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Determining Winter Hardy Apple Genotypes Prior to Seed Germination

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DETERMINING WINTER HARDY APPLE GENOTYPES PRIOR TO SEED GERMINATION

BY

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PRIOR TO SEED GERMINATION

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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INTRODUCTION

The objective of this research was to find a method of eliminating a high percentage of the tender genotypes in a population of apples before planting the seed.

The introduction of apple culture into the Great Plains and adjacent Canadian provinces confronted horticulturists with the problem of winter hardiness. Thousands of seedlings of standard varieties were planted in an attempt to find varieties sufficiently hardy for this region of North America. The results were discouraging in most cases. The lack of uniformity in severity of winters made the task of finding winter hardy varieties more difficult. Test winters, which seriously injure all but the hardiest varieties, may occur only once over a period of several years. Thus many varieties thought to be hardy were wiped out when a test winter occurred.

Fruit breeding to develop high quality apples adapted to the northern Great Plains is an important phase of horticulture. Each new variety developed must be subjected to long and expensive field tests for hardiness studies. A considerable amount of money is spent in growing large populations, only to find that a high percentage of the seedlings in the populations are not hardy. If these tender individuals could be eliminated prior to planting, the expense involved in land and labor could be greatly reduced, and more effort could be expended on the seedlings which would have the greatest chances of being winter hardy.

LITERATURE REVIEW

Many volumes have been published on the winter hardiness of plants during the past 200 years. The last 50 years has produced several volumes on methods for determining winter hardiness of plants. Some of these methods have proved practical in certain cases of hardiness studies.

Cultural methods have often been reported to be closely correlated to hardiness. Shutt (27) believed that hardiness was a quality that could be affected by cultural methods and was not limited to inheritance alone. It was his opinion that soil moisture and soil temperature in late summer and autumn probably play a more important role in determining a tree's resistance to cold than the severity of the succeeding winter.

Batchelor and Reed (2) found that winter drought was one of the factors responsible for winter injury of the Persian walnut (Juglans regia L.). The distal ends of the branches rather than the buds alone were killed. Winter injury was prevented under certain conditions by irrigating heavily late in the season after maturation had been induced by withholding water during late summer.

At the turn of the century Shutt (27) found that moisture content was lower in twigs of hardy apple varieties than in twigs of tender varieties. He believed that a distinct relationship existed between the moisture content of the twig and its power to resist the action of cold, and that those trees which contain the largest amount of water in their new growth in late fall are most tender. In 1919 Johnston (18) found that the water content of fruit buds of the Elberta peach was much greater than in the more hardy variety Greensboro.

Strausbaugh (31) in 1921 reported on the moisture determinations made on plum trees to find if moisture content and hardiness were correlated. A much lower moisture content was found in both fruit and leaf buds of the hardy varieties than in the tender varieties.

Previously Beach and Allen (3) had obtained similar results with apples. They explained the lower moisture content in hardy varieties was due to the lower rate of evaporation in hardy varieties. They found that all twigs tend to dry during freezing. The twigs of tender varieties dry more rapidly than twigs of hardy varieties, and after very cold temperatures the hardy twigs generally have a higher moisture content.

Hooker (16) investigated the correlation between winter hardiness and pentosan content by comparing shoots of tender varieties of apples with those of hardy varieties. The tissues of hardy varieties were found to contain more pentosan than those of tender varieties indicating that hardy varieties have a greater water-holding capacity. It had been observed by Beach and Allen (3), Johnston (18), and Shutt (27) that tender plant tissues usually contain more water than tissues of hardy plants. Hooker explained this apparent contradiction by pointing out that water in plants exists in several different forms. His theory was that pentosans or a specific pentosan in plant tissue holds water in the colloidal or absorbed form which does not freeze under ordinary winter conditions. The hardier varieties contain more water in the absorbed form while tender varieties contain water mainly in the form of free water which freezes at a higher temperature.

Steinmetz (29) made chemical studies of the roots of two alfalfa varieties. He noted that the hardier variety had more sugar in terms

of total carbohydrates.

Many workers have devised and redesigned equipment to suit specific requirements in hardiness determinations. Beach and Allen (3) built a special machine to test the hardness of twigs in their study on correlation between hardness of twigs and hardiness. The hardness of the wood was measured by the pull required to cut the twigs. The results showed that there is some correlation between hardness of wood and hardiness of variety. Using specific gravity tests on dry wood, they also found that density correspond quite closely with the mechanical tests showing hardness.

