THE EFFECT OF MIST AND ROOT PROMOTING SUBSTANCES ON ROOTING OF GRAPE CUTTINGS

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GRAPE PROPAGATION

HAQ

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AN ABSTRACT OF THE THESIS OF

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Title: The effect of mist and root promoting substances on rooting of grape cuttings.

Rooting of hardwood cuttings of the grape root stock Rupestris Du Lot was examined at the American University of Beirut under out door condition during 1966-67. The effects of mist and root promoting substances were studied. The cuttings were planted in vermiculite. Mist, IBA, alone or in combination with vitamin C, or soaking the cuttings in water, induced greater amounts of callusing and rooting than the control.

In general organic acids, enzymes, and urea reduced callusing and rooting. Mist tended to delay callus and rooting.

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I. INTRODUCTION

Most ornamental and fruit plants are reproduced by vegetative or asexual methods of propagation. Vegetative propagation is the reproduction of new individuals from a parent plant material without any change in the genetic constitution of the new plant as compared to the parent plant from which it is reproduced (28).

Vegetative propagation is done by means of cuttage, layerage, and graftage. Among these methods cuttage is extensively used. It has the advantage of being simple, successful, and overall has a low cost of production.

Reproduction of grapes by means of cuttage, in addition to the above mentioned advantages, makes it possible to obtain uniform stocks to be used for budding or grafting and which are resistant to pests such as Grape Phylloxera (*Phylloxera vitifoliae*) and root knot nematode (*Heterodera maroni*) (24, 44).

The main shortcoming with regard to this method of propagation has been the difficulty of rooting of some fruit and ornamental plant cuttings. With the discovery and the use of growth regulators, rooting of many previously difficult-to-root species has become possible. The application of specific growth regulators has been shown to favor rooting in cuttings of specific fruit and ornamental plants and has received more attention as a means of improving rooting than any other group of materials.

Grapes are grown wherever the environmental conditions are favorable and contribute much to the economy of many producing countries. The best examples are found in Europe where about five sixths of the world's wine is produced. In the United States, California is the greatest producing state with earnings of $111, 216,200 annually from raisin and wine grapes during the period of 1951-55 (52).

The production of grapes and the establishment of a well planned industry has tremendous possibilities in the Peshawar Valley in West Pakistan. The climate and environment of this region are highly suitable for economical grape production but at present not enough grapes are produced for local consumption. Many areas of land could be put under plantation and good quality grapes could be produced if more was known concerning proper production methods including propagation and use of disease and insect resistant root stocks.

Grape production is of paramount importance in the economy of Lebanon. Fourteen thousand six hundred sixty four hectares of land are annually under grape plantation. Lebanon produced 75990 metric tons of grapes in 1966 (5).

The present study was undertaken to determine the most efficient method of inducing rooting in grape cuttings. The various treatments tested included various growth regulators, enzymes, and organic acids both with and without mist.

II. REVIEW OF LITERATURE

The quantity of the reports of experimental works on methods of propagating plants by cuttings from the 17th Century onwards indicates the early and continuing recognition of the importance of these means of reproducing economic plants. Cuttings are portions of plants separated from the parent plant which are capable of reproducing the missing parts under the proper conditions. The type of cutting usually used for grapes is the hardwood cutting which is prepared during the dormant season from fully mature wood of the previous season growth (1, 28).

One of the phases of research, which has received a considerable amount of attention, has been the determination and the control of internal and external factors which affect rooting of cuttings. The amount of stored food, the age and stage of maturity of the tissues, the formation of callus, and the presence or absence of leaves and buds on cuttings are some of the more important internal factors. Among the external factors affecting rooting are rooting media, chemical and hormone treatments, mist sprays and humidity, light, temperature, and mechanical treatments (6, 7, 11).

The formation of callus at the basal end of cuttings was at one time considered to be a vital stage in the rooting of hardwood cuttings. Callus, a mass of irregular, thin walled, parenchyma cells arising primarily from the most recently differentiated cells in the region of the vascular cambium and especially those of the phloem, is commonly associated with injuries to the plant. It is thought to be beneficial to cuttings through its sealing of the opened ends of vascular tissues and its protective covering of exposed tissues (28). Recently, it has been pointed out that many cuttings root without callusing and many cuttings callus but fail to root. This has suggested that the relationship between callusing and rooting needs to be examined (1, 28).

In the present investigation the effects of callusing, certain organic and inorganic chemicals, growth regulators, and mist were examined. The medium used is of interest as it has only recently become available (26, 28, 33).

It has long been recognized that mist and humidification techniques are very useful in propagating plants by cuttings (6, 28). Most of the early work on mist was concentrated on the use of continuous mist during the day light hours only or for 24 hours.

