

**T H E S I S**

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**TRIPHENYLTETRAZOLIUM CHLORIDE  
FOR QUICK GERMINATION TESTS OF  
CUCUMBER, CANTALOUPE AND ONION SEED**

**Submitted by  
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**In partial fulfillment of the requirements  
for the Degree of Master of Science  
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WE HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER OUR  
SUPERVISION BY ..... ATA ALLAH AGAH .....

ENTITLED ..... TRIPHENYLTETRAZOLIUM CHLORIDE FOR QUICK  
..... GERMINATION TESTS OF CUCUMBER, CANTALOUPE AND CHICK  
SEED .....

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE  
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## TABLE OF CONTENTS

<u>Chapter</u>	<u>Page</u>
I INTRODUCTION . . . . .	9
The problem . . . . .	9
Problem analysis . . . . .	9
Delimitation . . . . .	9
II REVIEW OF LITERATURE . . . . .	14
III METHODS AND MATERIALS . . . . .	20
Accuracy and reliability of the tetra- zolium chloride test as compared to standard germination techniques. .	21
The relation between cantaloupe and cucumber seed weight and germina- tion within a variety. . . . .	26
The relation between cantaloupe and cucumber germination and seed color . . . . .	27
The relation between age of onion seed and germination . . . . .	27
IV ANALYSIS OF DATA . . . . .	34
Experimental results of reliability of the tetrazolium chloride test and standard germination in cu- cumber varieties . . . . .	35
Experimental results of reliability of the tetrazolium chloride test and standard germination in canta- loupe varieties. . . . .	36
Statistical methods used . . . . .	36
Relation of cantaloupe and cucumber seed weight and germination within a variety . . . . .	38
Experimental results. . . . .	43
Relationship between cantaloupe and cucumber germination and seed color . . . . .	45
Experimental results . . . . .	45
Relation between age of onion seed and germination. . . . .	46

## TABLE OF CONTENTS.--Continued

<u>Chapter</u>		<u>Page</u>
	Experimental results . . . . .	46
V	DISCUSSION . . . . .	48
VI	SUMMARY . . . . .	51
	APPENDIX . . . . .	53
	BIBLIOGRAPHY . . . . .	55

## LIST OF TABLES

<u>Table</u>	<u>Page</u>
1 THE EIGHT ONION SEED SAMPLES TESTED BY STANDARD GERMINATION AND TETRAZOLIUM TECHNIQUES. . . . .	28
2 COMPARISON OF THE STANDARD GERMINATION TEST WITH THE TETRAZOLIUM TEST IN DETERMINING SEED VIABILITY OF SEVEN CUCUMBER VARIETIES. . . . .	34
3 COMPARISON OF THE STANDARD GERMINATION TEST WITH THE TETRAZOLIUM IN DETERMINING SEED VIABILITY OF SEVEN CANTALOUPE VARIETIES . . . . .	35
4 SIMPLE CORRELATION COEFFICIENTS OBTAINED WHEN TESTING THE RELATIONSHIP BETWEEN SEED VIABILITY AS DETERMINED BY THE STANDARD GERMINATION AND SEED VIABILITY DETERMINED BY THE TETRAZOLIUM QUICK TEST.	36
5 WEIGHT IN GRAMS OF TEN RANDOM SEED IN THE CANTALOUPE VARIETY ROCKY FORD . . . . .	37
6 WEIGHT IN GRAMS OF TEN RANDOM SEED IN THE CUCUMBER VARIETY SNOW PICKLING. . . . .	37
7 THE WEIGHT OF EIGHT 100-SEED CANTALOUPE SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE TETRAZOLIUM TEST. . . . .	38
8 THE WEIGHT OF EIGHT 100-SEED CANTALOUPE SAMPLES WITH THEIR GERMINATION AS DETERMINED BY THE STANDARD GERMINATION TEST. . . . .	39
9 THE WEIGHT OF EIGHT, 100-SEED CUCUMBER SEED SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE TETRAZOLIUM TEST. . . . .	41

LIST OF TABLES.--Continued

<u>Chapter</u>		<u>Page</u>
10	THE WEIGHT OF EIGHT, 100-SEED CUCUMBER SEED SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE STANDARD GERMINATION TEST. . . . .	42
11	RELATIONSHIP BETWEEN PERCENTAGE GERMINATION AND SEED WEIGHT IN CUCUMBER AND CANTALOUPE AS MEASURED BY BOTH THE STANDARD GERMINATION AND TETRAZOLIUM TEST. . . . .	43
12	VIABILITY OF BROWN AND WHITE COLORED SEED SELECTED FROM THE SAME BULK LOT OF SNOW PICKLING CUCUMBER AND ROCKY FORD CANTALOUPE . . . . .	44
13	RELATIONSHIP BETWEEN VIABILITY AS MEASURED BY THE STANDARD GERMINATION AND TETRAZOLIUM TESTS AS USING BROWN AND WHITE SEED OF CUCUMBER AND CANTALOUPE . . . . .	45
14	PERCENTAGE VIABILITY OF EIGHT OLD ONION SEED SAMPLES AS DETERMINED BY THE TETRAZOLIUM AND THE STANDARD GERMINATION METHOD. . . . .	46
15	PERCENTAGE VIABILITY OF THREE LOTS OF OLD CANTALOUPE SEED AS MEASURED BY THE TETRAZOLIUM AND STANDARD GERMINATION METHODS . . . . .	47

LIST OF FIGURES

<u>Figure</u>		<u>Page</u>
1	NORMAL GROWTH OF CUCUMBER AND CANTALOUPE SEEDS. . . . .	23
2	ABNORMAL GROWTH OF CUCUMBER AND CANTALOUPE SEEDS. GROWTH OF MOLD IN DEAD SEED. . .	24
3	POSITION OF EMBRYO IN CUCUMBER AND CANTALOUPE SEED . . . . .	25
4	SECTIONED ONION SEED SHOWING POSITION OF THE EMBRYO . . . . .	30
5	EMBRYO ENLARGEMENT OF ONION SEED AFTER 50 MINUTES SOAKING IN TETRAZOLIUM. EMBRYO ENLARGEMENT OF ONION SEED AFTER 24 HOURS SOAKING IN WATER . . . . .	32

## Chapter I

### INTRODUCTION

Seed analysts need a quick and accurate method of determining the viability of seed which would reduce costs of running germination tests and would also provide better service to farmers and seedmen during rush periods in the laboratory. The seed laboratories use a standard method for determining seed viability which consists of planting 200 or 400 seeds from a sample in sand or between blotters. After a given time the sprouts are counted and percentage germination determined. In such specific cases as that of Bahia grass, three months are required to obtain maximum germination (2).

In recent years there has been considerable interest in developing a new method of measuring seed viability which would reduce the time and labor required by the technique now in use. It is possible with certain crops to predict the germinability of their seed by observing the seed embryo which stains red in the presence of a weak solution of triphenyl tetrazolium chloride. This staining method has been used to some extent in Germany (4), (6), (7), (9) and, although it has not been generally adopted in the United States, shows promise as

a rapid method for testing seed viability.

