Technical Bulletin No. 921 • October, 1946

UNITED STATES
DEPARTMENT OF AGRICULTURE
WASHINGTON, D. C.

Studies on the Germination of Guayule Seed[1]

By HARRIS M. BENEDICT, formerly *plant physiologist*, and JEANETTE ROBINSON, formerly *junior plant physiologist, Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration*[2]

CONTENTS

INTRODUCTION

When the Emergency Rubber Project was started early in 1942, very little was known about the factors affecting germination of guayule seed. Lloyd (*7*)[3] and Kirkwood (*6*) had published some findings which they had made in Mexico and which recognized the difficulty of getting the seed to germinate. Pissarev (*11*) had shown that light favored germination and that the seed should be planted when soil temperatures were between 23° and 25° C. McCallum (*9*) had stated that the delayed germination of guayule seed had been worked out and a treatment developed which would overcome this dormancy. This treatment, described

---

[1] Submitted for publication January 22, 1946.

[2] The authors express their thanks to Mr. Carl A. Taylor, chief seedsman, Emergency Rubber Project, U. S. Forest Service, for valuable assistance in furnishing and cleaning various seed accessions and for access to his many reports on the behavior of guayule seed. They also acknowledge the valuable assistance of Mrs. Zelda Mae Koger, who made the statistical analyses, and of Mrs. Thelma Milstead, Miss Margaret Klipstein, and Mr. Andrew W. Krofchek for their help with the germination tests.

[3] Italic numbers in parentheses refer to Literature Cited, p. 47.

in United States Patent No. 1,735,835 (*8*), was available to the project and with certain modifications has become the so-called standard treatment, which so far has been given to all the seed planted in the production nurseries. It consists in washing the seed in water for 18 hours, then soaking for 2 hours in a solution of sodium or calcium hypochlorite [4] containing 1.5 percent available chlorine at the rate of 4 ml. per 100 seeds (2½ gallons per pound), rinsing off the hypochlorite, and then drying it for storage or sowing while still moist if so desired. Whenever seed is spoken of as having received the standard treatment, it means it was given the treatment just described. This treatment, though expensive, insures the germination of over 90 percent of the filled seed. In order to devise a cheaper treatment, experiments were designed to find out how the hypochlorite breaks dormancy and how such factors as temperature, storage conditions, age of the seed, and conditions under which the seed was produced affect germination. This bulletin presents the results of these experiments.

METHODS AND MATERIALS

Cleaning Seed

CHARACTERISTICS OF SEED AT HARVEST

When guayule seed is harvested, two structures are usually gathered in addition to the usual debris of leaves, twigs, and other plant parts. One of these consists of the achene supporting the shriveled corolla and three short awns to which are attached two sterile disk flowers and an involucral bract. This structure, which is generally regarded as the guayule seed, will be referred to later as the complete fruit, achene plus florets, or seed. The second structure obtained consists of the flower centers, the remainder of the disk flowers adhering to each other in a conical mass (*1; 7, p. 46*). The flower centers can be removed by running the collection over a slotted screen.[5] Slots ½ by $\frac{1}{12}$ inch have been found satisfactory for most collections, but their size could be varied with the size of the seed and flower centers. Most of the remaining chaff can be removed by screening and fanning. Collections have been cleaned in this manner to a purity of 90 percent without discarding any light seed.

A very low percentage of guayule seed is filled, or contains mature embryos. For example, in bulk collections made in California in 1943 an average of about 20 percent of the seed was filled; in some as few as 5 percent and in one almost 60 percent was filled. There are several reasons for the low percentage of filled seed. First, because of impotent pollen and other factors, embryo development seems to be stimulated in only about 50 percent of the ovules (*13*). Some of the other factors which

---

[4] Calcium hypochlorite was originally used, but the sodium compound was found to be equally effective and is now used entirely.

[5] The use of the slotted screen for this purpose was first suggested by Dr. H. P. Traub, formerly principal physiologist, Special Guayule Research Project.

influence the percentage of filling are nutrient supply and temperature (*16*) and the presence of seed-feeding insects (*14*).

Thus, the only sure way to determine the potential germinability of a sample of guayule seed is to cut open a given number of achenes and count the embryos present. Because the seed is quite variable, at least 4 samples of 100 seeds each should be cut to give an estimate of the percentage filled.

Making numerous cuttings afforded an opportunity to observe the structures in filled and empty seed. A good or filled seed contains an embryo that almost completely fills the ovarian cavity, and only very small remnants of the inner layer of the pericarp or endocarp (*1*) remain. In an empty seed just the shriveled ovule can be found and the endocarp almost completely fills the space occupied by the embryo in filled seed. What becomes of the endocarp tissue as the embryo develops is not known; it may be mashed against the hard mesocarp or resorbed, or its development may be curtailed when embryo growth is stimulated. The endocarp is white, granular, and starchy in appearance, whereas the embryo is colorless, or hyaline. These differences make it easy to distinguish the two when cutting tests are made.

SEPARATING FILLED AND EMPTY SEED

It is necessary to remove as many of the empty seed as possible from bulk collections in order to obtain samples with sufficiently high percentages of filled seed for germination tests and to save storage space. Fairly satisfactory methods have been worked out by Taylor [6] for separating the empty from the filled seed (achenes plus florets) without throwing away too large a proportion of the latter. Several methods have been tried for removing the empty seed from small quantities to be used for studies of germination. These have all been based on the greater density of the filled seed.

The first of these methods consisted in placing the achenes plus florets in liquids of various specific gravities with the idea that the filled would sink and the empty would float. Whitehead and Mitchell (*16*) reported satisfactory results with Skellysolve F. In the hope of obtaining a still better separation, other liquids were tried. These included different concentrations of ethanol and methanol in water, ethyl ether, petroleum ether, chloroform, n-pentane, and isopentane. The results showed the lower the specific gravity of the liquid the better the separation. Thus, the pentanes, whose specific gravities are among the lowest of materials that are liquid at room temperatures (*3*), and Skellysolve F, which is composed largely of pentanes, gave the most satisfactory results. Table 1 shows the separation of two lots of seed from different sources and with different original percentages of filled seed obtained with n-pentane, isopentane, and Skellysolve F. In one lot practically all of the filled seed sank and the empty floated, but in the other lot a fair proportion of good seed did not sink.

[6] TAYLOR, C. A. SEPARATING GUAYULE SEED BY GRAVITY TABLE. Chief Seedsman, Emergency Rubber Proj., U. S. Forest Serv. Rpt. 1943. [Unpublished.]

TABLE 1.—*Separation of empty and filled guayule seed by flotation in various pentanes*

Flotation liquid and seed type	Seed collection 1			Seed collection 2		
	Weight	Proportion of original weight	Seed filled	Weight	Proportion of original weight	Seed filled
	Grams	*Percent*	*Percent*	*Grams*	*Percent*	*Percent*
Original sample	2.00	......	45.0	2.00	......	9.5
N-pentane:						
Heavy seed [1]	.73	36.5	50.0	.61	30.5	30.5
Light seed [2]	1.27	63.5	25.5	1.39	69.5	2.0
Isopentane:						
Heavy seed [1]	.73	36.5	82.5	.60	30.0	30.6
Light seed [2]	1.27	63.5	23.6	1.40	70.0	1.6
Skellysolve F:						
Heavy seed [1]	.68	34.0	87.0	.56	28.0	31.0
Light seed [2]	1.32	66.0	23.4	1.44	72.0	2.2

[1] Seed that sank. [2] Seed that floated.

In both lots many empty seed sank. It was found that soaking in pentanes for as long as 10 minutes had no effect on germination.

Attempts have been made to obtain seed samples with high percentages of filled seed with fanning mills of various sorts. By using a model B Clipper fanning mill, lots averaging 40 to 50 percent of seed filled could be obtained from samples originally with only 20 percent filled. Regardless of the percentage of filled seed obtained, much was discarded with the light seed. It is rather difficult to control accurately the air blast of a fanning mill of this type and to vary it. With this point in mind a blower was constructed from plans furnished by C. W. Legatt.[7] The air blast through the seed could be accurately controlled by varying the size of the air intake. By widening the opening, different grades of seed could be blown out. The larger the air opening the greater the weight of the seed and the higher the percentage of seed that was filled (table 2). As was true of the pentane separation, there was a large difference in the percentages of the heavy seed of the two collections that were filled; however, this blower was

TABLE 2.—*Characteristics of different fractions of guayule seed obtained by controlled fanning of 2 lots of seed*

Width of air inlet	Lot A					Lot B				
	Total seed	Seed filled	Proportion filled	Weight per seed	Proportion of total filled	Total seed	Seed filled	Proportion filled	Weight per seed	Proportion of total filled
	Number	*Number*	*Percent*	*Mg.*	*Percent*	*Number*	*Number*	*Percent*	*Mg.*	*Percent*
¼ inch	430	11	3	1.19	0.5	......	......	......	......	......
½ inch	1,850	190	10	1.34	7.9	2,305	48	3	1.20	5.9
9/16 inch	1,331	236	18	1.42	9.9	......	......	......	......	......
19/32 inch	1,105	381	35	1.64	15.9	......	......	......	......	......
⅝ inch	751	340	45	1.71	14.2	1,645	115	7	1.51	14.1
¾ inch	500	278	56	1.84	11.6	1,780	196	11	1.63	24.0
⅞ inch	......	......	......	......	......	898	256	29	1.75	31.3
Remainder	1,240	958	77	1.99	40.0	352	202	57	1.86	24.7
Total or mean	7,207	2,394	33	1.58	100.0	6,980	817	12	1.49	100.0

[7] Communication to Dr. H. P. Traub.

much more satisfactory than pentane or an ordinary fanning mill in removing empty seed.

Whenever a lot of seed was received for experimental purposes, with one or two exceptions it was run through the blower so that a fraction was obtained with at least 30 percent of the seed filled. It was desired to obtain one with 50 percent filled but often this was extremely difficult to do without throwing away practically all the filled ones.

It might be argued that the response of the heaviest portions of the seed to any treatment would not be representative of the whole. To obtain an indication on this point seed from some of the fractions of lot A (table 2) were germinated with and without receiving the standard treatment (table 3). When the seed was not treated, approximately the same proportion of the filled seed in all the fractions germinated. When it was treated, the germination of the filled seed in the heaviest and lightest fractions was increased by about the same percentage, and over 90 percent of the filled seed in all the fractions germinated. These results indicated that results obtained on the heavier seed would be applicable to all the seed in the original sample.

TABLE 3.—*Germination of standard-treated and untreated guayule seed from fractions of seed lot A of table 2*

Width of air inlet	Seed filled	Germination		Treated / Untreated
		Treated	Untreated	
	Percent	*Percent*	*Percent*	*Percent*
½ inch	10.3	10.0	7.0	143
19/32 inch	34.5	34.0	24.3	140
⅝ inch	45.3	45.3	37.0	122
¾ inch	55.6	54.3	42.0	129
Remainder	77.3	76.8	54.8	140

SEPARATION OF FILLED ACHENES FROM COMPLETE FRUITS

With various macerating machinery it was possible to break off the sterile florets and bracts and leave the achenes. Because of the ease of handling the achenes alone in seeding machinery and the reduction of storage space required, this practice has become more and more common but it is still not generally followed. However, the use of the achenes alone makes it necessary to consider them.

When it was desired to work with the simple achenes, lots with very high percentages of filled seed could be obtained. For small lots the complete fruits were placed in an ordinary seed flat lined with corrugated rubber matting; then they were rubbed by hand with a block of wood surrounded by the rubber matting. When all the fruits were broken up, this material was run through a Clipper fanning mill and the chaff and empty achenes were fanned out. Taylor [8] also described a method for obtaining

[8] TAYLOR, C. A. A RAPID METHOD OF DETERMINING THE FILLED PERCENTAGE OF GUAYULE SEEDS WITH ATTACHED FLORAL PARTS. Chief Seedsman, Emergency Rubber Proj., U. S. Forest Serv. Rpt. 1943. [Unpublished.]

filled achenes from very small lots of seed. The final step in his method involves placing of the achenes in acetone to separate the empty from the filled. It was found that soaking the seed in acetone for 10 minutes had no injurious effect on the germination. Both methods gave as good separation of empty and filled achenes as could be expected (table 4).

TABLE 4.—*Extraction and separation of filled achenes from complete guayule fruits by 2 different methods*

Method and seed lot	Fruits	Heavy achenes			Light achenes		
		Total	Filled		Total	Filled	
Rubber matting and fanning mill:	*Number*	*Number*	*Number*	*Percent*	*Number*	*Number*	*Percent*
Lot A	2,000	655	638	96.6	176	2	1.1
Lot B	2,000	578	550	94.5	206	5	2.4
Taylor method:[1]							
Lot C	500	108	107	99.1	106	3	2.8
Lot D	500	300	279	93.0	87	2	2.3

[1] See footnote 8, p. 5.

Lots of achenes extracted in 1,000-pound quantities also were obtained by Taylor[9] with a purity of 94 percent and averaging more than 98 percent of the seed filled. The purity and germination of these lots of seed compared favorably with those required of seed of other commercial crops.

OBTAINING SAMPLES

After a bulk lot of seed had been recleaned for germination studies it was well stirred and then set aside, and samples were taken as needed. Approximately the quantity of seed necessary for a particular experiment was taken from the large lot. This sample was then placed on a large sheet of paper and rolled back and forth several times to mix the seed. From this mixed sample, the necessary individual lots of 100 seeds each were counted.

The steps in obtaining seed to be used in germination tests may be summarized as follows:

(1) Pass sample over slotted screen to sieve out flower centers, large bits of stems, and other debris.
(2) Pass remaining seed over a fanning mill to screen and fan out the chaff.
(3) Eliminate as large a proportion of empty seeds as possible by separation in pentane or by blowing in a carefully controlled air blast.
(4) Mix remaining seed by stirring and set aside until needed.
(5) Withdraw at random approximately enough seed for the tests.
(6) Roll this seed on a large sheet of paper until thoroughly mixed.
(7) Count out as many lots of 100 seeds each as are needed.

CONDUCTING GERMINATION TESTS

For each treatment 4 lots of 100 seeds each were generally used as recommended in Rules and Regulations under the Federal Seed Act (*15*). At first it was thought that since only half of the seed had embryos that would germinate, 800 seeds should be used

[9] TAYLOR, C. A. TRIAL THRESHING OF 1,000 POUNDS OF UNTHRESHED SEED. Chief Seedsman, Emergency Rubber Proj., U. S. Forest Serv. Rpt. 1944. [Unpublished.]

in order to include roughly 400 filled ones in each test. Studies were made on the difference required for significance when 4 and 8 replications of 100 seeds each were used, and it was found that increasing the number of replications decreased the difference required for significance between means from between 7 and 8 percent to about 5 percent. This increase in accuracy did not warrant the increased work, as significant differences could be demonstrated without it.

In carrying out the germination tests each replication was handled separately throughout, beginning with any pretreatment that was to be applied. Thus, variation between replications included any errors involved in the technique of treating the seed and was not due just to sampling. After each replication was placed between moist germination blotters (4- by 4-inch), care was taken that the seeds were evenly spaced and not in contact with each other. The blotters were then placed on trays in germinators. The individual replications of the various treatments were randomized on the trays to avoid any possible effect of position. The use of blotting paper was decided upon after the seed had been germinated on different substrata (table 5). The complete fruits did not germinate as well on top of blotters as between them, but the various media tried had no effect on the germination of the achenes alone. Because blotters were much easier to handle than the other substrata, the between-blotter method was adopted.

