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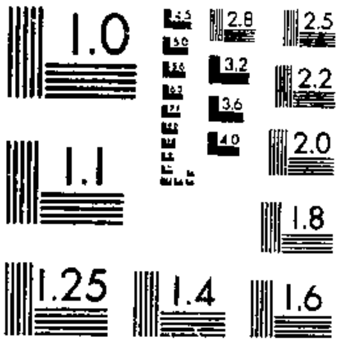

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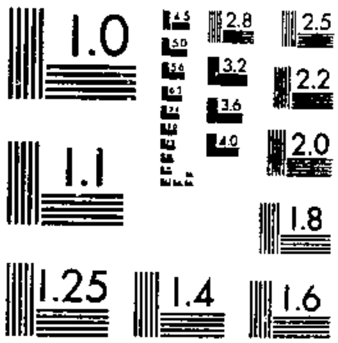

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Interspecific and Intergeneric Grafts, With Special Reference to Formation of Rubber in Guayule[1]

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APPLICATION OF GRAFT TECHNIQUES IN RUBBER PRODUCTION AND RESEARCH

By M. N. WALKER

RESEARCH on rubber until recently has been concerned mainly with production, field management, and initial processing on the one hand and with compounding and fabrication on the other. The more intimate aspects of physiology, particularly of the processes leading to rubber formation in the plant, have received secondary attention. In more recent years, however, increasing emphasis has been given to this important field. A good start has been made, but there is still much to be learned of the steps by which photosynthetic products formed in the leaves are translated into rubber. One of the first questions to arise from a consideration of this broad problem is whether the specific precursors of rubber are common to plants in general or only to those that form rubber, or whether they are further restricted within species or genera.

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The work reported here sheds some light on this question and, in addition, demonstrates a useful research tool. This publication describes methods of bringing together parts of non-rubber- and rubber-bearing plants by means of interspecific and intergeneric grafts, the histology of the graft unions, and the effects of the grafts on rubber formation. The success of the grafting work was largely due to the very early stage at which many of the grafts were made and to the delicacy of the technique. This technique has led to the application of the term "micrografts," defined on p. 7.

Beyond its contribution to an understanding of rubber formation in guayule and its usefulness as a research tool, the work here reported has interesting implications and some practical bearing on the work on hevea rubbertrees, since the use of two-way grafts produces high yield and controls the South American leaf blight caused by *Dothidella ulei* P. Henn.

Long used as a standard horticultural technique in propagating clonal scions, grafting has served also as a research tool in the investigation of physiological processes and of certain diseases, particularly those of virus origin. So far as is known, however, this is the first published report of the direct application of grafting to the investigation of rubber formation in plants.

In the Far East, the propagation of high-yielding clones by budding onto seedling rootstocks has long been the practical means of greatly increasing yields. Twofold and threefold increases in yield from budded stock as compared to seedling stocks are commonplace in plantation practice, and much greater increases are reliably reported from less extensive plantings. Some 15 percent of the plantations in the Far East are of grafted stock, and future progress will depend to a considerable extent on the success of efforts further to extend the use of grafted clonal lines. As the South American leaf blight is not, at present, a factor in the production of rubber from hevea in the Far East, major research has been directed to the development of high-yielding clones that are propagated and extended by means of budding onto seedlings, which, for the most part, are quite variable.

Some progress was made in developing true-breeding seed stocks of sufficiently high yield to justify distribution for planting in place by native growers, who are slow in accepting budded stocks. The best yields attainable by this means are less than those from the better clonal stocks, however, and on the larger plantations growers have concentrated on the use of budded clones, even though the initial cost of field establishment is somewhat greater. Actually, amortization over the productive life of the plantings makes the expense of using clonal material a minor cost item.

Although the development of true-breeding seed stocks is possible, there is little prospect that these stocks can soon compete with superior clonal material in obtaining maximum yields. The discovery of a superior tree leads immediately to almost unlimited asexual (clonal) propagation. In contrast, seed production may be limited, and the selection and testing of seed stocks take many years. It would appear, therefore, that the use of true-breeding seed stocks for field planting will continue to lag many years behind the use of clones.

On the other hand, there is a somewhat better prospect that, by the development of true-breeding seed, variability in seedling rootstocks can be reduced, though there is little likelihood that such material can be made available in the near future. The selection of seed stocks for this purpose on the basis of seedling vigor and disease resistance can be accomplished in a much shorter time than can selection, which requires yield measurements on trees 5 or more years old. As there is great variability in seedling stocks and the possible effects of such variability on the scions may be great, it is important to reduce variability as much as possible. However, this is not yet possible either by means of true-breeding seed stocks or by asexual propagation, or cloning, of rootstocks. The rate of multiplication by means of cuttings is not yet equal to research demands for uniform stocks on which accurately to measure the effects of different roots on clonal scions.

The variability of seedling stocks led to work in the Far East on the effect of stocks on the scions, in which certain stocks gave increases in yields up to 25 percent. This work, however, was not carried far enough to have much practical application. This type of research is, of course, handicapped by the lack of uniform stocks, the difficulties of developing them, the long period of testing before they can be proved, and the time required to multiply them for use after they have been proved.

A further complication arises in the hevea areas of South America and Central America, where the presence of the leaf blight has made necessary a second budding operation to put disease-resistant tops onto the high-yielding clonal trunks. This gives rise to the three-component, "sandwich," trees that are the basis of the expanded plantings being made in South American and Central American countries.

The present work shows that the final elaboration of rubber occurs in the storage site of the plant—in guayule, this occurs in the individual cells where rubber is found, whether in the leaves, the stem, or the root—and that the basic materials, or precursors, of rubber are common to several plants. The final polymerization of these basic materials, however, requires tissue that provides the special conditions or mechanism for the translation of the metabolic products of photosynthesis into rubber. As the manufacturing cells in guayule are also the storage cells, the yield of rubber from the grafted plants combining rubber and nonrubber components is obviously proportional to the amount of tissue developed by the rubber-producing part and could scarcely approach that from an ungrafted guayule plant. The rubber is released only by the rupture of the individual cells, each of which contains discrete globules of rubber, and the destruction of the plant is the basis of the extraction process.

This is entirely different from the situation existing in hevea, where the rubber is formed in latex tubules that by anastomosis are continuous throughout large areas of the cortex. The rubber globules are thus not confined to the cell of origin but are distributed within the liquid latex; thus the globules are capable of transport far from the point of origin. By the process of tapping, in which the laticiferous ducts are cut, the rubber formed by many cells over an area of several square inches is released for collection and, with proper management, the tree can be kept in practically continuous production for many years. There are, however, wide variations in the efficiency of rubber production of different trees, and this has given rise to the large number of existing clones, selected in the main for high rubber yield.

It is necessary to determine basic compatibilities of the various elements of the three-component trees. In addition, there is the greater and more difficult task of evaluating the various combinations, particularly for rubber production but also with respect to their relation to disease and their adaptability to different climatic and edaphic conditions. Each of these problems requires the investigation of many combinations of stock, bole, and top, not only within the single species (*Hevea brasiliensis* (Willd. ex A. Juss.) Muell. Arg.) but also among several other species that offer the prospect of greater vigor, resistance to various diseases, and improved adaptation to the soils and climate in which hevea can be grown. None of these desirable features can be considered apart from the basic factor of yield, which involves not only quantity of production but quality as well.

In the Far East, where a single budding operation is practiced, the boles and crowns of plantation trees are both from the same high-yielding clone. In Latin America a double budding operation is necessary because of the Dothidella leaf blight, and only about 6 feet of the trunk of the resulting tree, therefore, is from the high-yielding clone. Nevertheless, this has been found sufficient to produce yields approximating those from a tree all of whose length above ground is from the same high-yielding clone, even though the top may be from a tree producing little and inferior latex. The latex systems of the different components usually do not unite or they unite only to such a limited extent that there is almost no blending or degradation of the latex of the high-quality components except in a narrow band immediately surrounding the line of union between the two stocks. Even though unions of the laticiferous systems do not occur, or are extremely limited, other systems join in such a manner that a three-component tree with a panel of only some 6 feet of high-yielding tissue can function as efficiently as a tree having a much greater proportion of rubber-forming tissue.

There are, however, many possibilities of variation, and their evaluation in terms of efficient rubber production will take many years of painstaking effort. Infinite combinations of root and trunk, trunk and top, and of root, trunk, and top are possible among the large number of recognized clones, seedling selections, interspecific hybrids, and other species of *Hevea*. There are at present many combinations under test, and conspicuous lack of compatibility in certain combinations already has been demonstrated. This includes a reduction in the number of successful buddings and evident imbalance in growth of associated stocks, resulting in excessive breakage from overgrowth of certain tops. Evaluation of the less obvious variations in compatibility requires more time and more refined observational techniques, in which measurements of quantity and quality of production are among the most critical.

Although guayule and hevea are very unlike in many important and fundamental respects, they are alike in two ways: Rubber formation in both occurs in specialized tissues distributed throughout the plant, although it may be far from the source of basic photosynthetic materials (precursors); and the end product of synthesis is not directly used and is not recoverable for later use in the plant's economy. There is no positive proof of the function of rubber in plants. In these respects, both guayule and hevea differ from many other plants in which elaboration of chemical products is completed in the leaves or other organs and is then translocated to other parts of the plant for use or storage, either in the same or slightly different form. From storage they may again be transformed and translocated to other parts of the plant for measurable use as food or fuel.

The most obvious example is sugar, which is formed in the leaves and then transported to other parts of the plant, where it is stored. Less conspicuous examples are nicotine and anabasine, which are formed in the roots of tobacco and not in the leaves as was supposed earlier. The demonstration of the site of synthesis resulted from experiments by Dawson (*4*, *5*)[2] in which interspecific and intergeneric grafts involving *Nicotiana glauca* Graham, *Esculentum* spp., and *Datura stramonium* L. were tested in different combinations.

The synthesis of rubber from relatively simple basic compounds involves repeated polymerizations and is thus more complex than the few polymeric combinations required, as in sugar. The energy requirements for the development of the high molecular weights characteristic of high-quality rubber and the precise mechanism by which the polymerization is effected are not understood, but progress has been made recently by Arreguin and Bonner (*1*, *3*) in studies on guayule of the immediate precursors of the synthesis.

Rubber is always associated with resins, and the quality of rubber is influenced by the proportion and character of the resins with which it is associated. Resin is a generic term for materials associated with rubber and soluble in organic solvents. They are soluble in acetone, in which rubber is not soluble. They include an unknown variety of products. Though their association in some form or quantity with rubber is constant, their precise nature and their method of formation are even more poorly understood than those of rubber. Grafting together of various types of rubber-bearing plants, in which there are also variations in the resins, may provide a better insight into the nature of these products and of their relationship to rubber. It is possible that certain of the resins may, through further polymerization, give rise to rubber.

A further usefulness of work on intrageneric and intergeneric grafts may be in gaining a better understanding of the cis- and trans-conformation of the molecules of rubber and gutta percha, which empirically are identical.

The wide range of plants in which rubbers of varying quality occur, caused either by intrinsic nature or by associations with other substances (particularly the so-called resins, affords access to a practically limitless field of experimentation. Aside from their value in developing more vigorous and productive plant combinations of greater resistance to disease, such studies may also lead to a better understanding of the fundamental problem of rubber synthesis, particularly in conjunction with the more intimate biochemical studies.

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[2] Italic numbers refer to Literature Cited, p. 37.

INTERSPECIFIC AND INTERGENERIC GRAFTS OF GUAYULE

By CARL A. TAYLOR and H. M. BENEDICT

In guayule and other rubber-bearing plants, little, if any, rubber occurs in the leaves, most of it being in the stems and roots. All the rubber found in the plant, however, must come from the products of photosynthesis, which are first formed in the leaves. The question has often arisen as to whether the immediate precursors of rubber are formed in the leaves of high-producing species and then converted into rubber in the stems or whether the tissues of the stem or root, or both, are capable of taking the photosynthetic products of the leaves of any plant and converting them into rubber.