The use of mist for the rooting of cuttings was introduced by Rains (40) and Gardner and Fisher (reviewed by Synder, 45). Rowe-Dutton (37) has extensively covered the subject in her book Mist Propagation of Cuttings. She stressed the importance of good drainage of the rooting media and of the effectiveness of root promoting substances under mist condition.

Synder (45), in a review of the history of mist propagation, remarked that over one hundred different scientists and nurserymen had authored more than 300 articles and papers on mist propagation. He reported that the first use of mist propagation for cuttings was made by Spencer in 1936, but, that the first written report of its successful use was by Rains of Harvard University. Between 1942 and 1945, Pridham, Stoutmeyer, Gossard, and Cochran presented preliminary results of the use of mist for such diverse plants as rhododendrons, vacciniums, chaenomeles, symplococos, pecans, and peaches (45).

An increase in the interest in mist propagation started in the early 1950's when various research stations and commercial firms studied propagation factors, developed equipment and applied their results to the practical field. In 1955, papers by Floor, Hess & Synder, and Synder & Hess brought the mist technique to the attention of horticulturists and nurserymen in many parts of the world (45).

In mist propagation, an apparatus is used which disperses droplets or small particles of water in such a way that the surfaces of leaves or stems are covered with a thin film of water. Water evaporates from the surface film to the atmosphere, but little or no water is lost from the leaf or stem tissues. In this way mist helps to maintain the turgidity of the tissues of the cuttings (46). Suitable well drained rooting media, hormone treatments, and time of collecting cuttings have been shown to be the important factors affecting successful rooting under mist propagation (7, 29, 30, 34).

Sharpe (42) has found that Muscadine grape cuttings can be rooted quite readily by the constant mist method. Fahmy (18), using intermittent mist for 11 varieties of Muscadine grapes, has obtained very successful rooting of cuttings.

Dolle and Mitchell (15) wrote that, with mist propagation, the Black Shiraz grape produced uniformity in growth and a low failure rate (1 percent) in the field. In other studies Hartmann and Whisler (30) reported high percentages of rooting of cuttings of peaches, plum, grapes, olive, cherry, pear, apricot, lemon, and many woody ornamental species when propagated under mist alone or with 4000 ppm IBA treatments.

Root promoting substances can be applied to cuttings in a number of different methods (4). Three common methods used are the hormone powder, the concentrated solution dip method, and the prolonged weak solution soaking method. The hormone powder method (4, 7, 28) consists of wetting the basal half inch of the cutting and then dipping this portion into talc containing the chemical or hormone material. Powder containing IBA or NAA is especially effective in stimulating root formation of more easily rooted cuttings.

The concentrated solution dip method (7, 13, 27, 28), used by Hitchcock and Zimmer and described by Cooper (14), consists of dipping the bases of cuttings for a few seconds in a 50 percent Ethyl Alcohol solution containing a high concentration of the root promoting substances. The large percentage of alcohol used in these concentrated solutions makes the method helpful in preparing solutions of insoluble compounds such as Naphthalene acid amide.

Prolonged soaking with weak root promoting substances was widely used in early work. The duration of the treatments ranged from few to 24 hours (4, 7, 22).

Many chemicals have been used in efforts to induce root formation in plants difficult to root or to improve rooting quality (7, 11, 31). Sugars, nitrates, acetic acid, and compounds of zinc, boron, manganese, and iron have been reported to be effective (10, 23, 28). In some of the early studies (28), treatments of tomato and privet cuttings with sugar and compounds of manganese, iron, and phosphorous were tried. Improved rootings often resulted, especially with potassium permanganate ($KMnO_4$). Since these initial studies, only potassium permanganate has been considered to improve rootings enough to be useful.

For grapes, Winkler (51) reported that the greatest stimulation of root development by $KMnO_4$ was obtained by soaking for 24 hours with a concentration of 0.05 mols of the chemical. After using this method, he reported that the number of vigorous rootings of Champini grapes was appreciably increased.

In another report, the use of $KMnO_4$ was found to improve rooting of 41-B de Millardet Conderc grapes. However, the application of Beta-indole-3-butyric acid or Beta-indole-3-acetic acid at 5, 10, and 25 mg/1 was found to be more effective (48).

Soaking of cuttings in water has been reported to be effective in rooting of cuttings. Almela et al. (3) studied the effect of different periods of soaking cuttings in water for periods ranging from 24 to 120 hours. He showed that the rooting was increased by all treatments; but that, 24 and 48 hours were the best for Malbeck, and 48 and 96 hours were best for the Kober variety. Rooting capacity in Rupestris Du Lot, Malbeck, and Kober 5BB, according to Tizio (47), was greatly improved by immersing the bases of cuttings in running water for 48 hours.