TABLE 5.—*Germination of guayule seed on and between various substrata*

Substratum and seed position	Achenes of indicated lot germinating [1]		Achenes plus florets (lot 2) germinating [2]
	Lot 1	Lot 2	
	Percent	*Percent*	*Percent*
Between moist blotters	48	52	22
On top of moist blotters:			
Outside	44	55	15
In Petri dishes	48		
On top of moist filter paper in Petri dishes	45		
Between moist paper towels	48		
On top of moist paper towels	46		

[1] No significant difference between substrata and seed positions.
[2] Difference required for significance at 5-percent level, 7 percent.

The seed was germinated at alternating temperatures of 20° C. for 17 hours and 30° for 7 hours daily. In most instances, this alternation was obtained by switching the trays between two Minnesota germinators, one of which was kept at 30° and the other at 20° continuously. On rare occasions Keene and Mangelsdorf germinators were used. With those the alternating temperature was obtained by changing the setting on the thermostats. Although the change from one temperature to the other was not as rapid as when the trays were switched, this difference seemed to have no effect on the germination of the seed (table 6).

The germinated seed was removed and counted every 3 days for 21 days, which was usually as long as the tests were continued. When it was desired to determine how many filled seed had not germinated, the seed left on the blotters was dissected and the embryos were counted. These embryos added to the seed that germinated gave the number of filled ones originally present.

TABLE 6.—*Germination of guayule seed at alternating temperatures produced by switching trays or changing setting on the thermostat*

Lot and treatment	Seed germinating when temperature alternation was obtained by [1]—	
	Switching trays between germinators	Changing setting on thermostat
	Percent	*Percent*
Lot 1 (achenes plus florets):		
Seed standard-treated	46	43
Seed not treated	33	33
Lot 2 (achenes):		
Seed standard-treated	91	91
Seed not treated	91	95
Lot 3 (achenes plus florets):		
Seed standard-treated	42	40
Seed not treated	25	25

[1] No significant difference between methods of alternating temperatures.

ANALYZING DATA

At the end of the tests the number of seed germinated in each of the four replications was recorded, and the average for each treatment was then determined. An analysis of variance was made on the results of each experiment, and if the odds against the differences between treatments occurring by chance were greater than 19 to 1, the differences were considered as significant. If differences due to treatments were found to be significant, the differences required between means to give odds greater than 19 to 1 against these differences occurring by chance are given in the tables. Hayes and Immer (*5, pp. 375–379*) discussed the use of analysis of variance with percentage data; in accordance with their suggestion, the percentages were transformed by the angular transformation of Bliss ($p = \sin^2 \theta$) and the analyses were carried out on these transformed values. At first all the data were transformed, but it was found as suggested that when the values fell between 25 and 75 percent transformation was not necessary.

In some instances chi square (x^2) tests carried out according to the method of Powers (*12*) were substituted.

The results of treatments were generally so obvious that it was not necessary to run an analysis of variance on the data to be sure that real differences existed. In fact, if inspection of the results raised any doubts as to their significance, the experiment was repeated regardless of statistical analysis. From all the experiments it was learned that a difference of 8 percent between treatments can practically always be taken as an indication that the germinations differed significantly.

DESCRIPTION OF SEED LOTS

In the various experiments several different collections were used. To avoid the necessity of giving the history of each seed lot whenever it is referred to, the collections were given accession numbers. The characteristics of seed of the various accessions are shown in table 7. The percentages of seed filled represent the values after seed had been cleaned and some empty ones had been removed. From these percentages it is possible to determine approximately the maximum germination of any accession. Although most of the accessions are of strain 593, the one generally planted, enough tests were carried out on other strains to be fairly certain that the conclusions apply to guayule seed in general and not to strain 593 alone.

TABLE 7.—*Description of guayule seed lots used in the various experiments*

Accession	Strain	Year collected	Place collected	Seed filled	Remarks
				Percent	
1	406	1940	Salinas, Calif.	33.3	
1A	406	1940	....do.	95.5	Achenes from accession 1.
2	593	1942	....do.	28.5	
2R	593	1942	....do.	45.1	Refanned accession 2.
2A	593	1942	....do.	93.4	Achenes from accession 2.
3	130	1942	....do.	41.5	
4	406	1942	....do.	44.6	
5	593	1942	....do.	55.4	
6	406	1942	Indio, Calif.	43.8	
7	593	1942	Salinas, Calif.	41.4	
8	593	1942	....do.	22.1	
9	593	1942	....do.	46.7	
10	593	1942	Indio, Calif.	41.6	
11	593	1942	Salinas, Calif.	95.6	Achenes from accession 7.
12	593	1943	Indio, Calif.	72.1	
13	406	1943	....do.	70.3	
14	593	1943	Salinas, Calif.	44.4	
14A	593	1943	....do.	93.4	Achenes from accession 14.
15	593	1943	....do.	61.2	
16	593	1943	....do.	80.0	Achenes from accession 15.
17	593	1943	....do.	63.0	Achenes.
18	593	1942	....do.	69.0	
19	593	1943	Tracy-Newman district (Calif.)	96.3	Do.
20	407	1943	Salinas, Calif.	47.7	
21	593	1943	....do.	....	Embryos from accession 14
22	593	1943	Patterson, Calif.	32.5	
22A	593	1943	....do.	95.2	Achenes.
23	406	1943	....do.	40.6	
24	593	1944	Salinas, Calif.	40.9	
25	406	1944	....do.	47.6	
26	593	1944	....do.	40.3	
27	593	1944	....do.	29.3	

As a matter of convenience in the remainder of the bulletin, the accession and the strain numbers are presented as a hyphenated combination. Thus, 1–406 means that accession 1 of strain 406 was used.

EFFECTS OF VARIOUS FACTORS ON GERMINATION

FUMIGANTS

As a precaution against the introduction of various insects, plant materials brought into certain districts are fumigated. In order to determine the effects of fumigation, standard-treated and untreated seed of guayule was germinated 14 days after receiving the following fumigation treatments: [10]

[10] These fumigation treatments were carried out by the Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, U. S. Department of Agriculture, in cooperation with the California Department of Agriculture.

694351—46—2

(1) Check (no fumigation treatment).

(2) Three pounds of methyl bromide per 1,000 cubic feet for 90 minutes at 66° F. in a 27-inch vacuum sustained throughout the exposure.

(3) Three pounds of methyl bromide per 1,000 cubic feet for 90 minutes at 70° F. in a vacuum of 27 inches at the beginning but not sustained.

(4) Three pounds of methyl bromide per 1,000 cubic feet for 120 minutes at 70° F. under atmospheric pressure.

(5) Hydrocyanic acid gas (1 ounce of sodium cyanide per 100 cubic feet for 50 minutes at 60° F. in a 27-inch vacuum sustained throughout the exposure).

(6) Hydrocyanic acid gas (1 ounce of sodium cyanide per 100 cubic feet for 50 minutes at 60° to 65° F. in a vacuum of 27 inches at the beginning but not sustained).

(7) Hydrocyanic acid gas (½ ounce of sodium cyanide per 100 cubic feet for 50 minutes at 60° F. under atmospheric pressure).

These treatments were carried out in a tank of 137-cubic-foot capacity of which 1 cubic foot was occupied by the material (the seed and some guayule plants) being fumigated.

In only 1 instance was there a significant drop in germination of fumigated as compared with nonfumigated seed, and in several instances there were significant increases (table 8). According to the criterion for significance such a difference should occur by chance once in 20 times; inasmuch as there are more than 20 comparisons, this single significant drop in germination could well be a chance variation. The results indicated that fumigating as described had no injurious effect on the germination of the seed.

TABLE 8.—*Germination of guayule seed exposed to various fumigation treatments*

[Details of fumigation treatments appear above.]

Treatment	Seed of indicated accession and strain germinating					
	6–406	7–593	8–593	9–593	10–593	11–593
Seed not standard-treated: [1]						
Methyl bromide:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Vacuum sustained	27	22	2	15	18	54
Vacuum not sustained	27	23	3	19	20	50
Atmospheric pressure	30	22	4	21	18	56
Sodium cyanide:						
Vacuum sustained	27	25	3	27	21	63
Vacuum not sustained	27	23	4	26	24	61
Atmospheric pressure	28	26	4	20	17	66
Check (no fumigation treatment)	25	24	3	16	19	52
Seed previously standard-treated: [2]						
Methyl bromide:						
Vacuum sustained	39	44	19	33	30	83
Vacuum not sustained	37	39	15	46	28	86
Atmospheric pressure	39	41	18	43	27	82
Sodium cyanide:						
Vacuum sustained	47	38	21	40	31	87
Vacuum not sustained	43	41	21	42	33	86
Atmospheric pressure	48	40	24	38	31	86
Check (no fumigation treatment)	35	39	17	42	29	87

[1] Difference required for significance at 5-percent level, 7 percent.
[2] Difference required for significance at 5-percent level, 8 percent.

STORAGE CONDITIONS

STORAGE AT DIFFERENT RELATIVE HUMIDITIES

Guayule seed has been stored in climates that differ widely in their relative humidities, such as at Indio, Calif., which is very dry, and at Salinas, Calif., which is very humid. Experiments were set up to determine how the relative humidity in which the seed was stored affects its germination. Four accessions were kept in atmospheres of different relative humidities, and their germination was tested every month. The different relative humidities, approximately those listed, were obtained by placing the seed over saturated solutions of various salts, over water, and over dry calcium chloride in closed chambers. These chambers were aired once a week. The temperatures during the storage period ranged from 18° to 25° C.

The results for all the accessions are in general the same (table 9). When the accessions were stored at 80 and 100 percent relative

TABLE 9.—*Germination of guayule seed stored for various periods at different relative humidities*

[100 percent relative humidity, over water; 80 percent, over saturated ammonium sulfate; 50 percent, over saturated sodium bisulfate; 30 percent, over saturated calcium chloride; 0 percent, over dry calcium chloride; 30–50 percent, room humidity]

Accession, strain, kind of sample, and relative humidity	Moisture content of seed after storage for 7 months	Seed germinating after storage for—						
		1 mo.	2 mo.	3 mo.	4 mo.	5 mo.	6 mo.	7 mo.
12–593 (achenes plus florets): [1]	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
100 percent	....	0	0	0	0	0	0	0
80 percent	....	48	30	7	4	1	0	0
50 percent	....	33	21	38	22	40	43	47
30 percent	....	40	37	50	51	57	61	58
0 percent	....	29	23	31	18	19	21	17
30–50 percent	....	41	44	60	49	58	62	58
14–593 (achenes plus florets): [2]								
100 percent	....	0	0	0	0	0	0	0
80 percent	12.0	8	1	1	1	1	0	0
50 percent	11.5	8	6	23	12	12	12	13
30 percent	8.0	11	13	9	22	25	29	26
0 percent	4.2	11	7	7	9	9	9	8
30–50 percent	7.8	17	10	29	23	30	27	24
16–593 (achenes): [3]								
100 percent	....	0	0	0	0	0	0	....
80 percent	....	28	8	1	1	0	0	....
50 percent	....	28	26	30	37	26	34	....
30 percent	....	49	49	48	64	61	66	....
0 percent	....	27	18	22	23	34	18	....
30–50 percent	....	31	27	57	69	73	72	....
17–593 (achenes): [4]								
100 percent	....	0	0	0	0	0	0	....
80 percent	....	39	7	1	1	25	0	....
50 percent	....	42	27	56	34	39	38	....
30 percent	....	46	51	57	44	57	54	....
0 percent	....	40	36	34	32	36	43	....
30–50 percent	....	31	46	61	56	50	51	....

[1] Difference required for significance at 5-percent level: 10 percent between means; 14 percent for interaction.
[2] Difference required for significance at 5-percent level: 7 percent between means; 10 percent for interaction.
[3] Difference required for significance at 5-percent level: 11 percent between means; 15 percent for interaction.
[4] Difference required for significance at 5-percent level: 11 percent between means; 15 percent for interaction.

humidities, the germination decreased to zero in 1 to 6 months. When they were stored at 50 percent relative humidity, there was no consistent change in germination as the seed aged. When they were stored at the humidities prevailing in the room at Salinas and at 30 percent humidity, the germination of the seed in general continued to increase during the period of the tests. The germination of seed stored at 0 percent relative humidity remained low or actually decreased during the storage period. The analyses of variance showed the differences due to relative humidities and ages and the interaction relative humidity $\times$ ages to be highly significant.

After 6 or 7 months in the various relative humidities some of the seed was cut to determine the number of good embryos present. For example, 72 percent of the seed of accession 12 was filled at the start of the experiment. After it was stored for 7 months, the percentages of filled seed were 0, 1, 60, 71, 72, and 72 in 100 percent, 80 percent, 50 percent, 30 percent, 0 percent, and room relative humidities. This indicated that at the higher humidities there was a tendency for the embryos to break down possibly because of fungi present, whereas at relative humidities near 30 percent this break-down did not occur. At extremely low relative humidities the embryos not only do not break down but apparently go deeper into dormancy. When seed stored at 0 percent relative humidity for 6 months was given the standard treatment, well over 90 percent of the filled seed germinated, indicating that the poor germination of the seed stored at this relative humidity was not due to loss of viability.

The relative humidity together with the temperature at which the seed was stored affected their moisture content. At the end of 7 months' storage the moisture content of seed of accession 14 was found to vary directly with the relative humidity (table 9). Thus, the results obtained might have been due to the moisture content of the seed as well as to the relative humidities.

These results indicate that guayule seed should be stored with a moisture content of about 8 percent or under the saturation deficit prevailing in a relative humidity of approximately 30 percent at 20° to 25° C. if it is desired to obtain an increase in germination during a short period of storage. However, this increased germination might indicate a more rapid metabolic rate; for this reason the seed might not remain viable as long as seed stored under extremely dry conditions where the germination at least for 7 months of storage did not increase. Thus, if the seed is to be stored for a long period, perhaps the relative humidity or moisture content should be kept as low as possible. Only tests carried out on seed for several years can settle this point.

STORAGE IN AIRTIGHT AND OPEN CONTAINERS

In the past it has been the custom to store guayule seed in airtight containers. Table 10 shows the results of germination tests carried out on seed stored in sealed and open containers with and without the standard treatment after the storage period. Small screw-top bottles were filled with seed, and the tops were screwed on and dipped in paraffin to make a complete seal. Enough bottles were used so that after one was once opened no more seed was used from it, thus insuring airtight storage for the entire period. The open containers consisted of cheesecloth bags. The seed of both treatments were stored at room temperature (18° to 25° C.); they had a moisture content of about 7 percent at the time the storage was started.

