THE EFFECT OF STORAGE CONDITIONS ON GERMINATION AND VIABILITY OF SOUR ORANGE AND ROUGH LEMON ROOTSTOCK SEEDS

by

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A Thesis Submitted to the Faculty of the

DEPARTMENT OF HORTICULTURE

In Partial Fulfillment of the Requirements
For the Degree of

MASTER OF SCIENCE

In the Graduate College

THE UNIVERSITY OF ARIZONA

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ACKNOWLEDGMENTS

The author wishes to express his gratitude to Dr. Leland Burkhart, head of the Horticulture Department, University of Arizona, for making research facilities available. He wishes to acknowledge the assistance of Mr. Steve Fazio, Associate Professor of Horticulture, in supervising the experiment and his helpful suggestions in directing this thesis.

He also wishes to thank Dr. William P. Bemis, Professor of Horticulture, for his cooperation in outlining the plot design for the experimental germination tests.

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INTRODUCTION

Seeds are usually stored for varying lengths of time after harvest. Viability at the end of any storage period is the result of: (a) the initial viability at harvest, as determined by factors of production and methods of handling, and (b) the rate at which deterioration takes place. This rate of physiological change is associated with: (1) the species of seed, and (2) the environmental conditions of storage.

Barton (15) divided seeds into three biological classes according to their duration of life under favorable conditions: (1) short-lived or "microbiotic" seeds, with a life span not exceeding three years; (2) medium-lived or "mesobiotic" seeds which may live from three to fifteen years; (3) long-lived or "macrobiotic" seeds which may retain viability for fifteen to over a hundred years.

Although the longevity of many seeds in storage has been found to be increased by drying, there are some seeds which do not tolerate desiccation (12). Citrus seeds are among those which are injured by drying and hence deteriorate rapidly under ordinary conditions of storage. For the most part citrus seeds are stored in the fruit on the trees as long as possible with the exception of certain varieties. This means seeds are stored in this manner until just prior to planting in early April. Seeds from early maturing varieties are extracted during January and February and stored in water and placed in a refrigerator with the temperature set near 35 Fahrenheit degrees. This is the commercial method used in Florida for storing citrus seed and has produced excellent results with Cleopatra seed.

The Sour orange is used as a rootstock in the Salt River Valley area and the Rough lemon has been used in the Yuma area in recent years. The storage of seeds of these two rootstocks for extended periods, as is frequently necessary in shipment over long distances or when planting time is delayed for one reason or another, is usually attended by a serious reduction in viability during germination.

The objectives of this study were to re-examine some of the known storage conditions affecting germination rate and viability of the seeds, such as sealing the seeds, and chilling of fruits; to determine the germination rate and viability of citrus seed stored in undiluted juice extracted from the fruit; to determine whether treatment with boiled juice from the fruit would show better results than non-boiled juice on the germination rate and viability of the seed during storage; to determine the germination rate and viability of citrus seed treated with Arasan 75 prior to storage; to determine the effect of storage at room temperature on germination rate and viability of the seed.

Viability of seeds was represented by germination percentage which expresses the number of seedlings which can be produced by a given amount of seed. Seed vitality or germination power is represented by the germination rate which is the number of days required to produce a given percentage of germinated seeds. Coefficient of velocity $= \frac{\text{total number of seedlings x 100}}{A_1T_1 + A_2T_2 \ldots + A_xT_x}$ where A is the number of seedlings emerging on a particular number of days (T).

LITERATURE REVIEW

The morphology and anatomy of citrus seeds have been comprehensively discussed by Webber and Batchelor (40). The seed is made up of an embryo, the endosperm, and two seed coats. The embryo consists of the cotyledons which are the fleshy part and constitute by far the largest part of the mature embryo. They are attached to a very short hypocotyl, at the other end of which is the radicle. Lying between the cotyledons is the plumule. A seed with one embryo has two cotyledons about equal in size and shape, but where there are two or more embryos the size and shape of their cotyledons often vary greatly. The endosperm is merely a temporary nutritive tissue which is later absorbed by the embryo.

The two seed coats are the tegmen, or inner seed coat, and the testa, or outer seed coat. The tegmen is a thin rather delicate membrane which encloses the embryo or embryos. In most varieties the tegmen is colored; the area covering the chalazal end of the seed is brown, purple, pink, or yellow while the rest of the seed has a much lighter color, as clear brown, purplish or grayish white. The outer seed coat, or testa, is grayish white, cream, or yellow in color, and is tough and woody in texture.

The color of the intact seed depends chiefly upon the color of the tegmen and the embryos and on the extent to which their color is visible through the testa; for example, in a variety which has green cotyledons the green color may be visible through the seed coats. Fresh seeds in general range from grayish white, cream or yellow, to brownish or greenish.

Size and shape of the seeds between a variety are highly variable; for instance, common lemons have small, smoothly rounded, pointed seeds, while grapefruit seeds tend to be large and flattened, with conspicuous fin-like projections of the testa.

The germination process starts with elongation and emergence of the radicle through the micropylar end of the testa. The cotyledons are normally hypogean, remaining in the soil during germination. The radicle elongates rapidly to form a thick fleshy taproot which grows directly downward. Meanwhile, the plumule has been rapidly lengthening its first internode (the epicotyl) and developing the first true leaves. The cotyledons lengthen somewhat, and often the taproot has considerable growth when the plumule emerges from the burst testa and grows upward. Its tip is bent while pushing through the soil, but as soon as it emerges it straightens itself upward.

In many species and varieties of citrus the seeds may be polyembryonic. The seed produces two kinds of seedlings, namely the gametic and nucellar seedlings. The gametic seedling develops from the embryo after pollination and fertilization, and usually differs morphologically from the female parent. The other kind is the nucellar seedling which develops from the nucellar tissue of the ovule alongside the normal fertilized embryo. Nucellar embryos arise without fertilization, and are therefore identical to the female parent. These nucellar seedlings tend to be more vigorous than gametic seedlings.