Oprea and Puiu (36) has noted that cuttings of the root stock Berlandierix Riparia Kober 5BB gave maximum rooting after the cuttings were inserted in pots at soil moisture levels ranging from 5 to 100 percent.

During the past 25-30 years, growth regulators have been proved to be very effective in improving the rooting of cuttings (20, 35, 38, 50, 52). As Cooper (13) has shown, an initial basal treatment with a growth substance is effective in inducing roots on many plants which are usually difficult to root.

Some of the common materials in use are indolebutyric acid (IBA) and naphthaleneacetic acid (NAA). These materials were found to be effective with a great number of plants. A concentration of 10,000 to 20,000 ppm is required to root the more difficult cuttings. On the other hand IBA at 4000 to 10,000 ppm will induce rooting on most kinds of cuttings (7). For instance, Doran (16) found that 0.3 percent and 0.8 percent solutions of IBA, in water, usually resulted in a larger percentage of rooting in cuttings of woody plants than did lower concentrations.

On the contrary, Tureckaja (49) reported that cherry and grape cuttings produced satisfactory rooting after being soaked for 15 hours in 200 mg per liter of hetero-auxin or only 25 mg per liter of IBA. In addition, Aguirre et al. (2) obtained a good percentage of rooting, as compared to the control, in some difficultly rooting varieties of grapes treated with IBA or NAA at 5, 15, or 25 mg per liter for 12 hours.

Dragas and Arramov (17) reported that grape cuttings from three root stocks responded, on the whole, best to the hormonal treatment of 2 to 3 different concentrations.

Harmon (23) obtained good rooting by soaking grape cuttings of a number of varieties in IBA solutions of 0.001 percent, 0.005 percent, 0.01 percent, 0.015 percent, or 0.02 percent concentration for 8, 16, and 24 hours. Soaking for 24 hours in all concentrations gave good results as compared to the cuttings treated with distilled water which served as a check. In another experiment, neither IBA or NAA had any significant effect on the percentage of cuttings rooted, but the former increased the number of roots per cutting (39).

Organic compounds have been reported effective in rooting of cuttings when used in combination with growth regulators. In particular, attention has been centered upon vitamins. Tizio (47) found that a mixture containing 0.005 percent IBA plus biotin (vitamin) at 1 mg per liter most successfully promoted rooting of grape cuttings.

Untreated grape cuttings formed fewer roots than cuttings treated with a 0.01 percent solution of IBA. The presence of 0.2 percent ascorbic acid (vitamin C) with the growth substance failed to produce any further improvement in rootings (41). Cajlahjan et al. (10) studied the effect of bacterial secretions, IBA at 0.02 percent, and ascorbic acid at 0.2 percent on 3 varieties of grape cuttings after soaking the cuttings for 18 hours. He found that both root and shoot development was stimulated by all treatments.

Tizio (47) found that, at 25 and 50 ppm, both NAA and IBA increased rooting in grape cuttings. He also reported that combinations of 50 ppm IBA with 0.01, 0.1, and 1 percent yeast extract had a positive action on rooting. The action increased with the increase of IBA/yeast ratio.

For each material and species of plants, there is a critical level of effective concentration. Concentrations above this critical level for the species may result in injury. This may merely inhibit bud development or it may cause yellowing and dropping of leaves, blackening of the stem and eventually causing the death of the cuttings (28).

III. MATERIALS AND METHODS

Rooting of cuttings of the grape rootstock Rupestris Du Lot was examined using hardwood cuttings taken during the autumn of 1966 from 5-year old plants growing in the vineyard of the Agricultural Research and Education Center (AREC) of the American University of Beirut in the Beqa'a plain, Lebanon. The rootstock plants were pruned annually, irrigated regularly, and appeared to be in good healthy, growing conditions.

The terminal leafy and softwood portions of the canes were removed and discarded. Cuttings approximately 22 cm long and 1 to 1.5 cm thick were made from the hardwood portion of the canes with a sharp pruning shear. The cut at the basal end of each cutting was made straight across, just below a node. The cut at the top of each cutting slanted at an angle of 45^{o} and was one inch above the top node in order to avoid injury to the top bud.

Small bundles, consisting of 150 cuttings each, were made and were kept in moist sand to avoid drying before planting. Later, during handling and planting, the cuttings were wrapped in moist paper to avoid dessication.

A cutting bench was prepared on the Beirut campus of the American University of Beirut, in an open area near the western boundary of the campus. The metal framed bench was 280 cm long, 70 cm wide, and 50 cm above the ground. It was divided into two sections, one of which received a mist treatment and the other no mist application.

