

FACTORS AFFECTING THE GRAFT UNION OF MAMEY SAPOTE
(CALOCARPUM SAPOTA (JACQ.) MERR.)

By

MARY ANN HOLLINGSWORTH OGDEN

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Dedicated in Loving Memory of

My Mother, Mary Baxley Hollingsworth (1914 to 1982);

My Father, Roger Hollingsworth (1910 to 1983);

George N. Avery (1922 to 1983), botanist, naturalist, friend.

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FACTORS AFFECTING THE GRAFT UNION OF MAMEY SAPOTE
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By

Mary Ann Hollingsworth Ogden

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Mamey sapote is a tropical fruit from the lowlands of Central America grown in southern Florida. Mamey is difficult to graft, and supplies of grafted nursery stock of superior cultivars have limited planting and production. Seedling plants vary in quality and yield and have a long juvenility period. If seedling trees prove to be of inferior quality, topworking to change the cultivar is very difficult. Research was conducted to determine the best propagation methods, grafting period, juvenility, scion selection, interstocks, carbohydrate accumulations, possible

use of growth regulators, apical dominance, silica content, and anatomical characteristics relative to graft union formation.

It was found that grafting periods with warm days, cool nights, low rainfall, and low relative humidity were the best times to graft mamey sapote (late October to early December or late March to early May). Juvenility affected graft take, and scions that were reverted to a "juvenile-like" condition were better scion materials than mature wood. "Juvenile-like" scions with a modified veneer graft on rootstocks less than 1 meter tall were the most successful combination. Anatomical examinations showed an irregular vascular cambium and large bands of fibers surrounding the cambium that may inhibit graft formation unless proper grafting techniques are employed.

Measurements of carbohydrates indicated that starch accumulation enhanced graft take. Silica analysis on 3 scion maturities (seedling, "juvenile-like", and mature) indicated that mature scions with high silica content were the least desirable graftwood and "juvenile-like" scions with lower silica content performed best.

Growth regulator applications were not successful and auxins may inhibit graft take. Terminal bud removal to break apical dominance (and eliminate a source of auxins) enhanced graft take of mature scions during periods of active growth during the summer (the worst time to graft mamey) but did not improve take during the dormant periods.

CHAPTER I

INTRODUCTION

Calocarpum sapota (Jacq.) Merr. is difficult to propagate asexually (1, 68). Asexual reproduction is desirable because the seed do not come true to type and there is much variation in precocity fruit flavor, and yield (1, 10, 94). It is economically essential to asexually propagate nursery stock because of the long juvenility period that can last from 12-15 years (10, 94, 102).

All methods of asexual propagation are extremely difficult. These methods include marcottage, cuttage, and graftage (1). Once the tree has passed its juvenile period it is extremely difficult to marcott (10, 68, 102). Rooting of cuttings is hard at any stage, whether juvenile or adult. Grafting is possible, although it is not easy (68, 94).

The veneer graft is considered to be the best method for Florida conditions and in some of the Central and South American countries. It is possible to use a chip bud or a T-bud technique but the results are erratic (68, 94). Approach grafts have been recommended and were at one time the most common method of grafting in Florida nurseries (68). This technique is tedious and cumbersome, unless the

stock and scion are perfectly matched. The stock plant should be larger in diameter than the scion, or the graft will fail because the rootstock dies from vascular collapse (68, 88).

Once the tree is established and producing in the field, it is rarely possible to top-work if a different cultivar is desired (S. P. Lara, pers. comm.). Methods of top-working need to be developed because many of the trees in south Florida are seedlings and may be unacceptable to the homeowner or for commercial production.

All fruit produced in south Florida are sold locally with none being available for shipment to northern markets (11). Frozen mamey pulp sold in supermarkets is generally imported from Costa Rica and the Dominican Republic. Increased production would make local fruit available for processing.

Knowledge of the factors that affect the formation of the graft union would permit the plant to be more easily propagated and more grafted trees would be available. The factors investigated in this study include effects of grafting seasons, juvenility, topworking with juvenile interstocks, stored carbohydrates (total soluble sugars and starch), sugar pulsing, applications of growth regulators, apical dominance, anatomy (tissue type, silica inclusions, cambium location to determine regularity and anomalous secondary growth), and the formation of the graft union.

CHAPTER II

LITERATURE REVIEW

Origin and Distribution

The mamey sapote is a member of the family Sapotaceae that includes other tropical fruits such as the sapodilla (Manilkara zapota (L.) Royen),, green sapote (Calocarpum viride Pittier), caimito (Chrysophyllum cainito L., and canistel (Pouteria campechiana (H.B.K.) Baehni) (5, 35). Mamey sapote is widely distributed throughout Central America, northern South America, the Caribbean (especially Cuba) and south Florida (10, 33, 90, 94). Many centuries of cultivation have made the origin of this species somewhat obscure, but it is thought to be in the lowlands of Central America, including southern Mexico, Honduras, Guatemala, and Costa Rica (6, 34, 73). Popenoe (97) mentions that during Cortez's infamous march through Central America to the interior of Mexico, his army survived on the fruit of the mamey that was abundant and widely distributed. The trees can be found today growing around the ruins of ancient Mayan cities where they are still a part of the native diet (13, 97).

Taxonomy

There are many other members of the Sapotaceae that are indigenous to the lowland humid tropics of Central and South America. The family is an important part of the ecosystem in the neotropics making up 25% of the standing timber (55). Worldwide there are 70 genera, 800 species, with 3/4 of these species belonging to 6 genera (84).