Citrus seeds are easily injured by drying, and the germination of seeds decreases with desiccation. Fu (25) found that the moisture content of trifoliate orange seeds, and not the rate of drying is the critical factor, and that there was a sharp drop in the germination percentage when the moisture content was reduced below 70%. The moisture content was reduced to 70% in 31 hours at room temperature, but only 5 hours were required when seeds were dried in the sunlight. This emphasizes that a relatively short exposure of the seeds to sunlight may have pronounced effects on their viability.

Al-Rawi (2) presented data showing that drying reduced the rate and germination percentage of Rough lemon and Sour orange seed. Drying the Rough lemon seed for two days at room temperature reduced the germination percentage to 76% as compared with 98% germination for freshly extracted seed. Sour orange seed dried for 30 days gave 53% germination compared with 88% germination for freshly extracted seeds. Batchelor and Webber (16) have reported that if citrus seeds are allowed to dry they lose their viability. If the seeds are to be kept for some time they should be surface-dried quickly after being thoroughly washed, and then should be mixed with equal parts of ground charcoal, packed in a tight wooden box or tin container, and stored in a damp cool place, preferably between 38° F and 55° F.

Barton (12) has found that citrus seeds are among those which are injured by drying and hence deteriorate rapidly under ordinary conditions of storage. She pointed out that Florida dealers in these seeds have experienced difficulty in maintaining germination power of the citrus seeds. For the most part they have kept their seeds either in the original fruit juice or in moist sand until sold. She found that grapefruit and sweet orange seeds were injured by drying on blotters in the laboratory to 52% and 25% respectively, calculated on the basis of the dry weight of the seeds. However, seeds of both of these species remained viable longest in open storage in a humid atmosphere of 41° F, where moisture content of the seed was reduced to approximately 17%. Grapefruit seeds may be kept successfully for at least a year under these conditions. Forty-one degrees Fahrenheit (41° F) also proved best for maintaining the viability of Sour orange and Rough lemon seeds both of which tolerated more drying than grapefruit and sweet orange seeds. Room temperature and 23° F were deleterious to preserving viability.

Fu (25) pointed out that a more rapid rate of germination was obtained from trifoliate orange seeds collected later in the season than those collected earlier. Similar results were obtained by Al-Rawi (2) who showed that the stage of maturity or time of harvesting had a significant effect upon the rate and percentage of germination of seed from four rootstock species of citrus.

The effect of chilling the fruit of citrus or stratification of seed prior to sowing was investigated by several workers. Fu (25) presented data showing that chilling of the trifoliate orange fruit resulted in a more rapid rate of germination of the seeds; the rate increasing as the period of chilling was increased. The maximum was obtained after a chilling period of from 30 to 60 days. He mentions that chilling of intact fruit and stratification of seeds in moist sand at 42° F were effective in hastening germination. It seems likely that the response from chilling or stratification would be less marked with fruits picked late in the season since seeds collected later in the season ordinarily germinate more rapidly than those collected earlier.

Al-Rawi (2) found that cold storage of some citrus rootstock species at 40° F affected the rate and percentage of germination. His results suggest different citrus rootstocks show different responses in chilling.

Childs and Hrnciar (18) found that Semesan, 8-hydroxy-quinoline sulfate, puratized N5E, thiocyano analine, and Du Pont 1155HH were effective in controlling the growth of microorganisms and on viability of citrus seeds under moist conditions. In his experiments, citrus seeds were dipped in 1.0% solution of 8-hydroxy-quinoline sulfate and stored in moist sawdust or moss in non-sealed containers at 35° F for from 6 to 8 months. Of 34 varieties of citrus seed receiving this treatment, under Florida conditions, 29 gave better than 90% germination at the end of 6 months, and of the 33 varieties recorded at 8 months, 2 gave better than 80% germination.

Experiments by Fu (25) on storage of trifoliate orange seeds in moist sand, peatmoss, or sphagnum at 42° F showed that the seeds may be stored for at least 5 months without marked reduction in viability. He suggested that these orange seeds might be stored by burial in the ground, provided that they are removed before soil temperature rises sufficiently to allow germination during storage. He found that after 6 months 96% germination of the seeds was obtained, at which time 60% of the seeds had started to germinate in storage.

Richards' (31) preliminary germination studies indicated that seeds of Country Lime and Bible Sweet Orange showed a marked reduction in viability when stored at normal air temperature of 78° F to 80° F for more than 3 days after extraction, and that after one week's storage only about 50% of the seeds germinated. Seeds which have not been washed properly after extraction and treated with fungicide (copper), and surface dried tend to become moldy, and show poor germination. Seeds from decayed fruit also fail to germinate satisfactorily. He recommended that in the absence of cold storage facilities, the best results are obtained if the seeds are treated with a copper fungicide immediately after extraction and planted within 3 days after being surface dried quickly in the open.

Hartman and Kester (28) suggested that for storage of citrus species a temperature of 32° to 50° F should be maintained with seeds placed in a sealed container to maintain their moisture content, or that the seeds be mixed with a moisture-retaining material.

Observations of Milella (29) showed that Sour orange seeds sown immediately after harvesting on fortnight intervals showed respective germination percentages of 83.25, 78.50, and 76.25. These percentages were reduced to 41.75, 62.25, and 47.50 when the seeds were stratified in sand for 1 month before sowing.

Bacchi (3) found seeds of the two main rootstock species of Sao Paulo State stored best in closed containers at 35° F to 37° F in a very humid atmosphere. The Rangpur lime retained its viability for 14 months at 33-38% relative humidity and the Sweet orange for 11 months at 41-50% relative humidity.

Montenegro and Salibe (30) studied storage of seeds of the exocortis-resistant varieties of citrus rootstock. The seeds were washed, dried, and stored within 48 hours of harvesting. After 224 days the germinating capacity was satisfactory with seeds stored in plastic bags at room temperature. Fungicidal treatment was a great advantage, and in the untreated seeds fungal infection was correlated with moisture content. Germination was seriously checked at low moisture contents; the minimum was 27% for trifoliate orange and 10% for the other types. In seeds exposed to the atmosphere the values fell somewhat below these figures.