Each section of the bench was filled to a depth of 20 cm with a rooting medium of vermiculite, on top of a 5-cm layer of gravel spread to facilitate drainage of water from the bench. Vermiculite is one of the newer micaceous minerals which has been reported to be especially promising for rooting of cuttings. It is hydrated magnesium-aluminum-iron silicate which is heated until the moisture trapped within its framework expands and forces the various planes of the mineral apart. The result is a very light nearly inert material with a large water holding capacity but with good drainage and good aeration. In this media, cuttings are reported to produce a desirable type of fibrous and branched root system (12, 28, 33).

A non-automatic mist system was used. One section of the cutting bench received continuous mist, the other was shielded from the mist by a plastic sheet. The latter section was irrigated when necessary.

The experiment was layed out in a split plot randomized complete block design, in which the main (whole) plot treatments were mist application versus no mist application. The subplot treatments were different growth regulators or chemicals as follows:

1) Control - no treatment.

2) Soaked in water for 24 hours.

3) Urea at 100, 1000, and 10,000 ppm for 6 hours.

4) IBA at 50 ppm for 18 hours.

5) Vitamin C at 200 ppm for 18 hours.

6) Vitamin C + IBA at 200 and 2000 ppm respectively for 18 hours.

7) $KMnO_4$ at 20 ppm for 12 hours.

8) $KMnO_4$ + IBA at 20 and 2000 ppm respectively for 6 hours.

9) CCC (Cycocel) at 500 and 2000 ppm for 5 minutes.

10) Organic acids (Malic, Tartaric, and Citric Acid) at 200 ppm each for 18 hours.

11) Enzymes (Alpha-amylase, Beta-amylase, and Lipase) at 1000, 1000, and 500 ppm respectively for 18 hours.

Each subplot treatment consisted of 10 cuttings and was replicated four times in each of the main plot treatments. The cuttings were set on March 6, 1967, 2.5 cm from each other, in rows with a spacing of 5 cm between rows and at a depth of 15 cm each. Each row consisted of 3 subplot treatments for a total of 30 cuttings.

The cuttings were examined every 7 days and the following observations were recorded:

1) Number of cuttings callused.

2) Time of callusing to start.

3) Time for rooting to start.

4) Number of dead.

5) Number of cuttings rooted.

After every observation, the cuttings were replanted after discarding the rooted and dead cuttings. Observations were continued for a period of 91 days after which the experiment was terminated.

Statistical analysis of data was done according to the method appropriate to the design (32).

IV. RESULTS AND DISCUSSION

An experiment was conducted during the academic year 1966-67 on the campus of the American University of Beirut to find effective means of improving the rooting of grape cuttings under outdoor conditions. The results obtained are reported in Tables 1-4. The analysis of variance for each character studied is given in the Appendix in Tables 5-8. The experimental findings for the rooting of grape cuttings are reported for callusing and rooting.

Callusing

Callus formation and number of cuttings callused were affected by mist and chemicals. During the observation period it was noted that callus formation started earlier and that more cuttings callused under no mist than under mist at first but that ultimately significantly more cuttings callused under mist as compared to no mist treatment (Table 1).

Cuttings soaked in water or treated with the low rate of urea, all rates of IBA, vitamin C, vitamin C + IBA, $KMnO_4$ + IBA, CCC at the low rate, and malic acid highly significantly increased the amount of callus

Table 1. Effect of mist and chemicals on callusing of Rupestris Du Lot grape hardwood cuttings after 91 days.

Treatments	Mist[1]	No mist[1]	Average
Control	5.5	4.7	5.1
Soaking in water	8.7	9.2	8.9**
Urea, 100 ppm	8.0	4.5	6.2**
Urea, 1000 ppm	6.2	4.5	5.3
Urea, 10,000 ppm	5.0	3.5	4.2
IBA, 50 ppm	10.0	10.0	10.0**
IBA, 120 ppm	10.0	9.0	9.5**
IBA, 200 ppm	9.5	9.0	9.2**
Vitamin C, 200 ppm	7.2	5.5	6.3**
Vitamin C + IBA, 200 + 2000 ppm	9.2	8.2	8.7**
$KMnO_4$, 20 ppm	5.5	5.2	5.3
$KMnO_4$ + IBA, 20 + 200 ppm	8.2	9.5	8.8**
CCC (Cycocel), 500 ppm	7.7	6.2	6.9**
CCC, 2000 ppm	5.2	4.7	4.3
Malic acid, 200 ppm	7.7	6.0	6.8**
Tartaric acid, 200 ppm	5.0	4.0	4.5
Citric acid, 200 ppm	4.2	4.0	4.1
Alpha amylase, 1000 ppm	5.7	5.7	5.7
Beta amylase, 1000 ppm	3.7	3.0	3.3*
Lipase, 500 ppm	4.7	4.0	4.3
Average	6.8*	6.0	

* Significant at 5% level.
** Significant at 1% level.
[1] Mean of four replications with 10 cuttings in each treatment.

formation. Beta amylase produced a significantly lower number of cuttings callused than the untreated control (Table 1).

