EFFECT OF CENTRIFUGATION ON ROOT INITIATION IN *VACCINIUM CORYMBOSUM*, L. AND *SALIX ALBA*, L.

Thesis for the Degree of M. S.

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By

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AN ABSTRACT OF A THESIS

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ABSTRACT

EFFECT OF CENTRIFUGATION ON ROOT INITIATION IN *VACCINIUM CORYMBOSUM*, L. AND *SALIX ALBA*, L.

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The purpose of this study was to determine whether or not the centrifugation of cuttings would promote root initiation. Pieces 4 inches long of the hardwood cutting of *Vaccinium corymbosum*, L. and the softwood cutting of *Salix alba*, L. were centrifuged acropetally or basipetally with a force of 500 to 2000 g. for 30 minutes and with a standard force of 1000 g. on the various times of 15, 30, 60, and 90 minutes.

The centrifugal diffusate which diffuse from the cut ends of blueberry and willow cuttings into deionized distilled water in the centrifuge tube was saved and its effect on root formation was assayed using mung bean (*Phaseolus aureus* Roxb.). The diffusate which was obtained from both blueberry and willow cuttings did little to increase root initiation in mung bean cuttings. It was found that only the diffusate obtained from leafy willow cuttings increased the number of roots on mung bean cuttings.

The extract solutions from the willow leaves and stems promoted root initiation in mung beans. This indicates that the active material which promoted rooting was in the stem and leaves.

The results were obtained as accompanied by 12 tables.

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INTRODUCTION

This study was to investigate a centrifugal technique to stimulate rooting in some horticultural plants. Prior to this study, the similar technique has been undertaken in respect to rooting of cuttings. Kawase (11) working in Canada has reported that he could concentrate diffusate by centrifuging willow cuttings and that these cuttings rooted better than those which were not centrifuged.

Besides IAA, there are a number of other substances that have the ability to stimulate rooting or enhance the activity of IAA. Some of the substances are nutritional such as sucrose and organic and inorganic forms of nitrogen (22, 25); others may be considered to be growth factors such as vitamins K and H (7), nicotinic acid, thiamine, adenine sulfate (23), and biotin (21). However, even with this wide range of compounds, there are a large number of cuttings which are considered difficult or impossible to root regardless of what substances or combination of substances are applied.

According to above considerations, a study to obtain more information on the unknown factors which stimulate root initiation has been undertaken again.

Two varieites of plants were used in this study, Salix alba, L. and Vaccinium corymbosum, L. The studies were conducted at Michigan State University, Horticulture Laboratory and Greenhouse.

LITERATURE REVIEW

It has long been known that auxins, if applied in appropriate concentrations to stems, may induce the formation of roots. For this reason, the auxin have become generally known as the rooting hormones of plants.

In recent years (Went, 1938) it has been suggested that besides auxin an other hormone is necessary for root formation. This hypothetical substance was called "rhizocaline" and was considered to be a specific root-forming hormone which is produced in leaves in the light, stored in cotyledons, and activated or redistributed under the influence of applied auxins.

This concept of rhizocaline has been questioned by Van Overbeek et al. (25) who maintain that in Hibiscus, the stimulatory effect of leaves upon root formation is equal in light and darkness, and this effect may be duplicated by sucrose in combination with ammonium sulfate or arginine. They conclude that there is no reason to postulate the production of a special rhizocaline in the leaves of hibiscus.

The experiment on the physiology of root initiation in excised asparagus stem tip by Galston (5), the general results support the view that some substance other than auxin may become limiting to root formation. The substance active for asparagus is apparently synthesized in the light only, and is not identical with any compound found by Van Overbeek to be active for hibiscus.

It should be emphasized that work with rhizocaline has been done on markedly different types of plants. There is no evidence indicating that

the accessory factors in pea (material studied on rhizocaline by Went, 1938), Hibicus and asparagus are the same. The demonstration by Galston that substances effective for Hibicus are without effect in asparagus makes it seem likely that rooting may be limited by different materials in different plants. Therefore, rhizocaline, even if it is a specific rooting material, may be different in different species.

