SEASONAL CHANGES IN ROOTABILITY AND ROOTING SUBSTANCES IN MATURE AND JUVENILE PECAN CUTTINGS

By

HWEI-JEN JENNIFER CHIU

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National Taiwan University

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PECAN CUTTINGS

Thesis Approved:

Michael Smith
Thesis Adviser

James D Quinby

John T. Johnson

Earl E. Whitaker

Norman N Durham
Dean of the Graduate College

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

In 1934, an endogenous root promoting hormone was identified by Went and Thimann (52, 57). Since their discovery indole-3-acetic acid (IAA) and synthetic auxins have been used extensively to promote rooting of cuttings (6, 58). The response, however, is not universal; cuttings of some species still root poorly after treatment with auxin (24). On the other hand, it is generally found that juvenile wood will usually respond readily to auxins, whereas adult wood is much less responsive (21, 24). This difference has been attributed to naturally occurring substances, other than auxins, that either stimulate or inhibit rooting in such cases (8, 43, 56). This, together with the fact that there is a correlation between the presence of leaves and buds on a cutting and its capacity to root (55), suggest that the effect of auxin is connected with substances produced in leaves and buds (35). The production of rooting promoters or inhibitors does not seem to be consistent throughout the year; instead their production fluctuates with season. It is believed that seasonal responses in shoot rootability is associated with substances produced inside the plants, e.g., rooting co-factors (22, 53).

The pecan, Carya illinoensis (Wang), K. Koch, is propagated commercially by budding or grafting on seedling rootstocks. However, the development of pecan scion roots has been of interest for many years

because of the variability of seedling root systems and because of some mechanical and physiological difficulties that are involved in the graft union in some trees (60).

In propagation by cuttings, greater uniformity is obtained by absence of genetic variation. There is no problem of compatibility with rootstocks or of poor graft unions. It is inexpensive, rapid, and simple, and does not require the special techniques required in grafting or budding. Asexual propagation of material will also allow selection of rootstocks for specific traits such as salt tolerance, size control, drought resistance, etc. This will afford the same advantages that is associated with clonal rootstocks in apple.

Although some workers (14, 33, 36, 38, 42, 45) have succeeded in rooting pecan cuttings, the pecan appears to be one of the most difficult of plants to establish upon its own roots. It was proposed that pecan cuttings do not have enough food reserves to produce both roots and shoots (15).

The objectives of this study were: (1) to determine differences in rooting responses of juvenile and adult pecan cuttings; (2) to determine differences in seasonal rootability of pecan cuttings; (3) to determine optimum concentration of indolebutyric acid (IBA) for rooting; (4) to identify seasonal variations of rooting co-factors; (5) to identify differences in content of rooting co-factors in juvenile and adult pecan cuttings.

CHAPTER II

REVIEW OF LITERATURE

Age of Plant Selected for Cuttings

In species of plants which are difficult to root, the age of the stock plants can be an important factor. Probably one of the most consistent characteristics of juvenile plants is the relative ease of rooting cuttings when compared with cuttings from adult plants of the same species (41). After testing about 30 species of trees including apple, cherry, elm and pine, Gardner (12) reported that most cuttings rooted well from one-year-old seedlings. Two-year-old plants rooted fair, and as age increased the cuttings rooted with difficulty or not at all. Stoutemyer (44) obtained similar results with apple trees, as did Thimann and Delisle (50) with pine, maple and oak. Studies in Australia showed that stem cuttings taken from seedlings of a number of eucalyptus species root easily, but as the stock plants become older rooting decreases dramatically (34). Deuber (9) compared the behavior of cuttings taken from white pine ranging from 2 to 60 years old. Rooting was good in the earlier years but dropped sharply between the fifth and seventh seedling years. Sax (41) reported that stem cuttings taken from young seedling plants (in the juvenile growth phase) will almost always root much more readily than those taken from plants in the adult growth phase.

Since the ease of adventitious root formation is associated with juvenility, it would be useful to induce juvenile stage from adult plants. Gardner (12) cut one-year-old apple seedlings back to the ground and found that the sprouts arising the second year could be rooted, and in some cases even more readily than that of the first year. Stoutemyer (44) reported in 1937 that cuttings taken from water sprouts of apple failed to root, but by forcing adventitious shoots from root pices, juvenile forms that rooted readily could be obtained.

Any treatment which maintains the juvenile growth phase would thus be of value in preventing the decline in rooting ability as the stock plant ages (16).

Time of Year in Which the Cuttings Are Taken

Seasonal changes in rootability of cuttings have a considerable influence on the successful propagation of many plants. Since the rooting response can be seasonal, this increases the importance of timing in propagation. Several investigators (4, 5, 18, 30) have demonstrated the importance of timing in the successful propagation by cuttings.

In propagation of deciduous species, hardwood cuttings could be taken during the dormant season, or softwood cuttings could be selected during the growing season, using succulent or partially matured wood. Fadl and Hartmann (11) found a seasonal fluctuating rooting pattern in 'Old Home' pear hardwood cuttings. Rooting was high in late summer and fall, followed by much lower activity during November and December. Hartmann (17) indicated that softwood cuttings of woody species taken during spring or summer usually tend to root more readily than hardwood

cuttings taken in the winter.

For difficult to root plants, it is often necessary to use softwood cuttings. In testing cherries, Hartmann and Brooks (19) found that softwood cuttings in the spring gave satisfactory rooting, whereas hardwood cuttings taken in winter would not root. For azalea, Kraus (29) found these cuttings root readily if the cuttings were taken from succulent growth in early spring; by late spring, however, the rooting percentages decline rapidly. Stoutemyer (46) noted that the Chinese fringe tree is difficult to root, but by taking cuttings during a short period in mid-spring, high rooting ability could be obtained.

Brix (3) reported that Douglas fir could be rooted most readily in January and February. Roberts (37) considered this indicative of a dormancy relationship. He suggested that bud development and dormancy plays an important role in root regeneration of Douglas fir cuttings and that cold treatment to break dormancy releases one or more root promoters similar in action to the rooting substances composed of indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA). Thus, cuttings taken in late autumn-early winter (Oct.-Dec.) often show a very poor rooting response, whereas from January on the rooting response improves (27, 28). It is also known that the rooting of hardwood cuttings in the spring is stimulated by the presence of an expanding bud, and that disbudding reduces the rooting response dramatically (11). Clearly, the optimum time for taking cuttings must be established not by the calendar but by the season and the species of interest.

Plant Growth Substances

Indole-3-acetic acid (IAA) was identified in 1934 as a naturally

occurring compound (6) and was soon found to promote adventitious root formation (49, 52). Since then, indoleacetic acid as well as some synthetic auxins were subsequently tested for their activities in promoting roots on stem segments. In 1935, Thimann (48) and Zimmerman (63) reported that synthetic IBA and NAA were more effective than the naturally occurring IAA in promoting adventitious root formation. For general use in rooting stem cuttings, NAA and IBA are recommended. IBA is the most widely used in commercial preparations because it is non-toxic over a wide range of concentrations (17). It is also effective in promoting roots on a wide variety of plant species (40). The use of salts of some of the growth regulators rather than the acid may be desirable in some instances, owing to their comparable activity and greater solubility in water (61).

Pre-treatment of pecan scions with a root-promoting substance to obtain rooting has been done by some research workers. Stoutemyer (45) rooted dormant 'Greenriver' pecan cuttings by precallusing and treatment with IBA. Gossard (14) produced roots from pecan stems with considerable success by trench layering the tops of grafted or budded nursery trees, and by air layering shoots of older trees, in conjunction with IBA treatment by the toothpick methods of Romberg and Smith (39). Sparks and Pokorny (42) studied the effects of wound treatment and root-inducing chemicals on rooting of terminal pecan cuttings taken at four different dates. They found that: (1) rooting was inversely related to the maturity of the cuttings; (2) IBA plus a light wound gave the highest rooting percentage. In testing nursery pecan seedlings with IBA in toothpick, Romberg and Smith (39) found rooting responses from all treatments used, but the higher concentrations gave

the greatest responses.

It is clear that auxins are not the only root-inducing factor; there are many difficult-to-root plants which fail to respond to auxins and the other known root-promoting substances (6, 10, 23, 31). Several workers (2, 7, 13, 59, 62) proposed that two factors are necessary for successful rooting of stem cuttings. One is auxin, and the second is a factor or combination of factors produced by leaves of easy-to-root plants.

Hess (23, 24, 25, 26) isolated various rooting substances from Hedera helix cuttings, using chromatography together with mung bean (Phaseolus aureus) bioassay techniques. These co-factors are naturally occurring substances and act synergistically with IAA in promoting rooting. Dennis and Lipecki (32) noted that good rooting response of apple in June and July was associated with high levels of growth inhibitors, as determined by the wheat coleoptile assay, in the base of the apple cuttings. Fadl and Hartmann (11) isolated an endogenous root promoter from easily rooted 'Old Home' pear cuttings, but extracts of difficult-to-root 'Bartlett' cuttings did not show this rooting factor.

Endogenous chemical inhibitors have been proposed as a principal reason that certain difficult-to-root plants fail to form roots readily (51). Hemberg (20) first proposed that inhibitors may be involved in bud dormancy. Van der Lek (54) indicated that the low rooting in winter of cuttings with buds may have been due to the accumulation of inhibitors. These substances may not only arrest bud development but may also inhibit the formation of endogenous root-promoting substances (43). Taylor and Odom (47) extracted a compound similar to juglone from leaves and

stems of the pecan stock plants. This compound was associated with one of the areas of root-inhibitory activity. The presence of root inhibitory substances appears to play a significant role in the rooting complex of pecan stem cuttings.

Although auxins play a very important role in rooting, they are not the complete answer. The more difficult a cutting is to root, the less it responds to auxin alone. In grape (44), it is apparent that inhibitors make the rooting difficult during summer. In other cuttings which are difficult-to-root, the difficulty seems to be due to the lack of certain substances or co-factors. The co-factors are present in the easy-to-root forms, but are absent in the difficult-to-root forms.

CHAPTER III

METHODS AND MATERIALS

Rooting Responses Experiment

The type of cuttings, time of year, and concentration of auxin were used in factorial combination to determine their effects on rooting of pecan cuttings. Plant materials were taken in 1978 from pecan trees growing at the Oklahoma Pecan Research Station near Sparks, Oklahoma. One-year-old lateral stem cuttings, 15 to 20 cm. long, were taken from juvenile sprouts of seedling pecan roots, and mature compensatory growth of 'Western' pecan trees. Collection dates for the cuttings were as follows: (1) February 15; (2) April 15; (3) June 15; (4) August 15; (5) October 15; and (6) December 15. The basal ends of the cuttings were dipped for 3 minutes in IBA (potassium salt) solutions of 0, 5,000, 10,000, 20,000 ppm and placed under intermittent mist at 27° C. air temperature. Propagation medium was equal parts of Canadian spagnum peat moss and horticulture grade perlite, in 946 ml size bottomless containers.

A randomized complete-block design with 7 replications and 3 subsamples was used in this study. Statistical analysis was by Fisher's F-test and the protected LSD. Cuttings were given 90 days in the rooting bench, then lifted and evaluated by the number of roots per cutting and a rooting index from 1 to 10. The scale was based on: one to

four being no callus formation to well callused with five indicating few roots progressing to well rooted cuttings give a value of 10.

Rooting Co-factors and Inhibitors Experiment

Samples of juvenile and adult cuttings were selected on each date to determine the presence of root promoters and inhibitors. Five replicates with three subsamples of each treatment were analyzed using the mung bean (Phaseolus aureus) bioassay described by USDA Agriculture Handbook #336 (1) and based on research by Hess (24, 25) with some modifications.

Pecan stem sections were frozen, lyophilized and ground in a Wiley mill to pass through a 20-mesh screen. One gm. of ground tissue was extracted three times using 25 ml. portions of absolute methanol. The extract was evaporated to dryness under reduced pressure at room temperature. The extract was redissolved in chloroform and water, and partitioned in a separatory funnel, to obtain the chloroform fraction. The remaining aqueous layer was acidified to a pH of 3.0 with 0.5 M citric acid and partitioned with petroleum ether to obtain the water and ether fractions.

