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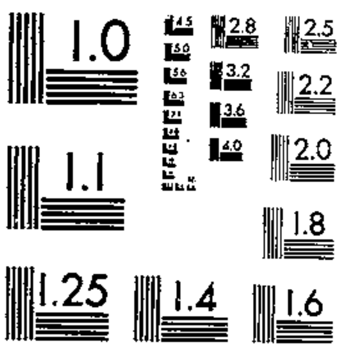


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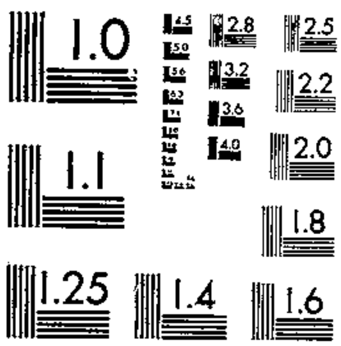


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# THE RUBBER CONTENT OF TWO SPECIES OF CRYPTOSTEGIA AND OF AN INTERSPECIFIC HYBRID IN FLORIDA

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CONTENTS



## INTRODUCTION

In studying the possibilities of rubber production in the United States, two species of rubber vines from Madagascar, *Cryptostegia grandiflora* R. Brown and *C. madagascariensis* Bojer, have received special attention. It was known that these plants would grow and reproduce in southern Florida, and experiments have shown that they are adapted to a wide range of conditions in southern California.

Rubber has been produced from *Cryptostegia* plants in Madagascar and India and marketed as " palay " or " pulay." Samples were on display at the Exposition of Madras as long ago as 1856. Both in India and in Madagascar the rubber was obtained from wild plants by crude native methods, and no system of cultivation was developed. Notwithstanding this early interest in the rubber-producing possibilities of these plants, *Cryptostegia* was first introduced into the United States as an ornamental rather than as a rubber plant.

*Cryptostegia grandiflora*, the palay rubbervine, was introduced into Mexico by a German sea captain who presented the seeds to friends in Mazatlan as those of a garden ornamental. The plant soon became established and spread throughout southern Sinaloa where it became known locally as " Clavel d'España " or " Clavel Alemán." From Sinaloa the plant spread to other parts of Mexico and to Florida and the West Indies. The introduction into the West Indies was made by Charles S. Dolley, who had become interested in the rubber-producing possibilities of *Cryptostegia* through contacts in Sinaloa.

*Cryptostegia madagascariensis*, or Madagascar rubbervine, sometimes erroneously referred to as " purple allamanda ", was introduced into Florida early in the twentieth century as an ornamental. As early as 1904, this species was listed in the catalog of a Florida nurseryman, though under the name of *C. grandiflora*. It proved popular in southern Florida, and today the species is well represented in garden and street plantings, especially in the Miami section.

To determine the cultural requirements of the two species of *Cryptostegia* and of an interspecific hybrid which was developed at the United States Plant Introduction Garden, at Coconut Grove, Fla., various methods of cultivation and exposure have been tried at this garden. Methods of propagation, both sexual and vegetative, have been tested and compared. Morphological variations within the two species have been studied, and also differences between the two species and the hybrid.

Investigation of the rubber-producing capacity of *Cryptostegia* has included comparisons of the rubber content of the two species and the hybrids, determination of seasonal variation in rubber content, and determination of individual plant yields.

## BOTANICAL DESCRIPTION

The names of the two species of *Cryptostegia* in many cases have been interchanged. This has occurred not only in Florida but in other parts of the world. Even from Madagascar, material received as *C. madagascariensis* later has been found to represent *C. grandiflora*. There appears to be no occasion for confusing the two species, since the essential differences are outstanding and easily recognized.

*Cryptostegia grandiflora* was originally described by Roxburgh (*9, v. 2, pp. 10–11*)[1] as *Nerium grandiflorum*, thus placing it in the botanical family Apocynaceae. His description is as follows:

9. *N. grandiflorum* R.

Shrubby, twining. *Leaves* oblong, polished. *Flowers* terminal. *Nectaries* bifid. *Follicles* three-sided horizontal.

A native of the Peninsula of India. In the botanic garden at Calcutta it is in flower a great part of the year though the seeds do not often come to maturity.

*Stem* stout, and woody. *Bark* smooth, greenish ash colour. *Branches* twining up and over trees of very considerable size, every part abundantly lactescent when wounded. *Leaves* opposite, short petioled, oblong, entire, obtuse-pointed, polished on both sides; underneath minutely reticulated, about three inches long, by one and a half broad. *Flowers* terminal, from one to many, forming a dichotomous raceme with one in the fork, very large, pale pink. *Bracts* conically-lanceolate, opposite, caducous. *Calyx* five-leaved. *Leaflets* oval-lanceolate, with ample, thin curled margins. *Corolla* campanulate, half five-cleft. *Nectaries* five, not alternate with, but attached to the tube of the corolla immediately above the stamina; each divided into two long, filiform, coloured segments. *Filaments* short, inserted on the contracted base of the tube of the corolla. *Anthers* cordate, incurved in form of a dome over the stigma. *Germs* two, one celled, each containing many ovula attached to a large projecting fleshy receptacle on the inside. *Style* at the base double and coalescing

[1] Italic numbers in parentheses refer to Literature Cited, p. 21.

into one body near the top. *Stigma* single, large, globular, with the vertex two-toothed, and five glands round the side, which are firmly attached to the inside of the five anthers near their base; between these are five dark-coloured, spoon-shaped scales, which become detached by age. *Follicles* horizontal, three-sided, with the angles sharp; tapering to a long, incurved, rather obtuse beak.

In 1819 Robert Brown determined that the plant described by Roxburgh as *Nerium grandiflorum* possessed specialized floral parts for transferring the pollen from the anthers to the stigma.[2] Brown therefore placed this species in the family Asclepiadaceae, rather than Apocynaceae, and created the new genus *Cryptostegia.* His original Latin description appeared in the Botanical Register (*1*) and is translated as follows:

*Cryptostegia.*

Corolla infundibuliform, tube with five included, bipartite, subulate scales, alternate with lobes of limb. Stamens included, inserted in lowest part of tube, filaments distinct, anthers coherent with base of stigma. Translators five, spatulate, collecting the granular pollen in the angles of the stigma. Ovaries two. Styles two. Stigma pentagonal. Seeds tufted. Climbing glabrous shrub. Leaves opposite. Peduncles terminal, three-parted. Flowers showy. Corolla contorted in aestivation. Follicles angular, very divaricate.

W. Bojer in 1837 made brief mention of another species of *Cryptostegia* which he named *C. madagascariensis*, and although his announcement could scarcely be termed a description, he is credited with having described this species.

A translation of the notice which appeared in Bojer's Hortus Mauritianus (*2, p. 212*) follows:

*C. madagascariensis* Boj.

Native of the island of Madagascar. Grows on the seashore, especially on the Bay of Bombetok. Cultivated in gardens and on the River Noire. Shrubby vine. Flowers March to May.

The first record of a detailed description of *Cryptostegia madagascariensis* is that of De Candolle (*3, v. 8, pp. 491–492*) in 1844. A translation from the original Latin follows:

*C. madagascariensis* Boj.

Leaves ovate-elliptical, rounded at the base, at apex short acuminate, whitish pubescent on under surface. Calyx lobes ovate, acute (when dry), with undulate-reflexed margins. Corolla lobes ovate, acuminate, spreading, twice as long as tube. Corolla scales linear-lanceolate, pointed, entire. Follicles triangular, widely divergent. Native of the island of Madagascar.

Miquel (*8*) seems to have been the first botanical authority to recognize the confusion of species in Cryptostegia. His accounts were based on observations of both species growing in the Botanical Garden at Buitenzorg, Java, and clearly recognize the basic differences between the two species.

In addition to the two universally accepted species of *Cryptostegia*, other more or less diverse forms of this genus have been recognized. Jumelle and La Bathie (*7, p. 295*) have emphasized the widespread occurrence of polymorphism among many different species and families of plants growing in Madagascar. They state:

One of us has observed in the northwest of Madagascar more than twenty species, apparently of very different families, which show similar variations.

[2] These organs, known as translators, are generally spoon-shaped with a sticky disk at the narrow end which becomes attached to visiting insects. The translators are alternate with the stamens, and each one receives pollen from the two adjacent anther halves. The presence of translators is a constant characteristic of the family Asclepiadaceae (pl. 5).

These species maintain themselves, in spite of brush fires, on the laterite hills which were formerly wooded. Arborescent elsewhere, they form here only small bushes not more than 1 or 2 meters in height, with a monstrous stump, flattened and subterranean. The pubescence, the leaves, the flowers, the fruit, even, are, at the same time, diversely modified, but in an inconstant manner, and rarely offer the certain characters of heredity. Among the *Landolphia*, *Landolphia perrieri* is one of the species which is very polymorphous in all parts and is able to become pubescent. Among the *Cryptostegia*, we recognize today a similar hairy form of *Cryptostegia madagascariensis*.

One of these divergent forms has been described by Hochreutiner (*5, pp. 89–90*) and tentatively given specific rank as *Cryptostegia glaberrima* (Hochr.). His description is as follows:

*Cryptostegia glaberrima* Hochr. sp. nov.—*Cryptostegia madagascariensis* Bojer ex DC. Prod. VIII, p. 492, pro parte.

Climbing. Stems pale brown, glabrous, with prominent and more or less quadrangular lenticels, cylindric, flattened a little at the apex and with the lenticels fewer and smaller. Leaves ovate-oblong, even the young ones glabrous on both sides, entire, rounded at the base, strongly acuminate at the apex, finely reticulate; petioles short, glabrous. Inflorescence corymbose, mostly few-flowered (I have seen 6–12 flowers); peduncle very glabrous, pedicels glabrous or minutely and scantily pilose. Sepals lanceolate, puberulous outside, very slightly so inside at the apex; calyx with about 10 small lanceolate glands at the base. Corolla infundibuliform, large and broad, outside, especially at the base, minutely puberulent, lobed to about 3/11 its length from base, with the lobes broadly ovate, acute, imbricate, dextrorsely imbricate. Crown formed by 5 appendages, lanceolate-subulate, glabrous, inserted above the androecium. Staminal column ovate-conical, anthers with short acute apex. Translators spatulate. Stigma conical, style divided to the base. Ovary bilocular, with the walls contiguous, grown together along the margins and strongly diverging in the middle. Follicles when mature diverging, woody, ovate-lanceolate, acute, subtriquetrous with two prominent foldings on the sides, and one dorsal folding which is narrow. Seeds brown, with pappus; pappus long, silky, very white.

