment over etiolation treatment (Table 1 and Fig. 1). These results agree with those of Luckan (45) who failed to obtain a successful rooting of Walnut cuttings after being girdled.

In the second experiment, the effect of etiolation plus ringing on rooting of both rootstocks cuttings was insignificant (Table 3 and Fig. 7). These results are in agreement with those of Vierheller (64) who found that girdling of apple shoots during the summer months resulted in no superior
rooting of cuttings over the control.

In discussing the effect of ringing on both rootstocks in regard to their IAA and root promoting and inhibiting substances activity, it should be made clear that ringing treatment in this study was accompanied with etiolation. In other words, no ringing treatment alone was made.

a. Ohio 2 clone

The significantly improved rooting of Ohio 2 cuttings in the first experiment by ER treatment can not be related to the endogenous IAA levels or to root promoting substances activities that occurred in tissue extracts of these cuttings. This is because the endogenous IAA levels were reduced by ringing (Fig. 18), and there was not any noticeable change in root promoting substances activity (Fig. 22, E, ER) compared to etiolation.

The results of IAA determination in ER treated tissue agree with those of Kato and Ito (38) who found that girdling of apple shoots reduces the endogenous IAA content in the apical buds of the girdled shoots. Also the results agree with those of Stoltz and Hess (60) who reported that ringing resulted in reduced IAA levels in many hibiscus cultivars.

In discussing the results of root promoting substances activity, it is necessary to point out that the author has found no other published data in regard
to the effect of ER on the rooting substances activity in the tissue extracts. Ringing alone has been shown to increase these activities in the extracts of the tissue above the girdle. Stoltz and Hess (60) have shown that the changes in cofactor levels above the girdle vary with the time after girdling and with the plant species. For example, cofactor 1 in red hibiscus increased through 10 days after girdling and decreased thereafter. Similar trends occurred with the difficult to root hibiscus, with the highest concentration occurring at 5 days from girdling and decreased at 20, 30 days after girdling. Cofactor 3 was absent and cofactor 4 in the easy to root hibiscus tissue increased with time of ringing. Furthermore, they suggested that this cofactor may be a significant one in determining the differences in rooting ability of the two hibiscus cultivars since it did not accumulate above the girdle in the difficult to root cultivar. On the other hand, Cooper (13, 14) demonstrated that ringing (removal of a complete ring of bark tissue) of lemon cuttings at 1.5 cm. from the base of the cuttings inhibited rooting. Van Overbeck and Gregory (63) also demonstrated that ringing stops the downward movement of root promoting substances in hibiscus. These two studies showed that ringing of shoots or cuttings interferes with the downward movement of these substances and accumulated above
the girdle. However, this effect was not demonstrated in this study. This could be due to the fact that girdling (removal of a complete ring of bark tissues) was not made in this study, but a small piece of a copper wire was used for ringing the base of the shoots, thus not resulting in a complete separation of the bark tissues as in those of Cooper and Van Overbeck.

The effects of ER on rooting of Ohio 2 cuttings of the second experiment did not cause any beneficial outcome over etiolation (Fig. 7). This is probably due to the low IAA levels that occurred in tissue extracts of ER treated shoots (Fig. 19). This is because the root promoting substances activities contained all the four rooting cofactors which were discussed earlier in their relationship to rooting of cuttings. Also, no rooting inhibitors were found in tissue extracts when they were tested on mung bean cuttings (Fig. 23, ER). In addition, the growth inhibitors were reduced remarkably by the treatment (Fig. 21, E, ER) in comparison with etiolation.

A possible explanation of the role of ringing in rooting enhancement of etiolated cuttings might be due to carbohydrate accumulation in the tissue above the girdle. There are numerous reports which indicate this fact. For example, Stoltz and Hess (59) with red hibiscus, Higdon and Westwood (33) with pears,
Weaver (65) with grapes and others (1, 2, 58, 61). Therefore, it seems very unlikely not to have the carbohydrates accumulated above the girdle.