Grafting scions of high-rubber-bearing species on stocks of species that normally produce no rubber (and the reciprocal procedure), then determining the rubber content of the plants above and below the graft, should throw a great deal of light on this problem. For example, if a guayule stem could form rubber from the products of mariola leaves (a plant that normally produces little or no rubber) or if a mariola stem could form rubber from the products of guayule leaves, certain conclusions could be drawn as to the origin of the rubber. Grafting more distantly related species with guayule should determine whether precursors of rubber common to the species of *Parthenium* might be absent in the photosynthetic products of other genera.

It is generally believed that grafts between unrelated plants are very difficult or impossible to make, and very little literature is available on the subject. Jones (*7*) mentions a number of intergeneric graft combinations that generally succeed, such as peach on plum, pear on quince, tomato on potato, medlar on hawthorn, sweet chestnut on oak, and discusses the morphological and physiological factors that limit the compatibility of scion and stock. Peacock, Leyerle, and Dawson (*11*) made reciprocal grafts of *Datura stramonium* with tobacco and tomato, and Dawson (*6*) also grafted *Nicotiana tabacum* L., *N. glutinosa* L., and *N. glauca* with tomato. Molisch (*9*) described the experiments of Lindemuth wherein the perennial *Abutilon thompsonii* Veitch was grafted on the herbaceous annual *Modiola caroliniana* L. More recently Nickell (*10*) described a grafting method wherein a scion of small diameter is inserted into the pith of the understock. By this method, he accomplished unions of *Melilotus alba* Desr. on *Nicotiana tabacum*, cowpea on tomato, clover on geranium, and tomato on geranium. Nickell's work was unknown at the time the grafting of guayule was started, but his method would not have been usable for reciprocal grafts of guayule because the pith of guayule is too small to contain the scion.

As no information was available as to whether or not successful grafting of guayule could be expected, the first phase of the work had to do with the development of a technique for obtaining successful grafts of guayule with guayule and with other species.

EXPERIMENTAL TECHNIQUES

Efforts to graft guayule with various species were carried out along two main lines: (1) The grafting of older tissues such as the woody stems of guayule (*Parthenium argentatum* A. Gray) and mariola (*P.*

incanum H. B. K.), and (2) the grafting of very young seedlings not much beyond the cotyledonary age and composed mostly of primary tissues.

The hardwood grafts between two species of *Parthenium* (*P. argentatum* and *P. incanum*), made as simple side or cleft grafts and held in the greenhouse under conditions favorable for growth, provided unions in 30 to 65 percent of the grafts. When made outdoors in early spring or when held under conditions ordinarily favorable for callusing prior to growth, the grafts were unsuccessful. The successful grafts included some in which the scions of dormant wood from old plants were put onto seedlings only a couple of months old and growing actively. Other combinations of dormant and active stems were successful when the faces of the graft were well matched and the plant was held under conditions favorable for prompt growth.

The grafts of the older and larger tissues were made in the conventional manner. Some were made by binding with surgical tape, some with rubber bands, and some with cinched ties of thread or string, sealed with asphalt emulsion. The rubber bands tend to give excellent unions, but they cannot be wound on very soft, limber stems of sizes approaching the micrografts described later. However, a tool was devised whereby a stretched rubber band may be applied to small semiwoody grafts, as shown in figure 1.

The continued success with younger and younger tissues led to trials with very young seedlings just past the cotyledon stage. This class was believed to be promising in that the less differentiated tissues of the hypocotyl might be more amenable than older tissues to union with more distantly related species. Also, the rubber found in the respective parts of the final plant would, for the most part, have been formed subsequent to grafting.

For convenience, the term "micrografting" has been applied to this class of grafts, wherein the scion and stock are very young, succulent seedlings, in or recently past the cotyledon stage and with stem diameters of about 0.8 to 1.5 mm.

The first trials of micrografting guayule on guayule were failures, apparently because of methods of binding and sealing that physically injured the succulent tissues or introduced substances which were toxic.

Accordingly, a trial was run to find a substance that would be soft enough at room temperature to apply with a fine brush or needle, adhere to wet surfaces, and be nontoxic to succulent plant tissues. The materials tested were applied to freshly scarfed stems of young seedlings that were later sectioned and examined under a microscope. The compounds tested were asphalt emulsion (Henry's Standard B); collodion; freshly collected guayule exudate; grafting wax made from rosin, linseed oil, and beeswax; Lastiseal (a plastic lacquer); shellac gasket compound; bentonite jelly; mucilage; and lanolin.

Compounds producing no injury were the asphalt emulsion and guayule exudate. As the latter substance had some practical limitations of supply and keeping qualities, asphalt emulsion was adopted for general use. The grafting wax was one of the most injurious, and lanolin one of the least injurious.

The procedure used in the micrografting of very small seedlings is as follows: The seedling to be used as a scion is cut near the ground line and the cut end promptly dipped in distilled water, then pruned to one-fourth to one-half square inch of leaf area. The stem is sliced to a wedge shape having one-third- to one-half-inch length of cut surface, then placed in distilled water until the stock incision can be made. The stock is pruned of part of its leaves, and an incision made downward from a point just below the cotyledons. The cut slants inward until the knife reaches nearly the center of the stem, thence parallel to the central axis and long enough to permit embedding all the cut surface of the scion. Immediately, the cut is flushed with a

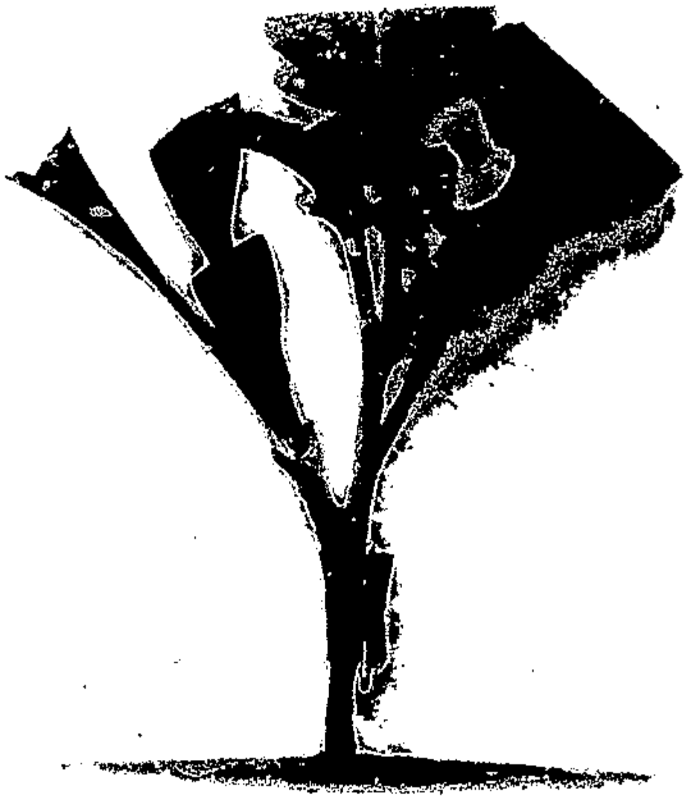

FIGURE 1.—Newly made graft of guayule scion (left) on mariola, with clipped rubber binder.

few drops of distilled water from a laboratory wash bottle to leave a water film on the cut surfaces while transferring the scion to the stock.

The scion is shoved downward into place, care being taken to aline at least one side of the scion and the stock. The tension of the water film seems to help hold the scion in place until the first tie can be made. Waxed No. 40 cotton thread is made into a loop by crossing over and passing the loose end twice through the bight (sometimes called the surgeon's knot). The loop is made large enough to pass over the tops of scion and stock. Three such ties are made, one each at the top, middle, and bottom of the flap cut from the stem of the stock. Considerable pull is applied to the thread in cinching up the ties, as part of the pull is consumed in friction within the knot, but it is not pulled tight enough to embed the thread in the tissues.

Promptly after tying in the graft, asphalt emulsion (thinned with water, if necessary) is spread on with the point of a dissecting needle, which is used to lift a small blob of the asphalt from a paddle. Only three sides of the graft are covered, leaving the tie-threads exposed on the side opposite the scion. This permits periodic examination and severing of the ties when growth of the stem starts to embed them. Appearance of the finished graft is shown in figure 2.

FIGURE 2.—Newly made graft of *Parthenium stramonium* scion (indicated by arrow at left) on guayule.

Survival of the grafts is favored by covering them with glass jars or beakers to limit transpiration losses and putting them under partial shade to prevent overheating by accumulated solar heat under the glass. Cheesecloth makes a suitable shade. Before the grafts are put under the cheesecloth in the sun, however, it may be well to hold them for 1 to 2 days in total shade. When growth of the stem causes slight embedding of the ties, they are carefully cut.

Frequently guayule and occasionally other species of scions in micrografts produce adventitious roots that penetrate the understocks for a short distance, then emerge as aerial roots, which eventually reach the soil if not removed; this gives a false take and must be watched.

Experience is the guide as to when to remove shade and covers, cut off the tops of the stock, and let the scion function solely with the stock. The length of time required for this establishment varies considerably. In the case of compatible species, the young scions may function in 1 or 2 weeks.

A head-band-type binocular magnifier of 6- to 8-inch focal length is a convenient aid to the accurate work needed for micrografting. A grafting knife made by binding a sliver from the edge of a safety razor blade into a small doweling stick proved more satisfactory than other types tried.

RESULTS

The percentage of unions to total number of grafts made varied rather widely in different trials of the same species combinations, but except in earliest trials, fair to excellent percentages were obtained. Table 1 summarizes the results on the basis of the number and percentage that apparently became established and made reasonable growth. Longevity of the grafted plants is discussed later by species. In several series, grafts that were slow to take eventually were lost by attacks of disease on the understocks at the ground line. This was the principal type of loss other than losses caused by simple failure of the stock and scion to unite.

The species grafted successfully were:

1. Guayule on guayule—different strains and age classes.
2. Guayule (*Parthenium argentatum*) on mariola (*P. incanum*), and reciprocal.
3. Guayule on *Parthenium stramonium* Greene, and reciprocal.
4. Guayule on Jerusalem-artichoke (*Helianthus tuberosus* L.).
5. Guayule on sunflower (*Helianthus annuus* L.), and reciprocal.

GROWTH BEHAVIOR

Growth of the grafted plants is summarized in table 2 by dry weights. Other growth aspects and their interpretation will be discussed by species combinations. Detailed analyses and the effects of grafting on rubber accumulation in the plants are covered in another section of this bulletin (p. 26).

GUAYULE WITH GUAYULE

Emergence of guayule seedlings follows sowing of the seed under greenhouse conditions in 4 to 10 days. A hypocotyl from ½ to 1 inch long is formed during the first few weeks. Three to five weeks after the seeding date, depending somewhat on the moisture and nutrient supply, the hypocotyl starts to become woody and attains a diameter of 0.8 to 1.5 mm. This late prewoody stage is the one commonly used for micrografting.

Grafts of succulent 1-month-old seedlings and of hardened 6-month-old seedlings were grown in the greenhouse in comparison with ungrafted controls. Growth of the scions lagged somewhat for about 2 weeks, then accelerated so that at the end of 4 months there was no significant difference between the dry weights of the grafts and their controls.