Leaf blade 9×5.2 to 5×3.4 cm. long and wide; petioles about 0.8 cm. long, acumen hardly 1 cm. long. Inflorescence up to 10 cm. in diameter; pedicels 0.3 to 0.7 cm. long. Sepals about 1×0.3 cm. long and broad. Corolla 4 to 5 cm. long and 3 to 4 cm. broad at the apex. Appendages of the crown 0.7×0.075 cm. long and broad. Staminal column 0.35 cm. in length. Follicles about 8 cm. long, and with the foldings, 4.2 cm. broad; lateral foldings up to 1 cm. and the dorsal about 0.3 cm. broad. Mature seeds about 0.9×0.35 cm. long and broad; setae of the pappus more than 3 cm. in length. Habitat: District of Vatomandry, concession of Sakarive, rubber vine, flower a clear mauve.

Hochreutiner himself apparently is not certain that the type which he has described is deserving of specific rank, for he states that he is defining it as a species only to serve "ad interim", or until further studies of the geographical distribution are reported. However, no record was found of further studies for the purpose of establishing Hochreutiner's *glaberrima* type as a natural species. Hochreutiner states that his description of *C. glaberrima* is based on a single branch which he found in the Prodromus Herbarium side by side with the branch upon which De Candolle based his description of *C. madagascariensis*. The two branches evidently are quite different as regards pubescence, for De Candolle describes the leaves as being tomentose on the under side, while Hochreutiner stresses the glabrous character of both sides of both young and old leaves in the *glaberrima* type.

In view of this evident diversity of herbarium material as contrasted with the marked uniformity of a fairly large population of *Cryptostegia madagascariensis* introduced from various sources, it appears that both De Candolle and Hochreutiner may have worked with material of hybrid origin.

The leaves of all *C. madagascariensis* plants that have been studied at the plant introduction garden are entirely glabrous at all stages of development. The young leaves of *C. grandiflora* are scantily pubescent, but the mature leaves are glabrous. The only plants that had definitely pubescent leaves were grown from seed received from Tananarive, Madagascar, in 1925. These plants evidently represent the second, or later, hybrid generation following an interspecific cross, as the individuals are very diverse and represent many different combinations of characters.

## DISCOVERY OF THE INTERSPECIFIC HYBRID

The interspecific hybrid referred to on page 21 was discovered in the spring of 1927 by Alfred Keys, associate horticulturist in the Office of Cotton, Rubber, and Other Tropical Plants. In 1926 he harvested two pods from a normal plant of *Cryptostegia grandiflora*. The seeds from the two pods were sown separately, but both lots of seedlings were given identical treatment and at the age of 11 months were set out in field plantings.

Up to this time no difference in the two lots had been noticed, but when planted side by side in the field it was apparent that while all of the plants obtained from one pod (lot 1) were true to the *grandiflora* type, the plants from the other pod (lot 2) were much more vigorous and exhibited certain morphological characters which were distinctly different.

In most respects the plants of lot 2 resembled *C. madagascariensis* as much as they did *C. grandiflora*, and in several characters they were definitely intermediate between the two species, thus affording cumulative evidence that cross-pollination had taken place and that the new plants were natural interspecific hybrids; in other words, the first generation of *C. madagascariensis* × *C. grandiflora*. This indication was confirmed when the second generation was produced and its characters studied, showing the segregation and recombination of characters commonly found in the second generation following a specific cross.

The flower structure of *Cryptostegia* is apparently adapted for cross-pollination, as evidenced by the specialized organs for conveying the pollen, but other cases of interspecific crossing are not known to have occurred at the plant introduction garden, where the two species have been grown side by side for 8 years.

There is no record of any plant of *C. madagascariensis* having produced any seed which did not come true to type. One lot of *C. grandiflora* seedlings showed several outstanding plants which may have been interspecific hybrids, but no other off-type progeny of *C. grandiflora* has appeared, and all efforts to cross-pollinate the two species artificially have failed. The plants of lot 2 (see above) are the only ones which have been proved to be hybrids and which have exhibited a combination of characters superior to the parental types.

Some plants growing in a private botanic garden near Homestead, Fla., have been found to possess vegetative and floral characters very similar to the hybrids of lot 2. The origin of the seed from which these plants were grown is not known, but there can be little doubt of their hybrid origin.

As mentioned above, the hybrid was first noticed because of its exceptional vegetative vigor. A more detailed comparison with the parent types revealed numerous morphological differences that had previously escaped attention.

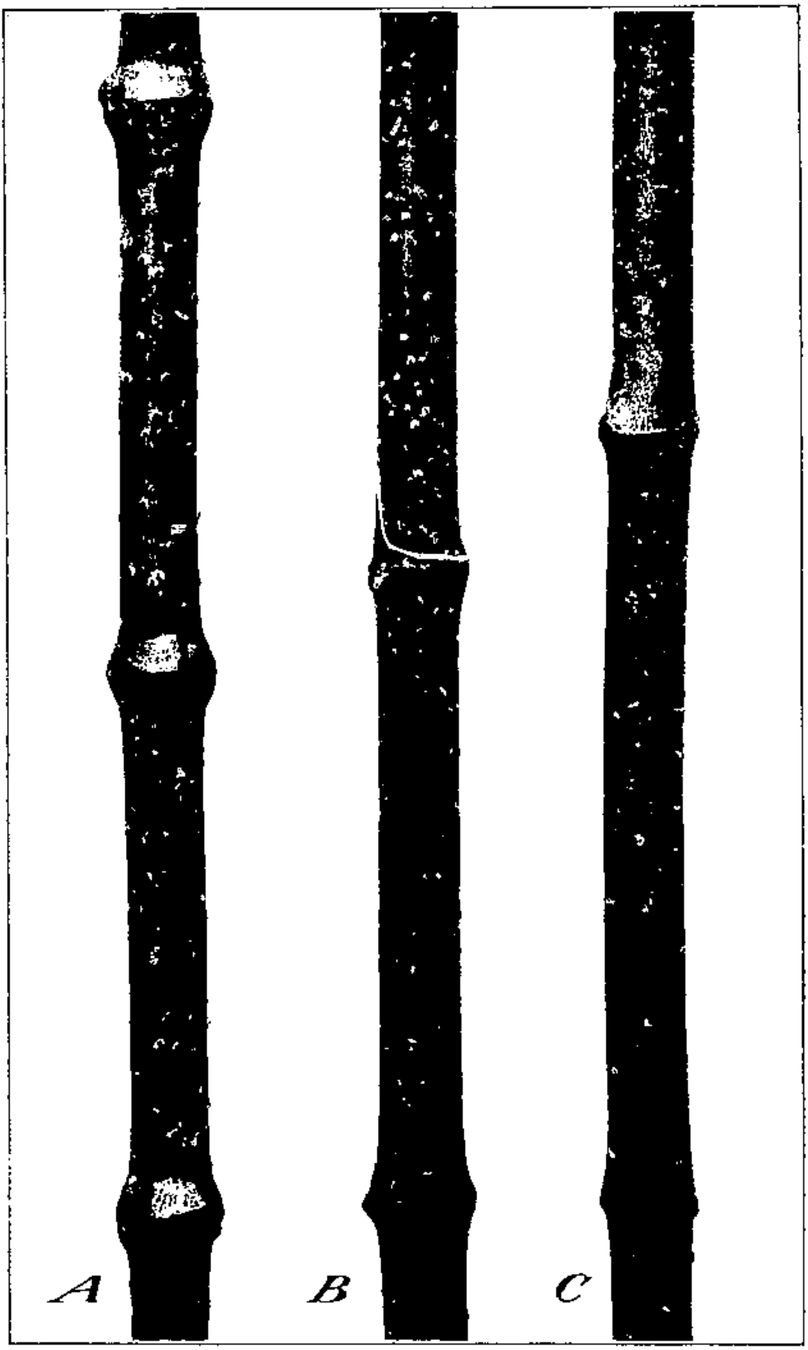
With the exception of three off-type plants, described on page 7, the hybrid population of 189 individuals is comparatively uniform. There is slight variation among the individuals in the expression of some of the quantitative characters, such as seed-pod size, amount of pigmentation in the leaves, depth of division of the petal appendages, rubber content, etc., but in general the hybrid type is well defined and easily recognizable. Individuals that have been transplanted to different environments show no great divergence in the expression of the character complex which constitutes the hybrid type.

The outstanding points of difference between the hybrid type and the parent species are shown in table 1 and in plates 1 to 6, inclusive.

TABLE 1.—*Comparison of plant characters of 2 species of Cryptostegia and an intermediate hybrid*