Sugars and starch are necessary as a source of energy and as a carbon source for the synthesis of other compounds including root promoting substances and hormones essential for the root initiation and development. In addition, there have been many reports where the rooting of cuttings was correlated with carbohydrate content of the cuttings. Stoutmyer (61) found that grape cuttings of high starch content rooted 63% and cuttings of medium starch content rooted 35% and those of low starch content rooted only 17%. Hartmann and Hansen (22) reported that quince cuttings taken from different parts of shoots were consistently less as the more terminal shoot portions were used, and this was associated with a slight reduction in total sugars. Herman and Hess (25) reported that etiolation reduced the sugar content of the etiolated shoots of red kidney beans. Therefore, it seems likely that ringing accompanied with etiolation could have acted in rooting enhancement of cuttings through the carbohydrate accumulation. Or perhaps through some other mechanisms which are unknown at the present time.
b. Myrobalan 29c clone

The lack of the beneficial effect of ringing on rooting of Myrobalan 29c cuttings in both experiments (Figs. 1, 5 and Tables 1, 3) can not be related to the decreased IAA levels and/or to the reduced root promoting substances activities and the presence of rooting inhibitors. This is because the IAA levels (Figs. 18, 19) and root promoting substances activities were higher with etiolation than ER treatment (Figs. 20, 21, ER) but no significant differences occurred in rooting of Myrobalan 29c due to E and ER treatments. The presence of rooting inhibitors in tissue extracts of ER treated tissues of Myrobalan 29c also seemed to have no effect on rooting of this clone cuttings. This is because their presence or absence did not result in any significant changes in rooting percentages (Tables 1, 3 and Figs. 1, 4).

c. Time of measurement

The results of the effect of etiolation plus ringing on rooting percentages and number of roots per cutting were similar to that of etiolation treatment in both experiments (Tables 1, 4). Therefore, the same discussions of the etiolation effect on time of measurement may apply here.
4. The effects of E and ER on number of roots

In the first experiment etiolation increased significantly the number of roots per cutting of the Ohio 2 clone over the control (Table 2 and Fig. 1). Thus these results agree with those of Herman and Hess (25) who found that etiolated red kidney bean cuttings had formed over 5 times as many roots as the nonetiolated after 4 days and about twice as many roots after 8 days. Also, the results are in agreement with those of Knight and Witt (41) with plum root-stocks when they stated that roots were developed at several points on the etiolated portions of the cuttings while the control cuttings produced roots only at the base. Etiolation plus ringing did not increase the number of roots of Ohio 2 significantly in comparison with etiolation (Table 2 and Fig. 1).

In discussing the increase in the number of roots per cutting of etiolated and ER treated cuttings, it is important to point out that root formation on cuttings is a complicated process and many factors, such as auxin, rooting substances, nitrogenous compounds and carbohydrates (14, 36, 67, 69) are involved. Auxin (IAA) has been shown to initiate the cell dividing process which is the first step in root development (12, 67, 68, 69). Also, it has been found by Went (68) that substances other than auxin are required for the growth and development of root primordia into visible roots. Since the auxin levels were increased by etiolation (Fig. 18),
it is possible that this increase in IAA levels have played an important role in increasing the number of roots per cutting. This effect might have been influenced favorably by the presence of more parenchymatic cells in etiolated tissues than in the nonetiolated. It has been reported by several workers (25, 48, 54) that etiolation induces anatomical changes such as the presence of more cells in less differentiated stage thus resulting in a larger number of roots. Also, the increased levels of root promoting substances in etiolated tissue extracts of Ohio 2 cuttings might have influenced favorably the number of roots per cuttings of the first experiment.

The lack of a significant increase in number of roots per cutting of Ohio 2 in the second experiment (Table 4 and Fig. 4) may be explained on the same basis as that of the rooting percentages. This is because a correlation existed between the rooting percentages and number of roots per cutting (Tables 1, 4).

The insignificant increase in number of roots per cutting of Myrobalan 29c in both experiments (Tables 2, 4) over the control for both etiolation and etiolation plus ringing might be explained by the findings of Went (68) who reported that the maximum number of roots per cutting that can be induced is a fixed amount. However, this number of roots being governed by the length of the cutting. Therefore, it seems likely that the number of roots per cutting of Myrobalan
29c had approached its maximum in the control cuttings. Thus an increase in root promoting substances and IAA did not increase this variable significantly.

5. Black paint experiments

The author believes that no comparison should be made between the results of both paint experiments. This is because in the second experiment the treatment killed a considerable number of shoots and retarded the growth of others, thus, resulting in nonuniform cuttings compared to those of the first experiment. It was thought that this was caused by a thicker layer of paint which was applied to the shoots in an attempt to minimize the cracking of the paint layer that occurred in the first experiment.

The black paint application method for etiolating shoots prior to taking of cuttings in the first experiment proved to be a successful means of enhancing rooting of Ohio 2 cuttings (Tables 5, 6 and Fig. 7). In addition, it provided time and material saving in comparison with the tape etiolation.