Each fraction was evaporated to dryness then dissolved in 0.5 ml. of 80 percent ethanol, and streaked on Whatman No. 3 MM chromatographic paper. The paper was developed with descending chromatography using isopropanol-water (8:2 v/v) as the solvent. Each developed chromatograph was divided into 10 equal segments, and placed in shell vials with 4 ml. of 5×10^{-6} M IAA. The control consisted of equal amounts of chromatography paper and IAA.

Mung bean seeds were grown in moist vermiculite for 8 days in a controlled environment growth chamber (light intensity, about 26,900 lux from a combination of fluorescent and incandescent lamps; temperature, 27° C.; photoperiod, 16 hr.). The cotyledons were removed leaving 2 primary leaves, and the hypocotyls were cut 3 cm. below the cotyledonary node.

Three cuttings were placed in each shell vial and returned to chamber for rooting. The solution was taken up within 18 hr. Distilled water was then added to the vials each day. Rooting response was determined after 7 days by counting the number of roots produced per cutting to identify the presence of rooting promoters and inhibitors.

CHAPTER IV

RESULTS AND DISCUSSION

Rooting Responses Experiment

The percent rooting of juvenile and adult cuttings without IBA was zero for all dates with the exception of juvenile cuttings taken in February (Table I). Application of IBA increased the number of cuttings rooting in February and August, but had no effect on the number of cuttings rooting during April and October. Response of juvenile cuttings to IBA application was greater than adult cuttings in February, and approximately equal in August.

There was a significant variation in the root number and root index influenced by IBA concentration and cutting source. Root number and root index were greater than the control for all IBA treatments in February, and 10,000 ppm IBA exceeded the control in August (Table I). In all instances that a response to IBA was noted, juvenile cuttings had more roots per cutting, and a higher root index than adult cuttings. A significant decline in the root index was found at 20,000 ppm IBA.

Juvenile and adult cuttings differed greatly in their responses during February. The percent of cuttings rooting, root number, and root index was greatest during February for juvenile cuttings. Adult cuttings had only 5 percent root at 5,000 ppm IBA, and 5 percent root at 10,000 ppm, and root number and root index were not significant from

TABLE I

EFFECT OF IBA CONCENTRATION, DATE AND CUTTING SOURCE ON ROOTING OF PECAN

Date	ppm IBA	% Rooted		Root No./Cutting		Root Index[1]	
		Juv.	Aud.	Juv.	Aud.	Juv.	Adu.
2/15	0	38	0	1.2 a1[2]	0.0 a1	3.0 a1	1.1 a2
	5,000	71	5	4.9 b1	0.1 a2	5.3 c1	1.9 a2
	10,000	52	5	5.9 b1	0.1 a2	4.8 bc1	2.0 a2
	20,000	29	0	5.2 b1	0.0 a2	4.2 bc1	1.5 a2
4/15	0	0	0	0.0 a1	0.0 a1	1.2 a1	1.0 a2
	5,000	5	0	0.1 a1	0.0 a1	1.1 a1	1.1 a1
	10,000	0	0	0.0 a1	0.0 a1	1.5 b1	1.0 a2
	20,000	0	0	0.0 a1	0.0 a1	1.1 a1	1.0 a1
8/15	0	0	0	0.0 a1	0.0 a1	1.0 a1	1.1 a1
	5,000	10	33	2.3 a1	1.1 a1	1.9 a1	3.2 b2
	10,000	29	19	8.2 b1	0.4 a2	3.5 b1	3.1 b1
	20,000	10	10	0.9 a1	0.3 a1	1.8 a1	2.3 b1
10/15	0	0	0	0.0 a1	0.0 a1	1.0 a1	1.0 a1
	5,000	0	0	0.0 a1	0.0 a1	1.5 b1	1.7 b1
	10,000	0	0	0.0 a1	0.0 a1	2.0 c1	1.7 b1
	20,000	0	0	0.0 a1	0.0 a1	1.8 bc1	1.8 b1

[1]Scale 1-10; 1-4 callus development, none to well developed; 5-10 indicates increasing root development.

[2]Means within rows followed by different numbers, or means within columns followed by different letters are significant by the protected LSD, 5 percent level.

the control. Rooting response in August differed from February. A greater percentage of adult cuttings rooted in August than juvenile cuttings. However, comparison of the root number indicates that the juvenile cuttings produced more roots per cutting than adult cuttings, indicating a greater chance for survival.

These data indicate juvenile pecan cuttings respond to application of IBA, with the optimum concentration being 10,000 ppm. Adult pecan cuttings will root, however, the number of roots per cutting is less than the amount produced by juvenile cuttings. These results are similar to those reported by Hess (21, 24). Easy-to-root juvenile wood will usually respond readily to auxins, whereas difficult-to-root adult wood is much less responsive. Many internal factors, such as auxin level, rooting co-factors, and nutrition level, can influence the rooting ability of cuttings. The rooting co-factor content, as determined by the mung bean bioassay, will be discussed in the next section.

The highest rooting percentage was obtained from cuttings taken February 15 and then decreased in April 15 followed by an increase in August 15 (Table II). Cuttings taken from October 15 showed no rooting response at all. Where there was rooting response, juvenile wood resulted in better or almost the same rooting percentage as compared with adult wood. The data suggest that pecan cuttings could be rooted most readily in February by using juvenile wood.

There was a significant difference in root number influenced by wood type on February 15. For both responsive dates, February 15 and August 15, juvenile wood had a higher number of roots initiated than the adult form. Adult cuttings had a low root number for all

TABLE II

EFFECT OF DATE AND CUTTING SOURCE ON ROOTING OF PECAN

Date	% Rooted		Root No./Cutting		Root Index[1]	
	Juv.	Adu.	Juv.	Adu.	Juv.	Adu.
February 15	48	2	4.3 a[2]	0.1 b	4.3 a[2]	1.6 b
April 15	1	0	0.0 a	0.0 a	1.2 a	1.0 b
August 15	12	15	2.9 a	0.5 a	2.1 a	2.4 a
October 15	0	0	0.0 a	0.0 a	1.6 a	1.6 a

[1]Scale 1-10; 1-4 callus development, none to well developed; 5-10 indicates increasing root development.

[2]Means within rows followed by different letters are significant by the protected LSD, 5 percent level.

cutting dates. Similar to the percent rooting, the highest root number occurred in cuttings taken February 15. No root initiation was found in April and October.

Rooting index was significantly greater for juvenile cuttings in February and April. The best root index was obtained from juvenile cuttings taken February 15. There was no marked difference in the root index for adult wood among the four collection dates. These data indicate that wood type has a significant influence on root initiation and root development.

In summary, there was a marked seasonal variation in the rooting of pecan cuttings. Cuttings taken in October show no rooting response, whereas in August and February they could be rooted readily.

A possible explanation for the differences in rooting response is related to bud activity. Cuttings taken in February were dormant, but had received adequate chilling for growth. When placed in the greenhouse, dormant buds began growth within 2 weeks. Cuttings taken in April were vegetative, but defoliated when placed under the mist. Dormant buds on these cuttings did not grow, possibly due to their immaturity. Cuttings obtained in August and October defoliated, but new shoots arose only from cuttings made in August. The difference in development of new shoots between August and October may be associated with their state of growth. During October the trees had begun to senescence, and when cuttings defoliated, no new shoot growth occurred because the chilling requirement had not been fulfilled. If the hypothesis that actively growing leaves are necessary for rooting of pecan cuttings is true, then the new leaves must be producing a growth substance necessary for rooting to occur.

A pattern of inhibitor levels or absence of rooting promoters in the wood seems to exist, increasing in summer, reaching a maximum in late fall, then decreasing during the winter. Minimum levels of inhibitors or maximum promoters occur in spring when buds are expanding. Thus, it is reasonable to assume that the non-dormant buds contribute the stimulatory effect on the rooting of pecan cuttings.

The higher rooting response shown by juvenile wood indicates that as the wood matures the rooting of cuttings declines. Although in August there is a higher rooting percentage in adult cuttings, juvenile cuttings had much higher root numbers than adult wood. This suggests that survival of juvenile cuttings may be greater than adult cuttings after transplanting and are better material for rooting when propagated by cuttings.

Propagating pecan by cuttings involves establishing juvenile sources and evaluating the interaction between IBA concentration and time of taking cuttings. In this study, juvenile cuttings taken February 15 with 10,000 ppm IBA gave the best rooting response.

Rooting Co-factors and Inhibitors Experiment

The activity in the mung bean bioassay of extracts from adult and juvenile pecan cuttings taken from six cutting dates is expressed by histograms (Figures 1-18). Bars above the horizontal line (control) indicate root promoting activity; bars below the line indicate root inhibiting activity.

Comparisons were made between juvenile and adult cuttings, and among six collection dates. Results show little or no difference between the activity of co-factors in adult and juvenile cuttings

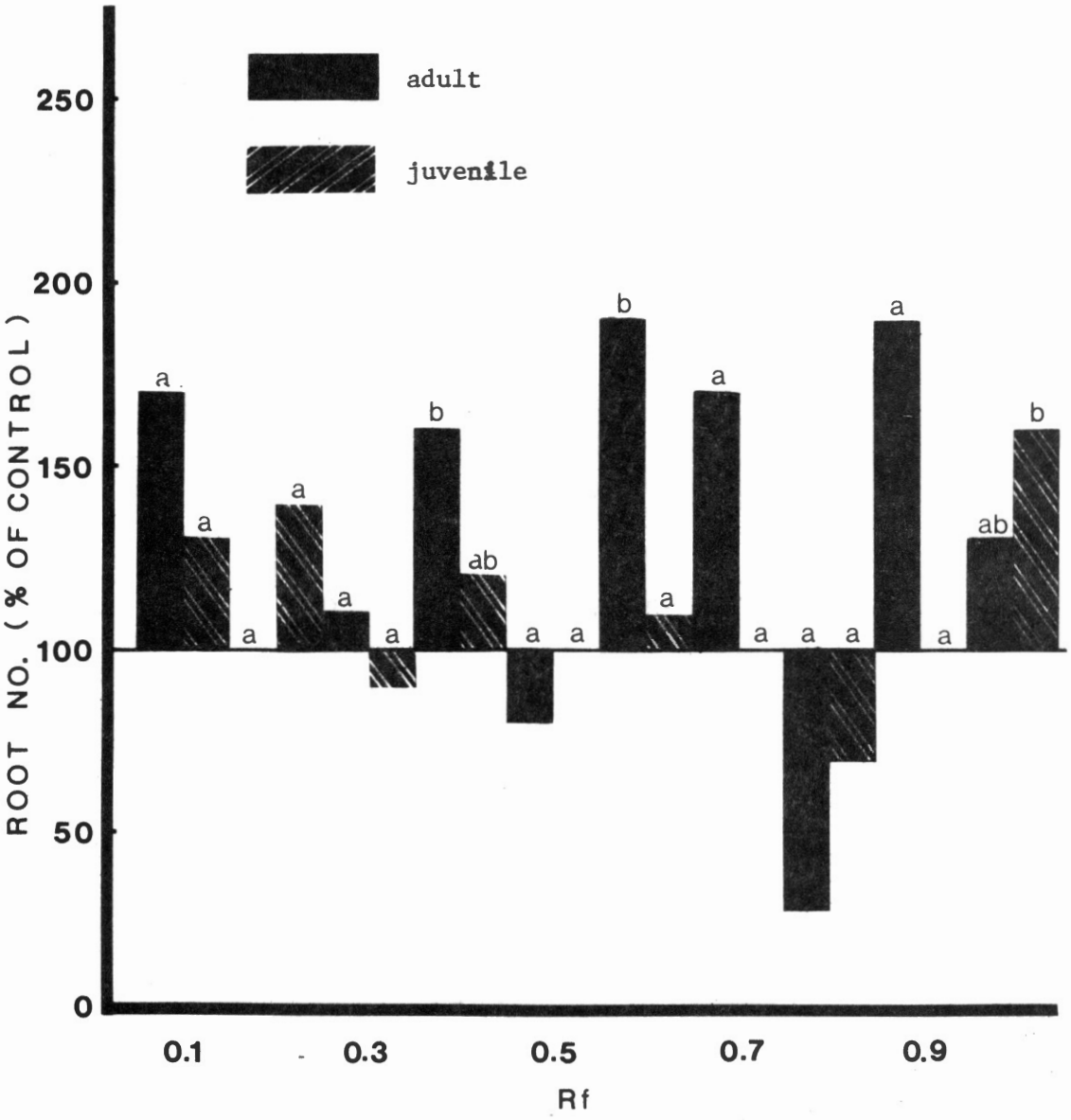

Figure 1. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected February 15. Significant Difference at 5% Level.