TABLE 10.—*Germination of guayule seed not treated and standard-treated after storage in sealed and open containers for various periods*

Accession, strain, and storage period	Untreated seeds germinating when previously stored		Treated seeds germinating when previously stored	
	Sealed	Open	Sealed	Open
	Percent	*Percent*	*Percent*	*Percent*
12–593:				
1 month	47	36	69	68
3 months	51	52	71	68
4 months	41	54	65	69
5 months	63	68	70	67
6 months	57	62	69	69
8 months	63	54	72	74
9 months	62	58	68	66
11 months	62	61	66	70
12 months	59	62	71	64
13 months	47	58	70	71
13–406:				
1 month	22	27	60	65
3 months	42	46	59	61
4 months	43	47	65	63
5 months	47	49	65	65
6 months	49	52	65	62
8 months	40	49	63	61
9 months	42	50	56	62
11 months	40	56	57	62
12 months	51	52	61	62
13 months	57	54	63	63
14–593:				
1 month	11	10	32	40
3 months	15	25	42	42
4 months	13	27	34	44
5 months	20	33	43	42
6 months	19	22	37	40
8 months	27	24	41	39
9 months	25	25	30	39
11 months	27	24	38	39
12 months	23	33	38	44
13 months	25	31	34	42
22–593:				
0 month	5	5	15	15
6 months	7	19	26	25
22A–593:				
0 month	32	32	59	59
6 months	69	89	88	94
23–406:				
0 month	4	4	22	22
6 months	7	30	26	45
27–593:				
0 month	4	4	11	11
6 months	10	26	25	28

The germination of seed stored in either sealed or open containers and not subsequently treated increased during the first 6 months of storage; the germination of the seed in open containers averaged higher than that of seed in the sealed containers for all accessions. This difference was not nearly so marked with seed of accessions 12, 13, and 14 as it was with the others, although x^2 tests showed it to be significant. The seed of these three accessions were poured into the bottle under their own weight, whereas those of the other accessions were packed in under pressure. This decrease in amount of air per seed may account for the difference in response of the various accessions to sealed and open storage. From the sixth to the thirteenth month, there was no significant difference in germination of seed held in the different storage conditions.

If the seed was given the standard treatment after being stored, under the two conditions there was generally no significant difference between the germination percentages except those of accession 23. A significantly lower percentage of its seed stored in the sealed containers germinated.

The results in table 10 indicate that storing in airtight containers tends to retard the germination of guayule seed for the first 6 months after it was harvested, but that such storage caused no loss in viability as seed given the standard treatment germinated as well as that stored in cheesecloth bags. Other effects of airtight and open storage may be brought to light as the seed is stored for longer periods.

Age of Seed

An outstanding result obtained from the storage experiments was the increase in germination of both the complete fruits and the achenes as the seed aged. In table 11 are given the germination percentages of untreated seed of different accessions when received and after being kept in the laboratory for various periods. The seed was stored in unsealed containers at room temperatures and humidities.

TABLE 11.—*Germination of different accessions of guayule seed after aging for various periods*

Kind of sample, accession, and strain	Source of seed	Age of seed when achenes threshed out	Length of storage	Seed germinating—		Filled seed germinating at end
				In beginning	At end	
		Months	*Months*	*Percent*	*Percent*	*Percent*
Achenes:						
1A–406	Salinas, Cal'f...	24	4	54	78	82
2A–593	.... do.	0	5	12	88	93
11–593	.... do.	3–4	6	4	85	89
11–593	.... do.	3–4	12	4	95	99
12–593	Indio, Calif......	2	6	40	74	100
14–593	Salinas, Calif....	0	8	13	43	96
16–593	.... do.	1	7	27	77	96
17–593	.... do.	2	6	31	58	92
22A–593	Patterson, Calif..	0	6	32	89	93
Achenes plus florets:						
1–406	Salinas, Calif....	....	28	....	19	58
2–593	.... do.	....	4	3	13	45
3–130	.... do.	....	7	4	19	45
4–406	.... do.	....	7	9	17	39
5–593	.... do.	....	7	5	22	40
6–406	Indio, Calif......	....	12	19	42	96
7–593	Salinas, Calif....	....	12	15	34	81
8–593	.... do.	....	12	2	11	51
9–593	.... do.	....	12	9	28	61
12–593	Indio, Calif......	....	12	4	62	86
13–406	.... do.	....	12	19	56	86
14–593	Salinas, Calif....	....	12	7	28	62
15–593	.... do.	....	5	8	32	53
20–407	.... do.	....	9	10	35	73
22–593	Salinas, Calif...	....	6	5	19	73
23–406	.... do.	....	6	4	30	75

The germination of all the seed lots increased with age during the first year. That of the achenes alone increased the most, 90 percent of the filled seed germinating after 6 months of storage.

The increase in germination of achenes plus florets was generally less than that of the achenes. However, there seems to be a definite relation between the percentage of filled achenes plus florets that germinated at the end of the storage period and the locality in which the seed was produced. After 1 year of storage filled seed of collections made at Indio averaged 89 percent germination; on the other hand, those made in the vicinity of Salinas and stored under the same conditions for the same length of time averaged only 64 percent. Collections made at Patterson averaged 74 percent germination after a storage period of 6 months, which is higher than that of the Salinas seed stored for a year.

During the time the seed is maturing, the climate around Salinas is much cooler and more moist than the hot and dry climates of Patterson in the San Joaquin Valley and of Indio in the Coachella Valley. It may be that these or other differences in the climates were responsible for the longer period of dormancy noted in Salinas seed. It is not known whether after aging long enough all of the filled seed of any collection will germinate without special treatment. However, only 58 percent of the filled seed of accession 1 germinated after being stored for 28 months.

These results indicated that guayule seed increases in germination as it ages at least up to 12 months and that the germination of achenes alone increases so rapidly that after 6 months over 90 percent of the filled achenes germinate without any special treatment. The results also indicated that seed produced in climates such as those prevailing at Indio after being stored in open containers at room temperatures for a year will show a germination of 90 percent of the embryos without treatment whether or not the florets and bracts are removed. Why the Indio seed has this shorter dormant period is not known at present. These results mean that, in so far as germination is concerned, the very expensive hypochlorite treatment now given guayule seed can be eliminated by using achenes alone that have been stored in the open for 6 months or by producing seed in climates such as that at Indio and holding it for a year before planting.[11]

Temperature During Germination

Germination tests were carried out in various constant and alternating temperatures. When the latter were used, the seed was kept at the lower temperature for 17 hours and at the higher one for 7 hours daily. Alternation of 20° to 30° C. seemed to be about the optimum for germination; temperatures below 15° and above 30° either constant or in alternation with those between these values reduce the germination somewhat (table 12). The benefit of alternation from 20° to 30° agrees with the findings of Roe.[12] The germination percentages at 15°, 20°, and 25° did not differ significantly from one another, but the germination was most rapid at the highest temperature. After 9 days the germination percentages of 1A–406 in the first test were 9, 42, and 51 percent, respectively. Because of the increased rate of germination 25° appears to be the best constant temperature for the germination of guayule seed.

[11] This does not necessarily mean that the Indio climate is the best for seed production, as seed gathered there may have a much lower percentage of seed filled than that gathered in cooler districts.

[12] Roe, E. I. FINAL REPORT ON TESTS OF GUAYULE SEED MADE AT ST. PAUL, MINN. Great Lakes Forest and Range Expt. Sta. Rpt. to Emergency Rubber Proj. 1944. [Unpublished.]

TABLE 12.—*Germination of guayule seed at various temperatures*

Constant temperature or alternating temperatures (° C.)	Seed of indicated accession and strain germinating					
	1A–406		2A–593 [1]	2R–593 [1]		14A–593 [1]
	Immediately after threshing [1]	4 months after threshing [2]		Standard-treated	Untreated	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
5°	0					
5° and 15°	12					
5° and 20°	24					
5° and 25°	23					
5° and 30°	11					
5° and 35°	8					
10°	0					
10° and 20°	23	45				
10° and 25°	29					
10° and 30°	21					
10° and 35°	13					
15°	55	72				
15° and 25°	49	64				
15° and 30°	56	66				
15° and 35°	44					
20°	51	69	64	24	7	84
20° and 30°	54	78	75	44	17	91
20° and 35°	45	52				
25°	53	70	61	28	11	
25° and 35°	50	53				
30°	21		43	22	9	67
35°	6					

1 Difference required for significance at 5-percent level, 8 percent.
2 Difference required for significance at 5-percent level, 9 percent.

Two tests were carried out on accession 1A–406: the first immediately after the achenes were threshed from the florets, and the second 4 months after the seed was threshed. This accounts for the increased germination of the second test at the same temperatures.

The results shown in table 12 were obtained when the lower of the alternating temperatures were maintained for 17 hours daily. In the vicinity of Salinas seedings were made under conditions in which the temperatures usually dropped below 15° C. for just a few hours during the night. Experiments conducted to determine the effect of low temperatures for short periods on germination indicated that exposures to 10° and 5° for as long as 2 hours had little or no effect on germination (tables 13 and 14).

TABLE 13.—*Germination of guayule seed alternated between 20° and 30° C. with short exposures to 5° and 10° in the middle of the 20° period*

Lowest temperature (° C.) and duration of exposure	Seed of indicated accession and strain germinating		Lowest temperature (° C.) and duration of exposure	Seed of indicated accession and strain germinating	
	2A–593 (untreated) [1]	5–593 (standard-treated) [2]		2A–593 (untreated) [1]	5–593 (standard-treated) [2]
	Percent	*Percent*		*Percent*	*Percent*
5°:			10°:		
0 hour	83	54	0 hour	83	54
½ hour	83	51	½ hour	86	53
1 hour	80	57	1 hour	88	53
2 hours	80	53	2 hours	79	41
4 hours	80	47	4 hours	74	46
			10° + 5° + 10° [3]	77	49

1 Difference required for significance at 5-percent level, 8 percent.
2 Difference required for significance at 5-percent level, 7 percent.
3 1 hour at each temperature.

Exposures for 4 hours to either 10° or 5° caused a considerable reduction in the germination of the seed when the temperature was not allowed to rise above 20°, but not so great a one when the temperature was allowed to rise to 30°. When the temperatures were held at 5° for 4 hours and 20° for 20 hours daily, the maximum reduction in germination noted (4–406, table 14) was about 32 percent; when the temperature was maintained at 5° for 17 hours instead of 4 and at 20° for 7 hours (table 12), a reduction in germination of 53 percent was obtained.

TABLE 14.—*Germination of treated guayule seed at 20° C. except for short exposures to 5° and 10° daily*

Lower temperatures (° C.) and duration of exposure	Seed of indicated accession and strain germinating [1]			Lower temperatures (° C.) and duration of exposure	Seed of indicated accession and strain germinating [1]		
	3–130	4–406	5–593		3–130	4–406	5–593
5°:	*Percent*	*Percent*	*Percent*	10°:	*Percent*	*Percent*	*Percent*
0 hour	32	34	52	0 hour	32	34	52
½ hour	32	35	50	½ hour	31	33	48
1 hour	33	35	51	1 hour	34	32	50
2 hours	32	31	51	2 hours	33	31	51
4 hours	30	23	40	4 hours	28	24	44

[1] Difference required for significance at 5-percent level, 5 percent.

These results indicate that daily exposures of guayule seed to temperatures of 5° and 10° C. during periods of otherwise favorable temperatures (20° or a 20° to 30° alternation) for 4 hours or longer are necessary to cause a reduction in the percentage of seed germinating. They also indicate that when the temperature is allowed to rise to 30° instead of 20° the reduction in germination will be lessened.

At Indio soil temperatures as high as 50° C. occurred while the nurseries were being planted. Table 15 shows the germination of accession 11–593 when exposed to this temperature for various periods each day. Exposures as long as 4 hours daily cut the germination in half, and shorter exposures reduced it still less. From a practical standpoint these results show that good germination of guayule seed may be expected where soil temperatures reach 50° for as long as 2 or 3 hours daily, but that if it is exposed for longer periods the stands obtained may be reduced to a point which would require replanting the nursery.

TABLE 15.—*Germination of accession 11–593 guayule seed alternated between 20° and 30° C. and exposed to 50° for various periods daily during the middle of the 30° exposure period*

	Germination temperature (° C.)	Seed germinating [1]
		Percent
1	20°, 16 hours; 30°, 8 hours; 50°, 0 hour (check)	94
2	20°, 16 hours; 30°, 7 hours; 50°, 1 hour	94
3	20°, 16 hours; 30°, 6 hours; 50°, 2 hours	77
4	20°, 16 hours; 30°, 4 hours; 50°, 4 hours	50
5	20°, 16 hours; 30°, 2 hours; 50°, 6 hours	18
6	20°, 16 hours; 30°, 0 hour; 50°, 8 hours	2

[1] Difference required for significance at 5-percent level, 8 percent.

694351—46—3

LIGHT AND DARKNESS DURING GERMINATION

To show the effect of light on its germination, guayule seed was germinated in glass-topped Mangelsdorf seed germinators at room temperature. The dark treatments were obtained by covering the glass top with lightproof material and sealing the door shut with brown, opaque, gummed paper. The light treatments were obtained by placing the germinators in a well-lighted room away from direct sunlight. The temperatures in both treatments were recorded and no appreciable difference was noted; for example, over one 24-hour period, the temperature of the dark germinator averaged 23.7° C. and of the light germinator 23.5°. Light favored the germination of untreated seed, whether achenes plus florets, achenes alone, or the embryos extracted from the fruit coat (table 16). These results agree with those of Pissarev (*11*). Light did not markedly favor the germination of seed given the standard treatment except in one instance, that of accession 20–407. This was freshly harvested and was germinated at a more or less constant temperature. Treated seed of refanned accession 2–593 responded markedly to alternation of temperature when germinated in the dark (table 12). At the time of these tests, seed of this accession was also freshly harvested. Thus, it may be possible that light and alternation of temperatures may have comparable beneficial effects on the germination of treated freshly harvested seed.

TABLE 16.—*Germination of guayule seed in dark and light*

Accession and strain	Type of sample of seed	Age	Untreated seed germinating in—		Standard-treated seed germinating in—	
			Dark	Light	Dark	Light
		Months	*Percent*	*Percent*	*Percent*	*Percent*
7–593 [1]	Achenes plus florets	10	20	25	36	40
9–593 [1]	.....do.	10	14	25	35	41
11–593 [1]	Achenes	7	32	48	88	85
13–406 [2]	Achenes plus florets	8	17	51	75	70
14–593 [2]	.....do.	7	15	36	42	41
20–407 [2]	.....do.	2	6	12	24	47
20–407 [3]	Naked embryos	2	42	66	....	....

[1] Difference required for significance at 5-percent level: 4 percent for untreated seed; 5 percent for treated seed.
[2] Difference required for significance at 5-percent level: 10 percent for untreated seed; 14 percent for treated seed.
[3] Difference required for significance at 5-percent level, 11 percent.

HYDROGEN-ION CONCENTRATION OF GERMINATING MEDIUM

Two experiments on the effect of hydrogen-ion concentration on the germination of guayule seed were carried out with series of buffers given by Gortner (*4*). The desired hydrogen-ion concentration was obtained by titrating one solution into a measured volume of the other while reading the pH values on a Coleman meter. In the first experiment the seed was placed on blotters soaked in the buffered solutions. The blotters were maintained at their original level of moisture by the addition of distilled water. Because some of the buffers proved toxic, a second experiment was carried out with a different series of buffers. In this experiment the achenes were soaked for 3 hours at the rate of

4 ml. of buffer solution per 100 achenes; then they were placed between blotters moistened with distilled water. The results were erratic (table 17). The first experiment showed considerable differences between percentages of germination at the different hydrogen-ion concentrations, but with the method employed in the second experiment no significant differences were noted. The boric acid buffers were toxic. Buffers containing sodium citrate and sodium hydroxide at pH 9. and 10 are very unstable; tests showed the pH values had dropped to 7.4 during the 3-hour soaking period. The difference in germination of accession 2A–593 between tests is related to the age of the achenes, those of the first being 2 months old and those of the second 6 months old. Although these results are not too conclusive, the evidence obtained indicated that the hydrogen-ion concentration of the solution from which the seed absorbs water in order to germinate has little effect on the percentage germination.