| Character | C. madagascariensis | C. grandiflora | Hybrid |
|---|---|---|---|
| Habit of growth | Compact, bushy shrub with slight vining tendency. Usually forms 2 or 3 main branches at or near the ground. | Large climbing shrub characterized by whiplike, twining shoots. Climbs to a great height if given a support. Usually does not branch so near the ground as *C. madagascariensis*. | Considerably larger and more vigorous than either parent. Makes fairly compact growth, but has pronounced vining tendency like *C. grandiflora*. Branching habit similar to *C. madagascariensis*. |
| Stem color and texture. | Dull grayish-brown bark of both young and old growth much rougher than *C. grandiflora*. | Main stem grayish brown and bark fairly rough. Younger branches greenish brown and smooth except for lenticels. | Similar to *C. grandiflora*. |
| Nodes [1] | Considerably enlarged | Less prominent than those of *C. madagascariensis*. | Intermediate. |
| Internodes [1] | Comparatively short | Comparatively long | Similar to *C. grandiflora*. |
| Lenticels [1] | Few and large | Small and numerous | Few and large. |
| Pedicels | Length 7 to 8 mm | Length, 8 to 9 mm | Length, 8 to 9 mm. |
| Corolla color and size.[2] | Light purple except enfolded portion of lobes, which are much lighter in color. Inside of tube darker, with white streaks where petals are united. Length, 55 to 60 mm; width, 60 to 70 mm; depth of tube, 25 to 30 mm; width of tube, 12 to 14 mm. | Inside almost pure white except for a purplish tinge along the median line of each petal near the base. Outside very pale purple, streaked with darker purple near base. Enfolded portion of lobes almost white. Length, 70 to 75 mm; width, 80 to 90 mm; depth of tube, 45 to 50 mm; width of tube, 11 to 13 mm. | Inside pale purple, darker at base, with white streaks inside tube where petals are united. Outside slightly deeper shade of purple except enfolded portion, which is very pale. Length, 65 to 70 mm; width, 80 to 90 mm; depth of tube, 35 to 40 mm; width of tube, 15 to 18 mm. |
| Corolla lobes | Length, 35 to 40 mm; width, 18 to 22 mm. | Length, 45 to 50 mm; width, 25 to 28 mm. | Length, 45 to 50 mm; width, 25 to 30 mm. |
| Sepals | Length, 7 to 8 mm; width, 3 to 4 mm. | Length, 13 to 15 mm; width, 6 to 7 mm. | Length, 13 to 15 mm; width, 5 to 6 mm. |
| Corolla appendages.[3] | Purple. Length, 8 to 9 mm. Not cleft. Do not converge above stigma head. | White at base, faint purple tinge toward apex. Length, 12 to 13 mm. Deeply cleft. Converge above stigma head. | Light purple. Length, 11 to 12 mm. Shallowly cleft. Converge above stigma head. |
| Translators [4] | Length, 2 mm; width, ½ mm. Narrowly triangular ovate. Abruptly contracted at base into short stipe. | Length, 2¼ mm; width, 1 mm. Spatulate-orbicular. | Length, 2¼ mm; width, 1 mm. Ovate, rounded at base into short stipe. |

[1] Comparisons of the nodes, internodes, and lenticels are shown in pl. 1.
[2] The size of the corollas is shown in pls. 2 and 3.
[3] See pl. 4.
[4] See pl. 5.



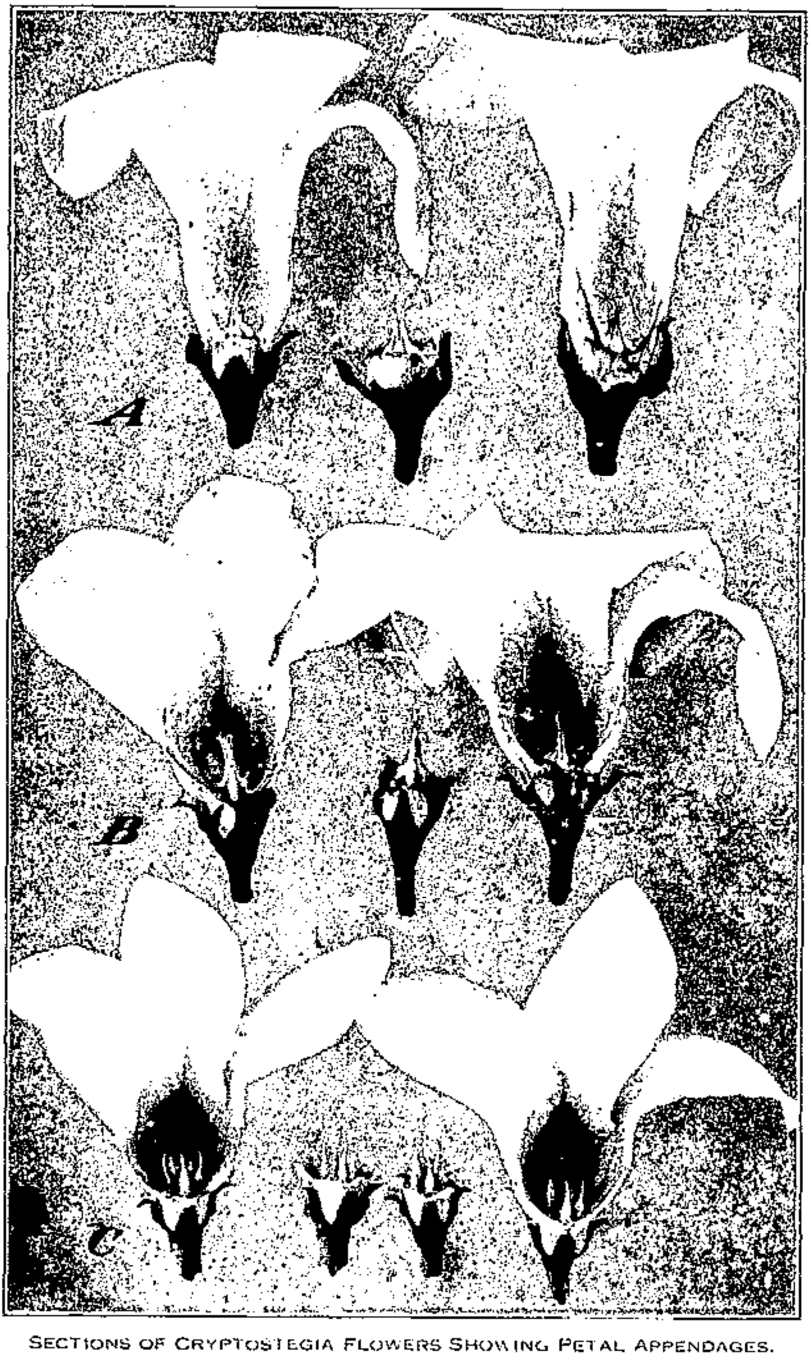

TWO SPECIES OF CRYPTOSTEGIA AND THE INTERSPECIFIC HYBRID

Comparison of lenticels, nodes, and internodes: *A*, *Cryptostegia madagascariensis*; *B*, *Cryptostegia* hybrid; *C*, *C. grandiflora*. (Natural size.)

FLOWERS OF THE TWO SPECIES OF CRYPTOSTEGIA.

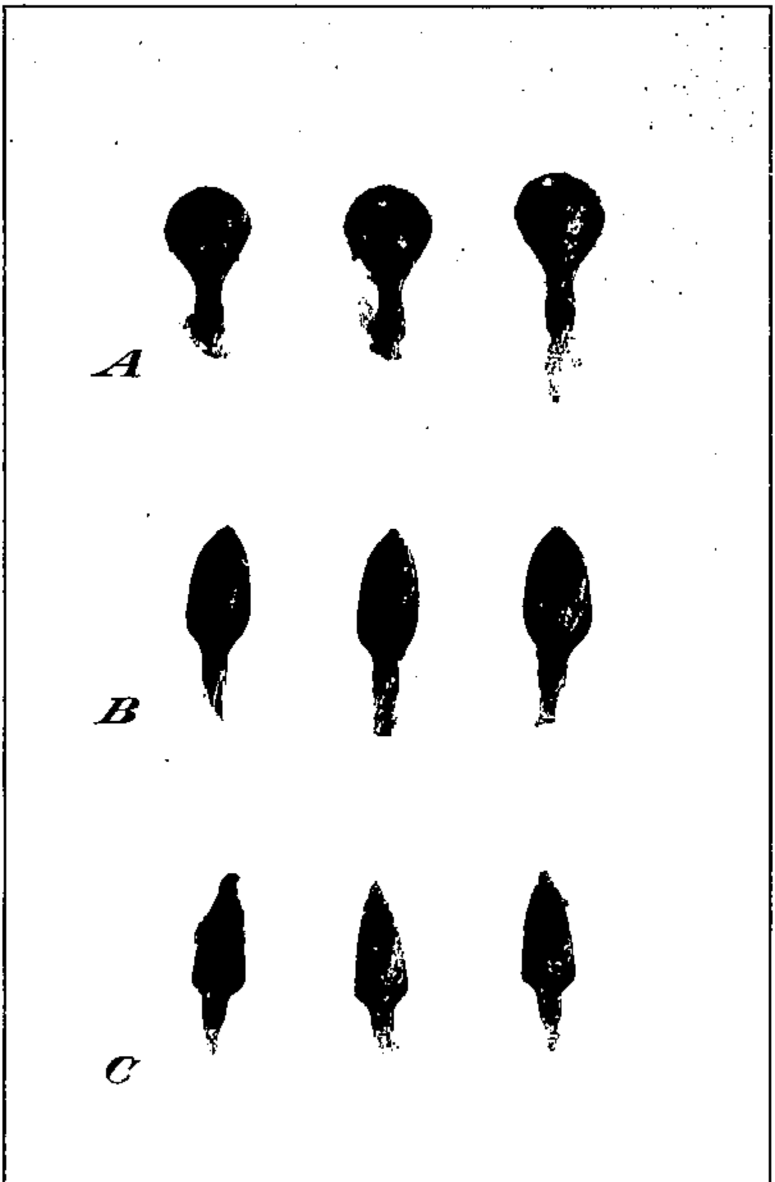
*A*, *Cryptostegia grandiflora*; *B* and *C*, *C. madagascariensis*. (Natural size.)

FLOWER OF THE CRYPTOSTEGIA HYBRID. (NATURAL SIZE.)