Also guayule scions consisting of the defoliated, woody, small stem segments from plants 6 months, 3½ years, and 7½ years old were grafted to 6-month-old guayule seedlings. All were grown in the greenhouse for 4 months, part of each class was harvested, and the rest

TABLE 1.—*Establishment of grafts of guayule with other species*

Composition of grafted plant	Age of— Scion	Age of— Stock	Diameter of— Scion	Diameter of— Stock	Plants grafted	Successful unions		Remarks
			Mm.	*Mm.*	*Number*	*Number*	*Percent*	
Guayule on guayule	1 mo	1 mo	1.1	1.1	6	6	100	Side-graft, glass cover, and no shade.
	1 mo	1 mo	1.1	1.1	9	7	78	Variation of technique.
	1 mo	1 mo	1.1	1.1	8	1	13	No cover and no shade.
	1 mo	1 mo	1.1	1.1	8	4	50	Cleft-graft, cover, no shade.
			0.8-1.0	0.8-1.0	12	12	100	Side-graft, glass cover, shade.
	7 mo	7 mo	4.5-6.0	4.5-6.0	20	20	100	Side-graft, rubber winding.
	6 yr	8 mo			20	14	70	Tips of old plants to seedlings.
	1 yr	1 yr			10	7	70	Cleft-grafted and taped.
	5 yr	1 yr			25	6	25	Losses by methods and disease.
	6 mo	6 mo			36	24	67	Side-grafted, rubber winding.
	3½ yr	6 mo		3.0	76	38	50	Tips of old plants to seedlings.
	7½ yr	6 mo		3.0	66	35	53	Do.
Guayule on mariola	6 mo	6 mo	3.5	3.5	18	16	89	Side-grafted, rubber winding.
			1.7-2.3	1.7-2.3	40	28	70	Side-grafted, rubber winding, clipped on.
Mariola on guayule	4 yr	1 yr			25	8	32	Cleft-grafted and taped in.
	3 yr	1 yr			35	12	36	Side-grafts wrapped with string.
	4 mo	4 mo	2.0	2.0	40	38	95	Side-grafted, rubber clipped on.
	6 mo	6 mo	3.5	3.5	23	15	65	Side-grafted, rubber wound.
Guayule on *P. stramonium*	5 wk	4 wk	.8-1.0	1.5-2.0	30	16	53	Regular micrografting.
P. stramonium on guayule	4-5 wk	5-6 wk	.7-2.0	1.0-1.5	50	27	54	Do.
Guayule on Jerusalem-artichoke.	4-5 wk	3-6 da	1.5-2.0	2.5	36	14	39	Harvested at 1 to 21 months.
Guayule on sunflower	3-4 wk	4-7 da	.9-1.8	1.8	31	31	100	Variations of technique used.
	4 mo	6 wk			4	0	0	Older tissues did not unite.
	1 mo	7 da	1.6-1.8	2.0	20	12	60	Mostly good growth until harvest.
	7 wk	8 da	1.9	1.9	29	29	100	
	3 wk	8 da	1.5	1.8	20	14	70	Disease losses later.
	4-5 wk	1-2 wk			143	143	100	Harvested periodically, starting at 2 weeks, so true survival unknown.
	3 wk	3 da	.9-1.1		56	55	98	Harvested periodically.
	3 wk	7 da			15	15	100	No asphalt seal used. Harvested periodically.
Sunflower on guayule	3-7 da	3 mo	1.6-1.8	1.8	17	17	100	9 died of disease later.
	7 da	1 mo	2.0	1.6-1.8	10	7	70	Later growth, see figure 7.
	10 da	3 mo	2.1	3.5-4.5	15	12	80	

TABLE 2.—*Summary of dry weights per plant*

Composition of grafted plant	Age of components			Time from graft to harvest	Grown in—	Number of plants	Dry weights per plant				Least significant difference [1]
	Scion	Scion	Stock or ungrafted plant				Leaves	Stems	Roots	Total	
							Grams	*Grams*	*Grams*	*Grams*	*Grams*
Guayule on guayule		5 months	6 months	4 months	Greenhouse	10	2.94	2.04	1.77	6.75	1.08
		3½ years	6 months	4 months	do	10	4.02	2.52	1.80	8.34	
		7½ years	6 months	4 months	do	10	2.92	2.34	1.47	6.73	
		5 months	6 months	14 months	do	10	20.70	59.30	36.20	116.20	9.36
		3½ years	6 months	14 months	do	12	20.70	61.30	31.20	113.20	
		7½ years	6 months	14 months	do	12	20.40	58.50	32.80	111.70	
		5 months	6 months	16 months	Field	3	24.10	52.30	[2] 14.10	90.50	60.50
		3½ years	6 months	16 months	do	15	52.00	95.80	[2] 17.70	165.50	
		7½ years	6 months	16 months	do	17	48.90	86.30	[2] 21.00	156.20	38.90
Guayule on guayule		4 weeks	4 weeks	18 weeks	Greenhouse	12	1.92	.51	1.45	3.88	.96
Ungrafted control			4 weeks	18 weeks	do	11	2.20	.54	1.88	4.62	
Guayule on guayule		7 months	7 months	22 weeks	do	11	4.05	2.10	3.15	9.30	2.28
Ungrafted control			7 months	22 weeks	do	9	3.97	1.86	3.20	9.03	
Guayule on guayule		5 years	1 year	5½ months	do	6	14.80	16.70	7.55	39.05	6.41
Mariola on guayule		3 years	1 year	5½ months	do	8	8.04	22.68	7.48	38.20	
Guayule on guayule		1 year	1 year	14½ months	do	6		28.73	20.67	49.40	5.53
Hybrid mariola on guayule		4 years	1 year	14½ months	do	7		19.97	5.43	25.40	
Guayule on mariola on guayule	6 months	6 months	6 months	14 months	do	6	8.70	43.73	13.92	66.35	No significant difference.
Mariola on guayule on mariola	6 months	6 months	6 months	14 months	do	8	5.10	53.37	18.79	77.35	
Ungrafted guayule			6 months	14 months	do	4	8.20	37.78	13.28	59.25	
Ungrafted mariola			6 months	14 months	do	12	7.11	42.16	24.91	74.12	
Mariola on guayule		6 months	6 months	1 year	do	12	21.16	43.84	12.74	67.66	1.98
Mariola and guayule on mariola.[3]	6 months	6 months	6 months	1 year	do	12	26.44	26.38	16.15	68.97	
Guayule on mariola		6 months	6 months	1 year	do	12	23.79	25.89	17.94	67.02	
Guayule and mariola on guayule.[3]	6 months	6 months	6 months	1 year	do	12	22.68	29.13	16.10	67.91	
Ungrafted mariola			6 months	1 year	do	12	21.09	29.83	14.80	65.63	
Ungrafted guayule			6 months	1 year	do	11	34.28	25.64	15.44	75.36	
Mariola on guayule		6 months	6 months	3½ months	do	6	2.75	4.83	2.13	9.74	No significant difference.
Mariola and guayule on guayule.[3]	6 months	6 months	6 months	3½ months	do	6	3.57	3.15	2.60	9.33	
Guayule on mariola		6 months	6 months	3½ months	do	2	3.74	1.97	3.35	9.05	
Guayule and mariola on guayule.[3]	6 months	6 months	6 months	3½ months	do	2	3.72	2.67	3.85	10.23	
Ungrafted mariola			6 months	3½ months	do	8	3.42	5.78	4.44	13.63	
Ungrafted guayule			6 months	3½ months	do	7	4.29	2.30	3.04	9.62	

Guayule on *P. stramonium*	4-5 weeks	4-5 weeks	9 months	Field	4	83.38	85.87	37.60	206.85	No significant difference.
P. stramonium on guayule	4-6 weeks	4-6 weeks	9 months	do	9	96.16	123.70	42.30	262.16	
Ungrafted guayule		5-6 weeks	9 months	do	8	62.98	71.54	20.75	155.28	
Ungrafted *P. stramonium*		4-5 weeks	9 months	do	7	262.93	372.94	180.93	816.80	
Guayule on *P. stramonium*	4-5 weeks	4-5 weeks	1 year	Greenhouse	4	24.42	32.91	17.80	75.21	Each significantly different.
Ungrafted guayule		5-6 weeks	1 year	do	3	14.23	31.23	12.20	57.66	
Ungrafted *P. stramonium*		4-5 weeks	1 year	do	3	16.77	34.50	50.50	101.77	
Guayule on *P. stramonium*	4-5 weeks	4-5 weeks	20½ months	Field	4	262.42	661.90	[2] 157.48	1,081.80	No significant difference.
P. stramonium on guayule	4-6 weeks	4-6 weeks	20½ months	do	8	74.03	741.50	[2] 159.40	974.93	
Ungrafted guayule		5-6 weeks	20½ months	do	8	172.41	452.22	[2] 78.23	702.86	
Ungrafted *P. stramonium*		4-5 weeks	20½ months	do	6	1,284.20	4,075.00	[2] 1,281.50	6,640.70	
Jerusalem-artichoke from:										
Whole tubers			5 months	Greenhouse	5	17.02	16.90	54.34	88.26	
Single eyes			5 months	do	2		9.75	22.70		
Guayule on Jerusalem-artichoke.	4 weeks	5 days	9½ months	do	2	13.48	15.17	[2] 1.49	30.14	
Do	4 weeks	5 days	11 months	do	1	9.60	15.40	[2] 15.33	40.33	
Ungrafted guayule		4 weeks	11 months	do	2	17.30	47.11	19.90	84.31	
Guayule on Jerusalem-artichoke.	4 weeks	5 days	21 months	do	1	21.50	92.40	51.10	165.09	
Ungrafted guayule		4 weeks	21 months	do	1	23.90	209.20	60.70	233.80	
Guayule on sunflower	4 weeks	4 days	3 months	do	2		2.25	2.30		
Sunflower on guayule	4 days	4 weeks	3 months	do	1		.50	.15		
Ungrafted sunflower		4 days	6 months	do	5		22.78	9.16		
Do		4 days	4 months	do	9		11.00	3.62		
Sunflower on guayule	4 days	4 weeks	6 months	do	1		2.30	.30		
Guayule on sunflower	4 weeks	4 days	6½ months	do	2		14.30	5.10		
Ungrafted guayule		4 weeks	6½ months	do	5		17.74	[3] 6.72		
Sunflower on guayule	2½ weeks	3 months	4 months	do	3		4.57	.37		
Ungrafted guayule		3 months	4 months	do	3		7.60	7.47		
Guayule on sunflower	4 weeks	1 week	5 months	do	4		7.80	6.63		
Ungrafted guayule		4 weeks	5 months	do	6		6.11	8.67		
Sunflower on guayule	1 week	4 weeks	5 months	do	3		4.10	.20		
Do	2½ weeks	3 months	4½ months	do	4		2.45	.33		
Ungrafted guayule		3 months	4½ months	do	4		9.50	9.38		

[1] Figures in last column represent the least significant difference in total dry weights per plant between items grouped, as determined by the formula $t\sqrt{2}\sqrt{V/N}$; the level of probability calculated being 0.05.
[2] Only stump and larger roots were harvested.
[3] The weights of leaves and stems represent the total of those components for both tops.

were grown for approximately 1 year longer in the greenhouse and in the field.

At the 4-month harvest, plants having tops produced from scions taken from 3½-year-old plants were heavier than those from scions of seedlings. In the later harvests, the plants grown in the greenhouse showed no difference in weight due to age of the scion parents. The apparent difference between mean weights of those grown in the field was open to question on account of competition of some of them with large plants of another species that adjoined the plots.

In the experiments described, the only losses were from failure of the scion to unite with the stock. There were no temporary unions. In several earlier series where the understocks consisted of approximately 1-year-old seedlings, which had been grown as a very dense stand in a very limited quantity of gravel and nutrient solutions, grafts that were slow to unite were lost by disease that attacked the stocks at the ground line. When once the grafts were established, no further losses occurred.

GUAYULE WITH MARIOLA

Micrografting guayule with mariola was difficult, because of the smallness of the mariola hypocotyls during the first 6 weeks. However, older seedlings in the semiwoody stage united readily and, with some care in selection for stem size, good matching of scion and stock was possible.

Reciprocal grafts of such seedlings were made, using the clipped rubber binding shown in figure 1. From half of each combination, the tops of the understock species were removed when the scions had become established, and in the other half the scions were allowed to grow in competition with the original tops of the stock. Ungrafted guayule and mariola served as controls. They were grown in the greenhouse in 2-gallon crocks until harvested at 3½ months and at 12 months after grafting.

At 3½ months, the plants had grown well but the mean dry weights of all combinations of grafted and of ungrafted plants lacked any significant difference. At 12 months, 12 plants were harvested of each of the combinations except ungrafted guayule, 1 of which had died, leaving 11. The average dry weights per plant were alike for all grafting combinations and the ungrafted mariola. The ungrafted guayule averaged somewhat heavier, but the differences between it and the other classes were mostly nonsignificant.