Stains have been used for determining the viability of cells since 1882 (9), but it was not until 1935 (10) that a report was made describing the use of this method to determine the percent of live seed.

Hosegawa (5), a Japanese worker, was the first to work on the new method. Further interest was aroused among German workers in 1936-37. Eidmann (4) made further investigation to confirm the work done by Hosegawa.

In 1941, a German scientist, Laykon (7), further developed Eidmann's technique and replaced the use of a selenium salt with triphenyl tetrazolium chloride.

In 1945 a supply of 2-3-5 triphenyltetrazolium chloride was obtained from Germany by R. A. Dutcher (3) and sent to the seed laboratory at the Pennsylvania State College (3) as well as the Iowa State College (3). It was here the validity of Laykon's work was checked by repeating his tests.

The tetrazolium test was compared to the standard laboratory germination tests for corn, rice, buckwheat, popcorn, soybean and bahia grass. The staining test was found to compare well with the standard laboratory tests as a measure of seed viability (10).

The review of literature on the tetrazolium technique indicates that no reports have been published on its use as a measure of the viability of cantaloupe,

cucumber, and onion seed. The seed of cantaloupe and cucumber crops represent a type which will maintain acceptable viability for a period of ten to fifteen years under average seed storage conditions, whereas onion seed loses viability very rapidly and is not acceptable after it is two years old even under optimum storage conditions.

Two classes of seeds were selected for this study one class which includes cantaloupe and cucumber seed that holds its viability for many years and the other class which includes onion seed that loses viability rapidly.

If the staining test for seed germination was found to be consistently accurate and reliable, it would be very useful to research workers, seed analysts and to the wholesale and retail seed trade. After careful research work, limitations of its value and use could be determined. Some of the possible uses of the methods are listed as follows:

1. A quick test would be valuable when there is little time for germination tests between selling or buying operations.
2. In studying the effect of various fungicides on the loss of germination of seeds held in storage.
3. It would be valuable as a means of reducing time, labor and other costs of running standard germination tests in seed laboratories.

4. Seed of some crops lose their viability within a year following harvesting of the seed. A quick test would be valuable in determining the rate of germination loss and total germination which would help seedmen and growers in adjusting planting rates per acre.

5. A quick test may show the degree of maturity or immaturity.

6. It could be used to test the affect of injury to seed from mechanical cleaning or heating while drying and curing.

7. The extent of freezing injury to seed before it has matured.

8. It may have a place in detecting diseases which are present within a seed coat or in the embryo. Virus, bacterial or fungus organisms might be detected.

9. Imperfect or partially developed embryos may be detected.

10. The materials and place for the test does not require special equipment and technicians could be quickly trained to use it in the field or office.

While many of the preceding uses of the staining method are possible the accuracy of the test must first be determined. Consequently the investigations initiated and reported here were limited to a study of the reliability of the tetrazolium test when compared to Standard seed laboratory germination technique, to de-

veloping cutting, sampling and staining methods, and to investigate some of the factors, such as variety differences, age of seed, weight of seed, and color of seed which might influence the accuracy of the staining test.

## Chapter II

### REVIEW OF LITERATURE

The literature on the use of triphenyl-tetrazolium chloride for testing seed viability is not extensive. Much of the early work on the problem was conducted in Germany and results were not readily reported due to the recent war. Research in the United States with the chemical has been of a limited nature and dates back only four or five years.

According to Peckman and Rung (9) Loew and Bokorny were doing research in 1882 which was closely related to this field. They determined the viability of the living cell by using an extremely dilute alkaline silver solution as a reagent. It was not until 1894 that Peckman and Rung (9) first prepared various tetrazolium salts including 2,3,5 triphenyltetrazolium chloride. The next step was taken in 1918 by H. Molisch (5) who used a silver sulphate solution in place of the alkaline silver solution previously used. According to Hasegawa (5) the staining method developed in 1929 was the most generally accepted at that time.

Eidmann's method was developed to correlate the degree of staining of excised embryos with seed viability.

A 2.0 percent sodium biselenite solution was used and the staining of the embryo was classified on the basis of surface area covered. When there was no color or not more than one-third of the surface of the embryo was weakly colored, the seed was considered dead. The results indicated a positive relationship between viability of seed and the degree of surface staining of the embryo.

In 1935, Hosegawa(5) developed and further refined the staining method of determining seed viability by using dilute solutions of telluric acid and selenic salts. His technique was called the "Reagent Method" and the results were obtained by staining the seed of evergreen species of trees. His staining procedures made it possible to distinguish between old and new seed of the plant species studied. Seed which stained dark indigo germinated well while those which stained dark brown were incapable of growth.

According to Hosegawa's report (5) "Among the Vital staining method hitherto published Mr. Neljobov's Indigocarmin method (1929) is recognized as the most successful. It appears however to present certain drawbacks, such as imperfect color reaction - contrary to the "Reagent Method" - and difficulties in determining the vitality of seeds." (5)

In 1947 Laykon (7) compared the result of

testing by regular germination tests on seeds of oats, barley, wheat, rye and corn with those obtained by putting the cut seeds in either a solution of 2-3 diphenyl-5-methyl tetrazolium chloride or 2-3-5 triphenyl tetrazolium chloride. The staining of the viable embryos correlated well with the germination results. Further information was published by Laykon on the use of solutions containing salts of selenium. Definite staining of the vital parts of the embryo was evidence of ability to produce a normal seedling. For cereal seeds he found that if both the embryo and the scutellum stained carmen red the seed would be strongly viable. Corn seed tested by this method at the Iowa State College (8) using a similar staining technique showed a close relationship between the red stained embryos and viability. A comparison of seed in which viability has been destroyed by placing in boiling water and then treated with tetrazolium chloride solution, showed no change in color of embryo. Color changes are then associated with live embryos when treated with tetrazolium chloride solution. It is possible to distinguish between viable and non-viable seed, as reported, using tetrazolium tests.

The colorless tetrazolium salt is reduced by the enzyme activity of the viable seed. Upon reduction this colorless salt forms a carmen red color and the

living embryo becomes stained deep red.

Samples of barley, oats, wheat, sorghum, buckwheat, popcorn, peas, soybean, and vetch were tested at the Iowa State College (10) with tetrazolium solution in much the same way as that used for corn with the soaking time in water and solution varied. The seeds of the small grains were cut medianly through the long axis of the embryo. The seeds of peas, soybean, and vetch were cut between the cotyledons, cutting the radicle longitudinally. These tests show that in three lots of barley, two lots of oats, wheat, buckwheat, soybean, and popcorn, there is a close relationship between the percentage of stained embryos and of normal sprouts produced in the ordinary laboratory germination test in sand.

The work done by Porter et al (10) confirmed that done by Laykon (7) on corn and the work done on yeast by Kuhn and Jerchel (6) as well as stimulated an interest in finding the potentialities of the tetrazolium salt as a test reagent for living tissues in general (3).

A group of worker (8) at Iowa State College published a report on the use of the Biselenite Reaction procedure as a measure of seed germinability. The method used was as follows:

a. Germination tests of small-seeded species were made on blotters in a glass germinator.

b. Large-seeded species were tested on cotton pads in covered enameled trays.