IBA at 50 and 120 ppm induced 100 percent callusing of cuttings under mist treatment and at 200 ppm showed the next highest number of cuttings callused. Beta amylase had an injurious effect on the cuttings callused. IBA at 50 ppm under no mist also produced 100 percent success in callusing.

Mist was found to improve rooting significantly. Out of 20 treatments, 16 produced a higher number of cuttings rooted under mist than no mist, 2 treatments gave the same results under either, and only 2 produced more callusing under no mist than under mist.

The interaction between mist and treatments was highly significant statistically as shown by the analysis of variance (Table 5).

The results show that the best treatment combinations, generally, were those in which IBA was applied in conjunction with mist. They were closely followed by IBA and no mist and soaking in water. The remaining treatment combinations, even though they might have produced greater amounts of callus than the control combination, failed, by an appreciable margin, to induce callus comparable to the better combinations. The combination of beta amylase and no mist produced significantly less callus than the control combination.

During the examinations of the cuttings for callusing and rooting, it was noted that callusing seemed to develop early and rooting occurred somewhat late. In order to sample this trend the average numbers of cuttings developing callus and roots under mist and no mist were determined. These data are presented in Figure 1.

This figure shows that callusing had reached its highest level just before the end of the fourth week of observations while rooting reached its highest level just after the fourth week. The data obtained on this date of observation appears to present the best picture of the early trends of both callusing and rooting. One of the most interesting aspects of the curves in Figure 1 is the striking similarities between the curves for callusing with those of rooting under both mist and no mist. It appears that in grapes callusing is closely related to rooting.

The number of cuttings developing callus, 28 days after planting, is shown in Table 2. Mist was found to have produced highly significantly more callus on the cuttings than no mist treatment.

Callusing was highly significantly increased by soaking cuttings in water or treating with IBA alone in all concentrations, vitamin C + IBA, $KMnO_4$ + IBA, and CCC at 5000 ppm. Vitamin C alone increased the callusing