Bakke, Radspinner, and Maney (1) analyzed the twig content of apples at five stages during the year as to depression of freezing point. The twigs were analyzed at dormant, bud swelling, blossoming, summer growth, and wood ripening stages. They found no correlation between depression of freezing point and hardiness.

Dunn and Bakke (11) used adsorption as a means of determining the relative hardiness in the apple. Using dyes as indicators, they found that the cortical tissue from hardy varieties adsorbed more than the cortical tissue from tender varieties. The higher adsorption in the hardy varieties was attributed to the higher hydrophilic colloid content in the hardy varieties than in the tender varieties. They concluded that the hydrophilic colloids which have relatively enormous adsorbing surfaces hold water within the cell and prevent death from dessication brought about by freezing.

Briggs (4) used the living bark of Black Locust in testing the validity of plasmolysis and dessication for determining frost hardiness.

The bark was plasmolyzed in graded series of salt solutions and deplasmolyzed in tap water. The tissues were stained with neutral red and again placed in the tap water. Those cells which retained neutral red had survived both plasmolysis and deplasmolysis. This method yielded comparative values of hardiness which were as dependable as those obtained from actual freezing tests.

Much has been done in devising and improving artificial freezing equipment and methods. Potter (23) in 1920 built one of the first cold chambers in which a uniform and known rate of temperature fall and rise was automatically controlled. This enabled him to freeze plant tissues under controlled conditions. A redesigned model of the 1920 machine was built by Smith and Potter (28) in 1937 but it was too complex for general use.

Cullinan and Weinberger (8) in 1934 placed dormant peach shoots in a freezer and allowed the temperature to drop to a predetermined minimum. They had difficulty in duplicating their results on different days because the rate of temperature drop was not controlled. Knowlton (19) in 1936 used a similar type of system for freezing fruit buds of peaches and apples. The rate of temperature fall was manually controlled.

Meader, Davidson, and Blake (21) in determining the relative cold hardiness of dormant peach buds, placed the buds in test tubes suspended in an antifreeze bath of 50 per cent alcohol in water. This alcohol-water solution cooled less rapidly than did the air in the chamber providing a relatively uniform rate of cooling. Chaplin (6) in 1948 placed dormant peach buds on a wire rack in a freezing chamber and used a fan to reduce air stratification. The temperature was dropped at a relatively

uniform rate by readjusting the thermostat manually.

Proebsting and Fogle (24) investigating fruit bud hardiness of peaches in 1956 built an artificial freezing apparatus capable of lowering the temperature automatically at a pre-set, uniform rate. A cartridge type thermostat was placed through the freezer wall into the interior of the freezing chamber providing a direct control of the interior temperature of the freezer. The thermostat was driven by an electric clock motor and controlled by five-minute interval settings on the timer.

Extensive use has been made of electrolytic methods of determining winter hardiness. The electrolytic method most often used is based on the theory that exosmosis of electrolytes from the frozen material is proportional to the amount of freezing injury. The procedure followed by Wilner (36) gives a typical outline of the method. Dormant twigs of previous season's growth were taken from the entire perimeter of each tree and exposed to artificial freezing. Each sample of twigs was cut into one-half inch segments and put into a 300 ml. Erlenmeyer flask. Distilled water, exactly five times the weight of the segmented twigs, was added to the flask and left standing for 24 hours at room temperature. The relative degree of injury was estimated by measuring the electrical conductivity of the water extract from the twigs.

Hildreth (15), using the terminal growth of the previous season, found in 1926 that hardiness of apples as determined by an electrolytic method of the type described agreed quite closely with hardiness shown by field experience. The hardy varieties were uniformly more resistant

to cold than the tender varieties although a great variation occurred from season to season in the killing point of hardy and tender varieties.

Swingle (35) and Stuart (32, 33, 34) used this electrolytic method very effectively in their studies on the hardiness of apples using root sections. Filinger and Cardwell (13) obtained satisfactory results in hardiness determinations of bramble canes using this method. However, Scott and Cullinan (26) found the electrical conductance method unsatisfactory in detection of injury to the fruit buds of peaches.