Elze (22) in investigating Palestinian sweet lime, Sour orange, Rough lemon, and Poncirus trifoliata determined that the storage of fruit had a delaying effect upon germination. He found that two weeks of stratification employing charcoal with washed wet seeds in closed containers gave satisfactory results, but that fungus infection grew worse if sand was used instead of charcoal for storage.

Storage temperature is a factor of vital importance in determining the life span of seeds. It was known that low temperature is more effective than higher temperature for viability of seeds, but above freezing temperatures, especially between 41° F and freezing, have been considered adequate. Barton (13) found in the region of Yonkers, N. Y., that a very moist atmosphere at 41° F brings more rapid deterioration in tree, vegetable, and flower seeds, than ordinary laboratory conditions.

The effect of moisture on the storage of seeds is closely related to temperature. Barton (13), (9) reported that high moisture content is more deleterious at high than at low temperatures. She also reports the more rapid deterioration of seeds stored in sealed containers which are repeatedly opened and re-sealed as compared with seeds in sealed containers which are never opened from the time of storage to the time of testing.

Barton (11) reported that the length of time at a harmful relative humidity (76%) was found to be directly related to the deterioration rate of some seeds regardless of the storage conditions before and after this exposure. When the period of exposure to 76% relative humidity was as long as 8 or 12 weeks, previous variations in relative humidity accelerated loss of viability.

The effect of a partial vacuum on longevity has been considered for several different varieties of seeds with varying results. Some benefits of a reduced oxygen supply may have been experienced by conifer seeds under certain storage conditions as reported by Barton (13).

Childs and Hrnciar (18) concluded that viability of citrus seeds is greatly depressed by microorganisms; the poor germination of seeds that become moldy at 35° F was due to treatment with a spent solution of 8-hydroxy-quinoline sulfate. The viability of citrus seeds is best maintained by storage at relative humidity levels approaching saturation, though not necessarily by the presence of free moisture.

It was found by Barton (10) that seeds of high initial vitality were much more resistant to unfavorable storage humidities and temperatures than those with low initial vitalities.

Data of Barton (14) showed that the lower subfreezing temperature (to -64° F) increased the survival of the short-lived conifer seeds in storage. Sealed storage at low temperatures (41° F or 23° F to 5° F) was effective for maintenance of vitality of coniferous seeds for considerable periods (6).

Germination data of Steinbauer and Steinbauer (34) relating to storage of American elm seeds indicated that 32° F is a better temperature for storage than either 50° F or 68° F. In regard to moisture content it appears that the more seeds were desiccated, the higher was the percentage of germination at any time and the longer viability is retained. Also, Barton (8) found that elm seeds can be kept viable for at least 16 months by sealing in containers kept at 41° F or below.

Results of Barton (5), (7) indicated that open storage at room temperature in regions of the same general temperature and humidity, would effectively maintain vitality of some vegetable seeds for 2 years. However, if the moisture content of the seeds is reduced to 6%, and sealed containers are used, vitality is assured for 3 years.

Toole and Gorman (37) found that seeds of vegetables sealed at various moisture contents in glass vials generally retained their vitality as well as, or better than, seeds at similar moisture contents that were exposed to the open air.

Results of Barton's (4) experiments on storage of delphinium seeds indicated that room temperature and sealed storage was much more effective than open storage. For keeping the delphinium seeds viable, 46° F and 5° F were superior to room temperature, especially in the case of open storage. Seeds from favorable storage conditions produced a good stand of seedlings even after 62 months of storage.

Toole (35) pointed out that increased moisture in the seeds speeds the changes that finally lead to loss of viability, that increased temperature further increases the rate of these changes, and that any exposure to high temperature and high moisture reduces the potential duration of life of seeds, even before the germination percentage is reduced.

Several explanations have been offered for the gradual degeneration of seeds in dry storage. The explanation that has the most facts in its favor embodies gradual changes in the chromosome system of embryo cells with duration of storage. Crocker (20) reported that the early changes are slight and may show little disturbance in the resulting plants except for producing new forms or even tetraploid plants. With further dislocations detrimental effects appear in the resulting plants, such as slower germination rate and growth, etiolation, sterility, and death of the plants before the set of flowers.

Weakening of the seeds may result from unfavorable conditions during ripening of the plant, during harvesting, or during curing immediately after harvesting; therefore age of the seed from harvest may not be a measure of ability to withstand further storage.

Rodrigo (32) found in the Philippines that vegetable and farm crop seeds stored in air-tight containers had outlived their corresponding counterparts in unsealed containers by 190 to over 620%. The life duration of seeds in air-tight containers varied with the kind of seed. For example, lettuce completely lost its viability in 27 months and soybean in 55 months.

Weibull (41) pointed out in Sweden the possibility of using a temperature of -4° F for the long term storage of certain vegetable and flower seeds. He indicated that storage at 32° F and with 90% humidity, and finally under normal conditions, (41° to 68° F and 70 to 80% relative humidity) gave considerably lower figures, and a useless product for onion and chive after 4 and 2 years respectively.

Goss (26) found that flower seeds of the same genera which were stored for 10 years in the California State Seed Laboratory, seem to retain their viability approximately the same length of time. The results of this experiment indicated that many kinds of flower seeds can be successfully stored under favorable conditions.

The cause of loss of viability in old seeds has been a matter of considerable investigation.

Duvel (21) stated that the seeds retain their viability longest in conditions which permit the least respiration.

Acton (1) by a careful analysis of old and new seeds, found that there was but a slight difference in condition of their food content. He discovered that there was a considerable diastatic and proteolytic enzyme action in new seeds, while in old seeds there was none. Acton (1) and Waugh (39) assumed that the loss of viability is related to the loss of enzyme action.