TABLE 17.—*Germination of guayule achenes in solutions of different hydrogen-ion concentrations*

pH	Experiment 1			Experiment 2	
	Composition of buffers	Achenes of indicated accession and strain germinating		Composition of buffers	Achenes germinating (2A–593)[3]
		1A–406[1]	2A–593[2]		
		Percent	*Percent*		*Percent*
2	Potassium chloride + hydrochloric acid	39	21	Sodium citrate + hydrochloric acid	79
	Potassium acid phthalate + hydrochloric acid	26	19	....	....
3	Potassium acid phthalate + hydrochloric acid	46	15	Sodium citrate + hydrochloric acid	83
4	do.	41	7	do.	78
5	do.	55	10	do.	81
6	do.	48	22	do.	78
	Potassium dihydrogen phosphate + sodium hydroxide	60	28	Potassium dihydrogen phosphate + sodium hydroxide	77
7	Potassium dihydrogen phosphate + sodium hydroxide	55	20	Sodium citrate + sodium hydroxide	74
8	do.	44	23	do.	75
	Boric acid + potassium chloride + sodium hydroxide	7	0	Potassium dihydrogen phosphate + sodium hydroxide	86
9	Boric acid + potassium chloride + sodium hydroxide	9	0	Sodium citrate + sodium hydroxide	76
10	do.	17	2	do.	76
9.5	....	....	....	Standard treatment	86
7.2	....	....	....	Tap water (check)	81

[1] Difference required for significance at 5-percent level, 12 percent.
[2] Difference required for significance at 5-percent level, 8 percent.
[3] No significant difference between means.

SODIUM HYPOCHLORITE TREATMENT

VARIATIONS IN TREATMENT OF ACHENES PLUS FLORETS

Except when achenes alone that had been threshed from their accessory parts for 6 months or more or achenes plus florets grown at Indio and stored for 12 months were used, it was not possible in the experiments described earlier to obtain better than 90-percent germination of the embryos by any alteration of the conditions under which the tests were made. These results indicated that some special pregermination treatment was necessary. When this work was started, a satisfactory treatment with sodium or calcium hypochlorite had been worked out. This treatment as finally evolved is the standard treatment described on page 1. How effective this treatment is can be seen by comparing the germination of treated and untreated seed in tables 10, 12, and 16. What characteristics of the hypochlorites were responsible for the increased germination and how they worked on the seed were not clearly understood, however. In this section are described numerous experiments carried out to determine how variations of this treatment affected the germination and what effect the different operations by themselves had on the germination.

LIGHT AND DARKNESS DURING TREATMENT

As guayule seed germinates better in the light than in the dark (table 16), experiments were set up to determine whether light had an influence on the treatment. Seed treated in the light was washed, soaked in the sodium hypochlorite, and rinsed directly under a 15-watt fluorescent daylight bulb. It was dried in the light in a closed greenhouse at a temperature of 40° C. during the day. The seed treated in the dark was washed, soaked, and rinsed in lightproof containers. The seed was dried in the dark in an oven at 40°. The temperature of the washing and soaking in both light and darkness was from 15° to 20°. No significant difference was found between the germination of the seed treated under any of the conditions shown (table 18), indicating that the treatment was equally effective in light and darkness.

TABLE 18.—*Germination of guayule seed standard-treated and dried in light and dark*

Treatment	Seed of indicated accession and strain germinating [1]		
	3–130	4–406	5–593
	Percent	*Percent*	*Percent*
Seed treated in light; dried in dark	14	6	22
Seed treated in light; dried in light	13	7	20
Seed treated in dark; dried in light	13	9	20
Seed treated in dark; dried in dark	14	7	18
Seed not treated	2	4	3

[1] Difference required for significance at 5-percent level, 5 percent.

TEMPERATURE DURING TREATMENT

The effectiveness of the sodium hypochlorite treatment carried out at different temperatures was studied. The temperatures were maintained by washing and soaking the seed in water or solutions in ovens or refrigerators operated at the various temperatures. The washing was carried out by changing with water already at the desired temperature. The water on the seed not washed by continuously running tap water was changed every hour for 9 hours, every 3 hours for the next 6 hours, and then every hour for the last 3 hours. A progressive increase in germination as the temperature of washing rose to 25° C. and a decrease as the temperature went above it was found (table 19). Thus, 5° is definitely too low, 39° is definitely too high, and 25° is the optimum temperature for treating the seed. The odds against the increase and subsequent decrease in germination with rising temperatures occurring by chance are well beyond those required for significance. Another interesting result is the poor germination of the seed washed in continuously running water compared with that of seed treated at the same temperature but with the water changed infrequently.

TABLE 19.—*Germination of guayule seed of accession 5–593 standard-treated at various temperatures*

Temperature of treatment (° C.)	Seed germinating [1]	Temperature of treatment (° C.)	Seed germinating [1]
	Percent		*Percent*
5°	26	30°	31
15°	27	39°	17
20°	32	15° (running tap water)	19
25°	34	Check (no treatment)	6

[1] Difference required for significance at 5-percent level, 8 percent.

MANNER OF WASHING THE SEED

Washing alone compared with washing plus soaking in sodium hypochlorite.—In order to ascertain whether washing in water without subsequent treatment with hypochlorite would be sufficient to stimulate germination, guayule seed was washed in water for 20 hours at the rate of 75 ml. per 100 seeds (3 changes of 25 ml. each) without soaking in sodium hypochlorite. Washed seeds of all accessions germinated better than unwashed ones, but in some cases the differences were very small (table 20). In all cases seeds given the complete standard treatment germinated much better than those just washed in water.

TABLE 20.—*Germination of guayule seed washed in water for 20 hours, standard-treated, or left untreated*

Accession and strain	Seed germinating after the indicated treatment			Accession and strain	Seed germinating after the indicated treatment		
	None	Washed in water 20 hours	Washed in water 18 hours and soaked in sodium hypochlorite 2 hours		None	Washed in water 20 hours	Washed in water 18 hours and soaked in sodium hypochlorite 2 hours
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
3–130	19	26	41	12–593	23	41	68
4–406	11	12	34	13–406	19	31	66
5–593	20	26	50	14–593	9	10	34

Amount of water used.—The germination of achenes plus florets of accession 5–593 washed in various amounts of water prior to soaking in sodium hypochlorite is shown in table 21. In these tests the seed was all washed and treated for the same length of time, the only variable being the amount of water used. Washing in running water instead of changing at intervals reduced the germination to a slight extent. Just why this reduction occurred is not known. The highest germination occurred when only 3 or 4 changes of water were made and only 75 or 100 ml. instead of a liter or more was used per 100 seeds.

TABLE 21.—*Germination of guayule seed of accession 5–593 washed with various amounts of water prior to being soaked in sodium hypochlorite*

Treatment	Seed germinating [1]
Seed washed in—	*Percent*
Running water (100 ml. per minute per 100 seeds for 18 hours)	46
Running water (67 ml. per minute per 100 seeds for 18 hours)	45
Running water (50 ml. per minute per 100 seeds for 18 hours)	49
900 ml. of water per 100 seeds (25 ml. changed every ½ hour for 18 hours)	47
450 ml. of water per 100 seeds (25 ml. changed every hour for 18 hours)	46
225 ml. of water per 100 seeds (25 ml. changed every 2 hours for 18 hours)	48
125 ml. of water per 100 seeds (25 ml. changed every 4 hours for 18 hours)	45
75 ml. of water per 100 seeds (25 ml. changed every 6 hours for 18 hours)	54
100 ml. of water per 100 seeds (25 ml. changed at 4 irregular intervals)	50

[1] Difference required for significance at 5-percent level, 4 percent.

In another experiment achenes plus florets were soaked for 18 hours in just enough water to keep them moist (1 ml. per 100 seeds) before they were treated with the standard amount of sodium hypochlorite. Seed washed in 4 changes of water germinated better than that kept just moist during the washing time, but this difference was not very great (table 22).

TABLE 22.—*Germination of guayule seed soaked in just enough water to keep the seed moist prior to treatment in sodium hypochlorite*

Treatment	Seed of indicated accession and strain germinating [1]		
	3–130	4–406	5–593
Seed soaked in just enough water to be kept moist before sodium hypochlorite treatment	*Percent* 37	*Percent* 30	*Percent* 50
Seed standard-treated [2]	38	35	54
Seed not treated	18	10	19

[1] Difference required for significance at 5-percent level, 7 percent.
[2] Washing prior to soaking in sodium hypochlorite consisted of 4 changes of 25 ml. of water per 100 seeds.

Length of washing.—Experiments were set up to determine the effect of the length of time of washing prior to soaking in sodium hypochlorite on the effectiveness of the standard treatment. Regardless of the time of washing, the seed was all washed with the same amount of water. In this experiment was included a treatment in which the seed was soaked in sodium hypochlorite and then washed in water for 18 hours, or just the reverse of the sequence in the standard treatment. Seed washed for 8 to 12 hours germinated as well as those washed for the 18 hours required by the standard treatment. Seed first soaked in sodium hypochlorite and then washed for 18 hours germinated somewhat better than the checks but not nearly so well as those given the standard treatment (table 23).

These results on the variations in the manner of washing the seed indicated that washing itself is of very little benefit as far as germination is concerned (table 20). They did show, however,

TABLE 23.—*Germination of guayule seed after being washed for various periods prior to being soaked in sodium hypochlorite*

Treatment	Seed of indicated accession and strain germinating [1]		
	3-130	4-406	5-593
	Percent	*Percent*	*Percent*
Seed not treated (check)	18	10	19
Seed washed 4 hours, soaked in sodium hypochlorite 2 hours	31	31	38
Seed washed 8 hours, soaked in sodium hypochlorite 2 hours	38	34	50
Seed washed 12 hours, soaked in sodium hypochlorite 2 hours	36	37	46
Seed washed 18 hours, soaked in sodium hypochlorite 2 hours	38	35	52
Seed soaked in sodium hypochlorite 2 hours, washed 18 hours	25	23	37

[1] Difference required for significance at 5-percent level, 7 percent.

that the washing seems to condition the seed so that the sodium hypochlorite can more readily perform its function in increasing the number of germinable seeds. Thus, seed kept moist without an excess of water being present for 18 hours and then treated with sodium hypochlorite germinated almost as well as seed washed with an excess of water for the same length of time prior to treatment with sodium hypochlorite. Seed had to be washed for 8 to 12 hours prior to soaking in sodium hypochlorite for 2 hours in order to obtain maximum germination; treating the seed with sodium hypochlorite and then washing in water for 18 hours was of little benefit so far as seed germination was concerned.

MANNER OF TREATING WITH SODIUM HYPOCHLORITE

In addition to studies on the effects of variation in the manner of washing the seed, experiments were also conducted on the effect of variation in the sodium hypochlorite portion of the standard treatment on germination of guayule seed. Whenever the concentration of the sodium hypochlorite solution is mentioned, the concentration of available chlorine in such a solution is meant.

Amount of sodium hypochlorite solution per seed.—It has become accepted practice to treat the seed at the rate of 4 ml. of the sodium hypochlorite solution per 100 seeds. In table 24 are shown the results of germination tests on seed washed in water for 18 hours and then treated with various amounts of 1.5-percent sodium hypochlorite per 100 seeds for 2 hours. There was no significant difference in the germination percentages of the seed soaked in the various amounts of sodium hypochlorite solution;

TABLE 24.—*Germination of guayule seed washed in water 18 hours before being soaked 2 hours in various amounts of 1.5-percent sodium hypochlorite*

Sodium hypochlorite per 100 seeds	Seed of indicated accession and strain germinating [1]			Sodium hypochlorite per 100 seeds	Seed of indicated accession and strain germinating [1]		
	2-593	14-593	22-593		2-593	14-593	22-593
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
1 ml.	....	40	....	8 ml.	27	45	31
2 ml.	26	46	29	10 ml.	28	42	32
4 ml.	27	43	28	12 ml.	28	....	....
6 ml.	23	43	33				

[1] No significant difference between treatments.

however, when the amounts used were above 4 ml. per 100 seeds, the seedlings showed indications of injury to the root tips and cotyledons.

Length of soaking time in sodium hypochlorite.—Germination tests were carried out on guayule soaked in 1.5-percent sodium hypochlorite solution at the rate of 4 ml. per 100 seeds for various periods after they were washed in water. Soaking the seed longer than 2 hours gave no further increase in germination, and soaking 1 hour was sufficiently long for seed of one accession but not of another (table 25), indicating that 2 hours of soaking in the sodium hypochlorite solution will give as good results as can be expected.

TABLE 25.—*Germination of guayule seed soaked for various periods in 1.5-percent sodium hypochlorite*

Treatment		Seed of indicated accession and strain germinating		Treatment		Seed of indicated accession and strain germinating	
Washing period	Soaking period	13–406 [1]	14–593 [2]	Washing period	Soaking period	13–406 [1]	14–593 [2]
	Hours	*Percent*	*Percent*		*Hours*	*Percent*	*Percent*
19 hours	1	55	40	16 hours	4	53	47
18 hours	2	58	44	15 hours	5	55	45
17 hours	3	52	46				

[1] No significant difference between treatments.
[2] Difference required for significance at 5-percent level, 4 percent.

Concentration of solution.—In connection with the treatment of the seed it was desired to know not only what effect the concentration of the sodium hypochlorite solution would have on the germination but also whether it should vary with the length of time the seed had been stored. In table 26 are shown the germination percentages of seed washed in water for 18 hours and then soaked in various concentrations of sodium hypochlorite at various periods after harvest. The germination of the seed of accession 14–593 was not affected by the concentration of the sodium hypochlorite regardless of the age of the seed. The seed of accession 15–593, however, showed a higher germination in the higher concentrations until it had aged about 8 weeks; after that the concentrations had little effect on the germination. A higher percentage of the seed of both accessions germinated when treated than when not treated. Neither collection approached its maximum germination until it had been stored for 6 to 10 weeks. (The drop in germination of the seed of accession 15–593 after 16 weeks' storage coincided with a change in location of the seed laboratory at the time the tests were running, which interfered with the accurate temperature control.) The results indicated that after the seed had aged for 8 weeks treatment with a solution of sodium hypochlorite containing 1.5 percent of available chlorine gave as good germination as could be expected. In the results presented even 1 percent of available chlorine gave satisfactory results. However, results of other tests showed that often a 1-percent solution is not strong enough to give maximum germination.

TABLE 26.—*Germination of guayule seed of different ages soaked in various concentrations of sodium hypochlorite*

Accession, strain, and concentration of sodium hypochlorite	Seed germinating when treated indicated weeks after harvest													
	0	2	4	6	8	10	12	14	16	20	24	28	32	36
	Pct.	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*	*Pct.*
14–593:														
None (check)	....	.....	.....	7	9	11	10	21	20	29	33	28	28	28
1-percent	32	32	36	38	32	34	32	43	42	45	43	41	39	42
1.5-percent	32	35	34	41	39	43	40	42	41	45	43	45	40	36
2-percent	35	29	33	37	41	37	41	40	42	40	43	46	40	44
2.5-percent	30	34	40	39	38	32	37	43	44	41	43	42	44	41
3-percent	33	32	37	38	38	39	34	40	44	42	38	35	40	40
15–593:														
None (check)	8	4	8	13	21	19	26	37	16	41	38	34	.....	.....
1-percent	27	13	28	42	52	54	56	57	46	50	60	54	.....	.....
1.5-percent	30	17	24	45	54	51	55	60	49	55	53	63	.....	.....
2-percent	34	19	28	55	58	51	53	55	47	58	60	54	.....	.....
2.5-percent	33	22	34	59	60	49	49	56	48	61	59	56	.....	.....
3-percent	40	22	38	51	53	47	47	55	51	58	54	58	.....	.....