The Sapotaceae is a well marked and natural family, but the familial floral characters are so uniform that generic identification is extremely difficult. This in turn is responsible for the extensive synonymy characterizing many taxa within the family. (55). Identification within this family is almost impossible without using material consisting of leaves, flowers, fruit, and seed. Unfortunately, species continue to be named on the basis of flowering or fruiting material alone and this continues to add to the already confused state of affairs (55, 56, 57, 58).

Family characters

Common family characters include well developed latex system (that produces chicle in the genus Manilkara); pubescence consisting of 2-armed hairs; mostly trilacunar nodes; vessels with simple perforations; silica bodies; calcium oxalate crystals; imperforate tracheary elements with mostly simple pits; wood parenchyma generally abundant and apotracheal, either in well defined ribbons or

reticulate, seldom diffuse or vasicentric. Leaves, alternate or seldom opposite, simple, mostly entire, occasionally with stipules. Flowers mostly small, mostly borne singly or in cymose clusters, nearly always perfect, sepals distinct, and imbricate or sometimes in 2 cycles; corolla usually sympetalous; carpels 2-14, ovules 1 per carpel, axile or axile-based placentation; fruit fleshy, indehiscent; seed large, commonly with a relatively large, excavated scar of attachment, seed coat shiny and usually thick and hard; embryo large, with thin, flat cotyledons, enclosed in a more or less well developed endosperm or with thick cotyledons and without endosperm at maturity (23, 24, 73, 99).

The mamey sapote (Calocarpum sapota) grows to a height of 30-35 m in the tropics and 15 m in Florida (12, 94) and may have large buttresses. Trunk diameter can be 80-100 cm (6, 23, 24, 99, 113). Leaves are broadly oblanceolate or narrowly obovate mostly abruptly acuminate, about 10-30 cm long, 4.5-15 cm wide, glabrous above with some appressed or loose hairs along the midrib and main veins beneath; primary lateral veins about 20-50 pair; secondary lateral veins regular, more or less perpendicular to the primary one, petioles subglabrous to densely pubescent or tomentose, 4 cm long or less. Flowers are clustered at defoliated nodes, cauliflorous on upper trunk and main branches, subsessile, or on short pedicels as much as 4 mm long; sepals 8-12, spirally arranged, increasing in size

centripetally; corolla 9-19 mm long, white, lobes about equal or a little exceeding the tube; filaments attached at or a little below the sinuses, 3-5 mm long; staminodes filiform, about 2 mm long; ovary 5-loculate. Fruit are brown (scurfy), ellipsoid to subglobose, about 8-20 mm long; pulp smooth to fibrous generally salmon colored to red and sweet; seed 1-4, commonly solitary, large with broad ovate scar (1, 24, 35, 99).

Separation of Pouteria and Calocarpum

On a generic level there are similar characters that link the genera Calocarpum and Pouteria and they are difficult to distinguish on a morphological level. Calocarpum has more numerous sepals arranged in an evident spiral. The woods are easily distinguished anatomically. Calocarpum has reticulate parenchyma with the corners rounded and showing more or less distinctive intercellular spaces or closely spaced uniseriate bands between the wood rays, rays 1-4 seriate, radially aligned pores, and very rarely to lacking tracheids. Pouteria has banded parenchyma, 1-2(3) seriate rays, and pores in diffuse arrangement, and vascular tracheids very common.

Synonymy

The scientific name of the mamey sapote has undergone many changes since it was first described in 1703 by Plumier (19). Sloan (19) described the mamey sapote in 1725 with a non-Linnean name Malus persica maxima foliis magnis.

Jacquin (19) placed the mamey in the genus Sideroxylum in 1760 with the first binomial description. Confusion of the synonymy dates back to the second edition of Linnaeus' Species plantarum that based the Linnaean name upon the type species of Plumier's sapota (96). Since Plumier had traveled in the West Indies he was familiar with both the mamey and its relative the sapodilla. The confusion exists because the drawings by Plumier combined characters of the 2 fruits. The nomenclature was based on the drawings and on a herbarium sheet that contained the 2 different genera for the type specimens (19, 96). A survey of the synonymy dating from the first binomial names includes:

- Sideroxylum sapota Jacq. 1760. (19, 23, 73, 95)
Achras mammosa (Jacq.) L. 1762. (19, 23, 34, 113)
Achras zapota var. major Jacq. 1763. (19, 73)
Sapota mammosa (Jacq.) Mill. 1768. (19, 34, 73)
Lucuma mammosum (Jacq.) Gaertn. f. 1807. (35, 113)
Lucuma mammosa (Jacq.) DC. 1884. (19, 34, 69, 73)
Vitellaria mammosa (Jacq.) Radlk. 1882. (19, 35, 34)
Calospermum mammosum (Jacq.) Pierre 1890. (19, 35)
Calocarpum mammosum (Jacq.) Pierre 1904. (19, 35)
Achradelpha mammosa (Jacq.) Cook 1913. (19, 35, 113)
Calocarpum sapota (Jacq.) Merr. 1923. (73, 99, 95)
Pouteria mammosa (Jacq.) Cronquist 1946. (35, 55, 73)
Pouteria sapota (Jacq.) Moore and Stearn 1967. (95)

Pouteria mammosa (Jacq.) Cronquist by Kukachka 1978.