All the tests were made at room temperature (75° to 80°F.). Seedling observation was made at the time of the germination counts (7 to 10 days).

c. Biselenite reaction tests were run simultaneously with the germination tests. Eidmann's method (4) was followed as a basic procedure, presoaking the seeds 16 hours, draining and aerating under moist conditions for 8 hours. All tests were on the basis of two replicates of 50 seeds each. Hard seeds with impermeable seed coats were classified as non-germinable.

The following conclusions were drawn from the above method.

The sodium biselenite method of seed testing with the presoaking step reduced to 16 hours aeration, proved generally satisfactory on seeds of a wide range of agriculture plants.

The biselenite test, suitably adjusted, may be used as an alternative for the standard seed germination test. It would have the advantage of being quicker, the time saved varying from 4 to 12 days in readily germinable seeds to perhaps several months in seeds which require a period of after-ripening assuming that the biselenite test could be made early in the after-ripening

period. When perfected, the biselenite test might prove more accurate for certain purposes than the germination test.

It has also been shown that the tetrazolium test may be used as an indirect measure of heating effects upon biological, physical, and chemical properties of peanut seeds (1).

Chapter III  
METHODS AND MATERIALS

The literature review indicated that there are no reports on the use of the tetrazolium as a test of viability on cantaloupe, cucumber and onion seed. No information was available on the reliability of the test or how such factors as variety, seed coat color, seed weight, and age of seed, would affect the accuracy of the test when used on the above mentioned crops.

Investigations were undertaken, (1) to determine the accuracy of the tetrazolium quick test when used on seed of cantaloupe, Cucumis melo L., cucumber, Cucumis sativus L., and onion, Allium cepa L., (2) to determine the most efficient seed cutting procedure so that the embryo will be properly exposed to the stain; (3) to determine if weight of seed within a variety has an effect on degree of staining or reliability of the tetrazolium test; (4) to determine if stained or discolored seed is an indication of lower germinability and if the tetrazolium stain is sensitive to this difference in viability; (5) to obtain information on the effectiveness and reliability of the staining method in testing viability of old seed.

I. Accuracy and reliability of the tetrazolium chloride test as compared to standard germination techniques.

Seed samples of seven cantaloupe varieties and seven cucumber varieties were used to compare the tetrazolium test with the standard germination test as measures of seed viability. The seed was obtained from a Denver seed company and varieties selected that are in commercial use. The seed was grown in 1949 and represented new seed. The following list of varieties were used in this study.

<u>Cantaloupe varieties</u>	<u>Cucumber varieties</u>
Jumbo Hales Best	Straight 8
Rocky Ford	No. A - C
Hales Best 9 - 36	Colorado
Pride of Wisconsin	Everbearing
Hales Best No. 45	Clarks Special
U.S.D.A. No. 5	Snow Pickling
Greeley Wonder	Cubit

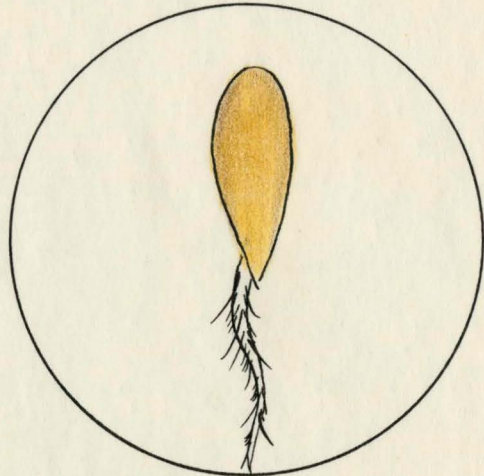
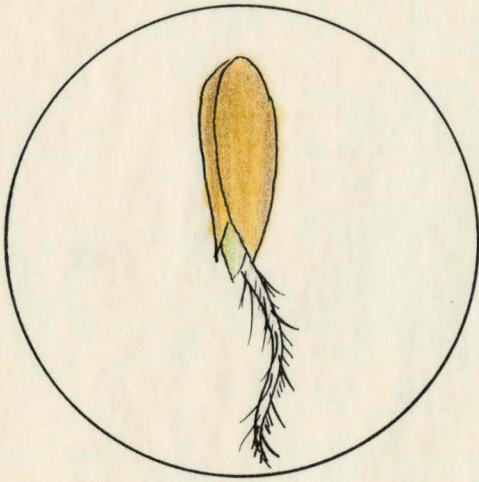
Four 100-seed samples of each variety were used for the standard germination test. The method used in determining viability by the standard germination method was as follows, each sample (100 seeds) was placed between blotting paper moistened with water and set in a germinator at a temperature of 20° C. from

5:00 P.M. to 8:00 A.M. and at 30° C. from 8:00 A.M. to 5:00 P.M. The blotting paper was kept moist through the entire germination period. Counts of germinating seed were taken after 4, 5, 6, 7, 8, and 10 days. Some of the seed, although germinating, produced abnormal growth and were not considered viable. Certain other seed became covered with a fungus growth and failed to germinate. Normal and abnormal germination and fungus covered seed are illustrated in Fig. 1 and 2.

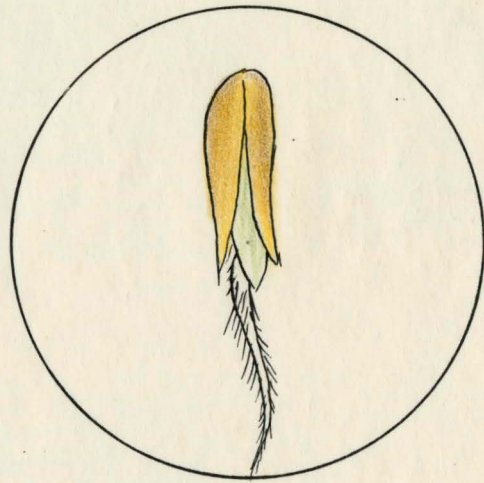
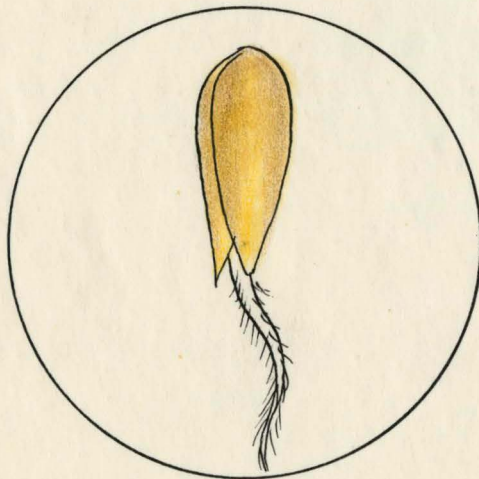
In the tetrazolium test four 100-seed samples of each variety were also used. Before beginning the tetrazolium test, it was necessary to find the position of the embryo and to determine the most efficient way in which to cut the seed so that the stained embryo would be clearly visible. By cutting the seed longitudinally the embryo was more clearly shown so this method was adopted. See Fig. 3.

