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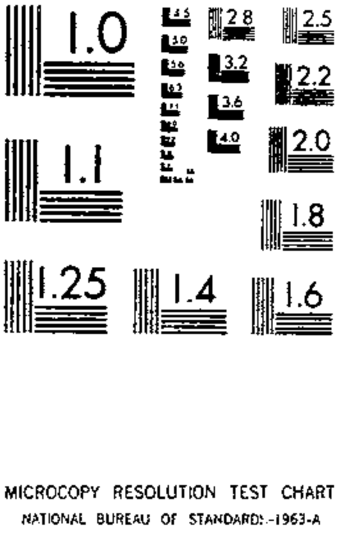

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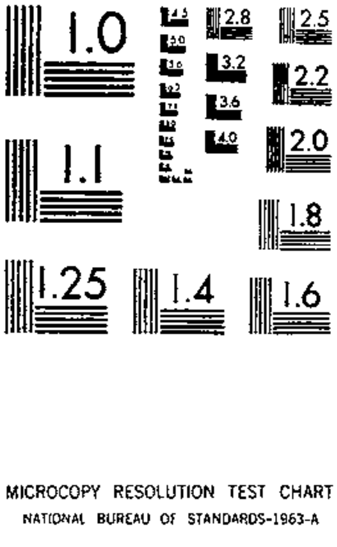

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RESEARCH ON GUAYULE
(Parthenium argentatum):
1942–1959

By
Bayard L. Hammond
and
Loren G. Polhamus

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Foreword

From 1922 to the beginning of World War II, the Department of Agriculture conducted research on native and foreign plants that might be relied upon for domestic production of natural rubber should our country be cut off from its chief sources of rubber in the Far East. Others interested in the problem included the late Thomas Alva Edison, who devoted the latter part of his life to a survey of native plants that might be useful for rubber production, and the Intercontinental Rubber Co., which conducted research on guayule in the attempt to commercialize the production of cultivated guayule rubber in California and Arizona. The Department of Agriculture, with only a small appropriation devoted to the study of rubber plants, did not attempt to duplicate the work of that company, but independently and in cooperation with the company, conducted sufficient research on guayule to keep informed regarding the accomplishments of the company.

After the outbreak of war in 1941, the Government of the United States took emergency action to develop new sources of natural rubber to replace that formerly available from the Far East. Efforts were made to increase supplies of wild rubber from Tropical America and Africa, and the Emergency Rubber Project was organized under the Forest Service, U.S. Department of Agriculture, to grow rubber domestically. Plants given particular attention included the Russian rubber-bearing dandelion kok-saghyz, goldenrod, the Madagascar rubber-bearing vine *Cryptostegia grandiflora* R. Br., and guayule. The major effort of the Emergency Rubber Project was devoted to guayule.

After acquiring the properties and records of the Intercontinental Rubber Company in the United States, a large-scale rubber production program was initiated, with active participation by the Department's research agencies on all phases of guayule shrub production, and the extraction and testing of guayule rubber. At the end of the war, the Emergency Rubber Project and all phases of the wartime rubber-production program were liquidated, but a program of research on shrub production and processing was continued. This report is a final summary of research by agencies of the Department of Agriculture on guayule shrub and its rubber from the initiation of the Emergency Rubber Project in 1942 until the final phase of the post-war research on guayule was discontinued in 1959.

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RESEARCH ON GUAYULE (*Parthenium argentatum*): 1942–1959

By Bayard L. Hammond, *research geneticist*, and Loren G. Polhamus, *collaborator*, Crops Research Division, Agricultural Research Service

INTRODUCTION

Guayule is the outstanding plant tested for rubber production within the continental United States. Under favorable conditions, it may contain more than 20 percent rubber on a dry-weight basis after 4 or 5 years' growth. The rubber yield at that time may exceed a ton per acre.

Species of Russian dandelion, particularly kok- and krim-saghyz, are capable of producing high-quality rubber in a relatively short time, but the yield is low. Cultural problems and harvesting difficulties make these plants costly sources of rubber. The Russian tau-saghyz and the Cryptostegia rubber vine of Madagascar have also been tested for domestic rubber production with unfavorable results. Polhamus (*175*)[1] shows that milkweed, goldenrod, and many other North American plants contain rubber; but difficulties have been experienced in domestication, and in producing an acceptable quality of rubber. Estimated costs of rubber production have been much higher than for domestic guayule rubber.

A private company, the Intercontinental Rubber Co., was primarily responsible for the initial cultivation of guayule for rubber production. This company conducted the first cultural tests in Mexico and later in the southwestern United States, and by the end of the third decade of the present century had planted some 8,000 acres of guayule shrub in the vicinity of Salinas, Calif.

This same company also considered making plantings in Italy, and conducted extensive tests and negotiations toward that end. The recession of the early thirties caused the company to harvest the shrub on rented properties at Salinas, and there was no further extensive field planting until the beginning of war in 1941. The U.S. Government planted some 32,000 acres of guayule in the United States during the war. Only a small portion of this acreage was harvested for rubber, the major portion being destroyed after the war when there appeared to be no further strategic need for the rubber.

At the same time, there was considerable interest in planting guayule in other parts of the world where conditions seemed favorable for its growth. The U.S. Department of Agriculture furnished seeds to Australia, Argentina, the Belgian Congo, Ceylon, Chili, China, Cuba,

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[1] Italic numbers in parentheses refer to References, p. 144. Those with an asterisk refer to unpublished material, or material not generally available in libraries.

the Dominican Republic, Egypt, England, French Equatorial Africa, the Gold Coast, India, Jamaica, Kenya, Mauritius, Mexico, Nyasaland, Palestine, Peru, Northern and Southern Rhodesia, Russia, St. Helena, Spain, Sudan, Uganda, the Union of South Africa, Uruguay, Venezuela, and the Virgin Islands.

Considerable success was reported for the plantings in Australia and the shrub produced had a very satisfactory rubber content. After the war, considerable seed of guayule was sold to the Governments of Spain and Turkey, and each embarked on a program expected to expand to some 30,000 acres. However, by 1962, notwithstanding the extensive plantings that had been made and the intensive research that had been conducted, there was no commercial production of cultivated guayule rubber anywhere in the world. Even production from wild shrub in Mexico had been discontinued.

HISTORICAL

Guayule was "discovered" in 1852 by J. M. Bigelow, a member of the Mexican Boundary Survey party. Dr. Bigelow submitted specimens of the plant, which he collected near Escondido Creek in Texas, to Harvard University where Asa Gray first described it and named it botanically, *Parthenium argentatum.*

The presence of rubber in guayule was known by the North American Indians who used it for several centuries in the production of bouncing balls for their games. The making of these balls apparently was effected by the communal chewing of the bark from which a small wad of rubber could be obtained after several minutes of chewing.

According to Lloyd's account (*145*), public attention had been drawn to guayule rubber in 1876, apparently for the first time, by an exhibit sent from Durango by the Mexican Government to the Centennial Exposition at Philadelphia. In 1888, the New York Belting & Packing Co. imported 100,000 pounds of the shrub, decorticated it, and extracted rubber from the finely ground-up bark by immersion in hot water. The quality of the rubber was regarded as equal "to the best grade of centrals."

In 1902, American capitalists financed a series of experiments by William A. Lawrence, which led to the successful mechanical extraction of crude rubber from guayule shrub in 1904 by the pebble mill extraction method. Extraction was accomplished by a rotating shell containing flint pebbles, which comminuted the shrub.

The first lot of rubber prepared was shipped to the United States and used by the Manhattan Rubber Co. As a result of the success of a mechanical method of extraction, the Continental-Mexican Rubber Co. completed a large factory at Torreon in 1906. Then followed the establishment of factories of various sizes in San Luis Potosi, Saltillo, Monterrey, Gomez Palacio, and Jimulco. In 1909, a mill built by the Texas Rubber Co. at Marathon, Tex. began operation. Production was stepped up to a point where, in 1909, Mexico exported 9,542 long tons of guayule rubber to the United States. Much of the rubber was used in the manufacture of automobile tires.

The limited, natural sources of guayule shrub were rapidly depleted to a point where most of the factories were forced to close down. The four owned by the Continental-Mexican Rubber Co. remained in operation, but their supply of shrub was getting low. They foresaw the need for carrying out cultural experiments and, in 1910, initiated such a program under the leadership of the late W. B. McCallum.

Threatened in 1912 by the Mexican Revolution, cultural operations were transferred to California under the name of the American Rubber Producers, Inc., of the Intercontinental Rubber Co. Headquarters were first established by Dr. McCallum near San Diego. Indicator plots were established throughout California and the southwest to determine areas best suited to guayule culture. Owing to the lack of sufficient acreage to support full-scale factory operations, his headquarters were moved in 1916 to southern Arizona where a large tract of land was purchased by the company and the township of Continental was established about midway between Tucson and Nogales. Although large shrub could be grown under irrigation at Continental, the very low rubber content was disappointing.

In 1925, headquarters were moved from Arizona to the Salinas Valley in California where conditions for rubber accumulation were more favorable. An extensive commercial development was initiated and, during four campaigns between 1931 and 1941, 3,068,630 pounds of rubber were milled from guayule planted largely in the Salinas Valley.

In a report dated June 6, 1930, Maj. Gilbert Van B. Wilkes and Maj. Dwight D. Eisenhower reported to the War Department on the state of the guayule industry and its significance to the War Department.

In March 1942, shortly after our rubber supply from the Far East had been cut off, the U.S. Government purchased the experimental records, seed stocks, and holdings of the Intercontinental Rubber Co. within the United States and assigned the task of carrying out the work to the U.S. Department of Agriculture.

The Department of Agriculture established the Emergency Rubber Project to administer the program, and assigned to the U.S. Forest Service the duty of organizing and directing the project. The Forest Service was authorized to call upon the research bureaus of the Department for any necessary assistance within their usual spheres of activity. The Bureau of Plant Industry, Soils, and Agricultural Engineering (now the Crops Research Division of the Agricultural Research Service) was called upon for research on crop production. The Bureau of Agricultural and Industrial Chemistry (now the Utilization Research and Development Divisions of the Agricultural Research Service) was asked to assist in improving methods of extracting guayule rubber from plants. The Bureau of Entomology and Plant Quarantine (now the Entomology Research Division of the Agricultural Research Service) initiated studies of insects affecting guayule.

Under Public Law 473, 77th Congress, signed by the President on March 5, 1942, the planting of 75,000 acres of guayule was authorized, and an extensive research program was initiated to study all phases of plantation guayule rubber production, including limited research studies in Mexico (*174*). Public Law 751, 77th Congress, increased the authorization to 500,000 acres and efforts to meet this expansion got under way immediately. Soon a highly coordinated organization was formed and ready to begin work. At this point, the expansion program was halted to minimize interference with food production in California, and the project was ordered to hold a standby position.

In the meantime, a special representative of the Rubber Director reviewed the rubber situation, thoroughly inspected the project itself, and contacted public officials in California and the Southwestern States concerning the attitude of the public toward the guayule program. Based on this report, the Rubber Director, on August 17, 1943, suggested to the Secretary of Agriculture that the program be expanded to produce 20,000 tons of rubber per year.

This time, project personnel centered attention on a program in Arizona and Texas where large areas of submarginal, irrigated land were available without much interference with food production. To sustain such a program, the necessary acreage was determined and extensive soil surveys were made, pending a sufficient appropriation of money to meet the suggested program. Five months later, Congress voted disapproval and the project reverted to its former position of maintaining the plantings (some 30,000 acres) already established in California.

Upon rejection of this proposed expansion program, the House of Representatives passed a resolution on February 15, 1944, to form a committee to make a complete investigation of the progress of the program. The report was made public on January 2, 1945. This committee reported favorably on the progress of the program and pointed out the need for continued, intensive Government research to determine the full possibilities of guayule with due consideration to postwar needs of agriculture. It recommended that the existing plantings be turned over to private interests, and that guayule production and processing be in private hands in the postwar period; and that the Government establish and guarantee a price floor for a period of from 7 to 10 years. A bill embodying these recommendations was introduced on February 26, 1945. It passed the House on May 14 and was reported out of the Senate committee in August 1946. No action was taken on the floor.

Early in 1945, the supply of natural rubber needed for heavy-duty truck tires and bullet-proof gas tanks for aircraft became dangerously low. The Rubber Director ordered a program to mill all of the guayule of an age suitable for rubber extraction. The Firestone Tire & Rubber Co. was given the task of designing, constructing, and operating the needed extraction plants. This project was well underway at the close of the war, but was terminated on V–J Day.

Upon cessation of war in the Pacific in August of 1945, the entire rubber outlook changed. Under the terms of the "Rescission Bill" passed by Congress on December 11, 1945, funds for the guayule project were frozen and the project was ordered liquidated in its entirety as of December 1946. All land, except for a few experimental areas, was released by June 30, 1946.

During the brief period of 3½ years, the Emergency Rubber Project produced and shipped to the Office of Rubber Reserve of the Reconstruction Finance Corp. a total of 2,974,272 pounds of rubber, leaving approximately 85 percent of the shrub unharvested. The liquidation of the project resulted in the destruction of an estimated 21 million pounds of rubber standing in the fields.

A careful appraisal of the rubber situation revealed that natural rubber was a critical material since synthetic rubber was not satisfactory for all essential purposes. Recognizing the importance of the guayule program, the Navy Department, through its Office of Naval Research, provided funds for an interim research program pending appropriations by Congress, and entered into a contract with Stanford Research Institute as an operating agency to continue research on guayule. On July 23, 1946, Congress directed the U.S. Department of Agriculture to conduct research on agricultural materials considered strategic and critical by the Munitions Board. Guayule rubber was one of these.

In consequence, a new research program was organized on August 1, 1947, at Salinas, Calif., to be conducted jointly by the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Bureau of Agricultural and Industrial Chemistry under the title of the Natural Rubber Extraction and Processing Investigations Project.

In December 1950, an emergency program of guayule seed and seedling stockpiling was initiated by the Department of Agriculture at the request of the Munitions Board for the purpose of stockpiling reserves of natural rubber. The overall administration was assigned to the Production and Marketing Administration with the Bureau of Plant Industry, Soils, and Agricultural Engineering acting in a technical guidance capacity. The stockpiling program was directed along two channels. In California, the Bureau initiated a stockpiling program by transplanting available seedlings to 337 acres of land in the Salinas Valley for seed production. In Texas, under the technical guidance of the Bureau, nurseries totaling 529 acres were established to produce seedlings for possible use in a production program.

The seedling stockpiling program in Texas was brought to a close on December 31, 1951, and all land and personnel were released. On December 31, 1952, the seed stockpiling program in California came to a conclusion. A total of 15,801 pounds of threshed seed had been harvested. Together with seed previously harvested, the stockpile consisted of 26,334 pounds of seed of variety 593, dried and stored in airtight metal containers.

On June 30, 1953, the Natural Rubber Extraction and Processing Investigations Project of the Bureau of Agricultural and Industrial Chemistry was liquidated. The cultural and breeding program conducted by the Natural Rubber Research Project of the former Bureau of Plant Industry, Soils, and Agricultural Engineering was discontinued on June 30, 1959.

BOTANICAL

Habitat

Guayule is a semidesert shrub native to the drylands of north-central Mexico in the States of Coahuila, Chihuahua, Durango, Zacatecas, San Luis Potosi, and Nuevo Leon, and to the adjacent Big Bend areas of Texas, usually at altitudes between 2,000 and 6,000 feet. Within its native habitat, it grows in scattered patches of from less than 1 acre to several hundred acres in size. It is rather restricted to outwash fans and slopes of calcareous soils (fig. 1) in regions having an annual rainfall of 10 to 15 inches, which occurs principally in late spring, summer, and early autumn. These sites are normally well drained but in addition to precipitation, they may receive water by runoff from more elevated areas. Actually, guayule will grow better in regions with higher rainfall, but there it may not survive in competition with other plants, particularly with the dense growth of grasses and shrubs on the alluvial soils. Temperatures throughout its range are fairly uniform, the daytime temperatures rarely exceeding 95° F., and the minimum seldom below 0°. The plants may live for 30 years or longer. In their native habitat they rarely exceed a height of 3 feet.

The genus *Parthenium*

A complete taxonomic revision of the genus has been made by Rollins (*103*) in a recent monograph in which he also discusses the morphology of the various species and the phylogenetic trends within the genus. The genus, now comprising 16 recognized species, in four sections, is native to the Western Hemisphere, and extends, with the exception of the Tropics, from Wyoming and Minnesota to northern Argentina.

In growth habit, the species range from annuals through perennials and woody shrubs to ligneous, treelike types. *P. tomentosum* D.C., var. *stramonium* (Greene) Rollins, native to northwest Mexico, may reach a height of from 15 to 20 feet or more. One of the smallest is *P. alpinum* (Nutt.) T. & G., an almost inconspicuous herbaceous

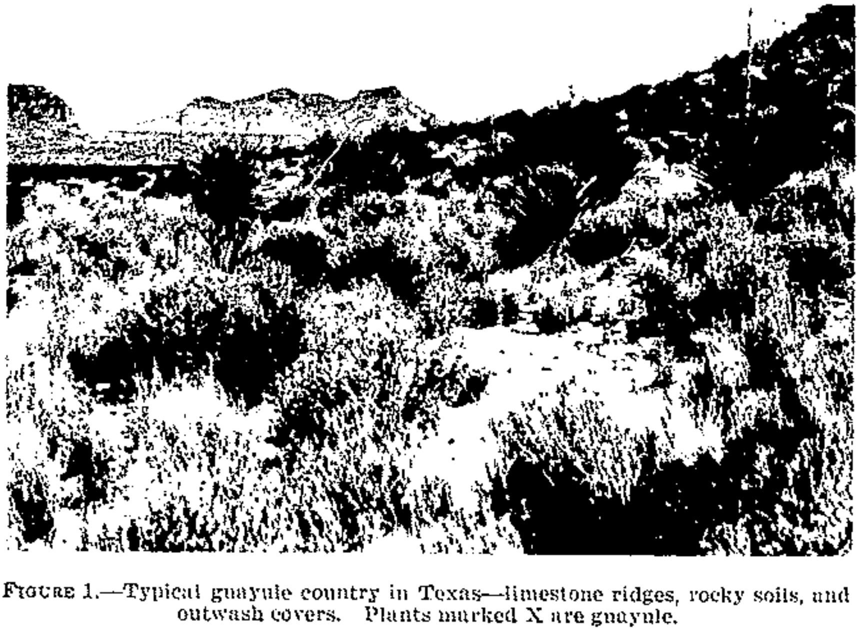

FIGURE 1.—Typical guayule country in Texas—limestone ridges, rocky soils, and outwash covers. Plants marked X are guayule.

and dwarfed perennial found in certain Rocky Mountain areas of the United States. *P. bipinnatifidum* (Ortega) Rollins, an ephemeral annual of the highlands of central Mexico, completes its life cycle from seed to seed in 8 weeks. Despite this great range in growth habit, there is a marked uniformity in floral and reproductive characteristics of the entire genus.

Parthenium appears not to be closely related to any other genus of the compositae. A unique feature of this genus is the achene-complex consisting, when it is shed, of the fusion of the basal portion of the two subjacent sterile florets, the basal portion of the achene and its subtending bract. Also unique is the shedding of the sterile, sessile, disk-florets as a unit, with disarticulation occurring on the receptacle.

P. argentatum Gray is the only species of the genus known to produce any significant amounts of rubber.

Morphology

The general morphology and anatomy of guayule has been reported in considerable detail in such publications as those of Lloyd (*145*), Artschwager (*20*), and Ross (*198*).

Root system

An exhaustive study of the root system of guayule as related to environmental factors is contained in the work of Muller (*165*).

The root system consists of the taproot which may lose its prominence and give way eventually to an intricate system of dense fibrous laterals and their branches. The depth of penetration and subsequent suppression of the tap root is determined by the penetrable soil. Roots of plants in wild stands investigated in Texas rarely penetrated the soil beyond 2 feet. The greatest concentration of fibrous roots is in the upper 6 inches of soil. They have a lateral spread up to 10 feet or more, enabling the plant to utilize the moisture of short, sporadic rains. These characteristics seem directly related to the very shallow water penetration and the imperviousness of the hardpans. The roots of cultivated guayule may reach a depth of 20 feet if not terminated by impenetrable layers.

Although guayule has many of the attributes of a desert species, it requires a moderate amount of moisture for active growth.

A significant factor in the survival of native guayule is the formation of adventitious shoots, called retoños in Mexico (Lloyd, *145*), on shallow roots exposed by erosion on the thin-soiled, rocky slopes which it most commonly inhabits. These usually arise from the roots of the parent plant at a distance of approximately 1 foot or more. The proximal portion of the mother root eventually becomes abstricted by decay, while the distal portion continues to thicken in keeping pace with the growing retoño. Adventitious roots arise later from the basal portion of the stem of the retoño, thereby increasing the extent of the root system.

Retoños often greatly outnumber the seedlings, particularly on very stony slopes where conditions, such as the absence of accumulated vegetable waste for holding moisture, make it difficult for seedlings to survive. They have a marked advantage over seedlings in a habitat of meager rainfall in the rate of growth and ability to flower as a result of the partially developed root systems already inherited at the outset from the parent plant.

The exposed ends of broken roots surviving the harvesting of guayule plants also may give rise to retoños, especially if an effective rainfall follows the harvesting period.

Stems

The first inflorescence, which terminates the monopodial growth of the seedling, is usually formed during the first year of growth while the stems are still in the herbaceous stage. Several of the uppermost branches then begin active growth. Each of these in turn ends its growth by the formation of an inflorescence, when usually the two or three upper buds begin to elongate. This system of branching continues, resulting usually in a symmetrical and closely branched shrub (fig. 2). The branching of biotypes may vary from rather spreading to fairly upright. Lignification of the stems begins during the first year of growth.

Leaves

The upper and under surfaces of the leaves of guayule are very similar but, with some difficulty, can be distinguished, at least superficially. Both upper and under surfaces are densely covered with T-shaped, nearly sessile trichomes laid parallel to the leaf axis, thus producing the light green-gray sheen characteristic of the plant.

FIGURE 2.—A 5-year-old guayule plant grown under field cultivation in the Salinas Valley of California.

As the seedling leaves approach the mature form, they are first characterized by a single tooth located near the middle of one margin only. Subsequently, a tooth appears on each margin. About half way between these and the more or less acute apex, a second pair may appear. All of these, by enlarging, attain lobate proportions (fig. 3).

In general, the amount of lobing is determined by the growth rate, which is evident in the sequence of leaf-forms during the growing seasons and subsequent dormant periods. The rate of lobing is much more pronounced during the growing season. As the dormant period approaches, which usually results from drought, the lower leaves become shriveled and are eventually shed, leaving only compact terminal clusters of small, elongate-ovate leaves tapering into the petiole, entire, or with one or two very much reduced teeth.

An abscission layer is imperfectly formed. According to Addicott (*4*), leaf fall from guayule may be considered a modified type of abscission, in which the abscission layer is not involved directly in the separation of the leaf from the stem. Leaf fall occurs only after the leaf dies. The separation is mechanical and is brought about by a break passing through the weak abscission zone at the base of the leaf. Since only part of the leaves are shed, guayule may be considered semideciduous.

Flowers and fruit

As stated earlier, an inflorescence terminates the growth of the primary shoot as well as that of subsequent branches. The inflorescence is a compound, one-sided cyme (fig. 3), and the flowers are borne in heads on a common receptacle. The head contains five fertile ray-florets, each with two attached subjacent sterile disk-florets. The ray-florets are unisexual with no visible remnants of stamens. The disk-florets contain an abortive pistil in addition to the fertile stamens and, with the exception of the outer row, are attached to each other at the base, and fall from the flower head as a unit. The achene-complex, when it is shed, consists of the achene to which are fused, at its base, the two subjacent sterile disk-florets and a subtending bract, together with the persistent ligule and the withered two-lobed stigma (fig 4).

The mature achene contains an embryo invested by two seed coats, an outer one that is thin, white, and soft and an inner one that is thin, white, and tough (*90*). The outer seed coat is a single cell in thickness except in the region of the vascular bundle and originates from the outer cell layer of the integument. The inner seed coat is composed of a membrane and a one- or two-celled layer of living, thick-walled endosperm cells, which at the micropylar end is usually several cells in thickness. The membrane, at least in the micropylar region, appears to be a wall material excreted by the endosperm cells.

Flowering in guayule is largely a response to active growth induced by favorable moisture conditions. In its native habitat, the flowering period normally occurs in the summer, although it may occur at any time of the year, depending upon the time and amount of rainfall. In the Salinas Valley, as a result of winter rains and rising temperatures, guayule begins to flower in April and produces seed in May and June.

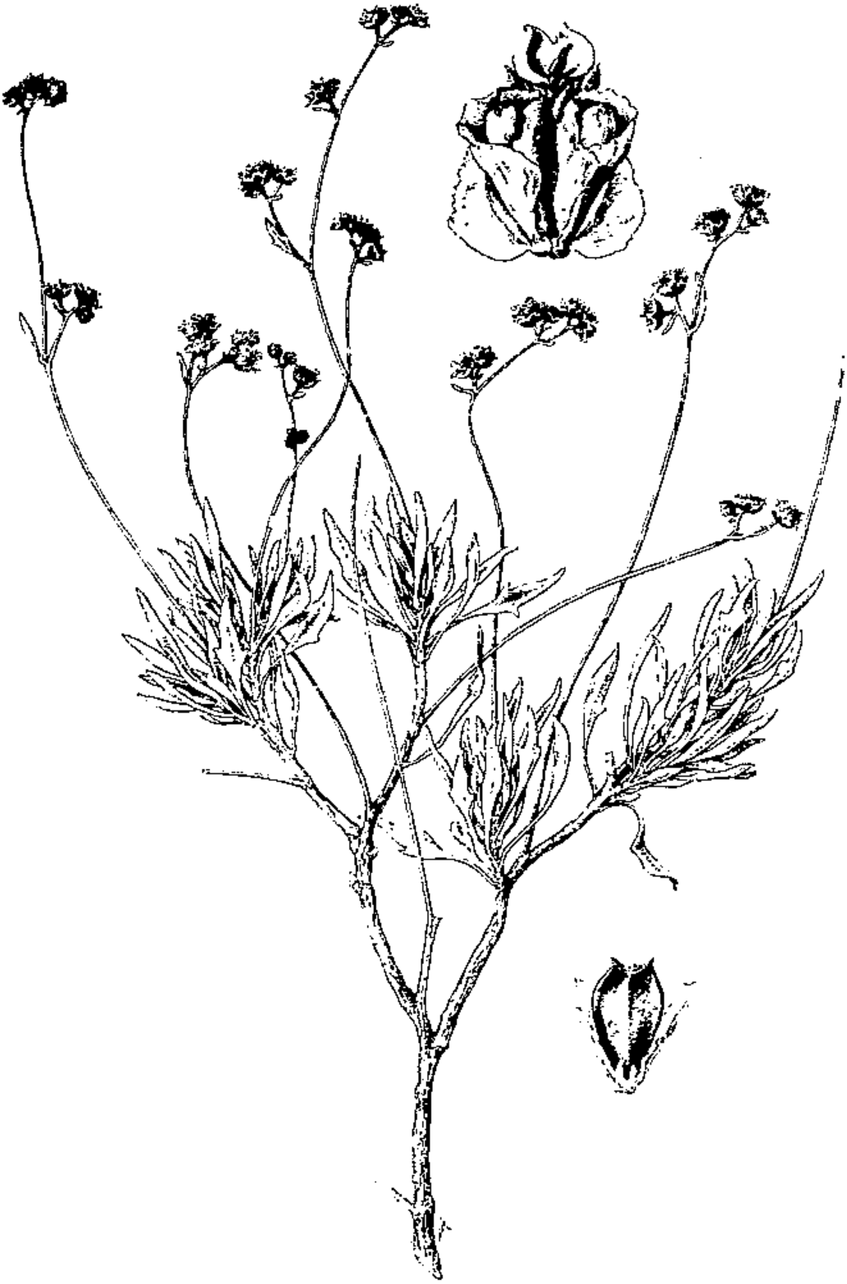

FIGURE 3.—Diagrammatic branch of guayule showing characteristics of leaves, inflorescences, and mode of growth.

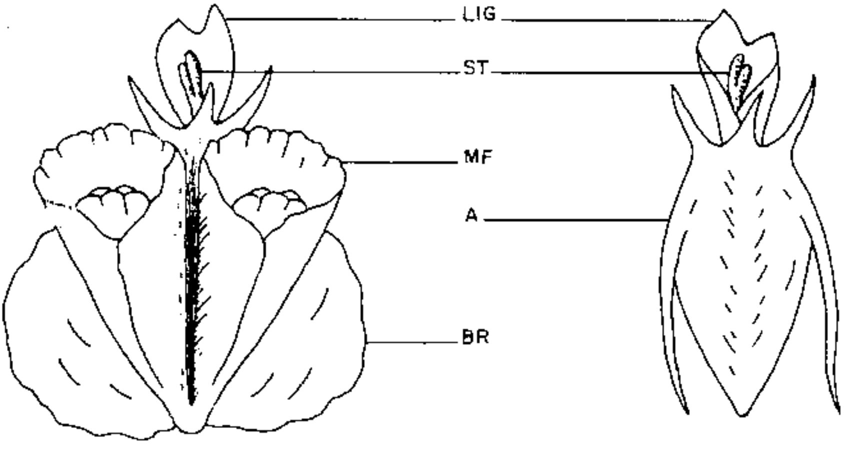

FIGURE 4.—Achene-complex in guayule. LIG, Persistent ligule (corolla); ST, persistent 2-lobed stigma; MF, attached male (disk) floret; A, achene; BR, subtending bract.

Under irrigated conditions, it may continue to flower from early spring to late fall.

Guayule is both wind and insect pollinated. Gardner (*103*) found that viable guayule pollen, which he classified among the heavier types of pollens, could be carried at least 850 yards by the wind at Salinas, Calif. The adhesiveness and spiny character of the outer surface of the pollen grains suggest also an adaptation to insect pollination. Gardner's experiments (*104*) demonstrated that insects, such as ladybird beetles, lygus bugs, and cucumber beetles, are effective carriers of guayule pollen. During the spring flowering periods of guayule at Shafter, Calif., the senior author frequently observed very large numbers of honeybees at work among the flowers, and it is not unreasonable to assume that they are also effective pollinating agents.

CULTIVATION OF GUAYULE

Few crops that are not produced commercially have received as much concentrated scientific attention as guayule. Even before the wartime effort of the U.S. Government, the Intercontinental Rubber Co. had domesticated guayule, had made selections of high-yielding types, had planted sizable acreages, and had developed efficient, standardized procedures for planting, cultivating, harvesting, and processing the shrub for rubber.

Cultivation by the Intercontinental Rubber Company

The specialized techniques developed by the Intercontinental Rubber Co. for the cultivation of guayule required also the development of special equipment. McCallum (*152*) summarized the practices of the company. These standardized practices were adopted in detail by the Emergency Rubber Project. Government experience during the war led to improvements and simplification of routine procedures but the basic concepts were little changed. The procedures involved planting the seed in nurseries where the moisture of the seedbed could be maintained at an optimum level and the young seedlings could be protected from drying wind and drifting sand. After 9 months to a year in the nursery, the young seedlings were topped, dug, and transplanted to the field. Both irrigated and nonirrigated fields were used. After some 5 years in the field, the plants were plowed up, windrowed, taken to the factory, and processed for rubber.

Intercontinental nursery operations

Normally the seed of guayule germinates slowly and irregularly. The company adopted two measures to obtain quick, uniform germination and an even stand of plants in the nursery. McCallum (*151*) found that treatment with sodium or calcium hypochlorite would speed standard practice by the company. To obtain even greater uniformity of stand, the seed was pregerminated before planting in the nursery.

The nursery beds adopted by the company were designed for maximum use of mechanical equipment. Each bed was 4 feet wide by 195 feet long. Mechanical seeders were designed to plant the guayule seed in seven narrow, equally spaced bands and to cover the seed with a thin coating of fine sand. The beds were separated by 12-inch paths in which 8-inch duckboards were laid to accommodate the wheels of seeding, cultivating, and digging equipment. Overhead irrigation equipment was installed and a light irrigation was given to each bed several times a day to insure constant but not excessive moisture during the emergence of the seedlings. Weeding in the nursery was accomplished by hand, and required large numbers of laborers. To facilitate weeding to the center of the nursery beds, with minimum damage to the guayule seedlings, wheeled platforms were provided. The laborers sat on these and hitched them ahead by foot as they proceeded down the bed. The digging of the plants, when they were large enough for transplanting to the field, was accomplished by heavy machinery with treads spaced to run on the duckboards between the beds. The plants were first topped with special equipment designed to retain the tops for rubber extraction. The plants were then undercut at a depth of some 8 inches with a heavy iron bar. After undercutting, the seedlings were pulled from the soil, graded, sorted, and transported to the field for transplanting.

Intercontinental field operations

Transplanting to the field was accomplished by mechanical transplanters designed especially for the company. A standard distance of 36 inches between rows was used and the plants were spaced at distances of about 15 inches within the row. Weeding and cultivation between the rows was accomplished by mechanical equipment, but hand hoeing was necessary to control weeds within rows. Hoeing out the weeds several times each year until the plants filled in the row was considered essential. The number of cultivations between rows was decreased to minimize injury to the plants as the rows began to close in from the spread of the plants. The company was conscious of the cost of hand labor and gave consideration to planting on the square rather than in rows to facilitate cross cultivation and minimize hand hoeing.

The company program involved harvesting the plants after some 5 years in the field by plowing them up to a depth of about 8 inches. It was recognized, however, that the time in the field could be lengthened materially without loss of rubber and that harvesting could be delayed if prices were unfavorable. The accumulation of rubber would continue for several years until the rubber could be sold profitably. After plowing, the plants were windrowed, both to facilitate loading on trucks for transportation to the factory, and to assist in shaking the soil from the roots.

Cultivation by the Emergency Rubber Project

When the Emergency Rubber Project was organized there was little time or opportunity to reassess the farm practices of the Intercontinental Rubber Co. The Department of Agriculture had given consideration to a possible program of guayule cultivation prior to Pearl Harbor and had developed tentative plans based on data furnished by the company. Prior to the war emergency, these plans remained only tentative and there was no plan nor congressional approval for their implementation. As described by Brandes (*39*), the war forced the immediate start on the domestic production of rubber based primarily on guayule, though other crops such as kok-saghyz, goldenrod, and cryptostegia were also given attention. The potential area of land suitable for guayule cultivation was described by K. W. Taylor (*218*), and Tysdal and Rands (*242*) described guayule cultivation in relation to agricultural development in the United States.

As quickly as possible after congressional authorization was obtained for the organization of the Emergency Rubber Project, the Department placed the Forest Service in charge. Chris Granger, Assistant Chief of the Forest Service, was given immediate responsibility for the organization and operation of the project. His executive officer was Gordon Salmond, and Evan W. Kelly, a regional forester, was made director of the guayule operations. Paul H. Roberts served as assistant director and later succeeded Mr. Kelly as director. Congressional action and the purchase of the domestic properties of the Intercontinental Rubber Co. were not completed until March 1942. Yet the first nursery and field plantings were made by the Emergency Rubber Project that spring and summer. This was not just a token planting to show that the project was underway but was a major planting program, field planting being limited only by the available nursery plants obtained from the Intercontinental Rubber Co. Gross and Perry (*114*) described the organization and operation of the Emergency Rubber Project.

Nursery operations by the Emergency Rubber Project

To prepare for the planting of guayule nurseries, the Forest Service imported large quantities of snow fence, then unheard of in the Salinas area, to protect the nurseries from drifting sand since the planting of live windbreaks would be too long delayed. Large quantities of sand were accumulated as cover for freshly planted seed (fig. 5). A special building was constructed with bins for the pregermination of seed to be planted in the new nurseries. All available overhead irrigation equipment was obtained.

Roberts (*186*) states, "Company sowing practices were used in the 1942 spring program. The sand that was used to cover the seed after it was placed in the seedbeds was screened, washed, and kiln dried. Large sand bunkers were necessary to protect the dried sand from excess moisture. The necessary 3,500 tons of dried sand were difficult to secure and transportation to the nurseries was expensive."

Nursery practices were improved considerably in successive campaigns including development of improved planting and cultivating equipment, reduction in the number of irrigations, use of stove oil and special spraying equipment to reduce weeding costs, and elimination of expensive pregermination procedures. Research conducted during the time of the Emergency Rubber Project indicated that row irrigation could supplant the overhead irrigation considered essential by the company. Planting seedlings in a single row on each side of a furrow (fig. 6) was tried and later adopted as standard practice in the post-war plantings of guayule.

It was thought that it should not be necessary to keep seedlings in the nursery for a full year before transplanting into the field. An attempt was made to double nursery production by planting twice a year. Fall transplanting of guayule in the Salinas district did not

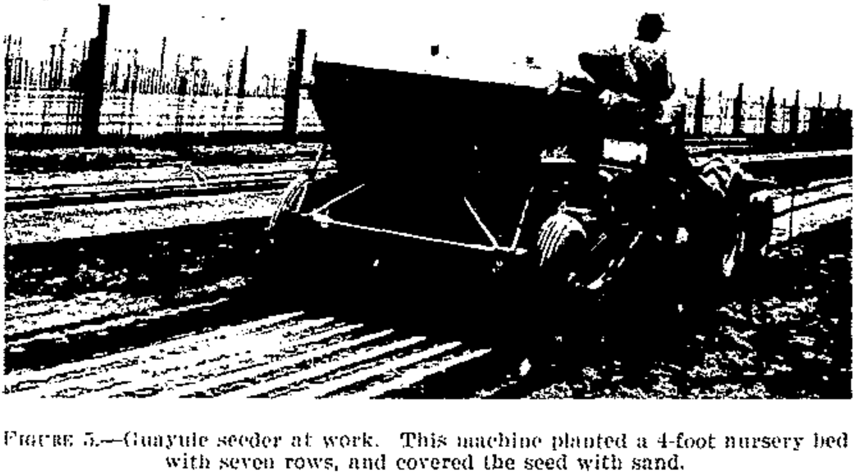

FIGURE 5.—Guayule seeder at work. This machine planted a 4-foot nursery bed with seven rows, and covered the seed with sand.

FIGURE 6.—Nursery plantings of guayule along the sides of irrigation furrows.

prove feasible and this program was abandoned. It was recognized, however, that under suitable conditions it would be possible to expand plantings quite rapidly by transplanting both fall and spring. Fall plantings, however, would need supplemental irrigation to become established.

Field operations by the Emergency Rubber Project

Roberts (*186*) states, "The need for utmost speed in the production of the greatest possible amount of rubber required that, in the beginning, the Emergency Rubber Project follow the practices of the Intercontinental Rubber Co. and the advice of Dr. McCallum, who had been head of the company's experimental work in the United States for a long period of years, with respect to ground preparation, planting, and care of field plantations."

Before the end of the war and the liquidation of the Emergency Rubber Project, new practices developed by the project became standard and new machinery and equipment replaced that developed by the company. The extent of these changes is indicated in a note received from J. J. Byrne[2] of the Forest Service in a review of the present report. Mr. Byrne states, "Considerable improvement was made in guayule culture by the Emergency Rubber Project. Mass methods of seed treatment, elimination of pregermination of seed, and the use of sandy loam for nurseries rather than heavy soils recommended by the Intercontinental Rubber Co., led to the abandonment of costly duckboards for nursery operation; mechanization of preparation of nursery soil to eliminate several operations thought to be needed by Intercontinental Rubber Co. scientists; fine-grading of plantation fields before planting eliminated water wastage and plant killing; the development of a 'rectangular contour' method of irrigation of steep lands to get better water distribution; considerably improved method of seed collection; weed killing in nurseries and plantations by oil emulsions eliminated thousands of laborers; the development of plantation machines; and careful selection of plantation sites by skilled soil scientists, all greatly improved the technology of guayule plant production."

These cultural improvements are illustrative of the many changes in procedure instituted by the Emergency Rubber Project, and adopted as standard practices in the nursery and field operations. A major contribution of the project was the development of standard practice guides based on the best available information, and the institution of these standard practices in field operations.

CROP RESEARCH

Agronomy

Guayule is a difficult crop to sample and compare. Because of major differences in shape, size, and composition of the individual plants and uneveness of most stands, the single-plant sample could not be considered representative of a plot, and efforts were made to perfect the sampling techniques to assure that the results of comparative tests were true. Much effort was put into devising sampling techniques that would be reliable. Federer (*95*) made statistical comparisons of samples of various sizes and concluded that the optimum size of sample would be 12 plants, 9 replicates would be necessary to give a variety mean for percentage of rubber, and 27 replicates would be necessary to give an adequate estimate of the standard error for dry weight of shrub and grams of rubber. While samples of this size and replications of this magnitude were not possible in the guayule research, this paper is important in pointing out the difficulties of sampling and comparing guayule.

Soils

When the Emergency Rubber Project was started in 1942, comparatively little was known about soil requirements. Because of pressure to produce as much rubber as possible in the shortest possible time, extensive soil surveys were immediately gotten underway.

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[2] Unpublished communication.

Youngs (*253*), in a reconnaissance survey of soils in the Sacramento Valley of California and adjacent areas, made a tentative classification of some of the more important soils as to suitability for growing guayule. His evaluation of soils was based on structure, drainage, presence of claypans and hardpans, height of water table, and other factors.

Vessel (*243*)* made a detailed survey of soils in the Tracy-Newman-Los Banos-Mendota area in the south-central part of California in the San Joaquin Valley to locate available areas suitable for growing guayule. Approximately 943,000 acres of land were surveyed. He divided the soils into the following five classes with respect to suitability:

"Class I soils are well adapted to guayule. They are coarse to medium textured, loose, permeable, friable, productive, and well-drained throughout. They occur on recent and young alluvial fans. The Mocho and Sorrento fine sandy loam, loam, and silt loam are in this group, as well as Esparto loam.

"Class II soils are less desirable for guayule than Class I soils because of finer surface textures, gravel, or less permeable and more compact subsoils. They occur mostly on recent or young alluvial fans and, to a lesser extent, on old alluvial fans. Included are the Mocho, Sorrento, and Panoche gravelly or sandy loams and clay loams, Esparto gravelly loams, and Lost Hills loam, silt loam, and clay loams.

"Class III soils are questionable for guayule because of slow internal drainage, fine surface and subsoil textures, or sandy or gravelly textures. They occur on the outer edges of recent alluvial fans, interfan areas, old alluvial fans, and old valley terraces. Included in this group are Sorrento and Mocho clays, gravelly loamy sands, Rincon clay loams, Ambroso clay loam, Esparto gravelly sandy loam and clay loams, and Pleasanton loams and clay loams.

"Class IV and V soils are unsuitable for guayule because they have slow internal drainage, fine surface and subsoil textures, steep topography, are subject to frequent overflow, or possess excess alkali. They occupy old alluvial fans, old terraces, and basin areas. Some of the heavier types of the following series are represented in these classes: Ambrose, Rincon, Lost Hills, Pleasanton, Positas, Herdlyn, and Denverton."

In this soil reconnaissance some 27,000 acres were classified as Class I soils, 131,000 as Class II, 126,000 as Class III, and the remainder (659,000 acres) as Classes IV and V.

Approximately 30,000 acres of guayule were planted in California under various climatic and soil conditions. Smaller acreages were planted in Arizona, New Mexico, and Texas. Retzer and Mogen (*184*) studied 1-, 2-, and 3-year-old plantation guayule to determine the effects of different soil types on shrub growth and rubber content. Most soils in the guayule belt (central valleys, central coastal areas, and southern part of California; southern Arizona; southwestern and southeastern New Mexico; and southwestern Texas) have a moderate to high level of natural fertility, few being considered poor for farm crops. They are commonly low in organic material and nitrogen as compared with soils of the more humid regions of the United States. Most of the soils are calcareous or have a neutral reaction.

These studies included 61 sites representing 34 soil types and 10 phases of soil types. The soils represented seven of the major profile conditions occurring in the guayule belt: (1) Good agricultural soils, (2) clay surface and clay subsoils, (3) sand and gravel surface and subsoils, (4) sand and gravel subsoils, (5) soils with claypans, (6) soils with hardpans, and (7) soils with high water tables.

Good agricultural soils. Soils of this nature—good in the ordinary agricultural sense—have nearly ideal moisture relationships and will produce in 2 years a uniform stand of guayule having a height of approximately 18 inches and nearly occupying the space between 28-inch rows. Roots of 2-year plants were found at a depth of 19 feet in Sorrento loam.