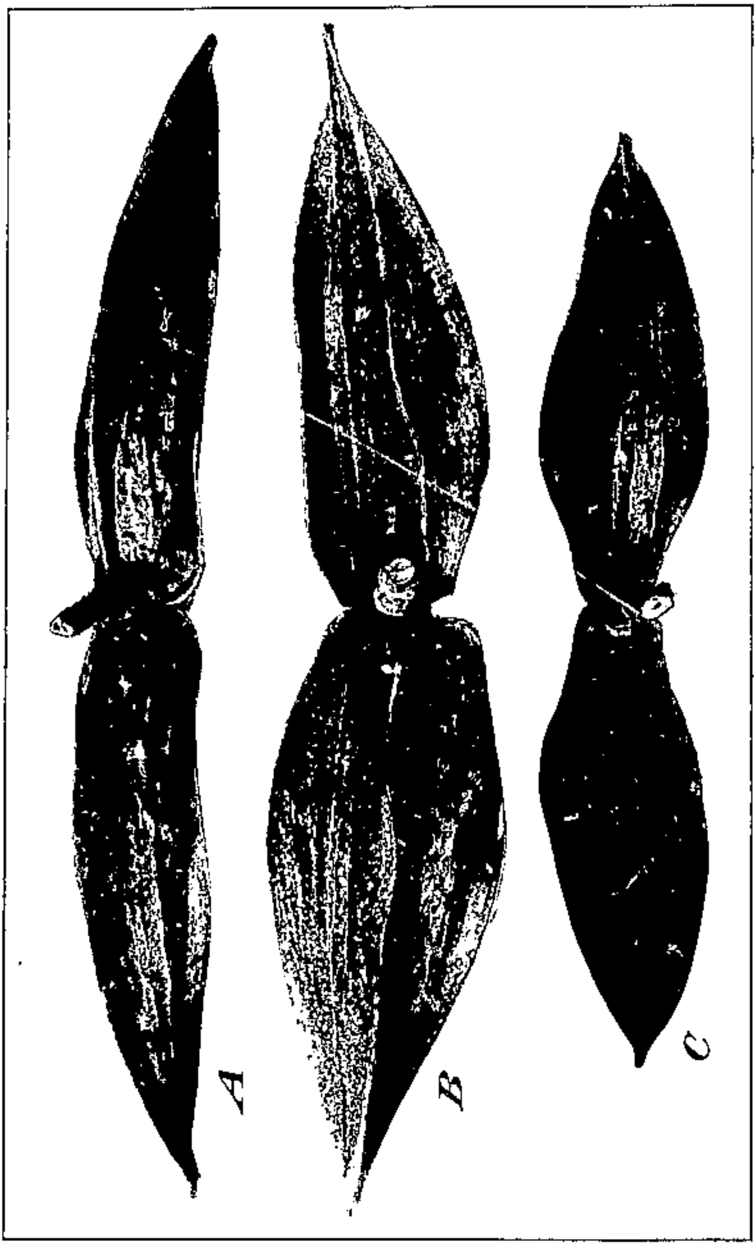

SECTIONS OF CRYPTOSTEGIA FLOWERS SHOWING PETAL APPENDAGES.

*C. grandiflora* (*A*) and the hybrid (*B*), converging above the stigma head, while the appendages of *C. madagascariensis* (*C*) are erect and separate. (Natural size.)




COMPARISON OF TRANSLATOR SHAPE.

*A*, *C. grandiflora*; *B*, hybrid *Cryptostegia*; and *C*, *C. madagascariensis*. (×10.)




COMPARISON OF RIPE SEED PODS.

*A*, *Cryptostegia grandiflora*; *B*, *Cryptostegia* hybrid; *C*, *C. madagascariensis*. (Nearly natural size.)


COMPARISON OF OFF-TYPE HYBRID CRYPTOSTEGIA SEED PODS WITH NORMAL HYBRID CRYPTOSTEGIA SEED POD.

*A*, Pod from plant B-15; *B*, pod from plant B-16; *C*, pod from normal hybrid plant. (Nearly natural size.)

TABLE 1.—*Comparison of plant characters of 2 species of Cryptostegia and an intermediate hybrid*—Continued

| Character | C. madagascariensis | C. grandiflora | Hybrid |
|---|---|---|---|
| Young leaves | Light green. Midrib and veins pale green-yellow. | Light green. Midrib and veins usually purplish. Basal portion of blade often brownish. | Light green, usually with pale green-yellow midrib and veins. Midrib and veins sometimes tinged with purple. |
| Mature leaves | Thick, slightly bullate, glossy, dark green. Midrib and veins pale green-yellow. Length, 70 to 90 mm; width, 40 to 60 mm. | Thinner, less glossy, and lighter shade of green than *C. madagascariensis.* Midrib and veins often purplish. Length, 100 to 120 mm; width, 50 to 70 mm. | Similar to *C. madagascariensis* in color and texture. Length, 100 to 120 mm; width, 50 to 70 mm. |
| Petioles | Yellowish green. Length, 5 to 7 mm. | Reddish purple. Length, 10 to 14 mm. | Yellowish green, sometimes tinged with purple. Length, 8 to 12 mm. |
| Seed pods [4] | Length, 75 to 90 mm; width, 35 to 40 mm. | Length, 110 to 125 mm; width, 30 to 38 mm. | Length, 110 to 120 mm; width, 45 to 55 mm. |

[4] Comparison of the size of the seed pods is shown in pl. 6.

As may be seen from table 1, the hybrid has some of the characteristics of each parent, being more nearly like *Cryptostegia grandiflora* in habit of growth, stem color and texture, internode length, corolla size, convergence of corolla appendages, and leaf size, but very similar to *C. madagascariensis* in regard to number and size of lenticels, leaf color and texture, petiole color, and seed-pod size. The expression of such characters as corolla color, node size, depth of division of corolla appendages, translator shape, and petiole length appears to be intermediate.

As mentioned on page 6, three off-type individuals—plants 15, 16, and 17, in row B—exhibited combinations of characters distinctly different from the normal hybrid type.

Plants B–15 and B–16 first attracted attention by the unusual shape of their seed pods (pl. 7). Plant B–17 had vegetative characters so nearly like the seed parent, *Cryptostegia grandiflora*, that it was thought at first that a plant of this species had been set out in the hybrid block by mistake. However, further observation and the conclusive evidence from a segregating generation have established its hybrid character.

The aberrant character of plants B–16 and B–17 is further emphasized by the fact that 6-parted flowers have been produced. Plant B–16 produced a flower which had 6 corolla lobes, 6 corolla appendages, 6 anthers, and a 6-lobed stigma. The ovary apparently was normal. Plant B–17 bore a flower which had a 6-lobed corolla, but otherwise was normal.

## METHOD OF ANALYSIS

Rubber is found in *Cryptostegia* in every part of the plant except the woody portions of the stem and root. Although the rubber occurs in latex it cannot be obtained economically by the bark-tapping method commonly employed in harvesting rubber from the *Hevea* tree, but can be extracted only by mechanical or chemical treatment of the rubber-bearing portions of the plant. Therefore, it is essential that in devising a system of evaluating the rubber-producing capabilities of the plant the methods of extraction be considered.

A direct comparison of the rubber content of any two plants would require that the entire plants be ground and the rubber extracted and weighed. Such a system would destroy the plants involved in the comparison and prevent the continuation of comparisons of the same individuals except through their progeny. To obtain an estimate of the rubber content of individual plants, it was desirable to devise a method of sampling which would cause the least possible injury to the plant and would be representative of the portions of the plant most likely to be harvested under a system of commercial production. It was also desirable that the sample should be comparable with samples taken from other plants at the same time and with samples of the same and other plants taken at other times.

It was found that mature leaves met the requirements of sampling more satisfactorily than any other portion of the plant, and comparable samples could be collected readily from many plants. Analyses showed that the highest percentage of rubber was in the leaves and that the rubber content of the other parts of the plants was relatively low. The taking of leaf samples had no apparent effect on the plants, and even complete defoliation caused no appreciable injury.

Except where otherwise noted, the analyses reported in this bulletin are of mature leaves taken at random from all portions of the plants. In the case of tests which were made immediately following periods of defoliation, when there were no fully mature leaves on the plants, the most mature leaves available at that time were selected.

Fifty leaves, including the petioles, selected from all parts of a single plant, were considered a standard sample. The samples were picked by hand, placed in cloth bags and, after being weighed preliminary to moisture determination, were placed immediately in a drying oven maintained at 65° C. The minimum length of time required for thorough drying at the above temperature was found to be 48 hours, although experiments have shown that leaves can be left in the oven as long as 30 days without appreciable change in rubber content. It was possible, therefore, to collect samples from a large number of plants on the same day and to hold them in the oven until ready for analysis. By this method of procedure, direct comparisons between as many as 150 plants could be obtained without possibility of error due to different harvesting dates.

In preparation for the chemical analysis, the thoroughly dried samples were ground in a small hand-power mill until practically the entire sample would pass through a 20-mesh wire screen. Five-gram samples of the ground material were then weighed out and subjected to the action of solvents in Bailey-Walker extractors. The process followed was essentially that described by Hall and Goodspeed (*4*) by which the sample is first treated with acetone to remove all benzol-soluble substances other than rubber. By this treatment the resins and similar substances which are soluble in acetone are removed, but the rubber which is insoluble in acetone remains and is extracted by the benzol in the next process.

In the work of Hall and Goodspeed an extraction period of 3 hours was considered sufficient. After experiments had shown that there was an appreciable amount of extractable material left in samples of *Cryptostegia* material after the 3-hour period, it was obvious that longer extraction was necessary. A 6-hour period gave optimum results and was adopted for both acetone and benzol extraction.

After experiments had shown them to be equally efficient and more easily handled, 25-ml Gooch filtering crucibles were used instead of the standard extraction thimbles supplied with the Bailey-Walker extraction apparatus.

Both in the acetone and the benzol extractions the weight of the extract was determined after the solvent had been evaporated and the residue dried overnight in an oven at a temperature of 65° C.

Whenever possible, analyses were made in duplicate or triplicate, but in some of the investigations it was advisable to analyze only one sample from each lot, so that it would be possible to obtain widely representative data from many plants.

## RUBBER CONTENT OF HYBRID AND PARENT SPECIES

The morpholgical differences that serve to distinguish the hybrid from its parent species have been stated. but from a practical standpoint rubber content is the most important characteristic. In this respect the hybrid has proved to be significantly superior to any individuals or groups of either parent species.

In May 1931 a series of analyses was undertaken to obtain comparable data on the rubber content of plants of the two species of *Cryptostegia* and the hybrid. Samples consisting of approximately 500 leaves each were picked at random from several plants of each species and the hybrid, at approximately the same date each month for a period of 10 months. The plants used to represent *C. grandiflora* and *C. madagascariensis* were approximately the same age as the hybrid plants, and care was exercised to obtain leaves of comparable maturity from all three populations.

No outstanding individual plants were used, since the object was to obtain a measure of the relative rubber-yielding capacity of large groups of individuals at the time of harvesting. Each 500-leaf sample was analyzed in triplicate to reduce the possibility of error.

The period covered by this series of analyses represents a complete annual-growth cycle, beginning in May with young leaves and including all stages of leaf maturity up to the defoliation period the following March.

The results are represented graphically in figure 1 and show that the rubber content of the leaves of the hybrid plants was higher than that of the leaves of the plants of either species in every month. In some months the rubber content of the leaves of the hybrid was more than double that of the leaves of either of the two species. The highest rubber content found in a leaf sample of *Cryptostegia grandiflora* was 3.13 percent, in *C. madagascariensis* 2.94 percent, and in the hybrid 5.97 percent.