Washing seed in water for 18 hours and then soaking in sodium hypochlorite for 2 hours takes considerable time. Attempts were made to shorten this period by simply soaking the seed for a short period in more concentrated solutions of sodium hypochlorite. Seed soaked for only 50 minutes in a 5.25-percent solution at the rate of 4 ml. per 100 seeds germinated as well as that given the standard treatment (table 27). This short treatment has some value in experimental work, but it is of little value from a practical standpoint. In handling large quantities of seed the drying process at the end makes up the greatest portion of the expense of the treatment, and it was found that it took as long to dry seed soaked for 50 minutes as that held in water for 20 hours. This, coupled with the increased amount of hypochlorite used, resulted in no reduction in cost of the treatment.

TABLE 27.—*Germination of guayule seed soaked for various periods in 5.25-percent sodium hypochlorite without previous washing in water*

Treatment	Seed of indicated accession and strain germinating		
	3–130	4–406	5–593
	Percent	*Percent*	*Percent*
Seed not treated (check)	23	17	10
Seed soaked:			
10 minutes	28	29	44
20 minutes	33	34	48
30 minutes	35	36	44
Seed soaked—Continued:			
40 minutes	39	34	49
50 minutes	35	40	52
Seed standard-treated	36	41	53

CHANGES IN CONCENTRATION OF SODIUM HYPOCHLORITE DURING TREATMENT

As a final study on the sodium hypochlorite part of the standard treatment, seed of 3 accessions was washed for 16 hours and then soaked at the rate of 4 ml. per 100 seeds in different concentrations for different periods. The seed was germinated after these treatments. The amounts of chlorine present as hypochlorite and as chloride at the beginning and end of the treatments were determined according to the methods of the Association of Official Agricultural Chemists (*2, p. 73*). The results of the germination tests and the analyses of the sodium hypochlorite solutions are shown in table 28. There was very little difference between accessions so far as changes in concentration of the solution were concerned; so the chemical analyses have been lumped together. The results show that the 0.5-percent solution of available chlorine was not strong enough to bring about maximum germination of accessions 3–130 and 4–406, and possibly 5–593. They also indicate that the 3-percent solution was not too strong for these particular accessions. The germination of the seed soaked for 2 hours in the solution with 1.5 percent of available chlorine did not differ significantly from the highest percentage germination obtained with any of the accessions, indicating again that such a treatment was adequate.

TABLE 28.—*Germination of guayule seed treated in different concentrations of sodium hypochlorite for various periods and changes in composition of the solution during treatment*

Concentration of solution (available chlorine)	Seed of indicated accession and strain germinating after treatment for indicated period (hours)								
	3–130			4–406			5–593		
	2	4	6	2	4	6	2	4	6
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
0.54-percent	30	32	33	34	29	33	47	49	45
1.55-percent	39	40	38	38	34	34	50	51	52
3.10-percent	39	42	37	40	40	41	45	50	53

Concentration of solution (available chlorine)	Chlorine as sodium hypochlorite per 100 seeds				Chlorine as chloride per 100 seeds			
	At start	After treatment for indicated period (hours)			At start	After treatment for indicated period (hours)		
		2	4	6		2	4	6
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.54-percent	10.8	1.1	0.8	0.7	6.8	15.2	15.4	15.0
1.55-percent	30.8	9.6	7.2	4.8	21.6	38.0	43.0	....
3.10-percent	61.6	24.0	21.2	13.2	43.2	75.2	83.2	90.0

Concentration of solution (available chlorine)	Decrease of chlorine as sodium hypochlorite per 100 seeds during treatment for indicated period (hours)			Increase of chlorine as chloride per 100 seeds during treatment for indicated period (hours)		
	2	4	6	2	4	6
	Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
0.54-percent	9.7	10.0	10.1	8.4	8.6	8.2
1.55-percent	21.2	23.6	26.0	16.4	21.4	....
3.10-percent	37.6	40.4	48.4	32.0	40.0	46.8

The results of the chemical analyses indicate that after being in contact with the seed for 2 hours practically all the sodium hypochlorite in 0.5-percent solution was broken down and that in all of the strengths used most of the loss occurred during the first 2 hours. It can also be seen that the more sodium hypochlorite present, the more was decomposed when in contact with 100 seeds. The increase in chloride chlorine accounted for approximately

90 percent of the loss of the chlorine in the sodium hypochlorite.

At the end of the treatment there was no difference between the chlorine contents of solutions exposed to the different accessions. About 50 percent of the seed of accession 5–593 germinated in contrast to about 35 percent of that of accessions 3–130 and 4–406. This indicates that the amount of sodium hypochlorite used up in treating is not related to the number of viable seed.

TEMPERATURE OF DRYING AFTER TREATMENT

After the standard treatment seed of three different accessions were dried at various temperatures to determine how high they could go without injuring the seed. Earlier trials had shown that drying at 40° C. did not injure the seed; so this was chosen as the lowest temperature in the tests. Seed dried at 55° and 70° did not germinate as well as that dried at 40° (table 29). There

TABLE 29.—*Germination of guayule seed after drying at various temperatures*

Conditions of drying (° C.)	Seed of indicated accession and strain germinating			Conditions of drying (° C.)	Seed of indicated accession and strain germinating		
	3–130 [1]	4–406 [1]	5–593 [2]		3–130 [1]	4–406 [1]	5–593 [2]
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
40°, 22 hours	36	37	54	45°, 12 hours; open room (20°), 10 hours	39	41	53
40°, 6 hours; 45°, 6 hours; 50°, 10 hours	35	39	52	50°, 22 hours	34	33	49
40°, 12 hours; open room (20°), 10 hours	38	41	52	50°, 12 hours; open room (20°), 10 hours	35	37	52
40°, 3 hours; 55°, 17 hours	....	....	46	55°, 20 hours	....	....	42
40°, 3 hours; 55°, 6 hours; 70°, 11 hours	....	....	42	55°, 3 hours; 70°, 17 hours	....	....	42
45°, 22 hours	32	41	49	70°, 20 hours	....	....	12
				70°, 3 hours; 55°, 6 hours; 40°, 11 hours	....	....	31

[1] No significant difference between treatments.
[2] Difference required for significance at 5-percent level, 5 percent.

was no significant difference between the percentage germination of seed dried at 40°, 45°, and 50°. However, fewer seed of all three accessions dried at 50° for 22 hours germinated than of those dried at 40°. Although this difference was not significant, it may indicate that some injury did occur at 50°. The results taken as a whole indicate that in drying the seed after treatment the temperature should not be allowed to rise above 45°.

TREATING ACHENES ALONE

The foregoing sections have considered variations in the manner of treating the achenes plus florets. Achenes alone germinate without treatment if aged for 6 months or more after harvest (table 11). The germination of unaged achenes dissected from fruits of accession 5–593 when treated in various concentrations of sodium hypochlorite after being washed for 16 hours in water is shown in table 30. Treating the achenes increased their germination, the 0.5-percent solution of sodium hypochlorite giving the highest germination; more than 90 percent of the filled seed present germinated. Thus, if it is necessary to germinate achenes alone before they have aged 6 months, they can be made to germi-

TABLE 30.—*Germination of freshly extracted guayule achenes of accession 5–593 washed in water for 16 hours and then soaked in different concentrations of sodium hypochlorite (4 ml. per 100 achenes) for 2 hours*

Treatment	Achenes germinating [1]	Treatment	Achenes germinating [1]
	Percent		*Percent*
Seed not washed or soaked (check).....	38	Seed treated with sodium hypochlorite after being washed—Continued:	
Seed washed in water 18 hours..........	41	0.75-percent	52
Seed treated with sodium hypochlorite after being washed:		1.00-percent	44
0.25-percent	42	1.50-percent	30
0.50-percent	53		

[1] Diffe ence required for significance at 5-percent level, 5 percent.

nate by washing in water for 16 hours and then soaking in a solution of sodium hypochlorite containing 0.5 percent of available chlorine for 2 hours at the rate of 4 ml. per 100 achenes.

CONCLUSION FROM STUDIES ON VARIATIONS OF STANDARD TREATMENT

The various tests and experiments described in the section on Sodium Hypochlorite Treatment indicate that any variation from the standard treatment will not increase the germination of achenes plus florets and may result in a decrease. Likewise, inasmuch as 90 to 98 percent of the matured embryos, or filled seed, germinate after application of this treatment, there seems little possibility of finding a better one, although a cheaper and less troublesome method is desirable.

If time is not a factor, the use of achenes alone that have been stored for 6 months or achenes plus florets produced in certain climates and stored for a year will make it unnecessary to use the expensive standard treatment to obtain germination of more than 90 percent of the filled seed.

CHARACTERISTICS OF SODIUM HYPOCHLORITE RESPONSIBLE FOR ITS EFFECTIVENESS

The results of the investigations on the standard treatment indicated that washing the seed in water prepared the way for the sodium hypochlorite to perform its function more readily. Solutions of sodium hypochlorite have a lower surface tension than water and are excellent sterilizing and powerful oxidizing agents. Because any one of these characteristics might be responsible for the increased germination, experiments were set up to test these possibilities.

REDUCTION OF SURFACE TENSION

It was argued at one time that the reason for the increased germination with the standard treatment was that the washing softened the various parts of the fruit and then the sodium hypochlorite solution, with its lower surface tension than water, allowed easier uptake of water by the fruit, so that the enclosed embryos absorbed sufficient water to germinate. This seemed unlikely because it was found that soaked fruits absorbed as much water in 4 hours as in 24 and the effects of the treatment persisted after the seed was thoroughly dried and had been stored for several months. However, if this hypothesis were true, soaking the seed in a solution of any substance that reduced the surface tension of the water should increase the germination; therefore, guayule seed was soaked for various periods in different solutions of Vatsol OS. None of the treatments (table 31) with material which reduces surface tension caused a significant increase in germination of the seed above that of the check. These results, together with the arguments just presented, indicate that the beneficial action of the sodium hypochlorite is not the indirect one of reducing the surface tension of the water and thus allowing the seed to take up sufficient water to germinate.

TABLE 31.—*Germination of guayule seed treated with Vatsol OS in different solutions for various periods*

Treatment	Seed of indicated accession and strain germinating [1]		Treatment	Seed of indicated accession and strain germinating [1]	
	14–593	20–407		14–593	20–407
	Percent	*Percent*		*Percent*	*Percent*
Seed not treated (check)	23	29	Seed soaked in water 18 hours and in 0.5-percent Vatsol OS 2 hours	27	27
Seed standard-treated	41	44	Seed soaked in water 18 hours and in 0.1-percent Vatsol OS 2 hours	15	28
Seed soaked in water 20 hours	22	31	Seed soaked in water 18 hours and in 0.01-percent Vatsol OS 2 hours	18	35
Seed soaked in 0.5-percent Vatsol OS solution 20 hours	12	19			
Seed soaked in 0.1-percent Vatsol OS solution 20 hours	23	29			
Seed soaked in 0.01-percent Vatsol OS solution 20 hours	21	28			

[1] Difference required for significance at 5-percent level, 8 percent.

STERILIZING ACTION

When untreated achenes plus florets are germinated between blotters, they invariably become covered with a fungal growth in a few days. This could prevent germination by destroying the young seedlings before they had come through the fruit coat. Solutions of sodium hypochlorite are excellent sterilizing agents; and when seed is given the standard treatment, this fungal growth does not occur. It seemed possible that at least part of the benefit of the sodium hypochlorite treatment resulted from the sterilization of the seed. Guayule seed of accession 5–593 was given standard seed-sterilization treatments, and the germination was compared with that of seed given the standard treatment. In this experiment mold appeared only on the checks. None of the seed given the standard sterilization treatments germinated as well as those washed and then soaked in sodium hypochlorite, although treating with two of the sterilizing agents increased the germination to a significant amount above that of the untreated checks (table 32). If the formaldehyde treatment had increased the germination, it might be argued that the fungal growth on the seed had reduced germination. The failure of this treatment to increase germination cannot be ascribed to any injury to the seed by the formaldehyde offsetting any increase that might have resulted from sterilization, as seed treated with formaldehyde and then given the standard treatment germinated as well as those given only the standard treatment. Furthermore, achenes, on which mold growth rarely develops, have to be treated before they will germinate. Also, embryos dissected from untreated seed that had been covered with mold for at least 15 days were intact and germinated promptly when given the proper treatment. All the evidence obtained thus indicates that the sodium hypochlorite does not increase germination by sterilizing the seed.

TABLE 32.—*Germination of guayule seed of accession 5–593 treated with various sterilizing agents*

Treatment	Seed germinating [1]	Treatment	Seed germinating [1]
	Percent		*Percent*
Seed soaked in 2-percent formaldehyde 10 minutes	16	Seed soaked in 0.2-percent mercuric chloride in ethanol 1 minute	30
Seed soaked in 6-percent calcium hypochlorite 20 minutes	27	Seed standard-treated	48
		Seed not treated (check)	17

[1] Difference required for significance at 5-percent level, 8 percent.

OXIDIZING ACTION

Sodium hypochlorite in solution can break down in at least two ways; viz, one liberates oxygen according to the chemical equation:

$$2\ NaOCl = 2\ NaCl + O_2.$$

The other, in the presence of an acid (carbonic acid is strong enough), liberates chlorine and oxygen according to the formulas:

$$2\ NaOCl + H_2CO_3 = Na_2CO_3 + 2\ HOCl$$
$$2\ HOCl = 2\ HCl + O_2$$
$$HCl + HOCl = H_2O + Cl_2.$$

As there is sufficient carbon dioxide in the air to create carbonic acid in the water and as the seed extract itself is acid, both reactions probably occur. Chlorine and oxygen are very powerful oxidizing agents; therefore, the action of sodium hypochlorite on the seed might be primarily oxidation and not related to any specific property of chlorine. If this were true, any oxidizing agent should have the same effect of increasing the germination as the sodium hypochlorite.

Hydrogen peroxide compared with sodium hypochlorite.—Experiments in which hydrogen peroxide was used instead of sodium hypochlorite were set up. These tests were carried out at different times, but the results of all are given in table 33. The seed was soaked in hydrogen peroxide at the rate of 10 ml. per 100 seeds for 20 hours. All of the accessions so treated showed more germination than untreated seed and that soaked in water for 20 hours. Germination of seed of all but one accession when treated with 1.5- or 3-percent hydrogen peroxide differed little from that of seed given the standard treatment. These results showed that hydrogen peroxide can take the place of sodium hypochlorite in treating guayule seed and indicated that the action of the latter in increasing germination was not due to any specific action of the chlorine.

It is known that once the seed is treated with sodium hypochlorite it can be dried and stored almost indefinitely without loss of the effect of the treatment. None of the effects of the hydrogen peroxide were lost when seed of four accessions was treated with 1.5-percent hydrogen peroxide, rinsed, and then dried at 40° C. and stored for 14 days before being put to germinate. This is further evidence that hydrogen peroxide can fully replace sodium hypochlorite in treating guayule seed.