Emmert and Howlett (12) made similar electrolytic determinations from the twigs of 55 apple varieties in fall, mid-winter, late winter, and early spring. The results of the fall tests agreed closely with the current hardiness concepts. However, no such agreement prevailed in the results obtained in the tests made in winter and spring.

Wilner (36), using the electrolytic method described to determine winter hardiness of woody plants grown commonly on the Canadian prairies, found as did Emmert and Howlett (12) that hardiness ratings varied at different times of the year. It was his opinion that hardiness ratings should be divided into three groups; those hardy in the fall, those hardy in mid-and late winter, and those hardy in early spring.

Cooper, Gorton, and Tayloe (7) using small trees and detached leaves of grapefruit measured the freezing injury by the electrical conductivity of the water extract from the frozen leaves. Results showed that the leaves on the trees sustained greater injury than the detached leaves in test tubes exposed to the same temperature.

Dexter (9), Dexter, Tottingham and Graber (10), Merrill (22), and Rodger, Williams and Davis (25) have successfully used this electrolytic method in determining the hardiness of agronomic crops such as alfalfa and oats. They used the roots of these plants for their study.

Filinger and Zeiger (14) in 1951 used another type of electrolytic method in which they inserted steel needles into the twigs of French crabapples commonly used as rootstocks and measured the electrical conductivity. The results compared quite favorably with the hardiness ratings obtained in the field. Campbell and Ghosheh (5) in 1957 found this method satisfactory in their hardiness studies of selected grape varieties. The advantage of this method is that the tests can be run in the field and the twigs or canes need not be detached from the plant.

Electrolytic methods of determining hardiness, either by measuring the conductivity of the solution in which frozen tissues or buds have been immersed or by directly measuring the conductivity of the twigs or canes have compared favorably with histological studies for determining winter hardiness. However, the histological examination of frozen tissue is much more tedious and time consuming than the electrolytic methods.

One of the more recent methods of determining winter hardiness, which is somewhat related to the technique used in this study, was used by Ivanoff (17) in his studies with oats. He activated oat seeds into a condition where internal growth commenced by soaking the seeds in running water at a given temperature for a given duration of time. The activated seeds were then subjected to cold tests. This approach offered the advantage that large numbers of plant entities could be tested for winter

hardiness. The activated seeds of both winter-hardy and non-winter-hardy oats gave results similar to the results reported for their respective counterparts in field trials.

Unpublished information at the South Dakota Agricultural Experiment Station indicates that seeds of Malus baccata (L.) Borkh., an extremely hardy epecies of crabapple native to Siberia, germinate at the stratification temperature after a much shorter period of time than seeds of hardy varieties of M. sylvestris Mill.[1] This has also been observed in comparing the seeds of Pyrus ussuriensis Maxim., a hardy species of pear, with seeds of hardy varieties of P. communis L.

The research work cited indicates that many methods have been used for determining winter hardiness. Many of these methods have been successful. However, they leave much to be desired in simplicity and reliability for determining the winter hardiness of large populations of woody plants. No simple method has been developed for either determining winter hardiness or eliminating a high percentage of the non-winter hardy plant entities in a plant population in early stages of growth.

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[1]Synonyms are Pyrus Malus, M. malus, M. communis, M. pumila.

PROCEDURE

This study on eliminating the least hardy genotypes in an apple seed population was carried on in three successive experiments. Experiments 1 and 2 were conducted during the winter of 1957-58 and experiment 3 was conducted during the winter of 1958-59. The common variables in each of the three experiments were genetic make-up of the populations, duration of stratification period prior to cold treatment, temperature of cold treatment, and duration of cold treatment. The total length of stratification period prior to and following cold treatment was eight weeks in all experiments. Modifications of variables in the second and third experiments were based on results obtained in the first and second experiments respectively.