In another experiment, guayule and mariola plants were twice grafted so that the top branching system was separated from the stump and roots by a trunk of the opposite species. The purpose of this was to study the rubber-bearing characteristics of the stem of one species when receiving translocated material from both the roots and leaves of the other species.

Eight out of 12 of the mariola-guayule-mariola and 6 out of 12 of the guayule-mariola-guayule plants survived the double grafting. The twice-grafted plants thrived as well as the ungrafted controls, as shown in figures 3 and 4.

Rectangular patches of mariola bark were set into the stems of guayule, and the reciprocal combination made, to determine whether

FIGURE 3.—Twice-grafted plant, guayule on mariola on guayule, 11 months after grafting.

FIGURE 4.—Twice-grafted plant, mariola on guayule on mariola, 14 months after grafting.

the secondary phloem formed from the cambium after the operation had the rubber content characteristic of the main plant or of the species from which the bark was taken.

GUAYULE WITH PARTHENIUM STRAMONIUM

Although *Parthenium stramonium* at maturity is a tree many times larger than the guayule shrub, the *P. stramonium* seedlings are smaller than guayule during the first month from seeding. Therefore, successful grafting of the seedlings depends on sowing the *P. stramonium* seed 1 week to 10 days earlier than the guayule and on grafting the two species of seedlings when they reach the same size. This stage is reached when both species of seedlings are very small (fig. 2).

Somewhat more than 50 percent of the grafts so made were successful, and 2 months later the grafts of both combinations were comparable in size and appearance with the ungrafted controls. They were then transplanted to the field. At 5 months the guayule on *P. stramonium* plants were still like the ungrafted guayule in size, color, and fruitfulness, but the grafts of *P. stramonium* on guayule varied more widely and were generally smaller than the ungrafted *P. stramonium*.

Parthenium stramonium normally has vastly greater vigor and size than guayule, but the influence of *P. stramonium* understocks on growth of guayule tops was slight. When the field-grown grafts were 9 months old, the guayule scion tops were somewhat more leafy but there was no significant difference in dry weights of the stems of scions and of ungrafted guayule. Influence of scions on root growth was much greater. Dry weights of guayule understock roots were about double those of ungrafted guayule. *P. stramonium* understocks for guayule tops weighed nearly twice as much as the ungrafted guayule roots but only one-fifth as much as the ungrafted *P. stramonium*.

The tops of *P. stramonium* grown as scions on guayule came into bloom earlier than the controls and bore flowers and seed heavily and continuously throughout the growing season. At harvest, there was little difference in the general appearance of guayule scions and ungrafted guayule, the only possible difference being a more upright habit of the scions. The appearance of the wood at the union is shown in figure 5.

GUAYULE WITH SUNFLOWER

In eight series of grafts of these species, two strains of guayule (593 and 1265) were grafted onto sunflower of Red and Sungold varieties, and reciprocals of these combinations were made. In some cases, the micrografting procedure previously described was varied by inserting the guayule scions into the split meristems of the sunflowers, by using guayule scions carrying a tip of root tissue, and by omitting the asphalt-emulsion seal.

Initial establishment of the unions was good, usually 60 to 100 percent, but 2 to 4 weeks later, when the tops of the stock species were removed, losses were moderate to very severe. Often the losses apparently started with attacks by fungi at the ground line of the under-

FIGURE 5.—Vertical section through a graft union of *Parthenium stramonium* on guayule. The darker colored wood near the bottom is guayule. Photographed at 9 months after grafting of very small seedlings, as shown in figure 2.

stock. A series of guayule-sunflower grafts was harvested at weekly intervals for microscopic examination by Artschwager, as described in another section of this bulletin (pp. 22 to 26). This section covers the anatomy of the unions and shows that true unions occurred between these distantly related species.

The two species showed marked unilateral compatibility. Grafts of guayule on sunflower thrived, both the guayule tops and sunflower roots making faster growth than the corresponding parts of ungrafted controls (fig. 6). When sunflower was grafted on guayule, both the tops and roots were severely stunted (fig. 7). The sunflower scion commonly had a stem diameter three or more times that of guayule understock, but very stunted in height as compared with ungrafted sunflower. Often the sunflower grew well for a time, then wilted suddenly as if the water supply were cut off. Sometimes death ensued, but often the plants gradually recovered, only to suffer one or more later recurrences. The extent of the two types of losses with guayule and sunflower grafts is indicated by the following tabulation:

	Grafted	*Established*	*Survived*
	10	10	3
Guayule on sunflower	21	21	8
	20	12	5
	17	17	8
Sunflower on guayule	10	7	7
	15	12	4

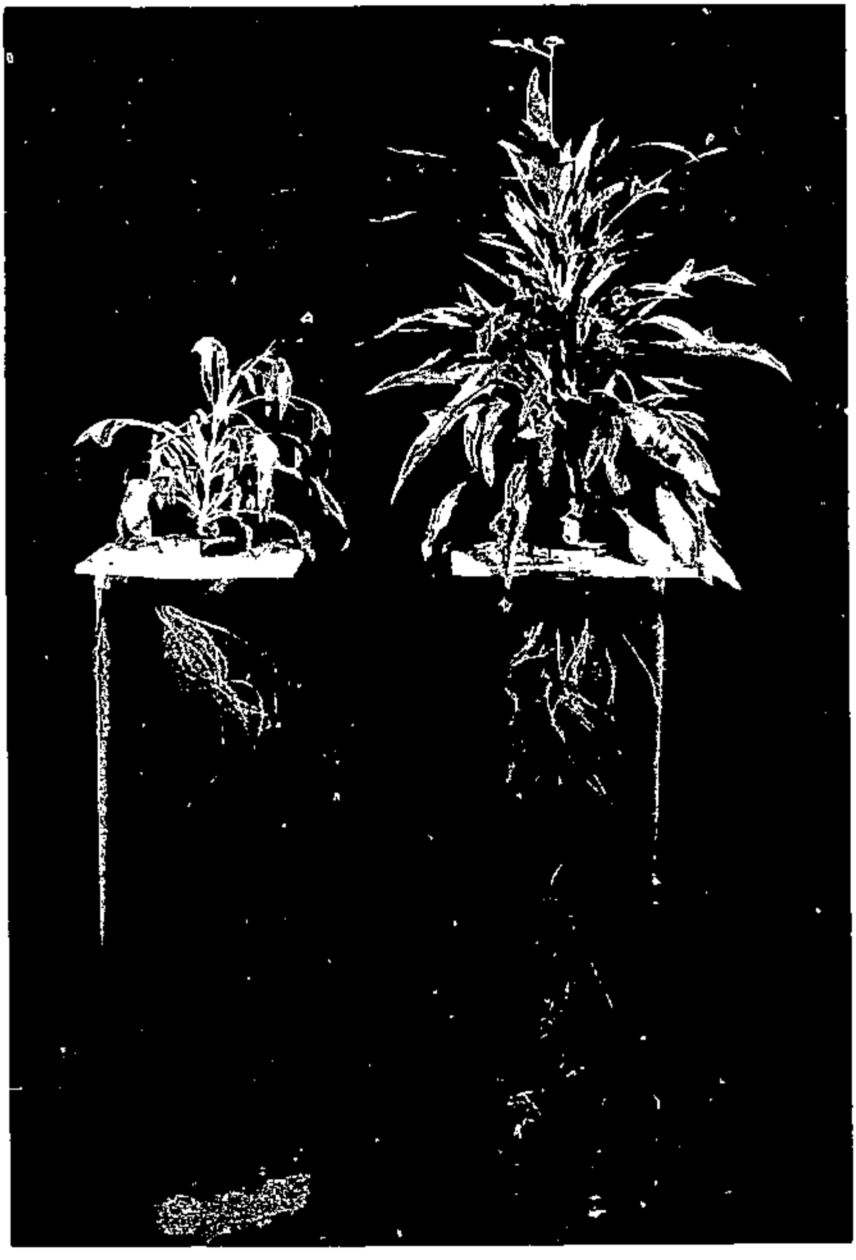

FIGURE 6.—Relative growth of ungrafted guayule (left) and guayule on sunflower (right), grown in aerated nutrient culture in midwinter for about 3 months after grafting.

FIGURE 7. Stunting of sunflower on guayule, in comparison with ungrafted guayule at right; grown in soil in the greenhouse for about 4 months during summer and fall.

Although sunflower is an annual plant, the species can function for more than one growing season as the understock for the shrub guayule. Two grafts of guayule on sunflower made in April lived until June of the next year. This agrees with the instance cited by Molisch (*9*) concerning the ability of an annual to function as understock for a perennial plant for several years.

GUAYULE WITH JERUSALEM-ARTICHOKE

Scions from very young guayule seedlings were grafted to the newly emerged shoots from the tubers and from single eyes cut from tubers of Jerusalem-artichoke. During the first few months thereafter, the foliage of the guayule s ions showed abnormalities of form and color. These effects appeared earlier and were more pronounced where the

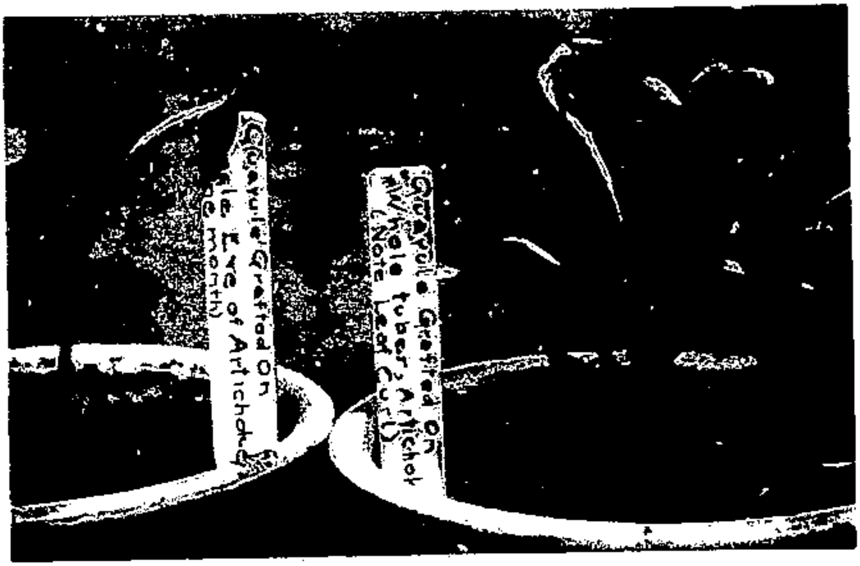

FIGURE 8.—Guayule grafted on Jerusalem-artichoke for about 1 month. Understock at left is a shoot from a single eye and that at right from a whole tuber. Note leaf-curing of scion of the latter.

shoots arose from whole tubers, and persisted in lesser degree throughout the lives of all the grafts (fig. 8).

The grafted plants were harvested at various periods from 1 to 21 months after grafting. Whereas the ungrafted Jerusalem-artichoke plants matured and died the first fall after planting, the same species as understocks lived for nearly 2 years. The assumption that the understocks at harvest during the second year were actually *Helianthus tuberosus* and not roots originating from the scions was supported by the facts that the roots were in appearance characteristic of *H. tuberosus*, had tubers attached, and contained very little material brominating as rubber.

SUMMARY

Guayule established unions readily and grew well when grafted by conventional methods with mariola (*P. incanum*).