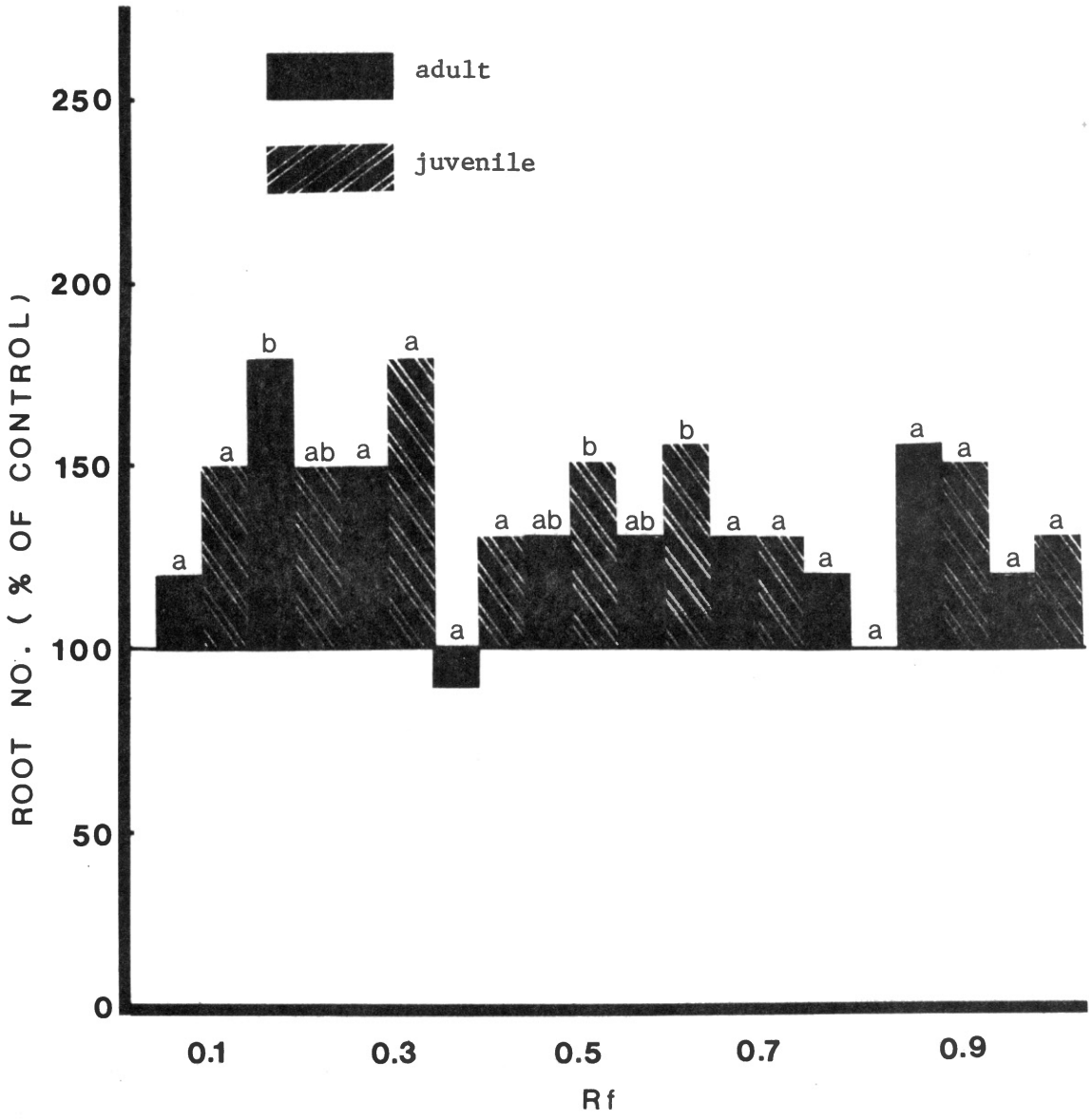

Figure 2. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected February 15. Significant Difference at 5% Level.

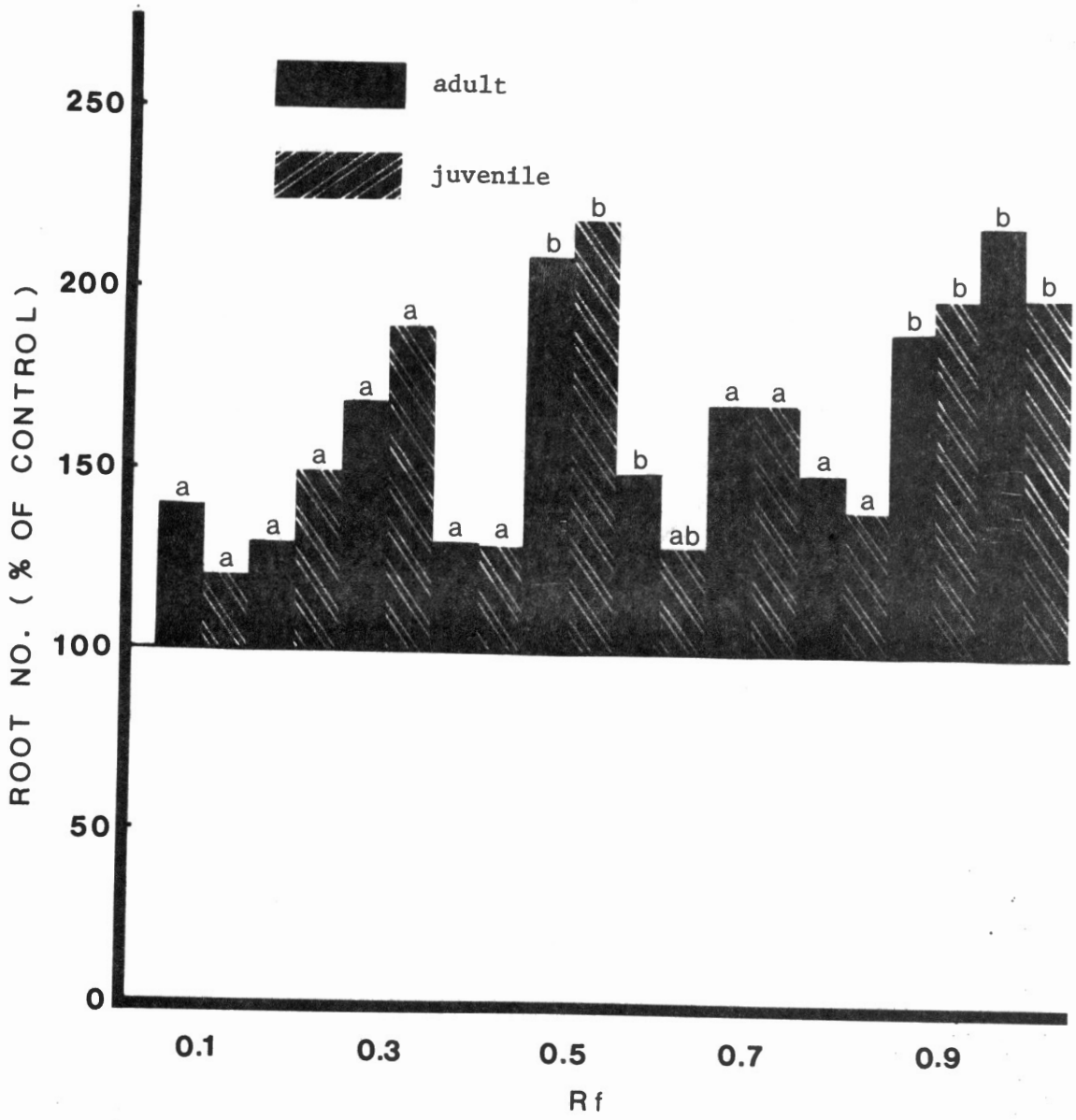

Figure 3. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected February 15. Significant Difference at 5% Level.

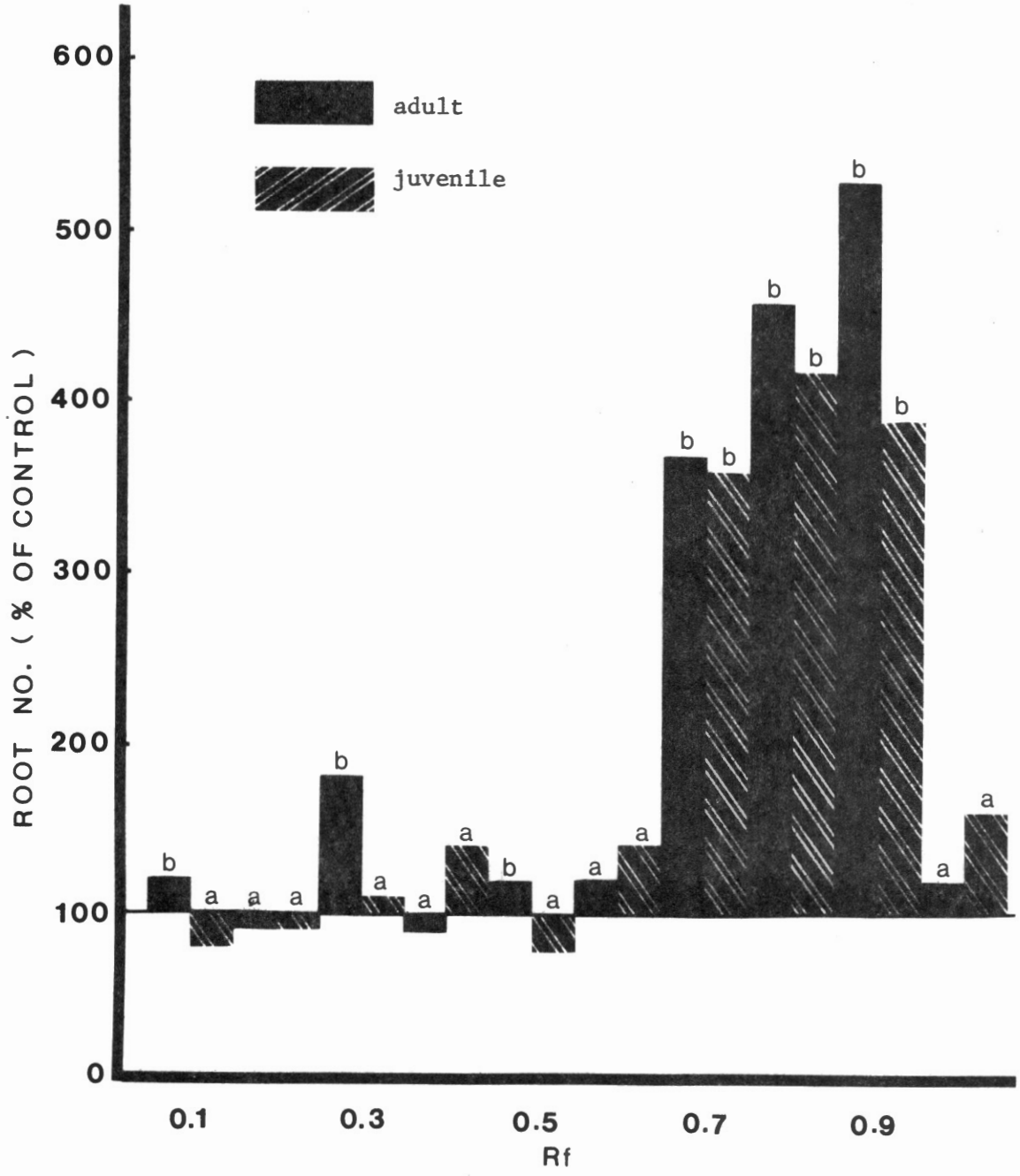

Figure 4. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected April 15. Significant Difference at 5% Level.