Calculated yields of 2-year shrub from irrigated fields averaged 52 percent greater than yields from dryland fields. The rubber content of the dried shrub from irrigated fields, averaging 6.85 percent, deviated widely and reflected the effect of irrigation practices, such as quantity of water and the time and number of applications. There was an inverse relationship between shrub weights and the percentages of rubber in the dried shrub. Shrub yielding at rates below 4,000 pounds of shrub per acre averaged 9.78 percent rubber; that yielding at rates between 4,000 and 7,000 pounds of shrub per acre averaged 7.82 percent rubber; and that yielding at rates above 7,000 pounds of shrub per acre averaged 5.44 percent rubber. Yields of rubber from 2-year shrub on irrigated land were estimated at 350 to 650 pounds per acre.

The rubber content of the dried shrub and that from dryland fields averaged 7.78 percent. Deviations from the average were small, indicating that physiological response was not greatly influenced by cultural treatments. Shrub yield ranged from 3,000 to 4,000 pounds per acre, and the estimated yield of rubber varied from 250 to 400 pounds per acre.

Clay surface and clay subsoils. Clay soils are not usually recommended for guayule because of difficulty in weed control, high disease hazards, and poor drainage. However, guayule may do well on very friable, calcareous, clay soils with good structure. The average rubber content of shrub in three fields that were studied was 10.28 percent, or 2 percent higher than shrub on good agricultural soils used as checks, but the plants were small and more rubber was produced per acre on the good agricultural soils. Estimated yields ranged between 250 and 600 pounds of rubber per acre but were usually lower than estimated yields of good agricultural soils.

Sand and gravel surface and subsoils. These soils are characterized by very poor moisture relationship and are often of very low fertility. Owing to low moisture-holding capacity, frequent irrigations are required. This results in rapid and succulent growth. Any quick changeover from low- to high-moisture stress results in death or damage since the plants are not able to change physiologically to meet the sudden stress. Root penetration in all cases observed was related to the amount of fine soil material present.

The rubber content of the shrub averaged 8.79 percent of the dry weight but the yields were very low because of the small size of the shrub. Estimated yields for 2-year-old irrigated shrub ranged from 100 to 200 pounds of rubber per acre. Production of rubber on these soils without irrigation was considered impractical. The quantity of shrub and rubber produced under irrigation depends upon the frequency of irrigation. Large yields are difficult to attain because of the need of frequent irrigations, which also add to the cost of production.

Sand and gravel subsoils. Although these soils occur extensively through the guayule belt, they seldom occur in large, continuous areas. The size of the 2-year-old, irrigated shrub depended upon the depth of the surface soils. The shrub ranged in height from 10 to 12 inches and in spread, from 13 to 16 inches. Plants grown without irrigation ranged from 6 to 10 inches in height and from 8 to 12 inches across. Roots were rarely observed to penetrate more than a few inches into the sand and gravel subsoils.

The irregularity of the physical makeup of these soils was reflected in the highly irregular rubber percentages of the plants from irrigated fields, which ranged from 3.05 to 10.96 as compared to 5.29 and 6.51 for the plants grown without irrigation. The rubber yield from irrigated, 2-year shrub was estimated at 150 to 300 pounds per acre, while that of nonirrigated shrub was estimated at between 20 and 100 pounds per acre.

Soils with claypans. These soils occur extensively throughout the guayule belt. They are characterized chiefly by poor drainage. Saturated conditions of the upper soil layers caused by winter rains and heavy irrigations often resulted in death or damage to guayule roots. Those plants that did survive usually had penetrated the claypan with thick, scantily branched roots which, in turn, branched again after reaching more favorable soil conditions below. In some cases, the development of a shallow root system rather than a deep, spreading one enabled the plants to survive.

Only one field with a claypan subsoil—near King City—was irrigated. In addition to effective rainfall, four irrigations were given, making a total of 38.8 inches. Rubber percentages were very high, averaging 11.51 percent, but the small size of the plants resulted in low rubber yields. The four irrigations given did not increase the rubber yield as compared with nonirrigated fields. Both the rubber percentage and shrub weight indicated a degree of stress similar to that of nonirrigated shrub. The dense claypan, having a high moisture-holding capacity and a correspondingly high wilting percentage, apparently supplied moisture slowly and under high tensions, and produced high rubber percentages under stressed but continuous growth. Estimated rubber yields for irrigated and nonirrigated 2-year shrub ranged from 20 to 200 pounds per acre.

Soils with hardpans. Hardpans in the subsoils are very extensive in the guayule belt and account for these soils being unsuitable, or of little value, for most farm crops. These impervious subsoils are found largely in the San Joaquin, Madera, and Exeter soils in California. During rainy seasons or after heavy irrigations, the soils above the hardpans become saturated and often remain in this condition for long periods, except in the Exeter soils where the hardpan is often discontinuous, permitting internal drainage by lateral seepage.

Two-year plants on Exeter soils were 11 inches high and from 9 to 13 inches wide. Most of the roots were near the surface of the soil, often matted on top of the hardpan, where most damage occurred from saturation. Since most of the moisture must come from the shallow surface, frequent and light irrigations were necessary.

The rubber percentage of shrub grown on Exeter soils was in the medium range but higher than on most fields in the Bakersfield area. The low yield and small size of the shrub was characteristic of shrub grown under considerable moisture tension. Rubber yield of 2-year irrigated shrub was estimated at between 100 and 200 pounds per acre. A high mortality might occur anytime between the establishment and harvest of the shrub in areas of high winter rainfall.

Soils with high water tables. High and fluctuating water tables are found in small areas throughout the guayule belt, and are often created by overirrigation or by seepage from irrigated lands at higher elevations.

The effect of water tables on guayule was studied in two fields near King City, Calif., where high water tables were considered permanent but fluctuated in depth during the year. In one of the fields, with a water table of 48 to 60 inches, 2-year plants were 17 to 19 inches wide and 15 to 17 inches high; in the other field, with a water table of 82 to 108 inches, the plants were 15 to 17 inches wide and 13 to 15 inches in height. Including rainfall, the former field received 54 inches of water and the latter, 62 inches. Roots were not observed below the moist fringe above the free water.

Although the rubber percentages of the dried shrub were nearly equal in the two fields, they were considerably lower than those of comparable shrub in the Salinas Valley on good irrigated soils. The shrub weight was higher than in any other field sampled in the entire valley. The high water tables undoubtedly permitted continuous growth without the benefit of summer moisture stress necessary for rubber storage, thus causing the plants to behave as would those under continuous irrigation. Water tables remaining at or near the soil surface for a few days or more would kill or severely damage guayule, especially during the hot summer months.

Dortignac and Mickelson (*83*)* studied the effects of continuous soil moisture in the Salinas Valley following winter rains on Chualar loam, Bryant loam—shallow phase, and Bryant loam—deep phase. They found that the rate of growth and, in some places, the death of guayule were directly related to soil profile characteristics and associated moisture conditions. The plants found on Chualar loam were larger and more rapidly growing than those on Bryant loam, either shallow or deep phase. Chualar loam is well drained and is permeable throughout its profile so that the roots penetrated easily. It has a high infiltration capacity to a depth of 6 feet. Dortignac and Mickelson noted that plants grew slowly on the Bryant soils because of the impermeable claypan, and that the shallower the claypan, the slower the plants grew. Indications were, however, that the entire root zone had to be saturated before considerable numbers of plants were killed by suffocation.

Observations by Mickelson and Mogen (*162*)* also indicated a direct relationship between soil properties and plant growth in the Salinas, Bakersfield, and Colusa Districts. Plant size was always directly correlated with the depth of soil overlying claypans and hardpans. The deeper the soil, the more vigorously the plants grew. The fine-textured soils, such as the silty clay loam and clay loam series in the Colusa area, were difficult to work in rainy seasons. Soils in which root penetration was easy and water-holding capacity was high produced the largest plants. In the Salinas area, most of the plants in low or poorly drained soils with a high clay content were killed by suffocation from standing water.

In 1951, plantings for producing nursery stock under irrigation by the Guayule Seedling Stockpiling Project in Texas were made on at least seven soil types (*45*).* The two outstanding soil types for seedling production were Uvalde silty clay loam and Regan loam. It was especially difficult to keep clay soils wet enough to obtain and retain stands during periods of high temperatures. Charcoal rot, especially on clay soil in one area, caused high mortality when the plants needed water. Difficulties were encountered in obtaining proper subirrigation of seedbeds on Frio silty clay loam.

Climate

Soils, soil moisture, and irrigation practices can be manipulated to increase yields of shrub and rubber. Climatic factors, on the other hand, do not lend themselves to manipulation, and affect not only the rubber content and growth of shrub, but also field production.

McCallum (*152*) noted the effects of variations in seasonal rainfall distribution on rubber accumulation in experimental plots extending from southern Texas to California, and found the coastal regions of California particularly favorable for dryland guayule. Here, the rains fall during the winter and early spring months with none during the summer months. Guayule could be planted in winter and early spring, the winter moisture being sufficient to allow the plants to attain considerable growth each year. Harvestable plants, 4 years old, had used up the moisture and stopped growth in early June, forcing the plants to undergo stress and to accumulate rubber during the remainder of the season.

The expanded program of the Emergency Rubber Project during World War II, requiring the planting of large acreages, necessitated the selection of areas outside the Salinas Valley. Extensive plantings were made in the Sacramento and San Joaquin Valleys of California. Indicator plots were established in California, Arizona, New Mexico, and Texas to determine, in addition to other factors, the climatic adaptability of guayule.[3]

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[3] These indicator plots were located and supervised by the Special Guayule Research Project of the Bureau of Plant Industry, Soils, and Agricultural Engineering as part of the research program of the Emergency Rubber Project.

Swenson and Bullard (*212*)* obtained climatic data in the areas where guayule was grown and outlined the acceptable limits of shrub growth. Bullard (*41*) later summarized available knowledge concerning the relation of climatic factors to both shrub behavior and rubber increment and defined the climatic limitations in guayule culture according to the latest information. On the basis of these studies, they prepared a map (fig. 7) showing the geographical location of the "guayule belt" in the Southwestern States.

Bullard concluded that:

1. The most critical aspect of temperature for guayule growth is the minimum temperature and that guayule should be grown in areas where the minimum temperatures do not drop below 15° F. unless the winters are dry and the plants are normally in a dormant condition for a considerable length of time before the minima occur. Guayule in a completely dormant state has withstood temperatures of zero degrees F. without injury. The plant makes its best growth around 90° to 100° F. Mean temperatures should be above 55°. Growth is slow and mortality may be high at mean temperatures below 60° F.

2. For dryland culture, the annual rainfall should not be under 15 inches. A minimum of 11 inches is satisfactory in areas with cool, foggy summers such as occur in the coastal valleys of California. Precipitation up to 25 inches per year is satisfactory. Areas of higher rainfall of year-long distribution produce large guayule with low rubber.

3. While Arizona and California have a winter rainy season in common, Arizona has in addition a late-summer rainy season. In New Mexico and western Texas, the heaviest rainfall occurs in September, the principal wet season occurring between July and Octo-

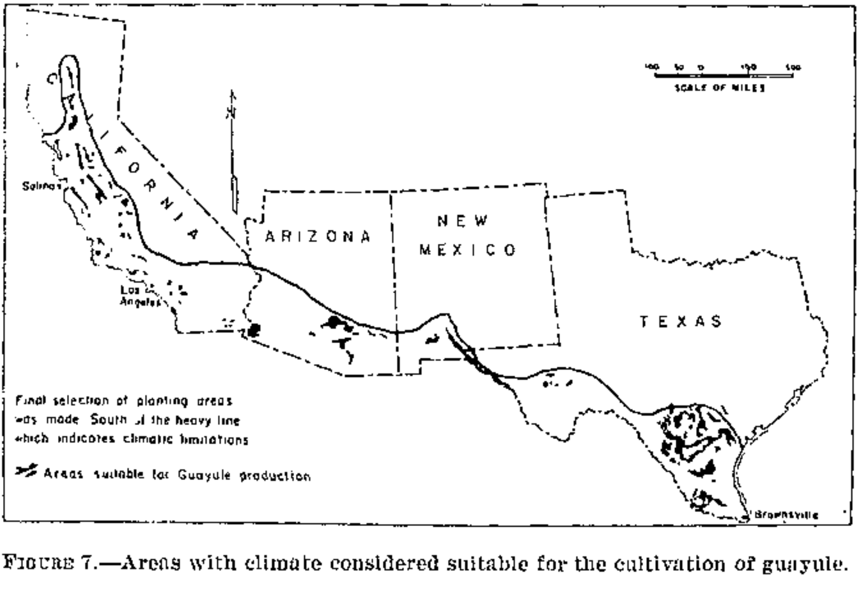


FIGURE 7.—Areas with climate considered suitable for the cultivation of guayule.

ber. The winters are quite dry. Southern Texas is characterized by moderately high precipitation with peaks in May and September, and there may be considerable rain in any month of the year. Colder winters occur in New Mexico and Texas (except in the extreme southern part) than in California and in Arizona. New Mexico and Texas are also windier. Humidity is low except in southern Texas and the coastal area of California.

4. Under dryland culture the largest shrub, having a relatively low rubber content, was grown in southern Texas where vegetative growth continued almost the year around. Under irrigation the largest shrub was grown in central Arizona and in the central valley of California. Shrub having the highest rubber percentage was grown without irrigation in the coastal area of California. However, the high rubber concentration did not always compensate for the high yield of shrub grown in the central valley.

5. For dryland culture, it was desirable to have a supply of moisture at the beginning and during the first half of the growing season and also to have a dry period at least 2 months' duration each year before the cold season.

6. In a windy climate, sandy soils drift easily and present erosion problems when the plants are young. In a wet climate, waterlogged, heavy soils may suffocate the shrub. These soils are also difficult to work for a considerable length of time when they are saturated. However, the moisture-holding capacity of heavy soils, together with the rate at which moisture is made available to the shrub, may make them highly desirable for dryland culture. In a climate of high-intensity rainfall, very light soils may provide serious erosion hazards. In a climate of limited rainfall, very light soils may also be undesirable because of their low moisture-holding capacity. Soils with claypans and hardpans, or soils having highly permeable layers at shallow depth, are greatly affected by the amount, intensity, and duration of rainfall.

Jenkins (*134*)* also made a study of the effect of low temperatures on guayule, and made observations during a 3-year period on the reaction of guayule to cold in the Texas and New Mexico indicator plantings. Although these observations did little more than show trends in cold tolerance, his generalized conclusions were:

1. The degree of injury is in inverse ratio to the degree of dormancy.
2. High soil moisture does not appear to be a primary hazard if the plant is dormant.
3. The determining factor in winter survival appears to lie in the temperature pattern that exists in late fall and early winter; i.e., a gradual and progressive onset of lowering temperatures provides a high degree of protection regardless of the presence of high soil moisture.
4. Especially hazardous areas are those subject to "winter warm spells" with temperatures high enough to induce plant growth which in turn are followed by freezing temperatures.
5. What would appear to give the plant the greatest insurance against winter injury is a climate pattern in which a fall dry period coincides with a gradual but progressive temperature decline.

6. With comparable temperatures and variable soil moistures, the degree of injury appears to be in direct ratio to the amount of moisture.

7. It would appear that the minimum temperature at which injury occurs is a fluctuating point whose value is determined by the fall and winter weather pattern.

8. In irrigated areas, where there is little or no rainfall in the late summer or fall, dormancy may be controlled by withholding irrigation, thus protecting the plant from injury by cold.

Bullard (*41*) studied the relationship of cultural practices to climate and concluded that "whether a given planting area will be dry-farmed or irrigated, of course, depends on the normal precipitation of the area. Variability in seasonal amounts and distribution through the year must be considered together with the average values. Drought frequency may have some bearing on type of culture; perhaps more on planning shrub conditioning. Storm frequency will determine the probable number of days for planting and cultivating and harvesting operations. Length of growing season and frost occurrence affect irrigation practice. Temperature and rainfall must be considered in connection with weed, pest, and disease-control measures. Long-term climatic trends, with respect to both rainfall and temperature, may determine whether or not an area can be devoted to guayule culture over a long period. The project period, in which trial plantings of guayule were made over a large area, coincided with the warmest part of a long-time climatic cycle; in the coldest phase of such a cycle, many of the otherwise successful plantings might be killed or greatly retarded by cold. Similar consideration must be borne in mind with respect to dry-land culture and long-time precipitation cycles."

Irrigation

The effect of irrigation on rubber production has been the subject of intensive study since it is one of the chief cultural factors influencing the yield of rubber. The Intercontinental Rubber Co. suffered many disappointments in their work with rubber production under irrigation. They could obtain large tonnages of shrub under irrigation in California, Arizona, and Texas, but low concentrations of rubber resulting from forced growth, greatly increased harvesting and milling costs. Their observations, and those of wartime and postwar agencies have indicated that, in general, conditions favorable to maximum growth adversely affect the accumulation of rubber. Although the per-acre yield of rubber may be increased by irrigation, the cost, together with increased costs of harvesting and milling, ordinarily would not justify irrigation except where the urgent demand for rubber and the element of time are the determining factors. Since guayule grows best on light soils that are susceptible to erosion, special methods, such as the use of the headland devised by Davis (*80*), were advantageous in irrigating guayule.

Kelley et al. (*141*), in their study of moisture stresses in nursery plantings, noted that low moisture stress produced lush plants with a lower rubber content than those grown under high moisture stress.

Tingey (*227*), in an experiment designed to show the effects of spacing and irrigation, noted the same trend after 21 and 33 months from seeding. Heavy irrigation (irrigating when approximately 50 percent of the available soil moisture in the first foot had been depleted) gave a consistently higher yield of shrub than a low level of irrigation (no irrigation the first season and only one the second). The low level of irrigation gave a consistently higher percentage of rubber in the shrub than did heavy irrigation. However, shrub receiving light irrigation (irrigating when all the available moisture had been depleted to a depth of 3 feet), while intermediate in shrub yield and in percentage of rubber, gave in general, the highest yield of rubber per acre. To facilitate the precise determination of soil moisture in these studies, Hunter and Kelley (*131, 133*) developed specialized techniques for recording soil moisture and for their control at various levels beneath the surface.

In 14-month-old guayule sampled on March 1, Tingey and Foote (*231*) noted that the maximum rubber production was obtained with one fall irrigation and fertilizer. Fall-irrigated shrub yielded at the rate of 213 pounds of rubber per acre. Nonirrigated shrub yielded at the rate of 161 pounds per acre. Plant spacing was 28 by 10 inches.

Olson (*172*)* and Rotty (*199*)* reported on the comparative yields from 2- to 3-year-old guayule grown with and without irrigation in the Salinas Valley. Bullard (*40*)* summarized their findings, and noted that "it was not until rather late in the study that the lack of significance in the results was fully appreciated, so that some of the trends brought out and some of the tentative conclusions reached are not properly founded." Although there were deficiences in their conclusions, nevertheless some of their results are important. Irrigation greatly increased the yield of rubber per acre, although it provided larger plants containing a relatively lower percentage of rubber. Rubber from 3-year-old shrub had a higher tensile strength than that from 2-year-old shrub. There was apparently no difference in tensile strength of rubber produced by irrigated, and by dryland shrub. There was a drop in rubber content in May and June during the season of accelerated growth. Moisture stress, caused by close spacing, high survival, or withholding irrigation, tended to increase the rubber yield. The highest yields were obtained from 3-year-old shrub irrigated the first 2 years only.

Hunter and Kelley (*132*) conducted irrigation experiments with 2-year-old guayule on two soil types, the Deland sandy loam and the Sorrento silty clay loam, in the San Joaquin Valley of California. Five moisture levels, ranging from very high to very low, were maintained on each soil type between April and October of 1944. Increases in shrub weight ranged from 1,000 to more than 10,000 pounds per acre during the year. Increases in rubber yield ranged from 190 to 650 pounds per acre on the sandy loam and from 350 to 515 pounds on the silty clay loam. The highest yields of both shrub and rubber were obtained on the sandy loam soil at the higher moisture levels. The highest yields of rubber were obtained on the silty clay loam at the lowest moisture levels. They found a possible explanation for this discrepancy in the differences in moisture characteristics of sandy and clayey soils. The available water in sandy soils is held at lower moisture tensions than in clayey soils, thus permitting the plants to absorb more water and to grow more vigorously. The effect of moisture-holding capacities of various types of soils on shrub and rubber production is discussed in the section dealing with soils pp. 16–21).

Retzer and Mogen (*184*) found that soil differences were dominant factors in influencing the yield of rubber per acre, and that these differences were primarly associated with variations in soil moisture stress in the types of soils they studied. Lesser but important variations in the quantity of rubber produced per acre were associated with the frequency and quantity of irrigations. From a survey of the plantings made by the Emergency Rubber Project, they concluded that the highest rubber percentages were obtained as a result of frequent but moderate periods of moisture stress and that the lowest yields resulted from shrub permitted lush growth under low moisture stress. Their study indicated that the best compromise would be to produce shrub having a rubber content of 7 to 9 percent dry weight and 4,000 to 7,000 pounds of dry shrub at 2 years of age, which could be obtained on good soils with the addition of 30 to 50 inches of water.

Dortignac and Mickelson (*84*) described the dryland production of guayule in California, and Dortignac (*82*) discussed the response of guayule to soil moisture in dryland farming in California.

Cultivation

Conditioning nursery stock for transplanting. In the Salinas Valley, the common practice of the Intercontinental Rubber Company was to keep guayule nursery seedlings supplied with abundant moisture during the summer to maintain good growth until early fall and then withhold irrigation to allow the seedlings to be hardened by moisture stress during the rest of the fall, and by low temperatures during the winter. Transplanting to the field was done in late winter and early spring. However, the large transplanting program undertaken by the Emergency Rubber Project necessitated extending the transplanting period over a major portion of the year. Survival in these stands varied considerably (from 50 to 90 percent) even under favorable soil and moisture conditions, and the rate of growth resumption was, at times, irregular.

With this in view, Kelley et al. (*141*) conducted experiments to determine the effects of soil moisture stresses, ranging from very high to very low, on the amount and type of growth in the nursery, and on the after-transplanting growth responses of guayule nursery stock at all seasons of the year.

The nursery beds were seeded on June 3, 1943. Prior to seeding, the soil was wet to a depth of at least 6 feet by irrigation to supplement any deficiency after the winter rains. The experiments were designed to grow seedlings under the following five ranges of soil-moisture stresses:

Treatment 1, between the field capacity and a tension of 850 cm. at 6-inch depth (very low moisture stress).

Treatment II, between the field capacity and a tension of 850 cm. at 12-inch depth.

Treatment III, between the field capacity and the wilting point at 6-inch depth.

Treatment IV, between the field capacity and the wilting point at 12-inch depth.

Treatment V, regardless of the moisture stresses developed, the plants were to receive no water after they were well established (very high moisture stress).

Treatment V plot received no irrigation after July 13. Identical moisture treatments were given to all plots until July 13, when the plants were considered well established. The differential moisture treatments were begun at this time and discontinued September 14.

Changes in moisture content were followed by means of oven-dried samples, Richard's tensiometers, and Bouyoucos plaster-of-paris blocks.

Nine field transplantings were made at intervals from September 1943 to July 1944 to determine survival and growth with relation to the previous moisture treatments in the nursery. The plants were topped to within 1½ to 2 inches of the crown, and all leaves were removed. With the exception of the fourth and fifth transplantings, the plants were transplanted to relatively dry soil and irrigated within 24 hours. The fourth and fifth transplantings were made during the rainy season, and the plants were not irrigated.

The first transplanting was made September 29. Thirty-five days later 96 percent of the plants of treatment V (grown under high moisture stress) showed new shoots or leaf growth and were growing vigorously, whereas only 19 percent of the plants of treatment I (grown under low moisture stress) showed any growth, and then only a few buds were evident. Renewed growth was observed on 60 to 70 percent of the plants of the other three treatments. There were no significant differences between the latter treatments until survival counts were made on the 115th day, at which time, and at all subsequent countings, there were significant differences between treatments.

The second transplanting was made on October 22. After 15 days, counts indicated that new growth for treatment V was significantly higher than for any of the other treatments. Survivals in treatments III and IV were significantly better than in treatments I and II. When counted after 265 days, the survival values for treatments I to V were in the order of 22, 33, 55, 56, and 93 percent, respectively.

The third transplanting took place on December 1. After 14 days, only 0.4 percent of the plants of treatment I had shown evidence of new growth, while 32 percent of the plants of treatment V had resumed growth. At all countings after 127 days, the survival values for treatment I were significantly lower than those for any of the other treatments. At 15 days and thereafter, the values for treatment V were always significantly higher than for any other treatment. After 200 days, the values for treatments I to V were 53, 68, 78, 71, and 92 percent, respectively.

The fourth transplanting trial was made on January 25. Twenty days later, only 0.8 percent of the plants of treatment I had resumed growth, while 34 percent of the plants of treatment V had new shoots or leaves. After 142 days the survival values for treatments I to V were 81, 88, 90, 98, and 92 percent, respectively. At all countings, the survival values for treatment V were significantly higher than for treatment I.

The fifth transplanting was made on March 8. One month later, 93 percent of the plants of treatment V had resumed growth. Survival in treatment I, resulting in 77 percent new growth, was significantly lower than in any of the other treatments. However, no significant differences existed between treatments after 93 days.

Fifteen days after the sixth transplanting, made on April 5, Treatments I and V resulted in 20 and 65 percent new growth, respectively. After 105 days, survival in all treatments except treatment I rose to 92–93 percent. Survival in treatment I, with 84 percent new growth, was significantly lower than in any other treatment.

The seventh transplanting was made on May 3. Sixteen days later, 86 percent of the plants of treatment V had resumed growth as opposed to 48 percent of the plants of treatment I. After 76 days, the survival values for all treatments had risen to 91 percent or higher, with no significant differences between treatments.

The eighth transplanting was made on June 2. Fifteen days later, 95 percent of the plants of treatment V had resumed growth as compared to 47 percent for treatment I and 60, 61, and 79 percent for treatments II, III, and IV. No significant differences in survival were evident after 34 days, all values being above 92 percent.

The ninth and final transplanting was made on July 8. Ten days later, the survival values for treatments I to V were, in that order, 30, 30, 45, 43, and 87 percent. When counted after 17 days, the values were 49, 53, 64, 60, and 90 percent, respectively. Subsequent counts were not made on this last transplanting trial.

These studies, made by Kelley et al. (*141*), demonstrated clearly that guayule seedlings grown under high moisture stress at Salinas may be transplanted any time of the year with satisfactory results. Regardless of the time of transplanting, more than 90 percent of the plants of treatment V survived, and in every case they resumed growth much more quickly and grew more vigorously than those of any other treatment. In fall transplantings, the percentage of survival was much higher for plants grown under high moisture stress. It was not until the January transplanting that plants of the other treatments survived satisfactorily, and it was not until the March transplanting that survival equalled that of treatment V. Even then, these plants were much slower in resuming growth. Although all of the experimental plots were exposed to identical moisture conditions after September 14, 1943, the effects of the differential moisture treatments previously given were still apparent more than 9 months later.

In their experiment it was evident that 1 or 2 months of "hardening" by withholding water and by cold improved the survival of transplanted, succulent plants previously grown under low moisture stress, and that 4 to 6 months of "hardening" were required to bring the percentage of survival up to that of plants grown under the high moisture stress of treatment V.

Kelley et al. (*141*) pointed out that the fact that well-conditioned nursery stock will grow when transplanted at any season of the year is of great practical importance, not only for irrigated lands, but more particularly for "dryland" areas, where yearly precipitation occurs during certain months of the year. In such areas it would be highly desirable to use nursery stock that would initiate growth within the shortest possible time after transplanting.

They indicated other advantages in favor of growing nursery stock under high moisture stress. The amount of irrigation water is brought to a minimum, which in turn reduces the weed problem. Diseases associated with high soil moisture are more easily controlled. Plants grown under high moisture stress are smaller than those grown under low moisture stress, which makes them more suitable for holding over into the second year, should this be necessary.

Erickson and Smith (*91*) conducted field trials to determine the extent to which guayule could be transplanted throughout the year under irrigation conditions at Salinas, Calif. Using properly hardened nursery stock, they concluded that guayule could be successfully transplanted during any month of the year. They found that by withholding irrigation during the second season, nursery plants could be held over at practically constant size and in good condition for 1 year or more.

Tingey and Foote (*230*) found that irrigation immediately after transplanting resulted in the plants showing quick growth. If irrigation was delayed for 2 to 3 months, there was no decrease in the resulting field stand, but the season's growth was diminished by the slowness of renewed growth.

Physiological changes brought about in guayule as associated with moisture stress are discussed elsewhere in the present paper.

Handling and transplanting nursery stock. In preparation for transplanting, nursery seedlings were topped in place, undercut, pulled, packed in boxes or paper-lined crates, and delivered to the planting sites.

The seedlings were topped to the desired height by machines equipped with a sickle bar or a rotary cutter (fig. 8). Digging was accomplished by passing a tractor-drawn blade under the plants at a specified depth below the soil surface (fig. 9). In addition to securing uniform root length, undercutting facilitated pulling by loosening the soil about the plants (fig. 10).

Topping was done at various levels above the root collar. The experiments of Erickson and Smith (*91*) indicated that removing seven-eighths by height of the top (all but about 1 inch) was the best topping level (fig. 11). Smith (*203*) showed that, in addition to facilitating the handling of nursery plants, topping, with resulting defoliation, was essential for successful survival. Smith (*205*) found that the leaves of guayule contained an auxin (3-indoleacetic acid) which retarded growth when they were left on the plant. Erickson (*89*) found that when the relative humidity was high (95–100 percent) little or no defoliation was necessary in order to obtain rooting of guayule plants in water. Erickson and Smith showed that topping of nursery stock had no deleterious effects on size, shape, or rubber content of shrub. Rather, topping tended to result in plants with an increased number of small branches, and a higher bark-to-wood ratio for storage of rubber in larger amounts.

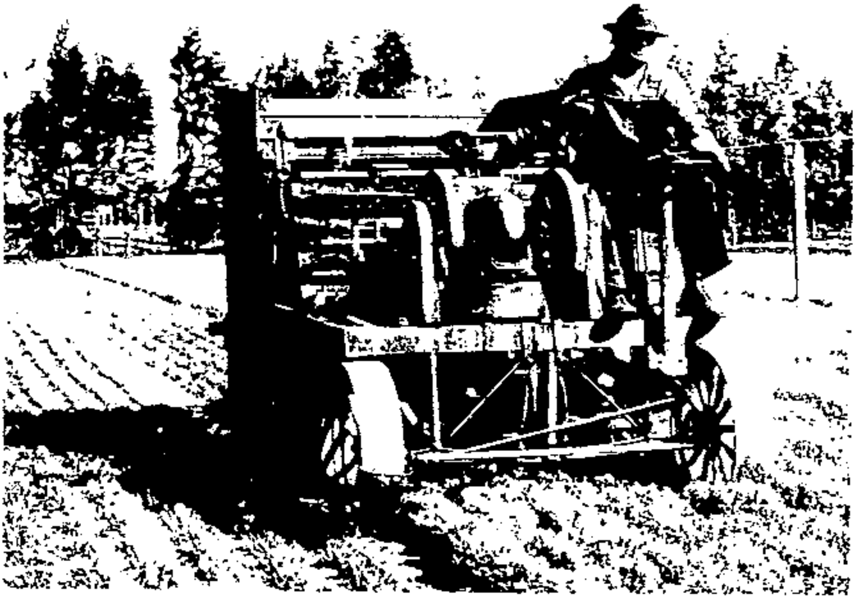


FIGURE 8.—Topping guayule nursery seedlings preparatory to transplanting. The clipped tops were collected within the machine for rubber extraction or disposal outside the nursery.

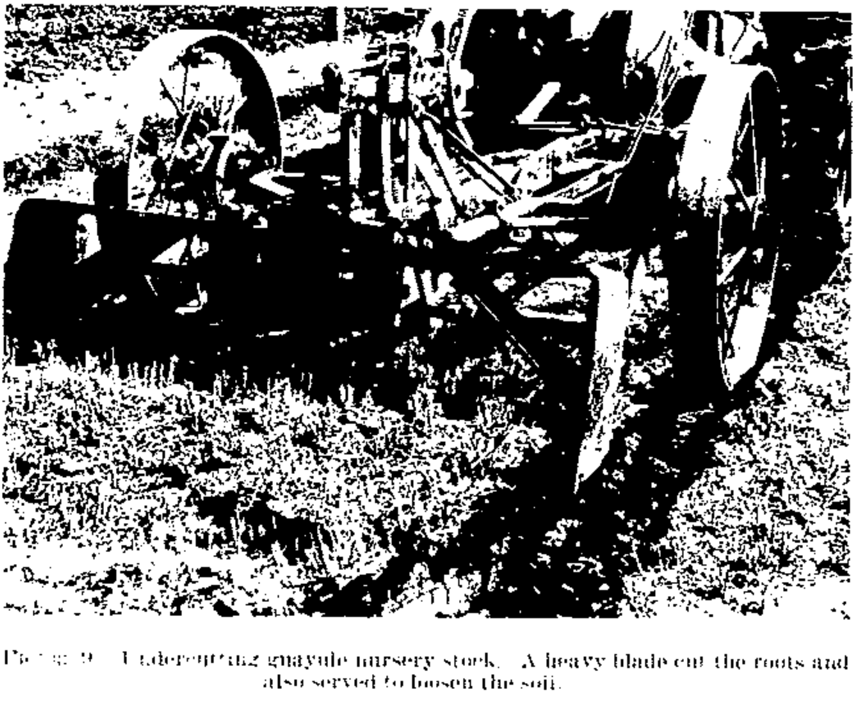


FIGURE 9.—Undercutting guayule nursery stock. A heavy blade cut the roots and also served to loosen the soil.

FIGURE 10.—Nursery crew removing undercut seedlings for transplanting to the field.

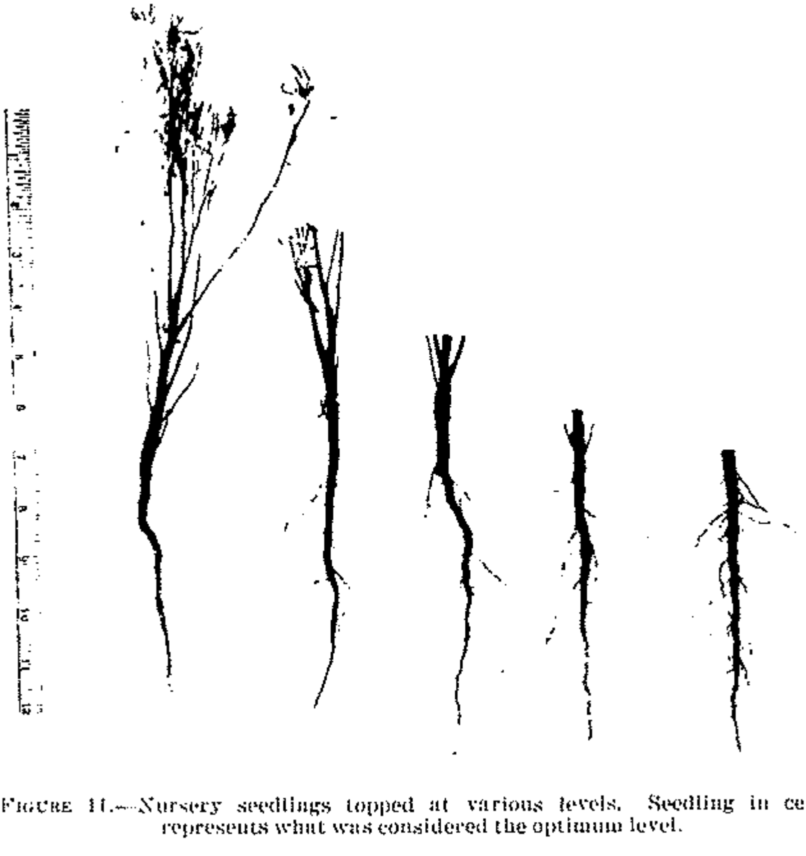


FIGURE 11.—Nursery seedlings topped at various levels. Seedling in center represents what was considered the optimum level.

Undercutting nursery stock 5 to 8 inches below the soil level was the usual practice. Erickson and Smith found that short roots, 5 inches or less, were less satisfactory from the standpoint of survival after transplanting than roots of about 7 inches.

Nursery plants most suitable for machine transplanting ranged from about 5⁄32 to 3⁄8 inch in root-crown diameter. Closely spaced plants, approximately 25 to 30 usable plants per square foot of nursery bed surface (the equivalent of approximately 21 linear inches of seed row), produced the most desirable type of stock for transplanting; these plants had less-bushy root systems, were more slender, less leafy and branched and, therefore, easier for the planters to handle than larger plants resulting from wide spacing. Erickson and Smith pointed out that the smallest plants in nursery stock should be discarded since these are likely to be aberrants or genetically slow growers. Their data indicated that size of transplants as such, within reasonable limits, did not result in shrub of greatly different size after a year's growth.

Nursery stock was usually undercut within as short an interval as possible before pulling. However, Erickson and Smith found that transplants tended to root better when they were topped approximately 3 days before digging rather than at the time of digging. The benefit of topping shortly before digging may have resulted from an increase in turgor in the taproot or the beginning of lateral-bud growth, which might increase the amount of root-promoting substances. The beneficial effects, were lost however, when topping preceded digging by more than 3 to 6 days.

Hardening by moisture stress or cold before undercutting and lifting resulted in the best survivals. Erickson and Smith (*91*) obtained a fair survival with partially hardened stock provided undercutting preceded topping 7 to 14 days before lifting. Six-month-old, partially hardened plants, undercut 7 days before lifting, resulted in 86.6 percent survival as compared to 30.0 percent for the controls. Undercutting 21 days before lifting gave no better results than the controls. Preundercut plants were more diffcult to top by mechanical means than intact plants. However, topping after undercutting was facilitated in two trials by giving at least one good watering to firm the soil. During warm weather, watering was also essential to prevent death of the plants.

Transplanting was done with a mechanical planter, such as the Holland celery transplanter or Kindorf planter. During the seed-stockpiling program initiated in 1951, the performances of the Lindeman and Holland transplanters were compared (*43*). The Lindeman transplanter (fig. 12), being equipped with rubber planting wheels, was found to be better adapted to a wider range in size of guayule plants. With the Holland transplanter, small plants often fell out of the holder before setting, and the large plants often wedged and locked the planter chain. The Holland transplanter required the operators to synchronize the feeding with the planting chain to avoid skips in the row, whereas with the Lindeman transplanter, the operators could vary the spacing at will within the row, thereby reducing the number of skips.

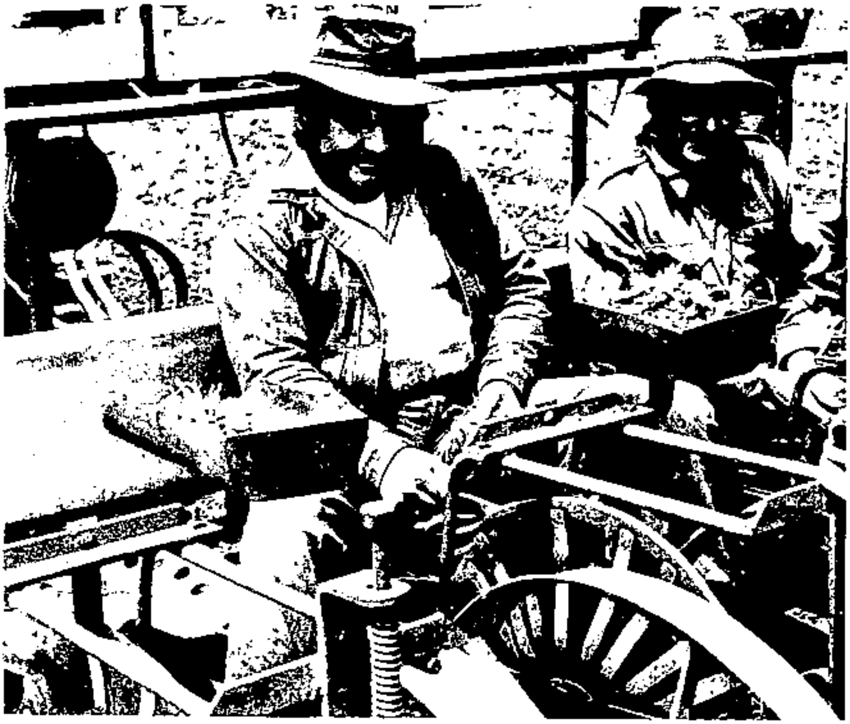


FIGURE 12.—The Lindeman transplanter. The rubber planting wheel is adaptable to a wide range of plant sizes.

Delayed transplanting, occasioned by adverse weather conditions, [illegible] or other cause, often resulted in losses of plants, largely because of excessive drying, sprouting, or attacks by fungi—especially *Sclerotinia* and *Botrytis*. Erickson and Smith (*91*) undertook a detailed and extensive study to determine methods of packing, shipping, and storing that would minimize losses of nursery stock. [illegible] factors contributing to losses were moisture for sprouting of plants, which occurred at 50° F. or above, and the rapid growth of storage fungi, which occurred at about 38° or above. There was little danger of excessive heating in well-hardened and relatively [illegible] stock, which survived best in transplanting. Surface-dry, [illegible] plants, without packing material, were found to survive several weeks in storage provided waxed paper was used to completely enclose [illegible] to reduce the loss of moisture. Topped guayule nursery stock in [illegible] hardened condition was found to recover from considerable [illegible].

Effect of spacing. The Intercontinental Rubber Co. chose a spacing of [illegible] 28 inches for transplanting guayule on dry land. Their [illegible] spacing produced the maximum tonnage [illegible] harvest between the fourth and seventh year after transplanting. The company also investigated closer spacings to determine maximum production of rubber within a shorter cycle of 2 to 3 years. Their experimental spacings ranged from 36 by 36 inches (4,840 plants per acre) to 36 by 6 inches (29,000 plants per acre). Olson (*171*),* in reviewing their data, which was somewhat fragmentary, was able to find definite indications that rubber yield could be substantially increased by the closer spacings, within a 3-year period.

For nonirrigated land, the Emergency Rubber Project adopted a spacing of 28 by 24 inches, since it was believed that closer spacings would not produce as high an ultimate yield of rubber because of competition for moisture (*186*). On irrigated land a closer spacing (28 by 20 inches) was used extensively in the belief that additional water would increase the tonnage at the end of 2 or 3 years, since more plants could be supported per unit of land surface.

In view of the need for producing the maximum yield of rubber in the shortest possible time, considerable experimental work on stand densities was undertaken to obtain more information on this important subject.

Reynolds (*185*)* reported the effect of spacing on shrub grown in numerous indicator plots established during the spring of 1942. At the end of the first season's growth, and at midsummer of the second year, there was a greater increase in yield of shrub and rate of rubber per acre in 12-inch rows than in either 24- or 30-inch rows. He felt that this condition might not necessarily exist in subsequent years because of a greater increase in crown volume and shrub weight in increased row spacing.

Davis (*79*),* in comparing rubber yields at the end of 21 months from plants grown at spacings of 28 by 20 inches and 28 by 10 inches in an experimental planting at Yuma, Ariz., noted a greater rate of yield of rubber per acre on plants spaced 10 inches apart than on plants spaced 20 inches apart. The grand average of plants given three levels of fertilization and three levels of irrigation was at the rate of 121 pounds of rubber per acre in the 10-inch spacing as compared to 101 pounds in the 20-inch spacing. Hilgeman (*124*),* in reporting his experimental findings on 1-year-old shrub at Gila Bend in the Salt River District of Arizona, found that 28- by 12-inch spacing produced at the rate of 210 pounds of rubber per acre as compared to a rate of 139 pounds for the 28- by 24-inch spacing. McAfee and Miller (*150*),* in an experimental planting at Edinburg, Tex., used spacings of 28 by 10 inches, 28 by 20 inches, and 28 by 40 inches, from which they obtained rates of 279, 197, and 105 pounds of rubber per acre, respectively, after 13 months of growth.

Guayule plantings for emergency production were discontinued in the latter part of 1943. A number of nurseries contained nursery stock that would no longer be needed. The possibility of harvesting such unused nurseries for rubber production lead Kelley et al. (*140*) to investigate and compare plant densities in relation to yield of rubber. The stands in these nurseries were sufficiently dense to permit thinning to almost any desired density.

Accordingly, exploratory experiments were established in the spring of 1944 at four nurseries in sourthern California, seeded in the spring of 1943. Two of these were in the hot interior valley near Indio.

The other two—the San Mateo nursery at San Clemente and the Carlsbad nursery at Oceanside—were within a half mile of the Pacific Ocean. Here, the temperatures and light intensity are lower and the relative humidity higher than in the Indio area, a condition more nearly approximately that of the Salinas Valley.

The plants in each nursery were grown in rows 7 inches apart with 7 rows per bed, the beds being spaced 56 inches from center to center. Before thinning to the desired stands, there were 10 to 20 plants per linear foot of row. By eliminating certain rows and thinning others, six densities were obtained, ranging from the original, unthinned stand to a spacing approximating 24 by 14 inches between hills, with two plants per hill. Moisture and fertility variables were also imposed in these experiments. The plots were sampled when the experiments were begun and again between 19 and 23 months from time of seeding.