In propagation blueberry by cutting, O'Rourke (14) conducted some experiments in an attempt to determine the effect of the presence of flower buds on the rooting of hardwood cuttings. The average percentage of rooting of cutting with only vegetative buds present was about 40 per cent while cuttings bearing both vegetative and flower buds rooted less than 5 per cent. The author states, however, that it is not the presence of the flower bud that makes the differences but it is the condition in the wood that favors the set of flower buds that retard rooting. Chadwick (2) in a discussion of the use of flowering wood versus vegetative states that the apparent effect of flower buds on cuttings is to increase a rate of respiration markedly over that of the vegetative buds, such that considerably more stored food is converted in the rooting process. This decrease in the stored food supply of the cutting appears to be contributing cause of the poor rooting of cuttings bearing flower buds. De Boer (4) also found that Rhododendron cuttings rooted better when the existing flower buds were removed from the cutting, than when they were allowed to remain; she suggested that this result may have been connected with the plant's natural hormones and inhibitors.

There have been several reports on the influence of certain hormone-like substances on rooting of blueberry cuttings, one by Johnston of Michigan (10) and another by Myhre in Washington (17), where synthetic growth materials have been used on hardwood cuttings of several varieties of blueberries. These reports would indicate very little, if any, response from synthetic materials on these types of cuttings.

The great majority of propagators have reported that there was no beneficial results from the use of synthetic materials on deciduous hardwood cuttings. Chadwick (1) made the statement that "....to the commercial aspects, I think by and large, results would indicate that root-inducing substances have been used to greater advantage on leafy cuttings than on leafless hardwood materials."

Stene and Christopher (18) working with hardwood cuttings of blueberry recommended that granulated peat moss be used as the rooting medium. The addition of sand with the peat was of no advantage. In contrast to this report, Schwartze and Myrhe (17) reported that Canadian peat and clean sand in the ratio 3:1 was the best rooting medium for blueberry cutting. O'Rourke (14) also reported that the most satisfactory way of handling hardwood cuttings of the Atrococcium variety of blueberry was to take cuttings 4 inches long, of one year old, uniform wood, in the last half of March and hold them in moist peat in cold storage until April 5 when they should be stuck in a cold frame containing 5 inches of a medium consisting of 1/3 peat and 2/3 sand. These reports show little, if any, consistence in respect to the best rooting medium for

blueberry cutting. The main conclusion that can be derived is that rooting medium incorporated with suitable environment may play an important role in the rooting of blueberry cuttings.

Hess (9) suggested that the environment plays an important role in the ability of a cutting to root. Some environmental factors which have a major role in rooting are light, nutritional, and several complex elements. He also pointed out that the problem in plant propagation is varietal differences in the rooting ability of the cutting. Part of the cause of varietal differences can be attributed to differences in the ability of the cuttings to manufacture substances essential for rooting.

Cooper (3) studying rootings of apple and lemon reported that cuttings were treated with auxin, and after analysis for auxin in the two groups of cuttings, it was found that there was little difference in the amount recovered, yet none of the apple cuttings rooted whereas the lemon cuttings did. It was assumed that the apple cuttings were lacking in certain unidentified internal substances necessary for root formation. On the other hand, the lemon cuttings were believed to have this substance, or substances, in abundance. Many investigators (14, 16, 17) concluded that internal and external factor interactions of these influence the initiation of roots on cuttings.

Hess (8) in discussion of the physiological comparison of rooting in easy- and difficult-to-root cuttings suggested that auxin, such as indoleacetic acid (IAA), do not seem to limit the difficult-to-root cuttings. However, the other substances which may act as cofactors with IAA are found in smaller amounts in the difficult-to-root cutting. Pearse (15)

treated the basal ends of dormant willow cuttings with lanolin paste and water solutions containing indolebutyric acid and reported that the treatment greatly stimulated the formation of roots. Treating the apical ends of cuttings accelerated root formation throughout the length of cuttings.

Many investigators tried to find out where the place is on the shoot, buds and/or leaves that produces the substance or substances essential for root formation in the cutting. In Went's pea test (27) for root-forming activity of various substances, it is significant that the presence of at least one bud on the pea cutting was essential for root production. A budless cutting would not form roots even when treated with an auxin-rich preparation. This indicates that a factor other than auxin, presumably produced by the bud, is needed for root formation. The presence of buds often greatly promotes root formation in cuttings, especially if the buds are starting growth. Removal of the buds has been shown (26) in certain plants to stop root formation almost completely, especially in species without performed root initials. It was shown (12) by van der Lek in 1925 that strongly sprouting buds promote the development of roots in cuttings of such plants as the willow, poplar, currant, and grape. It was assumed that hormone-like substances were formed in the developing buds and transported to the base of the cutting through the phloem where they stimulated root formation.

It has long been known, and there is considerable supporting experimental evidence (3, 16, 26), that the presence of leaves on cuttings exerts a strong stimulating influence on root initiation. The carbohydrates resulting from the photosynthetic activity of the leaves undoubtedly contribute to root formation.