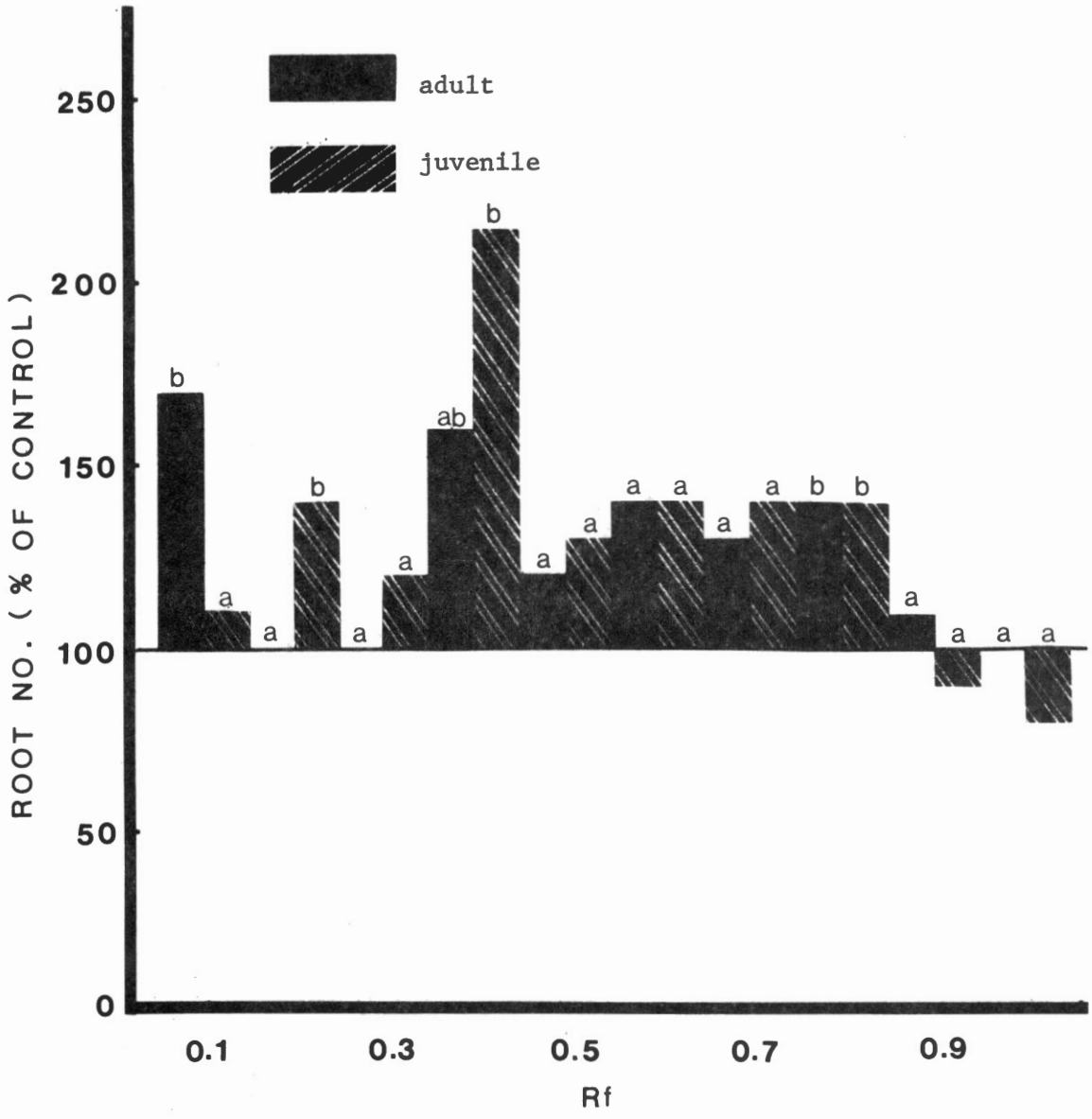

Figure 5. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected April 15. Significant Difference at 5% Level.

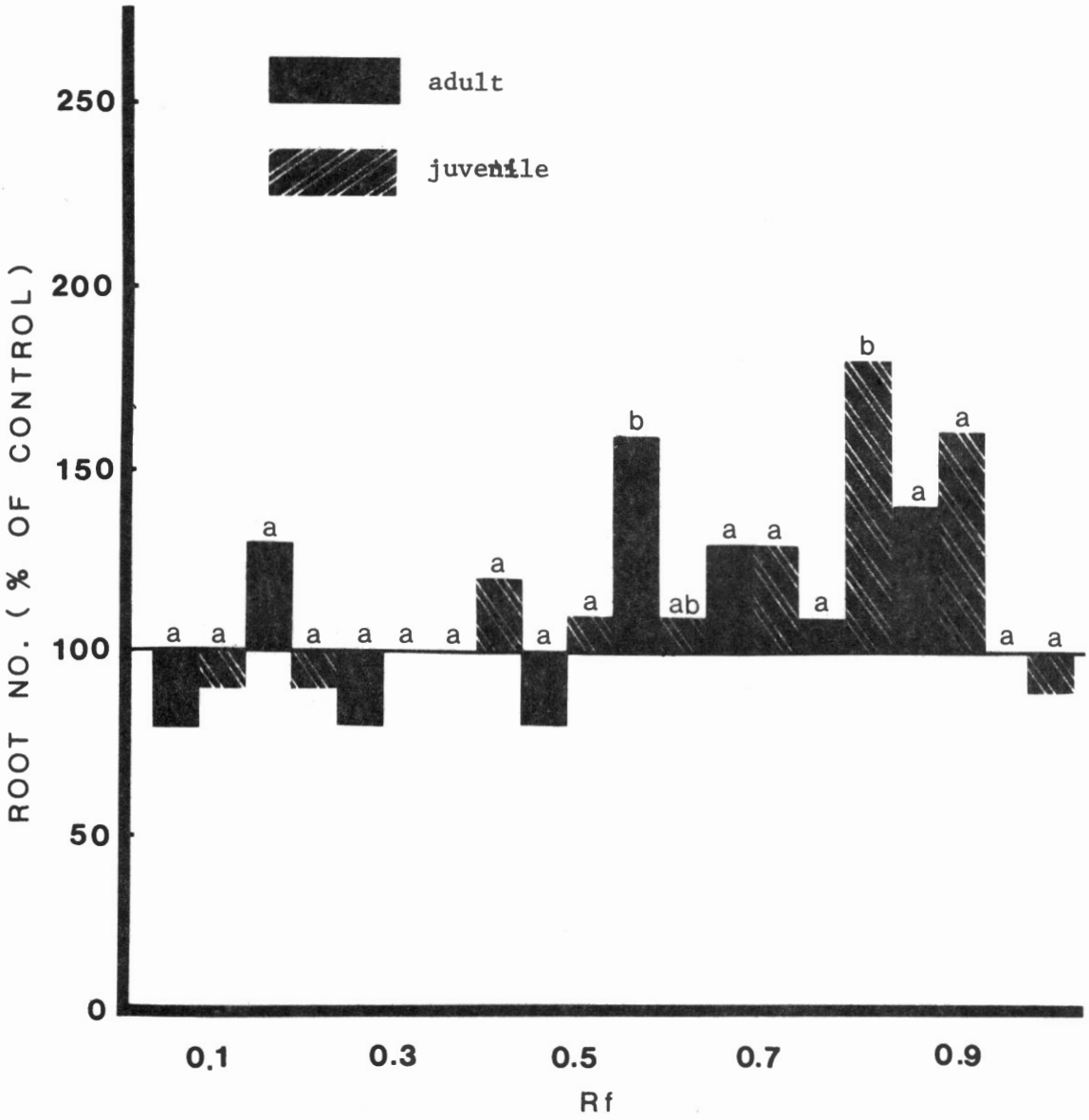

Figure 6. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected April 15. Significant Difference at 5% Level.

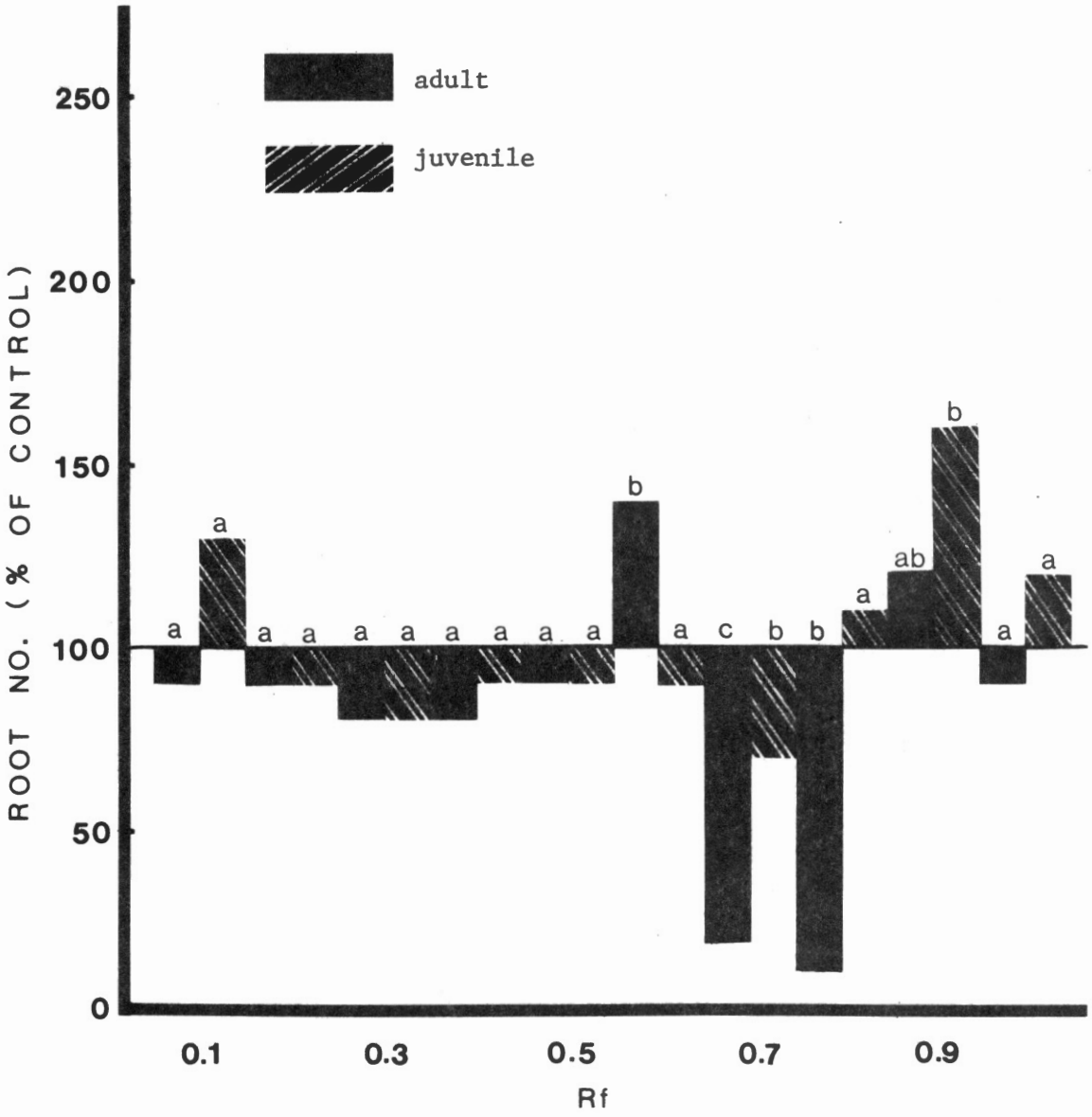

Figure 7. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected June 15. Significant Difference at 5% Level.