Groves (27) found that enzymes gradually disappear with age. He tested 300 species of seeds and found no peroxidases in any of the samples secured before the 18th century. In some cases the retention of enzymes was attributed to the hard coats of the seeds, and the loss of viability was stated to be due to some cause other than degeneration of enzymes. Also, he found no increase in the germination of seeds soaked in enzyme solutions, but rather a decrease due to an increased fungal action. According to his work the enzyme theory of the loss of viability is not tenable.

Toole, et al. (38) have concluded that the presence of germination-inhibiting substances in plants seems to be a wide-spread phenomenon. Evenari (23) reported that they occur in all parts of plants, in fruit pulps, fruit coats, endosperm, seed coat, embryo, leaves, bulbs, and roots. Besides inhibitors, high osmotic pressure and acid pH are often partly responsible for the germination inhibition caused by sap juices and extracts. Evenari mentions that Fruchten was the first who observed that fruit juices of Sour orange and Rough lemon contained the inhibitors. Cohen (19) mentioned that citrus juice contains inhibitors to germination which are citric and malic acids.

Scott (33) found, in the case of peach juice, that the injurious agent present in the fermented peach juice destroyed viability of the seeds. The same injurious effect on seeds was obtained by permitting the seeds to remain in decaying pulp for 10 days or in the fermented peach juice for 7 days.

Germination of citrus seeds is influenced by the temperature of the medium. Camp (17) reported that minimum temperature of the soil for germination of citrus species seeds is 59° F, the maximum is 104° F, and the optimum is between 87° and 95° F. He found that grapefruit and sweet orange seeds appear to have slightly lower optimum temperature than Sour orange and Rough lemon.

Fawcett (24) found that the most vigorous growth of seedling occurred after 90 days for the sweet orange seedlings at room temperature of 75° and 85° F. Batchelor and Webber (16) indicated that good germination is obtained when the mean daily temperature range is between 58° and 65° F. He reported no germination when the soil temperature was below 55° F or above 104° F.

Toole (36) found in crop seeds that under unfavorable storage conditions slight mechanical injuries predispose the seeds to more rapid deterioration than occurs in uninjured seeds, although preliminary results do not confirm this view. Abnormal seedlings are caused not only by mechanical injury, but also by other factors that result in the death of organs, or parts of organs, before the death of the entire seed or seedlings.

MATERIALS AND METHODS

Seeds for the germination tests were obtained from Sour orange (*Citrus aurantium* L.) and Rough lemon (*Citrus Lemoni* L.), commonly used rootstock species. This experiment was conducted in the greenhouse of the Department of Horticulture. Sour orange fruit were obtained from the University of Arizona campus, Tucson. Rough lemon fruit were obtained from the University of Arizona, Citrus Experiment Station, Yuma, Arizona.

Fruits used in the experiment were harvested on December 1, 1962, and on January 15, 1963. They were divided into two groups after harvesting; one group was stored in the cold room at 40° F for six weeks before they were removed for seed extraction. The other group received no storage treatment and the seed was extracted immediately after harvest and subjected to various treatments.

The seeds were extracted from the fruit by cutting each fruit carefully in half with a sharp knife in order not to injure the seeds. The seeds from the cut fruits were extracted by hand into a seive having a coarse mesh to retain the seeds and allow the juice and pulp to be washed through. The seeds were washed thoroughly in water, and soaked temporarily in a container with fresh water. Rudimentary seeds in which the embryos had not fully developed were light and these were discarded when they were floated to the surface during soaking in clear water. Then the seeds selected for storage and planting were dried on blotters for four hours after they had been thoroughly washed.

Seeds of Sour orange and Rough lemon were stored in sealed containers in cold storage at 40° F, and at room temperature at 70° F for six weeks. Other seeds were stored in the original undiluted juice of the fruit, and other seeds were stored in the original boiled juice of the fruit at room temperature, also, for six weeks. Other seeds, also, were dusted with Arasan 75, and stored in plastic bags at room temperature for six weeks. One group of seeds, used as a control, was stored at room temperature for six weeks.

Moisture determinations were made as follows: duplicate lots of 10 grams each were weighed and then dried in a vacuum oven at 78° C for forty-eight hours, after which the dry weights were determined. Calculations of moisture content were made on the basis of the dry weight of the seeds. Germination tests after each storage period were conducted in the green house. The germination tests were repeated twice, namely: January 15, 1963, and March 1, 1963.

The experimental design based on Split-pot Design with three replications and seven treatments was arranged in a randomized manner. The germinating medium consisted of a mixture of sand and peatmoss in equal parts by volume. Triplicates of twenty seeds each for each test were used. The germination medium was kept moist throughout the duration of each test.

The seeds were planted by hand, and were placed about an inch apart each way in the flats, and then pressed into the medium with a board. This pressed the seed down firmly in the soil in the normal position. The seeds were covered with a layer of clean river sand to prevent both the spread of damping-off fungi and the formation of a surface crust which might hinder the emergence of the tender young shoots.

Germination counts were taken for an 11 week period. Only the number of seeds that germinated was counted, and not the number of seedlings, since some polyembryonic seeds produced more than one seedling and the weak seedlings eventually died. The time required to reach a germinating percentage of 50%, and the total number of seedlings appearing above ground were recorded. The data are presented in tables.

Storage of Citrus Seed in Sealed Containers

In order to obtain information on the effect of sealing on germination of citrus seeds, the seeds were treated and stored in the following manner: thoroughly washed seeds from Sour orange and Rough lemon at two stages of maturity

(Table 1) were dried at room temperature on blotters for four hours. One group of seed was stored in sealed glass containers in the greenhouse at room temperature for six weeks; the other group was stored at 40° F for six weeks.