Experiments by Van Overbeek et al. using an easily rooted red hibiscus variety and a difficult-to-root white variety have given considerable information on the internal factors controlling root formation in cuttings (24, 25). Abundant roots could be obtained on leafy cuttings of the red variety by indolebutyric acid treatments, but this did not cause rooting of the white variety. It was concluded from this that the white hibiscus failed to root because it not only lacked auxin, but its leaves failed to produce the other factor or factors, which in addition to auxin, were necessary for root initiation.

It seems clear that auxin is only one of perhaps several substances that are required for root initiation. There are other necessary factors, such as those of a nutritional and perhaps a hormone nature. Rhizocaline may be one of these factors. In any event, it seems certain that the leaves and/or buds are the source of the substances (6).

The most recent work has been done by Kawase (11) to study the effect of axillary buds and leaves on rooting of willow cuttings. His experiments were designed, based on the rhizocaline concept, by using the centrifugation technique. The results on the effect of axillary buds and leaves on rooting of willow cutting basipetal centrifugation with an approximate force of 640 g for 1 hr. when leaves remained on cuttings, rooting was strongly suppressed. However, he suggested that this retarding effect, due to leaves, was modified by the presence of axillary buds. This tendency occurred in both centrifuged and non-centrifuged cuttings. Generally, better rooting was obtained in those that were centrifuged.

He concluded that neither leaves nor axillary buds were likely to have participated in the centrifugal promotion of willow rootings, indicating that the stem of the cutting received the primary effect of centrifugation. He also found that axillary buds had a strong effect on root formation if they were left intact after the centrifugation.

In the same study, he demonstrated that some substance or substances in the shoot was (were) accumulated at the basal end of the cutting by means of gravitational force and rooting of cutting were enhanced. Rooting of willow cuttings was enhanced by the longer duration of centrifugation, if centrifuged basipetally the rooting effect the basipetal centrifugation reached a maximum after 90 minutes treatment and declined when treatment was continued.

He also showed that the centrifugal diffusate from willow cuttings strongly increased the number of roots in mung bean cutting. The higher the concentrations of willow cutting put in the centrifuge tube 3, 10 and 30 cuttings/vials resulting in 8.6, 17.2, and 43.2 roots per cutting. It is, however, most interesting that the root-forming activity in the centrifugal diffusate as tested in the rooting of mung bean cuttings also increased with the greater force of centrifugation. The results clearly suggested that there was an accumulation of a root-forming substance (or substances) at the proximal ends by the basipetal centrifugation and also that a part of it diffused into water.

EXPERIMENTAL PROCEDURES

Experiment A: Centrifugation and Rooting in Blueberry with Mung Bean Bioassay

Materials: Two clones of blueberry, Bluecrop and Jersey, were used in the experiments. In both clones, the canes were about two feet in length, having been ordered from a nursery. All canes were pencil size in diameter. They were wrapped with plastic and stored in a cold room at 40°F. To make cuttings, only the basal 12 to 15 inches of each cane was used in order to eliminate the presence of flower buds on the upper part of cane. All cuttings had been prepared as hardwood cuttings. Each cutting was exactly 4 inches long and contained approximately 3-4 leaf buds.

Methods: Centrifugation - Five cuttings were placed in a centrifuge tube, 50 ml. in capacity. The tube contained 15 ml. of deionized distilled water. An international centrifuge, size 2, model K with attached indicating tachometer, was used with speeds of 1500 to 3000 rpm. Calculating the approximate gravitational force applied to the basal end of the cutting, the centrifuge provided 500 to 2000 g by using the formula $rpm = \sqrt{\frac{RCF\ (10)^5}{1.119\ r}}$ (13). The cuttings used in all experiments were separated into three groups according to the section of cuttings: lower, medium, and apical. Each section was used in all three replications of this experiment.

There were two kinds of the centrifugal force on the cuttings. One in which the cuttings were inserted into the centrifuge tube with proximal

or basal ends down, the direction of centrifugal force applied to the cuttings was from the distal or apical downwards toward the proximal ends. This case will be called basipetal centrifugation. When cuttings were placed in the centrifuge tubes with distal ends down, this will be referred to as acropetal centrifugation.

Centrifugations were operated in a cold room at 42°F. Various experiments were performed based on the times of centrifugation and different gravitational force. Times of centrifugation were 30 to 90 minutes and the gravitational force ranged from 500 to 2000 g.