In other tests of individual plants of the two species the highest rubber content found in the leaves of any plant of *C. grandiflora* was 3.34 percent, while in *C. madagascariensis* it was 3.14 percent. The highest rubber content found in the leaves of a single hybrid plant was 8.60 percent.

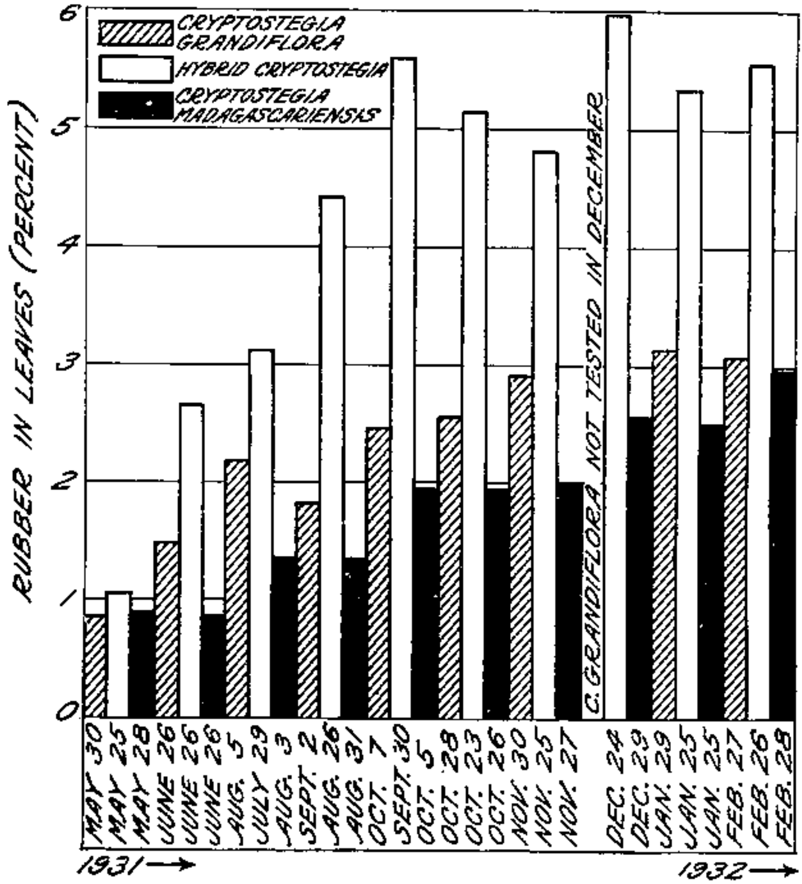



FIGURE 1.—Rubber content in percentage of the dry weight of leaves of plants of *Cryptostegia grandiflora*, *C. madagascariensis*, and the hybrid *Cryptostegia*, harvested on the specified dates from May 1931 to February 1932, inclusive.

## SEASONAL VARIATION IN RUBBER CONTENT OF THE HYBRID

Under southern Florida conditions, the hybrid *Cryptostegia* normally sheds all of its mature leaves in the early spring, i.e., March or April, and immediately puts out a vigorous flush of new growth and leaves. A limited amount of new growth is formed during the summer and early fall months and some leaf-shedding occurs at all seasons of the year, since the temperature is seldom low enough to induce complete dormancy. However, the main growth cycle normally is completed in the early spring.

In order to determine the stage in the growth cycle at which the leaves would be likely to yield the greatest amount of rubber, approximately 500 leaves averaging 1 cm in length, and less than 3 days old were tagged on September 22, 1931. One month later, and at 10-day intervals thereafter until the supply was exhausted, 25 of these leaves were harvested and their mean rubber content determined. Each leaf was measured when harvested, and the mean green weight and mean dry weight were determined for each lot. A similar experiment was inaugurated May 20, 1932.

The scope of these tests was limited by the supply of leaves of known age, which became exhausted on February 18, 1932, in the first test and on October 3, 1932, in the second test. The results of the tests that were started September 22, 1931, and May 20, 1932, respectively, are shown in table 2.

There was a consistent increase in the rubber content of the leaves of the hybrid *Cryptostegia* between the ages of 1 and 3½ months. Approximately 3½ months were required for leaves to attain their maximum rubber content. Leaves between the ages of 3½ and 5 months failed to show any significant increase in rubber content.

TABLE 2.—*Rubber and resin percentages, mean green and dry weights, age and dimensions of leaves of the hybrid Cryptostegia harvested on the stated dates at the United States Plant Introduction Garden, Coconut Grove, Fla.*

LEAF MATURITY TEST NO. 1[1]

| Date of harvesting | Age of leaves harvested | | Mean width | Mean length | Rubber content | Resin content | Mean green weight | Mean dry weight |
|---|---|---|---|---|---|---|---|---|
| | *Months* | *Days* | *Mm* | *Mm* | *Percent* | *Percent* | *Grams* | *Gram* |
| Oct. 15, 1931 | ... | 23 | 46.70 | 85.50 | 0.71 | 6.83 | 0.88 | 0.16 |
| Oct. 26, 1931 | 1 | 4 | 51.84 | 96.88 | .76 | 6.38 | 1.18 | .26 |
| Nov. 6, 1931 | 1 | 15 | 48.98 | 90.56 | .80 | 7.11 | 1.00 | .24 |
| Nov. 16, 1931 | 1 | 25 | 50.24 | 96.30 | .99 | 8.60 | 1.32 | .28 |
| Nov. 27, 1931 | 2 | 6 | 54.23 | 100.86 | 1.49 | 7.69 | 1.48 | .36 |
| Dec. 7, 1931 | 2 | 16 | 48.88 | 95.48 | 1.87 | 8.02 | 1.33 | .36 |
| Dec. 17, 1931 | 2 | 26 | 49.20 | 97.08 | 1.64 | 8.45 | 1.52 | .40 |
| Dec. 29, 1931 | 3 | 8 | 50.22 | 106.52 | 1.80 | 8.02 | 1.40 | .28 |
| Jan. 8, 1932 | 3 | 13 | 45.52 | 89.08 | 2.21 | 9.61 | 1.16 | .32 |
| Jan. 18, 1932 | 3 | 28 | 46.92 | 95.28 | 2.04 | 8.92 | 1.08 | .28 |
| Jan. 28, 1932 | 4 | 8 | 49.08 | 96.20 | 2.11 | 8.26 | 1.20 | .32 |
| Feb. 8, 1932 | 4 | 19 | 48.15 | 94.22 | 1.97 | 8.63 | 1.36 | .40 |
| Feb. 18, 1932 | 4 | 29 | 48.48 | 94.09 | 2.27 | 8.27 | 1.36 | .32 |

LEAF MATURITY TEST NO. 2[1]

| Date of harvesting | Months | Days | Mean width | Mean length | Rubber content | Resin content | Mean green weight | Mean dry weight |
|---|---|---|---|---|---|---|---|---|
| June 18, 1932 | ------- | 29 | 48.02 | 91.40 | 0.59 | 7.08 | 1.00 | 0.20 |
| June 29, 1932 | 1 | 10 | 48.12 | 91.52 | .68 | 7.42 | 1.00 | .24 |
| July 9, 1932 | 1 | 20 | 45.72 | 91.80 | 1.15 | 8.40 | .96 | .16 |
| July 19, 1932 | 2 | 0 | 48.84 | 94.12 | 1.09 | 10.21 | 1.08 | .24 |
| July 29, 1932 | 2 | 10 | 48.40 | 95.56 | 2.18 | 9.35 | 1.12 | .28 |
| Aug. 9, 1932 | 2 | 21 | 50.96 | 103.08 | 2.62 | 10.62 | 1.20 | .32 |
| Aug. 19, 1932 | 3 | 1 | 49.92 | 96.96 | 2.80 | 10.25 | 1.16 | .28 |
| Sept. 1, 1932 | 3 | 14 | 52.56 | 98.60 | 3.50 | 8.89 | 1.32 | .36 |
| Sept. 10, 1932 | 3 | 23 | 49.20 | 94.12 | 3.68 | 9.76 | 1.20 | .36 |
| Sept. 22, 1932 | 4 | 5 | 50.44 | 95.44 | 3.61 | 9.32 | 1.20 | .32 |
| Oct. 3, 1932 | 4 | 16 | 50.16 | 97.00 | 3.63 | 9.76 | 1.24 | .30 |

[1] Leaves used in this test were from 1 to 3 days old at time of tagging. The age of leaves shown in the table was calculated from time of tagging.

Leaf size is apparently no index of rubber content, as it was found that the leaves had attained their full size at the end of the first month. There was, however, a consistent increase in both green and dry weight during the period when the leaves were increasing in rubber content.