At all four nurseries there was an increase in yield of rubber with an increase in number of plants per acre, the unthinned plots producing the highest yield. The average yield of rubber for all four nurseries ranged from 763 to 176 pounds per acre as the spacing was increased. The highest yield of rubber was obtained in unthinned plots at the San Mateo nursery. With light irrigation and no fertilizer, a rate of 1,336 pounds of rubber per acre was produced in 21 months from seed. This was aparently the highest yield of rubber ever reported for guayule for a comparable period of time. More commonly, rates of 250 to 400 pounds of rubber per acre per year were obtained from field plantings.

Kelley et al. (*140*) point out that the differences between spacings and yields might have been smaller had the stands been thinned shortly after seeding, and that the differences in yield of rubber as a result of variation in plant density might also have diminished had the experiment been continued over a longer period of time.

Tingey (*227*) designed an experiment in 1943 to determine the effect of spacing on rubber yield in guayule sown directly in the field. Seeding was done in rows spaced 28 and 14 inches apart. Two months after emergence, the 28-inch rows were thinned to within-the-row spacings of 20, 12, and 6 inches, some plots being left unthinned. The 14-inch rows were thinned to 12 and 6 inches, some plots being left unthinned. Prior to thinning there were approximately 11 plants per linear foot of row. Fertilizer and irrigation variables were also imposed.

In 21 months, the 14-inch, unthinned rows averaged a rate of 825 pounds of rubber per acre for all treatments, whereas the widest spacing of 28 by 20 inches (the standard spacing adopted by the Emergency Rubber Project) averaged a rate of 256 pounds of rubber per acre. After 33 months, the rubber yields of the 14-inch unthinned, and the 28- by 20-inch spacings averaged rates of 1,483 and 535 pounds of rubber per acre, respectively, the yields for the other spacings falling between these two extremes. The highest yield of rubber (a rate of 1,708 pounds per acre after 33 months) was obtained from the 14-inch, unthinned planting with light irrigation and no fertilizer.

Direct seeding of guayule. A major item of expense in the production of guayule rubber has been the maintenance and operation of nurseries and the establishment of field plantings by transplanting nursery seedlings. This cost, insofar as it relates to reestablishment of plantings, may be partially overcome by harvesting only the upper portion of the plants. The aboveground parts may be mowed at or near the surface of the ground, leaving the roots in the ground to start a new crop. This would eliminate the cost of nurseries and transplanting for reestablishment of these plantings. However, the initial planting would still require the transplating of nursery seedlings, and reestablishment of stands after one to several mowings would need to be by transplanting. Tingey and Clifford (*229*) found that at the end of 12 to 18 months the yield of shrub and rubber from unthinned guayule seeded directly in the field was little less than the yield from guayule planted 36 by 24 inches, or 28 by 20 inches, and pointed out that the saving in time by not going through the nursery stage would be material.

The planting of guayule could be greatly simplified and the cost reduced if seeds could be planted directly in the field. Several factors, however, have made it extremely difficult to obtain stands of guayule by direct seeding, except under the most favorable conditions. Guayule seed is highly light-sensitive and, in planting, usually cannot be covered more than ¼ inch without a decrease in the number of seedlings produced. It is not like lettuce or celery in this respect. Seeds of these crops can be covered as much as 1 inch without material loss of emergence. Such shallow cover makes it difficult to provide the constant moisture needed for germination of the guayule seed. In nurseries, this moisture can be supplied by overhead irrigation or by repeated surface irrigations. In field plantings, repeated irrigations to assure constant moisture are relatively expensive, even on land with irrigation facilities. In dryland farming, plantings would be limited to seasons when rainfall was adequate for seed germination and plant establishment.

Direct seedling of guayule in the field, as differentiated from close planting of the seeds in nurseries, for the production of seedlings for transplanting has been somewhat more successful than direct seeding for rubber production. Such planting is similar to direct seeding for rubber production, but is limited to areas equipped for irrigation. Close spacing of the plants in the row justifies the increased cost of irrigation to aid in establishment of the planting and to assure rapid growth of the seedlings. Extensive production of field-grown seedlings has proved superior to intensive nursery production and thus represents a step toward field planting of seed.

Direct seeding for field production of seedlings. In 1943, Tingey (*224,* 225,* 228**) developed a method of direct seeding of guayule in Greenfield and Bryan loams near Salinas, Calif., with promising results. His method consisted of planting seed on well-prepared seedbeds during the summer within the temperature ranges favorable for guayule—an average maximum of 71° and an average minimum of 53° F. The method of planting was similar to that for planting lettuce. A Planet Jr. seeder was mounted on a sledlike frame designed to cover two beds. Seed was drilled in rows 14 inches apart along the edges of irrigation furrows spaced 28 inches apart (fig. 5). The press wheels were offset to avoid packing the soil over the seed, which might result in crusting. Seed planted no deeper than ¼ inch gave the highest emergence.

At least two initial irrigations were required for good stands, and occasionally daily irrigations were required to maintain at the soil surface high moisture levels necessary for good emergence of the relatively slow-germinating and shallowly planted guayule seed. There was no difference in emergence from seeding in preirrigated moist soil and seeding in dry soil (*224*).*

Tingey (*225*)* showed that guayule and celery are more sensitive than lettuce to seeding depth and irrigation frequency. The frequency of irrigation was partly associated with the rate at which the three types of seed emerged. Good stands of lettuce could be obtained in 6 days, whereas a period of from 10 to 14 days was required for pregerminated guayule seed, and several days longer for dry guayule seed. Celery required from 17 to 20 days. In contrast to guayule, lettuce could be planted to a depth of 1 inch without showing differences in emergence. Celery, seeded in dry soil, was somewhat lower in emergence for the 1-inch depth.

Davis (*74*,* *76*,* *77*,* *78**) conducted further direct seeding experiments in the Salinas area to determine the effects of tillage and land preparation, irrigation practices, seeding on dryland, and the types of nursery beds best suited for field growing of transplants. Data in these experiments largely confirmed the findings of Tingey cited above.

Davis found that deep or shallow ground preparation for seedbeds did not affect emergence in initially poor areas, nor did it affect the number of seedlings emerging after different numbers of irrigations. Using various types of seedbeds and spacings, he succeeded in getting seedlings at high density for transplanting, although his experiments were designed primarily to determine the limits of spacing for use in nursery production of seedlings under furrow irrigation. Rows seeded 4 inches apart on the bed could be cultivated by machinery.

Various attempts at seeding on dryland usually resulted in failures. In the Salinas area, the temperatures during the rainy season are too low for good germination, and the summers are too dry when the temperatures are optimum for germination.

In view of frequent failures and inconsistency of results with direct seeding for the purpose of producing nursery transplants in the field at the Edinburg irrigated unit in the lower Rio Grande Valley of Texas, Cowley (*65*)* conducted a small experiment to determine if pregermination of seed was practical where irrigation water is available. The general practice in the area had been to plant dry seed, with two to four subsequent irrigations. Cowley planted both dry and pregerminated seed at a depth of ⅛ to ¼ inch on single-row beds 6 inches high and spaced 28 inches apart. Seeding was done on January 12, 1945, with a Planet Jr. planter, at the rate of 10 pounds per acre. The plot was row-irrigated immediately following the planting. One subsequent irrigation was applied on January 27, and 2.19 inches of rain fell between January 5 and February 13.

Counts were made on January 26 and February 7 and 24. These indicated that pregerminated seed was vastly superior to dry seed, and the emergence was far in excess of a field stand. The emergence from the dry seed did not take place until January 18 and continued for almost 2 months after the planting was made, whereas the emergence of the pregerminated seed was nearly complete when the first count was made. Cowley stated that the gradual increase in the emergence of the dry seed was possibly the result of gradual increase in the average temperatures during the test. Pregermination of seed apparently modified the influence of pre-emergence temperatures in the field.

Later in the spring, Cowley (*67*)* designed a small, exploratory test to determine the possibility of establishing nursery stock during the hot summer months in the Lower Rio Grande Valley by direct seeding in the field. This plot was seeded on May 25 at the rate of 5 pounds each of pregerminated and dry seed on small beds 17 inches apart and approximately 5 inches high. The initial irrigation was followed by eight additional irrigations over a period of 3 months, during which intermittent rainfall totaled 8.64 inches. The maximum temperatures during the germination period ranged from 93° to 98° F. Stand counts were made on June 16, July 2, and August 28. The initial emergence of the pregerminated seed was vastly superior to that of dry seed. Counts made on July 2 demonstrated that seedlings at the rate of 1,325,000 per acre could be established during the hot season. Losses of seedlings from June 16 to August 28 amounted to 41.3 percent from pregerminated seed and 52.2 percent from dry seed.

Taylor (*213*) mentions a later trial made by Cowley in July when the maximum temperatures ranged from 95° to 114° F., during the germination period. Poor results were obtained, and the stands were so uneven as to mask the effects of the treatments.

Taylor (*213*) summarized the experiments of Hilgeman designed for the production of nursery seedlings in the Salt River Valley in Arizona.

Experiments in the Salt River Valley were made on a rather heavy clay loam soil having a field moisture capacity of 21 to 35 percent and a wilting point of 10 to 15 percent. Problems of tilth in seedbed preparation and crusting during emergence in such soil apparently were not serious.

High summer, and low winter, temperatures limited the seasons feasible for stand establishment to spring and fall. However, fall seedings were found impractical because the temperatures after November 1 were too low for growth, and the young seedlings were highly susceptible to disease at this time. The most feasible sowing season was late March to late May. The maximum-minimum temperature limits for direct seeding in the Salt River Valley were given as 100° F. and 40° F., respectively.

Best stands were obtained by sowing threshed seed at a depth of ⅛ to $\frac{3}{16}$ inch with a loose soil cover in rows located on the shoulders of the seedbed 1 to 2 inches above the irrigation water level of the furrow. Irrigation was begun immediately after sowing and continued until the beds were saturated across from furrow to furrow, after which light irrigations were given at sufficient intervals to keep the soil moist until the stand was established. Irrigations were then reduced in frequency. It was observed that, when the primary leaves were appearing on the seedlings, the roots had penetrated to a depth of 8 to 10 inches. The surface soil could then be allowed to become dry, thus reducing damping-off diseases. Although this drying caused a loss of some of the weaker seedlings, the loss was less than that which occurred from disease at higher moisture levels. To compensate for post-emergence losses, a minimum sowing rate of 50 viable seeds per lineal foot was recommended.

To avoid the effects of alkali in seeding beds for the experimental production of nursery stock in the Mesilla Valley (Anthony) of New Mexico. Davis (*77*),* in the summer of 1944, devised a bed shaper which formed a sharp ridge of soil with narrow, level shelves on the two sides of the ridge about midway up the slope and about 3 inches above the bottom of the irrigation furrow. The seed was sown on these shelves, the drill rows being 10 inches apart on the ridge and 18 inches apart across the irrigation furrows. Thus, the water "subbed" up to the seed and the wicklike action of the soil caused a concentration of the salts along the crest of the ridge. From seeding 32, 64, and 128 pounds of seed per acre, production rates of 513,701, 1,163,923, and 2,617,958 seedlings per acre, respectively, were obtained.

Wartime experimental work thus indicated the practicability of growing nursery stock under furrow irrigation in the field, eliminating the expensive overhead irrigation practices inherited from the Intercontinental Rubber Company. The growing of nursery stock on a large scale under furrow irrigation was demonstrated in 1948 in plantings established at Salinas and at Shafter, Calif. (*8*). This material reduction in cost of nursery plants reduced the margin between transplanting and direct seeding to the point where the advantages of direct seeding were less obvious than they had appeared earlier (*7*). Furthermore, developments in transplanting machinery permitting the spacing of nursery stock as close as 4 to 6 inches apart in the row removed the difficulties of securing stands of any desired density.

In 1948, Hunter (*127*)* established at Salinas approximately 2 acres of guayle planting for the purpose of determining the practicability of growing nursery stock by furrow irrigation. On June 2, two rows, 12 inches apart were seeded approximately ½ inch deep on low ridges spaced 28 inches apart. Seeding was done with tractor-mounted Planet Jr. seeders, at the rate of 6 pounds per acre of dry, threshed seed of variety 593 (germinability about 45 percent). Daily irrigations were given during the first 6 days to insure germination. Weeds were largely controlled by oil sprays. Stand counts during the fall indicated an average of 10.7 transplantable seedlings per linear foot of row, the equivalent of approximately 400,000 plants per acre.

The stock from this nursery was used in January transplanting trials at Shafter, Calif. and at Dilley, Tex. The unused portion of the nursery was held over in 1949 as reserve planting material (*9*). After 2 years of growth, it was still of satisfactory size for transplanting. The cost of maintaining this nursery throughout the second year was small. Irrigation was not required and only two cultivations were necessary. No hand labor was involved.

Satisfactory results were also obtained in a furrow-irrigated, nursery-seeding experiment involving approximately 1 acre at Shafter, Calif. in late May of 1948 (*127*).* Seeding at the rate of 3 pounds of threshed seed per acre was done with Planet Jr. planters at a depth of ¼ to ½ inch. Single rows were planted on ridges 30 inches apart. Three furrow irrigations were given during the first week following seeding, and further irrigations were given as needed. Stand counts made in October on unthinned plots indicated an approximate rate of 240,000 transplantable plants per acre. It was estimated that, by planting two rows instead of one on the ridge (thus doubling the seeding rate), well over twice this number of plants might have been produced.

Preliminary nursery seeding studies were initiated on approximately ½ acre of irrigated land assigned to the U.S. Department of Agriculture by the Texas Agricultural Experiment Station at Winter Haven, Tex., in the fall of 1948 (*127*).* Biweekly plantings were made from September to December and less frequently until March. Variables under study included date of seeding, depth of seeding, and frequency of irrigation. All plantings were made on ridges 16 inches apart, with furrow irrigation. Some good stands were obtained in the November and December plantings. Densities of 8 to 40 plants per lineal foot were obtained where two or three irrigations were given and dry, threshed seed planted at less than ½ inch deep at rates of 3 to 9 pounds per acre. A low temperature on the night of January 30 killed all plants except some of those in the September and October plantings.

Test plantings for the production of nursery stock were continued every month at rates of 6 and 9 pounds of seed per acre, with two or three irrigations (*9*). Summer plantings were not successful. January plantings were spotty. Good stands were obtained in the other months.

During the spring and summer of 1951, the Guayule Seedling Stockpiling Project (*45*)* seeded 1,155 acres of field nurseries with row spacing varying from one row 16 to 20 inches apart on 16- to 20-inch beds to two rows 12 inches apart on 36- to 40-inch beds, depending upon the available equipment of the farms where land was leased. Some 637 acres had to be abandoned before the end of the year because of adverse factors, including washing of seeded beds by heavy rains, damage by hail, excessive temperatures during summer plantings, weeds resistant to selective oil sprays, and poor soil types. Some soils were not suitable for subirrigation, and it was impossible to retain stands on clay soils.

Direct seeding for rubber production. In the fall of 1943 and spring of 1944, Hansen (*121*)* made direct seeding trials in the Bakersfield area of California. The design and results of these experiments were briefly summarized as follows:

"Direct seeding trials were made at Wasco and Lamont in the Bakersfield District in the fall of 1943 and the spring of 1944, to compare with nursery propagation and transplanting for effectiveness in field production of guayule. The trials were made on representative soils, a loamy fine sand at Lamont and a sandy loam at Wasco. The trial sites were preirrigated to bring the soils to field capacity. Threshed dry seed and unthreshed pre-germinated seed were used, and the irrigation treatments following sowing were varied, as was depth of sowing. Emergence was general regardless of treatment; but was best where sowing was shallow and the covering uniform.

"Weeds presented quite a problem, as they grew faster than the guayule seedlings. Hand weeding was slow and very expensive, and machine cultivation difficult to accomplish without damaging the seedlings. Weak oil sprays gave fair weed control and minimum damage. Wind storms caused damage to the fall plantings, and severe rainstorms, to the spring plantings. Damping-off affected the spring plantings seriously in humid weather.

"Irrigation almost daily from sowing to emergence was found necessary to get a good stand. At least five irrigations in 30 days were required for establishment. Under the local conditions, preirrigation was also a necessity.

"The plantings on the lightest soils were considered failures. Water facilities were critical. As to costs, the establishment of the direct seedlings is far more expensive than the establishment of transplants on irrigated lands."

Direct seeding trials in the Indio area of California were considerably more successful than those made in the Bakersfield District. Following preliminary seeding trials made by Clifford *(62)** at the U.S. Date Garden, Indio, Calif., Haise *(116)** made plantings on 24 acres of newly reclaimed desert land on the Bell Ranch north of Indio. The soil type was classified as "Coachella Sand" and the surface soil consisted of a heterogeneous mixture of dune sand and poorly pulverized silt and clay clods. Organic matter content was extremely low.

Periodic plantings were made between September 29 and October 31, 1943, both pregerminated and dry seed being used. To satisfy one of the requirements of the experiment, since it was designed to test the feasibility of growing guayule as a row crop by direct seeding, the plants had to be thick enough to block out a spacing of 7 inches within the row. Seeding was done with a Planet Jr. seeder at a maximum rate of 15 pounds per acre. Side dressings of nitrogen fertilizer at the rate of 250 pounds per acre, which later proved to be beneficial, were applied. Following an initial irrigation, it was found necessary to irrigate at least every other day to a total of six irrigations.

Observations were made when the seedlings became well established. Satisfactory emergence was obtained from seedings made between September 29 and October 22 in spite of high soil and air temperatures, which often reached 110° F. Good emergence was apparent for pregerminated seed after 3 or 4 days and from 5 to 7 days for dry seed. Sowings made on October 30 and 31 resulted in a very poor stand, which was later abandoned. The failure was attributed largely to the low soil temperatures which dropped to somewhat below 50° F. Some of the stands were considerably damaged by birds.

Haise concluded that dry seed was as satisfactory as pregerminated seed and that it could have been sown at a much lower rate than the 15 pounds per acre used, and that the sowing should be made from $\frac{1}{4}$ to $\frac{1}{2}$ inch deep with daily irrigations until germination is complete. In the Indio area, from the standpoint of winds and frequency and intensity of storms, the best time to plant is in the fall of the year rather than in the spring. Successful stands are doubtful when minimum soil temperatures fall below 50° F.

According to Cowley *(68)**, earlier results indicating that direct field seeding was not practical in Texas were in agreement with results obtained in the planting season of 1943–44, in which direct seeding trials on irrigated land were erratic and those on dryland areas failed.

He attributed these failures to climatic conditions throughout the year in southern Texas that were generally unfavorable for the establishment of guayule seedlings, particularly on dry land. The rainy periods around the months of May and September are also characterized by high temperatures and resulting high evaporation losses, and heavy weed infestation normally occurring at this time. Weed competition together with heat injury seemed to be the adverse factors for summer planting. Although temperatures are lower during the winter, this period is characterized by scanty rainfall and high winds which tend to intensify the moisture deficiency.

Cowley was able to overcome these adverse climatic conditions by clean fallowing during the spring, summer, and fall months of 1944 to conserve a maximum of the water that fell during the rainy periods. In November, there was an excellent supply of stored moisture to within 2 inches of the soil surface, and small exploratory tests were initiated and continued throughout the winter months. A modified planting technique was used, involving the drilling of the seed at various depths in the bottom of furrows of various widths made with lister shovels. Best stands were obtained from pregerminated seed at a depth of ¼ inch in the bottom of narrow, clean furrows from 2½ to 3 inches deep. In one trial he obtained approximately 10 plants per foot of row with 8 pounds of seed per acre. The stands in general were somewhat irregular because of the difficulty in maintaining a uniform shallow soil cover.

Cowley *(64)** found that planting in a water furrow was not practical under irrigation, owing to the lack of control of the water, which washed soil into the furrow, covering the seed to an excessive depth.

Taylor *(213)* summarized the experimental work of Davis for the establishment of field stands in the Yuma Mesa and Poston areas of Arizona.

The Yuma Mesa occupies a strip of true desert land hummocky, wind-blown sand from 3 to 7 miles wide along the Colorado River south of the Gila River. The principal limiting factors in this area were excessively high temperatures, sandstorms, and difficulties in maintaining moisture during germination.

The seasons which gave the only promise for successful direct seeding were a fall period from September 20 to November 15 and an early spring period between February 1 and April 15, when the maximum temperature limit of 90° F. was not ordinarily exceeded. Even then, the instability of the sand made the handling of irrigation water without eroding or flooding the seeded rows, difficult. In general, stands obtained from direct seeding at the Yuma Mesa Station were reported to be better than those from transplanting at any time.

On heavier soils at Poston, Ariz., a direct seeding trial, made in October, yielded an average uniform stand of about 10 seedlings per linear foot. Stands of 98 to 99 percent were obtained in plots where the seedlings were thinned to spacings of 6, 12, and 16 inches. By the following April, these plants were about 4½ inches tall, while those seeded at the same time at the Yuma Station ranged from 2 to 7 inches in height.

Taylor *(213)* also summarized the direct seeding trials by Crain and Davis *(70)** for field production of guayule in the Mesilla Valley (Anthony) of New Mexico.

The soil of the tract used for these trials was Gila silt loam, moderately to very alkaline. Temperatures were too low for direct seeding in winter or for survival of seedlings from late fall plantings. Summer temperatures were not excessive. Crain, after having investigated many of the sowing techniques employed in other areas, succeeded in obtaining stands only by sowing seed in the bottom of irrigation furrows. At first, this method did not appear practical because of scouring in some places and silting in others. He was able to overcome these obstacles by seeding at a depth of ⅛ to ¼ inch, or just enough to prevent the seed from washing away. By controlling the water to minimize scouring and silting, good stands were obtained on 12 acres seeded in the month of September.

Upon resumption of the guayule experimental work after the liquidation of the wartime Emergency Rubber Project by the U.S. Department of Agriculture in August 1947, interest was again centered on direct seeding. Experiments were established at Salinas and Shafter, Calif., and at Winter Haven, Tex., to determine the feasibility of direct seeding as a means of quickly and cheaply establishing field stands, as well as field nurseries. Earlier investigations (*7*) had shown that direct seeding was feasible under some conditions, but its general usefulness had not been demonstrated.

Earlier work had also indicated that closer spacing in the row yielded more rubber per acre for short-cycle production than wider spacing. The planters available at the time permitted 18-inch spacing which was about the closest that could be obtained. Therefore, attention was directed to the possibility of establishing dense direct seedings which could be thinned to the desired spacing, thus eliminating the heavy expense of filling in by hand. Direct seeding, however, presented difficulties because of special conditions required for the germination of the seed, the lack of vigor of the young seedlings, and the need for special or hand care in thinning.

During the summer of 1948, Hunter (*127*)* planted approximately 12 acres by direct seeding under row irrigation, for the purpose of establishing field stands for thinning to desired spacings. Two-thirds of the area was planted with one row on 28-inch ridges and one-third was planted with two rows, 12 inches apart, on similar ridges. The stands of guayule plants were among the best ever obtained by direct seeding.

In the fall of 1949, studies of direct seeding on dry land were begun on approximately 4 acres of non-irrigated Webb fine sandy loam assigned by the Texas Agricultural Experiment Station at Winter Haven, Tex. Variables included rate of seeding, depth of seeding, furrow, flat, and ridge planting, presence and absence of windbreaks, and covering material for seeds such as soil, wood shavings, and sand. Beginning October 7, five monthly plantings were made. In November a wind completely destroyed the October planting by covering or abrading the plants with sand. Stands obtained from the November and December seedings were killed by freezing on January 30. The planting made on January 10 had not emerged on January 30 and was apparently destroyed by low temperatures before emergence.

Further tests of direct seeding on dryland in Texas continued to give poor and uncertain stands regardless of the different rates of seeding, different methods of seedbed preparation, and different methods of covering the seed (*9*). Crusting of the soil and variable weather conditions appeared to make direct seeding on dryland very hazardous. It was concluded that transplanting of seedlings from irrigated nurseries is the only feasible means of planting dry land areas in southwest Texas.

Direct seeding in wastelands. Direct seeding of wastelands, either for the reestablishment and enrichment of native stands, or the establishment of guayule for the production of a reserve supply of rubber for emergency use, has also been attempted, but without significant success. This effort has included the sowing of improved strains of guayule in native stands, airplane sowing of guayule in brush lands, and hand sowing of guayule seed with or without some preparation of the soil surface before planting. In several instances, the weather conditions proved almost ideal for the initial establishment of the seedlings. However, even good initial stands of young seedlings failed to survive the plant competition and drought that followed.

McCallum (*153*), in 1929 and in the following several years, made serious efforts to reestablish guayule in Mexico. Approximately 500 pounds of seed were scattered in various places over cleared-off ground, a portion of which was harrowed to cover the seed. The results obtained were from nothing to an average of two or three seedlings per square yard. By the end of the first summer, most of these were gone, and the remainder died from subsequent drouths. Best initial results were obtained on good valley soil in a cooler climate where 50 acres were sown. Here the entire stand averaged 10 to 15 plants per square yard and grew well during the first year and following summer, but all were killed by frost during the next winter.

Attempts at direct seeding at various places in Texas also failed. Seedlings came up only on prepared land, cleared and plowed, and these were unable to compete with the weeds and grass that followed the first rains. In his account, Dr. McCallum stated that "in the end we did not have a single plant that had grown from the seed we sowed."

In more recent years, emphasis was placed on tests to determine the possibility of establishing stands on wastelands to serve as living reserves of rubber (*9*). In December 1948, an airplane seeding test was made on 80 acres, including four types of terrain and cover, on the reservation at Camp Pendleton, near Oceanside, Calif. In the spring of 1949, a few seedlings were found, but in November 1949, no seedlings were observed in the entire area.

In February and March 1950, wasteland plantings of both broadcast seed and nursery stock were made in each of the following unused Federal land areas in California: Los Padres National Forest, near Pozo; Fort Ord, near Monterey; Camp Pendleton, near Oceanside; Camp Roberts, near Paso Robles; and San Joaquin Experimental Range, near O'Neals (*10*). Of these five locations, only the Los Padres National Forest and Fort Ord plantings survived the summer, both transplants and seedlings appearing well established in disked areas at Fort Ord in the fall of 1950. By the summer of 1951, however, the grass and brush competition, destroyed by the disking, had become reestablished and had crowded out most of the guayule. The remaining few plants appeared to be dying. In the

Camp Roberts and San Joaquin areas, disking helped to get the seedlings started, but these did not survive the summer.

Since the spring of 1950 offered favorable growing conditions, the failure of these trials indicated the unlikelihood that guayule seedlings or transplants could establish themselves well enough to survive the competition with brush and grass under the dry summer conditions in California (*12*).

Eight trial plantings, involving both seed and transplants, were made in March 1950 on uncultivated rangeland under different soil and grazing conditions at the Texas Agricultural Experiment Station, Sonora, Tex. Observations were made in the spring of 1951. Despite favorable rainfall and good initial stands, survival was very sparse, owing to grazing and grass competition (*10*, *11*). The pelleted seed used in these experiments did not prove superior to unpelleted threshed seed, and in most cases it was inferior.

Many of those who visited native stands of guayule during, or shortly after, rainy seasons observed thousands of young seedlings which disappeared during subsequent periods of drought. Established plants in native stands often appeared alike in size or age, leading one to a possible conclusion that the stand had become established as a result of a fortuitous period of moisture together with other favorable conditions sufficiently prolonged to allow the plants to become established.

Figure 13, a photograph taken in 1948 by Hammond (*119*) during a seed-collecting trip in Mexico, is a view of a dense stand of young guayule, approximately 4 to 6 inches tall, established at an old guayule baling campsite in the State of Zacatecas, presumably from seed brought in on harvested guayule. Unharvested guayule plants were found in the area of this site. Of a number of observed baling sites operated by rubber companies during World War II, this was the only one where established seedlings were seen. Apparently here, an ideal set of growing conditions prevailed over a period of time. Ecological studies could add materially to the understanding of how guayule establishes itself in its native habitat.

Weed control

Weed control in nurseries. The control of weeds in nurseries is one of the most expensive operations in the growing of guayule. This is partly because of the small size and tenderness of the seedlings for the first few weeks following emergence, which make them unable to compete with the more aggressive, or faster growing weeds. The seedlings are easily damaged or pulled up in weeding if the intermingled weeds are allowed to develop many lateral roots before they are removed.

Hand weeding, the only method of weed control used by the Intercontinental Rubber Co., was not a serious problem in small nurseries such as those owned by the company. However, the weeding of 540 acres of nursery of the Emergency Rubber Project in the spring of 1942 presented such a serious situation that it was necessary to consider whether further expansion of nursery plantings should be made if the resulting seedlings were to die in competition with weeds (*186*).

[illegible] 13.—Dense stand of young guayule established at an old baling campsite in the State of Zacatecas, Mexico.

[illegible] a tremendous recruiting job was begun, and sufficient [illegible] was finally obtained to control the weeds and expand [illegible] plantings.

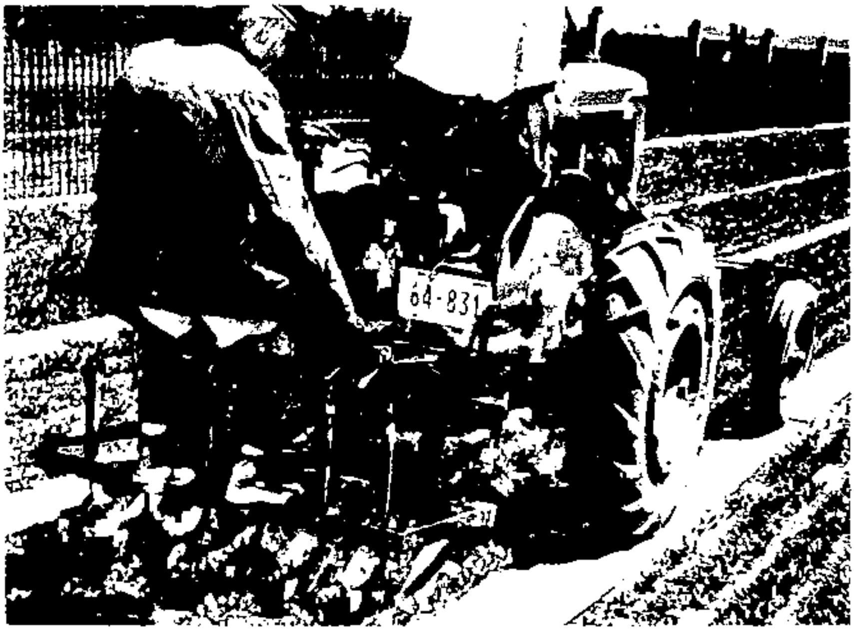
[illegible] mechanical weeding devices were developed [illegible] to cultivate the seedbeds between the seeded bands [illegible] and the use of oil spray as a selective weed killer was [illegible] effort to reduce costs and labor requirements. As a [illegible] were required in the weeding operation of [illegible] acres requiring [illegible] workers at the peak of the [illegible]

[illegible] sprays, used quite extensively for controlling weeds [illegible] crops, such as onions and carrots, were thor[illegible] [illegible] The earlier results from trial and error meth[illegible] [illegible] on guayule were fairly successful but sometimes [illegible] found that uncontrolled weed growth [illegible] plantings of [illegible] acres resulted in the loss of 50 percent [illegible] plants and a reduction of [illegible] percent in the rate of [illegible] stove oil sprays and undertook a detailed study [illegible] effects of stove oil on guayule and weeds. In his [illegible] experiments he used a thoroughly agitated mixture of 3 parts

FIGURE 14.—Hand weeding from carts in nursery beds. The carts relieved strain and facilitated weeding in the center of the beds.

water to 1 part oil by volume at the rate of 6 gallons of mix (or 1.5 gallons of oil) per bed 4 by 100 feet. The amount of oil applied was a little greater than that normally used in the nurseries so as to insure some injury to the guayule to determine differences in the resistance of seedlings of different ages, if such existed. In oiling nursery plots, the spray was applied from a 150-gallon Bean sprayer, at a pressure of 200 pounds per square inch through fan-shaped nozzles (fig. 16). The speed of the tractor controlled the amount of oil applied.

Benedict's results indicated that oiling at the rate used in these experiments resulted in a *kill of 14 to 55* percent of the guayule seedlings when they were 2 weeks old, but that oiling at the age of 4 weeks or more did not result in any killing. The greatest kill of weeds *occurred when they were oiled 2 weeks after seeding.* A considerable kill was also obtained 4 weeks after seeding. Oiling 2 weeks after seeding resulted in a 72- to 92-percent reduction in weeds. Two oilings at 2 and 4 weeks resulted in a total reduction from 86 to 98 percent, respectively, a point from which the weed population did not increase substantially during the remainder of the season (fig. 17). Oil-*treated guayule plants were retarded somewhat in growth.* However, if oil was applied in the early part of season, the plants made as much growth by the end of the season as the untreated plants in hand-weeded plots. Addicott *(3)* studied and described the injuries to guayule seedlings that resulted from oil sprays.



[illegible] Rear view of special cultivator developed by the Emergency Rubber [illegible] cutting [illegible] of soil between rows of guayule seedlings [illegible].

[illegible] were also designed to determine the effects of temperature [illegible]. Oiling tests were conducted on four dates in [illegible] of guayule seedlings with an intermixture of [illegible] relationship between [illegible] and temperature [illegible] at the time of oiling, but with the minimum [illegible] prior to oiling. The highest weed [illegible] number of guayule seedlings killed occurred when

[illegible] sprayer used for weed control in guayule nursery beds.

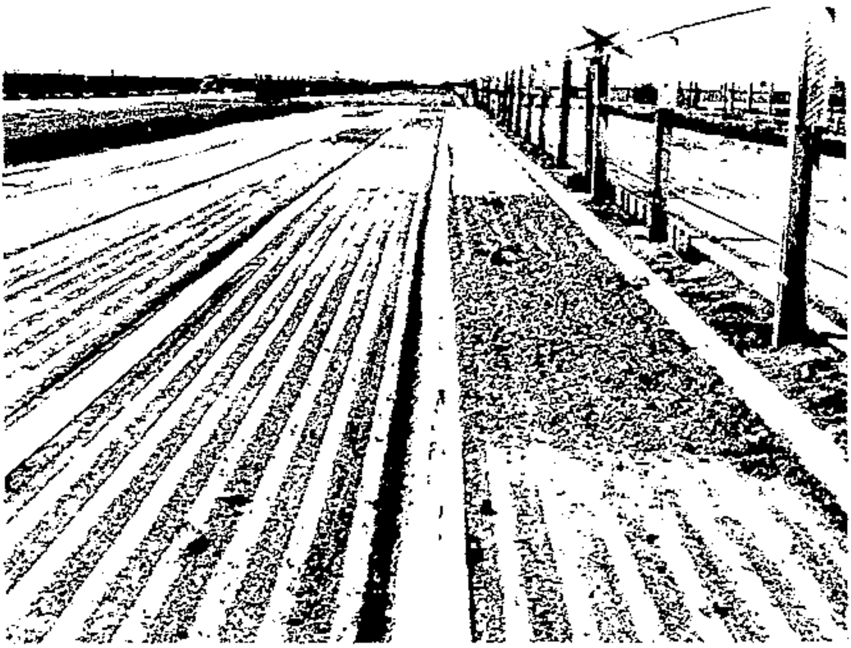


FIGURE 17.—Guayule nursery beds that have been treated with oil for weed control. Untreated check plots are crowded with weeds and elsewhere the guayule seedlings can be seen in the sand-covered planting strips.

the beds were oiled following a minimum temperature average of over 50° F. The poorest weed kill and the greatest injury to guayule seedlings occurred when oil was applied following a period during which the minimum temperatures averaged below 50° F.

From the standpoint of guayule, Benedict (*25*) concluded that oiling should not be done until 4 weeks after planting since practically no killing resulted after that time. However, from the standpoint of weed kill, oiling 2 weeks after the seed beds were prepared gave the best results. Therefore, he suggested preparing the seedbeds 2 weeks before planting time, irrigating to promote weed growth, and then, the day the beds were to be planted, spraying the beds with oil to kill the weeds, after which seeding should be done without disturbing the seed bed. Following these procedures, weed control would not be necessary until at least 4 weeks after seeding when the guayule seedlings would be old enough to withstand the oiling.

Results of pre-killing of weeds by oil in the manner recommended above were reported later by Benedict and Krofchek (*31*) and found successful. In fact, when the beds were prepared 2 weeks before seeding and then oiled, they obtained better stands of guayule than when seedings were made in freshly prepared beds. They attributed the increased emergence to the firm bed of soil on which the seed was placed.

In 1944, they used a lighter application of oil than was used in their other experiments with the result that there was no killing of even 2-week-old guayule seedlings. The spray was a mixture of three-fourths stove oil and one-fourth diesel oil, applied at the rate of 1.2 gallons per nursery bed 4 by 400 feet. It was necessary to make the first application about 2 weeks after seeding when both the weeds and guayule were in the cotyledonary stage. At this time, both were susceptible to the oil, but guayule was much more resistant. As a result, there was an almost complete killing of the weeds with only a temporary set-back in the growth of the guayule. In their later tests, three or four applications of oil during the summer were necessary to give good weed control.

Greenhouse tests indicated that guayule seedlings should be oil-sprayed at air temperatures between 70° and 80° F. Temperatures of 50° and 94° F. at the time of oil applications, resulted in the death of a great many plants.

All of their results with selective oil sprays indicated that weeds 2 weeks old were most easily killed. Some weeds 4 weeks old were killed, but weeds 6 weeks old usually were not killed. They found that certain weeds, after the development of the first true leaf, were impossible to kill with the strength of oil used. Among these oil-resistant weeds were common groundsel (*Senecio vulgaris* L.), scarlet pimpernel (*Anagallis arvensis* L.), and certain species of grasses.

Taylor and Champagne (*216*) furnished detailed working instructions on the use of oil sprays and types of oil rigs for weed control in guayule nurseries in their nursery handbook.

Selective oils for weed control were extensively used in Zavala and Maverick Counties, Tex., by the Guayule Seedling Stockpiling Project, where 1,166 acres of field nurseries were seeded in 1951 (*45*).* Varsol Naptha (Humbolt Oil Co.) was selected as the best of the local oils available. It was applied at the rate of 30 to 40 gallons per acre. Just as Benedict (*25*) had demonstrated earlier in California, guayule subjected to minimum night temperatures of 50° F. or below for 5 days prior to oiling was quite susceptible to oil injury. Continuous high temperatures, 100° F. and above, produced guayule seedings highly susceptible to oil injury. There was a survival of 97 percent in one planting of 23-day-old guayule oiled with 30 gallons of oil per acre when the temperature stood at 90° to 92° F. Recommendations were that oil should be applied only when the temperatures were 60° to 90° F.

Pre-emergence treatments, i.e., the application of oils immediately after sowing guayule in newly formed beds, did not retard weed emergence satisfactorily without killing the germinating guayule.

Weeds found resistant to selective oils at all stages of growth in Texas were drug fumitory (*Fumaria officinalis* L.), London rocket (*Sisymbrium irio* L.), common sunflower (*Helianthus annuus* L.), puncturevine (*Tribulus terrestris* L.), and annual and perennial species of ragweed (*Ambrosia*). Weeds susceptible at or near the cotyledon stage but resistant thereafter were redroot pigweed (*Amaranthus retroflexus* L.), common purslane (*Portulaca oleracea* L.), barnyardgrass (*Echinochloa crusgalli frumentacea* (Roxb.) Wight.), Texas millet (*Panicum texanum* Buckl.), and red deadnettle (*Lamium purpureum* L.). Seedlings of johnsongrass (*Sorghum halepense* (L.) Pers.) were susceptible to the oil, but the rootstock was not. This was the most troublesome weed in the nursery fields. A 52-acre field had to be abandoned entirely because of a heavy infestation of johnsongrass.

Weed control in plantations. The methods of guayule cultivation, being essentially the same as those employed in any row crop, permitted the use of standard cultivating implements (*186*). Cultivation tools, such as shovels, sweeps, et cetera, varied considerably according to the size and age of the plants and in accordance with local ground conditions.

The finding that petroleum oil sprays could be used successfully in controlling weeds in nursery plantings without serious injury to guayule led to investigations of its use in field plantations, with the result that in the spring of 1943 oiling was begun as a major part of weed-control operations. Lighter grades of diesel or stove oil were used. Further investigations in cooperation with the University of California resulted in the use of straight oil in amounts previously used in oil sprays but without mixture with water. In the spring of 1944, Freeman (*101*)* reported oil injury on new succulent growth of guayule planting. He partially solved the problem through a combination of diesel oil and stove oil.

Sprayers were developed with tanks mounted integrally on small crawler tractors capable of spraying four rows at a time. The crawler-type tractor had the advantage of being able to operate during the rainy seasons in California when weed growth was most active and when the soil was too wet to permit the use of cultivating implements or hand hoeing crews.

The removal of weeds not killed by oil within the row was one of the major problems in cultivation. The scope of the planting program required the use of mechanical planters which did not permit "checking" to facilitate cross cultivating. Hence, hand hoeing was the only method of removing weeds in the row. In 1944 an "in-the-row-cultivator" was developed by the project (fig. 18), consisting of a series of four to six units of small blades in pairs, each manually controlled by a pair of handles so that plants in the row could be dodged (*186*). The series of units was tractor-drawn, each unit being operated by a man on foot. This method of cultivating eliminated the need for hand hoeing to the extent that the output per man-day exceeded that by hand hoeing by 300 to 600 percent.

More recent experiments on weed control (*46*)* showed that the Ferguson Pencil Weeder, when used frequently and at the right time, would destroy young seedlings without harming newly transplanted guayule. It was also used successfully on larger plants. A second innovation which gave promising results was the use of overlapping sweep cultivator shovels on large, established guayule. These shovels slightly overlapped in the row, but the guayule was usually sufficiently tough to spread the shovels as they passed.

Harvesting for rubber

Harvesting by digging. In harvesting cultivated guayule, the Intercontinental Rubber Co. followed the practice of digging the shrub with a specially designed, two-row digger mounted integrally on the

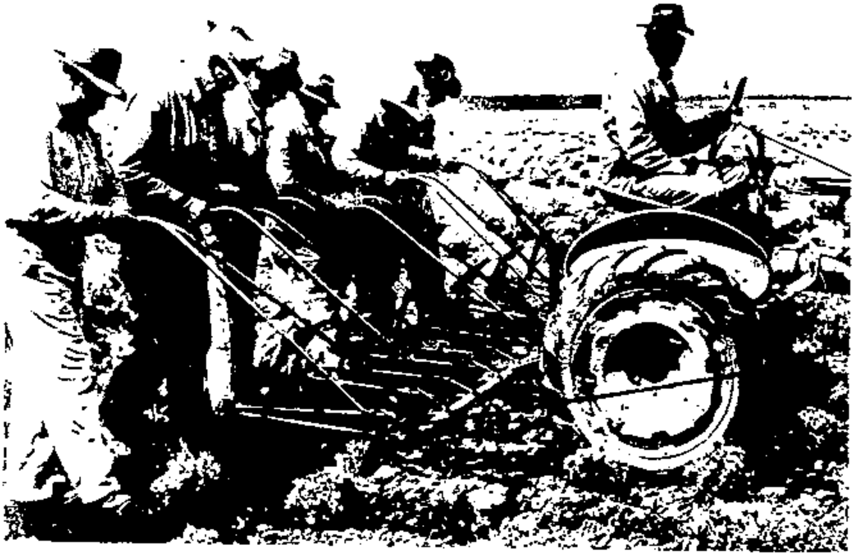


FIGURE 18.—Mechanical hoe for controlling weeds in guayule plantings. The handles could be manipulated to avoid the guayule plants while the rows were being cultivated.

front of a crawler tractor (*186*). The shrub was bunched by the digger into windrows, each containing two rows of plants. After drying for 3 to 5 days, the shrub was gathered with a special heavy-duty, side-delivery rake into larger windrows, which were rolled with a cultipacker to dislodge dirt from the roots not removed in the digging operation. The windrows were turned a second time and rolled again to remove additional clods or dirt. A special heavy-duty chopper, on the order of a portable ensilage cutter and equipped with a pickup attachment, was used to chop the shrub. From this machine, the chopped shrub was delivered by a blower fan and overhead pipe conduit into tight wooden bins mounted on trucks, at [illegible] auled to the mill where it was stored in large bins for later milling.

The Emergency Rubber Project discarded the practice of chopping the shrub in the field as impractical and adopted a method of digging the shrub and then baling it with standard baling equipment after certain modifications were made in its design (figs. 19, 20). This method greatly facilitated shipping the shrub to the mill by trucks, or over long distances by rail without deterioration. The baled shrub was stored either in the field or at the mill. These basic methods were followed in the 1942 harvesting operation and, with some modifications, they were used by the project in all later operations.