The centrifugal diffusate was obtained after the centrifugation of the cuttings in centrifuge tubes containing 15 ml. deionized distilled water served as diffusion medium. The solution obtained or diffusate from each centrifuge tube which represented one treatment, was filtered through No. 1 Whitman filter paper into vial 20 ml. capacity. The diffusates were measured to 10 ml. in all treatments. This volume of diffusate brought up the level in vial about one inch. So, each vial or treatment contains 10 ml. of diffusate derived from 5 blueberry cuttings. The diffusates were tested for rooting activities with mung bean (Phaseolus aureus Roxb).

Number of Experiments: There were three experiments performed based on times of centrifugation, gravitational forces and the position of cuttings in centrifuge tubes, as following:

Experiment A1: Centrifugation for 30 minutes, basipetal centrifugation with gravitational forces of 1, 500, 1000 and 2000 g.

Experiment A2: Centrifugation at standard gravitational force, 1000 g, basipetally with various time periods: 15, 30, 60 and 90 minutes.

Experiment A3: Centrifugation at standard gravitational force, 1000 g, acropetally with various time periods: 15, 30, 60 and 90 minutes.

Mung Bean Test: Mung bean cuttings were used as a bioassay for the detection of substances capable of stimulating root initiation. The mung bean test described by Hess and adapted by Kawase (11) was modified. Mung bean seeds were planted in moist vermiculite using a standard flat placed in a greenhouse at approximately 70-80°F. The seedlings were ready to use in 9-10 days. They were 8-12 cm. in length. The cuttings were prepared by cutting off the seedling root system 3 cm. below the cotyledonary node. The cutting consisted of 3 cm. of hypocotyl, approximately 5-8 cm. epicotyl, the primary leaves, and the trifoliate bud. The cotyledons were removed if they had not abscissed at the time the cuttings were prepared. Five cuttings were placed in 25 x 45 mm. vials containing 10 ml. of centrifugal diffusate. Deionized distilled water was used as a control. All treatments were placed in a controlled environment chamber with a light source of 400 foot candles at plant level, a temperature of 27°C., and to give daily 16-hrs. light period. By daily addition of deionized distilled water, the original volume was maintained throughout the experiment. The number of roots on each cutting was counted 6-7 days

after the cuttings were made. The number of roots initiated on treated cuttings in comparison to the control was used as the measurement of biological activity.

Blueberry Cutting: Centrifugation blueberry cuttings and control blueberry cuttings were put in a cold frame propagating bed for rooting. The peat moss was used in a cutting bed which was filled on top of sand about 5-6 inches. Plastic shades covered the cutting bed to decrease the light intensity during the sunny day.

Experiment B: Centrifugation and Rooting in Willow with Mung Bean Bioassay

Materials: Willow cuttings were taken from Salix alba, L. plants, approximately 18 feet high on the MSU campus. Soft-wood cuttings were obtained in mid June, taken from new growing shoots approximately 6-10 inches long. Only the middle section, 4 inches long, of each selected shoots were cut. These cuttings had approximately 5-6 nodes with leaves on. For convenience of centrifugation, all leaves on the lower part of cuttings were removed. There were 3 leaves on each cutting. The leaves on the cuttings were pressed upwards against the cutting and cut off at the level of the apex of the cutting. The cuttings thus obtained had a short petiole at the tip and different occurring from the tip towards the base. These were designated as leafy cuttings. Similar cuttings without any leaves were referred to as leaf-less cuttings. Two kinds of cuttings will be compared in results in regard to their rootings.

Methods: Centrifugation - Exactly 10 cuttings of both kinds, leafy and leaf-less cuttings, were placed in centrifuge tubes. Before centrifugation, all cuttings from each treatment had been tied with a rubber band into a bundle. The reason for this was because the willow cuttings were softer and more flexible compared to the blueberry cuttings. In addition, the leafy cuttings needed a piece of glass rod about the same length of the cutting for supporting the strength of the bundle.

Mung Bean Test: The same as in the blueberry experiments.

Willow Cuttings: All treated willow cuttings were put in a standard flat containing sand as a rooting medium. Untreated cuttings were also placed in sand and served as control treatment. All treatments were placed in a greenhouse. The results of willow cuttings were counted and evaluated within 2-3 weeks after the cuttings were put in the medium.

The willow rooting was scored based on characteristics of roots formed: 5 scores for heavy roots, 3 scores for medium roots, and 1 score for light roots, and no score for unrooted cuttings or dead cuttings. A perfect score in each treatment could have 150 from 30 cuttings.