TABLE 33.—*Germination of different accessions of guayule seed after standard treatment and treatment with hydrogen peroxide*

Treatment	Seed of indicated accession and strain germinating							
	3–130 [1]	4–406 [1]	5–593 [1]	6–406	7–593	8–593	9–593	10–593 [2]
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Seed not treated (check)	22	10	24	23	26	4	23	20
Seed soaked in water 20 hours	24	15	31	......	......	......	......	......
Seed standard-treated	36	34	50	32	37	16	39	25
Seed soaked in hydrogen peroxide 20 hours:								
5-percent	......	......	......	31	33	7	23	26
3-percent	35	37	48	36	36	17	26	28
1.5-percent	34	34	43	32	36	11	28	28
Seed soaked in 1.5-percent hydrogen peroxide 20 hours, rinsed, dried at 40° C., and stored 14 days	......	......	......	37	35	......	25	25

[1] Difference required for significance at 5-percent level, 7 percent.
[2] Difference required for significance at 5-percent level, 5 percent.

Other oxidizing agents compared with sodium hypochlorite.—If the action of sodium hypochlorite is one of oxidation, then treatment with any strong oxidizing agent should increase the germination of the seed. Guayule seed was treated with different concentrations of many oxidizing agents for different periods. In table 34 is shown the highest germination percentage obtained with each agent and the treatment used. For example, seed was soaked for 1, 10, 20, and 90 minutes in each of 5-, 25-, 50-, and 95-percent solutions of sulfuric acid. Soaking for 1 minute in the 95-percent solution gave the highest germination, and this percentage is given in the table. When oxygen gas was used, the volume of gas at 60 pounds' pressure per 100 seeds was twice the volume that could be liberated by the complete release of oxygen from the sodium hypochlorite surrounding 100 seeds in the standard treatment. The tests were carried out on seed of the same accession at different times after the seed was harvested. This accounts for the differences in the germination of the checks, especially of accession 14–593, and is the reason the germination of the untreated seed is given for each experiment.

Seed treated with calcium hypochlorite germinated as well as that treated with sodium hypochlorite. This was to be expected, as McCallum (*8*) used the calcium compound when he first developed the treatment for guayule seed. Potassium permanganate was also tried, but it proved toxic in solutions as dilute as 0.1 percent.

TABLE 34.—*Maximum germination of guayule seed obtained when treated with various oxidizing agents (percent)*

Oxidizing agent and treatment	Seed of indicated accession and strain germinating after receiving indicated treatment										
	6–406	7–593	8–593	9–593	10–593	12–593	13–406	14–593	15–593	20–407	23–406
	Seed washed in water 18 hours; soaked in 1.5-percent NaOCl 2 hours; 4 ml. per 100 seeds										
Sodium hypochlorite:											
Seed not treated	15	16	3	7	15	23	19	20	33	28	20
Seed treated as described	40	38	14	49	41	68	66	40	58	43	43
	Seed washed in water 18 hours; soaked in 1.5-percent $Ca(OCl)_2$ 2 hours; 4 ml. per 100 seeds										
Calcium hypochlorite:											
Seed not treated	....	....	....	....	....	....	....	25	....	33	21
Seed treated as described	....	....	....	....	....	....	....	[1]43	....	[1]43	[1]43
	Seed washed in water 18 hours; soaked in 1.5-percent $HClO_4$ 2 hours; 8 ml. per 100 seeds										
Perchloric acid:											
Seed not treated	....	....	....	....	....	....	....	29	33	....	....
Seed treated as described	....	....	....	....	....	....	....	[1]41	[2]44	....	....
	Seed washed in water 18 hours; soaked in 1.0-percent HNO_3 2 hours; 10 ml. per 100 seeds										
Nitric acid:											
Seed not treated	....	....	....	....	....	....	....	25	....	26	20
Seed treated as described	....	....	....	....	....	....	....	[1]39	....	[1]45	[2]33
	Seed soaked 1 minute in 95-percent H_2SO_4; 10 ml. per 100 seeds										
Sulfuric acid:											
Seed not treated	....	....	....	....	....	....	....	25	....	27	20
Seed treated as described	....	....	....	....	....	....	....	[2]32	....	[2]34	[2]28
	Seed washed in water 18 hours; soaked in 0.5-percent $K_2Cr_2O_7$ 2 hours; 4 ml. per 100 seeds										
Potassium dichromate:											
Seed not treated	....	....	....	....	....	23	19	9	....	....	....
Seed treated as described	....	....	....	....	....	[2]47	[2]45	[2]18	....	....	....
	Seed soaked in 0.5-percent solution of KNO_3 for 20 hours; 10 ml. per 100 seeds										
Potassium nitrate:											
Seed not treated	....	....	....	....	....	....	....	20	....	27	....
Seed treated as described	....	....	....	....	....	....	....	25	....	33	....
	Seed washed in water 10 hours; placed in O_2 gas 60 pounds' pressure 2 hours										
Oxygen:											
Seed not treated	15	16	3	7	15	....	....	....	....	....	....
Seed treated as described	17	16	2	[2]13	15	....	....	....	....	....	....

[1] These values do not differ significantly from those of the sodium hypochlorite treatment.
[2] These values are significantly higher than those of the checks.

Seed treated with strong oxidizing agents, such as hydrogen peroxide (table 33), perchloric acid, and nitric acid, germinated as well as those treated with sodium hypochlorite; and seed treated with sulfuric acid and potassium dichromate germinated significantly higher than the checks but not as high as those given the standard treatment. No significant benefit was derived from treating with potassium nitrate and in only one instance was treating with oxygen gas beneficial. In general, the stronger the oxidizing agents used the greater the benefit to germination.

End products from decomposition of hypochlorites.—In a solution of sodium hypochlorite or calcium hypochlorite, hydrochloric acid and sodium or calcium chloride are formed and remain after chlorine and oxygen have been released. Tests were carried out to see whether these compounds alone would affect germination. Seed of accessions 14–593 and 23–406 was washed in water for 18 hours and then soaked in various concentrations of sodium chloride and calcium chloride for 2 and 4 hours. Treatment of seed of accession 23–406 with calcium chloride resulted in a barely significant increase in germination over that of the checks, but this increase was not nearly as great as that resulting from the standard treatment (table 35). Treatment with sodium chloride was of no benefit.

Seed of accession 20–407 was treated with hydrochloric acid in different ways. When soaked in a 0.5-percent solution for 20 hours or for 2 hours after being washed in water for 18 hours,

TABLE 35.—*Germination of guayule seed washed in water for 18 hours and then soaked in solution of sodium chloride or calcium chloride*

Treatment	Seed of indicated accession and strain germinating	
	14–593 [1]	23–406 [2]
Calcium chloride for 2 hours:	*Percent*	*Percent*
1-percent	22	26
2-percent	21	22
3.5-percent	20	21
5-percent	24	20
7-percent	16	29
Calcium chloride for 4 hours:		
1-percent	20	26
2-percent	15	21
3.5-percent	18	27
5-percent	21	26
7-percent	24	23
Sodium chloride for 2 hours:		
1-percent	18	20
2-percent	15	26
3.5-percent	20	26
5-percent	15	16
7-percent	12	16
Sodium chloride for 4 hours:		
1-percent	18	26
2-percent	14	21
3.5-percent	15	19
5-percent	15	15
7-percent	14	14
Water for 20 hours	14	20
Check	20	23
Standard treatment	37	37

[1] Sodium chloride treatments not significantly different from checks.
[2] Difference required for significance at 5-percent level for calcium chloride treatments, 6 percent.

it germinated slightly better than the checks (table 36). This increase, though just significant, was not nearly as great as that which resulted from the standard treatment.

All of these results indicate that the action of the sodium hypochlorite in increasing germination of guayule seed is due to its oxidizing characteristics and is not related to its effect on surface tension of solutions, to its sterilizing action, or to any of its decomposition products that remain after the oxygen and chlorine have been released.

TABLE 36.—*Germination of guayule seed of accession 20–407 after treatment with hydrochloric acid*

Treatment	Seed germinating [1]
	Percent
Seed not treated (check)	19
Seed washed in water 20 hours	17
Seed washed in water 18 hours, soaked in acid 2 hours:	
0.5-percent	24
1-percent	20
2-percent	23
4-percent	24
Seed soaked in acid 20 hours:	
0.5-percent	26
1-percent	20
2-percent	10
4-percent	1
Seed standard-treated	45

[1] Difference required for significance at 5-percent level, 6 percent.

PRECHILLING

One of the common ways of obtaining the germination of dormant seed is to keep it under otherwise favorable germination conditions but at a temperature of 4° or 5° C. for various periods and then to allow it to germinate at favorable temperatures.

Three lots of guayule seed were prechilled for various periods. Seed of accession 2A–593 and 26–593 was freshly harvested, whereas that of accession 14–593 was 1 year old at the time the tests were carried out. In no case did a significantly higher percentage of prechilled seed than of the check germinate (table 37). With two of the accessions a lower percentage of prechilled seed than of the check germinated. These results thus indicated that prechilling for as long as 60 days is of no value in inducing germination of guayule seed and may be injurious.

TABLE 37.—*Germination of guayule seed prechilled at 5° C. for various periods*

Prechilling period	Seed of indicated accession and strain germinating			Prechilling period	Seed of indicated accession and strain germinating		
	2A–593 [1]	14–593 [2]	26–593 [3]		2A–593 [1]	14–593 [2]	26–593 [3]
	Percent	*Percent*	*Percent*		*Percent*	*Percent*	*Percent*
0 day	12	31	4	30 days	12	13	3
5 days	....	24	7	60 days	....	11	4
10 days	15	20	2	0 day (stored dry in room for 30 days)	28	....	....
20 days	10	23	4				

[1] Difference required for significance at 5-percent level, 6 percent.
[2] Difference required for significance at 5-percent level, 8 percent.
[3] No significant difference between means.

CHARACTERISTICS OF GUAYULE SEED RESPONSIBLE FOR DELAYED GERMINATION

The discovery that treatment with certain strong oxidizing agents results in the loss of dormancy of guayule seed, especially after the seed has aged for 8 weeks, raised the question as to what characteristics of the guayule seed are altered by this oxidation so that germination occurs. The failure of a seed to germinate under abundant air and moisture conditions is generally ascribed to two important features: (1) An embryo that requires after-ripening or is not mature; (2) a seed coat that is impermeable to gases or water or is so hard that the enlarging root and shoot cannot break through it. A third possibility has been postulated for guayule: under conditions favorable for germination, the sterile florets, bracts, and fruit coat surrounding the embryo give off a substance or substances that inhibits germination.[13] Tests were carried out to determine which of these factors is responsible for the delayed germination of guayule seed.

GERMINATION-INHIBITING SUBSTANCES IN ACCESSORY PARTS

If a germination-inhibiting substance (or substances) is present and active under germinating conditions, it should be extracted by soaking the fruits in water. Table 38 gives the results of several experiments conducted to determine the effect of water extracts from florets and bracts on the germination of guayule seed. The seed was soaked in these at the rate of 10 ml. per 100

[13] MIROV, N. T. REPORT ON GERMINATION OF GUAYULE SEED. Silviculturist, Calif. Forest and Range Expt. Sta. Rpt. to Guayule Emergency Rubber Proj. 1942. [Unpublished.]

seeds. The extract used on achenes of accession 1A–406 was made by soaking 120 gm. of chaff (obtained by rubbing the sterile florets and bracts from the achenes of this accession) in 1,500 ml. of water for 24 hours at 80° C., filtering, and cooling; that used on accession 2A–593 was made by soaking 20 gm. of its chaff in 500 ml. of water for 24 hours at room temperature and then filtering. In the indicated treatments 8 ml. of 1.5-percent sodium hypochlorite or 0.375 ml. of 30-percent hydrogen peroxide was added to 10 ml. of the extract. This amount was just sufficient to decolorize the extracts. The extracts for soaking the embryos of accession 21–593 were made by soaking 100 gm. of complete fruits in 1,000 ml. of water at 23° for 24 hours and filtering.

TABLE 38.—*Germination of guayule achenes and embryos after soaking in extracts from guayule florets*

Treatment	Seed of indicated accession and strain germinating		
	1A–406 [1]	2A–593 [2]	21–593 [3]
	Percent	*Percent*	*Percent*
Achenes not treated	52		
Achenes soaked in water for 18 hours	50		
Achenes soaked in floret extract for 18 hours	46		
Achenes soaked in water for 3 hours		85	
Achenes soaked in floret extract for 3 hours		75	
Achenes soaked in floret extract plus sodium hypochlorite for 3 hours		83	
Achenes soaked in floret extract plus hydrogen peroxide for 3 hours		88	
Embryos soaked in water:			
3 hours			77
12 hours			81
24 hours			80
Embryos soaked in extract from fresh florets:			
6 hours			79
12 hours			81
24 hours			73
Embryos soaked in extract from 1-year-old florets:			
1 hour			80
3 hours			75
6 hours			79
12 hours			81
24 hours			77

1 No significant difference between means.
2 Difference required for significance at 5-percent level, 9 percent.
3 Difference required for significance at 5-percent level, 7 percent.

In only two instances was the germination significantly reduced by soaking the seed in extracts and both reductions barely reached the value required for significance at the 5-percent level. In one of these instances, the germination-reducing action was overcome by adding sodium hypochlorite or hydrogen peroxide to the extract. These results indicate that under some conditions the germination of a few seed may be inhibited by material in the sterile florets but that treatment with oxidizing agents will eliminate the effects. However, the differences in germination between the seed soaked in the extracts and that soaked in water were very small; this indicates that, although a germination-inhibiting substance may be present, it accounts for a very small amount of the dormancy found in guayule seed. Moreover, embryos enclosed in fruits and held under favorable conditions for germination for 21 days without germinating sprouted overnight when given the proper treatment; thus, 3 weeks' exposure to whatever germination-inhibiting substance was present did not prevent germination.

Impermeable Seed Coat

Many members of the Compositae have impermeable seed coats (*10*), and the delayed germination of guayule seed might be due to the presence of such. To test out this possibility, achenes alone and achenes plus florets were scarified by passing a sharp needle through the cotyledonary end of the achene. For this experiment the achenes alone were obtained by pulling off the florets and bracts with forceps, care being taken not to crush the achenes. Thus, the percentages of filled seed in both types of samples were the same. The germination of the scarified achenes of two of the three accessions tested was not significantly less than that of seed receiving the standard treatment, and that of the other accession was barely significantly less (table 39). In all three seed lots a significantly higher percentage of the punctured achenes than of the unpunctured ones germinated. The scarified achenes plus florets had a much heavier mold growth than the unscarified ones, and their reduced germination may have resulted from the puncturing allowing the fungi to enter and feed on the stored food in the embryos.

Table 39.—*Germination of scarified guayule seed*

Type of sample and treatment	Seed of indicated accession and strain germinating[1]			Type of sample and treatment	Seed of indicated accession and strain germinating[1]		
	3–130	4–406	5–593		3–130	4–406	5–593
Achenes plus florets:	*Percent*	*Percent*	*Percent*	Achenes alone:	*Percent*	*Percent*	*Percent*
Not punctured (no treatment)	8	16	19	Not punctured (no treatment)	15	26	30
Punctured	8	7	15	Punctured	27	36	46
Standard-treated	33	37	53				

[1] Difference required for significance at 5-percent level, 6.5 percent.