TABLE 1

Species, Sources, Date of Harvesting and Color of Fruit from which Seeds for Storage and Germination Tests Were Obtained

Species	Sources	Harvesting Date	Color of Fruit
Sour orange (*Citrus aurantium* L.)	University of Arizona campus	December 1, 1962 and January 15, 1963	Not Fully Yellow Yellow
Rough lemon (*Citrus lemoni* L.)	Yuma Citrus Experiment Station	December 1, 1962 and January 15, 1963	Not Fully Yellow Yellow

Cold Storage of Citrus Fruits

Fruits of Sour orange and Rough lemon picked on December 1, 1962, and on January 15, 1963 (Table 1) were held in a cold room at 40° F for six weeks. The fruit was removed after storage and the seeds were extracted for the germination tests.

Storage of Citrus Seed in Undiluted Juice

Elze (22) stressed the importance of washing the seeds immediately after their extraction from the fruit. However, many nurserymen still prefer to store the fruits in barrels of water. Here the fruit undergo a slow process of decay which facilitates the separation of seed by softening the rind and pulp, but this process seems to exert an adverse influence on the viability of the seeds. Slight fermentation facilitates extraction and exerts a favorable effect on germination.

Sour orange and Rough lemon seeds were preserved in undiluted citrus juice pressed from the actual parent fruits for six weeks at room temperature (Table 2). Then the seeds were removed, washed thoroughly, and planted in the flats.

Storage of Citrus Seed in Boiled Juice

Sour orange and Rough lemon seeds were preserved in boiled citrus juice, in sealed containers, for six weeks at room temperature (Table 2). The juice was boiled, in order to test the influence of germination inhibitors, if such were present. The seeds were removed from the juice, washed thoroughly, and planted in the flats.

TABLE 2

Preservation of Citrus Seeds in Fruit Juice

Date of Immersion	Species	Treatment	No. of Seeds
	Sour orange (*Citrus aurantium* L.)		
12-1-1962		6 weeks in natural juice	60
		6 weeks in boiled juice	60
1-15-1963		6 weeks in natural juice	60
		6 weeks in boiled juice	60
	Rough lemon (*Citrus lemoni* L.)		
12-1-1962		6 weeks in natural juice	60
		6 weeks in boiled juice	60
1-15-1963		6 weeks in natural juice	60
		6 weeks in boiled juice	60

Dusting Citrus Seed with Fungicide Arasan 75

Many seed fungicides are quite specific in their toxic effect. Some are safe for the treatment of the seed of certain species of plants and are injurious to others. In order to investigate the possible reaction on germination, the fungicide, Arasan 75 (Tetramethylthiuram Disulfide), was applied in dust form to freshly extracted Sour orange and Rough lemon seeds that had been washed to remove juice and mucilaginous materials and then dried on paper towels for four hours. The seeds were dusted with an excess amount of the fungicidal dust, shaken gently to remove excess dust, transferred to cellophane bags, and were then stored for six weeks at room temperature, about 70° F. After storage the seeds were planted in the flats.

Citrus Seed Stored at Room Temperature

It is known that citrus seeds are injured by drying and hence deteriorate rapidly, and viability is impaired under ordinary conditions of storage. Freshly extracted Sour orange and Rough lemon seeds were washed and surface dried, and stored in plastic bags at room temperature for six weeks. They were then removed and sown in flats in the greenhouse.

The purpose of this test was to determine the range of loss in viability and rate of germination of the seeds, in comparison with other treatments.

EXPERIMENTAL RESULTS

Effect of Cold Storage on Washed Seed, Sealed and Stored at 40° F

Table 3 shows the rate and per cent germination of seed extracted from Sour orange (*Citrus aurantium* L.) harvested on December 1, 1962, and stored for 6 weeks at 40° F. Eighty per cent germination was obtained in 11 weeks with sealed stored seeds at 40° F for 6 weeks as compared with 5% germination which was obtained in 11 weeks from the control seeds (the use of the term control designates storage of extracted, untreated seeds at room temperature). A similar effect was noted for the rate of germination; it took six weeks to reach 50% germination for seed in cold storage, while 50% germination was not reached in the non-sealed seeds stored at room temperature (control).

Seed cold storage of Rough lemon seed from fruit harvested on December 1, 1962 also maintained the rate and per cent germination (Table 4). Eighty per cent germination was obtained in 11 weeks from sealed seeds stored at 40° F for six weeks as compared with 10% germination obtained in 11 weeks from non-sealed seeds stored at room temperature. The rate of germination was also affected by sealed cold storage; it took 5 weeks to reach 50% germination for cold storage seed, while this percentage was not reached in non-sealed seeds stored at room temperature.

Sealed cold storage greatly maintained the per cent germination of seed extracted from Sour orange and Rough lemon harvested on January 15, 1963. Data in Tables 5 and 6 show that 90% and 85% germination was obtained for Sour orange and Rough lemon stored at 40° F in sealed containers for 6 weeks, compared to 10% and 15% germination, respectively, obtained in 11 weeks from non-sealed seeds stored at room temperature. A similar effect was noted for the rate of germination: it took 5 weeks to reach 50% germination for Sour orange and Rough lemon sealed seeds stored for 6 weeks at 40° F while 50% germination was not obtained from the non-sealed seeds stored at room temperature during this same period.

Finally, the germination data for Sour orange and Rough lemon seeds show that sealed chilled seeds extracted from fruit harvested in December and January have a highly significant rate and percentage germination compared with non-sealed seeds stored at room temperature.