Number of Experiments: Four experiments were conducted with the willow which were similar to the blueberry experiments. In the experiments B 1-4, cuttings were prepared as leaf-less cuttings. The four experiments were as follows:

Experiment B1: Centrifugation for 30 minutes, basipetally with gravitational forces of: 1, 500,

1000 and 2000 g, in room temperature (80°F.) and cold room (42°F.).

Experiment B2: Centrifugation at standard gravitational force, 1000 g, basipetally and acropetally with various times of centrifugation: 15, 30, 60 and 90 minutes, in room temperature.

Experiment B3: Centrifugation at standard gravitational force of 1000 g for 60 minutes, basipetally with four different types of cuttings: leaves and buds on; leaves on, buds off; leaves off, buds on; leaves on, buds off; all at room temperature.

Experiment B4: The same as experiment 3, willow treated cuttings, basipetally at standard gravitational force of 1000 g for 60 minutes, were put in sand for rooting. Untreated cuttings were also put in sand and served as control treatment.

Experiment C: Willow Extracts

Since it was found by Kawase that the rooting substance found in willow cuttings is highly soluble in water, these experiments were designed in order to determine whether or not the solutions obtained from willow leaf extracts would promote rooting in mung bean. Also, an experiment

was conducted to determine in what part of the stem--basal, medium or apical section--that rooting substance was accumulated.

Experiment C1: Willow Leaf Extract Study

Willow leaves were collected in mid July. A 20 grams sample of fresh leaves was obtained and cut into small pieces. The tissue was homogenized with a waring blender containing 200 ml. of deionized distilled water for 5 minutes, then filtered through No. 1 Whitman filter paper. In order to obtain a clear solution, the filtrate was centrifuged at a speed between 8000-9000 rpm. Various dilutions of this solution were prepared by the addition of deionized, distilled water. The solutions were of five concentrations: Solution A 100%, Solution B 75%, Solution C 60%, Solution D 50%, Solution E 25%. Each concentration was prepared into 30 ml. for use in 3 vials. Each vial contained 10 ml. of solution with three replicates of each. Five mung bean cuttings were used in each extract solution. Deionized distilled water was used as a control treatment. The environment of mung beans during their rooting and root cuttings were performed the same as in previous experiments.

Experiment C2: Willow Stem Extract Study

Willow cuttings were taken in August from the same tree as in previous experiments for consistency of results. Cuttings were prepared exactly the same as previous experiments.

After centrifugation, the cuttings were cut into 3 equal sections--basal, middle and apical. Each section was about 1.3 inches in length. The weight of 10 such sections ranged between 6-7 grams according to the section. Normally the basal sections were a little heavier than the middle or top sections. The sections in each treatment were cut into smaller pieces and then homogenized with a waring blender containing 60 ml. of deionized distilled water for 5 minutes. Finally, 50 ml. of clear solution was obtained and dilutions made and the mung bean test was conducted as described in experiment C1.

Experiment D: Mung Bean Cutting Studies

This experiment was designed to study the mung bean cuttings that were used in the mung bean tests throughout the whole study. In order to see the effect of the length of cuttings, axillary buds and leaves on rooting of the cuttings.

Experiment D1: The Length of Cuttings and Their Rooting

Mung bean cuttings were taken from 10 day seedling. All cuttings were selected from the most uniform seedlings as possible. The cuttings were prepared into three groups: 11 cm., 9.5 cm., and 8 cm. cuttings. The cuttings had 3 cm. hypocotyl, including a pair of primary leaves, cotyledons were removed, and 8, 6.5, 5 cm. epicotyl respectively. Five cuttings were placed in glass vials containing 10 ml. of deionized distilled water in all treatments. The original water level about 2.5 cm.

was maintained throughout the experiment. After seven days, the roots on the hypocotyls were counted. The experiment has been performed in 3 replications.

Experiment D2: Study on the Axillary Buds and Leaves of Mung Bean Cuttings and Their Rooting

Two kinds of cuttings were prepared in this experiment. One had a pair of primary leaves; this was designated as leafy cuttings. The other without a pair of primary leaves was referred to as leaf-less cuttings. Each of these two kinds had 2 different lengths of the epicotyls, 4 and 5 cm. Four kinds of cuttings will be compared in results in regard to their rootings. The deionized distilled water was used in all treatments. The roots in all treatments were counted after 10 days of rooting in control growth chambers, due to the delays of rooting in leaf-less cuttings.