(55)

Pittier (96), Merrill (23), Baehni (4), and Lundell (63) considered the logical name for the mamey sapote as Calocarpum. Moore and Stearn (73) used Pouteria but said if the genus is considered in a narrow sense that Calocarpum could be retained. Using the anatomical characters of Kukachka (55) the genus should be Calocarpum over Pouteria (used by Cronquist (24) with a few morphological characters). C. sapota should be used instead of C. mammosum because Jacquin's name Sideroxylum sapota in 1760 is anterior to Miller's name Sapota mammosum in 1762. Calocarpum sapota (Jacq.) Merr. will be used for the remainder of this dissertation.

Common names

Common names for C. sapota are just as confusing as the lineage of the scientific names. Sapota is a derivation from either the Aztec (1, 19) or Nahuatl (35, 96) Indian word, tzapotl, a general name for all sweet fruit (19). Passing into the Spanish language, the "t" was dropped and tzapotl became zapote. When transferred to the English language "s" is substituted for "z" and becomes sapote (19).

Confusion of the common name sapote can arise because fruits other than C. sapota are called sapote, because of the usage of sapote to mean sweet fruit. Other fruits called sapote include, white sapote (Casimiroa edulis Llave) in the Rutaceae; black sapote (Diospyros digyna Jacq.) in

the Ebenaceae; green sapote (C. viride Pittier); yellow sapote (P. campechiana (H.B.K.) Baehni) in the Sapotaceae and (Casimiroa tetrameria Millsp.) in the Rutaceae (5, 35).

Other common names are numerous, and in the British and French West Indies, Cuba, Colombia, Venezuela, Panama, Costa Rica, southern Mexico (including the Yucatan peninsula) there may be several common names per country. A listing of the common names cited by 2 or more authors include: atzapotiquahuitl (35, 99, 113); Chac1 haaz (35, 99); grosse sapote (1, 35, 99); mamee sapote (1, 35, 99); mamey (1, 6, 34, 36, 69); mamey colorado (1, 6, 10, 12, 34, 36, 69); mamey sapote (1, 13, 34, 35, 86, 94); mamey zapote (1, 35); marmalade fruit (1, 35, 99); marmalade plum (1, 34, 123); tenzontzapotl (2, 32, 35, 36, 69, 96); tzapotl (23, 69, 96, 99); tsakas sabibu (35, 99); sapote (6, 10, 23, 34,); sapotier (35, 99); zapote colorado (34, 36); and zapote mamey (6, 34, 36, 69).

Culture and Propagation

The mamey sapote is best adapted to the lowland humid tropics (6, 34, 73). The mamey commonly grows at 600 m in altitude, less commonly at 900 m, and very rarely at 1200 m (113). The green sapote (considered by some authors to be the same species as the mamey) grows best at higher elevations around 1200 m (5, 35). Low temperature limits mamey distribution both in altitude and latitude (10,90). In Florida, -3.8 C is the lower limit for mamey (10).

Mamey sapote is tolerant of many soil types, including the heavy clays of Puerto Rico, the sandy clays of Guatemala, and the limestone and sands of southern Florida (1, 10). The tree is not tolerant of flooding so good drainage is essential (1, 10).

Cultural practices vary from country to country. Most of the trees that produce fruit for the market are dooryard rather than plantation crops (90), because the majority of trees are propagated by seed rather than grafting (91,94). Lack of knowledge of vegetative propagation of the mamey sapote has limited its culture (1).

Seedling mamey trees have a long juvenility period compared to grafted trees and this has fostered the myth that all mamey trees take a long time to bear (1). Seedling trees vary in size, fruit quality, yield and precocity (1, 10, 94).

Cultivation in Florida

In Florida, the mamey sapote has been cultivated as a dooryard fruit and in experimental plantings since the mid-1800's (13). It grows well in Florida and has relatives in the native flora (86). In 1970 Campbell (11) reported few small orchards of mamey sapote. By 1982 there were at least a total of 150 acres planted in Dade County, Florida, with thousands of trees in dooryard plantings.

There were no vegetatively propagated trees in Florida until the 1950s because of propagation difficulties (13). Previously, nursery stock was limited by a lack of

propagating materials (both seed for stocks and trees of superior cultivars for scionwood) and persons with experience in grafting mamey (91). Many Florida nurseries now regularly propagate the mamey sapote (13). There is much interest and demand for the fruit and trees, because of the large Latin population settled in Florida who hold the mamey in high esteem, and who are familiar with the mamey in their native countries (11).

Superior cultivars that are propagated in Florida include 'Magana', 'Copan', 'Pantin' or 'Key West', 'Tazumal', 'Chenox', 'Progresso', 'Francisco Fernandez', and 'Cuban no. 1'. The cultivars 'Copan', 'Tazumal', 'Mayapan' (or 'No. 2'), and 'Pantin' (or 'Key West') were developed in Florida (13).

Propagation of tropical fruit in the Tropics

Seasonal climatic variation in the tropics has much less effect upon graft take than in the temperate zone (92). Such variation is largely a matter of dry or wet conditions, and this can be largely controlled by irrigation. Most tropical species can be grafted most of the year. Major factors that inhibit propagation are flowering, leaf abscission, and growth suspension. Coordinating harvest season and seed extraction for planting rootstocks so as to have the rootstocks of proper maturity and grafting size is also a limiting factor. According to Pennock (92), moderate rainfall for field grafting is also desirable. Other factors that affect grafting of tropical plants are phenolic

substances and latex released by wounding, poor stock and scion selection, and tissue desiccation.