Effect of Room Temperature on Washed Seed, Sealed and Stored

The data which were obtained by this study and presented in Tables 3, 4, 5 and 6 indicate that sealed storage at room temperature yielded significant results for seeds extracted from fruit harvested in December and January as compared to the germination per cent obtained from non-sealed

TABLE 3

Germination of Sour Orange Seeds, in Sand-Peat Moss Media after Six Weeks Storage

Figures represent averages of triplicates for each test

Seed and Planting Date	% Germ. at Time of Storage	Pre-storage Seed Treatments	Rate of 50% Germ. Time in Weeks	% Germ. 11 Weeks from Planting
Sour orange January 15, 1963	85	Control (Open storage at room temp.)	...	5
(76% moisture calculated on basis of dry weight of seeds)		Sealed storage at 40° F	6	80**
		Sealed storage at room temp.	7	55*
		Fruit stored at 40° F	6	85**
		Seed stored in full strength fruit juice at room temp.	...	5
		Seed stored in boiled fruit juice at room temperature	...	25*
		Seed dusted with Arasan 75, sealed and stored at room temperature	5	85**

*Significant **Highly significant

LSD .05 = 3.05%
.01 = 3.97%

TABLE 4

Germination of Rough Lemon Seeds, in Sand-Peat Moss Media after Six Weeks Storage

Figures represent averages of triplicates for each test

Seed and Planting Date	% Germ. at Time of Storage	Pre-storage Seed Treatments	Rate of 50% Germ. Time in Weeks	% Germ. 11 Weeks from Planting
Rough Lemon January 15, 1963	70	Control (Open storage at room temp.)	...	10
(73% moisture calculated on basis of dry weight of seeds)		Sealed storage at 40° F	5	80**
		Sealed storage at room temp.	5	60*
		Fruit stored at 40° F	6	90**
		Seed stored in full strength fruit juice at room temp.	...	5
		Seed stored in boiled fruit juice at room temperature	...	15
		Seed dusted with Arasan 75, sealed and stored at room temperature	7	60*

*Significant **Highly significant

LSD .05 = 3.05%
.01 = 3.97%

TABLE 5

Germination of Sour Orange Seeds, in Sand-Peat Moss Media after Six Weeks Storage

Figures represent averages of triplicates for each test

Seed and Planting Date	% Germ. at Time of Storage	Pre-storage Seed Treatments	Rate of 50% Germ. Time in Weeks	% Germ. 11 Weeks from Planting
Sour orange March 1, 1963	90	Control (Open storage at room temp.)	...	10
(85% moisture calculated on basis of dry weight of seeds)		Sealed storage at 40° F	5	90**
		Sealed storage at room temp.	6	60*
		Fruit stored at 40° F	5	85**
		Seed stored in full strength fruit juice at room temp.	6	65*
		Seed stored in boiled fruit juice at room temperature	6	70*
		Seed dusted with Arasan 75, sealed and stored at room temperature	5	85**

*Significant **Highly significant

LSD .05 = 3.28%
.01 = 4.27%

TABLE 6

Germination of Rough Lemon Seeds, in Sand-Peat Moss Media after Six Weeks Storage

Figures represent averages of triplicates for each test

Seed and Planting Date	% Germ. at Time of Storage	Pre-storage Seed Treatments	Rate of 50% Germ. Time in Weeks	% Germ. 11 Weeks from Planting
Rough lemon March 1, 1963	80	Control (Open storage at room temp.)	...	15
(72% moisture calculated on basis of dry weight of seeds)		Sealed storage at 40° F	5	85**
		Sealed storage at room temp.	5	65*
		Fruit stored at 40° F	5	90**
		Seed stored in full strength fruit juice at room temp.	6	60*
		Seed stored in boiled fruit juice at room temperature	5	75**
		Seed dusted with Arasan 75, sealed and stored at room temperature	5	85**

*Significant **Highly significant

LSD .05 = 3.28%
.01 = 4.27%

storage at room temperature of seeds of the same two species.

The per cent germination for Sour orange seeds extracted from the fruit harvested on December 1, 1962 was 55% which was obtained in 11 weeks after sealed storage for 6 weeks at room temperature compared with 5% germination obtained in 11 weeks after open storage at 6 weeks at room temperature. A similar effect was noted for the rate of germination; it took 7 weeks to reach 50% germination for sealed storage at room temperature, while it did not reach 50% germination in non-sealed seeds stored at room temperature during this same period.

Sealed seeds of Rough lemon extracted from fruit harvested December 1, 1962 and stored at room temperature for 6 weeks, reached a germination percentage of 60% in 11 weeks, compared to 10% germination obtained in 11 weeks for the same species of seeds after open storage for 6 weeks at room temperature. The rate of germination was also affected by sealed storage of the seeds; it took 5 weeks for sealed seeds to reach 50% germination, while in the non-sealed seeds stored 6 weeks at room temperature this per cent was not obtained.

Sealed storage of seed at room temperature maintained the per cent germination of seed extracted from Sour orange and Rough lemon harvested on January 15, 1963, when compared to non-sealed seed stored at room temperature. Data in Tables 5 and 6 show that 60% and 65% germination was obtained in

11 weeks with Sour orange and Rough lemon respectively after having been sealed and stored for six weeks at room temperature. For the non-sealed seeds stored at room temperature the per cent germination obtained was 10% and 15% respectively. The rate of germination was also affected by sealing the seeds as it took 6 and 5 weeks for Sour orange and Rough lemon respectively to reach 50% germination when stored for 6 weeks at room temperature (control).

The data obtained show that sealed storage at 40° F for both species of Sour orange and Rough lemon seeds gave higher significant percentage germination than did the sealed seeds stored at room temperature for fruits harvested on December 1, 1962, and on January 15, 1963.

Effect of Cold Storage of Fruit on Germination

Comparison of rate and per cent of germination of seeds from the two rootstock species, Sour orange and Rough lemon, after storing the fruit at 40° F for six weeks is presented in Tables 3, 4, 5, and 6.

The data indicate that cold storage of Sour orange and Rough lemon fruits for 6 weeks at 40° F maintained the per cent germination of seeds harvested from fruits in December and January respectively. In spite of the fact that the fruit was yellow and appeared to be mature, this does not indicate that the seeds within the fruit were mature, because the appearance of fruit does not necessarily mean maturity of the seed.

Sour orange and Rough lemon fruits harvested on December 1, 1962 and stored for 6 weeks at 40° F gave highly significant percentages in germination over the control. Seeds from the stored Sour orange fruit gave 85% germination as compared to 5% germination obtained from the control seed storage. A six-weeks period was required to reach 50% germination, while the seeds from the control did not reach this percentage for the same period.