RESULTS

Centrifugation and Rooting: The results on shoot elongation of the blueberry cutting after 1 month in the propagation bed is presented in Table 1. The average number of growing shoots in experiments 1 and 2 was 85% for cutting that had been centrifuged in a basipetal position and 8% for those in experiment 3 that had been centrifuged in an acropetal position. The characteristic of shoot in experiments 1 and 2 were similar. All cuttings exhibited a black color about 1 inch from their ends. This appearance occurred on the basal part of cuttings in experiments 1 and 2--those cuttings that had been centrifuged basipetally--and occurred on the apical part of cuttings in experiment 3--which had been centrifuged acropetally.

The results of blueberry rooting were disregarded due to the fungus attached to the cuttings in the propagation bed.

To study the effect of axillary buds and leaves on the rooting of centrifuged willow cuttings, the results were obtained as shown in Table 2. In both cases, the centrifuged and non-centrifuged cuttings, there was a large increase in rooting when leaves remained on the cutting. On the other hand, the treatment without any leaves on, rooting was strongly suppressed. In any respect, rooting from the non-centrifuged cuttings showed higher scores than the centrifuged cuttings.

Centrifugal Diffusate and Rooting: The number of roots on the mung bean rooted tests in experiments A1, 2 and 3 were obtained within the range between 13-19, 13-21 and 13-17 roots per cutting respectively compared

TABLE 1

Blueberry Cuttings Having Shoots Growing After 1 Month in Propagation Frame. (Results Obtained from Experiments A 1-3)

Experiment	Number of Cuttings Having Shoots Growing	Percent
A 1	105	88
A 2	97	81
A 3	19	8

TABLE 2

Experiment B4: Effect of Basipetal Centrifugation at 1000 g for 60 Minutes at Room Temperature on Root Initiation in Willow Cuttings.

Treatment		Total Scores	Remarks
Centrifugation:			
Leaves	Buds		The characteristic of
+	+	76	root zone on centrifu-
+	-	89	ged cuttings. There
-	+	9	was no root on the end
-	-	2	of the cutting.
Control:			
+	+	110	The root zone on the
+	-	96	control cutting was
-	+	24	occurred beginning
-	-	13	from the end of the
			cutting.

Key to Table: + = Leaves or buds present
- = Leaves or buds absent

to the control treatment, 13 roots per cutting was obtained. The results were presented in Tables 3, 4 and 5. An analysis of variance showed that there is no difference between the treatments and the check in each of these three experiments.

The effect of the diffusates from the centrifugation of willow cutting at room temperature and cold room on root initiation in mung bean was shown in Table 6 and by analysis of variance it was shown that there was a significant difference among the treatments at the 5% level.

The effect of the diffusates from basipetal and acropetal centrifugation of willow cuttings on root initiation in mung beans is recorded in Table 7. The result of mung bean rooting was indicated by an analysis of variance that there was no difference among the treatments.

The effects of the diffusates from the centrifugation of different types of willow cuttings on root initiation in mung beans were shown in Table 8. They were found that the centrifugal diffusates strongly increased the number of roots on mung bean cuttings when the leaves remained on the centrifuged willow cuttings. An analysis of variance indicated that there was a significant difference among the treatments at the 1% level. From a comparison of the treatment means it was found that there was a difference between the treatments with the leaves on (with and/or without buds) and the check. It was also found that there was a difference between a leaf-less treatment with buds on and the check. That is, either leaves or axillary buds were likely to participate in the centrifugal diffusates to promote rooting on mung bean.

TABLE 3

Experiment A1: Effect of the Diffusates from Basipetal Centrifugation of Blueberry Cuttings for 30 Minutes on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Clone	Treatment Gravitational Force (g)	Number of Roots Per Cutting
Blue Crop	1	16
	500	18
	1000	18
	2000	19
Jersey	1	13
	500	16
	1000	18
	2000	17
Check		13

F value for difference between treatments was not significant.

TABLE 4

Experiment A2: Effect of the Diffusates from Basipetal Centrifugation of Blueberry Cuttings at Standard Gravitational Force 1000 g on Root Initiation in Mung Bean. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Clone	Treatment Centrifugation Time Duration (Min.)	Number of Roots Per Cutting
Blue Crop	15	21
	30	16
	60	16
	90	17
Jersey	15	17
	30	15
	60	13
	90	17
Check		13

F value for difference between treatments was not significant.

TABLE 5

Experiment A3: Effect of the Diffusates from Acropetal Centrifugation of Blueberry Cuttings for 30 Minutes on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Clone	Treatment Centrifugation Time Duration (Min.)	Number of Roots Per Cutting
Blue Crop	15	16
	30	15
	60	17
	90	16
Jersey	15	17
	30	16
	60	16
	90	13
Check		13

F value for difference between treatments was not significant.