Some grafting techniques are recommended over others for better and faster healing in fruit trees. In general, Skene et al.(109) recommends a chip bud over a T-bud for fruit trees because the xylem develops more rapidly.

Propagation of mamey sapote

Unlike many tropical fruits, mamey is difficult to graft and may be grafted only at certain times of the year. Most members of the Sapotaceae are difficult to graft, although some species are easier to graft than others (67, 68). An example of ranking in increasing order of grafting difficulty is 1) sapodilla , 2) canistel, 3) caimito and mamey sapote (C. W. Campbell and S. P. Lara, pers. comm.).

Numerous authors report that the mamey sapote is difficult to propagate (1, 10, 74, 88, 91, 98). One reason frequently cited for difficulty is that the latex exudate released from wounding interferes with graft take (a successful graft that has healed and sprouted (92, 98, 102). There are other authors who feel that latex does not interfere with graft take (68, 91). Lack of sufficient callus production is another problem in the formation of the graft union (1, 68).

Many methods have been reported for both sexual and asexual propagation, with the most common being seedage (1, 36, 74, 89, 91, 94, 98). Seed for either seedling trees or

rootstocks should be removed immediately from the fruit and planted quickly as they remain viable for only a short period (34, 74).

Asexual propagation methods that have been reported for mamey sapote include cuttage, marcottage, and graftage. Tissue culture has been unsuccessful due to a lack of callus initiation (R. Litz, pers. comm.). Insufficient callusing has also been reported for cuttage (1). Marcottage is possible (74, 122, 123) with generally poor success (1, 10). The best time to marcott is in the winter (123).

Graftage

Graftage has been the most successful method of asexual propagation for mamey. Grafting also shortens the juvenile period of 5-15 years (10, 61, 123) by almost half (10, 94). The types of graft reported for propagating the mamey are: patch (1); forkert (modified chip) (1); splice (60); and cleft, under both field conditions (59) and under mist (90). In many countries the approach graft is utilized but it is tedious, cumbersome, and expensive (45, 60, 68, 88, 94). One particular problem with approach grafting is that the scion diameter must not be equal to or larger than the rootstock diameter, or the scion will outgrow the rootstock (87, 88). Scion overgrowth of the rootstock causes the scion to transpire too rapidly and this results in vascular collapse of the rootstock and death of the scion (68, 88). A chip bud may be used during certain times of the year, but this method is not always dependable (10, 94).

Veneer graft

The veneer graft is the method of choice for grafting mamey under Florida conditions (10, 88, 90), and other countries as well (1, 34, 98). Veneer grafts are also used on other species of the Sapotaceae (12, 88, 102) and in other tropical families as well, for example mango (Mangifera indica L.) (45, 48, 49) and guava (Psidium guajava) (83).

Hussain and Buhariu (45) mention that the veneer graft is more successful for sapodilla because the vegetative top of the stock plant is not lost at the time of grafting and this supports the growth in the cambial layers of the graft union. Height of the graft on the rootstock can make a difference in mamey graft take when using a veneer graft (98).

Mamey sapotes are possible topwork but have low rate of success (S.P. Lara, pers. comm). The new new sprouts are veneer grafted much in the manner of topworking lychees (Litchi chinensis Sonn) (82).

A modified veneer graft used on annonas (Annona sp.) works well on the mamey sapote (89). This method is different from the standard veneer graft in that a shallow cut is made on the rootstock for the length of the scion to be grafted. The budstick is trimmed and the vertical cut removes only 1/8 of the diameter of the graftwood as compared to removal of greater than 1/2 of the budstick diameter in the standard veneer graft. This method

conserves more stored carbohydrates and moisture in the scion, which is beneficial, since the graft union formation takes a long time, 2 months or sometimes longer, in the mamey sapote (86, 90). The cinchona method is another type of modified veneer graft and is used extensively in southern Florida. This method does not use as shallow of a cut on the rootstock (20).

Scionwood that is selected must be from terminal shoots that are relatively short without fully expanded leaves (10). If the wood is too hard or too gelatinous, it is not suitable for grafting (90). Scionwood should be collected and used the same day and not stored overnight for the best grafting success (90).

Scionwood preparation

If the scionwood is not sufficiently mature or dormant, it can be prepared ahead of time. This has been done on annonas and guavas. The terminal buds are cut off on annonas and the lateral buds are subsequently able to swell and expand when the apical dominance is removed resulting in the proper stage for scionwood collection (89). Guavas are cut back severely, and the resulting sprouts when hardened off are used for scionwood (66, 81, 83).

Maturity of the scionwood makes a difference in grafting success for other species. Mango scionwood must be from mature branches (49, 64, 65, 75), while the immature scionwood is more successful in guava (81, 83).

Nelson (83) described the pruning back of guava from older parent trees and the resulting flushes as juvenile wood. This wood has larger numbers of scions with more desirable buds. Younger parent trees produce this type of flush without this procedure. The forcing of this "juvenile-like" growth furnishes a larger diameter stock of scionwood containing more scions than can normally be found on an older parent trees.

A definition of juvenility is considered by Westwood (121) and Zimmerman (126). Seedlings of most woody plants pass through a juvenile stage during which the seedling cannot be induced to flower. Actual production of flowers is the first sign of the adult phase. The end of the juvenile phase and the first appearance of flowers may not coincide. This intervening period can be referred to as the transition phase. Once the plant has reached the adult phase it cannot revert back to the juvenile stage. There are transitional tissues that are found between the purely juvenile and adult sections of the stems of seedling trees.