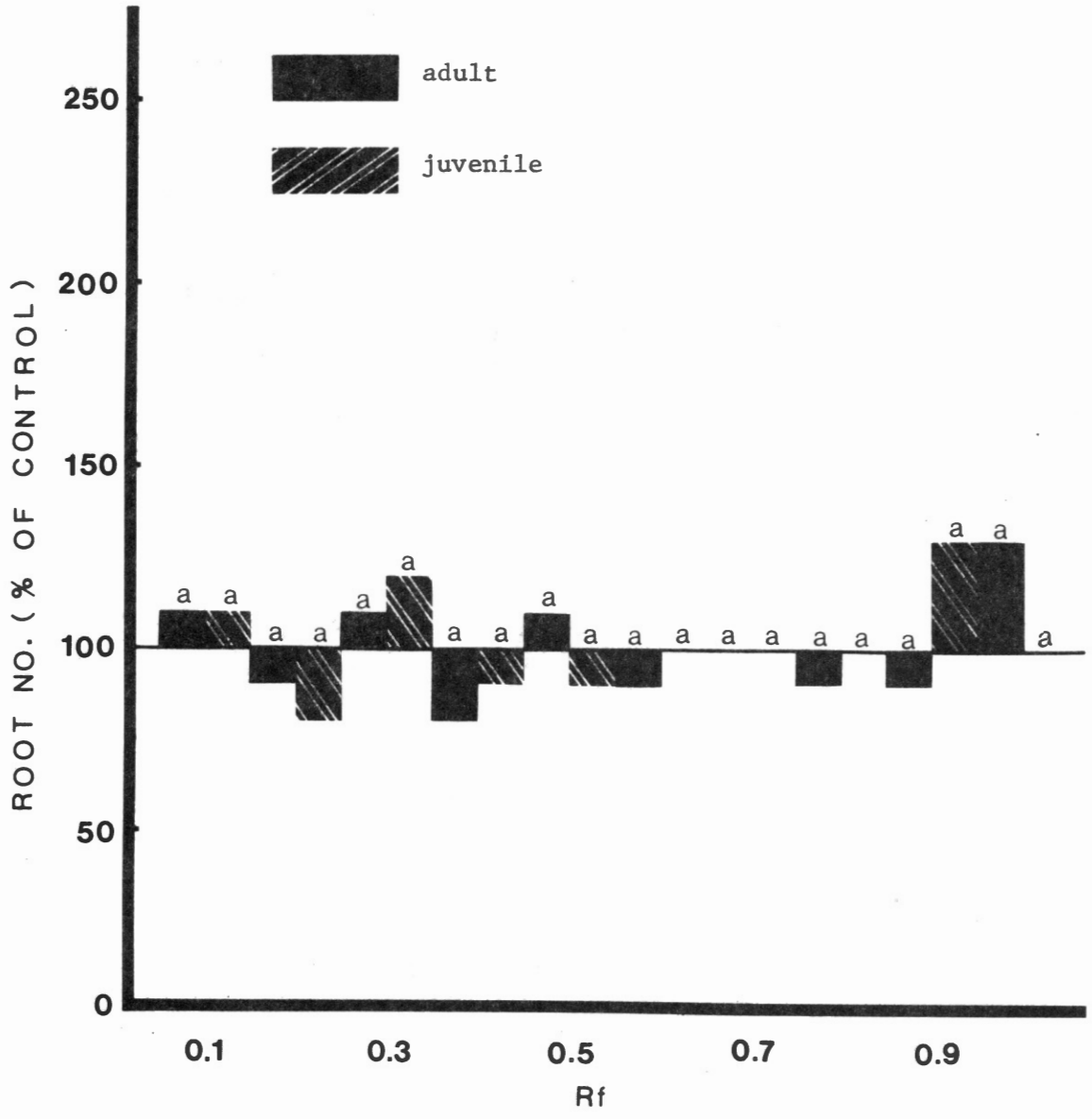

Figure 8. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected June 15. Significant Difference at 5% Level.

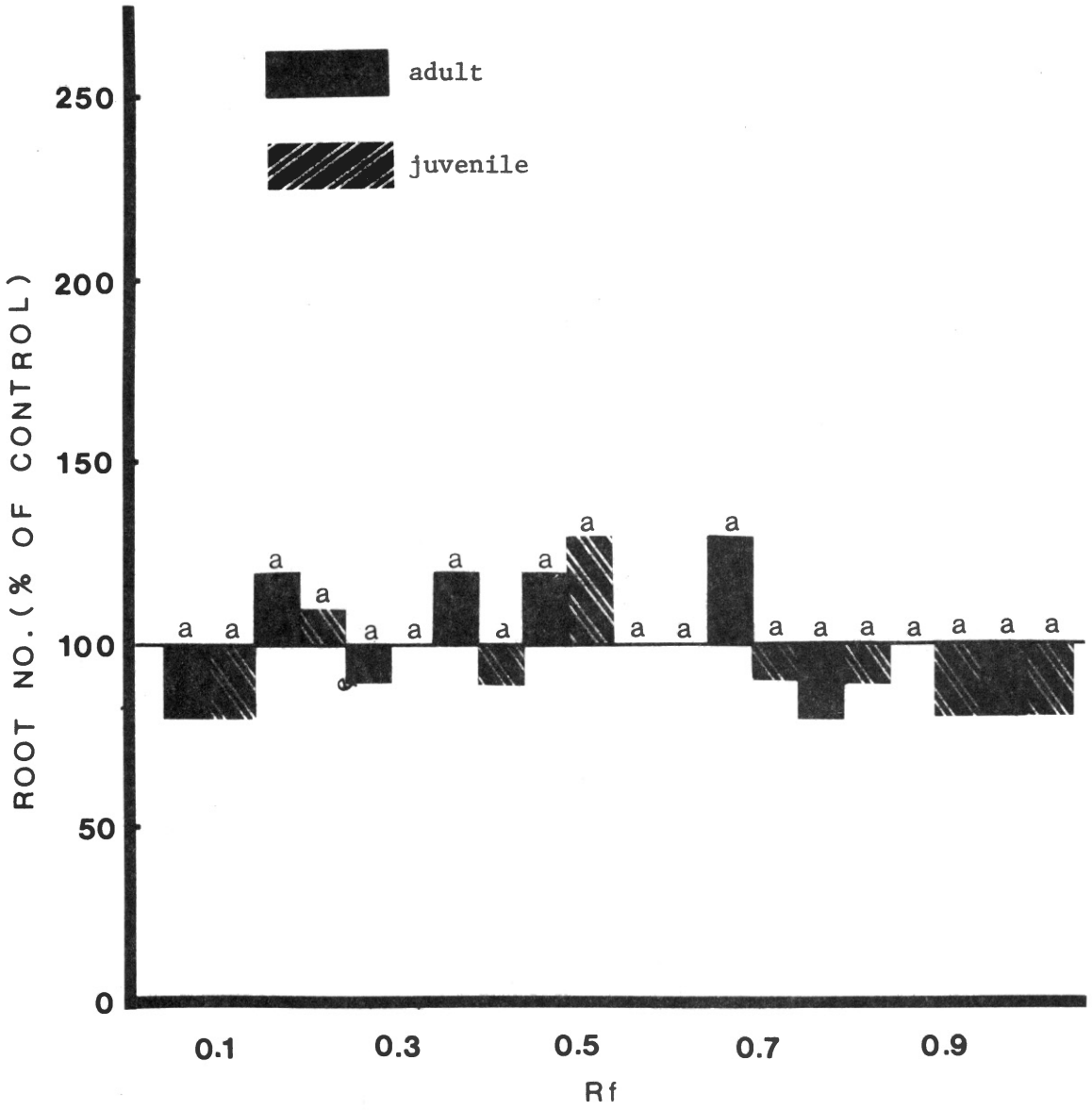

Figure 9. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected June 15. Significant Difference at 5% Level.

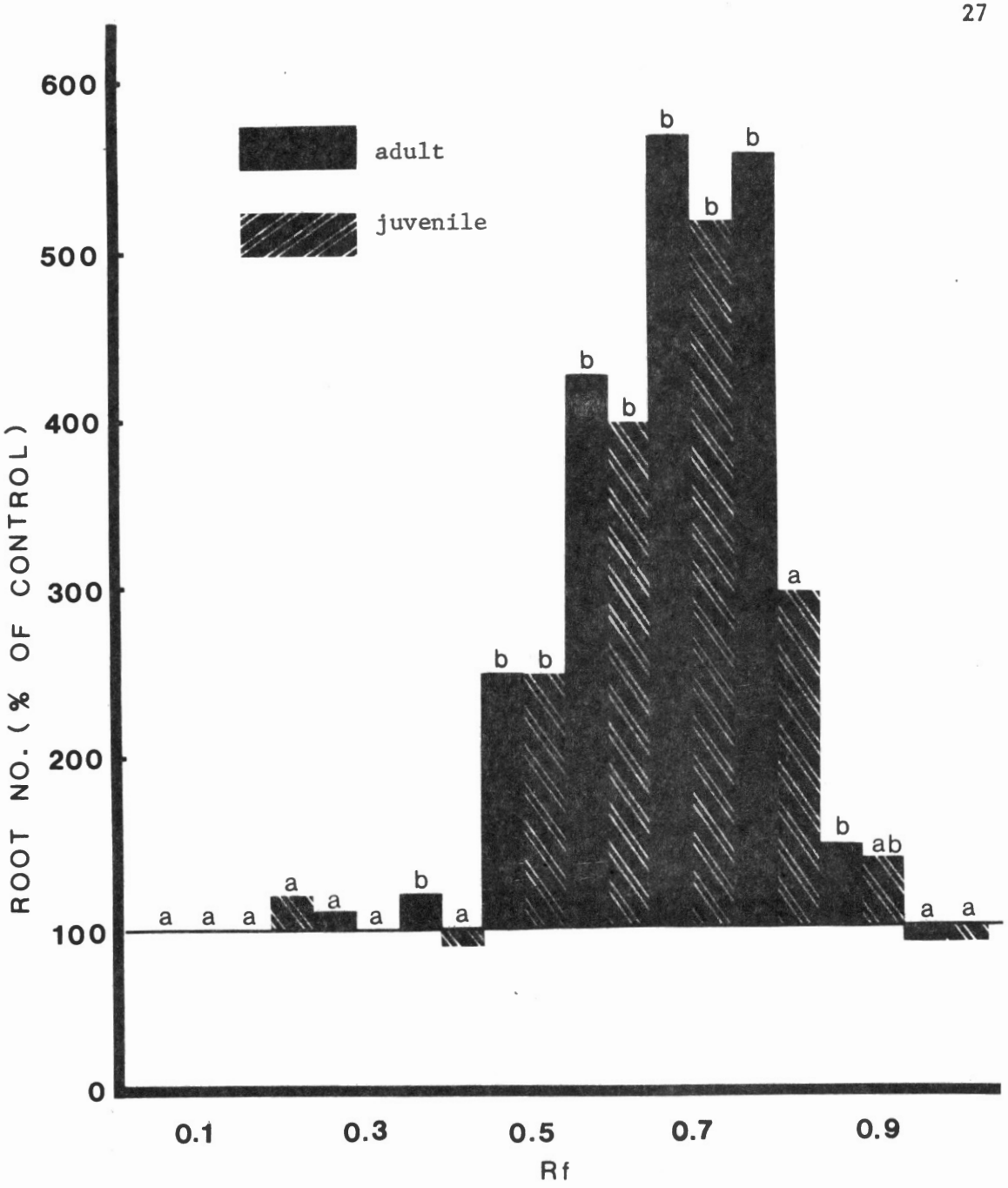

Figure 10. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected August 15. Significant Difference at 5% Level.