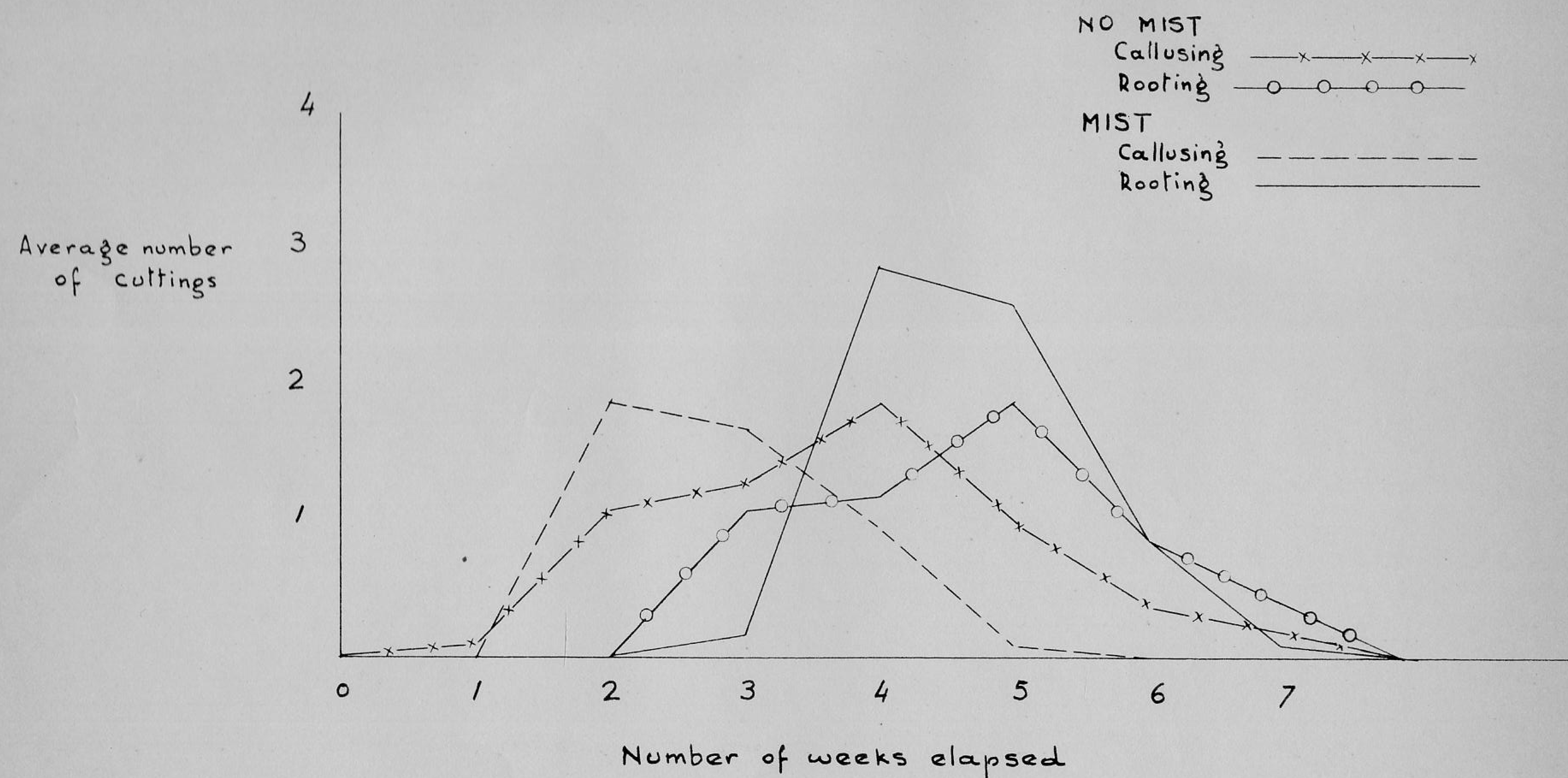

Figure 1. Effect of mist treatments upon callusing and rooting of grape cuttings during spring season 1967.

Table 2. Effect of mist and chemicals on callusing of Rupestris Du Lot grape hardwood cuttings after 28 days.

Treatments	Mist[1]	No mist[1]	Average
Control	5.5	4.0	4.7
Soaking in water	8.7	7.2	7.9**
Urea, 100 ppm	7.2	4.5	5.8
Urea, 1000 ppm	5.7	3.2	4.4
Urea, 10,000 ppm	4.7	2.2	3.4*
IBA, 50 ppm	10.0	9.7	9.8**
IBA, 120 ppm	10.0	8.0	9.0**
IBA, 200 ppm	9.5	8.2	8.8**
Vitamin C, 200 ppm	6.7	5.2	5.9*
Vitamin C + IBA, 200 + 2000 ppm	9.2	7.7	8.4**
$KMnO_4$, 20 ppm	4.7	4.5	4.6
$KMnO_4$ + IBA, 20 + 2000 ppm	8.0	7.5	7.7**
CCC (Cycocel), 500 ppm	7.7	5.2	6.4**
CCC, 2000 ppm	5.2	2.7	4.1
Malic acid, 200 ppm	7.7	2.5	5.1
Tartaric acid, 200 ppm	5.0	1.2	3.3*
Citric acid, 200 ppm	4.2	1.0	2.6**
Alpha amylase, 1000 ppm	5.7	1.5	3.6
Beta amylase, 1000 ppm	3.7	1.0	2.3**
Lipase, 500 ppm	3.7	0.7	2.2**
Average	6.6**	4.4	

* Significant at 5% level.
** Significant at 1% level.
1 Mean of four replications with 10 cuttings in each treatment.

significantly. Citric acid, beta amylase, and lipase caused highly significant reduction in the callus formation while urea at the high concentration and the tartaric acid treatment caused significant reductions.

IBA at 50 ppm and 120 ppm under mist, as already shown, gave 100 percent success whereas beta amylase and lipase affected the callusing adversely and produced the lowest number of cuttings callused. Under no mist treatment, IBA at 50 ppm gave the highest number of cuttings callused while urea, all organic acids and all enzymes had the most injurious effect on callusing. The interaction between mist and treatment was nonsignificant, statistically (Table 6).

Rooting

Statistical analysis shows that a highly significantly larger number of cuttings rooted under mist than under no mist (Table 3). Although root formation was observed to start earlier under no mist, the number of cuttings which rooted under the mist treatment gradually increased as time advanced and ultimately surpassed that of the no mist treatments.

Rooting of cuttings was affected differently by the different chemical treatments. Soaking in water and treatment with IBA alone or with vitamin C resulted in highly significantly greater numbers of cuttings rooted

Table 3. Effect of mist and chemicals on rooting of Rupestris Du Lot grape hardwood cuttings after 91 days.