Since the black paint used for etiolating shoots was chemically inert (AMCHEM - 100% asphalt base), whatever rooting enhancement occurred may be due to the effect of light exclusion similarly to that of the tape etiolation. There are other environmental factors which might have been influenced differentially by both techniques. For example, the relative humidity, temperatures and the air composition (CO₂, O₂ and
probably C₂H₄) of the etiolated portions are thought to be changed more in their magnitudes with tape application than with the paint. This is because of the fact that the black paint is in a direct contact with the environment. In the case of tape etiolation there is a plastic layer separating the environment and the black material on the tape. To what extent these changes might influence the effectiveness of etiolation on rooting of cuttings is unknown to the author.

The lower mean values that occurred with paint etiolation compared to tape etiolation might be due to a less etiolating action that occurred with the paint one. This is because the paint layer dried off and cracked because of the environmental conditions and the growth of shoots in diameter.

Paint etiolation plus ringing did not result in any beneficial effect over etiolation with Ohio 2 as it happened with the tape etiolation (Tables 1, 2, 5, 6). No explanation can be given at the present time to ringing effects on rooting and number of roots per cutting.

The method gave encouraging results with EM VII apple rootstock. Twenty shoots were etiolated with the black paint for four weeks under the field conditions. Eighteen cuttings formed roots as opposed to only four cuttings for the control (Yousif, unpublished data).

There are several possibilities for improving this method of etiolation such as shortening the time of etiolation under field conditions to avoid excessive drying off
and cracking of the paint layer. The method may be more effective with the plants having thicker shoots as in case of apple and pear.
SUMMARY AND CONCLUSIONS

The effects of etiolation and etiolation plus ringing on rooting percentage and number of roots per cutting of two Myrobalan plum rootstocks were investigated. Myrobalan 29c is propagated readily by stem cuttings while the Ohio 2 clone is difficult to root.

On May 15, 1971 shoots 3-4 inches in length were selected. The expanded leaves below the shoot tip were removed and a piece of black plastic tape 0.75 x 1.5-2.0 inches was folded lengthwise around the shoot starting as close as possible to the shoot tip.

Ringing was made with a small piece of a thin copper wire which was wrapped at the base of the taped area.

Similar procedures to those of the tape etiolation were followed in the black paint experiment except that paint was applied instead of tape.

On June 15, cuttings 5-6 inches in length having 4-5 leaves were taken and set under intermittent mist (5 sec./6 min.) in the greenhouse. The basal cut was made at the lower end of the etiolated area and the black tape was removed. The rooting medium consisted of 2:1 (v/v) perlite - peat moss, respectively.

Samples for the laboratory studies consisted of the
etiolated portions of the cuttings. The control samples were collected from similar portions of the shoots to that of the etiolated ones.

Auxin Study

The extraction and bioassay techniques used in this study were based on the methods of Nitsch and Nitsch (47).

A 50 mg sample was extracted three times for 30 min. each with 50 ml. absolute methanol at 0°C. The three extracts were combined, reduced in a flash evaporator at 36°C and spotted on a 2.5 cm. strip of Whatman No. 3 filter paper. Ascending paper chromatography was used. The developer consisted of isopropanol, ammonia and water (8:1:1 v/v), respectively. The chromatographs were left overnight for equilibration. When the solvent front reached 20 cm. from the starting line, they were removed, dried and used or stored at -20°C until they were assayed. Each chromatograph was sectioned into 20 equal pieces starting at the spotting line. The auxin activity of the section was assayed by the wheat coleoptile test. The coleoptile section length used in this study was 4.5 mm. The IAA equivalent was calculated from a synthetic IAA standard curve.

Rooting cofactors study

The mung bean (Phaseolus aureus) rooting test originally developed by Hess (30) was used with modifications.
The extraction and chromatography procedures were essentially the same as those described earlier for the auxin study.

Cuttings were taken from 9-10 day old seedlings and consisted of 4 cm. of hypocotyl below the cotyledonary node plus a pair of primary leaves.

Each chromatograph was sectioned into 20 equal pieces starting at the line of spotting. A one cm. section from below the spotting line was also tested. Five cuttings per chromatogram section were used. The sections were incubated with 4 ml. of $5 \times 10^{-6}$ M IAA solution. After 24 hours of incubation, glass distilled water was added up to the point where the cotyledonary node was maintained above the solution level. Later on, glass distilled water was added twice a day to maintain the original level of the solution in the vial. After 6 to 7 days the average number of roots per chromatogram section was compared with that of the control.