Rejuvenation may be obtained by differentiation of adventitious buds on the stems or the roots. This is because the juvenile tissue remains in situ at the base of the seedling tree. Heavy pruning of old, weak orchard trees is thought by some to induce juvenility. Such pruning brings about invigoration, not juvenility. The non-flowering phase of budded trees is referred to as the vegetative adult phase (121).

By definition, the tree cannot be reverted to a juvenile phase when pruned severely, even if the tree exhibits morphology of the juvenile stage, such as described in the work of Nelson and Lynch (81, 83, 66). This would be invigorating the trees and reverting them back to the vegetative adult transition phase as described for newly budded trees. This tissue with the juvenile morphology, that has been reverted from an adult phase to vegetative adult transition phase by severe pruning, will be referred to as 'juvenile-like' hereafter in this dissertation.

Another type of in-situ scionwood preparation is girdling, and it is employed with several species that are difficult to graft. Girdling is routinely done on macadamia (Macadamia integrifolia Maiden) as preparation for cuttage and graftage (22, 46, 51, 84). Girdled lychee branches show a 28% increase in carbohydrates and little nitrogen above the girdle (51). Using the prepared scions that are high in starch content, the graft take increased from 10% to 75- 80% (51).

Some other members of the Sapotaceae have shown a response to girdling. Canistel had an increase in graft take and an increase in carbohydrate accumulation (30). Sapodilla when girdled, showed an increase in carbohydrates but only in seasons other than spring was there an increase in graft take (30). The green sapote showed an increase in graft take after girdling (116). There have been tests with the mamey sapote and girdling does increase the graft take (117, 123) but it may be only as much as 5% (68).

Rootstocks

Rootstocks must be in good condition for optimum grafting. This is particularly true for the avocado (Persea americana) (41), mango (65) and sapodilla (87, 88). Stocks for the mamey sapote should have a height of 1 m or less, or approximately 1-1/2 years-old, under Florida conditions. Scion diameters should be well matched with that of the rootstock (60, 68, 88).

Other species have been tested for compatibility with mamey sapote. Canistel makes a graft union when used as a rootstock, but not as well as mamey itself (88). Sapodilla used as a rootstock does not form a union at all (38).

Growth regulators

Applications of growth regulators to the graft wound have been used on mamey sapote with little success (68). This is a general practice in some Florida nurseries (S. P. Lara, pers. comm.) but there have been no documented results. Kester (53) mentions that auxins, alone and in combination with adenine, stimulated callus. He maintains that factors such as time of year, scionwood condition, and correct technique are of greater importance than supplying external growth regulators.

Apical dominance

Apical dominance has been mentioned as a factor in grafting of several species in various ways, and it is usually mediated by auxins, although there may be growth

regulators involved other than auxins (93). Apical dominance can be transmitted through experimental grafts (25) and this may be part of the downward transport of auxins (32). Axillary bud break is suppressed by apical dominance, and when the dominance is broken, lateral buds can then expand and grow (93, 124). Axillary buds are inhibited by auxins and this inhibition can be released by kinetins (110).

Placing rose (Rosa sp.) stems horizontally caused bud break along the stem of all upward oriented buds and inhibition of the downward oriented buds. Upper buds that were uninhibited, were grafted on the basal part of the stem and then inhibited; basal buds that were budded on the upper part of the stem were still inhibited (124). Auxins tend to collect at the basal portion of the stem (125).

Cutter and Chiu (25) mention that in grafting a Hygrophila species, when buds were of unequal size at a node, the larger one predominated. When the nodes were split and grafted together again, the larger one grew out. When a foil block was placed across the node and the node was grafted, both buds subsequently grew out. When the terminal bud is removed from annona (Annona sp.) shoots, the lateral buds swell after the dominance of the terminal bud is broken (89). Bud dominance (dominance by buds over those lower on a stem) resembles apical dominance and is probably mediated by hormonal means (25).

Seasonal and environmental effect upon graft take

The environment affects graft take (53, 92). The mamey sapote responds to diurnal changes and levels of relative humidity (86). Warm days and cool nights, with relatively low humidity, are the best conditions for grafting mamey sapote (86). It has been found over a period of 5 years that the best grafting times in Florida for the mamey sapote are in the spring (March, April, and May) and in the fall (late October, November, and December). Times may vary according to when warm weather begins in the spring and cooler weather begins in the fall; this is variable in Florida (86, 88, 91). In Mexico, the mamey sapote can be grafted best in February and April, when the trees are defoliated (98).

The environment also affects mango grafting (49). In India, optimum grafting conditions are usually high humidity, with low night temperatures and medium day temperatures.

Anatomy and Graft Union Formation

There are several anatomical features that are characteristic of the Sapotaceae. These include presence of a white milky latex contained in laticifers, large amounts of silica in most genera, and scleriform pitting in the vessel members with simple perforation plates (24, 52, 77, 99). The laticifers in the Sapotaceae are articulated and anastomosing (77). Locations of the laticifers are in the

leaves, bark, and pith (24), in conjunction with phloem fibers, and oriented at random (28, 77). The cells of the laticifers are joined at pit connections at the center of the transverse wall and can be identified by the absence of a central vacuole in the protoplasm (104). In the genus Manilkara, the copious amounts of latex are tapped for the production of chicle (23).