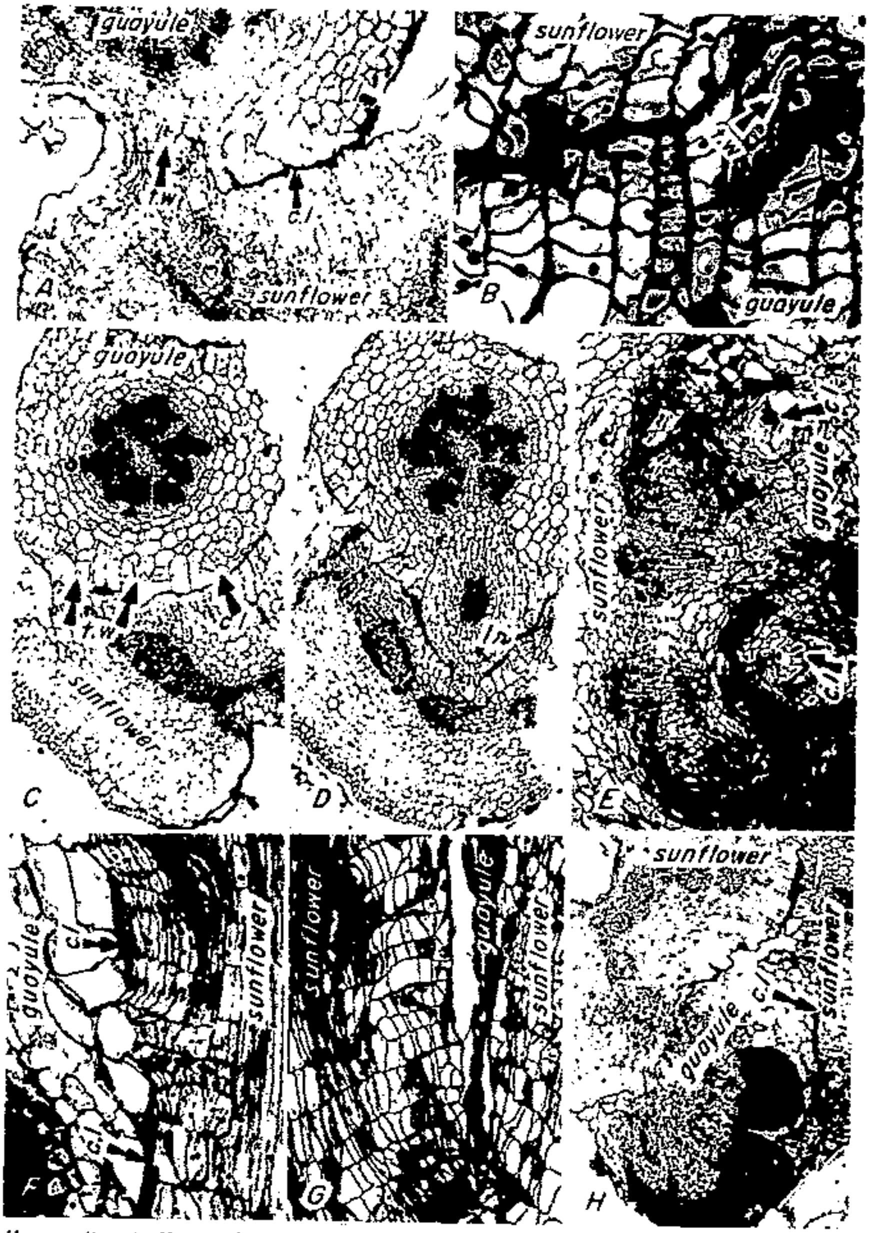

FIGURE 9. *A*, Formation of transfusion window in contact layer between scion and stock. × 100. *B*, Break and displacement in contact layer by invading parenchyma cells. × 340. *C*, Upper region of graft, 2 weeks old, showing break in contact through formation of transfusion window. × 45. *D*, Lateral rootlet of guayule scion penetrates contact layer in its outward passage. × 45. *E*, Cross section of graft 4 weeks old. Contact layer has disappeared except for two isolated pieces that have become walled off. Procambial strand passes

A micrografting technique was developed whereby very young seedlings of guayule were grafted successfully with *P. stramonium* and with more distantly related species such as sunflower (*Helianthus annuus*) and Jerusalem-artichoke (*H. tuberosus*).

The following growth effects were noted: (1) Self-grafted guayule grew at about the same rate as ungrafted guayule; (2) reciprocal combinations of guayule and mariola made about the same growth as ungrafted plants of those species; (3) in reciprocal combinations of guayule and *P. stramonium* (a tree species of the genus), guayule limited the growth of *P. stramonium* scions or understocks, and *P. stramonium* scions stimulated growth of guayule roots but *P. stramonium* understocks did not produce greater guayule scion tops than the tops of ungrafted guayule controls; and (4) in grafts of sunflower and guayule, there was evidence of marked unilateral compatibility, the guayule on sunflower thriving much better than sunflower on guayule. Sunflower became perennial when functioning as an understock for guayule.

ANATOMICAL STUDIES ON GRAFT UNIONS BETWEEN GUAYULE AND SUNFLOWER

By Ernst Artschwager

The structural changes that accompany the establishment of graft unions in different plants have been treated in detail in Krenke's work (*8*). That publication also contains a critical review of all pertinent literature.

According to Krenke, the anatomical changes involve the formation of a contact layer (Isolierschicht) between scion and stock; the elimination of this layer through resorption and formation of transfusion windows (Durchbruchfenster); and the establishment of vascular connections between scion and stock. In guayule-sunflower micrografts, the initial effect of the union is the formation of a contact layer. This layer consists chiefly of coagulated cell content derived from injured cells of the graft partners and is bordered by whole or injured cells. If the cut surfaces are smooth and under pressure, the contact layer is thin and resembles a thickened cell wall. If the contacting surfaces are uneven and not under pressure, the contact layer is broad and irregular and often breaks apart to leave an air-filled cleft between scion and stock.

As the contact layer forms a barrier to the ascending and descending sap flow between scion and stock, it must be eliminated before a graft can be considered successful. Permanent retention of the contact layer ultimately results in the death of the scion, or of both scion and stock. Elimination of the contact layer in guayule-sunflower grafts is effected through the formation of transfusion windows and localized resorption (fig. 9, *A*), processes that are usually complementary.

from phloem of sunflower toward guayule scion. × 100. *F*, Longitudinal section of basal region of graft, 2 weeks old, showing break in contact layer and development of a band of short cambiumlike cells in the sunflower tissue adjacent to the contact layer. × 120. *G*, Graft-union failure. Formation of broad concentric ring of cambiumlike tissue in sunflower stock. × 100. *H*, Section through base of graft. Guayule scion has given rise to numerous lateral rootlets. × 45.

Transfusion windows in guayule-sunflower grafts are localized regions of dividing and elongating cells that bridge the contact layer and establish direct connections between the tissues of the graft partners. Initials of such transfusion windows originate along the contact layer on the side of either scion or stock but most often on the sunflower side, which seems to possess the higher regenerative ability. As the transfusion windows develop, the contact layer is broken and displaced (fig. 9, *B*, *C*) and the isolated pieces are subsequently resorbed or walled-off by new tissue (fig. 9, *E*). Transfusion windows have a limited vertical extent (fig. 9, *F*), except in regions where favorable contact between scion and stock resulted in an early resorption of the contact layer. In guayule-sunflower grafts, transfusion windows take 7 or more days to develop. Krenke points out that initiation of transfusion windows is stimulated by the nearness of vascular tissue. This is true in guayule-sunflower grafts, but generally, because of the proximity of vascular elements in the tissues of both graft partners, no conspicuous correlation is noticeable.

Although transfusion windows arise only in localized regions, all parenchyma cells of the stock adjacent to the contact layer undergo periclinal divisions and form a broad band of cambiumlike tissue with the cells arranged in short vertical tiers. This layer is conspicuously broad in grafts where union between stock and scion is slow or unsuccessful (fig. 9, *G*). In successful grafts, cell divisions in this region cease after the contact layer has been eliminated. Cell activity in the scion, especially if its base is tipped with root tissue, gives rise to numerous lateral rootlets (figs. 9, *H*, and 10, *A*). Some of these grow diagonally outward, piercing in their passage the contact layer (fig. 9, *D*) and the jacketing sunflower stock. Others bend abruptly downward, penetrate the tissue of the stock, and if the graft union is close to the ground establish themselves in the soil and gradually replace the stock as the supplying organ for water and nutrients.

The partial or complete elimination of the contact layer permits some sap movement between scion and stock, but a normal flow is not established until the vascular tissues of the graft partners are united. The first vascular connections appear in the severed halves of the sunflower stock near the base of the graft (fig. 10, *B*) and somewhat later in the transfusion windows between scion and stock. They are represented by short parenchyma cells with scalariform thickenings and lignified walls. Differentiation of vascular elements proceeds progressively along the path of a transfusion window and in the direction of the nearest vascular bundle of the graft partner (fig. 10, *D*). The first xylem cells are short and irregular; elements formed later are more normal in length, wall sculpture, and end-wall opening. Often distinct procambiumlike strands extend from the tissue of the stock through a transfusion window into the scion (fig. 10, *C*). Such strands may occasionally be extensive and connect directly with the phloem of the sunflower stock (fig. 9, *E*). New phloem elements originate near the xylem. They are at first represented by narrow, elongated parenchyma cells that function provisionally as conducting units until sieve tubes make their appearance. The position of the parenchyma cells is usually irregular, but the normal collateral arrangement is eventually established. A return to complete normalcy in structure has not been observed, as the oldest series studied

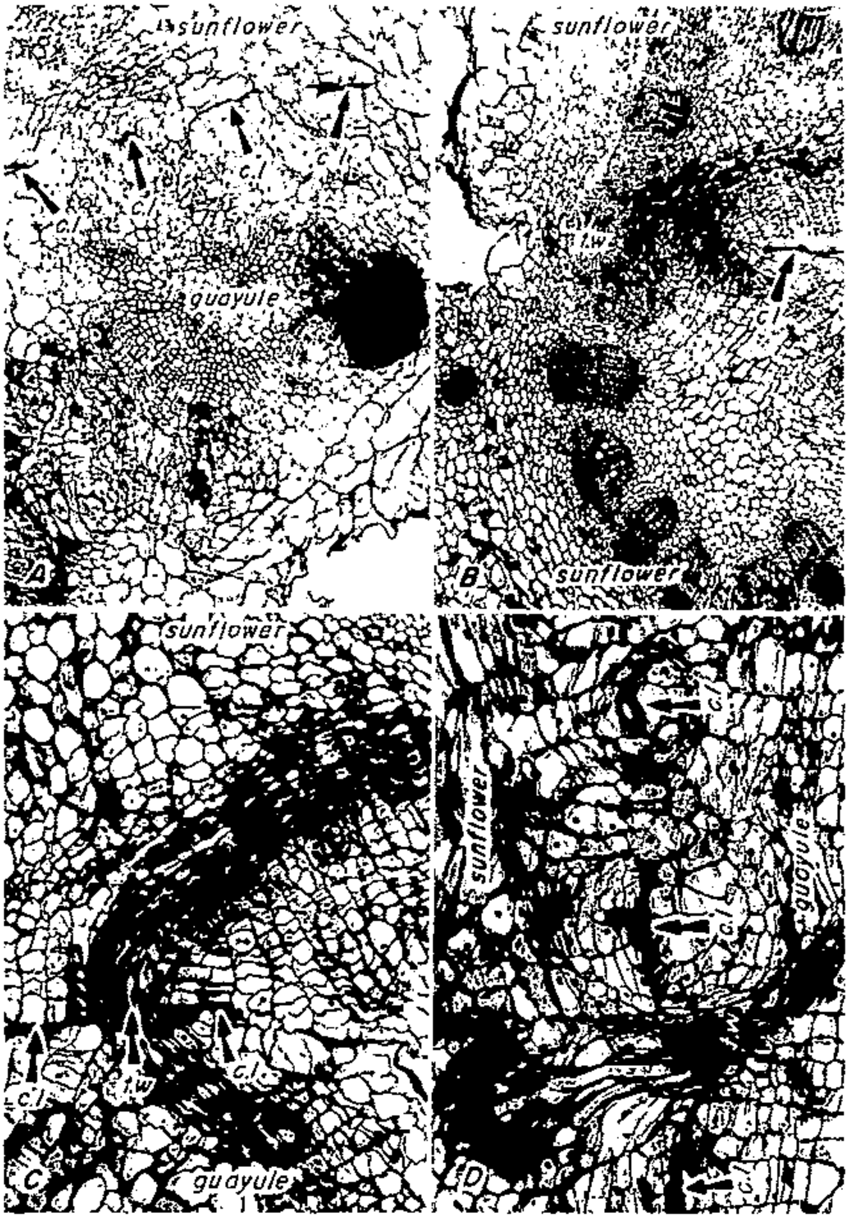

FIGURE 16. *A*, Break-up of contact layer through formation of transfusion windows and resorption. Lateral rootlet developing in guayule scion. × 100. *B*, Establishment of vascular connections between severed halves of sunflower stock. × 100. *C*, Strand of procambium bridges the contact layer between scion and stock. × 100. *D*, Differentiation of vascular elements in transfusion window. × 100.

grew only 6 weeks. The factors accelerating or delaying the normalization of the tissues are probably numerous and varied, but of these the complete and early elimination of the contact layer appears to be the most important.