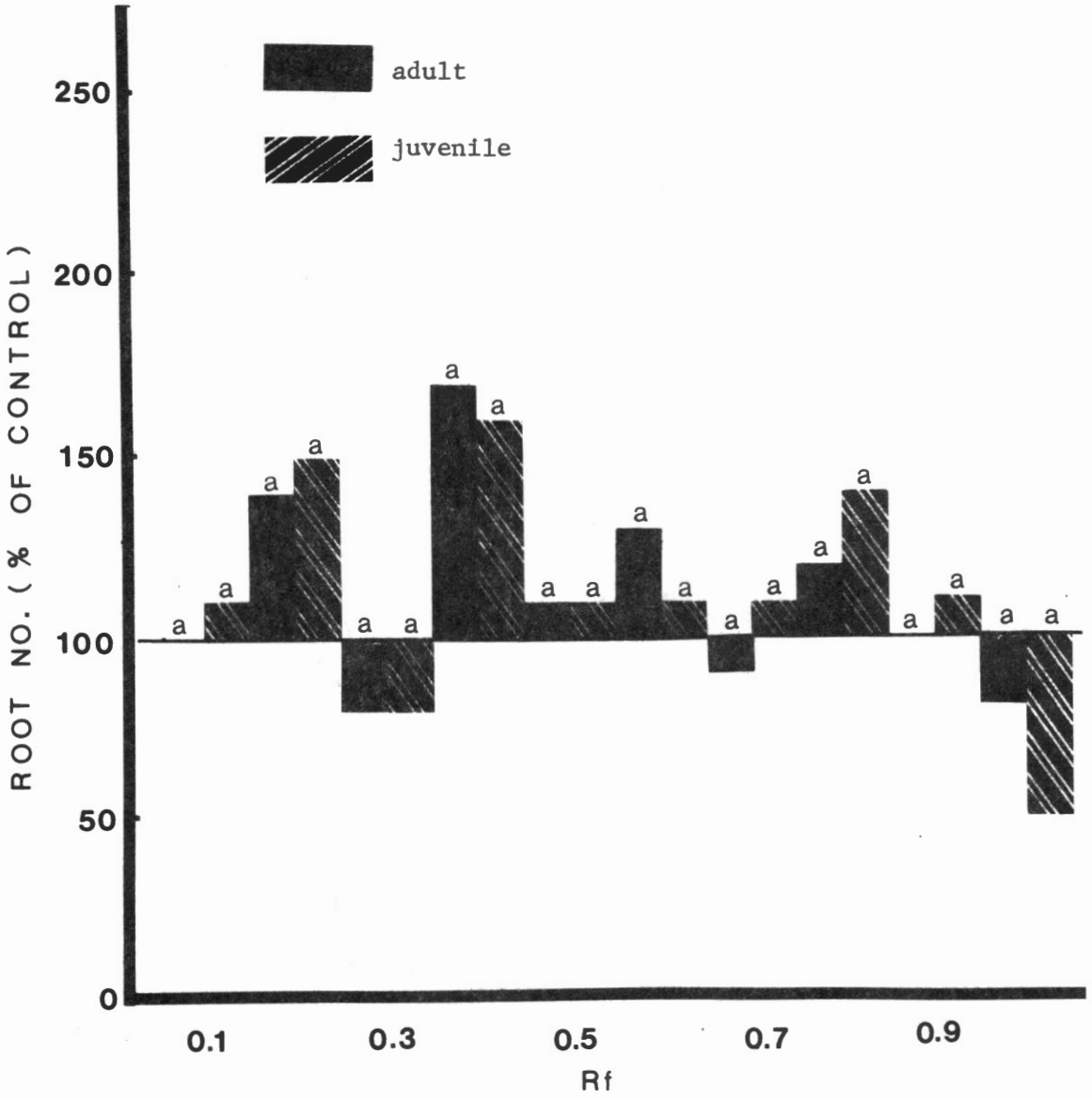

Figure 11. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected August 15. Significant Difference at 5% Level.

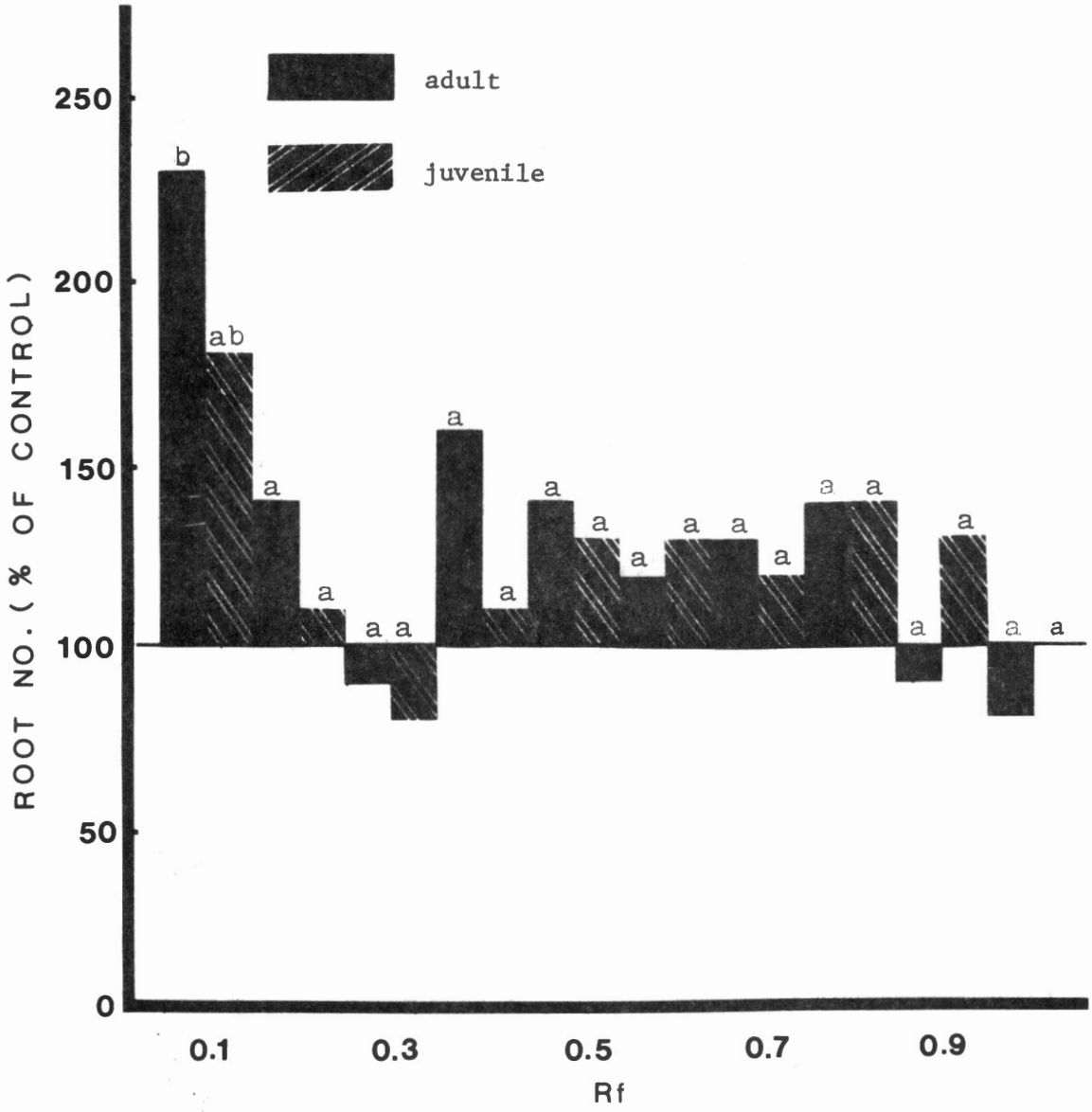

Figure 12. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected August 15. Significant Difference at 5% Level.

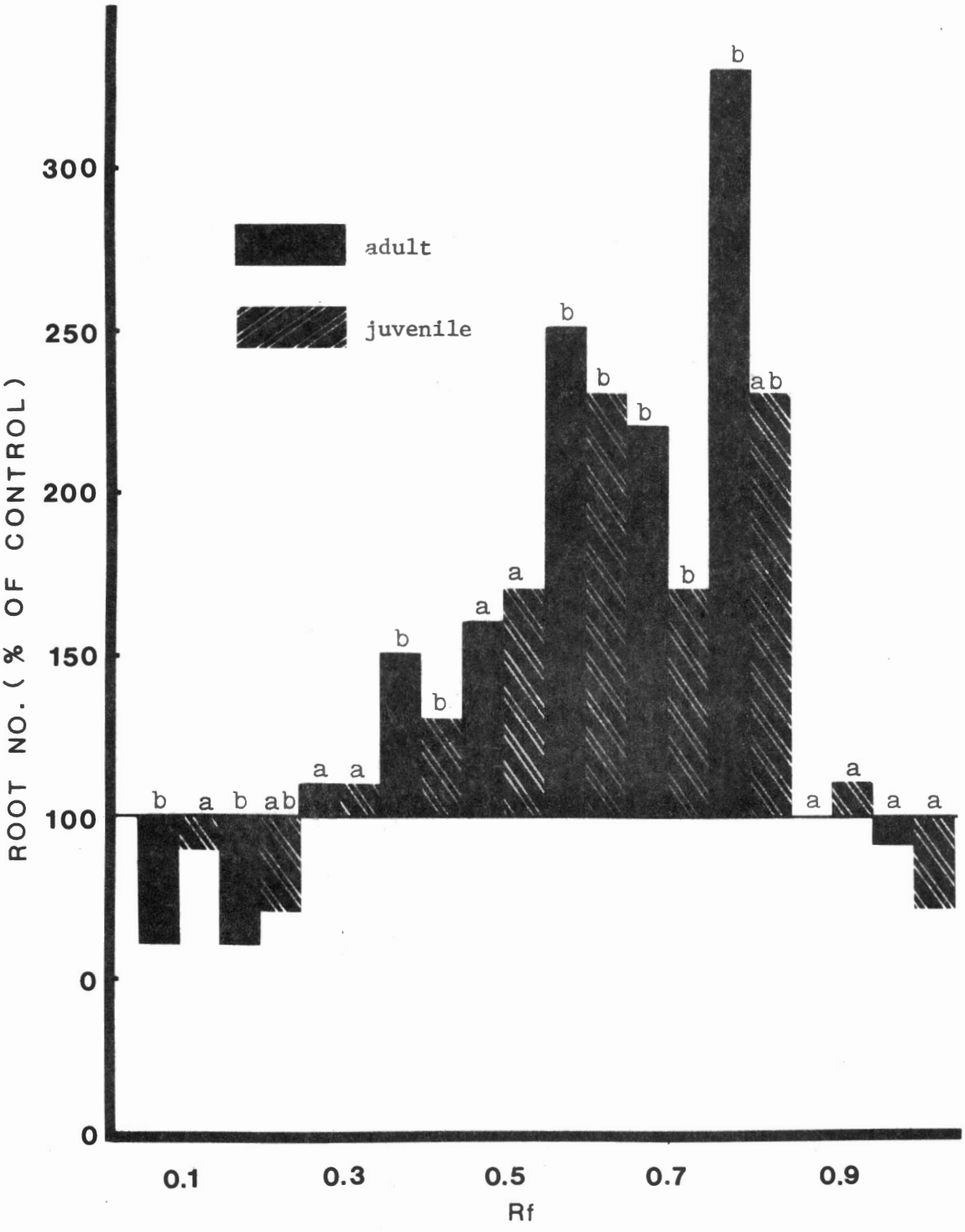

Figure 13. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected October 15. Significant Difference at 5% Level.

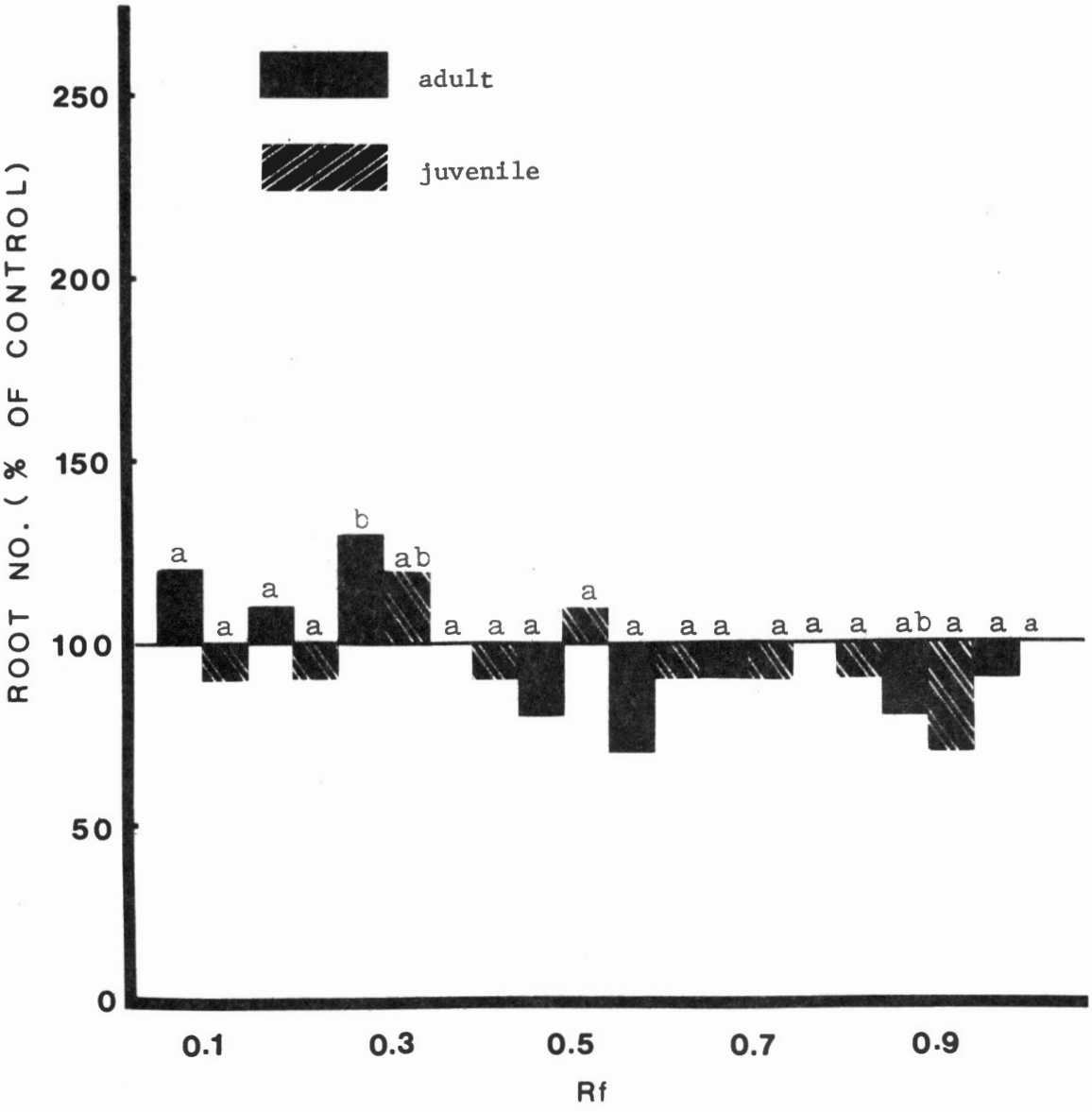

Figure 14. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected October 15. Significant Difference at 5% Level.