Harvesting by clipping (pollarding). Lloyd (*145*) suggested harvesting guayule by removing the upper portions of the plant and leaving the crown to generate new growth instead of digging the entire plant, as was customary. The first clipping trials by the Intercontinental Rubber Co. were inconclusive, and the company did not adopt this method of harvesting. Caldwell and Spence (*57*)[9] indicated that its usefulness made it worthy of further investigations, although results had been somewhat discouraging. The method, which was called "pollarding" by Hildreth (*123*), would eliminate the expense of ground preparation and replanting. At the same time, the new shoots should grow more vigorously because of an established root system. Furthermore, Traub (*234*) had found that there is no decrease in the amount of rubber once it is formed in the roots, and the rubber would be retained for a future harvest of the entire plant.

Curtis (*73*)* began some clipping experiments on guayule in 1944 but, owing to a curtailment of Federal funds, he was able to observe his investigations during only a brief period of 18 months. However, he made valuable observations. Tops clipped 1 to 2 inches above the ground level yielded about two-thirds of the rubber ordinarily obtained from uprooted plants. Rubber concentration in the tops was practically the same as in uprooted plants. The quality of the rubber from the tops was not inferior to that from uprooted plants, and the tops offered no difficulty in pebble-mill processing. Fairly comparable results were obtained by Tingey (*226*)*.

Upon resumption of research on guayule in 1948, Hunter et al. (*128*) laid out some carefully planned experiments to be conducted in existing stands at Salinas, for the purpose of observing the effects of clipping on yield of rubber, involving: (1) harvesting by digging the entire plant 6 to 8 inches below the ground line; (2) partial harvesting by clipping about 2 inches above the crown, and subsequent harvesting by digging; and (3) harvesting by digging, followed by replanting and harvest of the new stand during subsequent years.

Completely harvested plants 5 to 7 years old, with a spacing of 36 by 24 inches, yielded from 1,003 to 1,459 pounds of rubber per acre in 1948 and 1949. Tops clipped from comparable plants yielded from 686 to 1,064 pounds, which amounted to from 68 to 75 percent of the rubber in the entire plant.

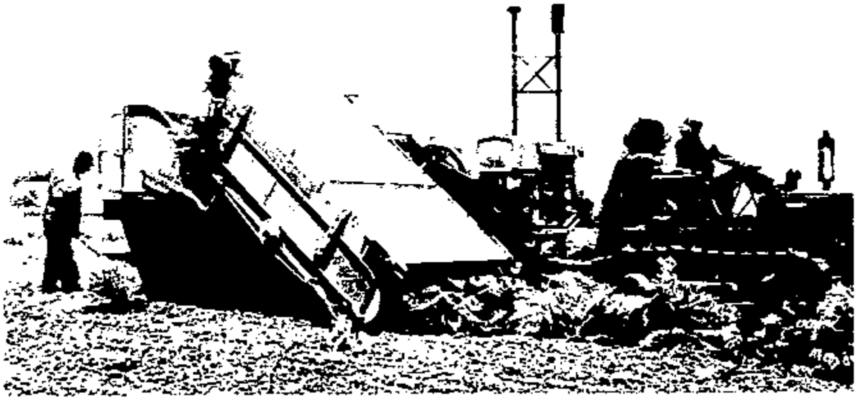


FIGURE 19.—Pickup-type hay baler remodeled for baling guayule shrub that had been dug, dried, and windrowed. The pickup attachment was designed and made in the U.S. Forest Service Shop at Salinas, Calif.

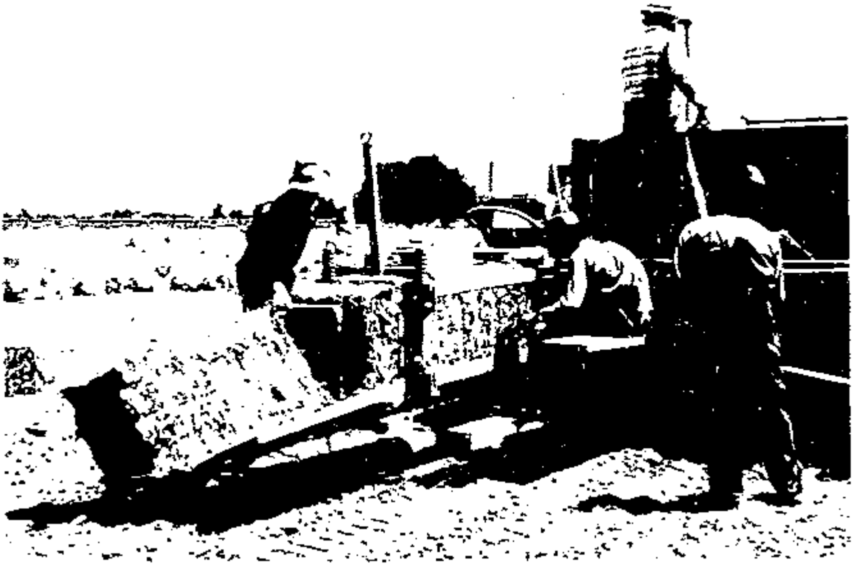


FIGURE 20.—Rear view of baler shown in figure 19.

After 5 or 6 years in the field, the cumulative yields in rubber from plants harvested by clipping and then completely harvested by digging 1 to 4 years later were greater than the yields from unclipped plants of comparable age. At the final harvest in 1953, the accumulated rubber yields of the plants first clipped and then dug exceeded a ton per acre. The yield of rubber of unclipped plants was significantly lower than that of clipped plants. The yield of completely harvested 5- to 6-year-old plants plus that of replaced transplants harvested 1 to 4 years later resulted in a cumulative total yield less than the yields of unclipped plants of an age equal to the combined ages of the two crops.

The time of clipping was an important factor in the survival of the plants. In general, the best survival resulted from clipping during the summer season when the highest yield of rubber could be obtained. Nonirrigated plants survived clipping better than irrigated plants, regardless of when they were clipped.

Although these experiments demonstrated that the cumulative total yields of clipped plants exceeded those of unclipped plants, data on actual costs of producing rubber by these methods were not available. However, it could be concluded that a single harvest of unclipped plants would involve less expense, without risking the loss of plants, than clipping followed later by harvesting the entire plant. Costs of additional harvesting operations and price of rubber would determine the feasibility of clipping.

Seed production[1]

Seed harvesting. Guayule tends to flower more or less continuously during the growing season when soil moisture is adequately maintained (fig. 21). In nonirrigated fields, slower growing plants of first year's growth mature their main crop of seed about the first of September. In subsequent years the main crop ripens in late June or early July. However, spotty yields may occur later if there is sufficient soil moisture to enable the plants to continue active growth.

The total yield of seed per acre varies greatly according to survival, age, vigor of the plants, and collecting methods. From 25 to 40 pounds per acre of clean, unthreshed seed were collected with a mechanical seed picker from irrigated plants in the San Joaquin Valley in their first growing season. Five hundred pounds of clean, unthreshed seed

FIGURE 21. Field of 2-year-old, irrigated guayule in flower in the Salinas Valley, California.

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[1] The Seed and Nursery Operations Handbook by Taylor and Champagne (*216*) contains information on the methods and equipment used in the harvesting, cleaning, and threshing of guayule seed. In his mimeographed report, Carl Taylor (*215*) gives a detailed account of these operations, and also cites many of his own unpublished reports, as well as those of others, on experimental work with guayule seed. The chapter on "Seed" in the Final Report of the Emergency Rubber Project compiled by Paul H. Roberts, Director, (*186*), also describes problems encountered and the development of working methods.

per acre, per season, was not uncommon in older, irrigated plantings. In the Salinas Valley, unirrigated plantings yielded 10 to 20 pounds of seed per acre during the first season after the plants were set out, and 100 to 200 pounds in the second and following years.

During the seed stockpiling program initiated in 1950, yields of 500 pounds of unthreshed seed were obtained from fields established for 1 year in rows 28 inches apart. This amount of seed was obtained from three harvests of the same field in 1 year. Careful attention was given to irrigation, fertilization, and insect control. Fertilizers (nitrogen and phosphoric acid at the rate of 60 pounds each) were applied in April, and the plants were kept in continuous bloom by irrigating every 2 weeks (fig. 22). Lygus insects were controlled by spraying the field twice during the summer with 10-percent DDT at the rate of 1 pound per acre. Harvesting was accomplished by means of a new type of seed harvester, described later.

The percentage of embryo-containing seeds in different seed lots varied considerably, the cause of which was not fully determined. Research studies indicated a possible interplay of a number of factors, any one of which could affect yield of filled seed. Lygus insects feeding on the flowers may reduce the yield considerably, and there was some evidence of seasonal and temperature influence. Viability of guayule seed is unusually low compared to other kinds of seed. The percentage of filled seed usually ranges between 10 and 45 percent, although in some instances it may be as low as 0 and as high as 70 percent.

Seed collection by hand methods was found practical for small quantities of seed, or when it was desired to obtain the maximum amount of

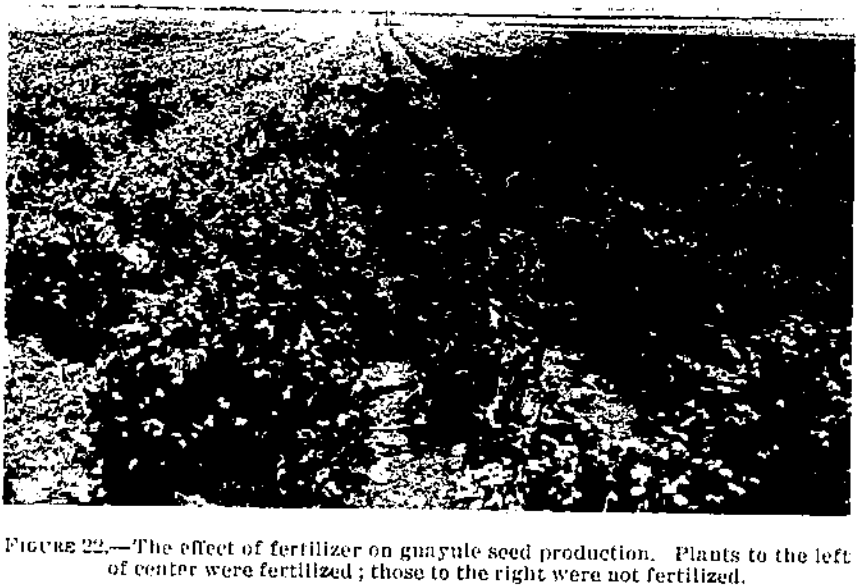

FIGURE 22.—The effect of fertilizer on guayule seed production. Plants to the left of center were fertilized; those to the right were not fertilized.

seed produced by the plant. There was a loss of up to 50 percent or more of the seed during machine harvesting operations, but hand methods could not compete in speed or cost per pound of seed. In 1942, hand-collected seed cost 30 cents per pound, whereas machine-collected seed in 1943 was under 10 cents per pound, uncleaned field weight (*186*).

Guayule seed is easily harvested by mechanical means since it loosens and shatters readily when ripe. Various types of harvesters were developed. The harvester used by the Intercontinental Rubber Company utilized a vacuum produced by fans, was complicated, heavy, and not very efficient. The Project developed several types of harvesters employing revolving brushes or beaters to dislodge the seed into trough receptacles. One model consisted essentially of a shaft carrying four rotary brushes, one for each row of plants, which was mounted on the chassis of a pull-type beet and bean cultivator, and driven by a belt from the cultivator wheels. The seed, as it was brushed from the plants, was caught in torpedo-shaped pans drawn along the ground between the rows. A canvas cover kept the seed from being tossed about.

An improved model was developed later by the Bureau of Plant Industry, Soils, and Agricultural Engineering for mounting across the front of a Ford-Ferguson tractor, to prevent the tractor wheels from knocking off the seed (fig. 23). This model consisted of a horizontal rotating brush partially enclosed in a metal box open at the front and long enough to span four rows. The height of this assembly was controlled by the power lift of the tractor. The seed was brushed directly into the box from which it was moved by a screw conveyor to a vertical bucket elevator and discharged into sacks. Although the

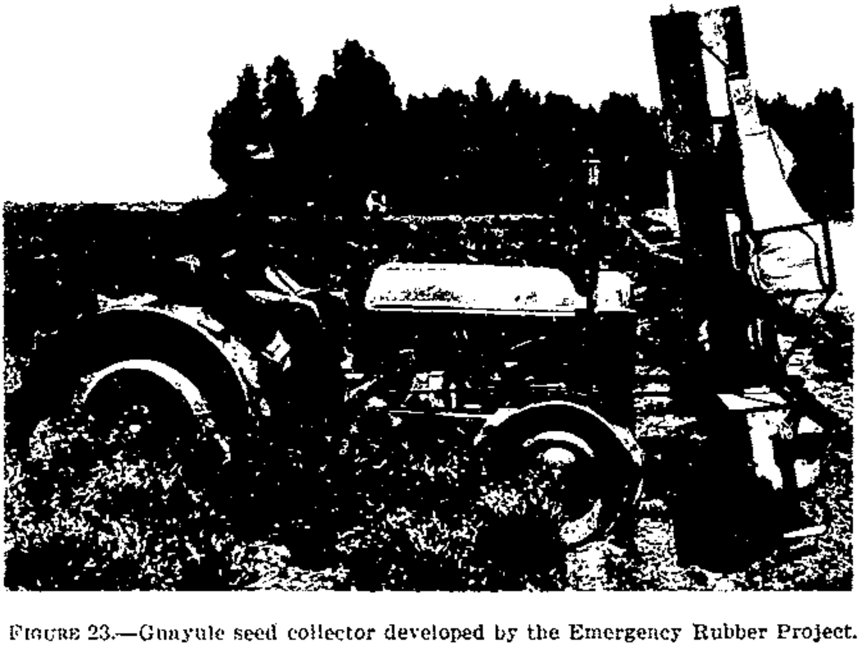

FIGURE 23.—Guayule seed collector developed by the Emergency Rubber Project.

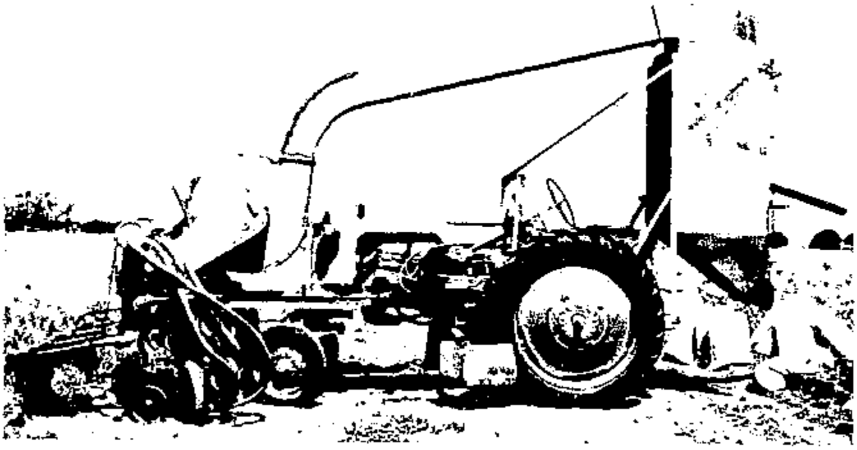

FIGURE 24.—Suction seed harvester for harvesting guayule seed. At the front of the tractor there is a suction and blower assembly, and at lower left a rotating rod with attached rubber strips for dislodging seed.

brush-type machine was light and covered acreage rapidly, it was very inefficient in gathering all the seed. The main objective of the earlier suction-type harvester was the sucking of seed directly from the plant. A lack of sufficient suction pressure made the machine inefficient in accomplishing this objective.

When ripe seed is dry, it is easily dislodged from the plants. Therefore, seed collections must precede cultivating, furrowing, or other field operations. Dampness or heavy dews cause ripe seed to tighten and prevent efficient harvesting. Heavy winds may cause dislodging or loss of large quantities of ripe seed.

When the seed stockpiling program was initiated in December 1950, it was apparent that improvements in seed harvesting equipment would be necessary to obtain maximum quantities of seed. After a considerable amount of research and improvements in design, a four-row, suction-type guayule seed harvester (fig. 24) was developed that permitted the harvesting of a higher percentage of available seed than any previously designed harvester (*44*).*

This new-type harvester departed entirely from the principle of the old-type suction harvester by sweeping the ripe seed from the ground after first dislodging it from the plants in front of the machine by a horizontal, rotating rod to which was attached a series of rubber strips. Upon falling to the ground, the seed was sucked up through large suction nozzles, or shoes, shaped to conform to the contour of the irrigation furrows. Two blowers were built in the machine—one a pressure blower, and the other a suction blower. The pressure blower delivered a stream of air on the seed at the base of the suction shoes which started the seed on the ground moving while, at the same time, the suction shoes readily forced the seed, dust, and trash into the machine and into the cyclone tank against a fine mesh screen. Material not passing through the screen fell into a hopper and was discharged into cloth sacks. Although this harvester was highly effective in collecting maximum quantities of seed, it included along with the seed a considerable amount of sand and soil particles, which made an additional seed-cleaning operation necessary.

Seed cleaning and threshing. Prior to 1942 a good method for cleaning guayule seed had not been devised. Harvested seed usually contains, in addition to central clusters of male florets, dried leaves, flower stems, and other extraneous material. The floral attachments make the seeds too light for separation by air blast. Leaf particles and clusters of male florets may be so near the size of the seeds as to make screen separation ineffective. The difference in shape of the male floral clusters and unthreshed seeds, suggested separation by a screen having ½- by $1/12$-inch slots. In the fall of 1942, a seed cleaner of this type was tried and found effective. Much of the seed in storage at the time was recleaned by this method.

Further experimental work showed that selective screening could be supplemented by a vibrating gravity separator commonly used for separating empty and filled seed. This method doubled the percentage of filled seed in low-quality guayule seed lots. Subsequent investigations indicated that the gravity separator was much more efficient in separating empty from filled seed when the floral parts [5] were removed from the seed in threshing. From several types of threshers and scarifiers tried, a seed huller was adopted that combined the qualities of high capacity and effectiveness with little or no injury to the seed.

A very efficient seed-cleaning and threshing system (fig. 25), using mostly standard equipment, was soon in operation. Essentially, the

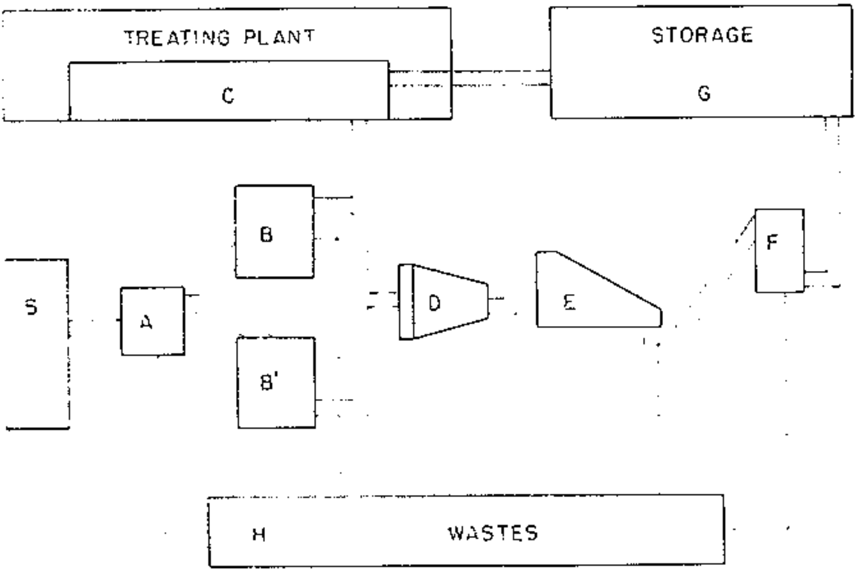

FIGURE 25.—Flow chart for guayule seed cleaning and threshing.

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[5] An unthreshed guayule "seed" is an achene with an attached bract and a pair of sterile florets. A threshed seed refers to the achene after the floral parts have been removed. See fig. 4, p. 11.

sequence of operations were: (1) preliminary cleaning, (2) threshing, (3) gravity separation, and (4) final cleaning.

The seed material, which usually contained much extraneous material such as stems and leaves, was passed first over a power-driven shaker or scalping screen of $\frac{1}{4}$-inch-mesh wire to remove the coarser trash, and then through a vibrating clipper cleaner. This cleaner contained a three-screen riddle, 42 by 60 inches, with a top screen of No. 10 round holes for removing oversize material, a middle screen of $\frac{1}{2}$ by $\frac{1}{12}$-inch slots for separating the clusters of disk florets from the seed, and a bottom No. 7 round-hole screen for removing the fine trash. Slightly different perforation sizes were occasionally required, depending on the average seed size of some lots.

Threshing was accomplished with a seed huller originally designed for hulling burclover. It consisted of a conical rubber-covered adjustable rotor turning inside a rubber-lined drum which removed the attached floral parts. With proper adjustment, it would thresh up to 450 pounds (unthreshed weight) of guayule seed material per hour.

The threshed material was then passed over a gravity separator. Usually with the proper adjustment of air, shaking speed, and pitch, the flow of threshed material across the table of the separator divided into two distinct streams, one containing chaff, empty achenes, and dust; the other containing filled achenes and particles of stems and leaves. In making the adjustments, the percentage of filled threshed seed, or achenes, was determined by flotation in acetone. The sinkers included nearly all of the filled seed, while the floaters consisted of empty or partially filled seed. Through proper adjustments, seed lots could be brought to 95 percent filled. The entire material from the thresher could be run over this separator at the rate of approximately 400 pounds per hour.

Final cleaning for removing any heavy trash such as gravel and stem fragments was accomplished by passing the filled, threshed seed from the gravity separator through a vibrating clipper cleaner (fig. 26). For this cleaning operation the top, middle, and bottom screens were $\frac{1}{2}$- by $\frac{1}{12}$-inch slots, and $\frac{1}{12}$- and $\frac{1}{20}$-inch round-hole perforations, respectively. The variable-speed fan supplied with the cleaner for air-blast cleaning was used.

Gravity separation followed by screening is the reverse of procedures usually followed in cleaning other kinds of seeds, and for unthreshed guayule seed. The reason for this is that the removal of chaff from the threshed seed by gravity separation greatly reduces the amount of material for final cleaning.

Guayule seed having a moisture content of 7 percent or less is more effectively threshed than seed with a higher moisture content. Threshing of the latter may be incomplete. However, the unthreshed portion can be returned from the gravity separator to the thresher and rethreshed. Seed containing higher than 9 percent moisture may require drying prior to threshing.

Some of the advantages of threshing were: (1) The reduction of material to approximately one-fifth of the original weight, and the reduction in storage space to one-tenth of the original requirements; (2) a much weaker solution of chlorine was required for seed treatment to break dormancy; (3) a higher percentage of open-stored, threshed seed would germinate after a 6 months' period without

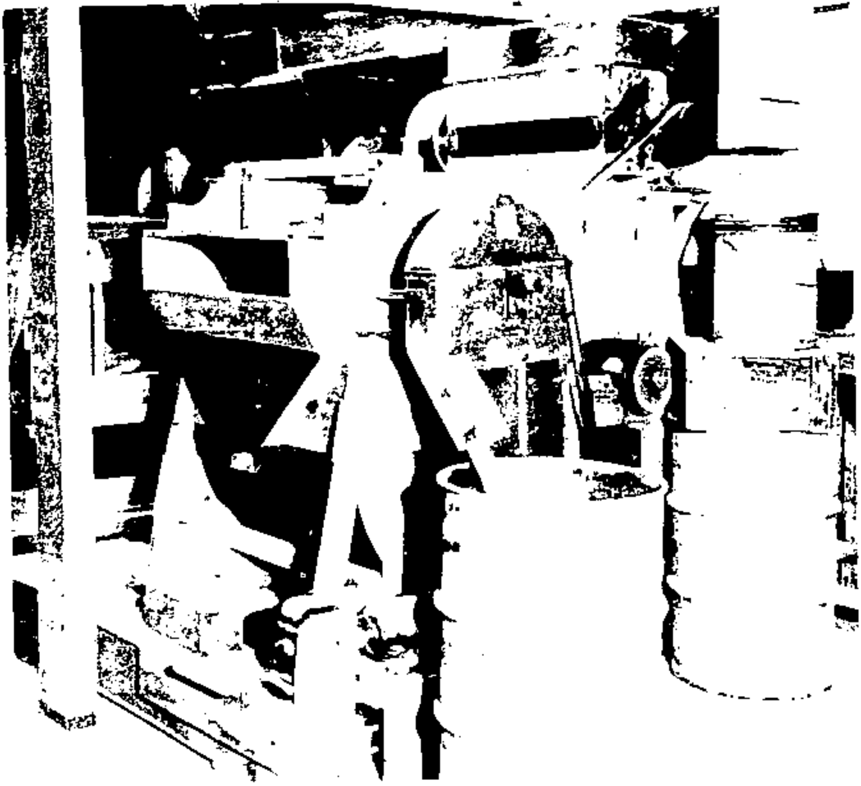

Figure 28.—Clipper cleaner used for final cleaning of threshed guayule seed.

chemical treatment; (4) being heavier and more uniform in size and shape, threshed seed flowed better through mechanical drills used for seeding; and (5) tests indicated that threshed seed germinated more quickly than unthreshed seed, and in seedling trials better stands were obtained from threshed seed.

Seed storage. Prior to 1942, the Intercontinental Rubber Co. had lost seed through deterioration after 2 or 3 years in storage at Salinas. They noted that seed stored at dry inland places retained its viability considerably longer and, therefore, adopted the practice of drying seed promptly after harvesting and cleaning to 4 percent moisture or less, and sealing it in metal drums. Most of the seed purchased by the Government was stored in this manner, and most of it was still good after 6 to 8 years.

Subsequent investigations by the Emergency Rubber Project indicated that the company's method was sound with respect to long storage, but that it was not the best method if the seed was to be used within 2 or 3 years after collection. Seed prepared for long storage

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Periodic germination tests have been made on cleaned, threshed seed stored in 1943 in sealed drums at 4 percent moisture. The last test was made in 1959 by the senior author which indicated no apparent loss of viability of the seed after 16 years in storage.

would have to be given the expensive hypochlorite treatment in order to break the seedcoat dormancy. Under conditions of fair aeration and moisture, the seed dormancy gradually disappears. The germination rate of open-stored, unthreshed seed increases up to approximately 12 months or longer, depending on seed lots, whereas the dormancy of threshed seed may completely disappear within 6 months. Seed stored at 4 percent moisture retains its dormancy for a greater length of time.

Benedict and Robinson (*33*) found that the relative humidity at which nondormant guayule seed was stored greatly affected its germination. The germination of threshed seed stored at 80 and 100 percent relative humidity decreased to zero in 1 to 6 months. There was no consistent change in germination of seed stored at 50 percent humidity. At 30 percent, and at relative humidities prevailing in the room at Salinas, the germination continued to increase during the test. At 0 percent humidity the germination of the seed remained low or actually decreased during the storage period. After being given the standard hypochlorite treatment, over 90 percent of the filled seed stored at 0 percent humidity germinated, indicating that the poor germination of seed stored at extremely low humidities was not due to loss of viability.

Taylor (*213*) reported that guayule seed is tolerant of a wider range of temperatures in storage than most kinds of seed. He subjected seed containing about 5 percent moisture to a temperature of 195° to 200° F. for 40 hours and to a temperature of 112° below zero for 72 hours, without any apparent injury. Wet seed exposed to temperatures above 120° F. for even a few hours was injured.

Physiology

Distribution and storage of rubber

Lloyd (*146*) indicated that rubber in guayule exists as a colloidal suspension or latex confined to individual cells, and concluded that there are no organized latex tubes since the freshly cut section of the stem of a living plant does not produce a flow of latex. However, if the freshly cut surface is dipped into a drop of water on a glass slide, the liquid takes on a milky appearance. Microscopic examination reveals a rich suspension of spherical particles in Brownian movement. The contents of uncut cells appear as densely milky fluid, also containing minute particles in Brownian movement. That the particles are mainly rubber was demonstrated by the use of differential stains and solvents. Particles of non-rubber constituents, including resins and fats, are also present.

The occurrence and distribution of rubber in the various plant organs has been studied in some detail and described by Ross (*198*), Lloyd (*145*), and Artschwager (*19*). In general, plants older than 1 year carry the major portion of rubber in the vascular rays of phloem and xylem. Smaller quantities are found in the primary cortex, pith, xylem parenchyma, and the epithelial cells of resin canals. In younger plants, much of the rubber occurs in some of these latter tissues. Very small amounts of rubber are present in the leaf parenchyma—0.3 to 0.5 percent, as recorded by Curtis (*72*). A similarly low concentration is found in the peduncles.

Curtis (*72*) made a thorough study of the distribution of rubber in terms of both concentration and weight. Defoliated plants of various ages and of known cultural histories were used. These were separated into segments of branch roots, root, and crown. The branches above the crown were separated into stem lengths, which were determined by growth increments separated by a collar of leaf scars of shortened internodes that appeared as cessation of bud growth in response to low soil moisture or low temperature.

The branch system above ground constitutes approximately two-thirds of the defoliated dry weight of the plant and contains an even larger proportion of the total rubber since it usually has a higher rubber concentration than either the root or the crown. The concentration of rubber in the branch roots is higher than in the main root.

The bark is the principal site of rubber occurrence. The difference between rubber concentration in the bark and that in the wood is most pronounced in the root, where the concentration in the bark may be 11 times as great as in the wood. The concentration of rubber in the bark of the branches is only two or three times that in the wood. Considering the plant as a whole, the concentration of rubber in the bark is three times that in the wood. The bark contains 75 to 80 percent of the total weight of rubber in the plant.

The proportion of bark to wood is low in the primary root, resulting in a lower rubber content in the primary root than in the aerial branch system in spite of the higher concentration of rubber in the root bark. The proportion of bark increases progressively upward from the root into the younger branches of smaller diameters; and, in general, there is a strong tendency for uniform rubber concentration in the stem segments, corresponding to successive annual elongation increments, owing to a greater proportion of bark in the younger stems.

Evidence indicates that rubber is concentrated in increasing amounts for several years in cells of xylem and pith, and that it is more highly concentrated in the older inner tissues than in the newly added xylem.

Factors affecting rubber formation

Individual guayule strains may respond differently to environmental conditions in their ability to accumulate rubber. A familiar example of this was McCallum's commercial variety 593. In the cool Salinas Valley of California, the plants of this variety had well-formed, fairly thick branches containing a fairly high concentration of rubber, whereas, in the warmer valleys of California and in southwest Texas, the plants were small and formed a number of relatively small branches just above the ground level. Rubber accumulation tended to level off abruptly after approximately 2 years' growth, whereas in the Salinas area, the accumulation of rubber continued for 10 to 15 years. As an opposite example, McCallum's variety 111, which was the poorest rubber yielder in the Salinas Valley, consistently yielded up to 50 percent more rubber in Texas than McCallum's other varieties.

Many workers have observed marked seasonal fluctuations in the overall concentration of rubber during the year. Concentration is lowest during periods of rapid growth under favorable moisture conditions, and highest during periods of moisture stress. Lloyd (*145*) and Artschwager (*20*) showed that the ratio of the radius of the wood to the bark is directly related to plant weight and water supply. Lush growth increases the production of xylem tissue, which produces little rubber, at the expense of phloem tissue, which has a much higher rubber-producing potential.

Growing plants at various temperature ranges under conditions of abundant moisture and high fertility, Bonner (*36*) showed that, when the air temperature was maintained constantly at 80° F., rubber accumulation was slow and there was no increase in rubber percentage. When the night temperatures were lowered to from 50° to 35° F., for more than 10 hours, rapid increases in rubber concentration resulted, the optimum temperature being 40° to 45° F. Low day temperatures of 40° to 45° F., in combination with low night temperatures, did not result in rubber accumulation.

When Guayule plants were grown at a constant air temperature of approximately 76° F., Benedict (*28*) found that the percentage of rubber decreased as the soil temperature was raised from 40° to 95° F. In one experiment, where growth seemed to be depressed, the rubber concentration increased as the soil temperature was raised from 65° to 90° F. He concluded that low soil temperatures tended to increase the rubber percentage but that high soil temperatures *per se* did not necessarily mean low rubber percentages. On a grams-per-plant basis, the highest yield of rubber was obtained from plants grown at soil temperatures of 80° to 85° F.

Mitchell et al. (*164*) found that plants grown at a relatively high nutrient level were extremely sensitive to reduced light intensities. A 25-percent reduction in light intensity reduced by 36 percent the absolute amount of rubber produced. Reduction in light intensities was associated also with decreases in rubber concentration. The percentages and absolute amounts of rubber produced by plants grown at a low nutrient level, on the other hand, were not greatly affected by reduced light intensities. The limited rubber output of plants subjected to reduced light intensities at a high nutrient level was associated with a decrease in dry weight, an increase in seed production, and a decrease in the number of functional leaves during the rubber-storing seasons.

Taylor and Benedict (*214*) found that shading the stems of guayule plants by burying them in dry soil or gravel, greatly increased growth of the stems, such increase being as much as 10 times that of unshaded stems.

Benedict (*29*) conducted various experiments to ascertain the effects of two levels each of light intensity, temperature, soil moisture, and available nitrogen, and various combinations of these on rubber percentages in guayule seedlings grown to an age of 6 months in sand and in soil. Strains used in these experiments were 4265 and 593, strain 4265 ordinarily being more vigorous and containing a higher rubber concentration than strain 593.

The results of these experiments at the end of 6 months indicated that dry weights and rubber percentages were generally higher on the high than on the low level of light intensity. In high light intensity, plants grown in soil cultures at the high levels of temperature, soil moisture, and available nitrogen always gave a greater dry weight and a lower percentage of rubber than those grown at the low levels. However, plants grown in sand cultures at the high levels of light, temperature, and available nitrogen showed the greatest dry weight, and next to the highest rubber percentage of those from all 16 treatments given and higher than any plants grown in soil cultures, indicating that a high rubber percentage is not always incompatible with rapid growth. It was interesting to note that two of the ten nurseries operated by the Emergency Rubber Project, in which there were large seedlings with 7 to 8 percent rubber hydrocarbon, were located on very sandy soils near Oceanside, Calif. These two nurseries were heavily watered, and had ample supplies of available nitrogen. Seedlings in the other nurseries contained only 2 to 4 percent rubber.

There was no evidence in these experiments that seedlings of strain 4265 either grew more vigorously or produced a higher rubber percentage than strain 593 at the age of 6 months.

By subjecting nursery plants grown in wooden boxes to alternating periods of low and high moisture stresses, Benedict et al. (*32*) were able to force rubber accumulation in guayule plants greatly above that of plants grown continuously under high moisture stress. Continuous high moisture stress tended to retard growth, whereas continuous low moisture stress favored growth but reduced the relative amount of rubber-bearing tissues. These experiments demonstrated two interacting factors affecting rubber yield. In addition to moisture stress, some condition other than seasonal temperature, such as length of day, light intensity, or hours of sunlight was apparently involved. The maximum rubber yields were obtained by alternating periods of low and high moisture stress during the season of the year other than the winter months. The most favorable periods of high stress were in the spring, when conditions favored both growth and rubber accumulation. Under the conditions of their experiment, a 4-month, high-stress period resulted in a much higher rubber yield than a 2-month period.

In practically all previous experiments dealing with the effects of temperature, soil moisture, light intensity, or available nitrogen on the growth, resin, and rubber content, one factor had been varied and the others held constant. As a result, no estimate of the relative effects of the various factors could be determined; nor was it possible to estimate how these would interact in their effects on the plant. Therefore, Benedict (*26*)* carried out an experiment in which guayule plants started from nursery stock were grown in three levels of temperature, soil moisture, light intensity, and available nitrogen, and in all possible combinations of the three levels of each factor. The plants were grown in nitrogen-deficient loam in three-gallon glazed crocks, two plants to a crock, under uniform conditions for 4 months to become established. The plants were then grown in the various treatments for 9 months, after which they were harvested.

From his experiment, Benedict concluded that "Soil moisture and nitrogen supply had the greatest effect on the dry weight of the stems, while temperature and light intensity had the greatest effect on the dry weight of the roots.

"The percentages of resins and rubber in both the stems and roots were most affected by temperature, next by light intensity, while soil moisture and nitrogen supply had the least effect.

"Several interactions between factors were noted. These usually resulted where one factor became limiting so that the effect of a second factor was not as great at one level as at another of the original factor. In only one or two instances did the effect of one condition change in kind with change in a second condition."

Origin of rubber

The question has often arisen as to whether the immediate precursors of rubber originate in the leaves and are then converted into rubber in the rubber-bearing tissues, or whether these tissues might take the photosynthetic products of leaves of any plant and convert them into rubber.

In a study of the biochemistry of rubber synthesis, Bonner and Arreguin (*37*) found that glycerol, acetate, acetoacetate, acetone, and β-methylcrotonic acid added to nutrient solutions increased rubber formation in guayule seedlings, and suggested that these substances be considered possible intermediates in rubber synthesis. These authors also found evidence that extracts of leaves of guayule plants actively engaged in rubber formation, when added to the nutrient solution, brought about increased rubber formation in guayule seedlings. Extracts of leaves of plants not actively engaged in rubber formation did not increase rubber formation.

Arreguin and Bonner (*18*) confirmed these results in another experiment in which isolated stem sections of guayule were grown in vitro and treated with the above mentioned substances. Since defoliation experiments reported by Bonner and Galston (*38*) had indicated that leaves perform an important role in rubber accumulation in guayule, Arreguin and Bonner suggested that the leaves may form and supply to the stem a substance or substances which act as precursors of rubber, and that β-methylcrotonic acid may be regarded as a possible precursor of rubber.

Taylor et al. (*215*) conducted a series of experiments involving interspecific and intergeneric grafts to determine if guayule rubber is formed from specific precursors derived from guayule leaves (fig. 27). Reciprocal grafts of guayule (*Parthenium argentatum* Gray) were made with mariola (*P. incanum* H.B.K.), *P. stramonium* Greene, sunflower (*Helianthus annuus* L.), and Jerusalem artichoke (*H. tuberosus* L.). All except guayule contain negligible amounts of rubber. Their results indicated that guayule tissue grafted to non-rubber-bearing plants contained rubber in the same percentages as comparable parts of ungrafted guayule, and that the rubber content of non-rubber-bearing plants grafted with guayule was as negligible as that of the ungrafted controls. Therefore, they concluded that the degree of rubber accumulation is a property of the rubber-bearing tissues and not of substances supplied to these tissues, and that the immediate precursors of rubber are formed only in these tissues.

Benedict (*28*) gave further evidence that rubber accumulation is a function of the tissues in which it occurs, by growing guayule plants where leaves were under like conditions. He found that the rubber content of the roots varied considerably at different temperatures.

Function of rubber

The function of rubber has been a source of considerable speculation. Earlier workers presented the view that rubber serves as a food reserve in guayule. Spence and McCallum (*208*) recorded losses in total rubber per plant of 10 to 15 percent during periods of vigorous growth. Other workers, however, have failed to observe utilization of rubber in guayule under conditions of mineral or carbohydrate exhaustion. Traub (*234*) carried out some carefully controlled defoliation experiments during the fall and winter months (from the middle of October to the middle of January) and showed that, even under conditions of

FIGURE 27.—Graft of sunflower on guayule.

severe carbohydrate starvation, there was no decrease in rubber per plant. Benedict (*27*) conducted a parallel series of defoliation experiments during the active growth period of the spring months. Weight per plant of free sugars, levulin, inulin, pentosans, resins, and rubber were determined in original stem and root sections at the beginning and end of this period. The plants were found severely depleted of their food reserves as a result of repeated defoliation from January 20 to June 1 with a loss of from 38 to 40 percent in dry weight. There was no loss in rubber hydrocarbon. Losses of rubber during periods of vigorous growth, as reported by other workers, were attributed to sampling errors in harvesting that may occur at this time of year.

The view that rubber serves as a protective mechanism against drought has been advanced from time to time, but no substantiating evidence has been offered. There is no positive evidence that rubber serves any physiological function in guayule.

Resins

In guayule, resin canals occur throughout the plant in well-defined systems. Artschwager (*19*) states that, in the root and hypocotyl, the primary resin canals are derived directly from the two-layered endodermis. Anticlinical divisions usually give rise to groups of four cells which, upon delamination of adjacent cell walls, form intercellular spaces which enlarge to become the resin canals. Subsequent periclinal divisions of these cells form two layers, the inner one being known as the secretory layer, or epithelium. There is no definite endodermis in the epicotyl or in the stem. Toward the end of the growing season, the stem tip of the seedling differs from the epicotyl in having resin canals in the pith. Secondary resin canals are also schizogenous in origin and are derived directly from the cambium. Leaves and peduncles also contain resin canals. For a detailed discussion of the origin and development of the resin canal system in guayule, the reader is referred to the publication of Lloyd (*145*) and Artschwager (*19*). Some of the latter's observations are not in agreement with those of Lloyd.

Curtis (*72*) made a detailed study of the distribution of resins (acetone soluble substances) in guayule. These are found principally in the bark and distributed rather uniformly here. The resin concentration in the bark of stems and roots of various ages in his material ranged from approximately 7 to over 10 percent on a dry-weight basis. The concentration of resin in the wood was considerably lower. Peduncles contained between 2 and 4 percent resin, and leaf samples showed a resin content of about 10 percent. Resin was at a low concentration in the root as compared with that in the stem. Stems no more than a year old showed higher resin concentrations than did older stems.

In greenhouse experiments beginning July 6, 1944, and ending May 6, 1945, Benedict, et al. (*32*) found a seasonal variation in resin percentage which was not affected greatly or in any consistent manner by moisture stress. The resin percentage in leaves and stems generally increased up to September and November harvests, respectively, and then decreased until the May 6 harvest. The resin percentage in the roots gradually decreased during the course of the experiment. There was an actual loss in resins, which could not be accounted for, between

November 6 and January 6, after which the plants continued to gain in resins until the final harvest.

When a branch dies, Curtis (*72*) found evidence that a considerable portion of rubber is converted to acetone soluble substances within a few months' exposure to field conditions. The proportion of dead twigs and branches increases with age as a result of competition within the plant, and perhaps to some extent from cultivation injuries. The effect of dead parts on whole defoliated plants of advanced age, as they are usually taken for analysis or commercial milling, is to lower the rubber concentration and to raise conspicuously the resin concentration.

Research has uncovered many components of the resin in guayule. They include essential oils, parthenyl cinnamate and partheniol (both apparently indigenous to guayule), betaine, fatty acids, an unidentified wax, and many other chemical constituents. A detailed discussion of the components of guayule resin is given in the Final Report, Natural Rubber Extraction and Processing Investigations, compiled by I. C. Feustel (*98*), project leader, U.S. Department of Agriculture.

Physiological changes associated with hardening of nursery stock

The nature of the carbohydrates in the guayule plant has been studied by McRary and Traub (*155*), McRary and Slattery (*154*), Hassid et al. (*122*), Traub and Slattery (*236,237*), and Traub et al. (*239*). Traub and Slattery (*236,237*) showed that the main reserve carbohydrates in guayule are the levulins, with inulin next in importance from the standpoint of total amounts present. Erickson and Smith (*91*) and Benedict et al. (*32*) made similar observations. Reducing sugars, pentosans, and inulin increased only slightly during periods of high moisture stress while levulins increased in large quantities. During periods of low moisture stress following high stress, the percentage and absolute content of levulins decreased, indicating that these were the main carbohydrates. Traub and Slattery (*236,237*) did not regard the monosaccharides as reserve materials since they are present in relatively small amounts and may be regarded as the chief carbohydrates of translocation.

Kelley et al. (*141*) conducted an experiment to determine the effects of soil moisture stresses on the amount and type of growth in the nursery and on subsequent growth responses of transplanted guayule nursery stock over a 1-year period beginning June 3, 1943. (The results of this experiment are summarized in the section, "Conditioning Nursery Stock for Transplanting" of the present paper.) Based on analyses of samples from these same experimental plots, Traub et al. (*238*) discovered certain correlations between changes in carbohydrate reserves and moisture stresses ranging from very low to very high during the growing period, and the carbohydrate reserves of plants and their subsequent growth responses and survival after transplanting in the field.

Traub et al. (*238*) found that "guayule plants grown under high moisture stress in comparison with plants produced under low and intermediate moisture stresses (1) in October, contained relatively greater percentages of total water-soluble carbohydrates, including

89 percent ethanol insoluble levulins and inulin, and pentosans, (2) retained a significantly higher pentosan content for the duration of the experiment (to June), (3) began to utilize the water soluble carbohydrate reserve earlier in the spring and to a relatively greater degree.

"For plants grown under high moisture stress, (1) the relatively higher percentages of water soluble carbohydrates in October was correlated in fall transplantings with more rapid growth resumption and greater percentage survival; (2) the retention of relatively higher pentosan percentages in the tissues was correlated with more rapid growth resumption throughout the experiment; and (3) the earlier and more extensive utilization of the water soluble carbohydrate reserves with earlier growth resumption, and relatively more vigorous growth, resulted in a relative production of nearly three times as much growth during spring as was produced by plants of any other treatment.