Puncturing the achenes with a needle resulted in a marked increase in germination (table 39). To determine whether the increase is due to the puncturing of the fruit coat (pericarp), of the true seed coat, or of both, untreated seed of various accessions was allowed to germinate for 3 weeks and the germinated seeds were removed. Then all of the ungerminated embryos were carefully dissected out of the achenes. Half of these were scratched with a sharp scalpel deep enough to penetrate the seed coat and the other half were not scratched; these embryos were then allowed to germinate. Whenever the seed coats were punctured more than 90 percent of the embryos germinated; but on the average only 40 percent of the embryos with unpunctured coats germinated, the percentage depending on the accession (table 40). Most of the scarified embryos germinated within 3 days after they were scratched, whereas only a little more than a third of the unpunctured embryos that were going to germinate did so in that time; it generally required 12 days for the latter to complete their germination.

Table 40.—*Germination of embryos extracted from guayule seed that had previously failed to germinate*

Length of germination period after extraction and treatment and seed-coat treatment	Seed of indicated accession and strain germinating							
	7–593	8–593	9–593	12–593	14–593	14–593	14–593	20–407
3 days:	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Not punctured	20	18	25	8	12	0	18	14
Punctured	90	92	85	92	80	80	77	98
12 days:								
Not punctured	68	46	56	22	33	13	35	57
Punctured	98	98	94	95	95	97	93	100

In another experiment, seed of accessions 23–406 and 26–593 was soaked in water overnight and the embryos from 800 fruits of each accession were carefully extracted. The seed coats of half of these were punctured with a sharp needle, whereas those of the other half were left intact. These embryos were then allowed to germinate. At the same time 400 complete fruits of both accessions were also germinated. In this test were included all the embryos present rather than only those that had failed to germinate while enclosed in the fruit coat under germination conditions for 3 weeks. At least 90 percent of the embryos with scarified seed coats germinated (table 41). These results, along with those given in table 39, indicate that the germination of the scarified embryos after being under germination conditions was not due to any preconditioning of the embryo during the 3 weeks they were in germinators prior to extraction and scarification.

Table 41.—*Germination of guayule embryos treated in different ways*

[χ^2 tests showed differences between treatments to be highly significant]

Type of sample and treatment	Seed of indicated accession and strain germinating	
	23–406	26–593
Embryos extracted from fruits:	*Percent*	*Percent*
Seed coats punctured	99	90
Seed coats not punctured	75	30
Embryos left in intact fruits	70	12

Removing the pericarp, or fruit coat, without scratching the true seed coat resulted in the germination of about 40 percent of the embryos (table 40). The results in table 41 also show that embryos removed from the fruits without being scarified germinated to a higher percentage than those left in the fruits. These results indicated that the fruit coat itself may have caused some reduction in the germination of the seed. The extraction of the embryos is a delicate task and many of those not purposely scarified may have accidentally been scratched while being extracted. This accidental scratching may account for some of the germination of the unscarified embryos. However, 68 percent of the unscarified embryos of accession 7–593 germinated (table 40), and it does not seem possible that over half were accidentally scratched while being extracted. Thus, the results indicate that the fruit coat itself may exert some slight inhibiting action on the germination of the seed.

Regardless of the effect of the fruit coat, more than 90 percent of the embryos did not germinate unless the seed coat was punctured or scarified in some way. The results in tables 39, 40, and 41 indicate that the guayule embryo is surrounded by a seed coat which must be broken or made permeable to gases or water before the seed will germinate under normal conditions. It would also seem that this hard seed coat is the factor most responsible for the delayed germination of guayule seed, especially after it has aged for a few months. It has been shown previously that the standard hypochlorite treatment results in the germination of more than 90 percent of the good seed. In the light of the results obtained with scarification, it seems that the hypochlorite must act in some way to soften the seed coat or increase its permeability to gases or water.

The seed coat passing around the end of the cotyledons is very distinct (fig. 1, *B*). It is composed of two layers. The outer layer is rather brittle and peels very readily from the inner layer. It is impossible to keep from breaking this outer layer in extracting embryos from achenes. The inner layer, which resembles an alligator skin in a surface view, is very tough; it must be punctured or made permeable before the seed will germinate.

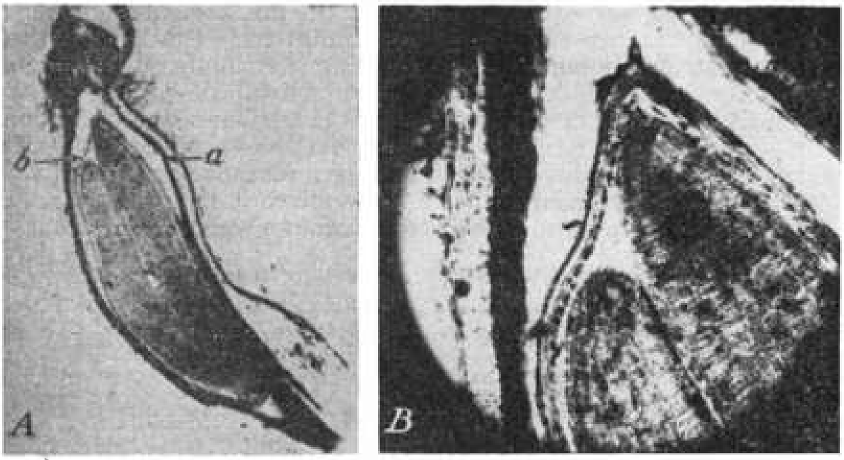

FIGURE 1.—*A*, Longitudinal section of a guayule achene showing pericarp, or fruit coat, (*a*) and seed coat (*b*); *B*, enlarged part of same section showing the double nature of the seed coat.

EMBRYO DORMANCY

When guayule seed is several months old, germination of more than 90 percent of the embryos can be obtained by scarifying the seed coat or by treating the seed with sodium hypochlorite (p. 24) or certain other strong oxidizing agents (p. 32). When treated with sodium hypochlorite at biweekly intervals beginning immediately after harvest, not more than 90 percent of the embryos of accessions 14–593 and 15–593 germinated for about 8 weeks. The concentration of hypochlorite used had no effect on the germination of the two accessions except for the seed treated up to a few weeks after harvest, and there was no significant difference in germination percentage between seed treated with 2.5-percent and 3.0-percent hypochlorite solutions. This indicated that stronger treatments would not have resulted in a further increase in germination. Thus, the results presented in table 26 indicated that guayule seed was not capable of complete germination upon being treated with sodium hypochlorite until 2 months or more after maturity. This strongly suggested the presence of an embryo dormancy of a rather short duration.

One of the ways of breaking embryo dormancy is by stratification, or by storing the seed in moist sand or peat at about 4° C. for various periods. In table 42 are shown the germination percentages of seed stratified in sand and unstratified seed of different ages and accessions. Trials were also made using peat as the stratifying medium, but the results obtained were similar to those obtained with sand.

TABLE 42.—*Germination of treated and untreated guayule seed of different ages after stratification for different periods and when unstratified*

Accession, strain, type and age of seed, and stratification period	Year of harvest	Seed germinating when treated as indicated			
		Standard-treated		Untreated	
		Stratified	Not stratified	Stratified	Not stratified
		Percent	*Percent*	*Percent*	*Percent*
1A–406 achenes 2 years old:					
7 days	1940	...	...	42	68
14 days	1940	...	...	33	...
28 days	1940	...	...	22	79
2A–593 achenes freshly harvested:					
7 days	1942	...	...	23	12
14 days	1942	...	...	12	...
21 days	1942	...	...	9	...
28 days	1942	...	...	7	23
20–407 achenes plus florets, 2 weeks old:					
3 days	1943	36	25	8	7
6 days	1943	34	29	11	8
14 days	1943	36	35	11	10
28 days	1943	38	39	6	11
24–593 achenes plus florets freshly harvested:					
3 days	1944	17	12	7	6
6 days	1944	19	15	12	9
10 days	1944	20	16	15	3
14 days	1944	20	11	15	13
28 days	1944	24	14	11	8
25–406 achenes plus florets freshly harvested:					
3 days	1944	12	9	4	4
6 days	1944	9	5	5	3
10 days	1944	16	5	7	3
14 days	1944	18	9	5	3
28 days	1944	20	10	1	1

The germination of 2-year-old achenes decreased with increased stratification time, whereas during the same period the germination of the unstratified seed increased. The germination of all freshly harvested seed was increased by stratification for a certain number of days regardless of whether the seed was given the standard treatment after the stratification period. The percentage germination of freshly harvested seed when stratified and treated with sodium hypochlorite was from 3 to 20 times that of unstratified and untreated seed, and for 2 accessions it was double that of unstratified but treated seed. The optimum time of stratification varied with the seed accession; 3 days was sufficient to insure the germination of 80 percent of the embryos of accession 20–407 when treated, whereas 28 days gave the highest germination of accessions 24–593 and 25–406.

If guayule seed has a short period of embryo dormancy, then even scarified embryos should not germinate as well when freshly harvested as several weeks later. Scarified and unscarified embryos of accession 14–593 were placed to germinate immediately on being extracted from the fruits at different periods after harvesting. Only about two-thirds of the scarified embryos germinated when they were 2 weeks old, but over 95 percent germinated when they were 14 weeks old (table 43). The germination of the unscarified embryos increased between the ages of 2 and 14 weeks, but their germination at the end of the experiment was much less than that of the scarified embryos. Thus, even though the seed coat was broken, freshly harvested seed did not germinate nearly as well as seed several weeks old.

TABLE 43.—*Germination of scarified and unscarified guayule embryos extracted from fruits of accession 14–593 at different times after harvesting*

[χ^2 tests showed differences between 6 and 14 weeks to be highly significant]

Age of seed	Embryos germinating when—	
	Scarified	Not scarified
	Percent	*Percent*
2 weeks	68	48
6 weeks	75	45
14 weeks	98	76

These results show that freshly harvested seed, even though given the treatment that insures complete germination of old seed, will not germinate readily until at least 8 weeks after it has been harvested and that stratifying fresh seed allows a great deal more to germinate, in one instance 80 percent of the matured embryos as compared with only 50 percent when the seed was not stratified. Such results indicate a period of embryo dormancy which lasts about 8 weeks. This embryo dormancy can account for the poor germination of guayule seed only for a short period immediately after the seed is matured but not after it has aged for several weeks or longer.

Because of the shortness of the period of embryo dormancy and the expense and trouble of stratification, seed is stored in open containers at room temperature for about 3 months after harvest before germination studies or seedings are attempted. After this storage the standard treatment will insure the germination of practically all the good seed.

PROBLEMS IN PREPARING SEED FOR PLANTING

During the season of sowing nursery beds near Salinas (April through June), it was difficult to obtain satisfactory stands even with treated seed (achenes plus florets) sown dry. The Intercontinental Rubber Co. overcame this difficulty by presprouting the seed. This involved soaking it in water at the rate of 1 pound of seed to 2.5 pounds of water and then placing it in wooden trays at 75° F. for 6 days or until radicles became apparent on a small percentage of the seed. The seed was kept moist and was stirred twice daily during the presprouting period.[14]

After this the seed while still moist was sown directly in the nursery beds. The Guayule Emergency Rubber Project followed this practice in 1942 but later reduced the time of presprouting to 3 days and termed the practice "presoaking." The time of soaking was reduced because it was found that no increase in germination was obtained by soaking longer than 3 days (table 44) and that little or no sprouting occurred after that time.

TABLE 44.—*Emergence of treated guayule achenes plus florets presoaked for various periods prior to seeding in the nursery*

Presoaking period	Seed sown (emergence 15 days after sowing) [1]	Presoaking period	Seed sown (emergence 15 days after sowing) [1]
	Percent		*Percent*
0 day (sown dry)	17	4 days	33
1 day	24	5 days	34
2 days	38	6 days	35
3 days	39		

[1] Difference required for significance at 5-percent level, 7 percent.

In connection with another experiment, seedings were made in the nursery on four different dates in the springs of 1943 and 1944. The same number of germinable seed (achenes plus florets) was sown per unit area, and the same amount of water was applied to all the plantings. The seed was presoaked for 1 day in 1943 and for 3 days in 1944 at the rate of 1 pound of seed to 2.5 pounds of water at a temperature of 70° F., except during the middle of the day when it rose to 80° or 90°; it was stirred twice daily while soaking. Continuous temperature records were kept in both years. In both years the poorest stands were obtained during the period of the lowest minimum temperatures and the best stands during periods of the highest minimum temperatures (table 45). Whenever the air temperatures stayed at about 50° or above (May 18, 1943, and July 7, 1944, plantings), the stands of guayule in the nursery were always greatly increased. Exposures to temperatures of 50° (10° C.) for 4 hours or longer results in a decreased germination of guayule seed (tables 12, 13, and 14). The highest count recorded in table 45 (35.5 plants per square foot) represents only 50 percent emergence of the germinable seed planted. There was little or no relation between the stand counts and the maximum temperatures in either year. These results indicated that the stands of guayule obtained in the nurseries at Salinas are closely related to the minimum temperatures occurring during the 2-week period after seeding.

In 1944 the lowest average minimum was 41° F. and in 1943 it was 45°; yet better stands were obtained in 1944 than in 1943.

[14] CARSONS, C. W. NOTES ON GUAYULE CULTURE. Regional Planting Insp., Region 5, U. S. Forest Serv. Rpt. 1942. [Unpublished.]

Whenever the minimum temperatures were comparable, better stands were obtained in the 1944 seedings when the seed was presoaked for 3 days than in the 1943 ones when it was presoaked for 1 day. When the seed is presoaked or presprouted, it is really allowed to germinate for a few days under favorable temperature

TABLE 45.—*Guayule seedlings per square foot in nursery plantings 14 days after seeding on various dates and the temperatures during this emergence period*

Date of seeding	Plants per square foot	Temperature			
		Average minimum	Range of minimum	Average maximum	Range of maximum
1943 [1]	*Number*	°*F.*	°*F.*	°*F.*	°*F.*
May 4	1.5	45	41–49	75	70–83
May 18	13.8	52	49–59	71	62–88
June 1	5.6	49	46–53	64	59–76
June 15	9.9	50	45–56	72	59–98
1944 [2]					
April 14	11.0	41	36–50	61	51–64
May 12	19.5	48	39–54	65	57–77
June 9	18.5	52	45–57	67	64–73
July 7	35.5	54	52–57	67	64–72

[1] Presoaked 1 day. [2] Presoaked 3 days.

conditions before being exposed to the conditions prevailing outside during the seeding season. These results indicate that presprouting or presoaking guayule seed tends to overcome any reduction in germination that might be caused by unfavorably low temperatures after seeding. Thus, at Salinas these practices were adopted to insure satisfactory stands in spite of unfavorably low temperatures prevailing there during the planting season. That temperatures under 50° can reduce germination is also brought out by the fact that seed held moist at constant temperatures below 50° failed to germinate after 21 days (table 12).

An experiment was set up to determine the effects of aeration and soaking the seed in different amounts of water on the subsequent emergence of presprouted seed. Treated seed was soaked at the rates of 1 pound of seed to 2, 2.5, and 3 pounds of water under aerated and nonaerated conditions. Aeration was accomplished by thoroughly stirring the seed twice daily. After being soaked for 6 days the seed was planted in flats outdoors. The greater the proportion of water to seed used the greater the number of seed that germinated during the presprouting period; also more of the aerated than nonaerated seed germinated during this 6-day period (table 46).