EXPERIMENT 1

Materials and Methods

The seeds used in this study were from Yellow Siberian crabapple, Haralson apple, Fireside apple, and a mixture of apple varieties consisting of Red Delicious, Yellow Delicious, Rome Beauty, and Jonathan. The crabapple named is *Malus baccata* and all the apple varieties named are *M. sylvestris*. The Yellow Siberian crabapple originated at the South Dakota Agricultural Experiment Station from seed obtained from Siberia. It is considered extremely hardy in the northern Great Plains region. The Yellow Siberian seed used in this experiment was collected at this station. The Haralson and Fireside apple varieties originated at the University of Minnesota Fruit Breeding Farm, Excelsior, Minnesota, and are commonly grown in this region. The seed of these two varieties was grown at the place of their origin. The mixture of seed of Red Delicious, Yellow Delicious, Rome Beauty, and Jonathan was obtained from the May Nursery Company, Yakima, Washington. These varieties are grown commercially in regions of milder climates but are considered very tender for growing in the northern plains. These four groups of open pollinated seed are considered as representing four genetic levels of hardiness.

A total of 39,600 seeds, 9,900 from each of the above named groups, was used in this experiment. The seeds of each group were divided into lots of 100 and stored at 7 to 13 degrees Centigrade until the experiment began. Three lots of each group served as checks. The checks were subjected to eight weeks of stratification as is required for germination, but did not undergo any other treatments (Table I.). Twenty-four seed

lots of each group were not stratified prior to cold treatment. The remaining 72 seed lots of each group were placed in Petri dishes between filter papers overlying eight layers of cheesecloth, moistened with distilled water, and placed into a cold chamber which was maintained at 2.8 degrees Centigrade for stratification.

Sixteen seed lots of each group were subjected to each of the cold temperature levels which were -40, -30, -20, -10, -7, and -3 degrees Centigrade. Four of the 16 seed lots at each temperature level had received no stratification, four had been stratified for one week, four for four weeks, and four had been stratified for six weeks prior to cold treatment. One of the four seed lots at each stratification level was subjected to a cold treatment of 6, 12, 24, or 48 hours duration (Table III.). The seeds were returned to the 2.8 degree Centigrade cold chamber after the cold treatment to terminate stratification. The seeds were placed at room temperature of approximately 21 degrees Centigrade for germination upon completion of stratification.

No replications were made except in the checks because of the preliminary nature of the study and because of the large quantities of seeds that replications would have required.

Results

Dry seeds which were exposed to the low temperature treatments had good germination regardless of the temperature, duration of exposure, or variety from which the seed came. All seeds which had been stratified for one, four, or six weeks prior to cold treatment were killed when exposed to -40 or -30 degrees Centigrade (Table III.).

Seeds stratified for one, four, or six weeks and then exposed to -20 degrees Centigrade were all killed except eight lots which had been subjected to only a six hour exposure following one or four weeks of stratification (Table III.). Fireside seeds had the poorest germination of the eight surviving lots.

Seeds exposed to -10, -7, and -3 degrees Centigrade gave germination in all cases (Table III.). However, the percentage of germination of Yellow Siberian, Fireside, and the mixture of tender seeds seemed to decline slightly as the length of exposure to -10 degrees Centigrade was increased. The Yellow Siberian, Fireside, and the mixture of tender seed showed a slight decline in germination percentage with increased exposures also at -7 degrees Centigrade cold treatment, and the tender seed showed this same trend at -3 degrees Centigrade. Seed lots of Haralson receiving the same treatment tended to have a uniform percentage of germination in all lots exposed to -10, -7, and -3 degrees Centigrade.

EXPERIMENT 2

Materials and Methods

The seeds used in this experiment were from Yellow Siberian crabapple, Haralson apple, Fireside apple, and a mixture of Red Delicious, Yellow Delicious, Rome Beauty, and Jonathan apples plus the crosses Minjon X Manchu, Minjon X New Jersey #12, Minjon X Red Delicious, and Minjon X Cox Orange. All varieties used in the crosses were of the M. sylvestris species with the exception of Manchu, which is a variety of M. baccata. Seeds of the varieties were from the same collection as used in experiment 1. The four crosses mentioned above were made at the South Dakota Agricultural Experiment Station in the spring of 1957. They are listed according to the supposed relative hardiness of the seed. Minjon was used as the common female parent in order to eliminate possible differences in the seed itself which could influence results. The number of seeds in the crosses was limited by the availability of seed. Weather conditions were unfavorable during the spring the crosses were made.