The testing procedure was as follows:

1. The seeds were soaked in water at room temperature for 15½ hours.
2. The seeds were taken from the water and cut in half longitudinally by use of a razor blade.
3. One-half of each seed was placed in a 0.5 percent solution of tetrazolium chloride for one hour and ten minutes.

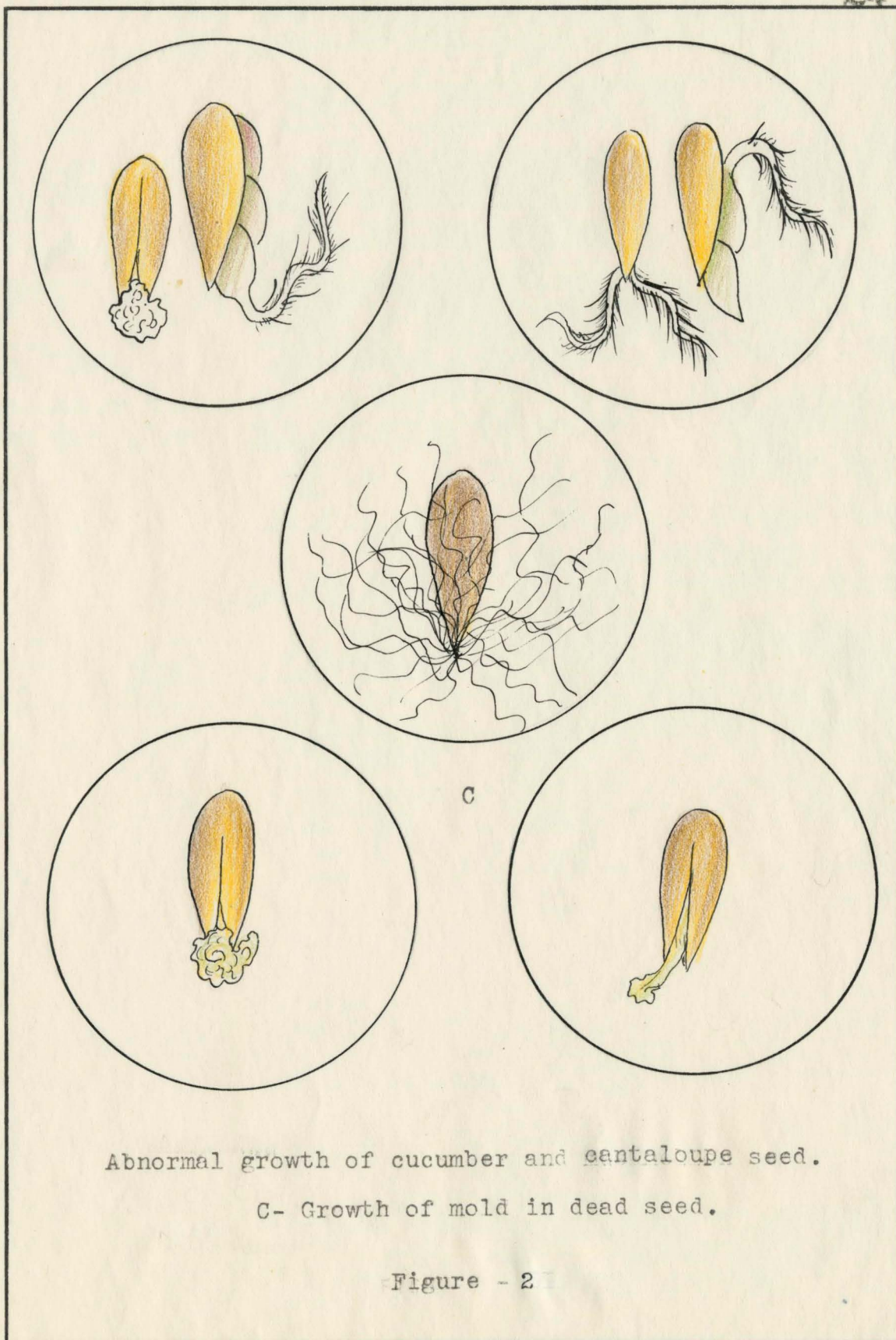


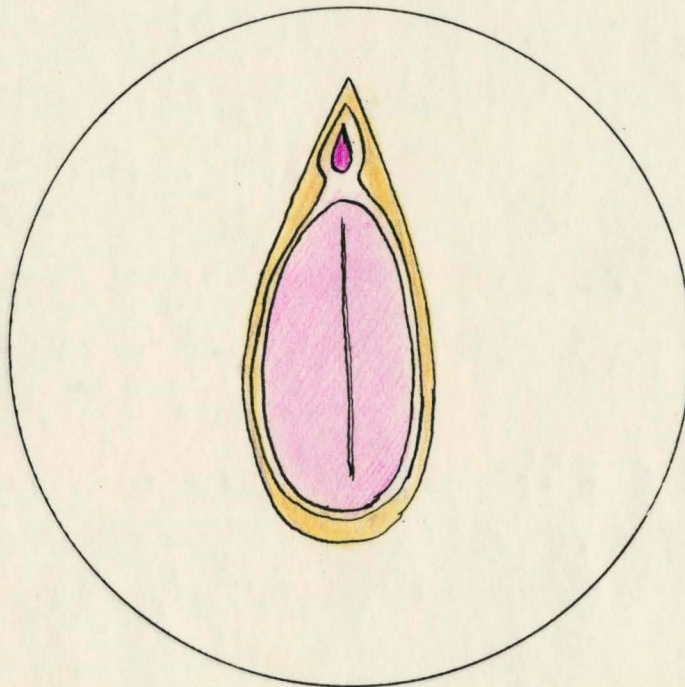
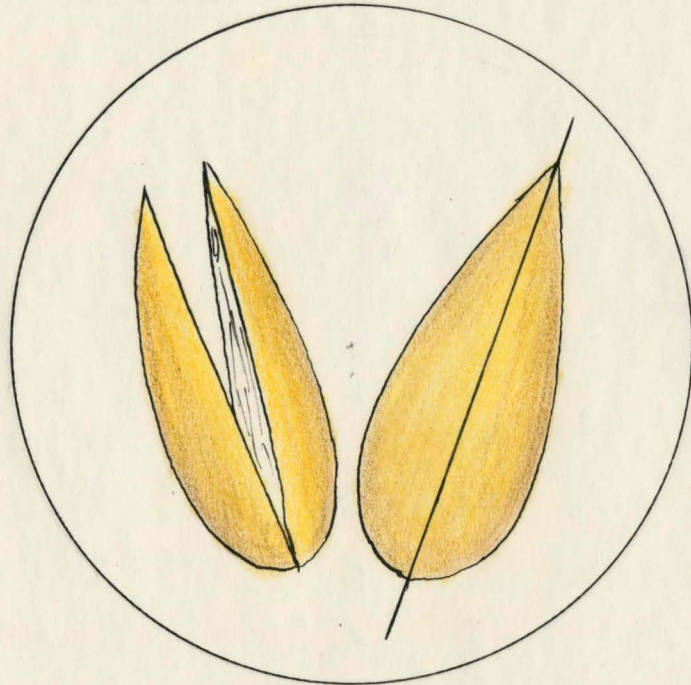
Normal growth of Cucumber seeds.