Summarizing, the anatomical changes that accompany the union of tissues in guayule-sunflower micrografts involve the elimination of the contact layer between the graft partners through resorption and formation of transfusion windows and the establishment of vascular connection between scion and stock. In grafts that failed to become established, the tissues of the graft partners had not united properly and did not provide adequate conducting channels for the movement of water and nutrients.

THE ACCUMULATION OF RUBBER IN INTERSPECIFIC AND INTERGENERIC GRAFTS OF GUAYULE

By Carl A. Taylor and H. M. Benedict

In initiating studies on the manner in which rubber is formed in plants, there arose the question of what effect grafting portions of rubber-producing plants to portions of non-rubber-producing species would have on their rubber accumulation. In other words, would a plant that normally produces rubber produce it at all or in normal quantities when grafted to a non-rubber-forming plant; or would a non-rubber-producing plant produce rubber when grafted to a rubber-forming plant?

The first step in gaining the answer was to develop methods for making such grafts between guayule (*Parthenium argentatum*) and non-rubber-forming plants. Taylor and Benedict (pp. 6 to 10) and Artschwager (pp. 22 to 26) have described the methods finally developed and the effects of such interspecific grafts on growth. This section presents the results of the rubber determinations on these plants and discusses them in relation to the question posed above. Only a few representative experiments are described.

Methods

The methods of procedure, the number of replications or plants involved, and the cultural practices used in growing the plants have been described by Taylor and Benedict on pages 6 to 22 of this bulletin. The rubber determinations were made according to the method described by Traub (*12*), except on the few occasions when the bromination method of Willits, Swain, and Ogg (*13*) was used.

Results

GUAYULE ON GUAYULE

Grafting per se did not affect the growth or impair the plants' ability to accumulate rubber. Guayule grafted on guayule when only 4 weeks from seed, as well as 7-month-old plants, had at harvest dry weights and rubber percentages, and hence total amounts of rubber, not significantly different from those of ungrafted controls, as shown in figure 11.

GUAYULE AND MARIOLA GRAFTS

Guayule was grafted in various combinations with mariola (*Parthenium incanum*), which normally accumulates very little rubber. All of the plants used were very young and were shown by analyses to contain very little rubber (0.10 to 1.24 mg. per plant) when grafted. Subsequent growth was made from the photosynthetic products of

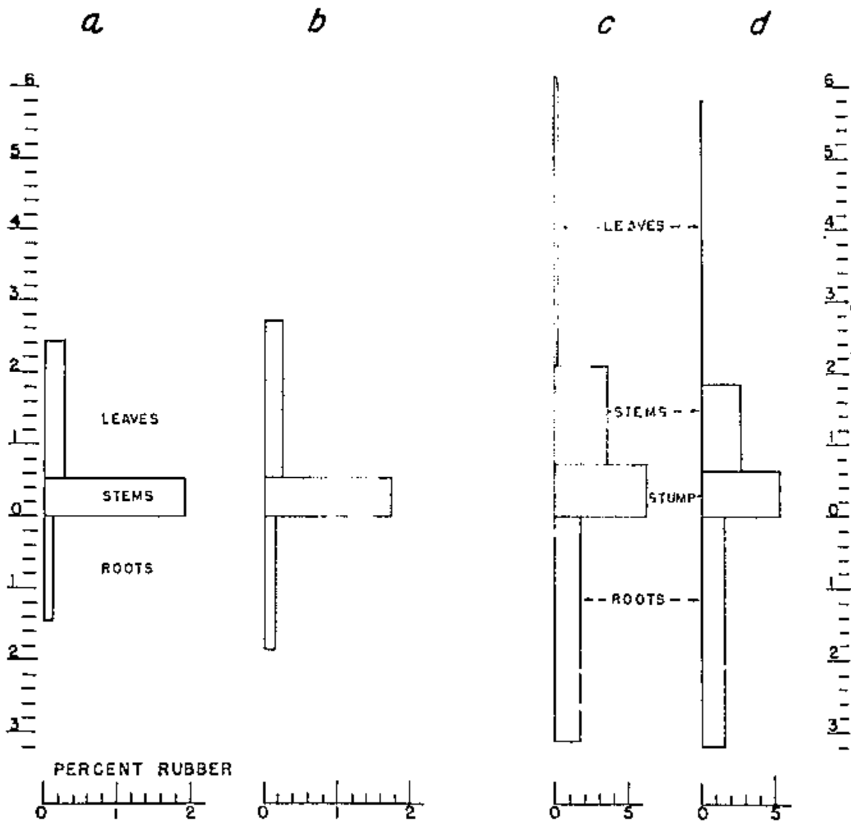

FIGURE 11.—Dry weights and rubber percentages of self-grafted guayule and ungrafted controls: *a*, Micrografts of very young guayule seedlings, harvested 18 weeks after grafting; *b*, ungrafted guayule controls for *a*; *c*, grafted 9-month-old plants, harvested 22 weeks after grafting; and *d*, ungrafted guayule controls for *c*. (Different scale of rubber percentages used for the two age classes.)

leaves of the opposite species, or from leaves of both species, depending on the grafting combination.

Harvests were made at 4 and 12 months after grafting. The harvest at 4 months contained varying numbers of plants ranging from 2 to 8 per treatment; but the 12-month harvest contained 12 plants of all treatments except 1, which contained 11 plants. Dry weights and rubber percentages found in the various parts of the plants are shown graphically in figures 12 and 13.

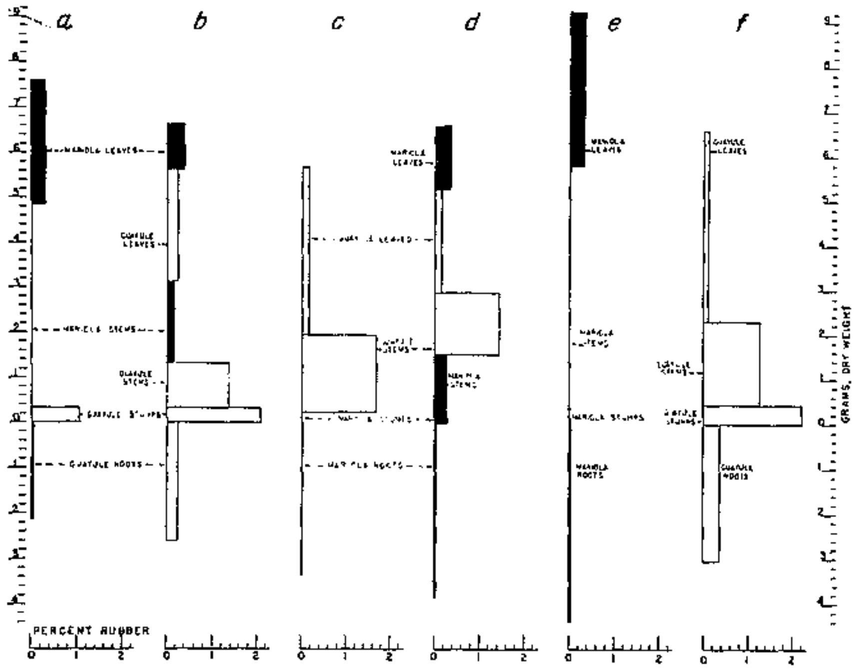

FIGURE 12.—Dry weights and rubber percentages of reciprocal grafts of guayule and mariola, grown in the greenhouse for 4 months: *a*, Mariola on guayule; *b*, mariola and guayule on guayule; *c*, guayule on mariola; *d*, guayule and mariola on mariola; *e*, ungrafted mariola; and *f*, ungrafted guayule. In *b* and *d*, the leaves and stems are shown above one another merely to indicate additive weights; actually, they were grafted near their bases and the tops of both species grew at the same levels.

When the plants were harvested, 4 months after they had been grafted, the guayule stumps below exclusively mariola tops had made considerable rubber, though the percentage was significantly less than when the stems and leaves were all guayule or a combination of mariola and guayule. The rubber percentage of roots of guayule understocks having exclusively mariola tops was very low.

The bispecies tops, the total dry weights of the leaves and of stems which were similar to those of ungrafted plants, had rubber percentages in the guayule stems, stumps, and roots as high as those in the ungrafted guayule controls. Thus, partial substitution of the guayule leaves with mariola leaves did not reduce rubber accumulation in the woody tissues of guayule.

Mariola growing as one component of guayule-mariola bispecies tops contained, at 4 months, significantly greater percentages of rubber in its stems than was contained in the mariola stems of single-scion tops or of ungrafted mariola. This held true whether the mariola was the scion or the stock species. The actual rubber percentages were very low as compared to guayule and were accompanied with considerable stunting, which may have contributed to the effect. Little is known of the influence of environmental factors on growth rates or rubber accumulation in mariola.

Guayule harvested at 12 months showed great increases in size and in rubber percentages, but, with a few exceptions, the general effects of grafting were the same as at 4 months. The rubber percentages of stumps and roots of guayule understocks with exclusively mariola-scion tops continued to be lower than in the ungrafted guayule but they had increased to such an extent that the difference was barely significant. Rubber percentage of the stems of ungrafted guayule had become slightly and significantly higher than in the grafts. Also, the rubber percentages in stems of ungrafted mariola and exclusively mariola-scion tops had increased to such extent that they were no longer significantly lower than the mariola stems in bispecies tops.

In this latter harvest, samples were taken of 1-inch segments immediately above the graft unions. Such guayule segments of grafts with bispecies tops showed slightly higher percentages of rubber than the average of the remaining stems. The mariola segments in the bispecies grafts and ungrafted mariola had significantly higher percentages of rubber than were found in the remainder of their stems, all the mariola rubber percentages being very low.

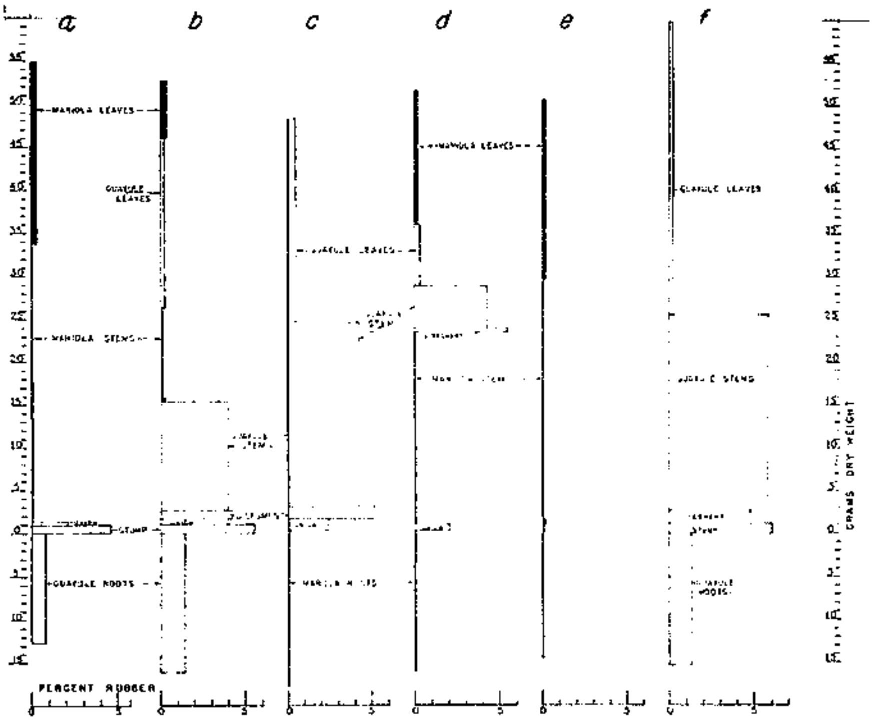

FIGURE 13.—Dry weights and rubber percentages of reciprocal grafts of guayule and mariola, grown in the greenhouse for 1 year: *a*, Mariola on guayule; *b*, mariola and guayule on guayule; *c*, guayule on mariola; *d*, guayule and mariola on mariola; *e*, ungrafted mariola; and *f*, ungrafted guayule. In treatments *b* and *d*, the leaves and stems are shown above one another merely to indicate additive weights; actually, they were grafted near their bases and the tops of both species grew at the same levels.