The tests just described are valuable only in determining the changes in rubber content due chiefly to differences in the age of the leaf, and are unfortunately confined to comparatively short periods. Data are available, however, which show the variation in the rubber content of the leaves of the hybrid plants from month to month for the 2-year period from August 1930 to July 1932, inclusive. A sample consisting of approximately 50 mature leaves was taken from each of 20 typical hybrid plants on the same date each month. No attempt

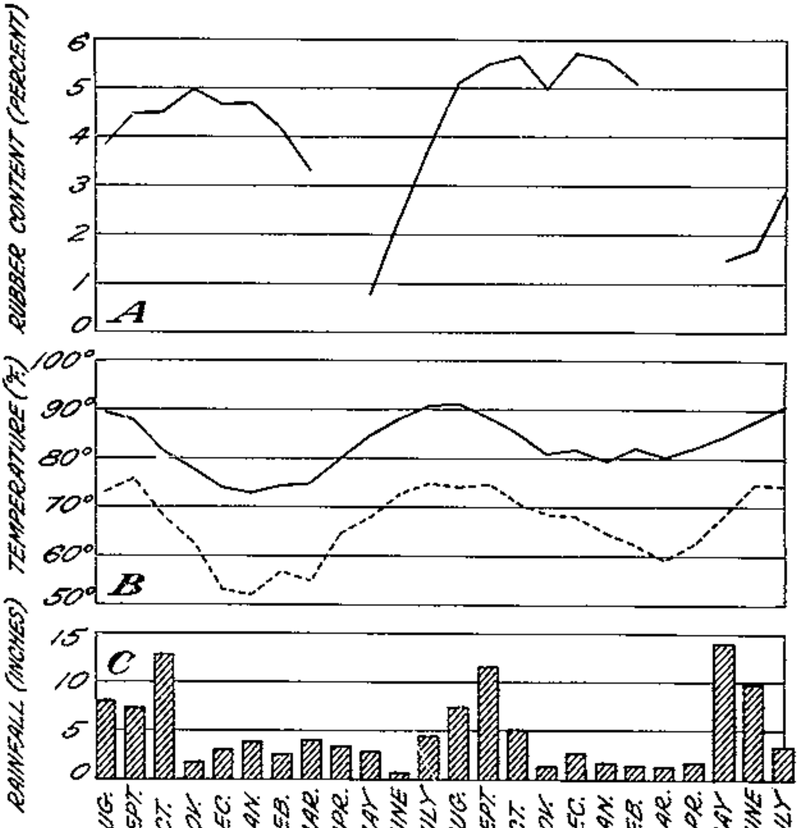



FIGURE 2.—*A*, Mean percentage of rubber in mature, dried leaves of 20 hybrid *Cryptostegia* plants analyzed at monthly intervals during the 2-year period August 1930 to July 1932, inclusive. *B*, Monthly mean maximum and monthly mean minimum temperatures for the 2-year period August 1930 to July 1932, inclusive. The maximum is represented by a solid line; the minimum by a dotted line. *C*, Monthly rainfall at the United States Plant Introduction Garden, Coconut Grove, Fla., for the 2-year period August 1930 to July 1932, inclusive. (Note.—Plants not analyzed in April 1931 nor in March or April 1932, owing to immaturity of the leaves.)

was made to keep a record of the age of leaves used, but in every case the most mature leaves available each month were utilized.

The data obtained are shown graphically in figure 2, which shows also the monthly mean maximum and minimum temperatures and the monthly rainfall for the same period.

Starting in August 1930 with a mean rubber percentage of 3.82, the trend of the curve was definitely upward with a marked increase to 4.49 percent in September. The rubber content in October was not significantly different from that of September, but in the following month, November, the maximum for the 1930–31 growth cycle was attained. Then followed a period of recession in rubber content which became more pronounced with colder weather until April, when the plants were completely defoliated and no material was available for analysis.

The analyses were resumed in May with leaf material a month or less in age, and the rubber content was very low. From this point the curve moved sharply upward through the months of June, July, and August, and more gradually upward in September and October. There was a noticeable drop in November, possibly because of lower temperature and lack of moisture. The maximum for the cycle was reached in December. A recession in rubber content, similar to that of the preceding year, characterized the months of January and February, but defoliation took place somewhat earlier in 1932 than in 1931, and there were 2 months when no material was available for analysis.

The rise in rubber content between May and July 1932 was not so abrupt as that of the corresponding period in 1931, but a definite increase at this stage of growth was evident.

From these data it may be concluded that the leaves of the hybrid *Cryptostegia* under southern Florida conditions are relatively low in rubber content during the spring and summer months when they are more or less immature, but as the season advances the rubber content rises consistently, reaching its maximum in November or December.

No attempt has been made to calculate the correlation between the seasonal changes in temperature and rainfall and the rubber content, but it is probable that the low temperatures and deficiency of moisture during the winter tend to lower plant vitality and cause leaf shedding in early spring, as described above, and that the warmer weather and more ample rainfall typical of the late spring and summer months promote more vigorous growth.

## INDIVIDUAL PLANT VARIATION IN RUBBER CONTENT

The inheritance of rubber-producing capacity in the *Hevea* tree has been studied in the East Indies, but no similar studies have been made of other rubber-producing plants. It has been found that *Hevea* trees grown from unselected seed vary widely in rubber production, in some cases as few as 25 percent of the trees on a plantation producing 75 percent of the total rubber crop. With *Hevea* it has been demonstrated that the relative yield of a tree determined at any time can be used as an index of its relative yield at any other time. Relatively high-yielding trees remain relatively high producers throughout their lives.

As stated above, the hybrid *Cryptostegia* progeny at the plant introduction garden has exhibited comparative uniformity of vegetative characters, hence if the rubber content were correlated with external plant characters the expectation of difference in rubber content between individuals would be very slight. Differences in soil and exposure would be expected to influence individual plant yields, irrespective of inherited rubber-yielding capacity.

In order to facilitate observation of plant characters and to provide material for individual rubber-content comparisons, 151 plants of the hybrid population were transplanted April 1, 1931, to an area where soil and exposure were relatively uniform. They were set in rows 8 feet apart and 6 feet apart in the rows. By June 1931 there was sufficient leaf material to warrant starting a series of analyses to determine the variation of the rubber content of the individual plants.

Because of the limited capacity of the laboratory equipment, it was necessary to restrict the number of plants used in this series of tests. Two blocks, of 12 plants each, were selected, block 1 consisting of four numbered plants, 11, 12, 13, and 14, in each of the three rows, A, B, and C; and block 2 consisting of plants numbered 1, 2, 3, and 4, in rows E, F, and G. Approximately 50 mature leaves were harvested from each plant at monthly intervals and analyzed. This series of tests was carried on from June 1931 to May 1932, inclusive, with the exception of April 1932, when it was impossible to sample the plants because of defoliation.

As a check on the selection of the two blocks to represent the entire area, and to obtain a more comprehensive measure of the range of individual variation, a leaf sample from each of the 151 plants in the area was analyzed in September 1932.

The rubber content of the monthly samples from blocks 1 and 2, together with the mean rubber content for each plant for the entire period, is given in table 3. Table 4 shows the rubber content of individual samples collected in September 1932 from each plant in the entire area.

TABLE 3.—*Rubber content in percentage of the dry weight of leaves of 24 selected hybrid Cryptostegia plants harvested in stated months during 1931 and 1932 at the United States Plant Introduction Garden, Coconut Grove, Fla.*

| Block and plant no. | Harvested during 1931 | | | | | | | Harvested during 1932 [1] | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | June | July | Aug. | Sept. | Oct. | Nov. | Dec. | Jan. | Feb. | Mar. | May | Mean |
| Block 1: | | | | | | | | | | | | |
| A-11 | 3.43 | 4.07 | 5.33 | 4.01 | 4.28 | 4.61 | 4.61 | 4.94 | 5.46 | 4.20 | 0.09 | 4.148 |
| A-12 | 2.11 | 4.54 | 4.88 | 5.80 | 5.24 | 3.68 | 4.57 | 5.17 | 5.54 | 3.03 | .61 | 4.169 |
| A-13 | 2.61 | 3.77 | 5.22 | 5.31 | 4.97 | 4.01 | 5.79 | 6.09 | 5.36 | 4.94 | .62 | 4.481 |
| A-14 | 2.73 | 4.13 | 4.75 | 5.01 | 4.58 | 5.02 | 5.33 | 5.77 | 6.24 | 4.83 | .70 | 4.545 |
| B-11 | 2.75 | 4.20 | 5.64 | 5.43 | 4.85 | 5.02 | 4.83 | 4.62 | 5.09 | 4.25 | .87 | 4.322 |
| B-12 | 2.65 | 3.38 | 4.02 | 5.31 | 4.51 | 3.08 | 4.93 | 4.90 | 4.68 | 4.09 | .70 | 3.923 |
| B-13 | 2.50 | 3.77 | 4.35 | 6.18 | 4.74 | 4.85 | 5.41 | 5.98 | 6.01 | 4.60 | .77 | 4.409 |
| B-14 | 2.25 | 3.71 | 4.00 | 6.63 | 5.70 | 5.83 | 4.93 | 6.72 | 5.95 | 6.09 | .74 | 4.837 |
| C-11 | 2.30 | 3.72 | 4.61 | 3.28 | 4.91 | 3.32 | 3.91 | 4.26 | 4.18 | 4.50 | .74 | 3.612 |
| C-12 | 1.84 | 3.29 | 3.72 | 4.21 | 4.26 | 4.20 | 4.98 | 5.00 | 5.52 | 4.50 | .00 | 3.807 |
| C-13 | 1.91 | 2.82 | 4.49 | 3.83 | 4.50 | 4.81 | 4.35 | 5.32 | 4.65 | 3.91 | .58 | 3.730 |
| C-14 | 2.02 | 3.11 | 4.30 | 4.74 | 4.28 | 3.93 | 4.13 | 4.99 | 5.17 | 5.05 | .62 | 3.849 |
| Block 2: | | | | | | | | | | | | |
| E-1 | 2.33 | 2.59 | 4.87 | 7.04 | 5.09 | 4.15 | 4.88 | 4.83 | 6.21 | 4.54 | .66 | 4.292 |
| E-2 | 2.60 | 3.35 | 4.72 | 4.20 | 5.58 | 4.20 | 4.77 | 3.98 | 4.75 | 3.48 | .81 | 3.875 |
| E-3 | 2.60 | 3.41 | 3.60 | 3.62 | 3.57 | 3.93 | 4.52 | 4.62 | 4.65 | 3.65 | 1.23 | 3.582 |
| E-4 | 2.71 | 2.77 | 4.63 | 6.30 | 5.67 | 4.14 | 4.75 | 4.33 | 4.10 | 3.81 | 1.01 | 4.028 |
| F-1 | 2.49 | 2.53 | 4.60 | 6.51 | 4.90 | 4.62 | 5.39 | 5.18 | 5.85 | 4.84 | .80 | 4.343 |
| F-2 | 2.28 | 3.55 | 4.23 | 5.45 | 5.17 | 4.01 | 5.23 | 4.01 | 4.09 | 4.80 | .72 | 3.957 |
| F-3 | 1.94 | 3.06 | 4.28 | 5.50 | 4.80 | 3.23 | 4.00 | 5.47 | 5.35 | 4.02 | .82 | 3.801 |
| F-4 | 2.81 | 2.83 | 3.96 | 6.12 | 5.21 | 3.50 | 3.77 | 4.54 | 4.90 | 3.74 | .70 | 3.836 |
| G-1 | 2.47 | 4.02 | 4.56 | 6.47 | 5.42 | 6.04 | 6.10 | 5.85 | 4.95 | 4.71 | .07 | 4.068 |
| G-2 | 2.25 | 3.45 | 4.22 | 5.56 | 5.03 | 3.40 | 4.71 | 5.82 | 5.75 | 4.53 | 1.27 | 4.268 |
| G-3 | 2.18 | 2.91 | 3.79 | 4.11 | 3.49 | 3.49 | 3.00 | 4.38 | 5.05 | 3.47 | .53 | 3.304 |
| G-4 | 2.74 | 3.73 | 4.41 | 4.84 | 3.90 | 3.43 | 3.67 | 5.20 | 4.53 | 3.24 | .80 | 3.697 |

[1] No analysis was made in April 1932.