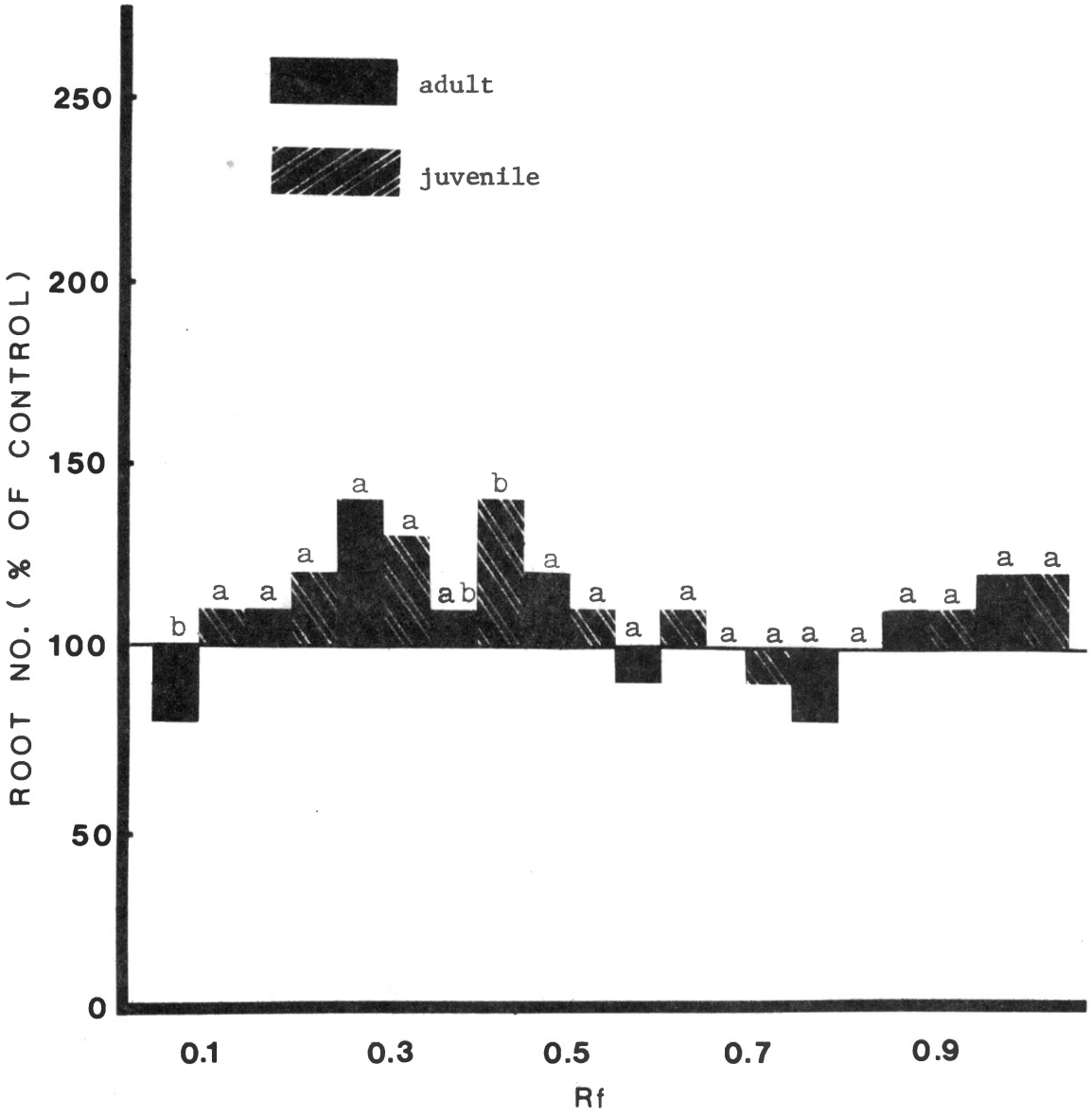

Figure 15. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected October 15. Significant Difference at 5% Level.

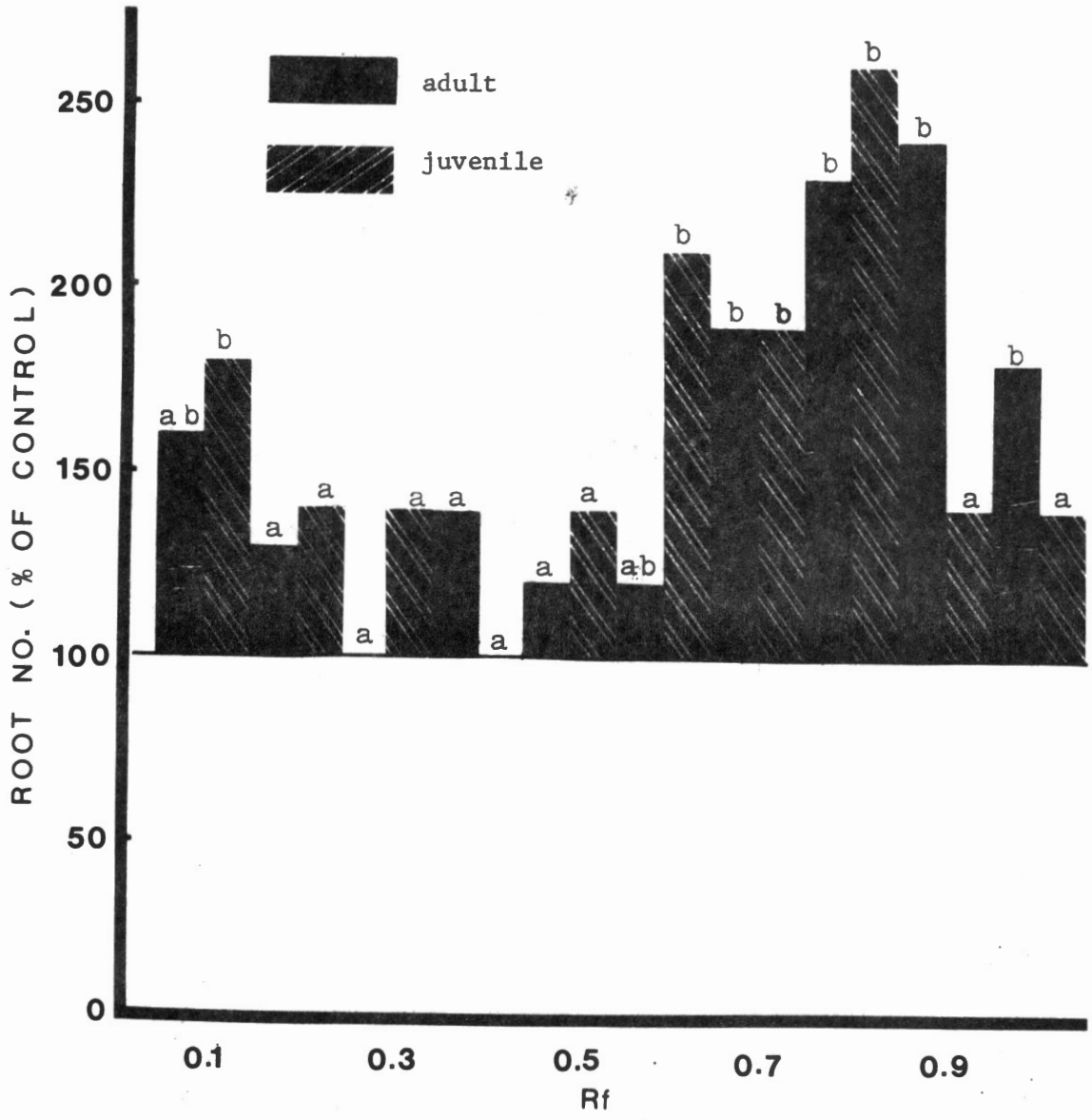

Figure 16. Response of Mung Bean Cuttings to Chromatographed Water Extracts of Pecan Cuttings Collected December 15. Significant Difference at 5% Level.

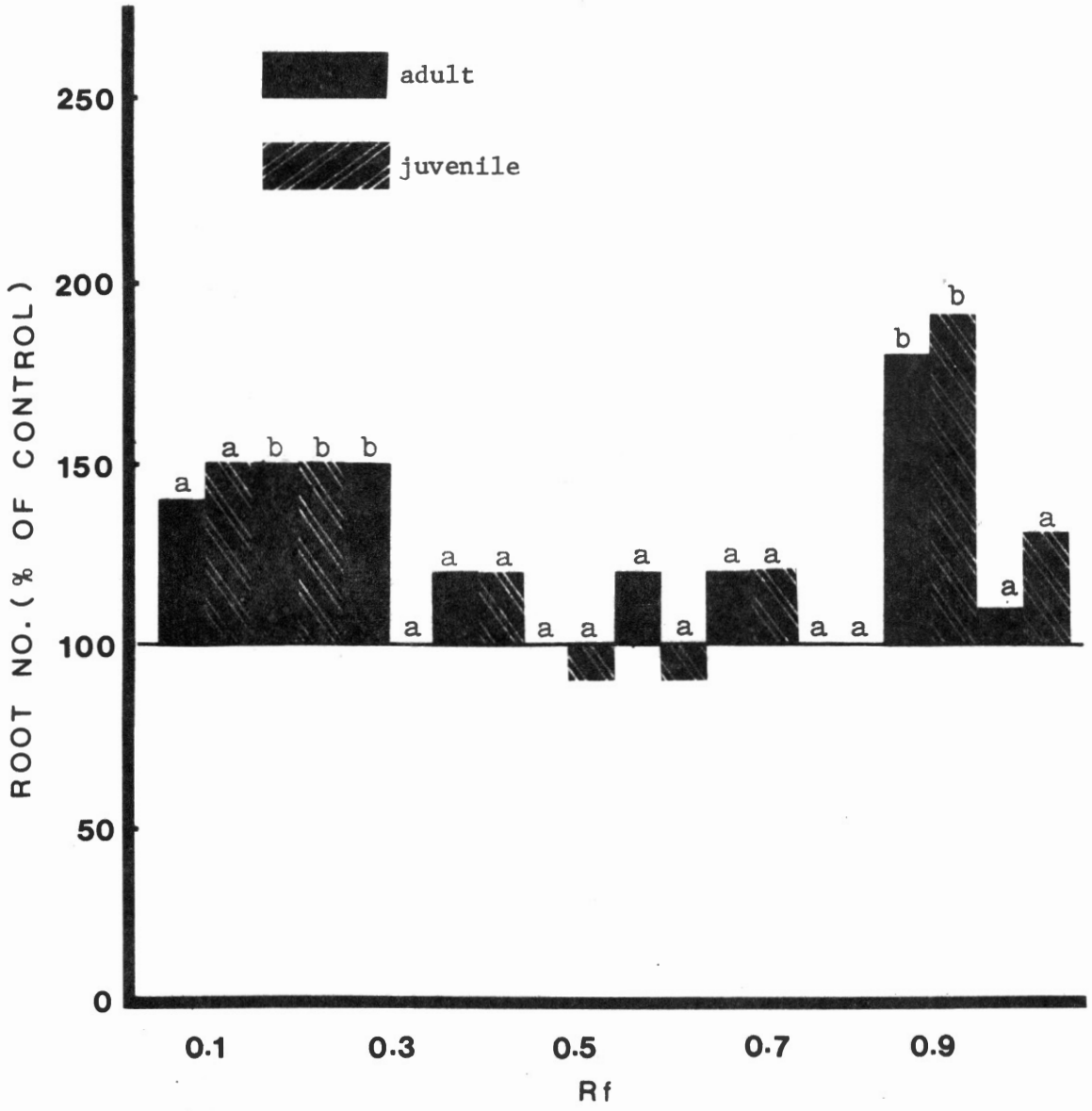

Figure 17. Response of Mung Bean Cuttings to Chromatographed Ether Extracts of Pecan Cuttings Collected December 15. Significant Difference at 5% Level.