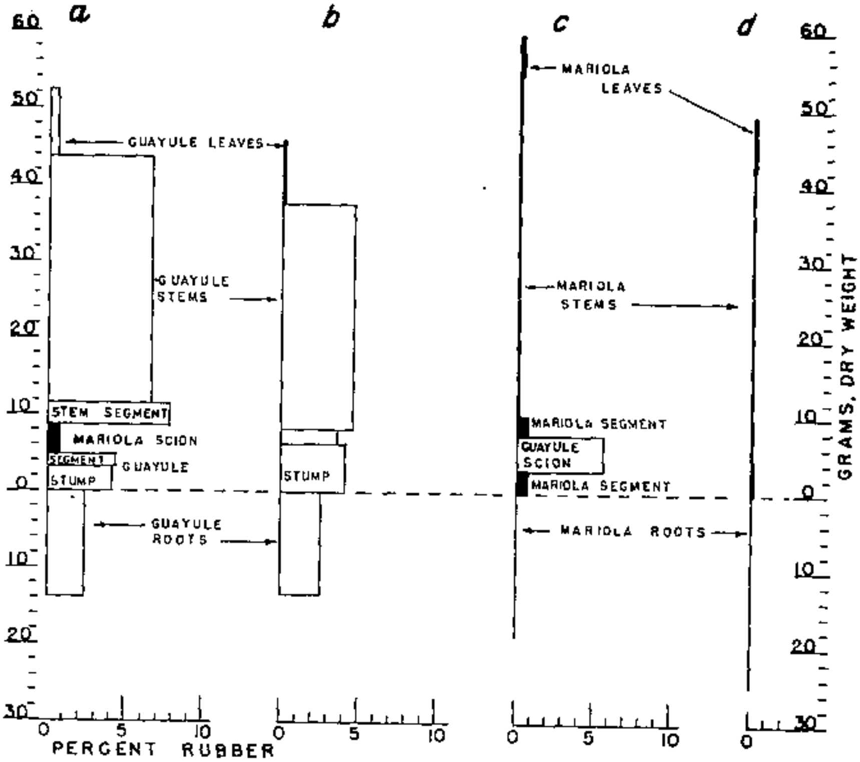

FIGURE 14.—Dry weights and rubber percentages of three-story grafts of guayule and mariola: *a*, Guayule containing a mariola stem; *b*, ungrafted guayule control; *c*, mariola containing a guayule stem; and *d*, ungrafted mariola control.

To investigate further the rate of accumulation of rubber in guayule and mariola, when each was making growth from materials furnished by the opposite species, plants were twice grafted so that in each plant a section of the trunk was guayule and the top, stump, and roots were mariola. The reciprocal combination was also made. Such grafts with ungrafted controls of both species were harvested at 14 months. Figure 14 indicates the dry weights and rubber percentages found in the various parts of the plants, including sections about 3 cm. in length located immediately above and below the ingrafted portion.

The guayule scions occupying a part of the main stem of plants otherwise entirely mariola contained about the same percentage of rubber as the stems of ungrafted guayule plants or the stems of guayule containing a mariola section, neither difference being significant at the 5-percent level. However, the stems and leaves of the guayule plants having a stem scion of mariola contained more rubber in percentage and total quantity than was in the ungrafted controls.

Surprisingly, the mariola wood growing within 3 cm. above or below the guayule unions was 15 to 20 times richer in rubber than the remainder of the mariola stems and about 8 times richer than the corresponding parts of 10 out of 12 ungrafted mariola. This enrichment was not coincident with any apparent stunting. The rubber enrichment of mariola was the same whether the graft was of mariola on guayule or the reverse combination. In the case of mariola adjacent to a stem section of guayule, any influence of the guayule on the rubber-making capacity of mariola could not be ascribed to photosynthetic products derived from guayule leaves, since there were no guayule leaves on one of the series of grafts. One of the mariola controls was somewhat higher, and another much higher, in rubber content than the other 10; but, even so, the influence of grafting with guayule was significant beyond the 1-percent level. All of the mariola rubber percentages were very low compared to those of guayule.

Grafting did not influence the rubber content of the roots of mariola or guayule.

GUAYULE AND PARTHENIUM STRAMONIUM GRAFTS

One group of reciprocal grafts of guayule and *Parthenium stramonium* was grown in the greenhouse for 1 year; a second group was grown in the field for 9 months; and a third group was grown in the field for about 21 months. The distributions of dry weights and rubber percentages found in the plants at harvest are shown in figures 15, 16, and 17.

Of the greenhouse series, the grafts of guayule on *P. stramonium* had significantly more rubber by percentage and total quantity in their stems than was found in the stems of ungrafted guayule controls. This was interpreted as being the result of better utilization of nutrients by *P. stramonium* roots in the limited quantities of soil employed in the greenhouse cultures, an effect that was lacking in the outdoor series. Traces of rubber, averaging 0.02 percent, were found in the *P. stramonium* roots of grafts, but none in the roots of ungrafted controls. However, such traces of rubber were considered of doubtful significance, because similar quantities were found in roots of other ungrafted *P. stramonium*.

Among the field-grown series harvested at 9 months, guayule-scion stems had slightly but significantly lower percentages of rubber than were found in the stems of ungrafted guayule. This effect is opposite that appearing in the greenhouse series and might be construed as the result of environmental effects rather than as a response to grafting.

There was no significant difference in the percentage of rubber in guayule roots of grafts and controls, though the understocks made significantly more growth than the roots of the controls.

Rubber in the stems and roots of *P. stramonium* amounted to traces, ranging from 0 to 0.07 percent. There was no apparent effect on rubber production from grafting with guayule.

In the harvest of field-grown plants at about 21 months after grafting, the plants were 5 to 8 times larger than those in the earlier harvest. There was little change in rubber percentages or size relationships that might be considered as effects of grafting combinations.

GUAYULE AND JERUSALEM-ARTICHOKE GRAFTS

Guayule was grafted on the Jerusalem-artichoke (*Helianthus tuberosus*), but the reciprocal combination was not made. The grafts and ungrafted controls were harvested, a few at a time, at periods ranging from 9 to 21 months after grafting. The stems, tubers, and

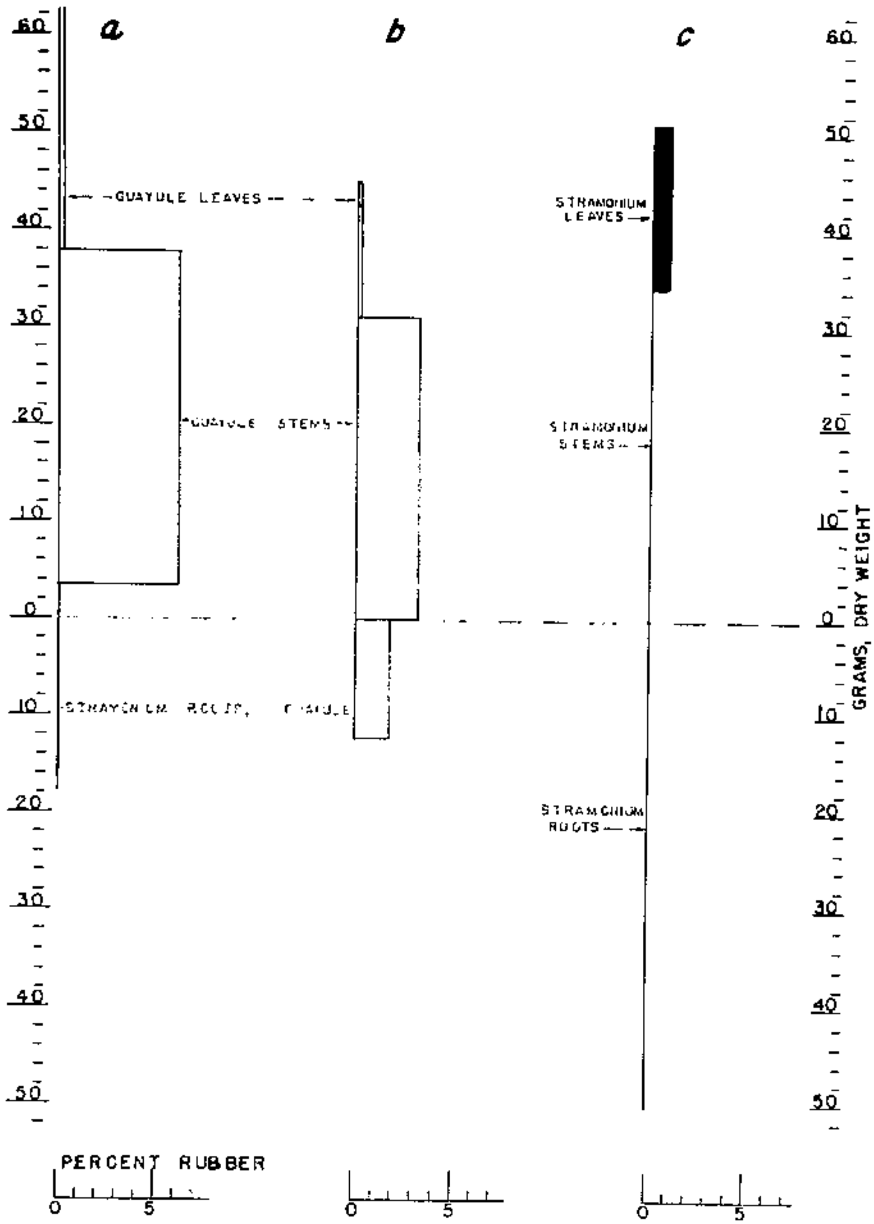

FIGURE 15.—Distribution of dry weights and rubber percentages of seedling grafts of guayule and *Parthenium stramonium*, grown in the greenhouse for 1 year: *a*, Guayule on *P. stramonium*; *b*, ungrafted control; and *c*, ungrafted *P. stramonium*.