"For plants grown under intermediate and high moisture stresses, the effect of the relatively lower temperatures in fall and winter was similar to that of very high moisture stress during the growing season, leading in both instances to increases in the percentage of total water soluble carbohydrates, 89 percent ethanol insoluble levulins and inulin of the plant tissues.

"Plants grown under very low moisture stress apparently lost the capacity to accumulate inulin during the following fall and winter in response to lower temperatures, but retained the capacity for the accumulation of relatively lower percentages of total water soluble carbohydrates and 89 percent ethanol insoluble levulins in response to lower temperatures."

Nutrition (fertilization)

The numerous experiments conducted to determine the response to fertilizers have indicated that guayule is a low user of the major elements. Cooil (*63*), however, was able to induce evidence both of potassium deficiency and potassium excess in guayule leaves. Hunter and Kelley (*120*),* in an experiment conducted on Chualar loam of the Alisal Nursery at Salinas, Calif, used N, P_2O_5, and K_2O applied to the soil at three rates, and with two placements in all possible combinations. In these nursery plantings, they found that nitrogen was the only element that gave a significant increase in dry weight of guayule. This response was obtained only after 3 months of growth after applying 40 and 100 pounds of nitrogen per acre.

In field plantings in the Salinas Valley, Kelley et al. (*142*)* found slight responses resulting from nitrogen applications. Nitrogen tended to increase growth, decrease rubber content, and increase the yield of rubber per acre. However, they concluded that it was doubtful that the use of fertilizer would be profitable on most soils of California and the Southwest where guayule might be grown.

Later experiments conducted in the Salinas Valley (*13*) have shown an increase in yield of rubber per acre with moderate irrigation and some fertilizer. Fertilizer was not beneficial on dry land.

Tingey (*227*), from an experiment conducted about 8 miles southeast of Salinas, concluded that commercial fertilizer had little or no effect on either shrub or rubber yields.

Hunter and Kelley (*132*) conducted field experiments on a light soil, the Delano sandy loam near Shafter, and on a heavy soil, the Sorrento silty clay loam near Crows Landing, in the San Joaquin Valley of California. They concluded that "when all plots are considered, the applications of fertilizer increased the weight of shrub but decreased the percentage and the absolute amount of rubber."

Nitrogen was the limiting fertilizer factor on soils of the Yuma Mesa in Arizona. On these sandy soils of low fertility, Davis and Abel (*81*)* found that guayule responded to nitrogen fertilizer only after the roots had reached layers of soil containing some clay, which retained the fertilizer lost from the sand by leaching. Nitrogen deficiency was associated with the absence of clay in the soil. This was demonstrated by mixing a small quantity of clay with the upper 6 inches or more of soil. A remarkable growth response to nitrogen took place within 2 months. Their data showed that nitrate and ammonium nitrogen produced increased growth, whereas phosphate with or without nitrogen produced no response. In his final report for this area, Davis (*75*)* pointed out that the best fertilizer was nitrate nitrogen applied as Chilean nitrate, and that the addition of the same amount of nitrogen, either as ammonium sulfate or as manure, did not produce as much growth as nitrate nitrogen. These results confirmed those of Bonner (*36a*) who grew nursery seedlings in nutrient solutions in 1.5-gallon cans containing 4-mesh gravel. Davis (*79*)* found that plants receiving most or all of their nitrogen as ammonium grew less and accumulated less rubber over an 8-month period than those receiving all or most of their nitrogen as nitrate.

Guayule at Yuma did not respond to the minor elements—calcium, iron, magnesium, and sulphur. Davis concluded that these minor elements were available in these soils in sufficient quantities for guayule growth.

Hunter and Kelley (*130*)* found that applications of the minor elements—boron, copper, manganese, zinc, iron, and magnesium—were not required to produce satisfactory nursery stock on the Chualar loam or the Greenfield coarse sandy loam soils of the Alisal nursery near Salinas, Calif.

Mitchell et al. (*163*) studied the effects of deficiences and excesses of boron on guayule in gravel culture and found guayule to have a relatively wide tolerance to that element. Boron deficiency was associated with reduced vegetative growth and lowered percentages of rubber.

Hilgeman (*124*)* concluded that the nitrogen and phosphate fertilizers used in his experiments on Gila clay loam and Papago fine sandy loam in the Phoenix area of Arizona did not increase growth or rubber production of guayule.

Davis (*75*)* reported that guayule grown in the Mesilla Valley (Anthony) of New Mexico did not respond to nitrogen, phosphorus, or a combination of the two.

On soils in the Pearsall area of Texas, Crain (*69*)* found that plots fertilized with 20 pounds of nitrogen and 40 pounds of phosphorus per acre produced more rubber than nonfertilized plots, owing to increased yields of shrub on fertilized plots. Fall application of fertilizer was more beneficial than spring application.

McAfee and Miller (*150*)* conducted numerous experiments involving the use of fertilizers in the lower Rio Grande Valley of Texas. In none of their experiments did fertilizers have any effect on guayule production.

A spacing and fertilizer experiment conducted by Cowley (*66*)* on newly cleared, Brennan fine sandy loam at Raymondville, Tex., indicated no increase in shrub or rubber from the fertilizer treatments.

Salt tolerance

The prevalence of excessive quantities of salts in soils of some areas in the arid Southwest is well known. Although the Emergency Rubber Project attempted to avoid soils of known high salt content, salty soil conditions were found in a few of the production and test plantings.

In a 160-acre planting near Coalinga, Calif., an irregular growth of the guayule soon became apparent. Small irregular patches were growing vigorously, whereas most of the plants were stunted in various degrees. This irregular growth pattern, together with discernible differences in the concentrations of soluble salts in the soil led Mickelson (*161*)* to investigate the relationship between plant vigor, as measured by volume growth, and salt concentration in a 10-acre plot in a portion of the field where there were plants of widely different growth characteristics. He found a pH range in the field of approximately 8.0 to 9.5 and a salt content in the soil profile down to 60 inches from the surface from less than 0.1 percent to more than 3.0 percent in some of the layers. The most vigorous plants were growing where the soil contained very slight amounts of salts throughout the profile (0.1 to 0.3 percent). The vigor of the plants was reduced somewhat where the average salt content was 0.5 percent or only slightly greater within 20 to 30 inches of the surface. A salt content of 0.5 percent or greater in the upper part of the root zone, 12 to 18 inches, greatly reduced the vigor of the plants. Relatively high accumulations of up to 1.0 percent soluble salts in the surface layer of ridges apparently had little or no effect on the vigor of the plants. The bulk of this accumulation was probably localized in one or two inches of the surface soil and took place after the guayule was irrigated, thereby reducing the salt content of the layer below.

Retzer and Mogen (*183*)* summarized later data compiled by Mickelson on subsequent treatments in the Coalinga plot and also reported their observations on salt tolerance in a second plantation site located near Bakersfield, Calif., together with a third site located in the upper Salinas Valley near King City, Calif. An indicator plot located near Coalinga on Panoche fine sandy loam was also studied by them. The dominant salts were usually the salines, especially NaCl and Na_2SO_4. In the Bakersfield site and in some deep subsoil samples of the Coalinga field, the strong reactions with phenophthalein were accepted as indicating the presence of Na_2CO_3. Segregated gypsum was present in the King City field and was especially pronounced in the Coalinga fields.

Their observations on growth behavior of 2-year old guayule grown in these fields indicated that the various salt concentrations had a marked effect on growth as well as on rubber concentration. Guayule was either killed or greatly retarded in growth where the concentrations in the first or second foot were 0.6 percent. Guayule grew well where the salts did not exceed 0.3 percent anywhere in any 5-foot profile layer. Concentrations between 0.3 percent and 0.6 percent in the surface 2 feet markedly retarded growth regardless of lesser degrees of concentrations occurring below this depth.

Retzer and Mogen also noted that the rubber percentages increased with increasing salt content and decreasing size of the shrub. At the same time, the total yield in pounds of rubber per acre was materially lowered because of the decreased tonnage of shrub. When compared to yields from salt-free soils, they concluded that production was not feasible when the salt content in the 5-foot profile exceeded 0.3 percent.

The increased deposition of rubber in plants in salty soils was attributed to stress brought about by the osmotic pressures of the salts. Although toxicity might have played some part in the death of plants in bare areas, physiological drouth seemed a more plausible explanation.

Wadleigh et al. (*245*) conducted a carefully controlled experiment in which guayule plants were grown in large containers of Panoche loam obtained near Coalinga, Calif., to determine the response of guayule to different concentrations of $NaCl$ and Na_2SO_4 under three different conditions of soil-moisture regime. Without the addition of salts, the rubber content increased with increased moisture stress. These results confirmed those of Kelley et al. (*141*) who were working with a nonsaline soil. Wadleigh et al. found an increase in rubber percentage at the lower ranges of moisture stress whether the stress was induced by moisture tension or by osmotic pressure of the soil solution. They presented evidence to show that really high stress resulting from a combination of both high soil-moisture tension and appreciable quantities of salts in soil solutions reversed the trend of relations between rubber percentage and moisture stress. These results suggested an incompleteness in the findings of Kelley et al. since the positive relation between rubber percentage and moisture stress appeared to hold only for the lower range of average moisture stresses.

Since high moisture stresses had been found to influence the photosynthetic reserves in other species, Wadleigh et al. believed it physiologically valid to expect a decrease in rubber concentration at very high levels of moisture stress. Furthermore, they observed that, when guayule plants were grown under very high stresses, the lower leaves of the plants died very rapidly. They also concluded that the inhibition of growth was related to total moisture stress regardless of whether it was due to moisture tension or to osmotic pressure of the soil salts in solution.

Retzer and Mogen (*183*)*, in comparing their results with those of Wadleigh et al. (*245*), suggested that, with the exception of the Coalinga field, the irrigation schedules of the indicator plot and the Bakersfield and King City fields might have been sufficient to prevent the attainment of moisture tensions required for a reversal in rubber accumulation. No explanation was offered for the lack of reversal on Levis clay in the Coalinga field. This field had received a total of 10 inches of water in May and June of 1944 and received no water the balance of the season.

Wadleigh and Gauch (*244*) grew guayule plants in sand culture with a control nutrient solution and also in culture with the same solution but with one, two, and three atmospheres of osmotic pressure of four salts studied separately, viz, Na_2SO_4, $NaCl$, $CaCl_2$, and $MgCl_2$. They found guayule very sensitive to magnesium. The plants were killed by the lowest concentration of $MgCl_2$ used. Fairly satisfactory growth was obtained with three atmospheres osmotic pressure of added $CaCl_2$. Although the plants were relatively sensitive to both sodium salts, they were more sensitive at higher concentrations to Na_2SO_4 than to $NaCl$ at equal osmotic pressures. Guayule being tolerant to $CaCl_2$ and extremely sensitive to $MgCl_2$ suggested that "the concentration of chloride ion *per se* would be a poor indicator of the inhibitive effect of a given substrate on growth unless information was available as to the cationic composition of the solutes present." In the light of their studies, Wadleigh and Gauch concluded that "guayule may not be regarded as a salt-tolerant plant."

Vegetative propagation from cuttings

Smith (*204*) made many attempts to root stem cuttings from various parts of the guayule plant. Cuttings usually failed to root satisfactorily in sand, soil, or peat-sand. Although high percentages of rooting (up to 90 percent) were occasionally obtained, the results were not consistent. The variable results were attributed chiefly to parasitism by several fungi, notably *Botrytis cinerea* Pers., and *Sclerotinia sclerotiorum* (Lib.) Mass. Best rooting was obtained with 2-inch terminal cuttings in sand.

In experiments to determine the proper frequency of water application, best results were obtained in sand when water was applied daily. These observations led Smith to test rooting in aerated water. The preliminary results showed exceptional promise. One thousand 4-inch cuttings were suspended on a wooden rack perforated by ¼-inch holes (125 per square foot) over a wooden tank equipped with carbon aerators and heating cables for maintaining a thermostatically controlled temperature of 76° F. Before placing in the tank, four-fifths of the cuttings were soaked for 12 hours in 1–50,000 indole butyric acid. Swellings of the treated cuttings occurred within 5 days and the first roots appeared in 9 days. Within 17 days, 80.0 percent of the cuttings were rooted and after 3 weeks the percentage of rootings was 88.2, with some of the unrooted cuttings showing swellings. At this time, none of the untreated cuttings had rooted, although a few showed enlargements at the nodes.

Another experiment was conducted with cuttings treated with indole butyric acid placed in sand and in aerated water, to compare the percentages of rooting, and the number of roots per cutting. Cuttings in sand were treated with a 1–1000 talc preparation of indole butyric acid, a treatment found superior to soaking in a water solution of this acid. Those in aerated water were previously soaked for 12 hours in a 1–50,000 solution of indole butyric acid. The temperature of both sand and water was maintained at 76° F.

Eighty percent of the cuttings in aerated water were rooted before any had rooted in sand. Final observations on those in water were made at 21 days, on those in sand at 42 days. At the end of 42 days, 52.8 percent of those in sand had rooted, whereas the percentage rooted in aerated water was 91.2 percent in 21 days. Only 3.6 percent of cuttings in sand had more than 5 roots per cutting, whereas over 63 percent of those grown in aerated water showed more than 5 roots per cutting. As many as 26 roots were counted on one stem. Four-inch cuttings in aerated water showed enlarged regions up to 3 inches above the cut end. In sand, the roots were confined to a swollen portion at or very near the base of the cutting.

At Manzanar in the Owens Valley of California, Nishimura et al. (*168*) obtained excellent results in rooting cuttings in sand provided the sand beds were properly prepared to maintain both aeration and moisture at the base of the cuttings. Three layers, each of a different grade of sand, were used in nursery flats 3 inches deep and 18 inches square. The bottom layer consisted of about 1 inch of 4-mesh gravel. Above this was a 1-inch layer of 8-mesh sand. To minimize excessive evaporation because of the high temperatures and low humidity occurring during the summer months, a ¾-inch layer of 16-mesh sand was used on top. Propagation was also carried out in sand beds 6 to 8 inches deep in large redwood frames built up off the ground. With such deep beds the use of a lower layer of gravel was unnecessary since good drainage was easily maintained. However, a surface layer of fine sand was used in order to maintain high humidity at the base of short cuttings. Bottoms of flats and frames had cracks wide enough to permit good drainage. To reduce evaporation further, cellophane covers were placed over the frames, and the nursery flats were kept either in frames covered with cellophane or in a glass house.

The technique for making desirable cuttings depends on the stage of growth of the parent material. In early spring growth, the first internodes are usually shorter than those of the previous season's growth. Best results come from cuttings taken before the stage of rapid elongation is reached. The cut should be made just below the node separating old and new growth, leaving a small portion of the second-year wood. The older leaves should be removed. These cuttings, including the leaves, are usually about 2 inches long. The cuttings should be inserted in the sand beds a little more than a half their length so that the cut ends pass through the surface layer of fine sand into the layer of coarser sand below. Cuttings made in the above manner later in the season are too long for best results. The cut should then be made 1 to 1½ inches below the growing point and immediately below a node. The lower leaves should be removed and the tops of the remaining leaves trimmed. These cuttings may root as well as those of early-season growth, but they require more care because they are more susceptible to damping off. Dormant shoots as well as woody cuttings may root without difficulty, but they are slower starting than herbaceous cuttings.

Except for the untreated controls, the cuttings were treated with the following hormones: indole butyric acid, naphthalene acetic acid, naphthalene oxyacetic acid, and naphthalene acetamide. These were used in both powder and liquid preparation. As a powder application, using talc as a carrier, the concentrations ranged from 0.50 to 2.00 mg. per gram. As a liquid medium, the hormone concentrations ranged from 10 to 60 mg. per liter. Cuttings were treated by standing them in the liquid media for 16 hours, or by dipping the cut ends in the talc preparations and shaking off the excess.

Cuttings treated with liquid hormone solutions were more susceptible to damping off. The talc treatments were less critically dependent on concentrations. Naphthalene acetic acid and naphthalene oxyacetic acid were notably toxic at the higher concentrations. In general, the treatment with naphthalene acetamide produced thicker, more vigorous roots, and more secondary roots than the other hormones, although sometimes the treatment with indole butyric acid produced roots that looked equally good.

By the use of these techniques, the rooting of more than 95 percent of the cuttings was obtained in 2 to 3 weeks with or without hormone treatments. This contrasts with the experience of Lloyd (*145*) who was unable to induce rooting of guayule stem cuttings except when a portion of root tissue was involved. In general, the optimum hormone concentrations used showed little advantage over the untreated controls provided the cuttings had been cut to the proper distance below the growing point to give best results. When long cuttings were made from plants that had undergone rapid elongation, the untreated controls gave a high percentage of failures, while those treated with hormones rooted as well as did short cuttings taken before elongation and rooted without hormones.

Rooted cuttings were easily transplanted to the field, where they started growth more rapidly than transplanted seedlings. They resulted in larger and more uniform plants during the first season's growth and produced seed earlier and more abundantly. There was some evidence that the cuttings contained more rubber than transplanted seedlings during the first year's growth.

Erickson and Smith (*91*) compared rooted cuttings and transplanted nursery seedlings with respect to growth and rubber and resin contents. After 4 months in the field, the rooted cuttings had produced a more extensive root system than the transplanted nursery seedlings. Samples were harvested after 8 months and again after 22 months from time of transplanting. In all respects the mean values for the cuttings were slightly higher than for the nursery seedlings. However, five replications did not show any statistical significance.

In another experiment, hardened 3-month-old greenhouse seedlings were included along with nursery seedlings and rooted cuttings. Sampling at the end of 22 months indicated no significant differences between dry weights of nursery seedlings and cuttings although the slightly larger size of the cuttings was again evident, but the results were highly significant for percentage of rubber and resin, and for yield in favor of cuttings. Greenhouse seedlings were better than either nursery seedlings or cuttings in all respects. But only in percentage of resin and yield of rubber per plant were they significantly better than cuttings.

They noted that the higher percentages of rubber were associated with the larger plants and that their results were not in line with those of Kelley, et al. (*141*), who found a negative correlation in growth of nursery seedlings under conditions of soil moisture stress; or with those of Federer (*96*), who found a negative correlation between plant size and rubber concentration for the commercial variety 593. Erickson and Smith (*91*) offer some possible explanations for the positive correlation they obtained. The positive relation between rubber percentage and size of the three types of plant material used might indicate that they had started to grow at different times, the early recovery giving advantage of size as well as age of tissues for rubber. Relative amounts of root system and reserve foods also could be important factors in determining size.

Although vegetative propagation of guayule may be of scientific interest in presenting opportunities to study homogeneous material, Erickson and Smith concluded that this method "is obviously impractical for commercial application in the production of rubber plantations."

Seed dormancy

Freshly harvested guayule seed quickly enters a period of dormancy and will germinate only up to approximately 6 percent. The failure to obtain good germination can be accounted for by two types of dormancy operating independently: (1) an inner seedcoat dormancy that may last 12 months or longer; and (2) an embryo dormancy of approximately 2 months' duration, first reported by Benedict and Robinson (*33*). The length of time required for the complete disappearance of the inner seedcoat dormancy varies with different seed lots and with the manner in which the seed is stored. The inner seedcoat is a thin, tenacious membrane enclosing the embryo, and in freshly harvested seed, it is highly impermeable to the exchange of carbon dioxide and oxygen gases.

Benedict and Robinson (*33*) reported that the breaking of seed dormancy may be hastened in several ways. Scarifying or puncturing the inner seedcoat of seed 8 weeks old or older was found to increase germination to approximately 95 percent, which seemed to indicate that the tough, impermeable seedcoat is the most important cause of delayed germination except in freshly harvested seed. Threshing the seed by removing the accessory floral parts from the achenes also hastens germination. Apparently the pericarp wall is weakened by threshing, thereby allowing greater access of gases to the inner seedcoat and thus more rapid oxidation. Complete germination of threshed seed was obtained after approximately 6 months. The embryo dormancy of freshly harvested seed (less than approximately 8 weeks old) could be partially broken by stratification or by storing the seed at about 4° C. in moist sand or peat prior to scarification. Roe (*187*)* indicated the possibility of guayule seed being sensitive to light during germination, and Benedict and Robinson (*33*) showed that a higher percentage of freshly harvested seed germinated in the light than in the dark.

An effective and expedient means of breaking seedcoat dormancy is the treatment with sodium or calcium hypochlorite, a method first described by McCallum under U.S. Patent No. 1,735,835 (*151*), and later made available to the U.S. Department of Agriculture. With certain modifications, it became the standard treatment in all nursery work involving dormant, unthreshed seed. The treatment consisted of washing unthreshed seed in water for 18 hours, after which it was soaked for 2 hours in a sodium hypochlorite solution containing 1.5 percent by weight of available chlorine, used at the rate of 2½ gallons per pound of seed (20 to 1 by weight). With threshed achenes, Benedict and Robinson (*33*) reduced the amount of available chlorine to 0.5 percent. Seed was then rinsed in water and either dried or planted while still moist.

Benedict and Robinson (*33*) demonstrated that other agents such as proper concentrations of hydrogen peroxide, perchloric acid, or nitric acid also would induce guayule seed to germinate, indicating that the oxidizing action of the sodium hypochlorite on the seedcoat was responsible for breaking the dormancy, and that the sodium hypochlorite itself did not have any specific property. In spite of these treatments the seed did not germinate fully until the embryo dormancy had disappeared.

Emparan and Tysdal (*87*) demonstrated that both embryo and inner seedcoat dormancy of freshly harvested seeds could be completely broken with the proper use of light and temperature in addition to the sodium hypochlorite treatment. They treated threshed seeds with a ¾-percent sodium hypochlorite solution for 2 hours, washed, and dried them. They then surface-planted the treated seeds in flats, where they were exposed to daylight for 4 days, during which they were kept constantly moist. Subsequently, a standard cover of 6 mm. (approximately ¼ inch) of sand was applied.

Hammond (*120*) reported that gibberellin also could break the dormancy of guayule seed, and compared the effects of gibberellin and sodium hypochlorite in combination with light and depth of planting.

In a preliminary experiment, light alone was found effective in breaking completely the embryo and inner seedcoat dormancy of freshly harvested achenes when these were germinated on moist sand in petri dishes and exposed to daylight for approximately 3 weeks.[7] Gibberellin, applied by soaking the achenes in an aqueous solution of 1,000 p.p.m. for 6 hours or longer, broke both embryo and inner seedcoat dormancy of freshly harvested achenes under an immediate ¼-inch sand cover without light, while sodium hypochlorite, an oxidizing agent, was equally effective but required a supplementary 4-day light treatment during germination. Progressively reducing the depth of immediate sand covers from ⅓ to ⅛ inch greatly increased germination of untreated achenes and achenes treated with sodium hypochlorite, mainly because of the effect of transmitted light through sand. These results confirm observations of Engstrom (*88*)* which indicated "that equally good or better stands were obtained in the beds with reduced depth of [sand] cover." However, he offered no explanation for the better stands.

Diseases of Guayule

Until after the Emergency Rubber Project was organized in 1942, comparatively little was known concerning the diseases of guayule. Lloyd (*145*) described briefly a disease fungus, *Puccinia parthenii* Arth., a rust of little importance found generally distributed through-

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[7] In native stands of guayule, the immediate breaking of seed dormancy by continuous exposure to daylight would be advantageous during favorable moisture periods sufficiently prolonged to allow young seedlings to become established.

out natural stands of guayule. He also reported it on plants grown under irrigation. The fungus appeared to attack only the older leaves that still remained attached from the previous year. Under dry conditions these leaves are usually shed, leaving only terminal clusters of small new leaves. The rust has never been reported or guayule north of the Mexican border.

Following the expanded planting program of the Emergency Rubber Project in California, Arizona, New Mexico, and Texas, several diseases of importance were found in cultivated stands, and studied extensively by numerous workers. A summary of the studies of these diseases and their control was made by Campbell and Presley (*50*). Addicott (*2*) described injuries of guayule seedlings resulting from splash caused by rain or wind.

Plantation and nursery diseases

Campbell and Presley (*50*) state that "on most irrigated and nonirrigated, well-drained soils, loss from disease was not an important factor in the production of nursery or field-grown guayule. However, serious disease losses occurred in localized areas on poorly-drained soils under irrigation and on well-drained soils when excessive amounts of water were applied. Careful control of irrigation was found necessary to avoid disease loss on many irrigated plantings."

Verticillium wilt. Verticillium wilt (fig. 28) is caused by the soil-inhabiting fungus *Verticillium albo-atrum* Reinke and Berth. The disease is distributed generally and is prevalent in the Mississippi Delta and the Southwestern United States. This disease attacks the roots of a wide variety of cultivated orchard and crop plants and, by interfering with the water supply, induces a wilting of the tops.

Guayule is highly susceptible to this wilt, both in the nursery and in the plantation. The disease is characterized by a yellowing and

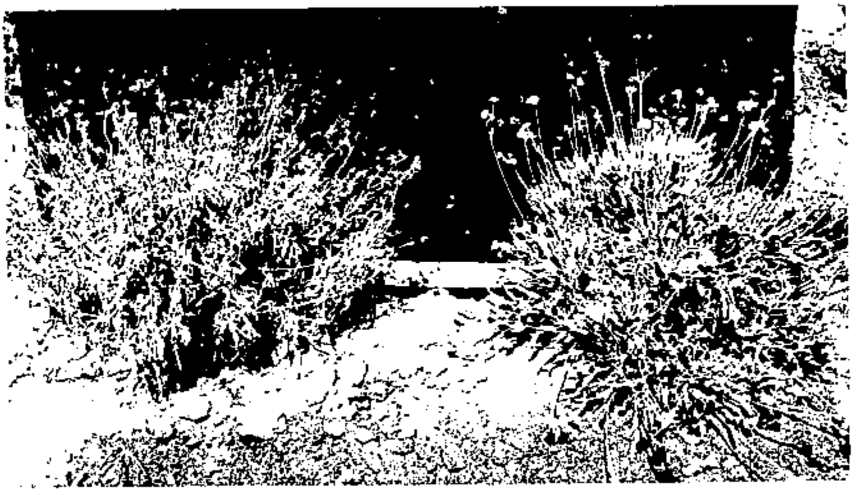

FIGURE 28.—Guayule plants affected by verticillium wilt. The plant on the right shows partial recovery from a spring infection.

dying of the lower leaves. Splitting the lower portion of the stem and tap root reveals a brownish streaking in the wood caused by discoloration of the cell walls and filling of the vessels with a brownish or yellowish insoluble gum (fig. 29).

The more severely infected plants, showing symptoms similar to plants suffering from drought, die. In less severely infected plants,

FIGURE 29.—Verticullium wilt on a guayule plant causing vascular injury.

occasionally a single branch or two will wilt before the symptoms appear in the rest of the plant. Under favorable conditions some of the less severely infected plants apparently recover. Mild infections in some guayule plantings result only in a stunting of the plants, in which case, if external symptoms are lacking, reductions in yield might not be attributable to verticillium wilt.

Considerable experimental work has been done to determine the influence of temperature and moisture on the development of verticillium wilt. Schneider (*200*) observed the behavior of plants grown in various places in California between 1943 and 1945 to test their reaction to verticillium wilt under different temperature and moisture conditions. His results seemed to show that the fungus is active in the soil at all moisture levels above the wilting point and that temperatures between 65° and 80° F. are most favorable for its growth. Summer temperatures in the Salinas Valley were considered optimum. The temperatures in the Bakersfield area were either above or below the range except in the spring and fall when the fungus became active. Prevention of infection in the Bakersfield area was obtained by holding the moisture content of the upper soil layers near the wilting point, a condition too dry for the satisfactory growth of guayule.

Campbell and Presley (*50*) concluded that there is at present no known method of effectively controlling verticillium wilt except by avoiding soils known to be infested, or using disease-resistant varieties.

Early investigations by Schneider (*200*) on some of Dr. McCallum's varieties showed highly significant differences in resistance to verticillium wilt. More recently, Gerstel's observations (*107*) on a planting made at Stanford University in 1947 indicated that diploids having 36 chromosomes were more severely affected by verticillium wilt than polyploids in an adjacent plot. In the following year, counts of diseased plants in field plantings made near Salinas showed highly significant differences in susceptibility to verticillium wilt between diploids, triploids, and tetraploids, the resistance increasing with chromosome number. The relative susceptibility of Dr. McCallum's polyploid varieties represented in these plantings followed the same trend as that of the same seven varieties observed earlier by Schneider (*200*).

The lack of representative selections from a larger number of natural populations led Gerstel (*107*) to doubt whether resistance to verticillium wilt in guayule was a function of polyploidy, or whether resistance might have developed independently of chromosome number in certain areas of Texas and Mexico where the original collections were made.

Although statistical data are lacking, observations in the guayule breeding nurseries at Salinas seemed to indicate, in general, that hybrid selections (of all chromosome levels) between guayule and *P. tomentosum* D.C., var. *stramonium* (Greene) Rollins were highly resistant to the wilt.

Phymatotrichum root rot. *Phymatotrichum omnivorum* (Shear) Dugg., the fungus causing this disease, also known as Cotton or Texas Root Rot, is widely distributed in calcareous soils throughout southwestern North America. It has been found on more than 2,000 different kinds of cultivated and wild plants. It is highly destructive to cotton in Texas, Arizona, and Mexico.

The disease was first observed on guayule in 1930 by Ezekiel (*94*) in a guayule experimental planting of the Intercontinental Rubber Company at Dilley, Tex., where he found a loss of about 6 percent of the plants as compared with a 70-percent loss of cotton plants in some nearby fields. Subsequent inoculation experiments confirmed the relative susceptibility of guayule and cotton to Phymatotrichum root rot.

The disease was again observed by Presley (*181*) in 1943 and 1944 in several of the Emergency Rubber Project guayule plantings in Texas. The most severe losses (fig. 30) occurred in the warm areas of southeast Texas, which also have a relatively high rainfall. Presley also observed in experimental plots having differential irrigation treatments that the rate of mortality from root rot increased in those which received the most water.

The disease is generally characterized by a sudden wilting of lush plants, drying and curling of the leaves, as a result of leisions girdling the taproot. Wilting is less pronounced in diseased plants not in a state of lush growth. If the roots are not too severely affected, the plants may recover during the winter when the fungus is in a dormant state.

Since similar symptoms are induced by other root-rot fungi, a positive diagnosis can be obtained only by isolating the causal organism,

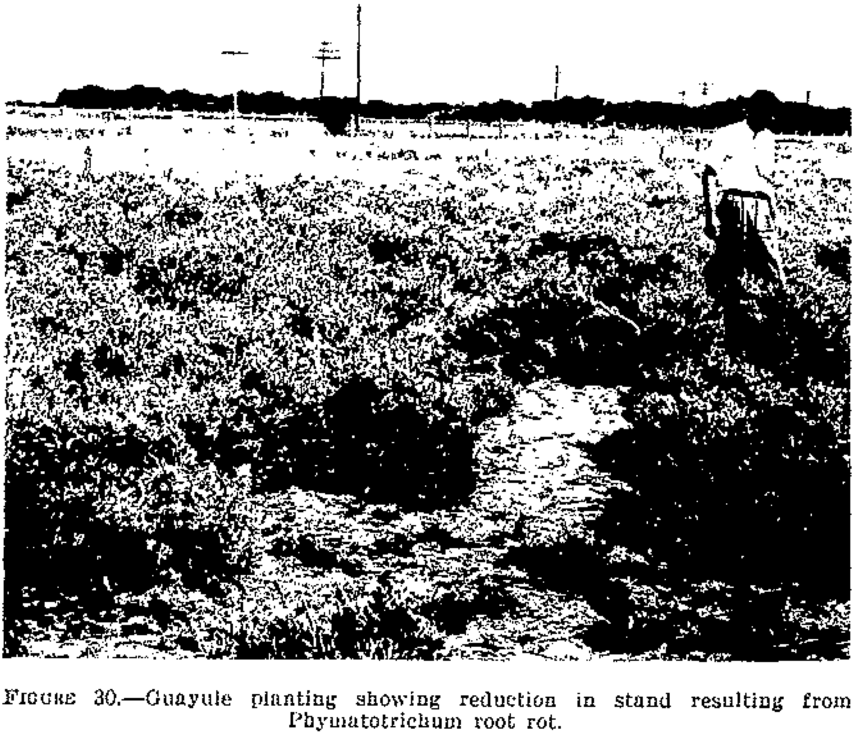

FIGURE 30.—Guayule planting showing reduction in stand resulting from Phymatotrichum root rot.

Diagnosis of Phymatotrichum in the field sometimes may be made if yellowish or brownish, fuzzy, mycelia are detected on the surface of the roots. Freshly diseased tissues are brownish and become darker and shredded with age.

Land known to be heavily infected by Phymatotrichum, especially in areas similar to those of southeast Texas, or any heavy soils under irrigation, regardless of location if the disease is known to be present, should be avoided. Light nonirrigated soils where moisture conditions are less favorable for the organism are to be preferred.

Charcoal, or crown rot. Charcoal rot is caused by the fungus *Sclerotium bataticola* Taub. It is widely distributed in warm climates and is prevalent in the drier central and southwestern United States, causing losses in a wide variety of cultivated crops, such as corn, sorghum, beans, sweet potatoes, and cucurbits.

The presence of charcoal rot in guayule often may be detected in the field by splitting the lower stem and root lengthwise. The internal tissues show a black discoloration, and dark brown, sunken lesions often appear at or below the soil line, followed by a progressive dying of the tops.

The first extensive outbreak of the disease on guayule was observed by Presley (*180*) in 2-year-old dryland plantings near Pearsall, Tex., after an abnormal period of hot, dry weather during the summer of 1944.

Norton and Frank (*170*) reported another serious outbreak that occurred during the summer of 1951 in guayule nurseries in the Winter Garden area of southwest Texas during a period of hot, dry weather. Damage was estimated to be around 5 percent, with losses up to 50 percent or more in some localities. It was noted that increased irrigation, despite high air temperatures, greatly reduced the loss from charcoal rot.

It was also noted that in areas where charcoal rot infection was comparatively light, damage was less severe in plantings of narrow spacing than in plantings of wider spacing. Similar observations were made by Presley (*180*) during the outbreak of the disease near Pearsall in 1944. Counts made August 22 on 10-, 20-, and 40-inch spacings indicated losses of 6.5, 8.9, and 10.3 percent, respectively.

The correlation between closer spacing and lesser degree of infection has been observed consistently. This correlation was difficult to explain. Tingey (*227*) showed that soil samples taken for moisture determinations around closely spaced plants became drier during irrigation experiments than those taken from the wider spacings. It was frequently observed that widely spaced plants grew more vigorously than closely spaced plants. Norton and Frank (*170*) suggested that the less vigorously growing plants possibly develop a greater thickening of the outer root layers, which might retard the entrance of the fungus. They also suggested that a greater storage of resins or other substances under these conditions could conceivably increase the resistance in the slower-growing plants.

Charcoal rot, which occurs in the nursery as well as in the plantation, has become a disease of increasing importance during recent years. It was found, apparently for the first time, in 1957, on guayule growing in California, although it is known to occur on various other crop plants in the warm valleys. Both pycnidia and sclerotia were found on the plants and the same organism was recovered in culture plates. Norton (*169*) reported that *Macrophomina phaseoli* (Maulb.) Ashby, the pycnidial form of the fungus, had never been found on guayule.

It is now well established that charcoal rot is favored by hot, dry weather following a period of lush growth. Applications of water will readily control the disease in spite of high temperatures. Clean cultivation to conserve moisture during the drier periods is also important (*13*). Breeding varieties of guayule resistant to charcoal rot seems to offer the best solution, since irrigation water is expensive and not always available or even desirable because conditions are not always favorable for high-rubber yields.

It had been observed repeatedly in Texas that Dr. McCallum's commercial variety 593 was more highly susceptible to charcoal rot than the more recently developed 4265–X, and there were also indications that other selections of guayule showed differences in susceptibility. Hybrids with *P. tomentosum* D.C., var. *stramonium* (Greene) Rollins grown in Texas, showed a high degree of resistance to charcoal rot (*13*).

Sclerotinia rot. *Sclerotinia sclerotiorum* (Lib.) DBy. and *S. minor* Jagger caused the greatest loss of seedlings in the Salinas nurseries, as well as many plants in storage in 1942 and 1943.

These fungi are most common in areas of continuously cool, moist weather. They are two of the most destructive diseases of vegetable crops in the field and in storage. Both species attack beans, lettuce, carrots, and other crops; the incidence of infection in the guayule nurseries was closely correlated with previous infections on such susceptible crops.

The affected plants are characterized by a soft, watery, odorless, rot of stem and root tissue. White cottony tufts of mycelium (fig. 31) form on the surface of the diseased plants, later giving rise to long-lived sclerotia, which are capable of perpetuating and increasing the infections.

As in the case of most pathogenic fungi attacking guayule, losses due to Sclerotinia are closely related to soil moisture and favorable growth temperatures. Excessive irrigations and poorly drained areas should be avoided, especially land on which susceptible vegetable crops have been grown.

Campbell (*48*) observed the loss of plants caused by *S. minor* and *S. sclerotiorum* in a 256-acre guayule planting near Salinas, Calif. over a 3-year period. Under moderate irrigation in 1943 the loss was less than 1 percent. Very few plants died in 1944 when no irrigation was applied, whereas in 1945, when heavy summer irrigations were applied, the average loss was 9 percent, ranging from 5.3 to 14.4 percent for different areas. Isolations from plants taken at random showed that 83 percent of the loss was caused by *S. minor* and 17 percent by *S. sclerotiorum*.

Phytophthora rot. *Phytophthora drechsleri* Tucker, the causal agent of this rot, has been observed in most areas where guayule has been planted. It affects both the root and crown, and it is characterized by a sudden wilting of the plant. The root lesions are black and are slightly sunken and firm. The diseased cortex is dark brown to green-

FIGURE 31.—Guayule plants affected by Sclerotinia rot.

ish black on freshly dug plants, becoming dull black upon drying. Woody portions near the lesions are also discolored.

This fungus requires abundant soil moisture and an optimum soil temperature of approximately 85° F. for its development. Since soil temperatures below 60° F. check the growth of the fungus, it, therefore, rarely develops during the period of dormancy in guayule.

The rot has been prevalent in guayule on irrigated land. It has been found occasionally in dryland plantings on heavy soils which have retained sufficient moisture from winter rains to initiate infections when soil temperatures become favorable.

In order to control this disease, it is obviously essential to avoid excessive irrigations. Conditions of oversaturation may be difficult to avoid on poorly drained or heavy soils.

Seedling root rot. *Pythium ultimum* Trow was the causal agent of a root rot of nursery and direct field seedlings of guayule reported by Campbell and Sleeth (*51*) in California. When plants in the cotyledonary stage were affected, the disease was known as "seedling root rot." When the organism attacked tap roots or root crowns of older seedlings from 6 to 16 weeks, the disease was commonly referred to as "pink rot," owing to the pinkish or reddish color of the woody cylinder at the lesion. If not too severely affected, the seedlings may recover under good growing conditions especially when there is a reduction of moisture in the upper 2 to 3 inches of soil. Recovery is brought about by the formation of lateral roots above the lesions of the tap root. However, if the lesions occur at or near the crown level of the tap root, sudden wilting and death of the plant may result.

Although the overall losses in seedlings were not serious, considerable mortality occurred in localized areas, especially in heavy, poorly drained soils.

Campbell and Sleeth (*52*) isolated three distinct types of *Pythium ultimum*, which they classified as O, OS, and S on the basis of relative abundance of oospores and sporangia when grown on Difco cornmeal agar. All were equally effective in causing preemergence loss of guayule seedlings.

Botrytis rot. *Botrytis cinerea* Pers, the causal organism of this rot, was commonly associated with *Sclerotinia* in the same areas in the nurseries. Being ordinarily a saprophyte, it attacks living guayule tissues only under the most favorable conditions. The infection starts on dead or nearly dead leaves and spreads to the living tissues, forming a soft rot which is soon covered by tufts of gray mold, the fruiting stage of the fungus (fig. 32).

Nursery stock dug and packed during the rainy season became infected in the same manner if sufficient moisture was present in the crates.

The disease is readily controlled in the nursery by avoiding excessive watering.

Diplodia dieback. Diplodia dieback (fig. 33) caused by the fungus *Diplodia theobromae* (Pat.) Nowell, was first observed by Presley (*183*) in guayule plantings in southern Texas following the summer rains of 1944. The disease was most serious where the plants were in a succulent and crowded condition after 2 years under irrigation. The disease was retarded during the following dry weather and the activity of the fungus ceased during the winter. The disease resumed activity

FIGURE 32.—Planting stocks of guayule attacked by Botrytis rot.

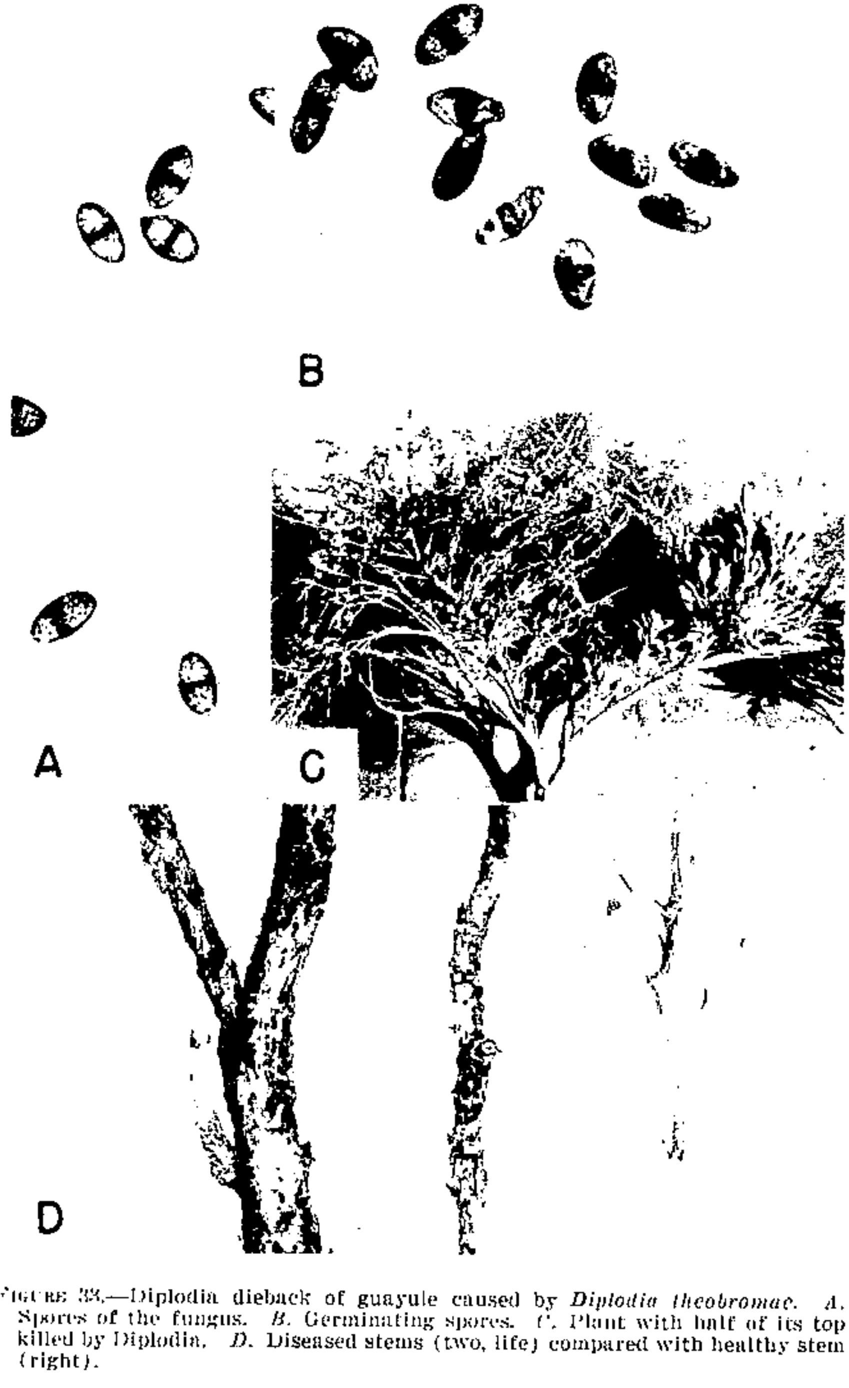

FIGURE 33.—Diplodia dieback of guayule caused by *Diplodia theobromae*. *A*, Spores of the fungus. *B*, Germinating spores. *C*, Plant with half of its top killed by Diplodia. *D*, Diseased stems (two, left) compared with healthy stem (right).

upon the return of high temperatures and rain during the following spring of 1945. Relatively little damage from the disease was done in dryland plantings and in 1-year-old irrigated plantings.