Normal growth of cantaloupe seeds.

Figure - I





Position of the embryo in cantaloupe and cucumber seed.

Figure - 3

4. Seeds with carmen red colored embryo were counted.

5. The seeds which had no color or were lightly colored were not considered viable.

The time required to complete a tetrazolium test on cucumber or cantaloupe seeds was 17 hours and 25 minutes.

The above methods were used in all further tetrazolium tests on cucumber and cantaloupe seed.

## II. The relation between cantaloupe and cucumber seed weight and germination within a variety.

To test the relation between seed weight within a variety and germination as determined by the two techniques the cantaloupe variety, Rocky Ford and cucumber variety, Snow Pickling were used.

The first step in this test was to find the average weight of seed in each variety. Ten seeds from each variety were weighed accurately to obtain this information.

After finding the average weight, 400 seeds heavier than the average and 400 seeds lighter than average were selected. Each sample (100 seeds) was again weighed.

Viability of the heavy and light seed was determined by the two methods.

### III. The relation between cantaloupe and cucumber germination and seed color.

To study the difference in germination between brown or discolored seed and white seed the same varieties of cantaloupe and cucumber were used as under II. Brown or discolored seed may be caused by a number of factors, such as light rain on seed during the drying process, excessive heating during the fermentation process prior to drying, excessive moisture while the seed is maturing, or freezing of vines and fruit before maturity.

Four hundred brown seed and four hundred white seed of each variety were used for the standard germination test. The same sized samples were also used for the tetrazolium chloride test. A germination count was taken on the 3rd, 4th, 5th, 6th, 7th, and 9th, day after planting.

### IV. The relation between age of onion seed and germination.

Onion seed was used for this phase of the work because it usually loses its viability very rapidly after the first year whereas cantaloupe and cucumber have a tendency to retain high germinability for many years.

In determining the effect of age on viability as measured by the two germination techniques it was desired to have samples of onion seed for which accurate

information was available. Unfortunately the germination information was not recorded for the old seed at the time it was harvested and stored. In one variety the name, age, and previous germination record was known. Three samples were named but the age and previous germination records were unknown.

The first four varieties listed in Table 1 were obtained from the Horticulture Department, Colorado A & M College and the last four samples listed were obtained from the seed laboratory, Colorado A & M College.

The "old" onion seed samples tested are listed in Table 1.

Table 1.--THE EIGHT ONION SEED SAMPLES TESTED BY STANDARD GERMINATION AND TETRAZOLIUM TECHNIQUES.

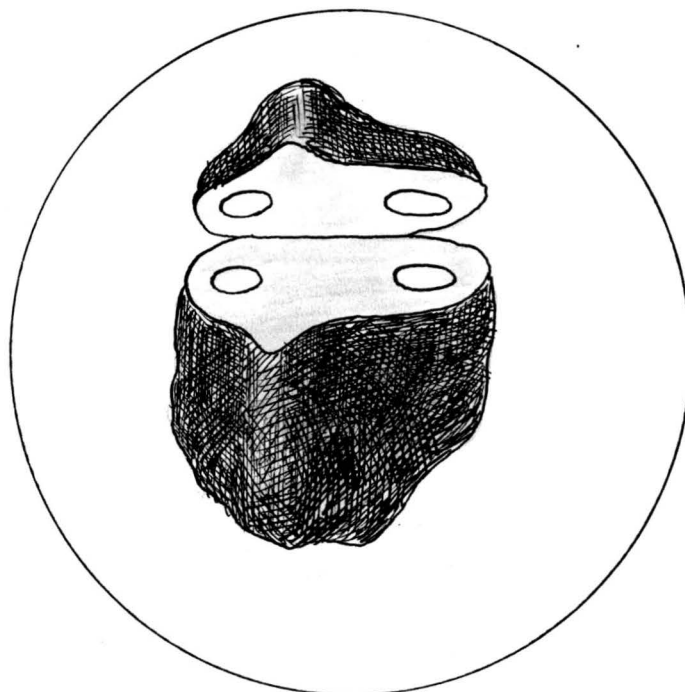
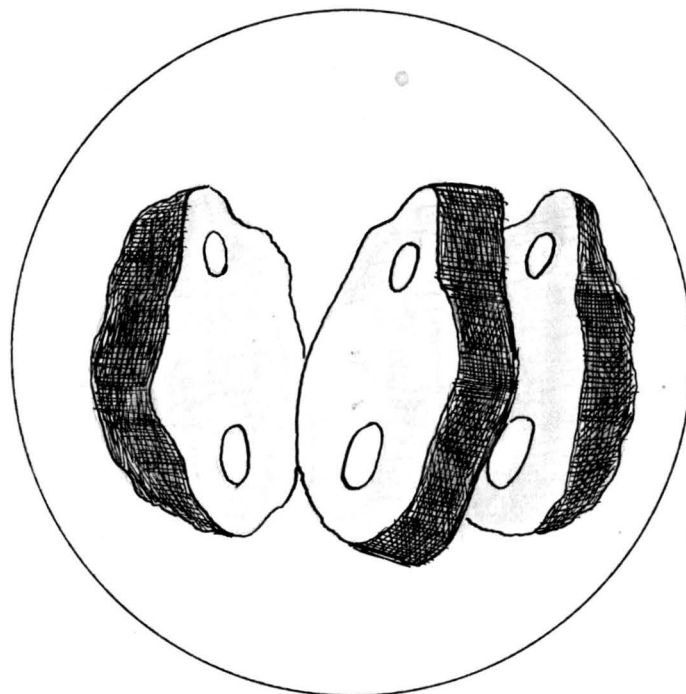
Sample	Age	Original germination
1. Early yellow Globe	1946	90.00
2. Mountain Danvers	1945	?
3. Brigham Yellow Globe	?	?
4. Mountain Danvers	?	?
5. Unknown A	?	?
6. Unknown B	?	?
7. Unknown C	?	?
8. Unknown D	?	?

Four samples of 100 seeds each from each of the eight onion seed lots were used to determine the viability by the standard germination techniques. Each sample of 100 seed was planted between blotters and set in the germinator at a temperature of 20° C. day and night. Sprout counts were made on the fifth day and every day thereafter until the tenth day.

Before beginning the tetrazolium test, it was necessary to locate the position of the embryo. It was found that the embryo was visible regardless of the direction in which the seed was cut as shown in Fig. 4.

In determining the viability by the tetrazolium test the following procedure was used.

1. Four hundred seeds of each variety were soaked in water at room temperature, from 5:00 P.M. to 8:00 A.M.
2. Seeds were removed from water and cut in half with a razor blade.
3. One-half of each seed was then soaked in a 0.5 percent solution of the tetrazolium for 50 minutes in the dark.
4. The embryos staining a carmen red color were counted. When the seed was removed from the solution some of the embryos were found to have grown 1 to 2 mm. Some showed enlargement in one point, others in



Section onion seed showing position of embryo.