TABLE 4.—*Rubber content in percentage of dry weight of leaves harvested from 151 hybrid Cryptostegia plants in September 1932 at the United States Plant Introduction Garden, Coconut Grove, Fla.*

| Plant no.[1] | Rubber content of plants in row— | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | D | E | F | G | H | I |
| 17 | 2.66 | 3.79 | 4.04 | 4.30 | 3.95 | 3.84 | 4.06 | 3.40 | 2.88 |
| 16 | 3.67 | 3.68 | 3.24 | 4.05 | 3.08 | 4.07 | 3.72 | 3.66 | 3.37 |
| 15 | 3.42 | 3.03 | 4.10 | 3.02 | 3.97 | 4.39 | 3.74 | 3.63 | 2.70 |
| 14 | [2] 3.13 | [2] 3.75 | [2] 3.34 | 4.11 | 4.00 | 4.16 | 4.19 | 3.44 | 2.81 |
| 13 | [2] 2.31 | [2] 3.60 | [2] 2.69 | 3.41 | 4.24 | 3.69 | 3.28 | 3.47 | 3.12 |
| 12 | [2] 2.59 | [2] 3.21 | [2] 3.54 | 4.19 | 3.04 | 3.92 | 4.03 | 3.46 | 4.25 |
| 11 | [2] 3.19 | [2] 3.09 | [2] 2.87 | 2.91 | 3.73 | 4.32 | 3.77 | 3.80 | 3.41 |
| 10 | 2.03 | 3.44 | 3.44 | 4.23 | 4.08 | 4.32 | 3.74 | 3.62 | 3.84 |
| 9 | 3.60 | 3.33 | 3.70 | 3.02 | 3.82 | 3.72 | 3.04 | 3.40 | 3.21 |
| 8 | 3.67 | 3.03 | 3.49 | 3.82 | 4.37 | 5.09 | 3.43 | 4.03 | 4.10 |
| 7 | 4.78 | 3.55 | 4.16 | 3.87 | 4.12 | 4.24 | 3.91 | 5.57 | 3.08 |
| 6 | 4.29 | 4.01 | 3.97 | 4.08 | 3.81 | 4.54 | 4.59 | 4.07 | 3.02 |
| 5 | 4.64 | 4.20 | 3.58 | 5.16 | 3.75 | 4.26 | 3.70 | 3.62 | 3.25 |
| 4 | 5.12 | 3.55 | 2.98 | 3.82 | [2] 3.68 | [2] 4.09 | [2] 3.50 | 3.62 | 3.17 |
| 3 | 3.72 | 3.31 | 3.30 | 3.60 | [2] 3.66 | [2] 3.05 | [2] 3.43 | 4.15 | 3.87 |
| 2 | 4.45 | 2.90 | 3.35 | 3.88 | [2] 3.70 | [2] 3.21 | [2] 3.28 | 3.55 | ------ |
| 1 | 4.87 | 4.25 | 3.38 | 3.13 | [2] 4.32 | [2] 4.06 | [2] 3.54 | 4.00 | ------ |

[1] The plants are shown in the order in which they were growing in the field.
[2] A sample from each of these 24 plants was gathered and analyzed each month.

Coefficients of correlation of the rubber content of the individual plants in each month, with the rubber content of the same plants in each other month, were calculated and are shown in table 5. Except when the rubber content is high the correlations are very low, indicating that selection for rubber production can be done only during periods of high rubber content.

TABLE 5.—*Intermonthly correlations of rubber content of 24 selected hybrid Cryptostegia plants for stated months in 1931 and 1932 at the United States Plant Introduction Garden, Coconut Grove, Fla.*

| Month | Correlations of monthly rubber content | | | | |
|---|---|---|---|---|---|
| | July | August | September | October | November |
| June | 0.270±0.130 | 0.375±0.121 | 0.060±0.140 | −0.009±0.141 | 0.136±0.138 |
| July | ------ | .445± .113 | −0.72± .140 | .056± .140 | .284± .120 |
| August | ------ | ------ | .225± .133 | .356± .123 | .423± .115 |
| September | ------ | ------ | ------ | .594± .091 | .346± .124 |
| October | ------ | ------ | ------ | ------ | .253± .132 |
| November | ------ | ------ | ------ | ------ | ------ |
| December | ------ | ------ | ------ | ------ | ------ |
| January | ------ | ------ | ------ | ------ | ------ |
| February | ------ | ------ | ------ | ------ | ------ |
| March | ------ | ------ | ------ | ------ | ------ |

| Month | Correlations of monthly rubber content | | | | |
|---|---|---|---|---|---|
| | December | January | February | March | May |
| June | 0.130±0.138 | −0.131±0.138 | −0.044±0.140 | −0.155±0.137 | 0.077±0.140 |
| July | .284± .120 | .200± .129 | .112± .139 | .156± .137 | −.031± .140 |
| August | .322± .126 | .154± .137 | .210± .134 | .172± .136 | −.233± .133 |
| September | .470± .110 | .410± .117 | .761± .059 | .410± .117 | −.053± .140 |
| October | .391± .119 | .104± .135 | .114± .139 | .348± .124 | .130± .138 |
| November | .673± .077 | .503± .105 | .340± .124 | .384± .120 | −.173± .136 |
| December | ------ | .413± .117 | .324± .126 | .511± .104 | −.212± .134 |
| January | ------ | ------ | .633± .084 | .550± .097 | −.024± .140 |
| February | ------ | ------ | ------ | .431± .114 | −.043± .140 |
| March | ------ | ------ | ------ | ------ | −.155± .137 |

After defoliation in the early spring, a period of from 3 to 4 months is required before the rubber content of the leaves reaches its maximum. During this period of leaf growth and increasing rubber content the correlation of rubber content with succeeding months is very low. As the rubber content increases and approaches its maximum, the intermonthly correlations increase.

Since the intermonthly correlations are so low, there is considerable question as to their significance. Any effect due to the location of the individual plants should be manifested by a larger correlation. To determine whether there was any consistent difference which might be ascribed to variation in soil conditions or other place effect, the correlation between the rubber content of each individual plant and that of the adjacent plants of higher serial number was calculated. By this method a measure was obtained of the tendency of adjacent plants to have similar rubber contents. A high correlation would indicate that there was a distinct place effect in the field with a strong tendency for adjacent plants to produce equally. A low correlation would indicate that there was no general tendency toward the grouping of plants on the basis of rubber content.

The correlation was found to be 0.280±0.027; very low, but possibly indicating slight place effects in the field as a whole. Such a small correlation probably indicates a spotting effect in the field, rather than a progressive place effect, that scarcely would affect the intermonthly correlations of the rubber contents of the selected plants.

A further estimate of the effect of location on the relative rubber content of the plants in this area was obtained by dividing the plot lengthwise into four 2-row blocks and one 1-row block. The mean rubber content of the leaves of the individual plants in each block in September 1932 was compared with the mean of each of the other blocks. This comparison is shown in figure 3. From this figure it will be seen that differences between blocks were 3 or more times their probable errors in 6 of the 10 comparisons.

| | B | C | D | E |
|---|---|---|---|---|
| A | 0.056±0.095<br>0.589 | 0.425±0.084<br>5.060 | 0.132±0.095<br>1.389 | 0.273±0.114<br>2.395 |
| B | | 0.369±0.063<br>5.857 | 0.076±0.077<br>0.987 | 0.329±0.100<br>3.29 |
| C | | | 0.293±0.063<br>4.651 | 0.698±0.089<br>7.843 |
| D | | | | 0.405±0.100<br>4.05 |

FIGURE 3.—Differences between each 2-row block and every other 2-row block in mean rubber content of leaves of hybrid Cryptostegia plants. These differences are based on the results of an analysis made in September 1932. The lower figure in each block is the difference divided by its probable error.

The plants in block C, constituting the inside rows of the area, being protected by 4 rows on one side and 3 rows on the other side, had a significantly higher mean rubber content than the plants in the other blocks. The rubber contents of the individual plants in block C also were much more uniform than those of the plants in any other block. The coefficients of variability were found to be as follows: Block A, 18.677±1.528; block B, 13.597±1.142; block C, 8.203±0.671; block D, 12.118±0.991; block E, 14.226±1.752. The coefficient of variability of individual rubber content of all plants in the entire plot was 14.559±0.565.

The coefficient of variability of the 24 plants used in the monthly individual variation tests was 14.112±1.374, that of block 1 being 13.452±1.852 and that of block 2 being 8.678±1.195. Block 1 is included in blocks A and B of the lengthwise division, and eight of the plants of block 2 are in block C of the lengthwise division of the area. The rubber contents of the plants in block 1 were comparable with those of the plants in the entire area in variability, but the plants in block 2 are in the favorable central location where rubber contents were higher and more uniform in September.

Since there were definite place effects manifested by differences in mean rubber content between plants in different portions of the area, and since the intermonthly correlations of rubber content were small, it appears probable that the differences in rubber content between the individual plants should be considered as due largely to effects of soil and exposure rather than to differences in rubber-producing capacity. The fact that the plants near the center of the field had higher rubber content and lower coefficient of variability than the plants in other portions of the area would indicate that the degree of exposure to wind may affect the production of rubber in the leaves of *Cryptostegia*.

## NONRUBBER CONSTITUENTS

In addition to the rubber in *Cryptostegia*, there is an appreciable quantity of material which is soluble in acetone and has been designated "resins." This material consists of true resins and small quantities of oils, fats, and acetone-soluble sugars. The resins in *Cryptostegia* have not been identified, and nothing is known of their economic value. Similar resins obtained in purifying *Euphorbia* rubber from South-West Africa have potential value in the manufacture of paints and varnishes. It also has been suggested that these resins may have value in connection with the manufacture of soap. Other uses possibly could be found if such a byproduct were available in quantity.