Damping-off fungi. The fungi commonly associated with damping-off of guayule seedlings are species of *Pythium*, *Rhizoctonia*, *Fusarium*, and *Phytophthora*. *Pythium ultimum* Trow was considered the most important of these in field and nursery plantings in California.

In the series of experiments with various fungicides, Sleeth (*202*) concluded that treating seed with sodium hypochlorite (primarily used in pretreating freshly harvested guayule seed to break dormancy) increased seedling emergence in the greenhouse and nurseries but that the protective effect of the sodium hypochlorite pretreatment was not as great as that of some of the commercial fungicides such as Arasan or Spergon when applied at the rate of 1 pound to 100 pounds of seed.

An effective method recently developed in the Salinas greenhouses to control both pre-emergence and post-emergence damping-off to guayule seedlings consisted of an initial treatment directly after seeding with a solution of Parzate (zinc ethylene bisdithiocarbonate) by dissolving 12 ml. in 5 gallons of water. After 2 or 3 weeks or when signs of damping-off occurred, the seedlings were again treated, this time with a solution of Semesan prepared by dissolving 1 tablespoon of fungicide in 1 gallon of water.

Bacterial rot. A bacterial root and stem disease caused by *Erwinia* sp., with symptoms differing from those caused by *Phytophthora drechsleri* Tucker was first observed by Campbell (*49*) in May 1944 in an irrigated planting of guayule near Bakersfield, Calif. M. P. Starr (*209*) identified the causal organism of this disease and suggested the name *Erwinia carotovora* f. sp. *parthenii* f. sp. nov. The disease was observed in 1944 and 1945 in other irrigated plantings in the San Joaquin Valley of California. Severe losses from the disease occurred in several plantings near Bakersfield following heavy irrigations in the late summer of 1945.

The first recognized symptom of the bacterial disease is wilting, the degree of which is determined by the vegetative condition when attacked and by weather conditions. Succulent plants succumb quickly, without recovery, during hot weather. Nonsucculent plants usually show a progressive wilting of the branches. In cool weather nonsucculent plants often do not show signs by wilting but, instead, the leaves gradually turn yellow and dry on the plant.

The disease is otherwise characterized at first by a yellowish or light brown resinous exudate on the lower part of the main branches which quickly turns dark brown and eventually black. An adhering layer of resin and earth may be observed on the upper tap root and root crown when a diseased plant is pulled. The root lesions differ from those caused by *Phytophthora drechsleri* in that the diseased tissue underneath the lesions does not become blackened or sunken. The diseased root cortex beneath the lesions is usually slightly discolored and is softer than healthy tissue. The freshly cut surface turns a dirty red or pink before finally becoming brown instead of the olive-green oxidation color of healthy root tissues under the same conditions. Older diseased tissue usually has a dull reddish brown color and may be almost black in the final stages of disintegration. In its earlier stages the pink pigment of the diseased tissue is water soluble. In the cambium and newly-formed wood a pronounced pink or reddish discoloration may be present for some distance in advance of the lesions.

To a limited extent, root lesions may form on 2- or 3-year-old plants which are not supplied with abundant moisture during the summer but are irrigated only once or twice during the season. These lesions may be checked by drying, but they become active again under subsequent irrigations, even in moderate amounts. Therefore, the initial losses are only a fraction of the eventual loss since further mortality continues after each subsequent irrigation. The original outbreaks causing severe losses from the disease were always observed after heavy irrigations.

Storage diseases

The most common fungi growing on crated seedlings in storage are *Sclerotinia* and *Botrytis.* If superficial molds appear, the plants should be scrutinized for these two fungi, which cause disease under the same conditions.

Sclerotinia rot. Storage rot caused by *Sclerotinia sclerotiorum* and *S. minor* was common and destructive in 1942 and 1943. Loss from rot was related to infections in the nurseries and to the time of year when the plants were pulled and packed.

Plants dug during the dry seasons were relatively free from disease. Plants packed under conditions of low humidity did not contain sufficient moisture for growth of these fungi, if present.

When conditions provided sufficient moisture for the development of apothecia of *S. sclerotiorum*, many plants became infected by ascospores. Plants in the initial stages of disease could not be detected in sorting for crating, and these served as sources of infection. When humidities were high, the surface of the leaves were often moist when packed. A combination of abundant moisture and storage temperatures that were frequently too high (above 32° to 34° F.) to check the growth of fungi caused a rapid spread of *Sclerotinia* from infected plants.

Sclerotinia under storage conditions develops at first a profuse, white, cottony mycelium, which spreads outward to neighboring plants, frequently forming characteristic cottony tufts. Later, these tufts become nodular and turn into black, irregular-shaped sclerotia.

Groups of diseased plants, or nests, are usually conspicuous because of the white mycelium that binds them together. *Sclerotinia* does not produce spores in the crate; the only means of spread from plant to plant is by mycelium.

When sclerotinia rot is present, the crated stock should be carefully examined before planting. If only one or two nests are found in a crate, these and neighboring plants may be removed. However, if several nests are present, or if a considerable portion of the plants are infected, the entire crate should be discarded.

Botrytis rot. Botrytis rot was also common and almost as destructive as *Sclerotinia* on nursery stock in storage in the spring of 1943. The spores are generally distributed throughout the nurseries and may be present on nursery stock when crated. These spores germinate under favorable moisture conditions, producing mycelia, which attack the crated plants and cause soft rot. Spore-bearing mycelia, producing millions of spores, quickly develop. Moving or jarring the crates may scatter the spores and cause new centers of infection.

Botrytis also requires moisture for its development and is most common on plants dug during rainy seasons when they cannot be dried to a desirable moisture condition before packing. The fungus is checked by temperatures around 32° to 34° F., but develops readily near 40° F.

The rot may be recognized by a gray fuzzy mold growing on diseased plants. All the plants in crates containing infected seedlings should be discarded. Even though many of them appear healthy, incipient infections may develop and kill the plants in the field.

Insects and Other Pests

Extensive investigations of insect pests found in the greenhouse, nurseries, and in fields were conducted by the Emergency Rubber Project. Cassidy et al. (*53*) summarized the literature pertaining to insects and mites infesting guayule with notes on control.

In general, the potentialities of damage from insects were apparently not serious. Of the several dozen species studied, the two types of most economic importance in guayule fields and nurseries were grasshoppers and *Lygus* bugs.

Grasshoppers were the most destructive to guayule in the Sacramento, San Joaquin, and Salinas Valleys of California and in Arizona, New Mexico, and Texas. Several species were involved. They bred within the guayule fields and also migrated in from range lands, alfalfa fields, roadsides, and ditch banks. The amount of damage inflicted by grasshoppers depended largely on the age of the plants. On young, sprouting transplants, the grasshoppers usually started at the base and fed upward, removing the cortex from the main branches. At this stage, very little feeding killed the plants. On established guayule in full leaf and in flower, the grasshoppers usually started to feed on the flowers and, moving downward, defoliated and removed the cortex from the branches. Plants completely defoliated usually died, but plants not completely defoliated usually recovered under favorable growing conditions. Grasshoppers were successfully controlled by poison bait recommended by the Bureau of Entomology and Plant Quarantine.

Romney et al. (*197*) reported that *Lygus* bugs were at times seriously detrimental to guayule seed crops in California. Since guayule remains in flower over a long period of the year under irrigated conditions, it was a very favorable breeding host for several plant bugs, the most important of which were *Lygus hesperus* Knight and *L. sallei* Stal.

Lygus hesperus occurred throughout the guayule plantings in northern and central California. Romney et al. (*197*) observed the behavior of these bugs under both cage and field conditions. They found that *L. hesperus* reduced the weight and viability of guayule seed. As long as seeds of pre-dough stage were available, the insects sucked the contents from them. When these were no longer available, they fed on the current season's shoots, thereby inhibiting growth and flowering.

Lygus sallei occurred only along the coastal areas of California. These bugs apparently did not feed on the seed of guayule, as did *L. hesperus*. They fed primarily on the growing terminals, causing the plants to become yellowed and stunted, as if diseased. These plants often failed to bloom. Upon removal of the insects, the plants resumed normal growth, but only after several months, indicating that the feeding of the insects was injurious to them.

Addicott and Romney (*5*) studied the anatomical effects of injury to guayule by *L. hesperus* in a controlled cage experiment. After 10 days, the leaves of branch tips of infested plants were gray and withered. Microscopic examination of the tissues at the stem apex showed collapsed cells and lack of detail in their structure. Lower down, the damage was less extensive, areas of injured cells being interspersed with areas of intact cells. Damage of tissues 10 mm. or more from the stem apex was largely confined to the phloem. Their observations indicated that *Lygus*, during its feeding, apparently injects a toxic substance into the host, which results in the death of the terminal meristem, young leaves, and phloem near the stem tip. These injuries were restricted to the area of feeding, as evidenced by the eventual development and growth of lateral buds near the stem tip.

Cassidy et al. (*54*) obtained reduction in *Lygus* populations and increased germination through the use of arsenical dusts. Romney et al. (*196*) were able to reduce *Lygus* bugs effectively and increase the viability of guayule seed by applying 5 and 2½ percent DDT dusts at an average rate of 33 to 34 pounds per acre.

During the flowering season of 1945, Romney (*194*)* conducted a field experiment to determine the effect of DDT dusts on seed quality and quantity by treating plots often enough to keep *Lygus* populations relatively low. Between May 28 and September 28, he made five applications of 2½ percent DDT dust at the rate of 30 to 35 pounds per acre to control two generations of *L. hesperus*. Two applications 7 to 10 days apart were necessary to control a given generation since the first application did not provide a lethal residual effect on nymphs that had not hatched. He obtained an average filled seed count of 40.6 percent in the treated plots, as compared to 14.3 percent in the untreated plots, and an increase of 26.8 percent of cleaned seed by weight.

Romney and Cassidy (*195*) reported an insect (*Anaphes ovijentatus* Crosby & Leonard), which parasitized the eggs of *Lygus hesperus* deposited in the receptacles of guayule flower heads. In a study of egg collections made during August 1944 in guayule fields near Salinas, Calif., from 10 to 70 percent of *Lygus hesperus* eggs were found parasitized. Previously, egg parasites of plant bugs of the genus *Lygus* had been reported only from New York and Canada.

A new species of mite, *Aceria parthenii* Keifer, was reported and described by Keifer (*130*) in 1952 as being a pest of guayule. These mites were first noticed during that year in the guayule greenhouses at Salinas, among surface hairs on twigs and leaves of guayule, sometimes causing a rather severe longitudinal curling of the leaves.

TB 1327 (1965) USDA TECHNICAL BULLETINS UPDATA
RESEARCH ON GUAYULE (PARTHENIUM ARGENTATUM) 1942-1959
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The so-called guayule aphid (*Cerosipha californica* Essig), reported and described by Essig (*93*) in 1944, is apparently synonymous with *Rhopalosiphum subterraneum* Mason, a species previously described by Mason (*149*). This aphid was occasionally found in small bark and root cavities made only on young guayule nursery stock by wireworms and other insects. It is fairly common in California, mainly infesting roots of cereal crops.

Because of the seriousness and wide distribution of nematodes in California, Allen and Thorne (*15*)* and Allen (*14*)* made surveys of the prevalence and importance of these organisms in nurseries and field plantings of guayule. They concluded that guayule was a very unfavorable host to rootknot nematodes, and that these were not of economic importance in guayule production. Fields known to be heavily infested with nematodes produced very satisfactory yields of rubber. The only serious aspect was the danger of introducing nematodes on transplants to infestation-free lands, and the condemnation of nursery stock through quarantine regulations.

Genetics and Plant Breeding

Modes of reproduction

Upon the organization of the Emergency Rubber Project in 1942, Powers, McCallum, and Olson made seed collections of guayule and mariola (*Parthenium incanum* K.B.K.) in Mexico. Powers (*176*)* and Federer collected seeds of these two species in Texas. Seeds of these collections, as well as those of commercial strains developed by McCallum prior to 1942, were germinated and planted at Salinas in 1943.

Bergner (*34*, *35*) began a detailed study of chromosomes and chromosome numbers in pollen mother cells to facilitate genetic and extensive breeding experiments. In native stands it soon became apparent that a chromosome series existed, which fell into three groups containing 36, 54±, and 72± chromosomes (*35*). Working independently, at approximately the same time, Stebbins and Kodani (*210*) came to the same conclusion. Stebbins and Kodani (*210*) and Powers and Rollins (*179*) also found in mariola a similar series. Since bivalents were found in 36-chromosome plants and trivalents in 54-chromosome plants, Bergner (*35*) assumed 18 to be the basic chromosome number.

Five collections of guayule seed gathered by Powers and McCallum in an area southwest of Mapimi, Durango, Mexico, were diploid ($2n=36$). Five triploid ($3n=54\pm$) collections were found, one in this same general area and the other four near San Bartolo, Durango. In the latter area, the senior author made two diploid collections in 1948. Nine tetraploids ($4n=72\pm$) were collected by Powers and McCallum, one in the Mapimi area, one in southern Nuevo Leon, one near Catorce, San Luis Potosi, and the remaining six in northeast Zacatecas. Only tetraploid stands were found in Texas. No natural stands of pentaploids or hexaploids were ever found. Chromosome levels higher than 72 were found only in individual plants in triploid and tetraploid populations, and in their progenies. The highest chromosome number was 144, which Powers (*177*) reported among offspring of 108±-chromosome plants. Guayule plants having less than 36 chromosomes were never found.

Bergner's investigations[5] indicated that meiosis is complete in the pollen mother cells at all polyploid levels of the series. Diploid plants showed 18 bivalents at metaphase I and the distribution of these at anaphase I was equal. In triploids, trivalents were always seen in addition to bivalents and univalents. Tetraploids had quadrivalents, trivalents, bivalents, and univalents in variable numbers. The tendency toward equal chromosome distribution in tetraploids at anaphase I was more pronounced than in triploids. In plants of higher chromosome levels, there was an excess of bivalents at metaphase I, and the distribution at anaphase I tended toward a more pronounced equality than in triploids, in spite of their larger chromosome numbers.

Powers and Rollins (*179*) in a comprehensive study of this material, reported for the first time the occurrence of apomixis in guayule, which they demonstrated in a series of breeding experiments. These experiments revealed a predominance of maternal types in F_1 generations of intra- and interspecific crosses. They also demonstrated that the mode of reproduction varied in relation to chromosome numbers. Plants having 36 chromosomes (diploid) were found to reproduce sexually. Plants of the polyploid series were predominantly facultative apomicts and were pseudogamous, as was shown by the absence of well-developed embryos when pollination was excluded.

Esau (*92*) confirmed the findings of Powers and Rollins in her studies of the morphology of reproduction in guayule. She presented evidence of both sexual and apomictic methods of reproduction. Plants characterized by sexual reproduction showed a normal sequence of events during megasporogenesis and formation of the 8-nucleate embryo sac, with subsequent fertilization of the eggs and triple fusion. Embryo and endosperm development were closely correlated, and no embryos were formed in the absence of pollination.

In apomictic reproduction, unlike sexual reproduction, there was no orderly sequence of stages in embryo-sac formation. The megaspore mother cells usually persisted in the ovules and assumed the appearance of uninucleate embryo sacs, indicating the ommission of meiosis (apomeiosis) and the development of the embryo sac directly from the megaspore mother cell (generative apospory). In nonpolli-

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[5] Bergner's material for studying guayule chromosomes consisted mainly of pollen mother cells augmented by dividing tapetal cells and root-tip cells. A 7:1 absolute-alcohol-acetic-acid mixture was used for fixing young flower heads. Chromosomes were stained in aceto-orcein. She encountered some difficulty in making chromosome counts during meiosis, owing to clumping or stickiness of the chromosomes. In later years, the addition of chloroform to the fixing solution in the ratio of 1 part acetic acid, 3 parts absolute alcohol, and 4 parts chloroform has given good results.

Meyer (*160*) encountered difficulties in studying root-tip chromosomes of guayule because they were small, numerous, and tangled. He found that pretreatment with paradichlorobenzene permitted precise chromosome studies. After pretreatment with paradichlorobenzene, Gerstel (*106*) fixed root tips of guayule directly in $^1/_{10}$ dilution of concentrated hydrochloric acid at 60° C for 10 to 14 minutes with good results, thus combining fixation and macerating hydrolysis into one step and saving considerable time.

nated material, small embryos were observed, indicating that fertilization of the egg of an unreduced gametophyte was not necessary to start the development of an embryo. However, pollination was apparently necessary for endosperm development or at least for the stimulation of normal growth of the embryo (unreduced pseudogamy). Meiosis also was observed in her material, indicating that apomixis in polyploid guayule is facultative.

Powers and Rollins (*179*) reported that diploid populations showed a considerable morphological diversity within different collections as a result of amphimixis, cross-pollination, and being largely self-incompatible. Triploid and tetraploid stands, on the other hand, were fairly uniform within themselves as a result of apomictic reproduction, although they differed from one another in morphological characteristics. Nevertheless, among triploid and tetraploid populations, three types of morphological variants were distinguished: polyhaploids, offtype normals, and aberrants. These variants were the result of amphimixis, since apomixis was considered only facultative.

A number of polyhaploids were found in tetraploid (72± chromosome) populations under cultivation. These resulted from the development without fertilization (parthenogensis) of eggs which had the reduced number of chromosomes. Esau (*92*) showed that normal meiosis could occur in plants predominantly aposporous, indicating that some eggs with the reduced number of chromosomes could be produced. Polyhaploids are, in general, poor pollen producers and slow growers but flower profusely. The flower heads are small, a characteristic which quite readily distinguishes them from other plants in a tetraploid population.

Offtype normals occasionally appeared in triploid (54± chromosomes) and tetraploid (72± chromosomes) populations as a result of normal meiosis followed by fertilization. Bergner (*35*) found that most of the offtype normals were aneuploids, the chromosome numbers of the triploid offtype normals ranging from 54 to 66, and the tetraploid from 65 to 75. Analyses of anaphase I in these populations revealed an unequal distribution of chromosomes in the pollen grains. Such a variation in chromosome numbers of offtype normals was explained on the assumption that there was an equivalent variability in the reduced chromosome numbers in the egg nuclei. Most offtype normals showed diverse morphological characteristics. They were usually smaller than the apomicts, presumably as a result of chromosomal unbalance.

Aberrant plants occurred in apomictic triploid (54± chromosomes) and tetraploid (72± chromosomes) populations, arising through abnormal amphimixis (nonreduction followed by fertilization). Bergner (*35*) reported 80 to 93 chromosomes for aberrants in the triploid populations, and 99 to 120 chromosomes for aberrants in the tetraploid populations. The aberrants were characteristically slow growing and had thick and irregularly formed leaves. The flower stalks and flowers were usually thicker and larger than those of the populations from which they were derived, and were often distorted. Federer (*96*) reported that the progeny of aberrants were mostly aberrant, but occasionally normal plants appeared among their progeny. Bergner (*35*) reported 12 offtype normals from aberrants. These varied in chromosome numbers from 74 to 93, and probably arose through amphimixis. Since these showed only a few of the phenotypic characteristics of aberrants, they probably indicated more successful genic balances despite the number of chromosomes.

As a result of reproduction by apomixis, the apomictic progeny of an individual plant should be expected to duplicate fully all the characteristics of this plant. However, even under carefully controlled environmental conditions, Rollins (*190*) found in guayule and mariola nearly always a few individuals which deviated from the mother plant to some degree. Since the majority of the plants of the progenies were strictly maternal, some type of genetic segregation apparently had occurred in the mother plants either before or during the formation of the seeds which produced the deviating maternal plants. Genetic segregation might have occurred in somatic cells giving rise to the female gametophyte. However, the possibility that segregation might have occurred as a result of atypical mitotic nuclear divisions with some features of meiosis during the formation of the gametophyte offered a more plausible explanation.

Inheritance of apomixis

Gardner (*105*) concluded from studies involving the progenies of two guayule-mariola hybrids, some of which reproduced sexually, that the apomictic processes, i.e., failure of reduction, failure of fertilization, and pseudogamous development, are controlled independently, and that the major factors controlling the sexual processes are few and apparently dominant. Powers (*177*) had found earlier in progeny tests of material studied by him that the number of major gene pairs differentiating fertilization from failure of fertilization are few, probably not more than two, and that fertilization is at least partially dominant to failure of fertilization.

The existence of both sexual and apomictic plants with the same chromosome numbers in guayule led Gerstel and Mishanec (*110*) to investigate and analyze more extensively the inheritance of apomixis. In these experiments they used a sexual diploid plant ($2n=36$), an apomictic polyhaploid derived from an apomictic tetraploid ($4n=72$) by reduction without fertilization, and the closely related 36-chromosome sexual *Parthenium stramonium* (*Parthenium tomentosum* DC., var. *stramonium* (Green) Rollins).

The cross, apomictic polyhaploid ♀ x sexual diploid ♂, produced maternals, diploid F_1, and triploids. The reciprocal cross, sexual diploid ♀ x apomictic polyhaploid ♂, produced only diploid F_1 plants. Diploid F_1 from both types of crosses were crossed with *P. stramonium*, a closely related sexual diploid species having widely different morphological characteristics. All plants obtained from these two crosses were shown to be interspecific hybrids by characteristics intermediate between those of the parents and were entirely sexual. Since all the diploid plants from both crosses were sexual, it was concluded that sexuality is dominant and apomixis recessive.

Triploids from the cross apomictic polyhaploid x sexual diploid which had originated by fertilization of unreduced eggs—two genomes from the apomictic parent and one from the sexual—were pollinated by *P. stramonium*. A few interspecific hybrids were produced from this cross, the majority being maternal facultative apomicts.

In another experiment, tetraploids obtained from colchicine-treated sexual diploids were crossed with *P. stramonium* to observe the effect of duplication of chromosome numbers on the method of sexual reproduction of their diploid progenitors. Almost all the progeny were triploid hybrids containing two genomes of *P. argentatum* and one genome of *P. stramonium*.

The existence of apomictic diploids (polyhaploids) and colchicine-produced tetraploids, together with the occasional occurrence of sexual polyploids in wild guayule indicated to Gardner (*102*) that apomixis is not an obligatory consequence of polyploidy. Rather, it appeared that the genotype determines the behavior of a given plant, and that the number of chromosomes is less important. These findings were supported by those of Powers (*177*), who observed that nonreduction is influenced by the genotype and not by changes in the polyploid level. Gerstel and Mishanec (*110*) concluded, however, that two doses of genes for apomixis are dominant over one dose of genes for sexuality, whereas in F_1 plants with equal doses of genes for apomixis and sexuality, sexuality is dominant. They stated, "While apomixis, on the whole, is determined by the genotype, chromosome numbers may have modifying quantitative effects."

In a test reported by Gerstel et al. (*109*), all female F_2 derivatives from a limited number of crosses between a diploid apomict and a sexual diploid arose sexually. These were crossed with *P. stramonium*. Only interspecific hybrids and no maternals were found among 52 progeny families, indicating the probability that apomixis in guayule is based on at least four recessive genes; a minimum of two controlling meiosis and two more, the requirements for fertilization. However, there was some doubt whether on the diploid level these two components of apomixis are independent as they were demonstrated by Powers (*177*) and by Kramer (*143*) to be in polyploid apomicts.

Powers (*177*) put forth a hypothesis as to the evolution of apomixis and polyploidy, suggesting that both had evolved together, and that either one would have had great difficulty in becoming established and surviving without the other. He listed three major steps involved in the evolution of apomixis from normal sexuality—namely, (1) failure of reduction in number of chromosomes, (2) ~~failure~~ of fertilization, and (3) development of nonreduced egg cells without fertilization. Evidence was given to show that it would be much more difficult for apomixis to become established in diploids than in polyploids and that, in agamic complexes such as those present in guayule, normal sexuality would have difficulty in becoming established in the polyploids.

Inheritance of self-incompatibility

The inheritance of self-incompatibility in diploid ($2n=36$) guayule, which is nearly always self-sterile, was intensively studied by Gerstel (*108*). Incompatibility reactions were observed mostly by microscopic examinations of pollen tubes in stigmata one to two hours after pollination, a technique developed by Gerstel and Riner (*111*).

Diploid parent plants numbered SP–1 to SP–15 were obtained from a collection presumably made in Mexico by the late Dr. W. B. McCallum, in which they occurred intermingled with polyploids.

The progeny of the cross SP–8 and SP–7 was most intensively studied. Sibs were tested for self-incompatibility and in all cases their own pollen failed on their stigmata. When they were tested reciprocally with both parents, four distinct classes resulted (fig. 34). These were designated A, B, C, and D, according to the following observations:

Pollen of parent SP–7 produced tubes in the stigmata of all plants placed in classes A and B, but the pollen of A and B did not cross with SP–7 either way. They could be separated by testing with SP–8 since C pollen functioned on SP–8, and SP–8 pollen failed on C stigmata. D was reciprocally compatible with SP–8.

All members of class A were incompatible with those of class B, and all members of class C were incompatible with those of D. Those of B with C and with D were also reciprocally compatible. Class A

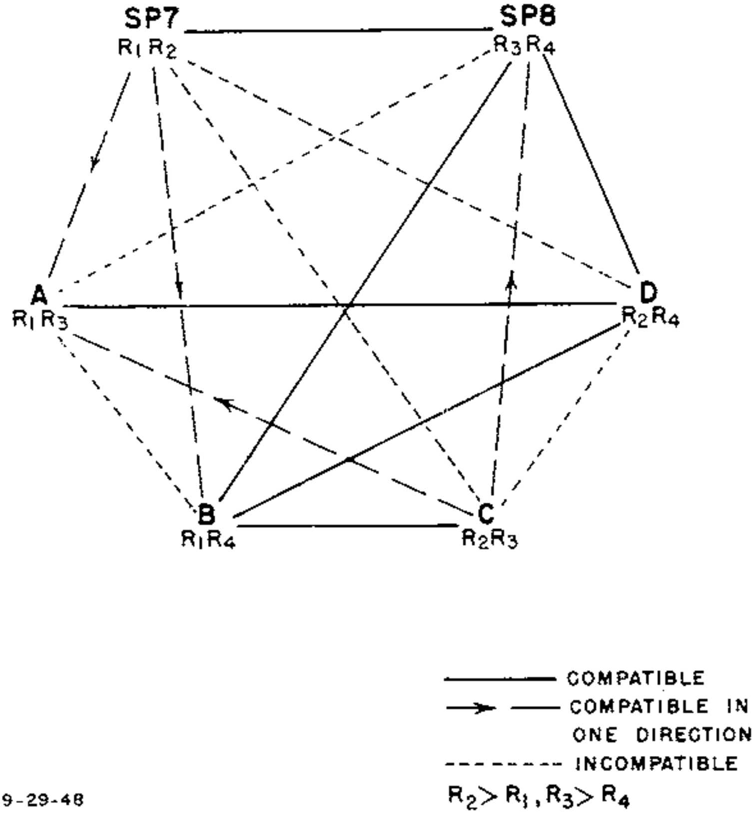

FIGURE 34.—Cross compatibilities and incompatibilities in one family of diploid guayule.

was compatible with class C provided class A was used as the male parent.

Analogous results with respect to incompatibility were obtained in a study of the progeny of the reciprocal cross SP–7 x SP–8. Tetraploid plants obtained by colchicine treatment of sibseedlings of the cross SP–8 x SP–7 were self-incompatible and gave exactly the same incompatibility classes as the diploid sibs. It was consequently postulated that there existed a series of multiple alleles which controlled self-incompatibility. The alleles were given the Mendelian symbols R_1, R_2, R_3, and R_4. Certain pairs of these were assigned to the following phenotypes: $SP–7=R_1R_2$; $SP–8=R_3R_4$; $A=R_1R_3$; $B=R_1R_4$; $C=R_2R_3$; and $D=R_2R_4$.

Incompatibilities were assumed to be controlled sporophytically; that is, the two alleles present in the pollen parent determined the reaction of all the pollen grains produced, and these acted alike regardless of which allele each grain contained. The differences in behavior between certain crosses and their reciprocals were explained by the dominance in the pollen (but not in the stigma) of R_2 over the other alleles and of R_1 and R_3 over R_4, which was recessive to the other three. R_4 is actually recessive in both stigma and pollen. The expression of dominance in the pollen but not in the style was "found to occur in only one other plant, a species of Crepis, which, like guayule, is a member of the Compositae."

Genetic variations

In material ordinarily regarded as belonging to *Parthenium argentatum* Gray, a considerable amount of heterogeneity was found throughout the species range, even in different sexual or facultatively apomictic populations of the same geographical area. Rollins (*192*) attributed these variations to the existence of sexuality, apomixis, polyploidy, and interspecific hybridization, which singly, or in combination, produced a very complex evolutionary pattern. A portion of the variability found was attributed by Powers and Rollins (*179*) and Rollins (*188, 189*) to introgressive hybridization with mariola (*P. incanum* H.B.K.), a related species (fig. 35), found coexistent throughout a large portion of the natural range of guayule. Considerable variation independent of introgression from mariola was found in facultatively apomictic, as well as in sexual, populations (*192*). Both genetic heterogeneity and heterozygosity were responsible for producing the variability found in sexual populations, whereas heterogeneity alone was involved in producing the variability found in the facultatively apomictic populations. Powers and Gardner (*178*) studied the occurrence of aborted pollen grains in all available guayule collections and found a high genetic variability in the percentage. On the basis of these studies, they concluded that both heterogeneity and heterozygosity were important factors in determining variability in the many collections of guayule.

Introgression from mariola was most common in the Texas portion of the range and in the southeast portion of the range in Mexico where the States of Coahuila, Nuevo Leon, San Luis Potosi, and Zacatecas adjoin. The least amount of introgression was found in the

FIGURE 35.—Blooming plants of guayule (*Parthenium argentatum* Gray) (left) and mariola (*P. incanum* H.B.K.) (right).

middle portion of the range in northeastern Durango, where the sexual diploid plants of guayule were found, and presumably predominated. Although these sexual diploid plants would cross readily with mariola in culture, Rollins (*191*) stated that hybrids apparently occurred rarely under natural conditions. On the other hand, where facultative apomixis was found to predominate, evidence of interspecific crossing frequently occurred. Rollins (*192*) presented evidence to show that polyploidy permitted wide interspecific crossing which otherwise would be impossible, and that fertility was higher than in hybrids on the diploid level. Hybridization between the diploid forms of the two species apparently was less effective in producing persistent new biotypes or introgressed populations. Polyploidy, on the other hand, facilitated initial interspecific crossing and allowed the progeny to survive, with apomixis acting as an isolating mechanism. Many populations showing introgression from mariola were found over widespread areas.

In populations not showing introgression from mariola, the range of variation in the facultatively apomictic populations was nearly as great as that in the sexual ones. A sufficient amount of segregation apparently was permitted by the less frequent sexual process in the production of seeds to keep apomictic populations nearly as heterogeneous as those of the strictly sexual ones.

It is difficult, if not impossible, to locate the geographical origin of most species of plants. Rollins (*193*) presents strong evidence that the area of origin of guayule is in the general region of eastern Durango in Mexico. Sexual diploid forms were found only in this area, whereas facultatively apomictic polyploids predominated elsewhere in the range. Obviously diploidy must have preceded polyploidy, and sexuality undoubtedly preceded apomixis.

Breeding for higher rubber yield and disease resistance

The major objectives of the guayule breeding work were to develop varieties (1) yielding more shrub per acre with a higher percentage of rubber in the plant, (2) having a greater resistance to disease, particularly to charcoal rot in Texas, and (3) having the ability to produce a higher percentage of rubber under hot climatic conditions such as occur in Texas and in other regions where guayule might be grown.

Since the breeding nursery at Salinas did not furnish all the genotypes necessary to attain these objectives, Powers (*176*),* McCallum, and Olson made extensive seed collections in 1942 in the Trans-Pecos area of Texas, and in Mexico in the States of Nuevo Leon, San Luis Potosi, Zacatecas, and Durango. Four hundred and forty-three collections were made from 44 locations. In 1948 Hammond (*119*)* made additional seed collections in Mexico. More than 175 individual plant and mass collections were made from 76 locations in the following six States: Nuevo Leon, Coahuila, Chihuahua, Durango, Zacatecas, and San Luis Potosi.

Upon examination of the progenies of the introductions made by Powers et al., it soon became evident that the predominant method of reproduction in guayule is facultative apomixis, although sexual reproduction may occur (see section, Modes of Reproduction). Strains of guayule having 54 or more chromosomes usually reproduce apomictically while those having 36 chromosomes reproduce sexually.

When guayule reproduces by facultative apomixis, a certain amount of reduction takes place, the frequency of which depends upon the particular plant or selection. It is through these chance reductions with subsequent fertilization that new combinations can be obtained. Occasionally an unreduced gamete will accept a pollen genome, resulting in a plant of a higher chromosome level. Table 1 gives the various chromosome combinations in guayule, all of which have been identified. Only 36- and 72-chromosome plants were used as pollen parents in controlled crosses.

Since the percentage of fertilization of unreduced eggs is small, as is also the percentage of reduced eggs which may be fertilized, it is necessary, when controlled crosses are desired to make large numbers of these. For this reason, the breeding work was supplemented by extensive selections within combined open-pollinated, facultatively apomictic and sexual populations.

The degree of apomixis plays an important role in breeding and selection. The higher the frequency of sexual off-types, the greater is the possibility of obtaining favorable combinations, whereas a low frequency of sexual off-types is more desirable for greater uniformity.

Guayule, therefore, presents an unusually fine opportunity for breeding and selection for higher rubber yields. A variety of types of combinations may be made in crosses, as shown in table 1. In selecting desirable progenies that will reproduce from seed without excessive

TABLE 1.—*Chromosome combination in guayule* [1]

Chromosomes of parent	Chromosomes of progeny			
	Nonreduction		Reduction	
	Not fertilized	Fertilized	Not fertilized	Fertilized
Number	*Number*	*Number*	*Number*	*Number*
36 (diploid)	----------	----------	----------	36, 54
54	54	72, 90	----------	----------
72	72	90, 108	36	54, 72
90	90	108	----------	----------

[1] The basic number of chromosomes is 18. Since unequal reduction takes place in 54- and 90-chromosome plants, these were rated as poor pollen parents.

segregation, crosses and backcrosses must be manipulated in such a way that a high degree of apomixis may either be maintained or introduced. Gerstel and Mishanec (*110*) have shown that two doses of genes for apomixis are dominant over one dose of genes for sexuality, whereas in plants with equal doses of genes for apomixis and sexuality, sexuality is dominant.

In his studies on self-incompatibility in guayule, Gerstel (*108*) has pointed out certain difficulties involved in breeding 36-chromosome sexual guayule, which is rarely self-compatible. In addition to self-incompatibility, which always interferes with the use of inbreeding methods, there may exist incompatibilities which place restrictions upon the use of sib crosses, and in certain crosses all of the progeny may be incompatible with one parent, an additional handicap placed upon the guayule breeder who may wish to depend upon the use of backcrosses in his work.

Tysdal (*240*) stated that there are several species of *Parthenium* which may be of value in breeding programs. Among these, the almost treelike *P. tomentosum* DC., var. *stramonium* (Greene) Rollins (fig. 36) was the most promising. It has 36 chromosomes (2n) and reproduces sexually, but contains little or no rubber. It is highly compatible with guayule, producing large and vigorously growing hybrids. Both sexual and highly apomictic hybrids were obtained.

Interspecific hybrids between these two species were tested in Texas and in California. Those tested in Texas were highly resistant to charcoal rot, a disease prevalent there, caused by *Sclerotium bataticola* Taub. Although these hybrids were always lower in rubber percent than high-rubber-yielding strains of pure guayule, indicating apparently a strong linkage between vigor and absence of rubber in *P. tomentosum* var. *stramonium*, some of them outyielded the commercial variety 593 because of their greater vigor. Owing to the lower percentage of rubber in the vigorously growing hybrids, the cost of milling the shrub would have been correspondingly greater. In a longer-range breeding program than was permitted, higher rubber-yielding

FIGURE 36.—Five-year-old plants of *Parthenium argentatum* Gray (guayule, left) and *P. tomentosum* var. *stramonium* (Greene) Rollins (right).

hybrids might have been obtained through more extensive hybridization between these two species.

The commercial variety 593, developed by the Intercontinental Rubber Company, was used as the standard of comparison with new strains and hybrids. Among the several hundred collections made by Powers in 1942, one group from Durango, identified as 4265, appeared to be superior to 593 in yield of rubber. However, this strain was extremely variable. Johnson (*135*) identified five different types in the original lot. Type I proved to be higher yielding in rubber than 593, but was still variable. It proved to contain 54 chromosomes. During a rather extensive greenhouse experiment with variety 4265–I, a superior plant was found by C. A. Taylor and H. M. Benedict, which proved to be a 72-chromosome segregate from 4265–I. In further progeny tests, it showed a higher rubber content and more uniformity than 4265–I. It was given the identification 4265–X and increased for additional testing. In Texas, variety 4265–X proved to be greatly superior to 593, particularly with reference to resistance to charcoal rot. It also accumulated a higher rubber content under both Texas and Salinas conditions than did variety 593. Both 4265–X and 4265–I were used extensively in crosses for higher rubber yields.

Collections made in Mexico by Hammond (*119*) in 1948 were planted at Salinas in the winter of 1948–49 and, where sufficient seedlings were available, some of them were transplanted in Texas. From these plantings, several outstanding selections were made. One of these was a highly apomictic, single plant selection from collection No. A–48136 obtained several miles south of Viesca, Coahuila, near the top of a hill along a road at Flores de Mayo Paredones. Cuesta de los Sorruedos. This selection proved to be not only more resistant to charcoal rot in Texas but also more vigorous, and contained a higher rubber percentage than variety 593. Tests over a 2-year period in Texas indicated a definite superiority of this selection, which was identified as N396, over both 593 and 4265–X. In the Salinas Valley, however, N396 did not show the outstanding vigor that it displayed in Texas. Furthermore, it was more highly susceptible to verticillium wilt under the Salinas Valley climate than any other of the introductions and advanced strains.

In the later years of the breeding program, most of the testing designed to select strains which accumulate rubber under hot climatic conditions was done at Shafter, Calif., where the climate is similar to that of southwest Texas. Plants in the 1955 Shafter variety test, were sampled again after 2 years, and N396 was found significantly higher in vigor and in rubber percentage than either 593 or 4265–X.

Plants in the 1956 Shafter variety test, which included 13 of the more promising 1948 Mexican introductions previously tested in the Salinas nurseries, were sampled in May 1958. Introductions A48118, A48115, and A48124 were significantly higher in yield of rubber than either 593 or 4265–X. (It may be stated here that in all variety tests conducted in Texas and at Shafter involving these as check varieties, 4265–X was always significantly higher than the old commercial variety 593 in vigor and in rubber percentage.) A48124 was particularly interesting from the standpoint of the possibility of its being a highly desirable variety for commercial planting. Among the five highest rubber-yielding selections in this test, it was significantly lower in dry weight, 682 grams per plant, as compared to an average of 827 grams for the other four, yet its significantly higher rubber concentration, 8.33 percent, as compared to 6.80 (the highest percentage of the other four), offset its lack of vigor, which would greatly reduce harvesting and milling costs.

Introductions A48116, A48121, and A48143, although not tested at Shafter, gave consistently better performance in the Salinas nurseries than varieties 593 and 4265–X. A seed increase of these introductions, as well as that of the four introductions described above, was made at Salinas. The seed was brought to 4 percent moisture and stored in air-tight, metal drums.

With the exception of A48143, these high-rubber-yielding introductions were obtained in the southeastern part of Durango. A48143 was collected nearby in northern Zacatecas. It may be recalled that N396 was collected in Coahuila in this same general area. 4265 also had been collected in the southeastern part of Durango. These introductions may be distinguished, for the most part, by differences in morphological characteristics and growth habits. Collections made elsewhere in Mexico were relatively low in rubber content. Collections made previously in Texas were also low in rubber content.

In a 2-year-old variety test sampled at Shafter in 1957, selections 11701, 11693, N565, 11634, and 11635, in that order, were significantly higher than the check varieties 593 and 4265–X in yield of rubber. Check variety 593 yielded an average of 30.2 grams of rubber per plant; 4265–X yielded 45.2 grams; 11701, 57.4 grams; 11693, 61.0 grams; N565, 64.9 grams; 11634, 65.4 grams; and 11635, 71.3 grams of rubber per plant.

Selection 11701 is a 72-chromosome descendant of an open-pollinated cross between a 54-chromosome plant of 4265–I and a 36-chromosome plant of unknown origin. 7.6 percent of the plants of this selection in this test were off-types; i.e., reduction in the number of chromosomes had occurred with subsequent fertilization by pollen from an unknown source. Selection 11693 is also a 72-chromosome cross. It was found among plants in a 54-chromosome 4265–I selection, 6.9 percent of the plants being off-types. N565 is a 72-chromosome, open-pollinated cross from strain S1811–206 of 4265–I origin. This selection produced 4.4 percent off-type plants. Selection 11634 has 72 chromosomes. It was traced through open-pollinated maternal descendants to an open-pollinated, 36-chromosome cross between SP–7 and SP–8 (N322 from an old nursery selection, S4838–74). Being of 36-chromosome sexual origin, it is interesting to note that this 72-chromosome selection produced, in the Shafter test, only 4.5 percent off-type plants, indicating a fairly high degree of apomixis. Selection 11635, the highest rubber-yielding strain of this test, is a 72-chromosome controlled cross between a 54-chromosome 4265–I plant and a plant of the 36-chromosome selection, N264. This open-pollinated selection produced only one off-type plant in a population of 648 plants, indicating an extremely high degree of apomixis.

In the 1956 Shafter variety test, sampled in 1958, selection 12229 (S2636–168) was significantly superior to 4265–X in rubber yield. It was from a single-plant selection from progeny of open-pollinated

S1873-143 (1950 Salinas nursery) derived from the open-pollinated accession 2106, supposedly a cross between 4265-Ig and a 36-chromosome hybrid between guayule and *Parthenium tomentosum* var. *stramonium*. It contained 56.9 grams of rubber per plant as compared to 40.5 grams for the check variety 4265-X.

Although not tested at Shafter, open-pollinated selections 11600, 11604, 11605, 11609, 11591, 11619, N566, and N565-II, all of 4265-I origin, were high-rubber-yielding in the Salinas nurseries. Seed increases of these slections, in addition to those of all other selections described above, were made. The seed was brought to a moisture content of 4 percent and sealed in metal drums.

Greenhouse and nursery operations

Seed obtained from the previous year's selections was usually planted in the greenhouse in December and January in flats to obtain sizeable seedlings for transplanting in May and June. The seed was first threshed in a small guayule air pressure thresher devised by Emparan (*86*), and then treated with Clorox to overcome any dormancy that might be present. (See section, Seed Dormancy.)

A soil-sand mixture in the ratio of 2:1 was used. The sand facilitated removing the soil in preparation for transplanting the plants bare-rooted. The mixture was poured into flats, carefully leveled with a metal, soil-leveling gauge, and then prewatered. The seed was scattered evenly over the surface. Nondormant seed was immediately covered with a ¼-inch layer of sand; dormant, treated seed was allowed to remain exposed to the light for 3 or 4 days before covering (see Seed Dormancy, p. 77, supra). The flats, as well as the soil and sand, were previously sterilized. For further protection against damping-off organisms, the plantings were immediately treated with a solution of Parzate (zinc-ethylene bisdithiocarbamate) prepared by dissolving 12 ml. in 5 gallons of water.

A commercial fertilizer was applied occasionally to obtain maximum growth. About 3 or 4 weeks prior to digging, fertilization was discontinued, and the amount of water used for maintaining optimum moisture conditions was reduced in order to harden the seedlings in preparation for transplanting.

At the time of digging, the plants were bare-rooted and grouped into hand-sized bundles. While these were held in the hand, the longer leaf portions and longer roots were trimmed off. The bundles, each containing an identifying pot label, were then wrapped in paper toweling, tied with rubber bands, and dipped in water.

The yearly nursery plantings usually consisted of some 6 to 8 acres of breeding material in rows 600 feet long. A standard 28- by 28-inch spacing was used. In early spring, the land was disked and mulched in preparation for planting in May or June. Just prior to planting, the field was cross-marked, furrowed lengthwise for irrigation, and then preirrigated, which was considered essential in order to keep the plants moist at all times. The transplants were set along the edge of the irrigation furrows at the intersections of the cross-markings, trowels being used to make openings in which the plants were inserted. The wet soil was then firmed around the plants.

This method of land preparation and transplanting often resulted in irregular or poor stands. Loss of plants was attributed mostly to air pockets left around the roots as a result of carelessness on the part of the planting crews.

Skips in the row had been observed frequently to have a marked effect on the growth of adjacent plants. The accelerated growth of these plants was attributed, at least in part, to lack of competition in response to delayed increases in soil-moisture tension in these areas. These conditions made difficult the selection of desirable plants, particularly in heterogeneous populations.