Treatments	Mist[1]	No mist[1]	Average
Control	7.2	5.7	6.4
Soaking in water	8.7	8.0	8.3**
Urea, 100 ppm	7.2	4.5	5.8
Urea, 1000 ppm	9.0	5.7	7.3
Urea, 10,000 ppm	6.2	4.7	5.4
IBA, 50 ppm	10.0	9.7	9.8**
IBA, 120 ppm	9.7	9.0	9.3**
IBA, 200 ppm	9.5	9.0	9.2**
Vitamin C, 200 ppm	7.2	5.0	6.1
Vitamin C + IBA, 200 + 2000 ppm	9.0	7.7	8.4**
$KMnO_4$, 20 ppm	5.5	4.2	4.8
$KMnO_4$ + IBA, 20 + 2000 ppm	7.7	6.7	7.2
CCC (Cycocel), 500 ppm	7.2	5.7	6.4
CCC, 2000 ppm	7.0	4.7	5.8
Malic acid, 200 ppm	7.7	5.7	6.7
Tartaric acid, 200 ppm	5.7	3.2	4.4
Citric acid, 200 ppm	4.7	3.0	3.8
Alpha amylase, 1000 ppm	5.7	5.5	5.6
Beta amylase, 1000 ppm	4.5	4.0	4.2
Lipase, 500 ppm	3.7	3.5	3.6
Average	7.1**	5.8	

** Significant at 1% level.

[1] Mean of four replications with 10 cuttings in each treatment.

than the control. On the other hand, citric acid, tartaric acid, beta amylase, and lipase reduced the number of cuttings rooted highly significantly. All others approximated the control (Table 3).

The interaction between mist and treatment was found to be statistically nonsignificant (Table 7). However, there appeared to be a trend in favor of the combination of IBA and mist treatment and mist and water soaking, with the best being the low rate of IBA plus mist. In addition, wherever it appeared that the chemical might be toxic,such as was observed with the organic acids, then no mist seemed to cause a more severe reponse than the mist treatment. After 28 days, the rooting of cuttings under the mist treatment was significantly better than under the no mist treatment (Table 4). However, much greater differences were obtained with some of the chemical treatments.

IBA alone or in combination with $KMnO_4$ or vitamin C induced the greatest number of cuttings to form roots while vitamin C and soaking in water induced fewer but still significantly more than the control. Nine of the chemical treatments seriously retarded rooting. Urea at the medium concentration, CCC at 2000 ppm, malic acid, and alpha amylase caused significant reductions in rooting and urea at the high rate, tartaric acid, citric acid, beta amylase, and lipase treatments reduced the rooting highly significantly.

Table 4. Effect of mist and chemicals on rooting of Rupestris Du Lot grape hardwood cuttings after 28 days.

Treatments	Mist[1]	No mist[1]	Average
Control	2.5	2.7	2.6
Soaking in water	3.5	3.5	3.5*
Urea, 100 ppm	2.7	3.5	3.1
Urea, 1000 ppm	2.2	0.7	1.4*
Urea, 10,000 ppm	2.0	0.2	1.1**
IBA, 50 ppm	5.5	5.5	5.5**
IBA, 120 ppm	5.2	4.2	4.7**
IBA, 200 ppm	5.0	3.7	4.3**
Vitamin C, 200 ppm	3.0	4.0	3.5*
Vitamin C + IBA, 200 + 2000 ppm	3.7	4.5	4.1**
$KMnO_4$, 20 ppm	2.5	3.2	2.8
$KMnO_4$ + IBA, 20 + 2000 ppm	3.2	4.5	3.8**
CCC (Cycocel), 500 ppm	4.0	2.7	3.3
CCC, 2000 ppm	2.2	1.2	1.7*
Malic acid, 200 ppm	3.0	0.7	1.8*
Tartaric acid, 200 ppm	2.0	0.0	1.0**
Citric acid, 200 ppm	1.7	0.2	0.9**
Alpha amylase, 1000 ppm	2.5	1.0	1.7*
Beta amylase, 1000 ppm	2.2	0.0	1.1**
Lipase, 500 ppm	1.7	0.2	0.9**
Average	3.1*	2.3	

* Significant at 5% level.
** Significant at 1% level.
[1] Mean of four replications with 10 cuttings in each treatment.

Some interactions were also significantly affected (Table 8). Primarily they help explain the responses noted for the chemical treatments as the same trends noted for them are to be seen in the interaction combinations. In particular, it is noted that when rooting was reduced by the treatments, it was more severely reduced under the no mist-chemical combinations and the best treatments for inducing roots seemed to be aided by mist.

Cuttings which died during the test were noted and then discarded. The highest mortality was observed for the cuttings treated with tartaric or citric acid and the enzymes beta amylase and lipase. These compounds gave poor results for both rooting and callusing. It is possible that too high a concentration of the chemicals was used causing direct injury to the cuttings treated with these compounds. Furthermore, organic acids and enzymes might not be effective at any concentration in inducing rooting of grape cuttings or might be antagonistic to rooting.