Wood anatomy relative to graft take

General wood anatomy of the Sapotaceae has been described by Kukachka in a series of 33 papers on the wood anatomy of the neotropical Sapotaceae. In particular the papers on the 4 economically important genera (Manilkara, Pouteria, Calocarpum, and Chrysophyllum) contain details for comparison of the wood anatomy that may give insight on the difficulty of grafting within these genera. These details include: vessel member length, vessel diameter, fiber length, pit diameter, ray height, presence of tracheids, parenchyma type, and parenchyma location. The comparisons and measurements are strictly for the secondary xylem and are listed in Table 2-1 (55, 56, 57, 58).

The genera that are easier to graft (Manilkara, and Pouteria) have tracheids, contain little to no silica, and have considerably more parenchyma in the rays and the secondary xylem. The genera that are more difficult to graft contain a great deal of silica, rarely if ever tracheids, very few if any bands of parenchyma in the secondary xylem and little parenchyma in the rays (55, 56, 57, 58).

Guridi-Gomez (40) describes the anatomy of Calocarpum as having simple pits in gelatinous fibers and the lumens of these fibers as being small (these are located in the cortex, xylem, and pith). Some of these simple pits are only 4 in diameter. The reticulated parenchyma is diffused and the rays are only 1-3 cells wide and 13-53 cells high (40, 99). Barajas (6) describes the gelatinous fibers as being 2040 long, 14 diameter with a wall thickness of 9 .

Pouteria and Synsepalum also have gelatinous fibers (3, 40). Vessel diameter in Pouteria is 226 with pit diameters of 2.5-5.0 There are distinct bands of parenchyma in the secondary xylem. The rays are 164-2500 high and 30 cells wide (6). This could be a factor in graft take since some parenchyma dedifferentiation to callus comes from the medullary rays (9, 28, 112).

Water conduction to facilitate graft take.

Lin (62) describes an unnamed species of Pouteria using factors that identify the wood as being of poor quality for use as a timber species. The inter-vessel pit diameters are very small with slit-like apertures and the vessel diameters are also small. There is a high percent of fibers with thick walls and irregular distribution of relatively small pits, not especially adapted for liquid conduction because flow movement between fibers is dependent primarily on simple pits (62). Table 1 illustrates that most members of the Sapotaceae have relatively similar sized pit openings

(55, 56, 57, 58). It is important in the formation of the graft union to have water conduction, as the primary need of the scion after budding is sufficient water supply, provided first by the callus bridge and later by the differentiation of the tracheid strands in the callus (71).

Silica content

Silica is one of the common characters of most genera of the Sapotaceae (55, 56, 57, 58). There are 32 families, 90 genera, and 300 species of tropical trees known to contain silica deposits (114).

Crystals of silica may be considered a taxonomic diagnostic tool on the familial, generic, and specific level (2, 16, 17, 70, 100, 106, 114, 115). Species with large amounts of silica often yield poor timber because they are so hard and tend to dull saws and other equipment (2, 6, 115). Timber may be soaked in water for a long period before an attempt is made to mill the logs. This same timber, because of its hardness, is almost impervious to attacks of marine boring worms (2, 115). The hardness of the wood due to silica also dulls grafting knives and in turn the jagged cuts will not heal as well as smooth cuts.

Silica crystals are present in vertical or ray parenchyma of many tropical families (16). In the Lauraceae, silica crystals may be in the axile or ray parenchyma and fibers and tyloses (100). Palms (Arecaceae) contain silica in many species (118). Silica crystals are found in the cells of the hypodermis and epidermis of

leaves, and as incrustations of lumens and lignified fibers in certain palms (118).

Several families that contain fruit and nut bearing trees contain silica and some of these are difficult to graft. These families include: Lethycidaceae (Brazil nut, Bertholletia, and paradise nut, Lecythis); Proteaceae, (Macadamia); Juglandaceae (Juglans); Sapotaceae (all genera but Manilkara, Dipholis, and Mastichodendron) (55, 56, 57, 58, 115).

Chattaway (16) notes that the presence of crystals in enlarged cells of idioblasts causes changes in cell walls and the walls become sclerosed. Silica, as opposed to calcium salts, is located not only in the cell lumen where it fills an entire cell, but as deposits in the cell wall, and lining the cell walls of the xylem (47). Silica may also be found in the form of silica sand either alone, or mixed with starch (106).

Differences in juvenile and mature stem anatomy

There are differences in the anatomy of the juvenile and mature stem anatomy in the Sapotaceae. Juvenile stems of the miracle fruit (Synsepalum dulcificum) contain 1-3 layers of fibers in the phloem and these fiber layers are traversed by parenchyma. The pith contains taniferous materials and many cells filled with starch grains. A few crystals are present in the cortex and pith (3). Mature wood in the miracle fruit has tyloses commonly present in the xylem, and thick walled vessels with sclerotic

tendencies. Many gelatinous fibers are present and they are thick walled with small lumina. Rays are exclusively uniseriate and many cells of the rays are filled with silica (3).

Juvenile wood is also different from mature wood in the Sapindaceae. Juvenile lychee stems have a continuous ring of sclerenchyma in the pericycle. In mature wood this band of sclerenchyma is interspersed with sclereids between the fiber bands. The mature wood is compact and of great hardness and the plant is difficult to graft (18, 119).