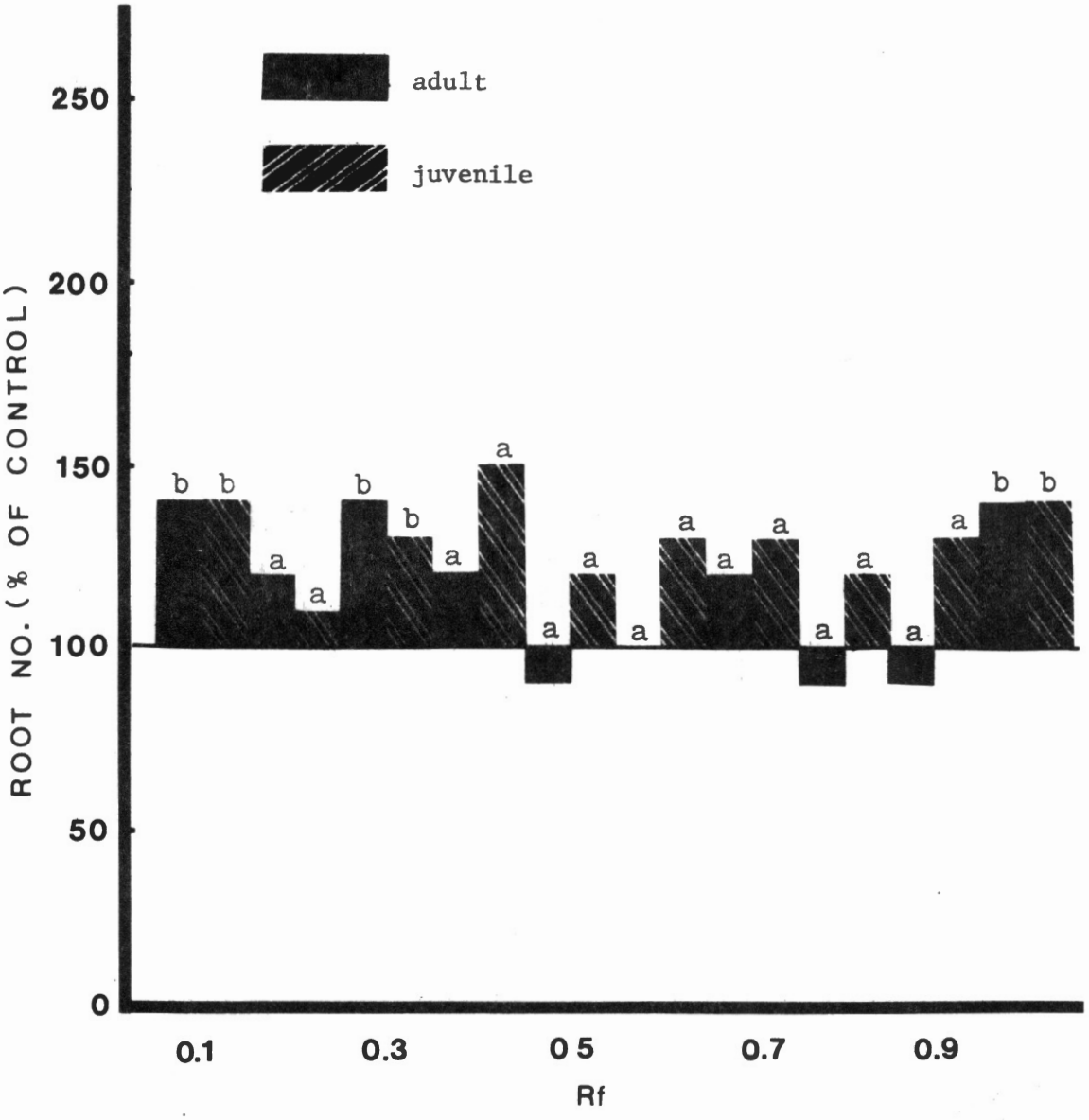

Figure 18. Response of Mung Bean Cuttings to Chromatographed Chloroform Extracts of Pecan Cuttings Collected December 15. Significant Difference at 5% Level.

which were different in their rooting ability. Rooting co-factor activity was even slightly greater in the adult wood than in the juvenile wood (Table III). This does not coincide with the result obtained from the rooting experiment which juvenile wood had higher rooting ability (Table IV).

Data from this study also show little or no significant activity at the four co-factor areas reported by Hess (21). The approximate Rf values of these co-factors using the isopropanol-water (8:2 v/v) solvent system are as follows: co-factor 1, 0.1 in water fraction; co-factor 2, 0.3 in water fraction; co-factor 3, 0.6 in ether fraction; and co-factor 4, 0.8 in chloroform fraction. Only extracts of juvenile cuttings taken from April 15 showed significant activity of co-factor 4 which is considered as the main co-factor involved in the endogenous regulation of rooting. Extracts from adult cuttings taken in April and juvenile cuttings taken in December showed activity in the area of co-factor 1. Activity of co-factor 2 occurred from adult cuttings taken in April. Co-factor 3 activity occurred in juvenile cuttings taken during February.

If the rooting co-factors were responsible for the seasonal rooting response one may expect to find a decrease in the bioassay activity of one or more of the co-factors in those seasons when rooting is low. But this is not the case in this study with pecan cuttings. It appears that changes in bioassay activity did not correlate with the seasonal response (Table IV).

Extracts of cuttings for mung bean bioassay were made at the time of collection. Thus one may suspect that some physiological changes

TABLE III

BIOASSAY ACTIVITIES IN WATER, ETHER AND CHLOROFORM EXTRACTS OF PECAN CUTTINGS

Date	Extract	% of Control	
		Juv.	Adu.
February 15	Water[1]	110	130
	Ether[1]	150	130
	Chloroform[1]	160	160
	W.+E.+C.[2]	140	140
April 15	Water	200	220
	Ether	130	130
	Chloroform	120	110
	W.+E.+C.	150	150
June 15	Water	100	80
	Ether	100	100
	Chloroform	100	100
	W.+E.+C.	100	90
August 15	Water	200	240
	Ether	100	110
	Chloroform	120	130
	W.+E.+C.	140	160
October 15	Water	140	160
	Ether	90	100
	Chloroform	110	110
	W.+E.+C.	110	120
December 15	Water	160	160
	Ether	130	110
	Chloroform	120	130
	W.+E.+C.	140	130

[1]All Rf zones are pooled.

[2]All extracts and Rf zones are pooled.

TABLE IV

SEASONAL CHANGES IN ROOTING ACTIVITY OF PECAN CUTTINGS AND ROOTING ACTIVITIES IN EXTRACTS OF THE CUTTINGS

Date	% of Control[1]		% Rooted		Root No./Cutting		Root Index[2]	
	Juv.	Aud.	Juv.	Adu.	Juv.	Adu.	Juv.	Adu.
2/15	140	140	48	2	4.3 a[3]	0.1 b	4.3 a	1.6 b
4/15	150	150	1	0	0.0 a	0.0 a	1.2 a	1.0 b
8/15	140	160	12	15	2.9 a	0.5 a	2.1 a	2.4 a
10/15	110	120	0	0	0.0 a	0.0 a	1.6 a	1.6 a

[1]All extracts and Rf zones are pooled.

[2]Scale 1-10; 1-4 callus development, none to well developed; 5-10 indicates root development.

[3]Means within rows followed by different letters are significant by the protected LSD, 5 percent level.

occurred before root initiation when cuttings were planted in the rooting medium. It is probable that these physiological changes contributed more to the response of the cuttings than the original state when cuttings were taken.

Taylor and Odom (47) reported that the presence of a root inhibitory substance similar to juglone which appears to play a significant role in the rooting complex of pecan stem cuttings. This type of material was found to move to near Rf 0.86 when isolated by using isopropanol-water solvent system. By further studies, the influence of varying concentrations of commercially purified juglone on rooting of mung bean cuttings has been determined. With a high concentration of juglone in solution, the outer tissues of the mung bean cutting stem were burned. The stem burning increased with increasing concentrations. Rooting of mung bean cuttings increased in the area above the burned tissue. This increased rooting continued with increasing juglone concentrations and tissue burning until a majority of the stem tissue was burned and little stem area remained for root initiation.

Data from this study showed that pecan cuttings varied in the number and intensity of inhibitory zones in each fraction on each collection date. Relatively high rooting of mung bean was obtained near Rf 0.7 to 0.8 in the water fraction on all cutting dates except February and June when rooting was inhibited.

It is possible that the inhibition of rooting found at Rf 0.8 resulted from low concentrations of a compound similar to juglone reported by Taylor and Odom (47), whereas increased rooting in the mung bean was the result of higher concentrations.

Seasonal changes in the area of Rf 0.8 indicate that concentration of this compound is lowest during February and June, and high during other months. No correlation between the activity of this compound in the bioassay and rooting of pecan cuttings could be found when extracts were made at the time of collection.

In summary, no relationship could be established between the co-factor and inhibitor level and the rooting response of pecan cuttings if the extracts were made at the time of collection. There was no significant difference between adult and juvenile cuttings in the content of rooting co-factor and inhibitor.

CHAPTER V

SUMMARY AND CONCLUSIONS

Although pre-treatment of pecan scions with a root promoting substance to obtain rooting has been done by some research workers (14, 39, 42, 45), the pecan appears to be one of the most difficult of plants to establish upon its own roots. It is clear that endogenous rooting factors, other than auxin, control rooting and are produced by leaves or buds or both (2, 11, 13, 59, 62).

The objectives of this study were: (1) to determine differences in rooting responses of juvenile and adult pecan cuttings; (2) to determine differences in seasonal rootability of pecan cuttings; (3) to determine optimum concentration of indolebutyric acid (potassium salt) for rooting; (4) to identify seasonal variations of rooting co-factors; (5) to identify differences in content of rooting co-factors in juvenile and adult pecan cuttings.

The higher rooting response shown by juvenile wood indicates that as the wood matures the rooting of cuttings declines. Although in August there was a higher rooting percentage in adult wood, juvenile wood had much higher root numbers than adult wood.

There was a marked seasonal variation in the rooting of pecan cuttings. Cuttings taken in April and October show no or almost no rooting response, whereas in February and August they could be rooted readily.

To obtain best rooting of pecan cuttings, juvenile cuttings taken February 15 with 10,000 ppm IBA is recommended.

No correlation could be established between the co-factor and inhibitor level and the rooting response of pecan cuttings if the extracts were made at the time of collection.

No significant difference between adult and juvenile cuttings in the content of rooting co-factor and inhibitor was found in this study.

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VITA

Hwei-Jen Jennifer Chiu

Candidate for the Degree of

Master of Science

Thesis: SEASONAL CHANGES IN ROOTABILITY AND ROOTING SUBSTANCES IN MATURE AND JUVENILE PECAN CUTTINGS

Major Field: Horticulture

Biographical:

Personal Data: Born in Taipei, Taiwan, Republic of China, November 29, 1954, the daughter of Mr. and Mrs. Yu-Hung Chiu.

Education: Graduated from Taipei Municipal First Girls' High School, Taipei, Taiwan, in 1973; received the Bachelor of Science in Agriculture degree in Horticulture from National Taiwan University in 1977; completed the requirements for the Master of Science degree in Horticulture at Oklahoma State University in May, 1979.

Professional Experience: Graduate Research Assistant, Department of Horticulture, Oklahoma State University, 1977-1979.