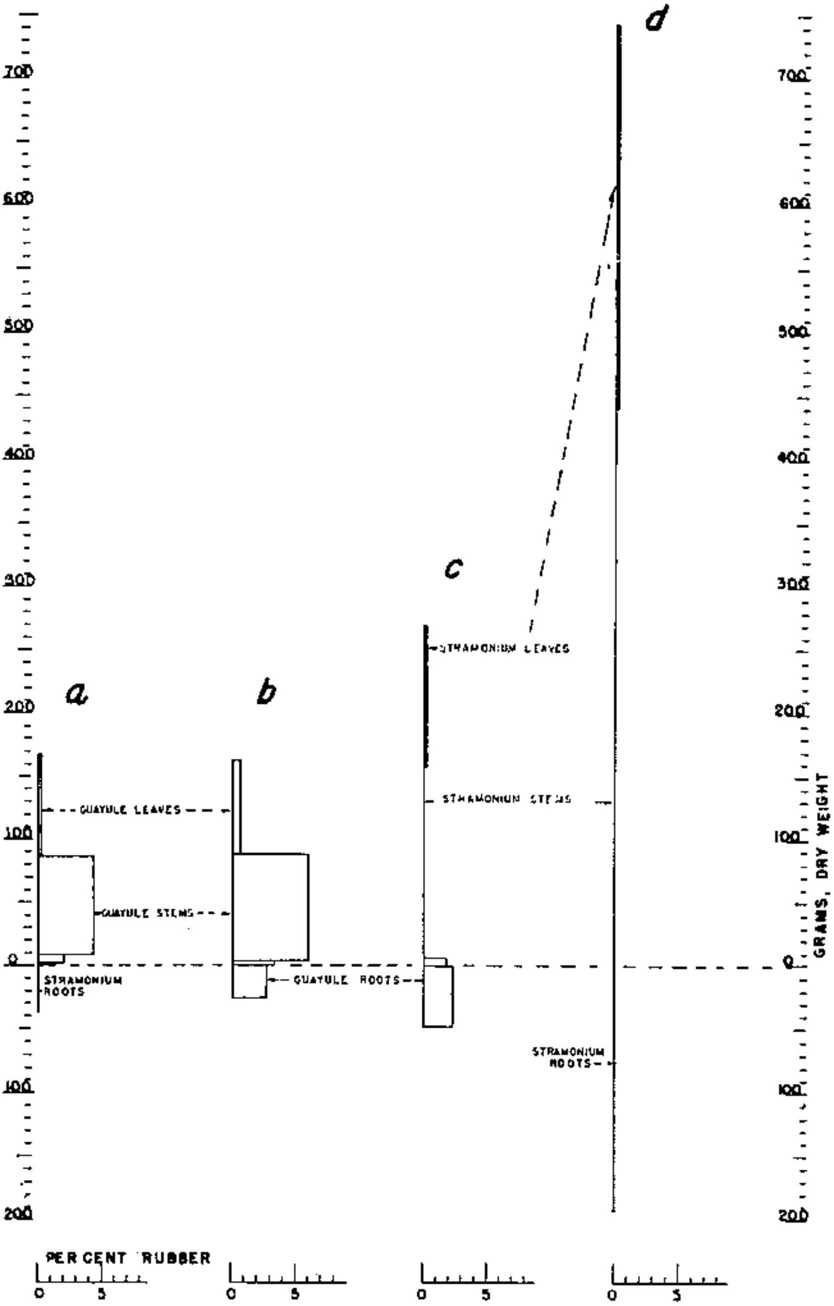

FIGURE 16.—Distribution of dry weights and rubber percentages in seedling grafts of guayule and *Parthenium stramonium*, grown in the field until 9 months after grafting: *a*, Guayule on *P. stramonium*; *b*, ungrafted guayule; *c*, *P. stramonium* on guayule; and *d*, ungrafted *P. stramonium*.

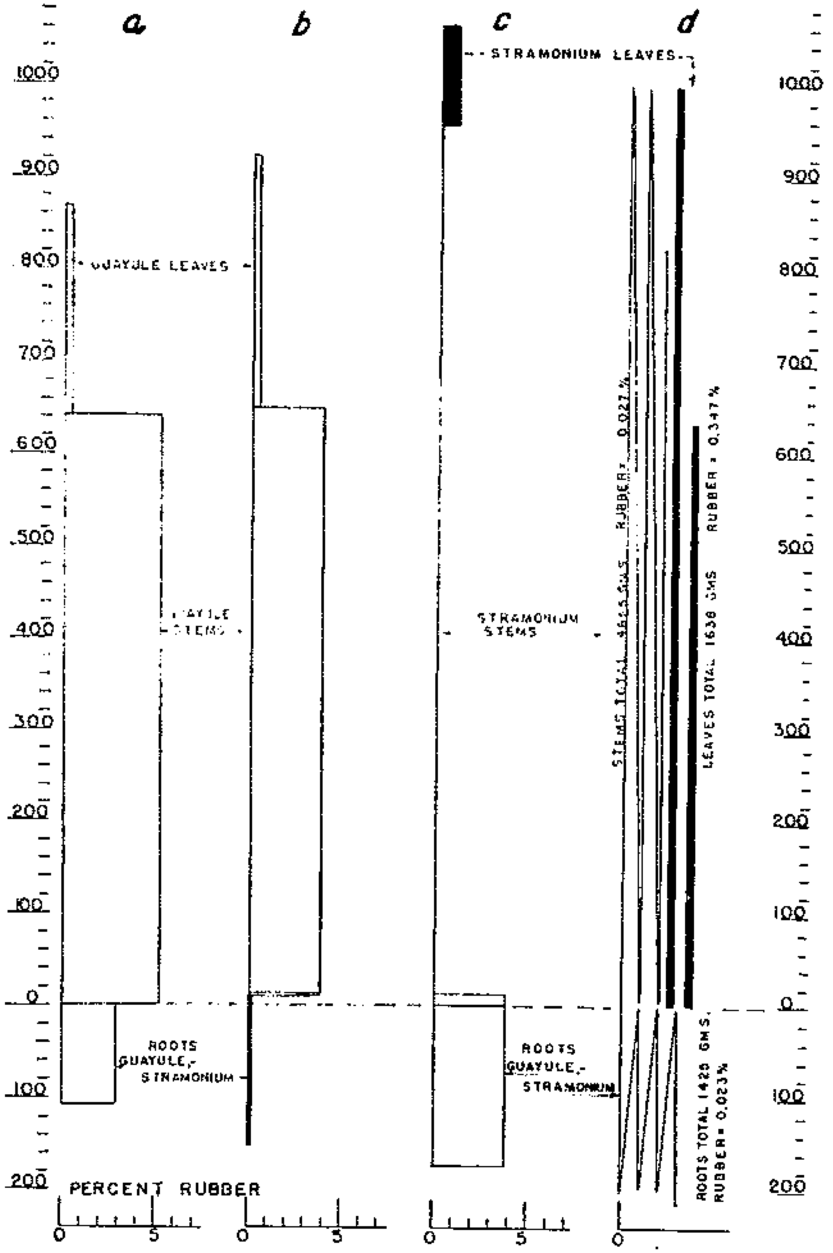

FIGURE 17.—Distribution of dry weights and rubber percentages in seedling grafts of guayule and *Parthenium stramonium*, grown in the field until about 21 months after grafting: *a*, Ungrafted guayule; *b*, guayule on *P. stramonium*; *c*, *P. stramonium* on guayule; and *d*, ungrafted *P. stramonium*.

roots of the Jerusalem-artichoke contained traces of materials that brominated as rubber and ranged from 0 to 0.04 percent. The percentages of such rubber in the leaves ranged from 0.52 to 1.10, which is considerably more than the 0.18 to 0.24 percent found in the leaves of guayule.

Rubber content of the guayule-scion stems ranged from 4.29 to 6.00 percent and was not significantly different from that of the stems of ungrafted controls. Likewise, the traces of indicated rubber in the roots, rhizomes, and tubers of Jerusalem-artichoke understocks were not of different magnitude than in corresponding parts of ungrafted controls.

GUAYULE AND SUNFLOWER GRAFTS

Reciprocal grafts of guayule and sunflower (*Helianthus annuus*) of several horticultural varieties were made and the plants harvested for analyses at various times, from 3 to 14 months, after grafting. Owing to difficulties of survival in some combinations, as discussed on pages 17 to 21 of this bulletin, only a limited number of grafted plants was available, and the rubber percentages shown in table 3 are the means of three to five plants per treatment in some cases and in other cases are single determinations.

TABLE 3. *Rubber percentages in tissues of grafted and ungrafted sunflower and guayule, at several ages*

Plant and age	Ungrafted stems	Grafted plants: Scion		Grafted plants: Understock		Ungrafted roots
		Stems	Burl[1]	Stump	Roots	
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Sunflower:						
3 months		0.03			0	
					0	
4 months	0.037	079	0.255		.089	0.022
		052	.024			
4¾ months[2]		.00	18	0.23	.015	
6 months	.037	.329				.03
	037					.02
14 months					013	
					.086	
Guayule:						
3 months	.10	.06				.06
		075			.308	
4 months	[3]3.23	923			.089	.876
		.918			.506	
4¾ months[2]	[3]3.71	[3]3.07		.71	[3].61	[3]1.97
	2.15					[3]1.90
6 months	[3]1.21	[3]2.04			.388	1.57
14 months	3.27	3.79		...		1.56
	3.13	2.30				1.95

[1] Burl indicates the enlarged burly grained sunflower tissue located immediately above the graft unions.

[2] The 4¾-month harvest was of a different series started later in the season than the others.

[3] Indicates means of from 3 to 5 plants, other items being individual plant values.

The data indicate that in most cases the guayule-scion stems contain about the same percentages of rubber as were found in the stems of ungrafted plants, the percentages of both classes increasing sharply after the first 3 months. Roots of guayule understocks contained lower percentages of rubber than were found in the roots of ungrafted controls. However, guayule seemed incompatible as an understock for sunflower, and the lower percentages of rubber were coincident with very poor growth.

Both the stems and roots of sunflower contained traces of material that brominated as rubber. Concentration of this material increased with plant age and was highest in the burl-like swelling immediately above the unions with guayule. The sunflower understocks survived for 14 months, at which time they contained only 0.04 to 0.09 percent rubber.

Discussion and Conclusions

Arreguin and Bonner (*1*) obtained evidence that rubber accumulation in culture of guayule tissues in vitro occurred more rapidly when the tissues were supplied with leaf extracts from plants presumably accumulating rubber rapidly than when they were supplied from plants accumulating rubber slowly.

Without exception in the present experiments, guayule tissues grafted to other plants contained rubber in the same percentages, and usually in as large amounts, as comparable parts of ungrafted guayule. Also, the rubber content of non-rubber-bearing plants grafted with guayule was, without exception, of the same low order of magnitude as that of the ungrafted controls. Thus, it would seem that the amount of rubber accumulation is primarily a property of the tissues in which the rubber accumulates and not of materials supplied to the tissues. In other words, guayule tissues are able to utilize the products of photosynthesis of leaves of non-rubber-bearing plants and to accumulate as much rubber as though they were being supplied by their own (guayule) leaves. Conversely, non-rubber-bearing plant tissues, even though supplied with the products of photosynthesis from leaves of rubber-bearing plants, are not able to accumulate more rubber than comparable tissues in ungrafted plants.

There are indications in the data that significant changes in the rubber content of tissues of non-rubber-forming plants were associated with proximity to guayule tissues within the graft. While these differences were relatively large, the actual magnitude of the largest values was very small as compared with the percentage changes common to rubber-bearing plants. For this reason, little importance is to be attached to these differences until further experimental work is done.

Traces of rubber were found in all species of plants used. For this reason, the accumulation of considerable quantities of rubber in some species and not in others cannot at this time be ascribed to qualitative differences in the precursors formed in the leaves. The evidence obtained here indicates that the leaves of both classes of plants produce the necessary precursors, but only the stems and roots of the so-called rubber-bearing plants are able to accumulate rubber in considerable percentage. Or, it might be that only the roots and stems of the non-rubber-accumulating plants are able to prevent the accumulation of rubber by the precursors to some compound that is of more value to the plant than rubber seems to be. For this reason, perhaps the distinction should be drawn between rubber-accumulating and non-rubber-accumulating tissues rather than between rubber-forming and non-rubber-forming tissues.

There is the possibility that the rubber found in the rubber-bearing plants is a different polymer from that found in trace amounts in other species. If so, both satisfy the requirements of being soluble in methyl isobutylketone and insoluble in acidulated ethanol; or soluble in benzene and their bromide insoluble in ethanol. No evidence has been obtained that the rubber in guayule is chemically different from that found in distantly related species. However, such a difference could mean that at least the immediate precursors of what we consider rubber are formed only in the tissues of plants that normally accumulate rubber.

Further evidence that rubber accumulation is a function of the tissues in which it occurs rather than of materials supplied from the leaves is found in studies described by Benedict (*2*). By growing guayule plants where leaves were under like conditions, the rubber content of roots at different temperatures varied considerably.

SUMMARY

Reciprocal grafts of guayule (*Parthenium argentatum*) were made with mariola (*P. incanum*), *P. stramonium*, sunflower (*Helianthus annuus*), and the Jerusalem-artichoke (*H. tuberosus*) to learn whether or not guayule rubber is made from specific precursors derived from guayule leaves.

The results indicate that the substances necessary for production of rubber in the stems and roots of guayule are not specific, since guayule understocks growing from infancy with scion tops of other species produced as much rubber as those of ungrafted guayule. Substances received from guayule leaves did not increase the rubber content of understocks of other species.

Theoretical aspects of rubber accumulation in plants are discussed.

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