A total of 10,200 seeds, 2,000 seeds of each of the four variety groups and 600 seeds of each of the crosses except Minjon X New Jersey #12 of which there were only 400 seeds, were used in this experiment. The seeds of each group were divided into lots of 100 and stored at 7 to 13 degrees Centigrade until the experiment commenced. Three lots of each of the four variety groups and two lots of each of the crosses except Minjon X New Jersey #12 served as checks (Table II.). The checks were subjected to the same treatment as in experiment 1. Stratification followed the method outlined in the previous experiment.

Modifications of variables were based on data obtained in the first experiment. It was found in experiment 1 that seeds exposed for six hours to -20 degrees Centigrade after one or four weeks of stratification prior to cold treatment showed survival whereas the seeds exposed for 12 hours to the same conditions were all killed. This second experiment was set up to determine whether or not it would be possible to eliminate the more tender seeds by short exposures to temperatures lower than -20 degrees Centigrade. The survival of seeds in experiment 1 was about the same when exposed to the cold treatment after either one or four weeks stratification. The four week stratification period was arbitrarily selected for this experiment. The temperatures used were -20, -23, -30, -33, and -36 degrees Centigrade. The duration of exposure to cold treatment was generally reduced (Table IV.). The seeds were returned to the 2.8 degree Centigrade cold chamber after cold treatment to complete the required eight weeks of stratification. The study was still in preliminary stages and therefore replications were limited to checks.

Results

The lots of seeds exposed to -20 degrees Centigrade for six hours showed some survival in all but Yellow Siberian, Fireside, and the mixture of tender seed. Lots of seeds exposed to -20 degrees Centigrade for $7\frac{1}{2}$, 9, and $10\frac{1}{2}$ hours all failed to germinate.

The lots of seeds exposed to -23, -26, -30, -33, and -36 degrees Centigrade for one and a half hours showed a high percentage of survival but all seeds exposed to the above temperature for three hours or longer

failed to germinate (Table IV.)

EXPERIMENT 3

Materials and Methods

The seeds used in this experiment were from the Red and Yellow Siberian crabapple, Minjon apple, and Macoun apple, and from the crosses Minjon X Manchu, Minjon X Haralson, Minjon X Wealthy, Minjon X Idared, and Minjon X Yellow Newton. The Red and Yellow Siberian crabapple is comparable to Yellow Siberian in hardiness. All varieties named, including those used in the crosses, are of the M. sylvestris species except Red and Yellow Siberian, Yellow Siberian and Manchu, which are of the M. baccata species. Minjon originated at the University of Minnesota Fruit Breeding Farm, Excelsior, Minnesota and is very hardy on the northern Great Plains. It is considered to be comparable to Haralson in hardiness. Macoun originated at the New York Agricultural Experiment Station and is considered tender for this area. The crosses mentioned above were made at the South Dakota Agricultural Experiment Station in the spring of 1958. They are listed in order of their supposed relative hardiness. All the seed except Macoun, which was obtained from apples grown along Big Stone Lake in Minnesota, was collected from apples grown at the South Dakota Agricultural Experiment Station. This experiment was started in January 1959.

A total of 10,750 seeds, 2,000 each of the varieties Red and Yellow Siberian, Minjon, and Macoun, and 950 each of the crosses were used in this experiment. The seeds of the varieties were divided into groups of 100 and the seeds of the crosses were divided into groups of 50. They were stored at 7 to 13 degrees Centigrade until the experiment began. Three lots of each of the varieties and two lots of each of the

crosses were used as checks (Table V.). Stratification methods used were the same as in the first two experiments.

The four week stratification period prior to cold treatment was retained. The temperatures were also the same as in experiment 2 but the duration of the exposure was changed to $1\frac{1}{2}$, 2, and $2\frac{1}{2}$ hours except at the -20 degrees Centigrade level. Lengths of exposure at -20 degrees Centigrade were 6 and $7\frac{1}{2}$ hours. The seeds were returned to the 2.8 degree Centigrade cold chamber after the cold treatment to complete stratification.

No replications were used except in the checks because the information available from the two previous experiments did not justify selecting a small number of variables for intensive study. It was considered wise to save some seed should the results indicate a promising area for further study.

Results

The seeds exposed to -20 degrees Centigrade for 6 and $7\frac{1}{2}$ hours duration all failed to germinate. Seeds exposed to the lower temperatures for $1\frac{1}{2}$ or 2 hours showed survival in all groups, but the seeds exposed for $2\frac{1}{2}$ hours all failed to germinate (Table VI.).