In the 1954 nursery planting, after first noting that well-hardened seedlings could withstand considerable dessication, a few rows were planted in dry soil without preirrigation for comparison with survival in adjacent plantings in preirrigated soil.[9] Irrigation immediately followed transplanting. Soon a marked difference was noted in the established stands. Survival in the rows in which the soil was dry at the time of transplanting and then irrigated was far superior to that in the preirrigated rows. The excellent survival was attributed to the absence of air pockets.

In view of these observations, the entire 1955 nursery and subsequent ones were planted without preirrigation. Other modifications were made in the older methods of planting. A brief description of the methods used in land preparation and planting of these nurseries is given below. (These methods are obviously valid only in areas requiring irrigation, such as the Salinas Valley.)

The land was disked and mulched soon after the winter rains ceased in order to conserve moisture up to planting time, usually in May or early June. Using the standard spacing of 28 by 28 inches, the field was first cross-marked with five 1-inch chisel points attached to standards of a cultivator bar at the rear of a tractor. In laying out the rows lengthwise, five 7-inch furrowers (crowder type) were substituted for the 1-inch chisel points to make irrigation furrows 3 to 4 inches deep. The crowder type was used to avoid throwing up too much soil at the sides of the furrows and obscuring the cross-markings. The 1-inch chisel points were replaced, this time for the purpose of lightly marking the rows lengthwise about 4 inches from the edge of the irrigation furrows. In marking the rows lengthwise, the rubber tires of the tractor served to round out and firm the irrigation furrows. The plants were set at the intersections of the cross and lengthwise markings.

In setting the plants, a trowel was thrust into the soil at approximately a 45-degree angle. A plant was slipped underneath the tilted trowel. The trowel was then withdrawn, which allowed the loose, dry soil to fall around the plant. No packing to avoid air pockets was necessary since the irrigation water, which immediately or soon followed, firmed the soil around the plants.

This method of planting had three chief advantages over the older methods: (1) Labor costs and time required for transplanting were reduced to approximately one-half; (2) the cost of preirrigation was eliminated; and (3) excellent stands resulted.

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[9] Unpublished report by the senior author.

TABLE 2.—*Guayule selections*[1] *included in latest comparisons*

Selection No.	Synonym	Applicable information
11591		Open-pollinated selection from 4265–I.
11600		Do.
11604		Do.
11605		Do.
11609		Do.
11619		Do.
11633	A–48124	Hammond collection having smaller plants than either 593 or 4265–X but higher total rubber.
11634		72-chromosome; from open-pollinated 36-chromosome cross between SP–7 and SP–8 (N–322 from an old nursery selection S4838–74).
11635	596	72-chromosome controlled cross between 54-chromosome 4265–I plant and a plant of 36-chromosome selection N–264.
11646	A–48121	Hammond collection with superior performance at Salinas.
11693	N–562	72-chromosome cross found among plants in a 54-chromosome 4265–I selection.
11701		72-chromosome descendant of open-pollinated cross between 54-chromosome 4265–I and 36-chromosome unknown.
A–48115	12231	Hammond collection.
A–48116	N–576	Hammond collection with good performance at Salinas.
A–48118		Hammond collection.
A–48121	11646	See 11646.
A–48124	11633	See 11633.
A–48143		Hammond collection from northern Zacatecas. Good at Salinas.
N–396		Single plant selection from Hammond collection A–48136 from several miles south of Viesca, Coah., Mexico. Resistant to charcoal rot; vigorous and high rubber content in Texas but not at Salinas.
N–566		Open-pollinated selection from 4265–I.
N–575	A–58143	See A–48143.
N–576	A–48116	See A–48116.
N–596	11635	See 11635.
12229		From single-plant selection from progeny of open-pollinated 2106 (supposedly a cross between 4265–Ig and a 36-chromosome hybrid) and guayule *Parthenium tomentosum* var. *stramonium*.
12231	A–48115	See A–48115.
593		Commercial variety developed by the Intercontinental Rubber Company.
4265		Collected by Powers in Durango in 1942. Highly variable.
4265–I		Selected from 4265 by Johnson; 54 chromosomes; variable.
4265–II		Open-pollinated selection from 4265.
4265–X		Selected from 4265–I by Taylor and Benedict; 72 chromosomes; higher rubber content and more uniform than 4265–I.
4265–XF		Seed increase of selected plants from 4255-X.

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[1] Seeds of most of these strains have been placed in storage.

Assaying of plant samples for rubber content

Without some method of assaying, the selection of guayule plants for high rubber content would be impossible. Morphological characteristics do not provide reliable clues as to the relative amount of rubber contained in the plant. The fact that most of the rubber is stored in the bark led to an investigation to determine if bark thickness of the guayule stem could be used as a criterion for rubber content (*9*). The results indicated that the relationship between percentage of rubber and thickness of bark was not sufficiently close for practical selection purposes. Earlier reports had suggested a direct relationship.

Predictions of rubber yield from guayule are needed to guide research and production, including both crop production and shrub processing, as well as studies of plant improvement. Such predictions depend on determinations of the rubber content of the material under study. Much effort has been put into finding adequate methods of sampling guayule and testing representative samples for rubber content to guide the various research and production activities.

Holmes and Robbins (*125*) made a study of methods used for the analysis of rubber-bearing plants, and summarized the background information.

Fendler (*97*) proposed the direct determination of rubber contained in guayule by extracting the plant juices with acetone, and dissolving the residue in petroleum ether: then filtering, precipitating the rubber from an aliquot with alcohol, filtering out the precipitate, and drying and weighing it as rubber. Spence (*206*) proposed a similar method but used benzene as the rubber solvent. He determined the rubber by simply evaporating the benzene, drying the residue in an atmosphere of carbon dioxide, and weighing. Fox (*100*) determined rubber in guayule by extraction, first with acetone, then with benzene; he then precipitated the rubber from the benzene with alcohol, and dried and weighed the precipitate. Whittelsey (*248*) determined rubber in guayule by extracting with carbon tetrachloride to remove the rubber; he evaporated the carbon tetrachloride on a steam bath, and boiled the rubber film with alcohol and water to remove any resin or water-solubles extracted with the rubber. After pouring off the water and alcohol, he dried the film and weighed it as rubber. Hall and Goodspeed (*117*) and Hall and Long (*118*), in their survey of the rubber content of North American plants, extracted the rubber by boiling samples for 3 hours in acetone and then for 3 hours in benzene. The acetone extract was evaporated and the residue weighed as resin: similarly the benzene extract residue was weighed as rubber.

In 1933, Spence and Caldwell (207) published the results of their work on the determination of rubber in guayule. They found carbon tetrachloride unsuitable for extracting rubber in analytical procedures, and used benzene instead. Their procedure consisted of the following steps:

1. Pass sample twice through differential laboratory mill rolls, set as tightly as possible.
2. Pass successively through a Universal grinder and corn mill. After each stage of grinding, quarter sample by a Jones riffler.

3. Weigh samples into special porcelain thimbles and boil in 1-percent sulfuric acid for 3 hours.
4. Autoclave at 13.6 kg. (30 pounds) pressure for 3 hours.
5. Extract for 12 hours with acetone.
6. Dry acetone from thimbles in vacuum oven.
7. Add antioxidant, evaporate the benzene on a water bath, dry, and weigh residue as rubber.

Holmes and Robbins (125) found that the treatment with sulfuric acid was not necessary if the samples were ground sufficiently fine, and determined that a satisfactory estimate of rubber content could be made as follows:

1. Immerse plants in boiling water for 8 minutes and shake off the leaves.
2. Dry at temperatures not exceeding 65° C. to about 10-percent moisture.
3. Chop through 5-mm. screen on cutter of Ball and Jewell type.
4. Take total weight, and remove subsample for determination of moisture by drying for 24 hours at 100° C.
5. Grind through 1.5-mm. screen in laboratory hammer mill.

Analytical procedures

1. Leach for 1 hour with water at 80° to 90° C.
2. Remove water from thimbles by suction.
3. Extract for 8 hours with acetone.

3a. If resin is to be determined, evaporate acetone on steam bath and dry extract in oven for 1 hour at 80° C.

4. Free thimbles of acetone by sucking air through them.
5. Extract for 16 hours with benzene.*
6. Evaporate benzene on steam bath and dry rubber film in oven for 1 hour at 80° C.

* Note. Tared flasks are used for benzene extractions and also for acetone extractions whenever resin is to be determined.

This process, with modifications suggested by Willits et al. (*251*), was used as the basic method of determining rubber content for the guidance of research and production programs. As research plots developed and multiplied, however, more rapid methods of analysis were needed to handle the increasing number of tests required. Traub (*235*) developed a method of partial precipitation of the rubber particles in a solution, and determination of the rubber content by measuring the turbidity of the resulting suspension. This process proved advantageous and, particularly in the post-war stages of the breeding program, made it possible to make thousands of analyses that would not have been possible with the limited personnel available at that time. The turbidometric system proved to be highly reproducible and relatively rapid. It did not provide any estimate of resin content nor of the quality of the rubber. An even faster method of testing was needed to meet the requirements of the breeding program.

Efforts directed toward developing a simpler method led to development in 1951 of a blendor method as a rapid and low-cost means of assaying rubber for plant selection (*201*).* The first machine used was the Waring Blendor, but the commercially available single-unit type was not sturdy enough to withstand continuous operation. It was soon replaced by a 4-spindle machine specially designed to eliminate the bearing in the bottom of the blendor bowl (figs. 37 and 38). The spindles were driven at the rate of approximately 13,000 r.p.m. by a ½-hp. motor and V-belt pulleys. Four multiple blendors of this design were placed in operation—three at Salinas and one in Texas.

Although the blendor method did not provide any estimate of resin content and was not as specific as the chemical methods, it proved satisfactory from the breeder's standpoint, both with respect to rapidity of test, and comparability of results. It was considered somewhat comparable to the commercial milling process. The method was essentially as follows:

One or more branches characteristic as to size were cut at the base of the plant. Thus, the plant was not injured or destroyed and, if found desirable, it could be utilized for seed collection or grafting. Sufficient material was taken to yield about 20 grams or more after trimming. The branches were bound with a heavy rubber band, and an identifying tag attached, which followed the sample through to completion.

In the laboratory, the samples were placed in boiling water for 15 minutes. This not only coagulated the latex, but after par-boiling, the leaves were readily removed by shaking. Green, lush wood, dead wood, and flower stems were trimmed off, and the branch samples rebound with a rubber band.

The branches were then ground in an intermediate Wiley laboratory mill. Fifteen grams of the ground material were weighed and transferred to a blendor bowl, together with 150 cc. of water and 95-percent ethyl alcohol in the ratio of 1:1. Zinc sulfate was added to the water-alcohol mixture to make a 0.25-percent solution to facilitate extraction. At the same time, moisture samples were taken to determine the moisture content of the assay material in order to convert later the weight of the extracted rubber to percent of rubber on a dry-weight basis.

The blendor bowl containing the sample was then placed in the blending machine, which was run for 15 minutes. At the end of this time the sample was thoroughly comminuted, and the rubber tended to agglomerate into small, spongelike masses called "worms." The contents of the bowl were than poured into a 500-cc. beaker and the beaker filled with a 0.5-percent table salt, deaerated water solution. The deaeration was essential to provide rapid settling of the bagasse, and the salt slightly raised the specific gravity to assure flotation of the rubber worms.

After a few moments the effluent was poured through a 30-mesh screen which retained the rubber worms along with some coarse particles of plant tissue. These were then rinsed under a water faucet. By moving the worms about in gathering them with forceps, they adhered to each other to form a single mass of rubber. This was placed on a dry blotter for 5 to 10 minutes to absorb the excess moisture and then deresinated in acetone for an hour, after which it was removed, dried and weighed. The average rubber so treated had a purity of

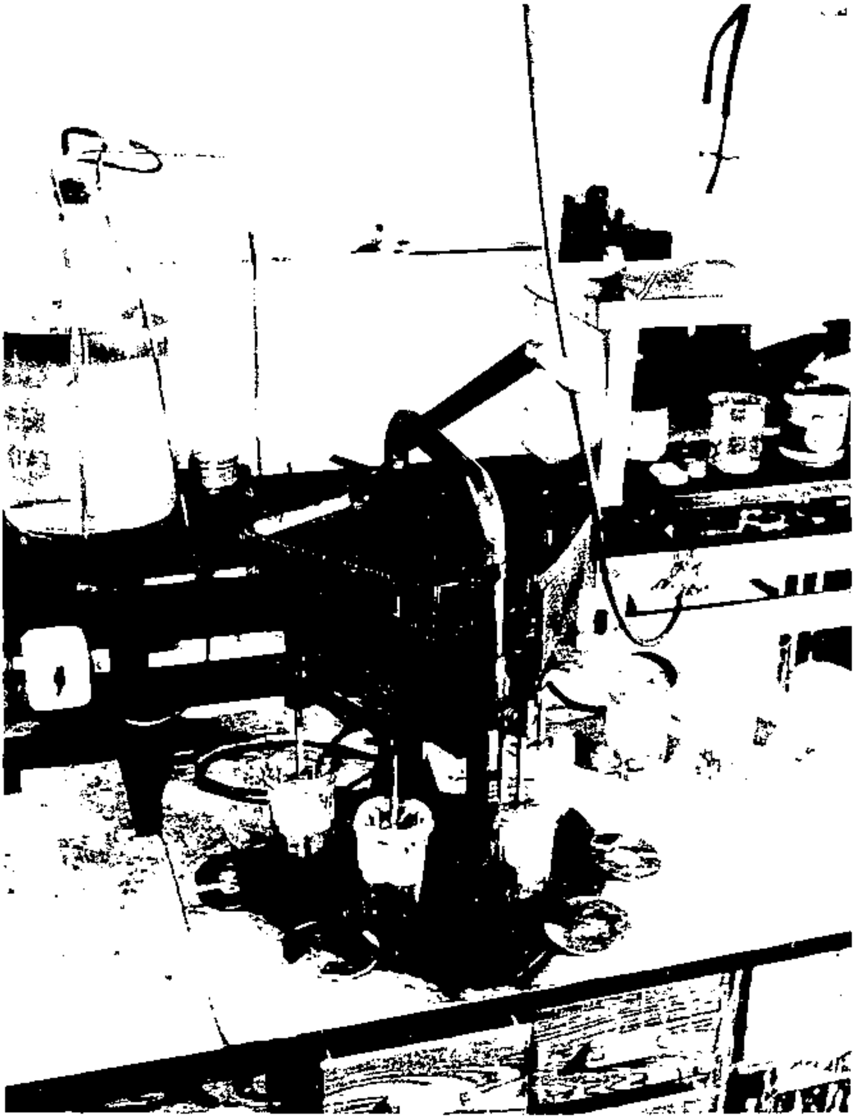

FIGURE 37.—Multiple blendor used in analysis of guayule for rubber content.

from about 85 to 90 percent as determined by the chemical method and could be graded primarily on color and texture.

Comparisons made of this method with the turbidometric method resulted in a high correlation of +.984. To convert weight of rubber worm to percent of rubber in the dry-weight shrub, the regression equation was $Y = 19.22X + 1.09$ where Y = percent of rubber in the shrub and X = grams of rubber extracted by blendor from 15 grams (wet weight) of shrub.

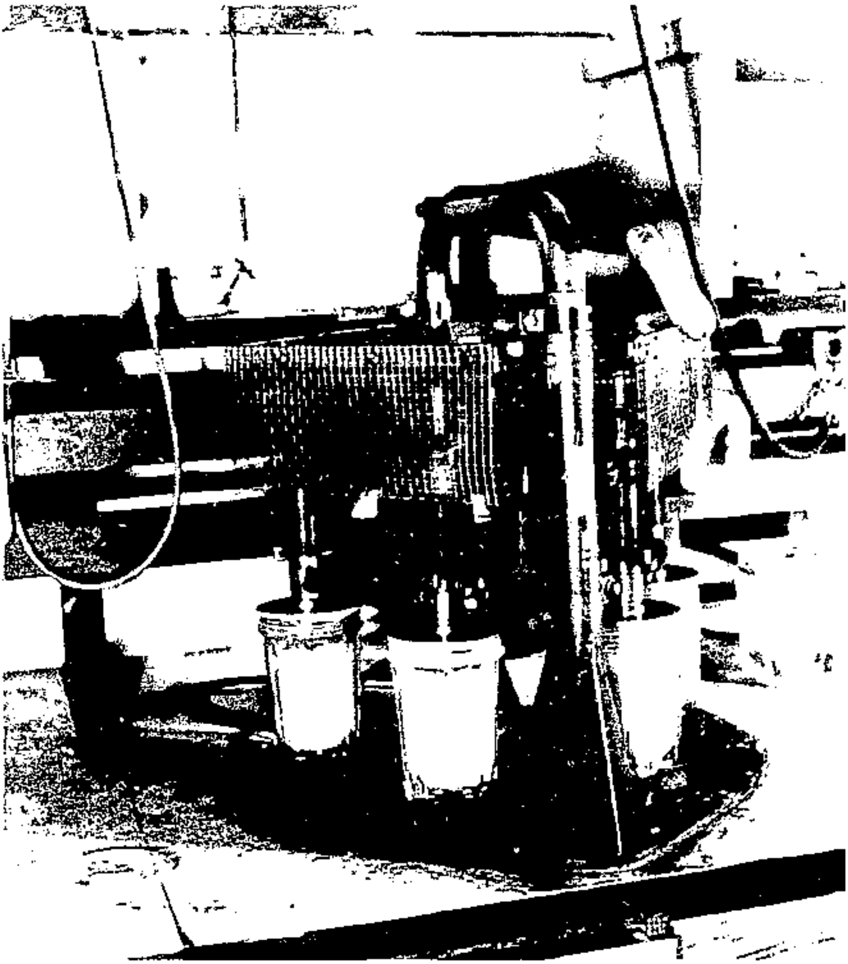

FIGURE 38.—Multiple blendor with blades lowered as in operation.

Since the shrub varied considerably from time to time in percentage of dry matter, it was necessary to make corrections on the weight of rubber (X) extracted by the blendor before applying the formula. Calculations were based on shrub samples having a dry-matter content of 48.4 percent. Thus, if the shrub had 40 percent dry matter, the rubber worm weight (X) was multiplied by the fraction 48.4/40, before using the regression equation. Likewise, if the shrub contained 60 percent dry matter, the fraction would be 48.4/60, i.e., 48.4/% D.M. of sample. For rapid calculations, tables were drawn up whereby percentage of rubber could be read directly from blendor worm weight and percentage of dry matter. For example, if the rubber worm weighed 1.59 gm. (after making correction for purity) and the dry matter content was 63 percent, the percentage of rubber in the shrub would read 17.38 percent. Tables were also drawn up whereby the dry weights of plants could be estimated from field height and spread measurements.

During the course of these investigations, it was found that plants differed with respect to ease of extraction of rubber mechanically. Tests showed that some plants or strains gave up to 85-percent extraction under the same conditions that others gave as low as 70-percent, when compared to the chemical method. Since the blendor method more nearly approached the method of commercial extraction, the mechanical assay would conceivably give a more practical result than chemical analysis. However, owing to lack of actual milling data, this was not possible to verify.

The blendor method of assay made possible the sampling and analyzing of as many as 300 plants per day with a staff of five people, none of whom had to be highly trained technicians whereas, in comparison, only about 100 chemical analyses could be made per day with four people. Progress in the breeding program for high rubber was largely dependent upon the number of samples that could be analyzed, and this method aided greatly in the breeding work.

PRODUCTION RESEARCH

Production research conducted directly by the Emergency Rubber Project personnel, or personnel of other agencies assigned to the Project, rather than by the research bureaus was of immediate importance to field operations. This research, under W. G. McGinnies, assisted by A. A. Nichol, cut across project lines, utilizing preliminary findings wherever necessary to obtain quick answers to field problems without waiting for the scientific checks and balances essential to careful detailed investigations, but exacting tests, required in the management of field practices, were also conducted. A good example of this type of research, and the close association between the research and production programs was that done on soils.

All Emergency Project lands were selected on the basis of detailed soil surveys. Thus it was necessary to organize this phase of the program at the very beginning. Soil surveyors were mapping and classifying land suitable for the cultivation of guayule almost immediately after the program was approved. Soil classification, of course, followed standard lines in accordance with well established principles.

In the beginning, the suitability of the soil for guayule culture was determined by information available from the Intercontinental Rubber Company. It was necessary, however, to have a continuing project to study the growth of guayule on the various soils, and to determine which soils gave the best response. It was also important to determine the cause of poor response on certain soils that appeared suited to guayule culture. Later it became necessary to record growth and rubber accumulation on the fields planted to guayule, and to determine the effect of soils on growth and rubber accumulation as a basis of estimating yields and planning cultural and harvesting operations.

Another example was research reported by Bullard (*42*). For field control, as well as for determining future farm practices in spacing, cultivating, and harvesting, it was necessary to establish a farm-by-farm inventory of the growing crop, including data on survival, growth, and rubber content.

This involved the establishment of a detailed inventory of shrub on more than 30,000 acres, some on irrigated, some on dry land, and on diverse soils with a considerable range in climatic factors. The first problem was to devise a sampling procedure to get maximum accuracy of estimation without excess cost. Shrub yield is, of course, derived from the amount of shrub on a given acreage. On the whole, the amount of shrub can be estimated by the size and weight of a single sample if that sample is large enough to represent the total variation; or a group of samples can be selected to provide as good or better a basis for estimation and at the same time give a measure of total variation. Bullard (*42*) found that a high degree of accuracy could be attained in the estimation of guayule shrub and rubber by selecting at random two test blocks from each 5-acre field in large plantings.

The test blocks consisted of four plants in each of five successive rows. Each of the 20 plants was measured with a yard stick. The height was measured from the root crown to the highest leaf. The diameter of each plant was measured along the row and across the row. Dead plants and skips were recorded by position within the plot. The plants in each plot were numbered from 1 to 20 and plant 6 in each block was pulled for chemical test for rubber. Should plant 6 be absent, number 7 would be chosen. The order of selection was then 10, 11, and on through 14 and 15 until a single living plant was found. The selected plant was weighed with a spring balance to 5 grams as soon as it was pulled, and then the diameter of the stem just below the lowest branch was measured to a millimeter with a small caliper. Two measurements were taken at right angles to each other. The samples were then forwarded to the laboratory, and the data sheets were forwarded to office personnel for calculation of the cubic content of the plants in the plot. The defoliated weight of the selected plant was obtained, and a general determination was made of the relationship of the nonrubber-bearing tissue to the entire weight of the plant. It was found that in irrigated shrub the leaf weight averaged 27 percent at the age of 1 year, 20 percent at 2, and 16 percent at 3 years of age. In dryland shrub, the leaf weight was somewhat higher, averaging 30 percent at 1 year of age, 25 percent at 2, and 21 percent at 3 years of age.

These data furnished a valid estimate of the volume and weight of the available shrub in the field. Laboratory tests provided information on the rubber content of the defoliated shrub, and this was used to make estimates of the amount of rubber in the field. This fulfilled the chief purpose of the sampling. Important aspects of guayule cultivation were revealed by the data collected in the sampling.

Survival in the fields sampled was seldom as high as 90 percent. Survivals as low as 65 percent were not uncommon. In the analysis of the sampling data, it was found that there was an inverse correlation of around −0.5 between survival rate and the crown volume of the individual plants. This showed that the remaining plants took advantage of the space not occupied by the dead or missing plants. The increase in growth of the remaining plants did not fully compensate for the missing plants in the 3-year period for which data were collected. In a longer program, full compensation might have been made. Bullard (*42*) states in regard to estimating error, "Ordinarily, the variation in survival found in one plantation is not particularly great. Where such is the case, any bias introduced by survival would be small."

Data collected in the sampling program indicated that the 28- by 20-inch spacing adopted in general for irrigated fields might be too close for adequate growth of the plants in a field rotation of more than 3 years. Even with the slower growth of dryland shrub, the adopted spacing of 28 by 24 inches would be inadequate if shrub maturity (maximum rubber) were not attained until the seventh or eighth year. It was suggested that possibly a return to a 36-inch between-row spacing might be indicated, with a greater density of plants within the row.

By coordinating the data from the sampling program with information obtained in the research programs, it was possible to obtain information on the effect of soils, climate, and culture on the yield of rubber. In general, it was adduced that close spacing, which increased the plant stress, should be practiced for early production of rubber, but that wide spacing would be better for long-time production. Mechanical cultivation and hand hoeing injured the plants. This injury could be minimized by the use of chemical herbicides. Diesel oil proved effective in field planting. The rubber content and condition of the shrub affected the efficiency of the milling of the shrub. These results indicated that fall harvest of shrub from dryland plantings would be best, while late fall and winter harvest would be best for shrub on irrigated lands.

Production research conducted directly by the Forest Service, or in close collaboration with the research agencies through assignment of research personnel to the Forest Service, also made a major contribution in the form of field surveys of native stands of guayule. Field surveys of the available shrub in the Big Bend district of Texas led to estimates that some 1,750 tons of shrub were available and that about 500,000 pounds of rubber could be obtained. Actual shrub collection resulted in 1,700 tons, dry weight, of shrub, which yielded a total of 510,086 pounds of rubber.

Another major contribution of production research was in improvements in the methods of processing guayule for rubber recovery. Campaigns for the utilization of shrub growing at Salinas, Calif., taken over by the Government from the Intercontinental Rubber Company, and that from native stands in Texas, necessitated major changes in plant harvesting and processing methods. Directly, and in cooperation with the appropriate research agencies, these changes were investigated and instituted in the Spence factory at Salinas and in a new factory constructed at Bakersfield, Calif. The extent and character of these changes are discussed on pp. 117–119. They represented major and concrete accomplishments by the production research unit in the guayule program.

SHRUB PROCESSING

Processing by the Intercontinental Rubber Company

In the beginning, guayule shrub was treated in individual ball or pebble mills for the separation of the rubber from other plant material. This treatment was accomplished in the presence of water. The action of the balls or pebbles was to comminute the shrub. In the process, the rubber gathered together in small particles that became known as "worms" while the woody and fibrous materials were ground finer and finer. After a calculated time, the comminuted shrub was discharged into a tank of water. The rubber particles floated to the surface and the other material, that had been ground fine and waterlogged by the grinding process, sank. The Intercontinental Rubber Company had improved this process by the introduction of long tubes into which measured amounts of shrub and water could be fed continuously and from which there was a constant discharge of processed plant material. As in the batch process, this comminuted material was discharged into an excess of water to float the rubber. The rubber from this first treatment contained "corky" material from the bark and woody fragments caught mechanically in the rubber. It was placed in a pressure chamber to waterlog the corky and other nonrubber material. A second flotation then served to furnish a somewhat purified rubber that was then dried and pressed into blocks for shipment to the market.

Processing by the Emergency Rubber Project

Among the properties taken over from the Intercontinental Rubber Co. was a 600-acre field, known as Arguello Field, containing mature guayule shrub that could be used immediately to gain experience in the processing of guayule for rubber. There was also an extraction factory, the Spence Mill, located at Salinas that had been used by the company in past rubber extraction campaigns and ready, after overhaul, for use.

Some of the company processes were recognized as unsatisfactory in the beginning. Chief of these were:

(1) Dependence on direct exposure of the harvested shrub to the sun as a means of curing.

(2) Chopping the shrub in the harvest field and hauling the chopped shrub to the mill for processing.

(3) Storage of the cut shrub in bins for several days prior to processing.

The disadvantages of such a system were immediately apparent. Harvesting and milling were frequently interrupted by weather conditions which disrupted harvesting, curing, and cutting operations. When chopped shrub was placed in bins at the plant, particularly in damp condition, compaction occurred and decomposition followed. This resulted in reduced yield and lowered quality of rubber. A rotary kiln installed by the company was only partly successful in overcoming this factor.

Processing changes by the Emergency Rubber Project

The general processes used by the Emergency Rubber Project to obtain rubber from guayule were described by Kenneth Taylor (*217*). The first changes in procedure instituted by the Emergency Rubber Project (*186*) involved principally the harvesting of the shrub and its transport to the factory. Shrub was dug and allowed to cure (dry) in a windrow for 3 to 5 days. This eliminated the surface moisture and reduced the water content of the shrub somewhat. The shrub was not chopped in the field because of the problem of storage in bins at the factory. Instead, the shrub was compressed into 200-pound bales and hauled to the mill as soon as possible. Undercover storage for 10 to 12 days of milling was provided.

Changes instituted at the mill included:

(1) A conveyor was provided to pass the bales of shrub through a soaking vat to soften any dirt clinging to the roots.

(2) Bales were then broken and the plants placed separately on a conveyor, which passed through high-pressure water sprays to remove the dirt.

(3) Washed shrub was then chopped on a rotary-blade cutter designed to furnish particles of uniform size.

(4) An improved drier, heated by steam, was obtained to reduce damage to the rubber, which resulted from older types that were directly fired.

(5) The primary crusher was installed after the drier, to increase efficiency, and to reduce further the exposure of the rubber to elevated temperature.

(6) A continuous weighing machine was installed in an effort to obtain better control over the amount of shrub fed to the mill.

(7) New crushing rolls were installed.

(8) Silt traps were installed before and after the primary flotation tank, and a mechanical skimmer was installed on the second flotation tank.

(9) A hydraulic ram was installed to give better control in dewatering the rubber worms.

(10) The dewatered worms were treated with 0.5-percent Tonox (an antioxidant) prior to final drying.

(11) Standardized operational specifications were adopted.

Milling of young shrub

The Bureau of Agricultural and Industrial Engineering initiated a program of research into the milling of young shrub at the age of 2 years. It was considered essential to be able to accomplish this should it become important to produce rubber before the shrub reached the normal age of 5 to 10 years, when maximum rubber content would be reached.

Since the rubber is contained in the guayule shrub in the form of latex in individual cells, it was considered important to bring about the coagulation of the latex before submitting the shrub to comminution in the presence of water. Much of the loss of rubber under such conditions was attributed to the loss of this uncoagulated latex. The curing or drying of the mature shrub prior to milling was primarily to prevent the loss of uncoagulated latex and it was even more important that the young shrub be given prior treatment to assure the coagulation of the latex.

One treatment for young shrub, also considered for old shrub, was retting before milling. It was considered that this would result in the coagulation of the latex, would reduce the amount of material to go through the mills, and might also serve to simplify the storage of shrub harvested during the optimum harvesting period but held to provide year-round factory operation to reduce the cost of extraction and make adequate use of the extraction facilities.

In these experiments, it was found that bringing the plants to the 25-percent moisture content, considered necessary for coagulation of the latex, might take as long as 55 days, and that even by parboiling and deleafing the shrub the time could not be reduced to less than 55 days. The retting experiments proved only partially successful and there was only a 40-percent recovery of rubber from retted shrub. From shrub that had been stored whole for 70 days and defoliated immediately before milling, there was 78-percent recovery of rubber in milling, while from shrub that was defoliated before storage, there was 88-percent recovery.

The rubber worms from the young shrub were sticky and more difficult to free from nonrubber particles than was rubber from old shrub.

Shrub defoliation

The leaves of young plants constitute 15 to 33 percent of the dry weight of the plant. These leaves contain little rubber, and none that could be recovered by the conventional methods of extraction. Compared to the rest of the plant, the leaves contain a large amount of saponaceous material that interferes with the formation of the rubber into worms in the extraction process. The leaves are high in manganese and copper, both deleterious to the rubber. The defoliation of the plants prior to extraction of the rubber is therefore highly important. It was not possible to defoliate the plants before harvest by the use of defoliant sprays since guayule does not produce an abscission layer and does not shed its leaves normally.

A standardized method of deleafing guayule shrub by boiling whole bales was worked out. The bales were then transported to a trommel, or rotary screen. At the entrance to this machine, the bales were torn open. The tumbling action released the leaves, which then fell through the screen. The guayule leaves were high in nitrogen, and found a ready sale to farmers in the Salinas area.

Other improvements in shrub processing

Improvements were made in nearly all of the operations of shrub processing for rubber—flotation, decorking and refining, dewatering, and drying. An experiment of great potentiality, that was started but not carried through to completion, was the use of Jordan mills, such as are used in the preparation of wood pulp in the paper-making industry. It appeared that use of these mills might revolutionize the guayule extraction process. Mills were obtained, but delays in receipt of certain fundamental equipment, and a shortage of skilled labor, made it impossible to test them adequately. The few tests made were highly promising but indicated that the limitations in the accessory equipment made it impossible to take full advantage of the capabilities of the Jordan.

Post-War Processing Research

The Bureau of Agricultural and Industrial Chemistry took over all research on the processing of guayule after the termination of World War II, the liquidation of the Emergency Rubber Project, and the reauthorization by Congress of research by the Department of Agriculture.

In the beginning of this phase of the research it was stated (*6*) that "Laboratory investigations will be devoted to the extraction and processing of rubber from guayule as the most promising of domestic rubber-bearing plants. In the conduct of these investigations it is felt that more emphasis should be placed upon fundamental aspects of the work in comparison with the previous Emergency Rubber Project extraction research program. The latter was of necessity largely centered around development of improvements in the existing process, owing to war emergency need for rapid production of rubber. For example, more intensive study will be directed toward the identification and physical and chemical characterization of resins and other constituents of guayule shrub which affect rubber recovery, either in the latex or coagulated form, and/or which affect quality of the vulcanized products."

Organization of processing research

The research work of the Bureau of Agricultural and Industrial Chemistry was divided among four Sections: Laboratory Extractions, Pilot Plant Operations, Analysis and Testing, and Latex.

Laboratory Extractions Section. This unit, under the direction of Dr. Ralph W. Planck, conducted chemical and technological research on the extraction of rubber and other constituents from rubber-bearing plants, research on methods of recovery of the rubber in coagulated form as "worms," retting, milling, solvent extraction, and separation of rubber hydrocarbons and resins.

Process Research and Development Section. This unit, under the leadership of Mr. Kenneth W. Taylor, conducted research on the operation of the pilot plant for recovery of rubber from various rubber-bearing plants, on the perfection of various steps for rubber recovery on a factory scale, and on the development of new and improved methods of processing. This unit also cooperated in evaluating samples of shrubs from various field tests and breeding experiments. This cooperation was designed to guide the agronomists and plant breeders in producing shrub with an optimum processibility in the extraction of the rubber after harvest.

Analysis and Testing Section. This section, headed by Mr. Frederick Clark, was organized as a service unit to perform analyses of plant material, bagasse, latex dispersions, crude rubber, et cetera, and to make physical tests of crude rubber and vulcanizates for other sections. It conducted research on the development of new improved methods of analysis and testing, and special formulations, and use applications of rubbers from domestic rubber-bearing plants.

Latex Section. The latex section, under the direction of Dr. E. P. Jones, undertook the improvement of the various processing steps for the recovery of guayule rubber in latex form, including the mechanical disintegration of shrub for release of rubber from individual plant cells, the separation of finely suspended plant solids from latex dispersions, and the concentration and creaming of the latex.

Milling research

The Emergency Rubber Project, following closely the lead of the Intercontinental Rubber Company, stressed the necessity of drying (curing) the guayule shrub before rubber extraction. Efforts were made to bring the shrub to a moisture content of not more than 25 percent in the expectation that this curing would help to coagulate the latex and avoid its loss in the water in the mill.

An early contribution of the post-war research was the demonstration that it was not necessary to dry the shrub before milling; that the lush shrub direct from the field could be milled successfully, and that the rubber from lush shrub was superior to that from dried or stored shrub.

The milling of fresh, lush shrub was first accomplished by adding acid to the water fed to the extraction mill. This served to coagulate the latex and prevent its dispersal in the milling water. Later, Taylor and Chubb (*219*) developed a method that did not require the use of acid. Under this method, the shrub was parboiled and then submitted to high pressure in crushing rolls or hammermills. Passing the shrub through the crushing roll a second time gave even better results, and successive crushing increased the yield, six passages through the rolls being the most reported. A recovery of 85.3 percent of the available rubber was reported after two passages of the shrub through the crushers.

A great handicap in the production of guayule rubber from wild shrub was the length of time required to get the shrub to the mill after harvest. The shrub had to be dug by hand and hauled to concentration areas on pack animals. Baling equipment was then used to compress the shrub into bales for transportation by truck to the nearest railroad siding. Uncertain railroad schedules often resulted in the baled shrub standing unprotected on the siding for months, awaiting transportation to the factory. The extracted guayule rubber was high in resins and was discounted on this basis. However, it was found that there were large amounts of plant debris in the rubber, and tests at Salinas indicated that this insoluble material was more deleterious to the guayule rubber than were the resins.

In milling lush shrub it was found possible to greatly reduce the amount of insoluble impurities. When very dry shrub is milled, fine particles of bark and wood are incorporated in the rubber, and are almost impossible to eliminate. This factor is minimized in milling lush shrub, and separation of the insolubles is greatly simplified. Improvement of guayule rubber by deresination is more easily accomplished if the insoluble constituents can be kept to a minimum.

Development of control milling

Taylor and Chubb (*219*) recognized that the results of comparative milling tests of guayule samples had been confusing and difficult to analyze. Methods of sampling, the vagaries of the weather during harvest, and variations in methods of handling the samples had introduced variables in the results, and obscured the basic comparisons. It was considered necessary to standardize procedures to assure reproducibility, and comparability of results. Experiments were instituted to determine the sources of variability so that uniform test procedures could be established. As a result of these experiments, the following procedures were adopted:

The block of plants to be used was carefully selected with respect to plant size, vigor, and growth characteristics. If desirable in respect to the type of test being conducted, the offtype plants were removed. Otherwise the individual plants to be harvested were selected by a process of randomization. In all cases, the harvested plants were taken to the pilot plant as quickly as possible.

The plants were parboiled for 15 minutes at 200° F. and then defoliated. They were then cut in a fly-knife cutter to pass through a ½-inch screen.

Samples of the cut shrub were taken for analytical determination of rubber content. The cut shrub was then passed twice through crushing rolls with a clearance of approximately 0.002 inch, and then through a hammermill with a ½-inch screen. This material was separated into the desired number of replicates, each of which was sampled for rapid moisture determination.

Mill charges were weighed out on a dry-weight basis determined by the rapid moisture test. Each mill charge was labeled and held overnight in a covered can.

The replicates were milled in 27-inch batch pebble mills with a water-to-solids ratio of 4.5–1 and a pebble-to-solids ratio of 20–1. The mills were run at 80 percent of their critical speeds, and with an initial temperature of 85° F. Milling time was 60 minutes, exclusive of time required after some 10 to 15 minutes to stop the mill, remove the cover, and replace in the mill the shrub that had become packed between the mill and lid.

When milling was complete, the mill was opened, filled about half full of water, and the contents, exclusive of the pebbles, discharged into the flotation tank. The mill was rinsed five times with copious amounts of water. On the fourth rinse, the lid was replaced and the mill run for five revolutions. After rinsing, hot or cold water was added to the flotation tank to provide a water-solids ratio of 100–1 at 100° F.

The floating rubber was then skimmed off and the flotation liquor agitated for 5 minutes with an electric mixer and allowed to settle for 5 minutes. After a second skimming, the agitation and settling were repeated and a third skimming performed.

Decorking was accomplished in a pressure tank (paila). The tank was preheated to 200° F. The rubber with about 3 times its volume of water was introduced and brought to a rolling boil. Hydraulic pressure of 500 pounds per square inch was applied for 90 minutes.

This pressure was adopted as standard rather than a pressure of 2,000 pounds per square inch, described by Cumming and Chubb (*71*). The rubber was discharged into a flotation tank with a water-to-solids ratio of approximately 400–1 and a temperature of 140° F. The rubber was skimmed off and held in warm water for scrub milling.

The scrub mill was preheated to 140° F. The rubber was placed in the scrub mill together with pebbles at a pebbles-to-solids ratio of about 20–1. Water and steam were introduced to give a water-to-solids ratio of about 20–1 and a minimum temperature of 140° F. After scrubbing for 15 minutes, the rubber was discharged into a flotation tank with a water-to-solids ratio of 400–1 and a minimum temperature of 140° F. The rubber was skimmed off and spread to uniform depth in drying trays.

Depending on the nature of the experiment, antioxidant could or could not be added before drying. The rubber was dried in a through-circulation drier at temperatures of 100° F. for 7½ minutes; 125° F. for 7½ minutes; 150° F. for 7½ minutes; 175° F. for 7½ minutes; and 200° F. for 30 minutes. The trays were then weighed and replaced in the drier for 5 minutes and reweighed. This was repeated until a constant weight was reached. The trays were then removed from the drier, samples taken for chemical analysis, and the weight of recovered rubber recorded.

Control methods were set up for determining the rubber lost in the bagasse and in the effluent (or flotation) liquors. It was found possible to control the temperatures and ratios with sufficient accuracy to give a high degree of reproducibilty of results.

Extraction of guayule rubber as latex

The Emergency Rubber Project demonstrated the possibility of extracting the rubber from guayule in the form of latex. Lloyd (*146*) had shown that the rubber was contained in the plant in separate cells but in the form of latex. Taking advantage of this fact, it was found possible to disrupt these cells into a liquid medium, dispersing up to 85 percent of the rubber available in the plant. By suitable centrifuging, it was possible to recover up to 90 percent of the dispersed rubber, giving an overall net recovery in the form of latex of some 76 percent of the available rubber.

Jones (*136*,*137*,*138*) improved the methods of disintegrating the plants to obtain maximum dispersion of the rubber latex. It was recognized that it would be desirable to eliminate the leaves before extracting the rubber. This, however, could not be done as successfully as was possible in processing the shrub to obtain solid rubber. The lack of a definite abscission layer in guayule made it impossible to use chemical defoliants on the plants in the field. Parboiling to release the leaves resulted in the loss of latex by coagulation. The leaves on the plants added much to the fine trash that was included with the latex. Centrifuging was made very difficult by the presence of this fine trash, and the batch-type centrifuges had to be closed down periodically during the centrifuging to eliminate the fine solids. In the later stages of the work, continuous systems of centrifuging were tested with some promise. Whittenberger and Brice (*250*) developed a method for the rapid estimation of the rubber content of guayule latex dispersions to guide the concentration procedures.

Rubber extracted in the form of latex compared favorably with *Hevea* rubber. Feustel (*98*) found that "Guayule latex rubber showed physical properties and chemical composition superior to those of regular commercial guayule rubber. The composition of guayule latex rubber is quite similar to the composition of Hevea latex film, except that the latter rubber is slightly lower in resin and slightly higher in insolubles."

Storage studies

Specific studies were initiated by Taylor and Chubb (*219*) to determine the effect of storage on the content of rubber, the quality of rubber, and factors involved in extraction. Standardization of the milling procedures made it possible to avoid some of the uncertainties associated with previous tests. The need for storage for limited or protracted periods to accommodate to milling capacities when harvesting of fresh shrub was impossible or undesirable, had to be considered in estimating the cost of producing rubber from guayule.

The first tests undertaken were to compare the results obtained by milling baled shrub and defoliated baled shrub held in storage with results obtained through milling fresh shrub not held in storage. The stored shrub consisted of (1) shrub dug and field cured for 7 days, baled with leaves on and stored up to 6 weeks, then parboiled, defoliated, and milled; and (2) shrub dug and hauled to the pilot plant at once, parboiled and defoliated, stored up to 6 weeks, and milled. The control consisted of shrub harvested and processed with a minimum of delay.

The tests left many problems of shrub storage unsettled but, in general, the fresh shrub yielded the best rubber with the highest molecular weight and lowest content of insolubles, some 50 percent lower than usually found. The shrub stored without defoliation gave the lowest yield of rubber and the lowest quality but had a slight decrease in resin content. The shrub that was defoliated before storage showed an increase in the rubber hydrocarbon in the crude rubber, a decrease in the resin content, but an increase in the content of insolubles. The total amount of rubber recovered increased with storage from 3 to 6 weeks, but decreased thereafter.

Storage as ensilage

In cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, a test was made of ground shrub stored (1) in a comparatively warm location without added water, (2) in a comparatively cool location without added water, and (3) in a comparatively cool location with added water. The conditions of the test made it impossible to compute specific yields, but as reported by Naghski et al. (*167*), the predominating organisms were studied and identified. In these tests, the quality of the rubber extracted deteriorated because of storage conditions, but in other tests, White et al. (*247*) and Allen and Emerson (*16*) reported that the quality of the rubber from guayule shrub could be markedly and consistently improved by retting before milling, the resins being reduced 50 percent and the tensile strength increased 50 percent. Allen et al. (*17*) described a method found useful in detecting resin-decomposing organisms, and improving the process of eliminating resins by retting.

Mill equipment studies

Shrub preparation. Parboiling and defoliating were found to be desirable in the preparation of shrub for milling. The problem of reducing the shrub to a size suitable for feeding to the pebble mills required extensive research which resulted in the determination to discard cutting machines which required high maintenance costs to keep the cutters sharp. Hammermills and crushing rolls did a superior job of shrub preparation and also served, through blunt maceration, to coagulate the latex and facilitate the extraction of the rubber.