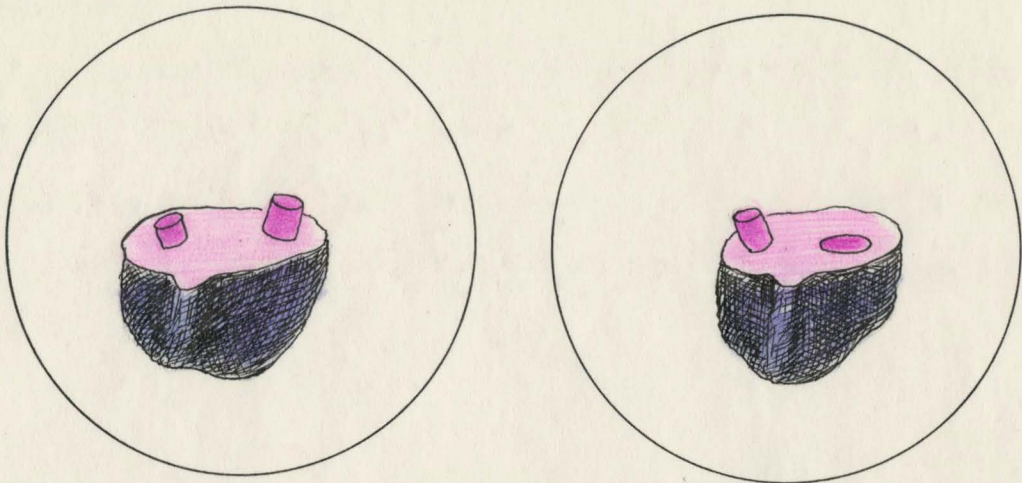
Figure - 4

both as shown in Fig. 5. There was a question at this point if this increase in size of the embryo was an indication of viability. To investigate this problem, the following procedure was used.

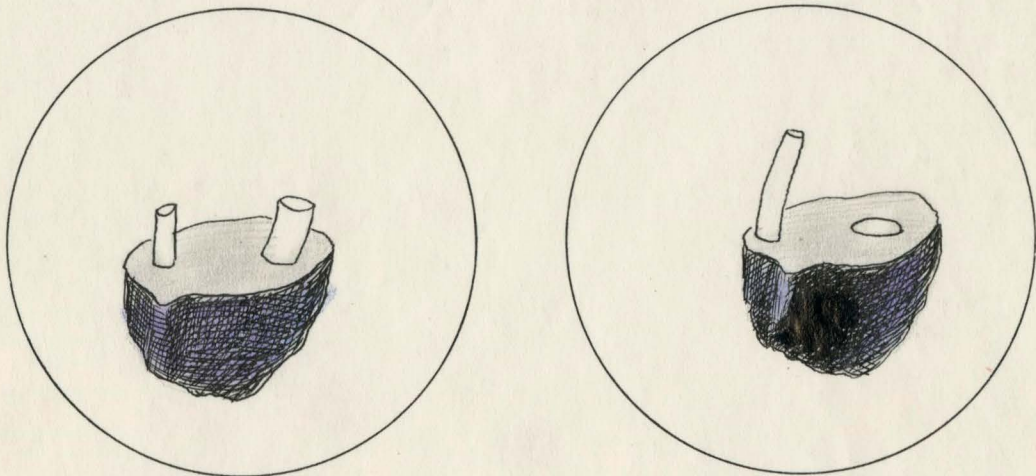
1. Two samples (100 seeds in each sample) were used.
2. They were soaked in water at room temperature for 15 hours.
3. After soaking the seeds were cut through the embryo.
4. 100 seeds were placed in the water at room temperature for 12 hours.
5. 100 seeds were placed between moist blotters.

Four samples of 400 seeds each were placed in boiling water for five minutes to kill the seed and then tested as follows:

1. One sample (400 seeds) from those which had been boiled were cut and placed between blotters.
2. Another sample of boiled seed was cut and placed in tetrazolium solution for fifty minutes.
3. A third sample was cut and then placed in water for 24 hours.
4. The fourth sample was placed in a blotter as a check to find if the seeds that had been boiled



Embryo enlargement of onion seed after 50 minutes  
soaking in tetrazolium solution.



Embryo enlargement of onion seed after 24 hours  
soaking in water.

Figure - 5

were dead.

The tetrazolium and standard germination tests were also made on old cantaloupe seeds. The three old cantaloupe seed samples were obtained from Mr. Raymond T. Farr, Sugar City, Colorado, who claimed they were grown in 1919, 1929 and 1932.

Chapter IV  
ANALYSIS OF DATA

1. Accuracy and reliability of the tetrazolium chloride test as compared to standard germination techniques.

The viability of the seven cucumber varieties as determined by the tetrazolium and standard germination tests are given in Table 2, and for the seven cantaloupe varieties in Table 3.

Table 2. <sup>XX</sup>---COMPARISON OF THE STANDARD GERMINATION TEST WITH THE TETRAZOLIUM TEST IN DETERMINING SEED VIABILITY OF SEVEN CUCUMBER VARIETIES.

Variety Name	Mean of four 100-seed lots	
	Standard germination percent viable	Tetrazolium test percent viable
A & C	94.00	95.00
Straight's	94.25	95.00
Colorado	92.00	92.50
Everbearing	94.00	94.25
Clarks Special	56.25	57.25
Cubit	96.50	97.00
Snow Pickling	93.75	93.25

Table 3.—COMPARISON OF THE STANDARD GERMINATION TEST WITH THE TETRAZOLIUM IN DETERMINING SEED VIABILITY OF SEVEN CANTALOUPE VARIETIES.

Variety name	Mean of four 100-seed lots	
	Standard germination percent viable	Tetrazolium test percent viable
Jumbo Hales Best	96.00	96.50
Rocky Ford	93.25	94.00
Hales Best 9 - 38	88.25	89.50
Pride of Wisconsin	93.25	94.00
Hales Best 45	89.00	91.50
U.S.D.A. 5	79.00	79.25
Greeley Wonder	92.75	91.50

\* Seed material of Rocky Mt. Seed Co., Denver, Colo. furnished

The germination percentages obtained with the tetrazolium compare very closely with those determined by the standard method for all varieties tested. The greatest mean difference obtained was 2.50 percentage points as shown by the cantaloupe variety, Hales Best 45. The significance of the difference was determined by the "t" test. The value for  $t = 1.29$  was not significant at the 5 percent level.

In order to determine the extent to which the two methods agree in determining seed viability for the cantaloupe and cucumber samples tested, simple correlation coefficients were calculated. Mean viability percentages obtained for each variety were used for the

calculations. The  $r$  values are given in Table 4.

Table 4.—SIMPLE CORRELATION COEFFICIENTS OBTAINED WHEN TESTING THE RELATIONSHIP BETWEEN SEED VIABILITY AS DETERMINED BY THE STANDARD GERMINATION AND SEED VIABILITY DETERMINED BY THE TETRAZOLIUM QUICK TEST.

Type of Seed	Degrees of freedom	Value of $r$
Cantaloupe	5	+ 0.885**
Cucumber	5	+ 0.992**

\*\*Exceeds 1 percent level of significance.