The results of this study indicate that:

1. Etiolation did increase the rooting percentage and number of roots per cutting of Ohio 2 in the first experiment only. However, etiolation did not influence noticeably Myrobalan 29c cuttings.

2. Etiolation plus ringing did not increase rooting percentages of Ohio 2 over etiolation except for experiment 1 with the black tape etiolation. However, the number of roots per cutting was increased by the treatment compared to etio-
lation alone. On the other hand, etiolation plus ringing did not increase either rooting percentages or number of roots of Myrobalan 29c over etiolation.

3. The differences in rooting ability of Myrobalan 29c and Ohio 2 cuttings were not consistently related to their contents of rooting cofactors. The endogenous IAA levels, growth and rooting inhibitor levels did not appear to be important factors in this regard.

4. Etiolation increased the endogenous IAA levels significantly in stems of Ohio 2 clone, but inconsistently in Myrobalan 29c.

5. Etiolation generally increased the root promoting substances in etiolated stem of Myrobalan plums. However, the growth inhibitor levels were decreased in Ohio 2 but not in Myrobalan 29c.

6. Etiolation plus ringing decreased the endogenous IAA levels consistently compared to etiolation in both rootstocks. The root promoting substances were reduced by the treatment in Myrobalan 29c but not in Ohio 2. However, a slight amount of rooting inhibitors were found in Myrobalan 29c due to the treatment.

7. Both etiolation and etiolation plus ringing shortened inconsistently the rooting time.

It was discussed that the modification in rooting of Myrobalan plum cuttings by etiolation and etiolation plus
ringing was not likely caused through the change in levels of IAA, rooting cofactors, growth inhibitors and rooting inhibitors.
LITERATURE CITED


41. _____ and W.A. Witt. 1927. The propagation of fruit tree stocks by stem cuttings. II. Trials with hard and softwood cuttings. J. pomo. 6:47-60.


APPENDIX

Tables showing the rooting results and the endogenous IAA content with the statistical analysis.
Table 1. The effects of black tape etiolation (E), etiolation plus ringing (ER) and time of measurement on the number of rooted cuttings of Myrobalan plums. (Exp. 1)

| Treatment          | Rootstocks |  | Means |          |          |
|--------------------|------------|  |       | T. x Rs. | T. x D   |
|                    | Ohio 2     | Myro 29c | Treat  |          |          |
|                    | Date       | Date     |        | 2         | 29       |
| Control (C)        | 0          | 1.0      | 7.0    | 9.7       |          |
| Etiolation (E)     | 2.3        | 3.0      | 7.7    | 9.7       |          |
| Eti. + Ring. (ER)  | 5.3        | 6.3      | 8.7    | 10.0      |          |
| Rs. Means          | 3.0 a      | 8.8 b    |        |           |          |

1- Each value in the body of the table is an average of three replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 2. The effects of black tape etiolation (E), etiolation plus ringing (ER) and time of measurement of the number of roots per cutting of Myrobalan Plums. (Exp. 1)

<table>
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<th>Treatment</th>
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1- Each value in the body of the table is an average of three replications of ten cuttings per replicate.

2- Numbers within each group of means not followed by a common letter are significantly different at 5 percent level.
Table 3. The effects of black tape etiolation (E), etiolation plus ringing (ER) and time of measurement on the number of rooted cuttings of Myrobalan plums (Exp. 2).

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1- Each value in the body of the table is an average of three replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 4. The effects of tape etiolation (E), tape etiolation plus ringing (ER) and time of measurement on the number of roots per cutting of Myrobalan plums (Exp. 2).

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1- Each value in the body of the table is an average of two replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 5. The effects of black paint etiolation (Ep), etiolation plus ringing (EpR) and time of measurement on the number of rooted cuttings of Myrobalan plums (Exp. 1).

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</tr>
<tr>
<td></td>
<td>Date</td>
<td>Date</td>
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</tr>
<tr>
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1- Each value in the body of the table is an average of two replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 6. The effects of black paint etiolation (Ep), etiolation plus ringing (EpR) and time of measurement on the number of roots per cutting of Myrobalan plums (Exp. 2).