Degradation by milling processes. Meeks and Feustel (*159*) reported that detailed studies of the extraction of rubber by the standard methods failed to show that any single process, of itself, resulted in any degradation of the rubber. The molecular weight of the rubber in the shrub was found to be 160,000 to 165,000. After passing through the pebble mill, the average value was 162,000. The mean molecular weight was 157,000 after paila treatment and scrub milling, but this reduction was not found to be statistically significant. Drying in a circulating draft oven at 200° F. lowered the molecular weight to 141,000—a significant decrease. However, lowering the temperature and adding antioxidant to the rubber overcame this factor.

Tube mills. Detailed studies of the operation of the tube mills used for the extraction of the rubber from guayule shrub showed that, while they are still the best devices available for this purpose, there is an unavoidable loss of rubber in the process. Feustel (*98*) reported that in tests designed to determine the efficiency of the tubes, there was a recovery of only 661.64 pounds of rubber hydrocarbon in the crude rubber extracted compared to a calculated content of 912.8 pounds of rubber hydrocarbon in the shrub fed to the tubes. This indicated a recovery of only 72.5 percent of the rubber available in the shrub. A total of 13.6 percent of the available rubber was found in the bagasse (11.8 percent) and in the sump and centrifugal filter (1.8 percent). This left a rather large discrepancy, some of which was undoubtedly attributable to errors in sampling. Continued operation of the tubes was not possible because of lack of time and shrub. The conclusion was "that tube mills may not be the best possible tool for the recovery of rubber, or considerable additional work may be needed to develop the optimum milling procedure."

Jordan mills. Preliminary tests by the Emergency Rubber Project had indicated that Jordan mills were promising for extracting rubber from guayule. These tests were made on a batch basis and were never carried on over a sufficient period to determine the efficiency of the mills. Feustel (*98*) reported that the Bureau of Agricultural and Industrial Chemistry converted the Jordan mills to continuous flow operation and conducted many tests of their efficiency, but it was determined that the Jordan mill did not give satisfactory yields of rubber hydrocarbon. The yields ranged from 69.8 to 78.8 percent of the available hydrocarbon, with a mean yield of 76.25 percent. Most of the losses consisted of unmilled rubber in the bagasse.

Guayule varieties and hybrids

Feustel (*98*) reported that, in cooperation with the Bureau of Plant Industry, Soils, and Agricultural Engineering, tests were conducted to determine the milling characteristics of certain selections of guayule and of hybrids between guayule and other species of *Parthenium*. Three selections of guayule were tested, including the standard strain 593, developed by William B. McCallum for the Intercontinental Rubber Company; 4265–I, a selection from 4265, collected by LeRoy Powers in the State of Durango, Mexico; and 4265–X, a selection from 4265–I.

Four hybrids between guayule and stramonium (*Parthenium argentatum* A. Gray and *P. tomentosum* var. *stramonium* (Greene) Rollins) were tested, as were also two hybrids with mariola (*P. incanum* H.B.K.). One of the mariola hybrids was with guayule and one with a guayule-stramonium cross.

These tests were too few and too limited to determine the basic characteristics of these strains and hybrids, but were highly important in indicating that in any breeding program to improve the yield of guayule the processibility of new strains must be determined before they can be considered for commercial use.

Notes were made on the individual tests as follows:

D–65 (Guayule 593, female X Stramonium 43700, male), 3-year-old shrub. Wood fibers were very long. Small worms of rubber were observed after one hour of milling. After milling, worms were bright olive green in color, very buoyant in the flotation tanks, but difficult to recover because of their fineness.

D–118 (Guayule 42268, female, X stramonium 43691, male), 3-year-old shrub. Wood fibers long and stringy. Flower stems profuse. No distinct worms discernible after 1 hour's milling. At end of milling, worms were very small, bright green, and difficult to skim because of their size. Buoyancy was average.

D–153 (Stramonium 43691, female, X Guayule 4268, male), 3-year-old shrub. Long wood fibers. No worms apparent after one hour's milling. At end of milling, worms were exceedingly fine, dull olive green in color.

D–155 (Stramonium 43691, female, X Guayule 4268, male), 3-year-old shrub. Wood tough and fibrous. Much more difficult to handle than guayule. Flower stems profuse. No distinct worms at one hour. At end of milling, worms were exceedingly small, difficult to handle, and dull olive green in color. Dried deresinated rubber not rubbery in texture; more like dried putty.

4265–I (Guayule), 3-year-old shrub. Very difficult to defoliate. Wood fibers comparable to guayule (593). At the end of milling the worms were typical grey-green and about the usual size. Rubber very buoyant and floated more readily than most.

4265–X (Guayule), 3-year-old shrub. Defoliation not difficult. Wood character ordinary. Worm characteristics same as for 4265–I and 593.

593 (Guayule), 3-year-old shrub. Wood fibers very much shorter than for any hybrid, but about the same as the two varieties of 4265. Worms formed at end of one hour of milling and at end of milling period exhibited normal characteristics.

Mariola hybrids, 2-year-old shrub. Too little material to process in mills. An attritor was used for milling. The resulting rubber aggregates were very fine, not wormlike, and could not be recovered by conventional skimming.

Deresination of guayule

Two methods of obtaining deresinated guayule rubber were tested. In the first method, the chopped shrub was subjected to treatment with acetone to extract the acetone-soluble materials prior to milling. In the second method, the rubber worms were treated with acetone to extract the resins. In both cases, it was found possible to make the extraction without drying either the shrub or the rubber. The acetone quickly displaced the water, and the efficiency of the extraction was not lowered; nor was the loss of acetone material.

Shrub deresination

Chubb *et al.* (*55*) reported on tests undertaken to determine the feasibility of deresinating the chopped shrub prior to extraction. Shrub of various ages was gathered throughout the year. In these tests both shrub age and seasonal climatic influences were compared on the basis of effect (1) on processing, and (2) on the quality of the extracted rubber. A special countercurrent extractor was devised for the tests. Milling of the deresinated shrub presented no difficulties and the rubber produced was distinctly superior to, and more uniform than, the ordinary resinous product produced by milling the shrub that had not been deresinated. The rubber from the deresinated shrub was reported comparable to *Hevea* No. 1 smoked sheet. U.S. Patent 2,459,369 was granted Tint and Murray (*233*) for a method of deresinating guayule shrub before milling for rubber.

Deresination of guayule rubber

Clark (*57*), Clark et al. (*85*), Banigan (*21*), and Clark and Feustel (*59*) investigated the factors involved in deresinating guayule after extraction. Much smaller amounts of acetone were required than in deresination of shrub, but there was the problem of obtaining adequate penetration of the acetone into the extracted rubber.

In the normal milling process, resinous guayule rubber is recovered from shrub in the form of small spongy particles known as "worms." The term "worm deresination" is therefore used to designate deresination methods wherein the milling operation precedes the acetone extraction step, in contrast to "shrub deresination" where the reverse order is followed.

The resin-containing rubber worms were prepared from freshly harvested 5- to 7-year-old guayule shrub, variety 593. Worms that had to be held were preserved with a small amount of formaldehyde and/or refrigerated. Acetone was used primarily in the studies.

The spongy worms were treated without dewatering since the presence of water helped to maintain their spongy character, and aided in obtaining efficient penetration of the acetone, which quickly replaced the water. Very little acetone is required for water removal once equilibrium has been established in a continuous countercurrent system. Equilibrium was maintained in a laboratory-scale-batch, countercurrent extractor at a resin level of 2 percent in the issuing rubber, and 10 percent in the withdrawn acetone solution for an acetone- to resin-free rubber ratio of 4.8 to 1. Hoover et al. (*126*) found that guayule "worms" could be improved by fermentation in aqueous medium with *Pseudomonas boreopolis*, *Aspergillus fumigatus*, or *Trichoderma* sp. Stemberger et al. (*211*) perfected a method of purifying guayule rubber by treatment with hot metallic hydroxides.

Analytical procedures

Determination of the efficiency of guayule processing depends on accurate methods of determining the rubber present in the shrub before processing, the precise measurement of the resulting rubber, and estimation of its quality. A constant effort was made to improve methods of sampling the shrub and methods for determining the amount of rubber present.

Estimation of the rubber content of plants. Numerous methods of determining the rubber content of plants have been developed and used successfully. The standard methods (see p. 109) in the past have depended primarily on a double extraction, (1) with acetone, to extract the so-called resins or material that would otherwise be extracted, and (2) with benzene to extract the rubber. After drying, the second extract was weighed as rubber. Many improvements have been made in the basic system to gain precision and reproductivity, and Meeks et al. (*158*) improved the methods of comminution of the sample by substituting grooved and smooth rolls for the laboratory grinders. They added trichloroacetic acid to the benzene and brought the rubber into solution rapidly by tumbling in the presence of pebbles. A major shortcoming of the method was that it did not differentiate between *cis*-polyisoprene (rubber) and *trans*-polyisoprene (gutta). In a general comparison of miscellaneous plants for rubber content, this would be a serious deficiency but it did not prove important in the case of guayule, which produces only the *cis* polymer. A minor deficiency that did apply to guayule was the existence in guayule of a small amount of rubber of low molecular weight that is soluble in acetone.

Low-molecular-weight fraction of guayule rubber. Meeks et al. (*157*) showed that an acetone-soluble low-molecular-weight fraction exists in guayule rubber. This was found to constitute only a negligible portion of the rubber in the plant, but represented up to 46.37 percent of the rubber in individual segments of the plant. It was not possible to determine with certainty the origin of this low-molecular-weight rubber. The authors state, "Whether this fraction is present as such in the live shrub where it may play a role in the synthesis of rubber, or whether it should be regarded as some degradation product of the higher polyprenes, resulting from an enzymatic or oxidative process, are subjects for speculation and further investigation."

Determination of rubber in plants by bromination. Edison (*85*)* used a method of converting the extracted rubber from plants to a bromide that could be precipitated from solution by alcohol and could then be determined gravimetrically. In Edison's laboratories, a factor of 0.285 was used to convert the weighed bromide to rubber. This method of analysis confirmed the presence of rubber in the benzene solution from plants and included the low-molecular-weight fraction that would otherwise have been lost in the acetone extraction. Willits et al. (*252*) studied the application of this method to the determination of rubber hydrocarbon in samples of raw rubber and found a factor of 0.292 rather than 0.285 determined in the Edison laboratories. Gowans and Clark (*113*) compared samples of crude guayule rubber and samples of *Hevea* smoked sheet. Their tests showed that the factor for guayule rubber was 0.301 while that for the *Hevea* rubber was 0.299.

Whittenberger (*249*) and Haasis (*115*) developed microscopic methods of testing ground plant material for rubber that could serve for the examination either of fresh samples or the effluent from extraction mills.

The pilot plant as a research tool. The pilot plant is normally thought of as the forerunner of the factory installation, and is designed and operated to guide factory design and to determine and solve the problems that may be faced in large-scale operations. In the guayule research, however, Kenneth Taylor and Chubb (*220*) showed that the existing pilot plant at Salinas had an immediate usefulness not associated with factory operations. In the field operations of the research on crop production and the study of the effect of growing or handling of the shrub on the amount and quality of the rubber, it was found desirable to make processing studies on a sufficient amount of shrub to determine the effect of the treatment under study. Thus, the pilot plant became a research tool, and the development and maintenance of precise controls and procedures had to be developed to gain standardization and reproducibility. Steps taken to assure this uniformity of operation are outlined under Development of Control Milling.

Rubber testing. The ultimate test of all field and factory operations in the production of guayule rubber is the amount and quality of rubber produced. Each step of the production of shrub and rubber must be checked, not only for its effect on the amount of rubber, but as to whether the rubber quality is improved or impaired by the treatment. Most of the methods of extracting rubber at the various stages of shrub growth or shrub processing could be standardized for maximum reproducibility. These processes were unique for guayule, and depended on the accumulated experience of the agencies that had had responsibility for guayule development.

In testing the rubber obtained from the shrub, standardized processes, developed in research laboratories of the rubber trade throughout the world, were available. These procedures proved adequate for most of the testing problems. Contributions to the techniques of rubber testing were made by the guayule project, including that of Rolla Taylor (*221*) who experienced difficulty in maintaining the dies used in cutting rubber samples for laboratory tests. He developed a fixture for holding the dies precisely while sharpening them so as to give a high degree of precision in the configuration of the die.

Gowans (*112*) experienced difficulty in obtaining reliable oxygen absorption measurements in accelerated aging tests of raw guayule rubber owing to the flow of the rubber as oxidation took place. A perforated stainless steel envelope was developed to hold the rubber sample during the test. This prevented the flow of the rubber and did not affect the rate of oxygen absorption.

Taylor and Ball (*222*) measured the temperature gradients in the Mooney viscometer during laboratory tests of guayule rubber. They reported that, owing to the assymmetry of the Mooney viscometer, the variations in temperature were great and suggested that, in the absence of a practicable method of making the temperatures uniform, actual temperatures should be recorded.

Byproducts of guayule processing

The leaves, cork, bagasse, and resins in guayule have a potential byproduct value of some importance in relation to the cost of producing rubber from cultivated shrub.

Resins. The identification and characterization of the acetone-soluble constituents of guayule received major attention. It was found that with shrub deresination of variety 593 roughly one-half pound of resin could be obtained for each pound of rubber produced. Deresination of the rubber extracted in the form of worms resulted in a yield of about half that amount. Some 37 percent of the resins obtained from the worms consisted of unsaturated long-chain fatty acids, notably linoleic acid, along with traces of linolenic and oleic acids. Linoleic acid has long enjoyed an established position in the paint and varnish industry. Banigan and Meeks (*22*) described the isolation of various organic acids from guayule resins.

Cinnamic acid is present in resin as the ester of partheniol. It can be released by saponification, and has an established value in the cosmetics and pharmaceutical industries.

A "drying resin" fraction readily obtainable from shrub resin is a hexane-insoluble, alcohol-soluble, shellaclike gum resin which can be easily polymerized to a heat-resistant, clear coating of good solvent resistance. This fraction constitutes 35 to 50 percent of the shrub resin but a much smaller proportion of the worm resin.

The terpenes of guayule constitute a potentially valuable "naval stores" type of byproduct. Volatile terpenes, comprising 3 to 5 percent of the worm resin and a much higher proportion of leaf resin, include alpha-pinene, dipentene, cadinene, partheniol, and others. Sesqui-, di-, and higher terpenes are also readily obtainable in significant quantities from the nonvolatile, unsaponifiable fraction.

Meeks et al. (*157*) developed a new process for the isolation of parthenyl cinnamate from guayule resin by a liquid-liquid extraction, and Walter (*246*) described the isolation of partheniol, parthenyl cinnamate, and other constituents from guayule resin.

Betaine is a constituent of guayule resins, and is also found in water extracts of guayule shrub. This substance is commonly obtained as a byproduct of the sugar beet industry, and has a limited market as a pharmaceutical and for other purposes. Murray and Walter (*166*) described the isolation of betaine from guayule. An improved procedure was developed by Banigan et al. (*24*).

The cuticle wax from guayule leaves constitutes about 0.25 percent of the fresh weight of the leaves. Its relative hardness, molecular weight, and melting point justify its consideration as a substitute for carnauba. The distribution of this and other waxes in the guayule plant was reported by Banigan et al. (*23*) in 1951.

Water solubles. The water solubles of guayule can be classified into three types—polysaccharides, amino acids, and inorganic salts. The polysaccharides comprise levulins (fructose polymers) and pentosans, including possibly xylan. The amino acids include betaine, discussed above. Inulin-derived levulins are present in the defoliated shrub to the extent of 8 to 12 percent, and are readily extracted by hot water. They are of interest as a possible source of low-cost alcohol.

Bagasse. This material could have some value as a source of fuel, particularly for fuel in the operation of guayule-extraction factories. Other uses suggested include filler for brick, pressed logs, pressed board, and compost. When used as compost, considerable nitrogenous material must be added as the bagasse alone ties up the available nitrogen in the soil.

Leaves. The leaves represent an important source of guayule resins, especially volatile terpenes, wax, and plant pigments. Much of the value of the leaves for resins would be dissipated in the parboiling process, however. Leaves constitute an excellent soil amendment when composted. After parboiling, the leaves can also be compressed into a building board that, due to the structure of the leaves, possesses a pleasing pattern.

Cork. The cork is conveniently obtained in a waterlogged condition from the discharge from the last flotation tank. It was suggested as a possible linoleum filler, but no tests were conducted.

The quality of guayule rubber

The quality of guayule rubber has been considered to be inferior to that of *Hevea* rubber. In the past, this difference has been attributed to the high resin content, and the custom has been to assume a commercial value directly related to the relative resin content. Owing to the softer character of guayule rubber, it found particular use for many years as a softener for *Hevea* rubber.

The Department's research on guayule rubber resulted in recognition that the inferior quality of guayule rubber was attributable to insoluble particles of dirt and plant trash, as well as to the high resin content. The milling method of extracting guayule rubber resulted in a product that contained much more extraneous matter than rubber made from latex obtained by tapping trees. As has been pointed out, it was found possible to reduce the insolubles, and to deresinate the rubber to produce a commercially acceptable product that could be compounded to equal the best grades of *Hevea* rubber.

The best index of advancement in the art of producing guayule rubber, as noted by Clark (*56*) and Feustel and Clark (*99*), is the quality of the rubber produced. The estimation of the quality serves a double purpose: (1) the comparison of the final product with other types of rubber, either from other botanical sources or synthetic products that are used in the general field of rubber technology, and (2) the examination of field and extraction procedures to determine the effect of each on the quality of the product. It was also necessary to determine the particular characteristics of guayule rubber with respect to testing procedures, and to develop new concepts such as those of Rolla Taylor et al. (*223*), who showed that the moisture content of the rubber and the compounding ingredients should be recorded for consideration in the final comparisons.

General laboratory procedures are available for the testing of small samples of rubber, and the comparison of rubber samples on the basis of physical behavior in standardized tests. Most of these tests could be performed in the testing laboratories established at Salinas, Calif. The facilities available at other locations in Government and commercial establishments were also utilized to take advantage of specialized techniques whenever the facilities were offered.

In addition to the small-scale testing of the rubber samples, larger-scale tests were conducted in cooperation with governmental and commercial interests. Many of these tests involved the fabrication of articles of guayule rubber using specially devised formulae, such as described by Clark and Place (*60*), and the testing of them in comparison with similar articles made from *Hevea* or suitable synthetic rubbers (Clark and Place, *61*).

Aging characteristics of guayule rubber

All rubbers have a tendency to oxidize and become tacky. This is called "aging" and is a measurable character that can be used in the comparison of rubbers. Rubbers with a low rate of oxygen absorption are considered to be superior to those with rapid absorption. Crude guayule rubbers, both resinous and deresinated, have relatively poor aging characteristics in comparison with high-quality *Hevea* rubbers. The aging of the guayule rubber is manifested by severe tackiness, the rubber becoming quite sticky with an accompanying loss in physical properties owing to molecular breakdown.

Marked improvement was possible in the aging of guayule rubber. Deresination of the crude guayule rubber improved the aging characteristics. Removal of the last resin in a complete deresination improved the aging of the rubber more than did the first resin removal. There appeared to be a fraction of the acetone-soluble material in guayule rubber that was deleterious to the rubber and that acted to facilitate oxygen absorption. Removal of this fraction improved the aging of the rubber. With *Hevea* rubber, extraction with acetone removes a natural antioxidant. Guayule rubber that has been deresinated with acetone compares favorably with *Hevea* rubber that has been treated with acetone.

The presence of a chelating agent (sodium salt of ethylenediamine tetra acetic acid) in the milling liquid when guayule rubber is being milled from shrub tissue significantly improves the aging of the resultant rubber. Stearic acid added to the rubber before scrub milling also improves the aging of the rubber. The use of hydrochloric acid in milling resinous "worms" improved the color of the resultant rubber and resulted in a 40-percent reduction in metal content. The rubber from this treatment had inferior aging characteristics. Rubber obtained in an open container, such as the blendor used for rubber content determinations, had inferior aging characteristics.

The use of antioxidants was found to improve the aging characteristics of guayule so that it was comparable in aging with *Hevea* rubber. The best antioxidant was Beta Conidendrol, followed by Alpha Conidendrol, Oxynone, and Agerite White, in that order. The latter had a negligible effect on the rate of cure in vulcanization, and was judged to be the best all-round antioxidant for use with guayule. It was found that when a chemical antioxidant such as Agerite White was added at a 1-percent concentration to guayule rubber, the latter showed aging characteristics superior to raw *Hevea* rubbers. Tint and Cumming (*232*) described suitable methods for applying antioxidants to guayule to give maximum protection in storage. They reported that the primary aromatic amine Tonox could be applied to wet rubber at a concentration of 0.5 percent (dry rubber basis).

Tensile tests

Deresinated guayule rubber was compared with other types of rubber in standardized breaking tests. It was found that guayule compared favorably with good grades of *Hevea* rubber in these tests. However, as shown by Clark and Place (*60*) and Place and Clark (*173*), the best tests required the use of a special formulation for guayule, and this usually was not the best formulation for use with the rubber being compared. Results of tensile tests of deresinated guayule rubber using two special formulas, are shown in table 3.

Cooperative tests

Cooperative tests conducted by cooperating Government agencies and commercial laboratories provided useful information on the value of the rubber. Many of the tests were significant in showing desirable or undesirable characteristics of the rubber and providing information regarding its use in normal manufacturing processes. In many cases, test procedures were applied with no regard to the special requirements for guayule rubber, and the results in such cases were not favorable to the guayule rubber.

One rubber manufacturer found that worm-deresinated guayule rubber would be useful in tire carcasses but that the guayule was slower curing and had lower physical properties than *Hevea.* In some categories—cut flex, aged modulus, aged tensile, and aged cut flex—the guayule rubber was superior, or equal to *Hevea*, but was inferior in heat buildup.

Another rubber manufacturer found that shrub-deresinated rubber was superior to the worm-deresinated sample, but not equal in modulus and tensile strength to first-quality *Hevea* rubber. These findings are shown in table 4.

A rubber manufacturer reported, ". . . the deresinated guayule is a high-grade form of rubber hydrocarbon, suitable for carcass stocks."

Another report from a rubber manufacturer, who compared deresinated guayule rubber with a good grade of *Hevea* rubber, indicated that when compound recipe adjustments were made to compensate for the absence of fatty acids in the guayule, vulcanizates of ex-

TABLE 3.—*Typical physical test results of deresinating guayule rubber in gum formulas*

Minutes cured at 275° F.	Stress		Tensile strength
	At 500 percent elong.	At 600 percent elong.	
	p.s.i.	*p.s.i.*	*p.s.i.*
		FORMULA A[1]	
30	280	480	2, 320
40	420	780	2, 930
60	520	1, 000	3, 420
80	570	1, 040	3, 245
		FORMULA B[1]	
25	340	610	2, 770
30	470	940	3, 150
40	640	1, 270	3, 720
60	670	1, 440	3, 450
80	730	1, 510	3, 410

[1] Compounding recipes:

	Formula A	*Formula B*
Rubber	100	100
Stearic acid	6. 0	4. 0
Zinc oxide	4. 0	4. 0
Sulfur	3. 5	1. 5
Benzothiazyl disulfide	1. 0	1. 0
Methyl thiuram disulfide	---	0. 15

cellent quality were obtained having stress-strain properties nearly equal to that of the controls. The guayule stocks were somewhat inferior in heat buildup and flex cracking. Stocks of both rubbers behaved similarly on aging, but the modulus of the guayule stock remained constant, while that of the *Hevea* stock increased.

A rubber manufacturer investigated the molecular weight of guayule in comparison with *Hevea* rubber and synthetic GR-S. After preliminary mastication (four passes through the mill) molecular weights reported were: Guayule, 491,000; *Hevea* Smoked Sheet, 472,000; *Hevea* Pale Crepe, 614,000; GR-S, 215.000 and 252,000. After 10-pass milling, the molecular weights were: Guayule, 254,000; *Hevea* Smoked Sheet, 382,000; and *Hevea* Pale Crepe, 321,000. Thus, the guayule had a quicker break-down on the mill than the *Hevea* rubber. This manufacturer attempted to determine if the molecular-weight distribution in guayule was responsible for its comparatively high heat buildup. A sample was fractionated and it was found that the high-molecular-weight fraction was superior to the low-molecular-weight fraction.

Since tests by Benedict et al. (*30*) and by Meeks et al. (*156*) had shown that the molecular weights of rubber from various parts of the

TABLE 4.—*Comparison of worm-deresinated and shrub-deresinated guayule rubbers with Hevea No. 1 ribbed smoked sheet in tire body stock (as reported by the Firestone Tire & Rubber Co.)*

Cured at 260° F.	30 min. cure		60 min. cure		90 min. cure	
	Mod. at 600% elong.	Tensile	Mod. at 600% elong.	Tensile	Mod. at 600% elong.	Tensile
Normal tests	*p.s.i.*	*p.s.i.*	*p.s.i.*	*p.s.i.*	*p.s.i.*	*p.s.i.*
Worm-deresinated guayule	1, 900	2, 525	2, 700	3, 625	2, 700	3, 650
Shrub-deresinated guayule	2, 275	3, 375	2, 625	3, 825	2, 650	3, 725
Hevea #1RSS	2, 675	4, 275	3, 275	4, 475	3, 350	4, 175
Aged 7 days at 158° F.						
Worm-deresinated guayule	2, 775	3, 975	3, 375	3, 875	3, 700	3, 700
Shrub-deresinated guayule	3, 700	4, 075	3, 800	4, 200	3, 650	3, 725
Hevea #1RSS	3, 825	4, 475	4, 175	4, 175	-------	3, 825

guayule plant were different, a cooperative test was undertaken to determine if rubber from different parts of the plant would have different freezing points for use where low temperatures caused failure of other rubbers. No difference was found in any of the rubbers.

Deresinated guayule rubber was furnished to manufacturing companies for the construction of passenger and truck tires. In a test of 9:00 x 20 heavy-duty truck tires, *Hevea*, *Hevea*/GR-S (55/45 blend), Guayule/GR-S (55/45 blend), and guayule tires were compared. Three tires of each class were tested by the Government Tire Test Fleet at Camp Bullis, San Antonio, Tex. Tire loads were 145 percent of the recommended maximum to increase the severity of the test. One *Hevea* tire and one guayule tire were still going at the end of the test after 50,900 miles. The guayule tires were considered fully equal to the *Hevea* tires in this test. The *Hevea*/GR-S and the Guayule/GR-S mixtures were definitely inferior.

Tests of passenger tires were instituted but the results were not considered satisfactory because of failures not related to the tests. In general, GR-S treads on *Hevea* carcasses were thought to be more resistant to tread wear than other combinations. The test was discontinued at 23,201 miles when most of the tires were worn to the fabric.

A manufacturer of electricians' or linemen's gloves reported that guayule rubber was even better than *Hevea* rubber with respect to electrical leakage at 10,000 and 16,000 volts. This report was only of a single test but was considered of some importance since *Hevea* rubber is essential in the manufacture of such gloves.

COST OF GUAYULE RUBBER PRODUCTION

Estimates of the cost of producing rubber from guayule have been based primarily on the experience of the Intercontinental Rubber Co. and the Emergency Rubber Project, the only agencies that have planted appreciable acreages of rubber in the United States. These estimates have been compiled carefully from the best available records but lack the precision that could be gained only by normal operational experience. Prevailing wage rates and other operational costs vary from time to time and from place to place. The quantity of shrub and rubber produced is largely determined by climate, soil types, cultural practices, water requirements, and varieties used. Milling costs depend upon capacity and efficiency as well as upon rubber content of the shrub. Any increase or decrease in rubber from a ton of shrub would tend to lower or increase the cost of rubber per pound.

These normal factors of cost estimates are made more difficult to assess by the fact that, despite the large acreages planted, no standard production procedure has been established, and not even a single crop has been raised, harvested, and the rubber extracted on the normal schedule visualized in the cost estimates. The 8,000 acres of shrub planted by the Intercontinental Rubber Co. were used for rubber production but on an emergency schedule dictated by the need to abandon the shrub on rented land because of the low value of the rubber. The short history of the Emergency Rubber Project hindered any harvesting and extraction studies except of old shrub that had been planted by the Intercontinental Rubber Co. and of young shrub that had not reached the age for most economic rubber production.

To serve as a basis for estimating costs of possible future operations by private enterprise, Lee (*144*)* presented data on actual production costs from the records of the Intercontinental Rubber Co. These represent the average of all cultural, harvesting, and milling costs, and thus represent an operational budget rather than a basic cost-of-production record, which would have to include additional items of capital costs, interest on capital investment, taxes, et cetera.

Table 5 gives the estimate prepared by Lee (*144*)* for the total cultural costs per pound of crude rubber on 598 acres in the Arguello field near Salinas, Calif. The planting was made by the Intercontinental Rubber Co. in 1931 and harvested in 1942 by the Emergency Rubber Project. Although the field costs represented the entire 598 acres, 550 acres only were harvested, the balance containing no harvestable plants at the time of harvest. The Project did not keep harvesting cost records of this field. The Arguello field was owned by the company, and the rental cost is shown arbitrarily.

During four campaigns between 1931 and 1936, the Intercontinental Rubber Co. harvested 2,612,400 pounds of crude rubber, at a total cost of $61,597.04 or 2.358 cents per pound. The factory cost of crude rubber during these campaigns was $140,142.83, or 5.3645 cents per pound. This included general expenses, shrub handling, crushing and milling, separating and refining, drying, boxing, and bagasse disposal. On

TABLE 5.—*Cultural and land rental costs from records of the Intercontinental Rubber Company on 598 acres (Arguello field)*[1]

Operations	Total costs	Costs per acre
Land preparation	$3, 934. 83	$6. 58
Planting	4, 905. 26	8. 20
Cultivation	7, 258. 58	12. 14
Hoeing	3, 018. 47	5. 05
Pest control	48. 00	. 08
General upkeep	1, 481. 59	2. 48
Cost of seedlings	8, 121. 08	13. 58
Supervision	1, 157. 66	1. 94
Autos	119. 73	. 20
Miscellaneous expenses	21. 70	. 04
Total cultural costs [2]	30, 066. 90	50. 29
Rental on 598 acres for 11 years at $10	65, 780. 00	110. 00
Total cultural and land-rental costs [3]	95, 846. 90	160. 29

[1] Crude rubber produced, 880,286 lbs. (1,472.05 lbs. per acre).

[2] Cultural cost per pound, $3.42.

[3] Total cultural and land-rental costs per pound, $10.89.

the basis of cost per pound of rubber produced, these four production campaigns can be summarized as follows:

	Cost per pound (cents)
Culture	3. 400
Land rental	7. 440
Harvest	2. 358
Milling	5. 365
Total production costs	18. 563

Assuming that salaries and labor costs had gone up 90 percent and that other costs had risen 20 percent, Lee estimated that the cost of producing rubber in 1946 would have been 25.414 cents per pound.

These estimated costs did not take into account the advancements and improvements made in the various phases of guayule activities since the Government took over the project in 1942. Proposed changes in factory operation would have reduced the milling cost per pound of crude rubber by 50 percent. Table 6 gives an estimate of project costs, other than milling, based on actual cost experience, except that the actual cultural costs covered four years only, the last three being estimated costs. This estimate did not include any overhead costs. Estimates of cost per acre were originally calculated on the basis of a potential yield of 1,900 pounds of guayule rubber per acre. For comparison, costs per acre are also included for a yield of 1,400 pounds of rubber per acre which approximates the yield actually obtained from the Arguello planting.

TABLE 6.—*Estimate of Emergency Rubber Project costs per pound of rubber produced (estimated) at the time of the liquidation of the project* [1]

Operations	Cost per acre	Cost per lb. at 1,900 lb. per acre	Cost per lb. at 1,400 lb. per acre
		Cents	*Cents*
Rental at $10 for 7½ years	$75.00	3.95	5.36
Culture	187.02	9.84	13.36
Harvest	37.78	2.10	2.70
Milling	81.65	4.30	5.83
Total cost per pound of crude rubber	----------	20.19	27.25

[1] These costs are based partially on actual costs and partly on projected costs for a continued 3½ years of operation.

Lobenstein and Champagne (*147*)* prepared estimates of guayule production costs from the time of ground preparation through harvesting on irrigated and on dry land in California, and Lobenstein and White (*148*)* prepared production cost estimates for guayule cultivation in Texas. Land rental costs were not included. These estimates are summarized in table 7.

TABLE 7.—*Estimated costs per acre for producing guayule shrub in California on irrigated land (4-year rotation) and non-irrigated land (5-year rotation), and in Texas (5-year rotation)*

Operations	Estimated direct costs per acre		
	California		Texas, dry land
	Irrigated	Dry land	
Ground preparation	$33.32	$11.78	$3.50
Planting stock	22.45	18.40	8.73
Planting	10.82	9.80	19.80
Weed control:			
Machine cultivation	9.26	11.55	14.26
Hand cultivation	61.88	57.91	38.35
Oil spraying	14.61	24.35	----------
Pest control	----------	----------	4.00
Irrigation	61.44	----------	----------
Harvesting	[1] 60.67	[2] 37.78	[3] 30.00
Total	274.45	171.57	118.64

[1] 9.4 tons (shipping weight) shrub per acre.

[2] 5.06 tons (shipping weight) shrub per acre.

[3] 7.5 tons (shipping weight) shrub per acre.

The cost of producing nursery plants had been estimated at from approximately $2.00 to $2.50 per thousand. The direct cost of producing seedlings in 1943 was about $2.50 per thousand (*186*).

During the seedling stockpiling program in 1951, an estimated total of 89,840,800 guayule nursery plants were produced on 461.70 acres under cultivation in Zavala County, Tex., at a production cost of $100,594.36 (*45*)*. The average cost per acre was $217.88 or $1.12 per 1,000 plants. Average number of plants per acre was 194,587. Many adverse conditions contributed to costs higher than budgetary estimates for establishing seedlings. A breakdown of costs is given in table 8. Land rental was not included.

Tysdal (*241*) presented cost data on the production of nursery stock, transplanting, and establishing guayule for a period of 3 years for seed stockpiling purposes. Table 9 indicates a total cost of $128.56 per acre at the end of the 3-year period. Nursery plants were produced at a cost of 75.6 cents per thousand. Land rental was not included for established guayule.

SUMMARY

Guayule (*Parthenium argentatum* Gray), a member of the Compositae, is a semidesert shrub native to the dry lands of north-central Mexico and adjacent Big Bend areas in Texas. The genus *Parthenium*, comprising 16 recognized species, is native to the Western Hemisphere, extending (with the exception of the Tropics) from Wyoming and Minnesota to northern Argentina. The species range from ephemeral annuals through perennials and woody shrubs to ligneous treelike types. *P. argentatum* is the only species of the genus known to produce significant amounts of rubber.

TABLE 8.—*Cost of producing nursery stock in Zavala County, Tex.*

Operations	Cost of producing nursery stock		
	Total cost	89,840,800 plants	On 461.70 acres
		Per 1,000 plants	*Per acre*
Land preparation and seeding [1]	$16, 175. 02	$0. 18	$35. 03
Water service	1, 683. 50	. 02	3. 65
Pre-irrigation	1, 726. 98	. 02	3. 74
Irrigation	20, 038. 30	. 22	43. 40
Cultivation	2, 950. 50	. 03	6. 39
Weed control [2]	50, 796. 78	. 56	110. 00
Insect and disease control	2, 151. 68	. 02	4. 66
Supervision	5, 071. 60	. 06	10. 98
Total	100, 594. 36	1, 12	217. 88

[1] These costs were high because initial plans and plantings were based on a larger program than that carried to completion. This factor also affected, but to a lesser degree, the next three items listed in the table.

[2] This charge includes $4,606.69 expended for weed-spraying oils, and approximately $2,200 for contract application of oils.

TABLE 9.—*Cost of establishing guayule for 3 years under dryland cultivation in the Salinas Valley*

Operations	Cost per acre of established shrub	Total cost, end of first, second, and third year
Nursery stock:		
Land rental	$70.00	
Land preparation	20.00	
Planting	4.00	
5 irrigations at $6	30.00	
8 cultivations at $1.75	14.00	
2 oil sprayings at $7	14.00	
3 hand hoeings at $25	75.00	
	227.00	[1] 7.56
First year of establishment:		
Digging nursery plants	9.00	
Shipping nursery plants	7.00	
Transplanting	15.00	
Land preparation, including fallowing	20.00	
3 cultivations at $2	6.00	
6 cultivations, spring tooth at 50 cents	3.00	
2 hand weedings at $15	30.00	
	90.00	97.56
Second year of establishment:		
4 cultivations at $2	8.00	
1 hand weeding at $15	15.00	
	23.00	120.56
Third year of establishment:		
4 cultivations at $2	8.00	128.56

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[1] Cost of producing 10,000 nursery plants required for 1 acre of field planting. One nursery acre, ordinarily producing 300,000 plants (in California), is sufficient for 30 acres of field planting.

Guayule requires good agricultural soils having nearly ideal moisture relationships. Heavy, poorly drained soils, and those with claypans and hardpans, are particularly undesirable since they permit continuous growth without benefit of periods of moisture stress, necessary for rubber storage.

Suitable areas for guayule production extend from the central valleys and coastal areas of California, through southern Arizona, southwestern New Mexico, to the southwestern and southern parts of Texas.

Guayule makes its best growth at temperatures around 90° to 100° F. The most critical aspect of temperature is the minimum. It should be grown in areas where minimum temperatures do not drop below 15° F., unless the winters are dry and the plants are normally in a dormant condition for a considerable length of time before the minima occur. When in a completely dormant state, guayule has withstood temperatures of zero degrees F. without injury.

Annual rainfall should not be under 15 inches for dryland culture, although a minimum of 11 inches is satisfactory in areas, such as the coastal valleys of California, with cool foggy summers. Areas having more than 25 inches of rain of year-long distribution produce large guayule with low rubber content.

Irrigation is one of the chief cultural factors influencing yield of rubber. Conditions favorable to maximum growth adversely affect the accumulation of rubber. Plants grown under high-moisture stress yield higher percentages of rubber but less shrub than plants grown under low-moisture stress. In general, shrub receiving light irrigations, while intermediate in shrub yield and in percentage of rubber, give the highest yield of rubber per acre.

Seedlings grown under high-moisture stress at Salinas, Calif., could be transplanted any time of the year with satisfactory results. Survival of seedlings grown under low-moisture stress was low.

In preparation for transplanting, nursery seedlings were topped in place, undercut, pulled, and packed in boxes or crates. The best topping level is attained when all but about 1 inch of the top is removed. The resulting defoliation is essential for successful survival of nursery stock, since the leaves of guayule contain an auxin (3-indoleacetic acid) which retards growth when these are left on the plant. Nursery plants tend to root better when they are topped approximately 3 days before digging. Losses of packed nursery stock caused by disease may be minimized by using well-hardened and relatively leafless stock, which survives best in transplanting. Topped nursery stock in a properly hardened condition may recover from considerable desiccation.

Experimental work was done on stand densities for maximum yield of rubber. If shrub is to be left unharvested for more than 4 years, wide spacings such as 36 x 24 inches between plants yield the maximum tonnage of shrub. For short-term production, narrow spacings yield the most shrub. Yields of rubber up to a rate of 1,708 pounds per acre were obtained after 33 months from seeding from 14-inch, unthinned plantings.

Attention was given to direct seeding as a means of eliminating the cost of growing and transplanting nursery seedlings. Direct seeding in the field for rubber production was less successful than for the production of closely spaced nursery seedlings. Guayule seed cannot be covered more than 1/4 inch, and constant moisture must be maintained by frequent irrigations until the slowly growing seedlings become established. Seeding on dry land is not feasible unless rainfall is adequate for seed germination and plant establishment. Direct seeding in wastelands was not successful.

Selective oil sprays were effective in controlling weeds in the nurseries, but were less effective in controlling weeds in plantations. Special cultivators were designed to remove weeds not killed by oil within the row. Some weeds were resistant to selective oils at all stages of growth.

In harvesting cultivated guayule, the Emergency Rubber Project discarded the former practice of chopping the shrub in the field for delivery to the mill and adopted a method of digging the shrub and baling it to facilitate shipping.

Research was focused on the possibility of harvesting only the upper portion of the plants by mowing, leaving the crown to generate new growth in order to eliminate the cost of reestablishing plantings. Under certain conditions, this method of harvesting is feasible.

Highly effective means of cleaning and threshing guayule seed were devised. Threshed seed has several advantages over unthreshed seed. Viability may be retained for more than 15 years by bringing the seed to 4-percent moisture or lower, and storing it in sealed metal drums.

Rubber in guayule exists as a colloidal suspension in individual cells instead of in organized latex tubes, as found in some other rubber-producing plants. In general, the major portion of the rubber is found in the vascular rays of phloem and xylem. Lesser amounts are formed in the pith, xylem parenchyma, and epithelial cells of resin canals. Very small amounts are present in the leaf parenchyma and in the peduncles.

Several factors affect the formation of rubber in guayule. Among these are differences in response of individual strains to different environmental conditions. Concentration of rubber is lowest during periods of rapid growth induced by low-moisture stress, and highest during periods of high-moisture stress. Under experimental conditions, light, temperature, and nutrient levels were shown to affect the formation of rubber. A series of experiments involving guayule in interspecific and intergeneric reciprocal grafts with non-rubber species demonstrated that rubber accumulation is a property of the rubber-bearing tissues, and not of substances supplied to these tissues, as it was formerly thought, and that the immediate precursors of rubber are formed only in these tissues. No positive evidence has been found that rubber serves any specific physiological function in guayule.

Resins are found principally in the bark, and are distributed rather uniformly there. There are seasonal variations in resin percentages that are not affected greatly, or in any consistent manner, by moisture stress.

The main reserve carbohydrates in guayule are the levulins. These increase during periods of high-moisture stress and decrease during periods of low-moisture stress. This may explain the greater survival rate of transplanted nursery seedlings grown under high-moisture stress. The monosaccharides were regarded as the chief carbohydrates of translocation.

Guayule is a low user of nitrogen, phosphoric acid, and potash. Commercial fertilizer had little or no effect on either shrub or rubber yields.

Various concentrations of salts in soils had a marked effect on shrub survival and yield of rubber. Guayule is not regarded as a salt-tolerant plant.

Methods were worked out by which guayule stem cuttings could be easily rooted on a fairly large scale. However, vegetative propagation from cuttings was considered impractical for commercial plantings.

Several diseases have been found in cultivated guayule. These are not important factors in the production of nursery or field-grown guayule on most well-drained soils. Excessive amounts of water on poorly drained soil may cause serious disease losses. *Sclerotinia* and *Botrytis* were the most common fungi growing on crated seedlings in storage. Losses caused by these fungi were usually attributed to high storage temperatures and high humidity.

Insects of most economic importance were grasshoppers and *Lygus* bugs. Grasshoppers were controlled by using poison bait. *Lygus* bugs were controlled by applications of DDT dust. *Lygus* bugs may at times seriously reduce the weight and viability of seed.

Guayule is an unfavorable host to rootknot nematodes, and these are not of economic importance in guayule production.

There is strong evidence that guayule originated in eastern Durango in Mexico, since diploid sexual forms are found only in this area, whereas facultatively apomictic polyploids predominate elsewhere in the range.

Seed collections were made in Mexico and Texas in 1942 to supplement the available breeding stock, which did not contain all the genotypes required in a breeding program. Additional seed collections were made in Mexico in 1948.

The highest rubber-yielding introductions were obtained in Mexico, in that region where the States of Durango, Coahuila, and Zacatecas adjoin.

Seed increases of all high rubber-yielding selections were made. The seed was brought to a moisture content of 4 percent and sealed in metal drums.

Analytical procedures were standardized to guide the research staff in laboratory and field studies. In addition to detailed research assigned to the research agencies of the Department of Agriculture, the Emergency Rubber Project initiated production research as an immediate guide in the operations of the project. Soil surveys to determine suitable lands for guayule culture proved highly valuable. Projects were set up to guide field work, and to provide accurate estimates of the amount of shrub being grown and its probable rubber content.

A major contribution of production research was guidance in the processing of shrub. This led to improvements in methods of harvesting and handling the shrub before milling. Time of "curing" in the field was reduced; shredding of the shrub in the field was eliminated; a method of defoliation of the shrub was developed; and improvements were made in milling procedures.

After liquidation of the wartime Emergency Rubber Project, detailed studies were made of the methods of extracting the rubber, and of the type and value of the byproducts that might be produced, including resins, water solubles, bagasse, leaves, and cork.

A high quality of rubber was obtained from fresh shrub by extracting it in the form of latex rather than as solid rubber. When the project was discontinued, many problems of purification and concentration had been solved and the production of guayule latex appeared promising.

The deresinated guayule rubber protected by antioxidants proved equal to regular grades of Hevea rubber in many tests.

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