Chemically the resins are closely allied to rubber and are found associated in all rubber-bearing plants. The evaluation of the quality of rubber is often no more than a determination of the quantity of resins associated with the rubber hydrocarbon in the crude sample. *Hevea* rubber has from 1.8 to 6 percent of resins, according to the manner in which it is prepared, while pontianak or jelutong, the product of species of *Dyera* and *Alstonia* in the East Indies, may contain from 70 to 80 percent of resins.

The method of analysis employed in the investigations included the determination of resin content as well as rubber content of all material tested. The record, therefore, includes data on the seasonal variation in resin content, resin content of leaves of different ages, and variation in resin content among individual plants.

These data indicated that resins were always associated with rubber in *Cryptostegia*, but that the proportion of resins to rubber was extremely variable.

The figures shown in table 2 indicate that a very high resin content is found in comparatively young leaves. Leaves harvested October 15 at the age of 23 days contained 6.83 percent of resin, or within 2.78 percent of the maximum found during a series of tests which included leaves up to 5 months of age. Leaves harvested June 18 at the age of 29 days (table 2) contained 7.08 percent of resin, or within 3.54 percent of the maximum attained during a series of tests which included leaves up to 4½ months of age. The high resin content of young leaves was in marked contrast to the rubber content, which was almost negligible in leaves 1 month of age. The rubber content increased gradually with age and reached its maximum in leaves approximately 3½ months old, while the resin content of month-old leaves was comparatively high and increased very little with age.

To determine the correlation between the resin content and the rubber content of the hybrid, the data gathered in the test of the seasonal variation of rubber content were used. In this test, running from August 1930 to July 1932, inclusive, leaf samples of 20 plants were gathered monthly and analyzed. It was found that there was no correlation between the mean rubber content and the mean resin content of the leaves of individual plants during this period, the coefficient of correlation being only $-0.078 \pm 0.154$. The plants were sampled in 22 of the 24 months of the period.

During the same period 20 plants of *Cryptostegia grandiflora* also were sampled. Due to defoliation at certain periods, these plants were sampled only in 10 of the 24 months. In these 10 months the correlation between the mean rubber content and the mean resin content of the individual plants was found to be $0.609 \pm 0.097$. Twenty plants of *C. madagascariensis* also were sampled and tested during 18 months of the same period. The correlation between the rubber content and the resin content of the individual plants for the 18 months was found to be $0.692 \pm 0.081$.

The significant correlations between resin and rubber content of *Cryptostegia grandiflora* and *C. madagascariensis* indicated a strong tendency for high rubber content to be associated with high resin content and low resin with low rubber in these two species. The lack of correlation found in the hybrid plants tested indicated an indiscriminate association of resin and rubber, with no regular relation of quantity or proportion.

In addition to the rubber and resins there is an appreciable amount of fiber in *Cryptostegia* which may have commercial value. This fiber is in the bark and possibly could be separated in connection with the mechanical extraction of the rubber. Jumelle (*6, p. 351*) reports that in Madagascar *C. madagascariensis* is used as a textile plant. He states:

> The Malagasy know it well in that character. In Boina and the Ambonga the Sakalavas use chiefly the bast to make string, fish lines, and thread. In the Southwest the Antandroy, too, use it in the manufacture of good cords.

In describing the method by which the fiber is obtained from the bark, Jumelle states (*6, p. 351*):

The Sakalava method of preparing this bast is as follows: The stems are decorticated by hand; and by hand, using his nails, the harvester removes from the detached bark, which is neither macerated nor beaten, the fibrous bundles which are found on the inside (more accurately, in the pericycle entwined in the bark), and which are easily seen by reason of their whiteness and their spacing.

In regard to quality, Jumelle states that while the fiber from *Cryptostegia madagascariensis* is inferior to hemp, it is superior to other types of native fiber which have been compared favorably with jute.

Among the natives of Madagascar, *C. madagascariensis* is considered poisonous. Jumelle states (*6, p. 355*):

In Androy, according to M. le Commandant Vacher, the Antandroy, before they had any suspicion that the latex might produce rubber, were already making use of the plant as a means of committing suicide or of ridding themselves of their enemies. M. Perrier de la Bathie tells us, however, that the poison cannot be a very virulent one, for in Boina the Sakalavas prepare decoctions of the roots as a remedy against chronic blennorrhagias.

Possibly, therefore, further search for material of byproduct value may bring to light a drug with pharmaceutical value.

## CULTURE AND PROPAGATION

*Cryptostegia* has proved well adapted to a variety of soils in southern Florida, although its younger growth is sometimes injured by low temperature and strong winds during the winter. Instances are known when stems of plants in exposed conditions were killed back to within a few inches of the ground by abnormally low temperatures of 20° F., but it has been cultivated successfully as an ornamental in the Miami section for many years. Its moisture requirements are not excessive, and while growth is checked to some extent by the deficiency of rainfall during the winter months, it apparently suffers little from this cause. Notable resistance to drought has been recognized in *C. grandiflora* in northwestern Mexico.

In southern Florida defoliation normally takes place in March or April, but seasonal conditions sometimes cause a complete defoliation as early as December or January. Also, the length of the defoliation period is affected by weather conditions.

*Cryptostegia grandiflora*, *C. madagascariensis*, and the hybrid all produce abundant crops of viable seed. However, the hybrid does not come true to type from seed, and several methods of vegetative propagation have been tested.

Hardwood cuttings have been rooted, but although the cuttings have been tried in several different mediums and have been given varying amounts of water and heat, it never has been possible to root more than a small percentage of them. It was found, too, that plants grown from cuttings usually lacked vigor and seldom developed normally.

Marcottage, or air-layering (pls. 8 and 9), has given much more satisfactory results in the vegetative propagation of the *Cryptostegia* hybrid. As many as 15 or 20 marcots have been placed on a large mature plant at one time, and the new plants produced in this way were larger and more vigorous than those grown from cuttings. If properly handled, the cutting-back incidental to marcotting is not injurious to the parent plant.

The method of marcotting *Cryptostegia* is to remove a ring of bark 1 to 2 inches in width and thick enough to reach the cambium layer from just below a node of a well-matured branch. This incision is then surrounded by a mixture of sand, peat, and sphagnum moss which is held in place on the branch in a specially designed cardboard marcot box, developed by Eugene V. May and Robert J. Bullen, of the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.[3] The marcot is kept moist at all times. It is examined occasionally to ascertain the state of root development, and the new individual is removed from the parent plant as soon as a good root system has formed.

After removal from the parent the young plants are placed in large pots or boxes for a few weeks, and, as soon as they are well established, they are set out in field plantings. Plants propagated in this way compare favorably with their parents in vegetative vigor and rubber content, and marcottage may be considered a successful method of increasing stocks of high-yielding hybrids.

By this means it will be possible to obtain a sufficient number of plants of identical parentage to extend the present plantings and evaluate the effects of exposure and soil conditions on the production of rubber. Also by using marcots from plants of differing parentage it will be possible to estimate more accurately the relative effects of heredity and of the environmental factors on the production of rubber as a basis for the selection of clones or strains with high-rubber producing capacity.

## SUMMARY

Two species of the genus *Cryptostegia* which were formerly utilized as sources of rubber by the natives of Madagascar and India have been introduced as ornamentals into tropical America, including the West Indies, Mexico, and the warmer districts of California, Arizona, and Florida. The range of adaptation and comparatively high rubber content of these plants has led to consideration of their commercial possibilities.

Confusion has existed in the nomenclature of the two recognized species of *Cryptostegia*—*C. grandiflora* R. Brown and *C. madagascariensis* Bojer.

A natural hybrid, *Cryptostegia madagascariensis* × *C. grandiflora*, has been studied at the United States Plant Introduction Garden at Coconut Grove, Fla., since 1927. This hybrid shows characteristics of both of the parent species, being more nearly like *C. grandiflora* in habit of growth, stem color and texture, internode length, corolla size, convergence of corolla appendages, and leaf size, but very similar to *C. madagascariensis* in regard to number and size of lenticels, leaf color and texture, petiole color, and shape of seed pod. In corolla color, node size, depth of division of corolla

[3] Public Service Patent 1,655,731.

SECTION OF A MARCOT BOX

Half of marcot box removed showing the root development of a hybrid *Cryptostegia* marcot. The branch has been removed from the plant and is ready for potting.

MARCOT BOXES IN PLACE ON THE BRANCHES OF A MATURE HYBRID CRYPTOSTEGIA PLANT.

appendages, translator shape, and petiole length the hybrid is intermediate between the parental types.

In a series of tests comparing the rubber content of composite samples of leaves taken from the hybrid plants with that of similar samples of the leaves of the parent species, the rubber content of the hybrid has been consistently higher than in either of the two species. In these tests the maximum rubber content found in any composite leaf sample of *Cryptostegia grandiflora* was 3.13 percent, while for *C. madagascariensis* it was 2.94 percent, and for the hybrid 5.97 percent.

The highest leaf rubber content in any individual plant of *C. grandiflora* was 3.34 percent; in *C. madagascariensis*, 3.14 percent; and in the hybrid, 8.60 percent.

A series of analyses made at monthly intervals over a period of 2 years established the fact that there was a significant seasonal variation in the rubber content of both species of *Cryptostegia* and of the hybrid, the rate of rubber production in the leaves being greatest during the period of maximum vegetative growth.

The rubber content of the leaves of hybrid *Cryptostegia* plants was found to increase in direct proportion to leaf maturity, the maximum being attained when the leaves were about 3½ months of age.

No significant differences were found in the individual rubber-yielding capacity to the hybrid plants, but soil conditions and exposure affected the relative rubber production in the leaves of the individual plants.

In addition to the rubber in *Cryptostegia*, the byproduct value of the resins and fiber may be worthy of consideration. Resin content was found to be proportionate to rubber content in the leaves of *C. madagascariensis* and *C. grandiflora*, but there was apparently no correlation between the rubber and resin content of the hybrid.

The hybrid *Cryptostegia* does not come true to type when grown from seed, but marcottage, or air-layering, has proved to be a satisfactory method of vegetative propagation.

Plantings of marcots are planned to give a basis for evaluating the effect in exposure and soil conditions on the production of rubber in *Cryptostegia* and to estimate the relative effects of heredity and of environmental factors on the rubber content.

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