Anomalous secondary growth

One of the grafting problems related to the anatomy of the lychee is that the vascular cambium has anomalous secondary growth (119). This cambium is actively growing at different points along the continuous cambial ring during different times of the year. It is hard to determine, when selecting a twig for scionwood, where the actively growing points are located within the vascular cylinder (119).

Fiber band location

Vascular groups in the xylem of the lychee are interspersed with a particular type of parenchyma that has a general appearance of tracheids in cross section. As the stem diameter increases, the parenchymatous cells between vascular bundles quickly differentiate into sclereids and the rays remain only one cell wide. There is a continuous ring of sclereids within the xylem. Scattered parenchyma

cells differentiate to the inside of the sclerenchymatous ring of pericycle at the same time (119). Roth (101) describes the anatomy of the Mimosaceae as having numerous fiber rings that are similar to the fiber distribution of the Sapotaceae.

Fibers do not dedifferentiate readily (28), and cell dedifferentiation is necessary for both differentiation of root initials and and callus differentiation for graft unions. Gomez (37) describes the difficulty of rooting within the 3 races of avocados. The West Indian race has thick, frequent fiber bands near the cambium where roots tend to arise and this sclerenchyma ring acts as a barrier to rooting. The Mexican race is easier to root and has fewer fibers. The Guatemalan race is intermediate in the numbers of fibers and relative ease of rooting. Mamey is also difficult to root and has many fibers in the cortex and in the xylem (1, 6, 40).

Wound healing and graft union formation

Graft union formation and wound healing is related to the anatomy of the plants. Differentiation is preceded by a proliferation of parenchyma tissues (28). Collenchyma will dedifferentiate, and only very rarely will sclerenchyma dedifferentiate (28, 31). Callus or wound tissue is produced by recent cambial derivatives, parenchyma within phloem and xylem rays (9, 104). Callus may develop from the medullary rays in some plants. In general, it is possible to derive callus from any meristematic tissue arising in the

pith, wood rays, recent secondary xylem, phloem, and cortex (9, 11, 111).

The vascular cambium within the graft union is formed by the mixed callus of the stock and scion. Vascular elements within the mixed callus may develop before the vascular cambium (28, 53).

Healing is related to tree vigor, age of the plant, seasonal variation, and size of the wound (9, 79, 80). Other factors in graft wound healing besides the environment and vigor would be the influence of the stock and scion (92, 112). Auxins may play a role in differentiation of the new xylem (120).

There are differing rates in graft union formation. The healing stages proceed as follows: formation of an isolation layer, an initial contact layer, a callus bridge, vascular tissue, vascular cambium, lignification, and a graft union (20, 92). Duration of these stages in the graft union development vary according to species. Douglas fir (Pseudotsuga) usually has initial callus formed at 10-14 days, cambium in 10-17 days, and a union around 35 days with good conditions (21). Juniper (Juniperus) forms callus in 10-20 days, and the graft union is completed around 60 days (29).

Citrus (Citrus sp.) appears to be faster in the initial callus production than gymnosperms. Mendel (71) noted callus initiation after 24 hours, and thick callus layers at 10 days was noted by Nanthachai (78), with the vascular

cambium is forming in 7-45 days (78). Xylem in citrus differentiates before the phloem and the cambium (72, 78). Callus may initiate from any of the outer layers of wood in citrus but not from the inner layers (71).

Avocado grafts had callus initiation in 5 days and a callus bridge by 11 days. This callus is derived from the inner cortex. By the 9th day, new vascular elements appear and a new cambium is forming. In 15 days the terminal bud swells and the scion begins to grow (41).

Mango grafts have a pre-callus stage of 4 days with obvious callus at 8 days and a callus bridge at 12 days. The cambium develops in 38-48 days and is completely healed in 6-8 months (112).

Little is known about the graft formation of the mamey sapote. Healing is faster using a veneer graft (2 months) than an approach graft (2-3 months) (88). During the summer months the wounds of the mamey sapote heal slowly. When the plants are placed in a cooled greenhouse, they heal more rapidly than in a slathouse where temperature cannot be controlled (88).

Table 2-1: Anatomical Characteristics in the Sapotaceae, Manilkara, Pouteria, Calocarpum, and Chrysophyllum (55,56,57,58).

Charc. ^z	Manilkara	Pouteria	Calocarpum	Chrysophyllum
VML	640	396	800	700
FL	1.49 mm	1.30 mm	1.85 mm	1.64 mm
VDi	117	84.3	165	197
RHi	244	500	590	395
Pits	6-8	6	6-8	8-10
Si%	0.0%	0-.25%	2.20% ^y	.25-1.13%
SG	1.05	0.62	0.76	0.88
Tr	common	present	v.rare	absent
Par	diffuse	diffuse	radial	diffuse
Par-R	present	absent	present	present
AxPar	banded	banded	rare	zones free

^z

Charc. (Anatomical characteristics)
 VML (Vessel member length)
 FL (Fiber length)
 VDi (Vessel diameter)
 RHi (Ray height)
 Pits (Pit diameter in vessel members)
 Si% (Percent silica in secondary Wood)
 SG (Specific gravity)
 Tr (Trachieds)
 Par (Parenchyma type in secondary xylem)
 Par-r (Contains reticulated parenchyma)
 AxPar (Axile parenchyma, banded, rarely banded,
 and parenchyma free zones)

^y

Kukachka (55) found 2.02% but Barajas and Echenique (6) reported 3-5%. (analysis of the secondary xylem)

CHAPTER III

JUVENILITY AND SEASONAL EFFECTS

Introduction

Summer, Fall, Winter, and Spring are somewhat nebulous time periods for determining grafting conditions in a subtropical climate (88, 91, 92). Several time periods for optimizing grafting conditions for mamey sapote have been determined using data from local nurseries in southern Florida. Preferred grafting periods are late October through early December and late February through early April (both periods have warm days and cool nights and low relative humidity and little rainfall). May through early October (hot days and nights, and high humidity) and late December through early February (cool days and nights with occasional frost) are the least desirable times. Experiments were conducted to determine the optimum season for grafting mamey sapote and determination of the effect of scion maturation or juvenility upon graft take.