Data indicate that storing Rough lemon fruits for 6 weeks at 40° F maintained the per cent germination of the seeds. Ninety per cent germination was obtained 11 weeks after planting from the seeds which were planted immediately after extraction from the fruits which had been stored for 6 weeks at 40° F; as compared with 10% germination for the control seeds. Six weeks was required to reach the 50% rate of germination for seeds from the stored Rough lemon fruit.

The per cent germination of freshly extracted seeds from fruits harvested on January 15, 1963 and stored for 6 weeks at 40° F was 85% and 90% after 11 weeks for Sour orange and Rough lemon respectively, as compared with 10% and 15% germination obtained from the control seeds of the same species, respectively.

Five weeks were required to reach a germination rate of 50% for Sour orange and Rough lemon seeds planted immediately after being extracted from fruits stored for 6 weeks at 40° F.

Cold storage treatment of these two species gave highly significant results as measured by both the high per cent and high rate of germination. Data obtained from this study and presented in Tables 3, 4, 5, and 6 indicate that cold storage of citrus fruits is an ideal method of storing citrus seed, particularly if such seeds are to be used for planting the following season.

Effect of Seed Storage in Undiluted Fruit Juice on Germination

Tables 3, 4, 5, and 6 summarize the results of preserving citrus seeds in unsterilized juice extracted from the parent fruits.

The per cent germination obtained was not significant for Sour orange and Rough lemon seeds extracted from fruit on December 1, 1962 and stored for 6 weeks at room temperature. Five per cent germination was obtained for both species as compared to 5% germination for the control seeds in open storage.

On the other hand, Sour orange and Rough lemon seed extracted from fruit harvested on January 15, 1963, and stored for 6 weeks in undiluted juice at room temperature gave a significant per cent germination as compared with the open storage at room temperature (control).

The per cent germination for Sour orange seeds stored in undiluted juice was 65% as compared to 10% for the control seeds after 11 weeks, and germination was 60% for Rough lemon as compared with 15% germination for the control seeds. A similar effect was noted for the germination rate: it required 6 weeks to reach 50% germination for both species of citrus stored in undiluted juice while this 50% rate of germination was not obtained from the control during the same time interval.

Effect of Storing Seed in Boiled Citrus Juice on Germination

Data obtained in this study, Tables 3, 4, 5, and 6, indicate that preservation of citrus seeds in boiled citrus juice yields higher per cent germination than from seeds preserved in non-boiled juice extracted from the parent fruit.

Per cent germination for seeds extracted from Sour orange and Rough lemon fruits harvested on January 15, 1963 and stored for 6 weeks in the boiled juice at room temperature was 25% and 15% respectively, after 11 weeks as compared to 5% for Sour orange and 10% germination for Rough lemon for the same period.

On the other hand, per cent germination of seeds extracted from Sour orange and Rough lemon fruits harvested on January 15, 1963 and stored for 6 weeks in the boiled juice at room temperature was 70% and 75% after 11 weeks respectively as compared to 10% for Sour orange and 15% for Rough lemon.

These data are significant compared with per cent germination of seeds extracted from fruit harvested on December 1, 1962 and preserved in the natural juice. The rate of germination also showed significance over the control; it took 6 weeks for Sour orange and 5 weeks for Rough lemon to reach 50% germination, while it was not reached by the control seeds.

The results of these experiments suggest that the juice of citrus fruits has an adverse influence on the germination percentage in the early stages of ripening, but this effect is less at the middle of the season. Boiling of the juice, however, changed the results at the middle of the season rather than in the early stages of ripening.

The Effect of the Fungicide Arasan 75 on Seed Germination

Preliminary experiments indicate that when citrus seeds were allowed to dry out their viability was impaired. However, if storage was attempted with seeds in a moist condition the growth of fungi and bacteria soon destroyed their viability. In order to overcome this difficulty, Arasan 75 was selected and applied, in dust form, to freshly extracted Sour orange and Rough lemon seeds that had been washed to remove juice and mucilaginous materials and then dried on paper towels.

Data in Tables 3, 4, 5, and 6 show the significant results which were obtained from using the fungicide Arasan 75.

Sour orange and Rough lemon seeds extracted from fruit harvested on December 1, 1962 were surface dusted with Arasan 75, and stored for 6 weeks at room temperature. They showed a highly significant per cent germination. The per cent germination was 85% and 60% after 11 weeks for Sour orange and Rough lemon respectively, while the 50% rate of germination was not reached by the control seeds of either variety.

Surface dusting with Arasan 75 maintained a high per cent germination for seeds extracted from Sour orange and Rough lemon fruits harvested on January 15, 1963. Data in Tables 3, 4, 5, and 6 show that 85% germination was obtained in 11 weeks for both Sour orange and Rough lemon seeds stored for 6 weeks at room temperature, compared with 10% and 15% germination in seeds of the control. The rate of germination was also affected by surface dusting with Arasan 75; it took 5 weeks to reach 50% germination for both Sour orange and Rough lemon seeds, while it did not reach 50% germination in seeds of the control.

DISCUSSION

Previous research workers have shown that stage of maturity, chilling of sealed seeds, and chilling of fruits are important factors which affect citrus seed viability and germination after seed storage. No research previous to this has shown effects of undiluted and boiled juice of the parent fruits or of treatment with surface dusting of Arasan 75, on citrus seed storage and germination. Data presented in this thesis have shown that undiluted and boiled juice and treatment with Arasan 75, may be useful tools in studying storage and germination of citrus seeds. The data presented for storage and germination of seed harvested at two periods of the year indicate that the color of the fruit cannot be depended upon to determine when the seeds are fully mature.

The rate and per cent germination after storage of Sour orange and Rough lemon seeds indicate the difference is probably due to environmental conditions during fruit maturation, for these two species. In Rough lemon and Sour orange the rate and per cent germination, for some treatments after storage, were high and have a satisfactory useful application. The application of various pre-storage treatments indicates further differences between Sour orange and Rough lemon seeds which probably are due to their inherent characters.