After this seed was planted, the rate of emergence and the final stands of seedlings were greater from the aerated than from the nonaerated seed regardless of the rates of soaking. This emphasizes the importance of aerating the seed well during the soaking period. The greatest number of seedlings was obtained in the shortest time from aerated seeds soaked at the rate of 1 pound to 2 pounds of water. At the higher rates of soaking more seed germinated during the soaking period, and in planting the seed the young roots and shoots were generally rubbed off so that those that germinated before planting were usually killed;

TABLE 46.—*Germination of standard-treated guayule seed after being aerated and nonaerated while soaked in various amounts of water for 6 days prior to seeding in soil outside*

Treatment per 1 pound of seed	Seed germinating		
	During presoaking [1]	After planting	
		8 days [2]	16 days [2]
	Percent	*Percent*	*Percent*
Seed not soaked (check)	....	2	25
Seed soaked in 2 pounds of water:			
Aerated	1	32	31
Nonaerated	0	3	21
Seed soaked in 2.5 pounds of water:			
Aerated	4	26	30
Nonaerated	1	23	26
Seed soaked in 3 pounds of water:			
Aerated	10	25	25
Nonaerated	4	16	21

[1] χ^2 tests show differences due to aeration and amount of water during presoaking period to be highly significant.
[2] Difference required for significance at 5-percent level, 5 percent.

hence they could not produce seedlings when planted in the soil.

Obtaining the best stands with seed soaked in the smallest amount of water is important because the drier the seed the easier it is to handle in the seeding machines. Seed soaked at the rate of 1 pound to 3 pounds of water is so wet that it does not pass through the seeders very readily.

It should be remembered that this procedure of presoaking seed in water at the rate of 1 pound of seed to 2 pounds of water at 70° F. and keeping it aerated for 3 days is followed in planting the complete fruits at Salinas, where temperatures may fall below those favorable for germination. Achenes alone are not presoaked when planted in the nurseries even at Salinas. At Indio no advantage from presoaking has been found when the seed is sown in the warmer parts of the year, but some increase in rate of emergence is observed if seed is presoaked before sowing during the winter months.[15]

DISCUSSION AND CONCLUSIONS

Achenes which have been detached from their sterile florets and bracts lose their dormancy in about 6 months, whereas those with these parts still attached retain their dormancy much longer (table 11). The methods employed in removing these accessory parts tear them away from the achene and in some cases actually rupture the pericarp wall, but in every instance of tearing away the florets a mark along the side of the achene can be seen where the floret was attached. As the results indicate that oxidation renders the seed coat permeable, it was wondered whether the rapid loss of dormancy of these achenes alone as compared with that of achenes plus attached florets could be accounted for on the basis that removing the florets weakened the pericarp wall and thus allowed for freer access of oxygen in the air to the seed coat; thus, a more rapid oxidation of the coat and loss of dormancy took place. To test this, the sterile florets and subtending bracts

[15] KELLEY, O. J. Unpublished data on experiments conducted at Indio, Calif. 1944.

were carefully cut away from achenes so that the pericarp wall was not disturbed in any way. It was necessary to work on 2,400 fruits before 800 satisfactory ones were obtained. These were stored at room temperatures in envelopes for 6 months along with achenes obtained in the normal manner and achenes plus florets. Ninety-five percent of the achenes threshed out of the florets by the usual method germinated without treatment after being stored for 6 months, whereas only 77 percent of the achenes with the florets carefully clipped off germinated after this period of storage (table 47). These results indicated that the rapid increase in germination of achenes as contrasted with that of achenes plus florets is probably related to the rupturing of the fruit coat that generally accompanies their extraction rather than to the removal of any effect that the presence of the florets themselves might have.

TABLE 47.—*Germination of guayule seed handled in different ways and then stored for 6 months*

Method of handling seed	Seed germinating [1]—	
	At start of experiment	After 6 months' storage
	Percent	*Percent*
Achenes threshed out with scarifier	76	95
Achenes carefully clipped away from florets	66	77
Entire fruits (achenes plus florets)	62	71

[1] Difference required for significance at 5-percent level, 9 percent.

Guayule seed which has been stored in airtight containers does not increase in germination as rapidly as that stored in containers that allow free access of air. The tighter the seed is packed in the airtight containers or the less air per seed, the slower the increase in germination (table 10). All of these results suggest that the increase in germination of untreated guayule seed as it ages beyond 2 months is due to the slow oxidation of the seed coat in air.

Data [16] showing that the seed coat of guayule is more or less impermeable to oxygen and possibly to carbon dioxide and that the germination of guayule seed is sensitive to the concentration of these gases in the atmosphere have been obtained. Thus, any factor which interferes with the gas exchange of the embryos may reduce the number of seed of a given collection that will germinate. In these experiments some achenes plus florets always germinated even without treatment. Embryos removed from the fruits (freed of the fruit coat or pericarp) without puncturing the seed coat germinated better than those left in the achenes (table 41). Puncturing the seed coat of the embryos removed from the achenes resulted in the germination of well over 90 percent of the embryos, an increase in the germination above

[16] BENEDICT, H. M. STUDIES ON THE IMPERMEABILITY OF THE GUAYULE SEED COAT. 1945. [Unpublished.]

that of the embryos with the unpunctured seed coats (table 41). These results can be accounted for by assuming different degrees of impermeability of the seed coats to oxygen and possibly to carbon dioxide.

Some seeds have coats sufficiently permeable to allow the gas exchange necessary for germination to take place regardless of any reduction that the mere presence of the accessory parts and fruit coat may impose. Other seeds may have coats of such permeability that, by removing the fruit coat and thus eliminating the interference to gas exchange which its presence imposes, the embryos will receive sufficient oxygen and be freed of enough carbon dioxide to germinate. The remainder of the seeds may have coats so impermeable to oxygen and carbon dioxide that, regardless of the presence or absence of the fruit coat, the seed coat must be punctured or made permeable by some treatment before germination can occur. Thus the permeability of the seed coat would govern germination after the dormancy of the embryo had been broken.

In conclusion, the experiments showed that delayed germination of guayule seed is due to several contributory factors:

(1) A period of embryo dormancy, which lasts for about 2 months after the seed is mature.

(2) A hard or impermeable seed coat, which must be broken or made permeable before the seed will germinate. This seed coat seems to be the principal cause of the failure of guayule seed more than 2 months old to germinate, as only by puncturing it was it possible to obtain germination of 90 to 100 percent of the embryos, regardless of what was done to the rest of the fruit.

(3) The presence of the pericarp, which encloses the embryo with its seed coat; however, this is not a factor if the seed coat is punctured or made completely permeable.

(4) The presence of germination-inhibiting substances, which seem to be present in small quantities in the sterile florets and bracts attached to the achene; the amount of germination inhibited by these substances seems to be so small as to be negligible.

The germination of over 90 percent of the embryos, or filled seed, can be obtained by either of the following practices:

(1) Forcibly remove the sterile florets and bracts and store the achenes in unsealed containers at room temperature for 6 months. During this time the embryo dormancy is broken and the seed coat becomes permeable.

(2) Break the embryo dormancy by stratification or simply by storing at room temperature for 2 to 3 months. As stratification is expensive and troublesome, the latter method is preferable. Then make the seed coat permeable by treating with strong oxidizing agents as sodium hypochlorite, calcium hypochlorite, hydrogen peroxide, perchloric acid, and nitric acid.

If time is not a factor, the first method is by far the easiest and least expensive. It is a simple operation to remove the florets and bracts from the achenes at the time of harvesting and cleaning, and 6 months usually elapse from the time the seed is gathered until it is to be sown. This means that so far as germination is concerned, the expensive and troublesome standard treatment described earlier can be done away with. However, the standard treatment seems to be the best available for seed with the florets and bracts still attached to the achenes.

SUMMARY

In this bulletin are presented the results of studies on the effect of various conditions and treatments on the germination of guayule seed. The indications of the data obtained are given in the following paragraphs.

Fumigating guayule seed with agents commonly used to kill insects such as methyl bromide and hydrogen cyanide had no deleterious effect on the germination of guayule seed at the dosages used and under the test conditions.

At temperatures between 20° and 25° C., the seed should be stored at a relative humidity of about 30 percent. Seed stored at humidities of 50 percent and higher tended to lose its viability. Seed stored at a humidity near 0 percent did not lose its viability but did lose some of its germinability.

A higher percentage of seed stored for 6 months in unsealed containers allowing for free exchange of gases germinated without treatment than of seed stored in sealed containers. There was no difference in germination, however, between seed kept in the different storage conditions and subsequently given the standard treatment, indicating that in 6 months no loss of viability had occurred in the seed stored in airtight containers.

The germination increases with the length of storage at least up to 12 months. If the sterile florets and bracts were rubbed off the achenes, the germination increased very rapidly so that after 6 months over 90 percent of the embryos germinated without special treatment. On the other hand, only 50 to 80 percent of embryos in achenes plus florets germinated after 12 months of storage except with seed produced at Indio, Calif., in which case over 90 percent germinated.

The optimum temperature for germination is a daily alternation of 20° C. for 17 hours and 30° for 7 hours, but excellent germination was obtained above 15° and below 30°. Under conditions otherwise favorable for germination, temperatures had to drop to 5° for 4 hours daily or rise to 50° for 2 hours daily before any significant reduction in germination occurred.

A significantly higher percentage of the seed germinated in the light than in the dark, especially if the seed was not given the standard treatment.

Studies were carried out to determine how many of the possible variations of the treatments used to insure the germination of guayule seed altered the effectiveness of the so-called standard treatment. This treatment consists in washing the seed in water for 12 to 18 hours, using 3 or 4 changes of water, and then soaking the seed for 2 hours in a solution of sodium hypochlorite containing 1.5 percent of available chlorine at the rate of 4 ml. per 100 seeds.

Sterilizing the seed with formaldehyde or other agents did not increase its germination to the extent that the standard treatment did.

Seed treated with the proper concentrations of hydrogen peroxide, perchloric acid, nitric acid, and other oxidizing agents germinated just as well as that treated with sodium hypochlorite.

These results indicate that the oxidizing action of the sodium hypochlorite on the seed breaks its dormancy and that the sodium hypochlorite itself does not have any specific action.

A very slightly lower percentage of extracted embryos soaked in extracts made from complete guayule fruits germinated than of those soaked in water for the same period. This finding indicates that, although some inhibition in germination might be due to the presence of growth-inhibiting substances in the fruits, they could not account for the great differences in the germination of standard-treated and untreated seed.

Embryos of untreated seed 2 months old or older that had failed to germinate after 3 weeks in the germinator quickly did so if the seed coat was punctured. The germination of the scarified embryos (approximately 95 percent) was about the same as that of seed given the standard treatment. These results show that the guayule embryo is surrounded by a hard or impermeable seed coat which must be broken or weakened before the seed will germinate. They also suggest that the standard treatment oxidizes the seed coat and changes its structure in such a way that the seed can germinate. Except in freshly harvested seed this hard seed coat seems to be the most important cause of the delayed germination of guayule seed.

Even when the coats were scratched with a needle or when the standard treatment was used seed did not germinate fully until about 8 weeks after harvest. The germination of freshly harvested seed could be increased by stratification prior to the standard treatment. These results indicate that in addition to having a hard seed coat guayule seed has a short period of embryo dormancy, which lasts for approximately 8 weeks after harvest.

It is postulated that the complete germination of achenes threshed from the sterile florets and bracts 6 months or so after the threshing is due to a more rapid oxidation of the seed coat in air than normally takes place when the seed is surrounded by all the accessory parts of the complete fruit. Removing the florets weakens the pericarp wall and allows for more ready access of the air to the seed coat.

LITERATURE CITED

(1) ARTSCHWAGER, E.
1943. CONTRIBUTION TO THE MORPHOLOGY AND ANATOMY OF GUAYULE (PARTHENIUM ARGENTATUM). U. S. Dept. Agr. Tech. Bul. 842, 34 pp., illus.

(2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1940. OFFICIAL AND TENTATIVE METHODS OF ANALYSES...Ed. 5, 757 pp., illus. Washington, D. C.

(3) CHEMICAL RUBBER COMPANY.
1941. HANDBOOK OF CHEMISTRY AND PHYSICS, A READY REFERENCE BOOK OF CHEMICAL AND PHYSICAL DATA. Edited by C. D. Hodgman and H. N. Holmes. Ed. 25, 2503 pp., illus. Cleveland, Ohio.

(4) GORTNER, R. A.
1938. OUTLINES OF BIOCHEMISTRY. Ed. 2, 1017 pp., illus. New York and London.

(5) HAYES, H. K., and IMMER, F. R.
1942. METHODS OF PLANT BREEDING. 432 pp., illus. New York and London.

(6) Kirkwood, J. E.
1910. PROPAGATION OF GUAYULE BY SEEDS. Amer. Rev. Trop. Agr. 1: 34–43, 77–84, illus.

(7) Lloyd, F. E.
1911. GUAYULE (PARTHENIUM ARGENTATUM GRAY), A RUBBER-PLANT OF THE CHIHUAHUAN DESERT. Carnegie Inst. Wash., Pub. 139, 213 pp., illus.

(8) McCallum, W. B.
1929. METHOD OF TREATING AND SOWING GUAYULE SEED. (U. S. Patent No. 1,735,835.) U. S. Patent Office Off. Gaz. 388: 520.

(9) ———
1941. THE CULTIVATION OF GUAYULE. India Rubber World 105: 33–36, 153–156, illus.

(10) Miller, E. C.
1938. PLANT PHYSIOLOGY, WITH REFERENCE TO THE GREEN PLANT. Ed. 12, 1201 pp., illus. New York and London.

(11) Pissarev, V. E.
1930. SELEKTSIIA I PRIEMY KUL'TURY GUAIULY. (BREEDING AND METHODS OF CULTIVATION OF THE GUAYULE.) Trudy Prikl. Bot., Genet., i Selek. (Bul. Appl. Bot., Genet., and Plant Breeding) 24: 3–84. [In Russian. English summary, pp. 83–84.]

(12) Powers, L.
1944. MEIOTIC STUDIES OF CROSSES BETWEEN FRAGARIA OVALIS AND × F. ANANASSA. Jour. Agr. Res. 69: 435-448.

(13) ——— and Rollins, R. C.
1945. REPRODUCTION AND POLLINATION STUDIES ON GUAYULE, PARTHENIUM ARGENTATUM GRAY AND P. INCANUM. H. B. K. Amer. Soc. Agron. Jour. 37: 96–112.

(14) Romney, V. E., York, G. T., and Cassidy, T. P.
1945. EFFECT OF LYGUS SPP. ON SEED PRODUCTION AND GROWTH OF GUAYULE IN CALIFORNIA. Jour. Econ. Ent. 38: 45–50, illus.

(15) [U. S.] Agricultural Marketing Service.
1940. RULES AND REGULATIONS UNDER THE FEDERAL SEED ACT . . . TREASURY. Serv. and Regulat. Announc. 156, 41 pp.

(16) Whitehead, M. R., and Mitchell, J. W.
1943. EFFECTS OF NUTRIENT, PHOTOPERIOD, AND NIGHT TEMPERATURE ON THE DEVELOPMENT OF GUAYULE SEEDS. Bot. Gaz. 105: 14–24, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1946—694351.

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price 10¢ cents