The germination percentage in the lots surviving the cold treatment was much lower than in the comparative tests in the previous experiment. However, the checks also showed a lower percentage of germination (Table V.).

The results indicate a slightly lower percentage of germination of seeds from the more tender varieties and crosses at each level of cold

treatment than of seeds from hardier varieties and crosses. The results do not follow this pattern in all cases, and in those instances in which they do the difference is not very great.

DISCUSSION

The results of the three experiments conducted in attempts to find a temperature and duration of exposure at which the majority of the seeds of the more tender genotypes would be eliminated but the seeds of hardy genotypes would survive were inconclusive. Various combinations of the common variables which included genetic make-up of the populations, duration of stratification period prior to cold treatment, temperature of cold treatment, and duration of exposure to cold treatment, were used.

The results of experiment 1 in which no seed except dry seed survived following exposure to -30 degrees Centigrade but in which seed stratified for one or four weeks and then exposed to -20 degrees Centigrade for six hours survived, indicated that possibly a selective killing point could be found by exposing the seeds to temperatures below -20 degrees Centigrade for short periods. Experiment 2 showed a relatively high percentage of germination for all seeds exposed to -23, -26, -30, -33, and -36 degrees Centigrade for 1½ hours but no germination of seeds exposed to these temperatures for three hours, with the exception of a single seed of the Yellow Siberian group which germinated after exposure to -23 degrees Centigrade for three hours. Experiment 3 was set up to determine if a temperature and a length of exposure between 1½ and 3 hours could be found at which the least tender seeds would be eliminated.

A factor which should be considered is that the temperature of cold treatment, one of the variables, was not completely under control. There was a fluctuation of plus and minus 2.5 degrees Centigrade at each

level of cold treatment. This fluctuation in temperature might have influenced the results.

Lowering the temperature of the freezing chamber to the desired level of cold treatment before the seeds were placed in the chamber is another factor that might have influenced the results. Different results might have been obtained if the seeds had been placed into the cold chamber and then the temperature gradually lowered to the desired level of cold treatment.

The results obtained in experiment 3 indicated a slightly lower percentage of germination of the more tender varieties and crosses at each level of cold treatment than in the hardier varieties. Possibly further study along this line would produce more conclusive results if very exacting equipment were available. The critical killing point of all seeds exposed to -23, -26, -30, -33, and -36 degrees Centigrade was between 2 and 2½ hours exposure to the cold treatment. Thus very accurate temperature control would be required to work at such a narrow range in duration of exposure.

The dry seeds were used in experiment 1 anticipating that possibly they might behave more like dormant mature plants than the seeds which had undergone partial stratification. The results indicated a high percentage of germination in all genotypic groups following exposure of dry seeds to even the lowest temperature and longest duration of exposure. The available equipment did not permit using lower temperatures in the following two experiments. Further study with dry seeds using much lower temperatures and longer exposures might be worthwhile.

The data in experiment 1 showing a decline in germination of Yellow Siberian, Fireside, and the mixture of tender variety seeds exposed to -10 degrees Centigrade after four or six weeks stratification prior to the cold treatment did not seem important as compared to the data obtained at the -20 degree Centigrade test, upon which the second and third experiments were based. Yellow Siberian, which is an extremely hardy variety, showed a decline in germination percentage as the length of the exposure to -10 degrees Centigrade increased. The same decrease in germination was noted in the two tender varieties. However, Haralson, which is classed as a very hardy variety, showed a high germination percentage at all exposure lengths.

It has been observed in unpublished reports at the South Dakota Agricultural Experiment Station that seeds of M. baccata varieties germinate at the temperature of stratification after a shorter period of time than seeds of M. sylvestris varieties. Possibly Yellow Siberian (M. baccata) seeds were so much further developed after the stratification periods prior to cold treatment in experiment 1 than the seeds of the M. sylvestris varieties that they were more susceptible to cold than seeds of the M. sylvestris. Therefore the low percentage of germination of the hardy Yellow Siberian seeds might have been due to their more advanced stage of development at the time of cold treatment. The seeds of the hardy Haralson variety showed a high germination percentage at all lengths of exposure to -10 degrees Centigrade in experiment 1 whereas seeds of less hardy M. sylvestris varieties showed a decline in germination percentage as the length of exposure increased. There is a possibility that within the M. sylvestris species seeds of tender genotypes

could be eliminated by increasing the length of exposure to -10 degrees Centigrade. This is worthy of further study.