To obtain a satisfactory per cent germination, citrus seeds must reach a minimum stage of maturity before harvesting the fruit for seed extraction and storage. There may be an increase in per cent germination beyond the minimum stage of maturity of citrus seeds because of physiological changes of the fruit and the seeds. Under these conditions maximum germination can be obtained after storing the seeds for an extended period of time.

Sealed storage of Sour orange and Rough lemon seeds is an important factor in seed storage and germination studies. A satisfactory per cent germination was obtained in sealed cold storage for 6 weeks at 40° F. The germination results of both harvest dates indicate that there is an interrelationship between harvesting date and the seeds exposed to cold storage at 40° F.

Chemical inhibitors associated with the seed coat may delay or inhibit germination (28). It is known that some of the chemical inhibitors associated with germination and present in seeds are anti-auxins. Chilling of seeds probably causes chemical changes of the inhibitors in the seeds.

Data obtained in this study show that cold storage treatment maintains a high per cent germination for sealed seeds of Sour orange and Rough lemon when compared to seed stored at room temperature.

Data presented in this thesis indicate that in the case of Sour orange and Rough lemon seed, a highly significant increase in per cent germination can be expected if the fruits from which seed is extracted are stored in a cold room at 40° F for at least six weeks. The increase in germination percentage is probably the result of a chemical change in seed inhibitors. Chilling of the fruits maintains a high germination percentage after storage, as indicated by the results obtained in the experiments. It may be expected that there will be a gradual increase in germination of seed obtained from fruit placed in cold storage since additional physiological changes take place, and it may help to break the dormancy of the stored seeds.

The rate and per cent germination is high for seeds extracted from fruit chilled at 40° F for six weeks, which from a commercial nursery standpoint would be considered satisfactory.

Data obtained in this study with seeds of Sour orange and Rough lemon stored for 6 weeks in the parent fruit juice showed a low germination percentage due to the possible influence of inhibitors in the juice; the presence of which was demonstrated by Elze (22) for tomatoes and other fruits.

Seeds extracted and planted immediately from fruits harvested in January showed a higher percentage germination than seeds extracted from fruits harvested in December. This difference in per cent germination is probably due to a lack of full seed development.

The seeds of Sour orange and Rough lemon stored for six weeks in boiled juice, resulted in a low germination percentage. Seeds extracted from fruit harvested in January and stored in boiled juice showed a higher rate and per cent germination than those seeds extracted and stored in December. This difference in per cent germination could be attributed to the different harvesting dates of the fruit.

Seeds stored for 6 weeks in boiled juice showed a low germination per cent probably because of no fermentation and hence no breakdown of seed coat inhibitors.

Treatment with surface fungicide Arasan 75 seems to have a good commercial nursery value. Data obtained in this study gives evidence that microorganisms affect the maintenance of viability of Sour orange and Rough lemon seeds in storage. Tables 3, 4, 5, and 6 show that a high per cent germination was obtained from both varieties harvested in December and January when treated with Arasan 75 and stored for 6 weeks. It has been shown in two instances that viability of Sour orange and Rough lemon seeds is greatly increased by surface dusting Arasan 75. Untreated seeds gave a low rate of germination.

Evidence seems to indicate that low temperature promotes longevity of stored citrus seeds through its depressing effect on germination of seed in storage, or re-respiration of seed, or growth of fungi and bacteria, thus supplementing in some measure the action of the fungicides. The fact that dry storage conditions cause serious reduction in viability of citrus seed suggests that they are in a more active state of metabolism than most seeds. Accordingly, access to sufficient oxygen must be essential to the prolonged storage of citrus seeds (18).

The results obtained in this thesis indicate that sealed seeds in cold storage and treated with a fungicide will maintain a high per cent germination after storage. Cold storage brings about certain physiological changes within the embryo (after ripening) which enable germination to take place promptly under normal growing conditions (28).

The difference in per cent germination is due, in most cases, to differences in harvesting dates of the fruits because seeds from the first harvest date were not fully ripe while the seeds from fruit of the second harvest date were completely ripe.

SUMMARY

Storage and germination conditions for Sour orange and Rough lemon seeds used for citrus rootstock have been investigated.

This study was designed to provide specific information concerning storage and germination of these two species of seeds.

Chilling the fruits for 6 weeks at 40° F maintained both the rate and percentage germination of both Sour orange and Rough lemon seeds extracted from the fruit.

Chilling was most effective in maintaining the rate and percentage germination of Sour orange and Rough lemon seeds after 6 weeks of storage at 40° F. There appears to be an interrelationship between chilling and the stages of maturity with respect to citrus seed germination.

Sealed cold storage of seeds of both species resulted in a higher rate and per cent germination for seeds extracted from fruit harvested in December and January and stored for 6 weeks at 40° F, than for sealed seeds extracted from the same two species and harvested at the same respective dates and stored for the same period of time at room temperature.

A low percentage germination was obtained from seeds of both Sour orange and Rough lemon when stored for 6 weeks in undiluted fruit juice.

It is difficult to explain why seeds of both these species showed a relatively low percentage germination when stored for 6 weeks in boiled citrus juice. While no conclusive explanation is offered, it does not seem that boiling might affect the inhibitors.

The Arasan 75 used as a surface dusting was found to be effective in controlling the growth of microorganisms on Sour orange and Rough lemon seeds. A highly significant increase in germination was obtained after treatment of the seeds of both species and storage for six weeks after the Arasan 75 application when compared to non-dusted seeds.

Citrus seed should never be extracted from the fruit and dried before planting. This method of handling citrus seed lowers the viability each day it is held in storage.

Evidence indicates that citrus fruit should be allowed to reach full maturity before harvesting the crop for seed purposes.

Since the time elapsing between sowing and germination of citrus seeds is rather long, their initial germinability may decrease due to adverse edaphic conditions.

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