TABLE 6

Experiment B1: Effect of the Diffusates from Basipetal Centrifugation of Willow Cuttings for 30 Minutes at Room Temperature (80°F.) and Cold Room (42°F.) on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment Gravitational Force (g)	Number of Roots Per Cutting
Room Temperature:	
1	11
500	12
1000	12
2000	15
Cold Room:	
1	14
500	16
1000	16
2000	15
Check	8

F value for difference between treatments was significant at 5% level.

L.S.D.	.05	3.0
	.01	4.4

TABLE 7

Experiment B2: Effect of the Diffusates from Basipetal and Acropetal Centrifugation of Willow Cuttings at Standard Gravitational Force of 1000 g at Room Temperature on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment Centrifugation Time Duration (Min.)	Number of Roots Per Cutting
Basipetally:	
15	13
30	13
60	11
90	10
Acropetally:	
15	12
30	14
60	12
90	11
Check	8

F value for difference between treatments was not significant.

TABLE 8

Experiment B3: Effect of the Diffusates from Basipetal Centrifugation of Willow Cuttings at Standard Gravitational Force of 1000 g for 60 Minutes at Room Temperature on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment	Number of Roots Per Cutting
Leaves on, buds on	49
Leaves on, buds off	34
Leaves off, buds on	32
Leaves off, buds off	26
Check	16

F value for difference between treatments was significant at 1% level.

L.S.D.	.05	11.5
	.01	16.8

Mung Bean Test on Willow Leaf and Stem Extracts: The result of mung bean test from the willow leaf and stem extracts were obtained as shown in Tables 9 and 10. The concentration of solutions in both extracts were obtained from an approximately equal weight of tissues. In willow leaf extract, the 125 ml. of extracted solution contained 20 grams of willow leaves or 0.16 gram of leaf tissue in 1 ml. solution. Whereas in willow stem extract a treatment of each stem section weighed about 6-7 grams and extracted into 50 ml. solution. So the concentration of solution in stem extract was about 0.12-0.14 gram of stem tissue in 1 ml. solution.

It was found that the mung bean cuttings put in the solutions of leaf extract have shown a slight toxicity within the concentrations that range between 60-100% solutions. The concentrations less than 50% showed normal growth of roots as well as stimulated the number of roots. In contrast, the result of mung bean rooting in the willow stem extract, the 100% concentration of extracted solution from all three sections--basal, medium and apical--showed very high toxicity that caused the death of all mung bean cuttings after 36 hours in the solution. The concentrations of stem extracts less than 50% showed normal growth of roots as in the leaf extract.

The mung bean rooting in willow leaf extract data is presented in Table 9. An analysis of variance indicates that there was no difference between the treatments and check. Whereas in the willow stem extract, Table 10, it was found by an analysis of variance that there was a difference among the treatments and the check. There was no difference

TABLE 9

Experiment C1: Effect of Willow Leaf Extracts on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment		Number of Roots Per Cutting	Remarks
Solution A	100%	29	No elongation, no root at the base
B	75%	27	No elongation, no root at the base
C	60%	38	No elongation, no root at the base
D	50%	41	Normal root growth
E	25%	48	Normal root growth
Check		24	Normal root growth

F value for difference between treatments was not significant.

TABLE 10a.

Experiment C2: Effect of Willow Stem Extracts on Root Initiation in Mung Beans. The Data Represent the Average Number of Roots from 12 Mung Bean Cuttings.

Treatment		Number of Roots Per Cutting	Remarks
Centrifugation:			
Section	Conc.%		
Basal	50	18	5 wilt cuttings, yellow leaf
	25	33	
	12.5	24	
Medium	50	22	7 wilt cuttings, yellow leaf
	25	34	
	12.5	26	
Apical	50	28	4 wilt cuttings, yellow leaf
	25	36	
	12.5	25	
Control:			
Basal	50	24	1 wilt cutting, 4 falled leaf cuttings
	25	37	
	12.5	25	
Medium	50	30	2 falled leaf cuttings
	25	28	
	12.5	28	
Apical	50	32	1 wilt cutting, 1 falled leaf cutting
	25	33	
	12.5	25	
Check		19	

F value for difference between treatments was significant at 5% level. L.S.D. .05 11.4
.01 15.4

NOTE: The results of mung bean rooting in 100% concentration of extract were not shown in this table because all mung bean cuttings were dead due to high toxicity of the extracts.

TABLE 10b.

Experiment C2: Effect of Willow Stem Extract Concentrations on Root Initiation in Mung Bean. The Data Represent the Average Number of Roots From 12 Mung Bean Cuttings.