The  $r$  values were positive and highly significant for both the cantaloupe and cucumber varieties indicating a good agreement between the two methods of testing.

II. Relation of seed weight and germination within the Snow Pickling variety of cucumber and the Rocky Ford variety of cantaloupe.

To test the effect of seed weight on germination, the cucumber variety, Snow Pickling and cantaloupe variety, Rocky Ford were used. It was first necessary to find the average weight of a single seed in the two varieties. To determine this, ten seeds in each variety were taken at random and weighed to the nearest milligram. The individual seed weight for the cantaloupe variety are given in Table 5, and weights of the cucumber seed are given in Table 6.

Table 5.--WEIGHT IN GRAMS OF TEN RANDOM SEED IN THE CANTALOUPE VARIETY ROCKY FORD.

Seed	1	2	3	4	5	6	7	8	9	10	Ave.
Wt.	.018	.022	.014	.019	.017	.025	.020	.012	.026	.014	.018

Table 6.--WEIGHT IN GRAMS OF TEN RANDOM SEED IN THE CUCUMBER VARIETY SNOW PICKLING.

Seed	1	2	3	4	5	6	7	8	9	10	Ave.
Wt.	.022	.033	.032	.018	.034	.020	.029	.025	.024	.022	.025

Eight 100-seed samples were selected from the same lot of Rocky Ford cantaloupe seed. Four of the samples were heavier and four were lighter than the average. The weight and viability as measured by the tetrazolium test are given in Table 7.

Table 7.--THE WEIGHT OF EIGHT 100-SEED CANTALOUPE SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE TETRAZOLIUM TEST.

Sample	Weight of 100 seeds in grams	Percent viable
1	3.652	97.0
2	3.605	96.0
3	3.400	95.0
4	3.320	95.0
<hr/>		
Total-----	9.977	383.0
Mean-----	2.494	95.75
<hr/>		
1	1.720	93.0
2	1.640	92.0
3	1.615	92.0
4	1.600	90.0
<hr/>		
Total-----	6.575	367.0
Mean-----	1.643	91.75
<hr/>		
Difference between viability means		4.00
<u>t</u> Value		2.33**

\*\*Significant at 1 percent level.

The percentage viability of the heavy seed was 4.00 percentage points greater than that of the light seed.

Eight 100-seed samples were selected from the same lot of Rocky Ford cantaloupe seed. Four of the

samples were heavier and four were lighter than the average. The weight and germination as measured by standard germination test are given in Table 8.

**Table 8.--THE WEIGHT OF EIGHT 100-SEED CANTALOUPE SEED SAMPLES WITH THEIR GERMINATION AS DETERMINED BY THE STANDARD GERMINATION TEST.**

Sample	Weight of 100 seeds in grams	Percent viable
1	3.560	96
2	3.483	96
3	3.403	94
4	2.315	93
Total	9.759	379
Mean	2.439	94.75
5	1.733	95
6	1.650	92
7	1.637	91
8	1.603	89
Total	6.613	376
Mean	1.653	91.75
Difference between viability means		3.00
t Value		1.68

The heavy seed on the average germinated 3.00 percentage points higher than the lighter seed. This difference was not significant at the 5 percent level.

Four 100-seed samples heavier than average, and four 100-seed samples lighter than the average were selected from the same seed lot of the cucumber variety Snow Pickling. The weight of each sample with viability percentages as determined by the tetrazolium are given in Table 9.

Table 9.--THE WEIGHT OF EIGHT, 100-SEED CUCUMBER SEED SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE TETRAZOLIUM TEST.

Sample	Weight of 100 seeds in grams	Percent viable
1	2.995	96
2	2.992	95
3	2.990	94
4	2.883	93
Total	11.860	378
Mean	2.965	94.50
5	2.320	87
6	2.290	86
7	2.198	86
8	2.192	83
Total	9.000	342
Mean	2.250	85.50
Difference between viability mean		9.00
<u>t</u> Value		4.24**

\*\*Significant at 1 percent level.

The heavy seed on the average was 9.00 percentage points higher in viability than the lighter seed. This difference was significant at 1 percent level.

Germination percentages were determined by the standard method, on four 100-seed samples lighter than average, and four 100-seed samples heavier than average, selected from the same seed lot of Snow Pickling cucumber. The seed weights and germination percentage are given in Table 10.

Table 10.--THE WEIGHT OF EIGHT 100-SEED SAMPLES WITH THEIR VIABILITY AS DETERMINED BY THE STANDARD GERMINATION TEST

Sample	Weight of 100 seeds in grams	Percent viable
1	3.007	97
2	2.985	97
3	2.973	93
4	2.832	91
Total	11.797	378
Mean	2.949	94.50
5	2.308	89
6	2.398	88
7	2.240	87
8	2.183	83
Total	9.037	347
Mean	2.256	86.75
Difference between viability mean		7.75
t Value <sup>1</sup>		3.76**

\*\*Significant at 1 percent level.

The heavy seed on the average germinated 5.25 percentage points higher than the lighter seed. This difference was significant at 1 percent level.

The relationship between seed weight and viability as measured by the standard germination and tetrazolium test were determined by calculating simple correlation coefficients. The  $r$  values for the various relationships are shown in Table 11.

Table 11.--RELATIONSHIP BETWEEN PERCENTAGE GERMINATION AND SEED WEIGHT IN CUCUMBER AND CANTALOUPE AS MEASURED BY BOTH THE STANDARD GERMINATION AND TETRAZOLIUM TEST.

Variety	Test By	Degrees of freedom	Value of $r$
Snow Pickling	Tetrazolium	6	+ 0.960**
Snow Pickling	Standard germination	6	+ 0.914**
Rocky Ford	Tetrazolium	6	+ 0.950**
Rocky Ford	Standard germination	6	+ 0.712*

\*\*Exceeds 1 percent level of significance.

\*Exceeds 5 percent level of significance.

Seed viability as determined by both the standard germination and tetrazolium test showed a strong positive correlation with seed weight in both varieties tested. The  $r$  values were either significant or highly significant.

### III. Relationship between germination and seed color.

To test the effect of seed color on germination,

800 brown colored seed and 800 white colored seed were selected from each of the varieties Snow Pickling cucumber and Rocky Ford cantaloupe. Four hundred seed of each of the brown and white sample were tested by the standard germination method. The same sized sample were tested by the tetrazolium test. The results are given in Table 13.

Table 13.--VIABILITY OF BROWN AND WHITE COLORED SEED SELECTED FROM THE SAME BULK LOT OF SNOW PICKLING CUCUMBER AND ROCKY FORD CANTALOUPE.

Variety	Percent Viability					
	Tetrazolium			Standard germination		
	Brown	White	t	Brown	White	t
Snow Pickling	90.50	93.75	1.99*	92.75	96.00	2.36**
Rocky Ford	89.25	94.25	2.54**	90.50	94.75	2.29**

\*\* Exceeds 1 percent level of significance.

\* Exceeds 5 percent level of significance.

The percentage viability of the white seed was greater than the brown seed in all tests. Differences in all cases were either highly significant or significant.

Simple correlation coefficients were calculated to show the relationship between the two methods of testing when used on brown and white seed. The results are given in Table 13.