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<th>Means</th>
<th>Treat</th>
<th>T. x Rs.</th>
<th>T. x D</th>
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<tr>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Control (C)</td>
<td>0</td>
<td>2.5</td>
<td>3.4</td>
<td>8.1</td>
<td>3.5</td>
<td>1.3 a</td>
</tr>
<tr>
<td>Etiolation (E)</td>
<td>3.5</td>
<td>4.6</td>
<td>3.2</td>
<td>9.8</td>
<td>5.3</td>
<td>4.1 c</td>
</tr>
<tr>
<td>Eti. + Ring. (EpR)</td>
<td>2.0</td>
<td>3.0</td>
<td>4.1</td>
<td>8.4</td>
<td>4.4</td>
<td>2.5 b</td>
</tr>
<tr>
<td>Rs. Means</td>
<td>2.6 a</td>
<td>6.2 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1- Each value in the body of the table is an average of two replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
### Table 7. The effects of black paint etiolation (Ep), etiolation plus ringing (EpR) and time of measurement on number of rooted cuttings of Myrobalan plum (Exp. 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rootstocks</th>
<th>Means</th>
<th>Treat</th>
<th>T. x Rs.</th>
<th>T. x D</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ohio 2</td>
<td>Myro 29c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Date</td>
<td>Date</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (C)</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
<td>4.5</td>
<td>1.5</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29</td>
<td>D1</td>
<td>D2</td>
<td></td>
</tr>
<tr>
<td>Etiolation (E)</td>
<td>0.5</td>
<td>0.5</td>
<td>0</td>
<td>4.5</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29</td>
<td>D1</td>
<td>D2</td>
<td></td>
</tr>
<tr>
<td>Eti. + Ring. (EpR)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>4.0</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>29</td>
<td>D1</td>
<td>D2</td>
<td></td>
</tr>
<tr>
<td>Rs. Means</td>
<td>0.41 a</td>
<td>2.16 b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1- Each value in the body of the table is an average of two replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 8. The effects of black paint etiolation (Ep), etiolation plus ringing (EpR) and time of measurement on number of roots per cutting of Myrobalan plums (Exp. 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rootstocks</th>
<th>Means</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ohio 2</td>
<td>Myro 29c</td>
<td>Treat</td>
<td>T. x Rs.</td>
<td>T. x D</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Date</td>
<td>Date</td>
<td>2</td>
<td>29</td>
<td>D1</td>
<td>D2</td>
</tr>
<tr>
<td>Control (C)</td>
<td>0 a</td>
<td>1.8 a</td>
<td>0 a</td>
<td>3.4 b</td>
<td>1.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Etiolation (Ep)</td>
<td>0.5 a</td>
<td>0.5 a</td>
<td>0 a</td>
<td>5.5 b</td>
<td>1.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Eti. + Ring. (EpR)</td>
<td>0 a</td>
<td>0 a</td>
<td>0 a</td>
<td>4.3 b</td>
<td>1.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Rs. Means</td>
<td>0.5 a</td>
<td>2.2 b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1- Each mean value in the body of the table is an average of two replications of ten cuttings per replicate.

2- Numbers within each group of means not having a common letter are significantly different at 5 percent level.
Table 9. The estimated endogenous IAA content (ug. x 10^-3 / 50 mg. FDT) of plum rootstocks (Exp. 1).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rootstocks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ohio 2</td>
</tr>
<tr>
<td>Control (C)</td>
<td>10^-3 x 3.9 a</td>
</tr>
<tr>
<td>Etiolation (E)</td>
<td>10^-3 x 29.9 b</td>
</tr>
<tr>
<td>Etiol. + Ring. (ER)</td>
<td>10^-3 x 14.9 ab</td>
</tr>
</tbody>
</table>

1- Each value in the body of the table is an average of three replications of 50 mg. FDT per replicate.

2- Means not having a common letter are significantly different at 5 percent level.
Table 10. The estimated endogenous IAA content (ug. x 10\(^{-3}\) / 50 mg. FDT.) of plum rootstocks (Exp. 2).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ohio 2</th>
<th>Rootstocks</th>
<th>Myrobalan 29c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (C)</td>
<td>10(^{-3}) x 2.8 a</td>
<td>10(^{-3}) x 1.95 a</td>
<td></td>
</tr>
<tr>
<td>Etiolation (E)</td>
<td>10(^{-3}) x 51.5 b</td>
<td>10(^{-3}) x 4.8 a</td>
<td></td>
</tr>
<tr>
<td>Etio. + Ring. (ER)</td>
<td>10(^{-3}) x 5.4 a</td>
<td>10(^{-3}) x 3.0 a</td>
<td></td>
</tr>
</tbody>
</table>

1- Each value in the body of the table is an average of two replications of 50 mg. FDT per replicate.

2- Means not having a common letter are significantly different at 5 percent level.