The three experiments did not indicate any definite temperature and duration of exposure at which the majority of the tender genotypes could be eliminated. However, results suggest that further research should be conducted on various phases of this problem.

SUMMARY

Three experiments were conducted from the fall of 1957 through the spring of 1959 in an attempt to eliminate the least hardy genotypes in an apple population prior to planting the seed. Seeds of different genotypic groups were stratified for one, four or six weeks prior to exposure to temperatures ranging from -3 to -40 degrees Centigrade. The length of exposure varied from 1½ to 48 hours. A total of 60,550 seeds in 653 separate lots were used in the study.

No definite duration of exposure or temperature in combination with the stratification treatments used was found at which one could eliminate the more tender genotypes within a population. The first experiment indicated that exposing the seeds to -20 degrees Centigrade or lower for short periods of time might eliminate the more tender genotypes. This approach later proved to be unsuccessful.

There was a decrease in the percentage of germination of open pollinated seed of Yellow Siberian, Fireside, and the mixture of tender varieties when exposed to -3, -7, or -10 degrees Centigrade as the duration of exposure was increased from 6 to 12 to 24 to 48 hours. Haralson, a hardy variety, had a high percentage of germination at all comparable exposures. This indicates that within the M. sylvestris species further studies using longer exposure periods at these temperatures may possibly be worthwhile.

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TABLE I. GERMINATION PERCENTAGE OF APPLE SEED LOTS USED AS CHECKS IN EXPERIMENT 1.*

	Germination Percent			
Replication	Yellow Siberian	Haralson	Fireside	Tender**
1	81	98	93	92
2	62	98	97	84
3	87	95	93	77
Mean	76.6	97	94.3	84.3

*Seeds of varieties used in both experiment 1 and experiment 2 were from fruit gathered at the same time from the same source.
**A mixture of Red Delicious, Yellow Delicious, Rome Beauty, and Jonathan.

TABLE II. GERMINATION PERCENTAGE OF APPLE SEED LOTS USED AS CHECKS IN EXPERIMENT 2.*

	Germination Percent						
Replication	Yellow Siberian	Haralson	Fireside	Tender**	Minjon X Manchu	Minjon X Red Delicious	Minjon X Cox Orange
1	67	93	87	55	99	77	87
2	85	93	93	73	96	66	88
3	80	96	88	86			
Mean	77.3	94	89.3	71.3	97.5	71.5	87.5

*Seeds of varieties used in both experiment 1 and experiment 2 were from fruit gathered at the same time from the same source.
**A mixture of Red Delicious, Yellow Delicious, Rome Beauty, and Jonathan.

TABLE III. GERMINATION PERCENTAGE OF APPLE SEED LOTS SUBJECTED TO COLD TREATMENT IN EXPERIMENT 1.

Cold Treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage			
			Yellow Siberian	Haralson	Fireside	Tender
-40	6	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	4	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	1	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	0	6	89	96	86	82
		12	88	95	95	91
		24	77	94	89	89
		48	82	88	95	77
-30	6	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	4	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0

TABLE III. (Continued)

Cold Treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage			
			Yellow Siberian	Haralson	Fireside	Tender
-30	1	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	0	6	83	84	88	83
		12	80	90	91	76
		24	77	84	80	75
		48	63	77	73	81
-20	6	6	0	0	0	0
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	4	6	82	68	20	53
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	1	6	77	85	2	68
		12	0	0	0	0
		24	0	0	0	0
		48	0	0	0	0
	0	6	67	98	92	67
		12	76	97	97	88
		24	88	91	91	94
		48	60	90	93	87

TABLE III. (Continued)