Treatment		Number of Roots Per Cutting
Centrifugation	Conc. %	
	50	23
	25	34
	12.5	25
Control		
	50	29
	25	32
	12.5	26

F value for difference between treatments was significant at 5% level.

L.S.D.	.05	9.5
	.01	13.5

among the section of the stem extracts on the rooting of mung bean. The significant difference was found on the concentration of the extracts, especially in the centrifugation, the concentration of 25% was strongly increased the number of roots on mung bean.

The Effect of the Length and the Leaf of Mung Bean Cutting on the Number of Roots on Their Own Cuttings: In the study the relationship between the length of mung bean leafy cuttings and their rooting, it was found that the longer the cuttings the greater the number of roots initiated. The data, presented in Table 11, revealed that there was a relationship between the length of the mung bean cuttings and their rooting. The significant difference was indicated by an analysis of variance with 99% confidence.

The effect of the leaf on mung bean rooting showed a difference in the number of roots between leafy and leaf-less cuttings (Table 12). The leaf-less cutting had shown not only the less in the number of root but also the delay of the root initiation.

TABLE 11

Experiment D1: Effect of the Length of Cuttings on Rooting of Mung Bean Cutting. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment	Number of Roots Per Cutting
11 cm. cutting	22
9.5 cm. cutting	18
8.0 cm. cutting	14

F value for difference between treatments was significant at 1% level.

L.S.D.	.05	2.5
	.01	4.2

TABLE 12

Experiment D2: Effect of Axillary Buds and Leaves on Rooting of Mung Bean Cuttings. The Data Represent the Average Number of Roots from 15 Mung Bean Cuttings.

Treatment	Number of Roots Per Cutting
7 cm. leaf-less cutting	4
8 cm. leaf-less cutting	5
7 cm. leafy cutting	21
8 cm. leafy cutting	22

DISCUSSION AND CONCLUSION

The centrifugation of cutting did little to increase root initiation in willow as determined by the method of ranks (Table 2) nor was the diffusate, obtained from centrifugation of blueberry cutting, effective in increasing root initiation in mung bean cuttings (Tables 3, 4 and 5). A small difference was observed in one experiment (Table 6), but it is felt that this was not a significant increase.

It would appear, from these studies, that centrifugation of hardwood cutting of blueberry or soft wood cutting of willow as a method to increase rooting was not successful. These findings do not agree with those of Kawase (11) who proposed that the endogenous basipetal transport of rooting substance (or substances) in willow cuttings can be enhanced by basipetal centrifugation. Thus, the basipetal centrifugation promoted root formation in willow cuttings and the resulting diffusate increased rooting in mung bean cuttings.

However, it was observed that root formation and root quality in centrifuged willow cuttings showed a different characteristic from those of the control treatment. The root zone on the centrifuged cutting usually occurred in an area above the end of the cutting while in the control cuttings the roots were initiated exclusively at the base. This characteristic of root initiation may be explained:

(1) The centrifuged willow cuttings might have been injury from the gravitational forces and caused death to the cell at the basal part. As the results, most of the roots occurred

on the centrifuged cutting were above the basal end where the cells were not damaged.

(2) The basal end of the cutting may have accumulated a substance (or substances) which moved downward due to the gravitational force and accumulated to a toxic level which caused injury to the cells in that region.

It was found that the roots of mung bean in the solution of the willow leaf and stem extracts showed the same characteristic as the roots of the centrifuged willow cuttings. That is, the roots initiated on the mung beans in the extract solutions occurred above the basal end while those that were rooted in water initiated roots only at the base. In this case, it might be due to a disease contamination that was deposited at the basal end of the cutting thus killing the cells in that area.

It was found that the high concentration of willow leaf extracts increased root initiation in mung beans but inhibited root elongation.

The most interesting observation was that when leaves were left on the cuttings the quality and quantity of rooting was much improved (Tables 2, 8, 12). These results agree with previous reports (3, 16, 26) that the presence of leaves on cuttings exerts a strong stimulating influence on root initiation.

It also has been suggested that a hormone or hormone-like material is manufactured in the leaves and is transported into the base when it has an effect on root initiation.

To conclude the study of this work, it should be emphasized that the centrifugation of blueberry and willow cuttings, from the commercial

standpoint, was of no value since it did not enhance root initiation. Interesting results were obtained in the study with the willow cutting which had their leaves on. The leaves of the willow contributed some substance (or substances) to the cuttings that results in increasing root initiation. Further studies are necessary to identify the active material.

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