Table 13.—RELATIONSHIP BETWEEN VIABILITY AS MEASURED BY THE STANDARD GERMINATION AND TETRAZOLIUM TESTS USING BROWN AND WHITE SEED OF CUCUMBER AND CANTALOUPE.

Variety Name	Degrees of freedom	Value of $\bar{r}$
Snow Pickling <sup>2</sup>	6	+ 0.966**
Rocky Ford <sup>3</sup>	6	+ 0.735**

\*\*Exceeds 1 percent level of significance.

The highly significant positive correlations indicate a good agreement between the two methods of testing viability.

VI. A comparison of the tetrazolium test and the standard germination method in measuring viability of old onion seed.

Eight seed samples of old onion seed were tested for viability by the tetrazolium and the standard germination method. Four, 100-seed samples from each of the eight seed lots were used for the tests. The significance of the differences between the two methods were determined by the  $\bar{t}$  test. The results of the test are given in Table 14.

Table 14.—PERCENTAGE VIABILITY OF EIGHT OLD ONION SEED SAMPLES AS DETERMINED BY THE TETRAZOLIUM AND THE STANDARD GERMINATION METHOD.

Sample	Age	Percent Viability			t
		Original	Standard	Tetrazolium	
Early Yellow Globe	1946	90.00	80.25	79.00	0.438
Mountain Danvers	1945	?	87.75	86.00	0.733
Brigham Yellow Globe	?	?	43.00	35.00	2.036*
Mountain Danvers	?	?	55.50	50.25	1.414
Unknown A	?	?	49.75	30.25	5.627**
Unknown B	?	?	8.25	0.00	--
Unknown C	?	?	7.50	0.00	--
Unknown D	?	?	10.55	0.00	--

\*\*Exceeds 1 percent level of significance.

\*Exceeds 5 percent level of significance.

The percentage viability determined by the tetrazolium test was lower than that obtained by the standard germination test for all samples tested. The differences, however, were not statistically significant in three of the eight cases. In two other samples, the differences were statistically significant and in the remaining three samples, the stain test failed to detect any viability. This has been considered in the discussion of table. It will be noted from the table that where the viability of the sample was high, the two methods of testing were in agreement, but where the viability percentage dropped

below 50 percent, the tetrazolium stain was less accurate.

Three lots of old cantaloupe seed were also tested by the tetrazolium and standard germination techniques. The size of sample used for the tests was the same as in the previous test. The results are given in Table 15.

Table 15.--PERCENTAGE VIABILITY OF THREE LOTS OF OLD CANTALOUPE SEED AS MEASURED BY THE TETRAZOLIUM AND STANDARD GERMINATION METHODS.

Sample	Age	Percent Viability		
		Standard germination	Tetrazolium test	$t$
Pink	1919	68.00	65.00	0.919
Pink	1932	65.00	63.00	0.579
Nugget	1939	0.0	0.0	--

The differences between the two methods were not significant, indicating that the two methods of testing gave very similar results.

Chapter V  
DISCUSSION

The results of the tetrazolium stain tests were closely in agreement with those obtained by the standard germination method when used as a test for seed viability of seven cantaloupe and seven cucumber varieties. The largest difference was 2.50 percent obtained when testing seed of the cantaloupe variety Hales Best 45. This difference was not statistically significant and was therefore considered as being due to chance.

When the two methods were correlated  $r$  value of 0.865 was obtained for the cantaloupe and 0.992 for the cucumber varieties. These strong positive simple correlation coefficients were further indication of the close agreement between the two testing methods.

Cucumber and cantaloupe seed heavier than the average for a particular seed sample had a higher viability than seed lighter than average when tested by the tetrazolium stain and the standard method. The differences were highly significant except for the standard germination test of the cantaloupe seed. The difference in this case approached but failed to reach the level required at the 5 percent point.

The strong positive t values obtained when correlating seed weight with germination was added evidence that heavier seed had greater viability than light seed from the same sample.

The two testing methods showed close agreement in all tests on heavy and light seed. For the cantaloupe sample, the tetrazolium gave a 4.00 percent difference between heavy and light seed and by the standard germination method a 3.00 percent difference. The difference in mean viability between heavy and light seed of cucumber was 9.00 percent for the tetrazolium and 7.75 percent for the standard method. These results indicate that the tetrazolium stain is comparable to the standard germination method in detecting differences in seed viability as affected by seed weight.

White colored cantaloupe and cucumber seed had a somewhat higher percentage of viable seeds than brown colored seed selected from the same seed lots. The white cucumber seed was 3.25 percentage points higher in viability than the brown colored seed in both methods of testing. The white cantaloupe seed was 5.00 percent more viable than the brown when tested by the standard technique.

The tetrazolium gave consistently lower viability percentages than the standard method when used on old onion seed. Where the standard germination test

indicated 50 percent or higher viability the differences obtained by the two methods were not significant. However, when the seed viability was less than 50 percent the tetrazolium stain gave significantly lower tests than the standard method and in fact failed to detect any viable seed in samples showing less than 10.55 percent.

The two methods of testing viability gave almost equivalent results when used on old cantaloupe seed. The differences obtained were not significant at the 5 percent level. The germination of the old seed 68.00 percent for the 1919 sample and 65.00 percent for the 1932 sample seems high for seed of that age and it is possible that there was some error in labeling the seed.

The results of the series of tests conducted in these investigations indicate that the tetrazolium stain is a reliable method of testing the viability of cucumber and cantaloupe seed. Reliable results were obtained when testing onion seed of greater than 50 percent germination but with samples of lower germination the accuracy and reliability of the tetrazolium is in doubt. Further tests should be made on other vegetable seed and on old seed samples of cantaloupe, cucumber and onion.

## Chapter VI

## SUMMARY

1. The tetrazolium chloride stain compared well with the standard germination method as a test for seed viability when used on year old seed of seven cucumber and seven cantaloupe varieties. The largest mean difference obtained was 3.50 percent.

2. When results of the two tests were correlated an  $r$  value of +0.885 was obtained for the cantaloupe varieties and +0.993 for the cucumber varieties. These strong positive simple correlation coefficients indicate a close agreement between the two testing methods.

3. Cucumber and cantaloupe seed heavier than average showed consistently higher viability than lighter than average seed when selected from the same seed lots. There was close agreement between the two methods when used to test the heavy and light seed.

4. White colored cantaloupe and cucumber seed showed a higher percentage of viability than brown seed selected from the same sample. The tetrazolium gave viability percentages closely comparable to the standard method in this test.

5. The viability percentages of old onion seed

determined by the tetrazolium stain were lower in each case than those determined by the standard technique. Where viability percentages were above 50 percent the differences were not significant but below 50 percent the tetrazolium was not reliable. The three samples showing 10.55 percent germination or lower showed no stain reaction with the tetrazolium chloride.

6. Embryo enlargement was shown by viable onion seed after immersion in water for 24 hours and after being exposed to the stain for 50 minutes.

## APPENDIX I

Original data are on file in the Horticulture  
Department, Colorado A & M College, Fort Collins,  
Colorado.

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