Rooting and callus formation was delayed by the mist treatment. This is, perhaps, due to the continuous spray of water throughout the day and night, low temperatures and a lack of bottom heat. Hartmann (28, 29) has observed that, in general, better results were obtained when bottom heat was used, under conditions of high humidity, in rooting of cuttings.

V. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

A study was conducted at the American University of Beirut during the year of 1966-67 to determine the effect of some chemical treatments and misting on the rooting of grape (Rupestris Du Lot) cuttings.

Chemical treatments consisted of urea at 100, 1000, 10,000 ppm; IBA at 50, 120, 200 ppm; vitamin C alone at 200 ppm; vitamin C + IBA at 200 + 2000 ppm; $KMnO_4$ alone at 20 ppm; $KMnO_4$ + IBA at 20 + 2000 ppm; CCC at 500, 2000 ppm; malic acid, tartaric acid, and citric acid at 200 ppm each; alpha amylase and beta amylase at 1000 ppm each; lipase at 500 ppm; and cuttings soaked in water for 24 hours.

From the current season branches of Rupestris Du Lot rootstock, cuttings 22 cm long and 1 to 1.5 cm thick were obtained. The cuttings were wrapped in moist paper during their handling to prevent dessication. The treatments were applied using the solution soaking method. Planting was done in a bench filled with a vermiculite medium. Cuttings under mist conditions received a continuous fine spray of water.

The cuttings were examined weekly for callusing and rooting.

Cuttings planted under the no mist treatment started callusing and rooting earlier than those under the mist conditions but the total number of cuttings ultimately callused and rooted were higher under the mist treatment.

The highest mortality was observed in the cuttings treated with the organic acids, tartaric and citric acids, and the enzymes, beta amylase and lipase. The mortality percentage was higher in the no mist treatment as compared to the mist treatment.

Mist, IBA, and urea at 1000 ppm, and vitamin C + IBA produced a marked effect on callusing and rooting of cuttings. IBA at 50 ppm without mist and at 50 ppm and 120 ppm with mist produced one hundred percent callusing of cuttings, while at 50 ppm, with mist, it produced 100 percent rooting.

Cuttings, soaked in water for 24 hours, under both mist and no mist, produced the next best callusing and rooting.

The results of the experiment indicates that misting; chemicals like IBA, vitamin C + IBA, and urea; and water soaking all enhance the phenomenon of rooting on the cuttings. However, as the economical considerations are of prime importance in all the agricultural enterprises, it is recommended, on the basis of this experiment, to follow the practice of water soaking of cuttings for 24 hours prior to planting for the better propagation of grape cuttings.

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A P P E N D I X

Table 5. Analysis of variance for callusing of hardwood cuttings after 91 days.

Due to	D.F.	M.S.
Replications	3	1.06 N.S.
Mist treatment	1	25.60*
Error (a)	3	1.10
Hormonal treatment	19	35.79**
Treatment x Mist	19	2.17**
Error (b)	114	0.61
Total	159	

	L. S. D.	
	5%	1%
Hormonal treatment	0.77	1.02
Interaction	1.3	1.8

* Significant at 5% level.
** Significant at 1% level.

Table 6. Analysis of variance for callusing of hardwood cuttings 28 days after planting.

Due to	D.F.	M.S.
Replications	3	1.56 N.S.
Mist treatment	1	209.30**
Error (a)	3	0.43
Hormonal treatment	19	46.21**
Treatment x Mist	19	0.11 N.S.
Error (b)	114	1.45
Total	159	

	L. S. D.	
	5%	1%
Hormonal treatment	1.18	1.57

* Significant at 5% level.
** Significant at 1% level.

Table 7. Analysis of variance for the rooting of hardwood cuttings 91 days after planting.

Due to	D.F.	M.S.
Replications	3	1.53 N.S.
Mist treatment	1	77.00**
Error (a)	3	1.23
Hormonal treatment	19	27.50**
Treatment x Mist	19	1.41 N.S.
Error (b)	114	0.86
Total	159	

	L. S. D.	
	5%	1%
Hormonal treatment	0.91	1.20

* Significant at 5% level.
** Significant at 1% level.

Table 8. Analysis of variance for the rooting of hardwood cuttings 28 days after planting.

Due to	D.F.	M.S.
Replications	3	5.46 N.S.
Mist treatment	1	19.60*
Error (a)	3	1.50
Hormonal treatment	19	15.90**
Treatment x Mist	19	2.72**
Error (b)	114	0.79
Total	159	

	L. S. D.	
	5%	1%
Hormonal treatment	0.87	1.15
Interaction	1.1	1.5

* Significant at 5% level.
** Significant at 1% level.