Cold Treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage			
			Yellow Siberian	Haralson	Fireside	Tender
-10	6	6	90	93	74	78
		12	79	86	58	59
		24	69	87	65	50
		48	56	84	45	54
	4	6	81	93	92	90
		12	25	96	90	82
		24	78	90	76	68
		48	74	87	75	79
	1	6	86	91	88	76
		12	75	93	87	74
		24	78	96	72	81
		48	85	96	67	73
	0	6	86	92	93	92
		12	91	96	88	91
		24	82	91	90	84
		48	88	95	82	82
-7	6	6	77	92	93	73
		12	79	90	78	79
		24	85	90	82	67
		48	76	88	77	41
	4	6	64	88	86	74
		12	85	94	84	65
		24	90	90	85	70
		48	70	86	85	60

TABLE III. (Continued)

Cold treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage			
			Yellow Siberian	Haralson	Fireside	Tender
-7	1	6	78	98	96	87
		12	74	100	83	89
		24	80	95	88	90
		48	83	88	82	86
	0	6	84	96	97	63
		12	78	89	95	72
		24	70	86	78	83
		48	59	99	91	62
-3	6	6	91	85	93	72
		12	90	85	91	77
		24	76	89	89	57
		48	80	88	85	55
	4	6	81	95	96	87
		12	76	86	95	89
		24	81	91	94	78
		48	79	91	97	78
	1	6	82	99	91	84
		12	82	89	80	75
		24	81	78	91	84
		48	71	91	90	77
	0	6	87	97	84	84
		12	88	97	91	72
		24	74	93	84	88
		48	74	94	93	88

TABLE IV. GERMINATION PERCENTAGE OF APPLE SEED LOTS SUBJECTED TO COLD TREATMENT IN EXPERIMENT 2.

Cold treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage							
			Yellow Siberian	Haralson	Fireside	Tender	Minjon X Manchu	Minjon X New Jersey #12	Minjon X Red Delicious	Minjon X Cox Orange
-20	4	6	0	15	0	0	19	9	1	11
		7½	0	0	0	0				
		9	0	0	0	0				
		10½	0	0	0	0				
-23	4	1½	75	77	63	52				
		3	1	0	0	0				
		6	0	0	0	0	0	0	0	0
		7½	0	0	0	0				
		9	0	0	0	0				
-26	4	1½	81	37	66	79				
		3	0	0	0	0				
		6	0	0	0	0	0	0	0	0
-30	4	1½	71	95	7	62				
		3	0	0	0	0	0	0	0	0
-33	4	1½	37	95	89	47				
		3	0	0	0	0				
-36	4	1½	58	96	1	71				

TABLE V. GERMINATION PERCENTAGE OF APPLE SEED LOTS USED AS CHECKS IN EXPERIMENT 3.

Replication	Germination Percentage							
	Red & Yellow Siberian	Minjon	Macoun	Minjon X Manchu	Minjon X Haralson	Minjon X Wealthy	Minjon X Idared	Minjon X Yellow Newton
1	75	69	43	63	29	59	41	59
2	48	78	29	39	51	46	37	41
3	66	56	51					
Mean	63	67.7	41	51	40	52.5	39	50

TABLE VI. GERMINATION PERCENTAGE OF APPLE SEED LOTS SUBJECTED TO COLD TREATMENT IN EXPERIMENT 3.

Cold treatment (in degrees Centigrade)	Stratification prior to cold treatment (in weeks)	Duration of exposure (in hours)	Germination Percentage							
			Red & Yellow Siberian	Minjon	Macoun	Minjon X Manchu	Minjon X Haralson	Minjon X Wealthy	Minjon X Idared	Minjon X Yellow Newton
-20	4	6	0	0	0	0	0	0	0	0
		7½	0	0	0	0	0	0	0	0
-23	4	1½	66	71	30	49	39	21	35	49
		2	43	65	28	47	31	39	34	45
		2½	0	0	0	0	0	0	0	0
-26	4	1½	69	65	23	37	53	48	31	47
		2	71	58	39	52	41	44	37	41
		2½	0	0	0	0	0	0	0	0
-30	4	1½	51	43	28	41	39	43	35	31
		2	56	41	25	44	48	37	31	27
		2½	0	0	0	0	0	0	0	0
-33	4	1½	59	49	31	52	44	47	17	45
		2	56	48	26	48	39	48	37	29
		2½	0	0	0	0	0	0	0	0
-36	4	1½	63	23	27	43	36	39	13	38
		2	50	31	19	39	21	38	24	23
		2½	0	0	0	0	0	0	0	0