Materials and Methods

The general time periods described by Ogden and Campbell (91) and Campbell (88) for the optimum and least desirable time periods were followed. Precise dates were chosen when the rootstocks were ready and the graftwood had sufficiently matured. Dates selected for grafting in 1982 were 13 December, and for 1983, 23 February, 6 April, 12 August, and 15 November.

Scionwood was selected on the basis of tissue maturation. The scion types were, mature (from flowering and fruiting trees), "juvenile-like" (from grafted trees reverted to a vegetative adult phase by pruning), and seedling tops (truly juvenile, collected from nursery stock plants).

Modified veneer grafts were used on seedling rootstocks as described by Ogden et al. (90), except that on the scion the shallow cut did not extend down to the vascular cambium and was well within the cortex, and the wound was wrapped with clear polyethylene tape. A shallow cut was likewise made on the rootstock that is $3/4$ to 1 m high or around 1 to 1-1/2 years old. All stocks and scions were chosen randomly from groups of plants similar in size and age. Replication numbers were according to the numbers of available rootstocks that were sufficiently mature and availability of suitable scionwood.

Results and Discussion

Grafting results are listed in Table 3-1 by scion maturity and month grafted. Mature tissue was always lower in graft take than either seedling or "juvenile-like" tissue. Mature scions had a graft take ranging from 0.0-47% with the average for the year of 20.9%. The seedling tops and "juvenile-like" scions had a high range of 80% graft take.

Seedling tops had more consistent graft with a range in graft take from 52%, 60%, 68%, 66%, and 80 % and a the average for the year of 65.2%. "Juvenile-like" scions had more variation in graft take than the seedling tops or the mature scions and the range was 36%, 80%, 72%, 26%, and 80% with an average for the year of 58.8%. Mature scions are the least desirable scions to use at any time of the year. Both "juvenile-like" scions and seedling scions are good graftwood but since the "juvenile-like" scions are clonally propagated, they would be the graftwood of choice.

Juvenility is a factor in the graft take of many scions since the scions that are morphologically juvenile consistently have better graft take than the mature scions. The best grafting period would be during the spring months of February and April and the worst times of the year would be in the summer month of July and the followed by the cool months when the plants are dormant in November and December.

TABLE 3-1: JUVENILITY AND SEASONAL EFFECTS

Date	Stage ^z	y	Gfts	%Take
Dec.	mature		25	11
	"juv"		25	36
	seedling		25	52
Feb.	mature		15	47
	"juv"		15	80
	seedling		15	60
April	mature		15	24
	"juv"		15	72
	seedling		15	68
July	mature		15	0
	"juv"		15	26
	seedling		15	66
Nov.	mature		15	20
	"juv"		15	46
	seedling		15	80

^z
"juv" ("juvenile-like")

^y
Gfts (number of grafts per scion maturity)

CHAPTER IV

USE OF JUVENILE INTERSTOCKS TO FACILITATE TOPWORKING

Introduction

Topworking a mature mamey sapote is extremely difficult once the tree has flowered and fruited. It would be advantageous to topwork mature trees if they have fruit that are an undesirable cultivar in quality, yield, or pest and disease resistant. If grafted trees are frozen below the graft union, topworking would put bearing groves back into production.

Experimental objectives were to use seedling tops (truly juvenile material) as interstocks and to facilitate the use of mature scions and "juvenile-like" scions as graftwood.

Materials and Methods

Three large trees of inferior cultivars were cut back to a stump, a height of approximately 1 m in June, 1982. The trunk was painted with white latex paint to prevent sun-scald. Sprouts from the stump were permitted to grow until they obtained a size suitable for grafting and danger of frost had passed.

On 25 March, 1983, 45 sprouts were grafted with seedling tops as scions, using a modified veneer graft and wrapped with polyethylene tape (as described in Chapter III). The seedling tops were collected from plants grown from seed in the nursery for use as rootstocks.

When the seedling scions had sprouted and obtained a stem diameter of 2 to 3 cm, they were grafted with the second graft on 8 August, 1983, using mature graftwood and "juvenile-like" graftwood. Both types of graftwood were from the cultivars 'No. 2' and 'Magana' in replications of 10 each, for a total of 40 grafts.

Results and Discussion

Seedling tops that were used as interstocks were evaluated on 8 March and had a combined graft take of 92% (Table 4-1). The mature and "juvenile-like" scions were evaluated on 13 September, 1983. The ones still alive had sprouted and were growing vigorously. The "juvenile-like" scions had slightly different results: 100% for 'No. 2' and 80% for 'Magana', for an overall average of 90%. Both 'No. 2' and 'Magana' mature scions had 80% graft take. Juvenile (or seedling tops) scions are the method of choice to be used as interstocks for topworking mature mamey trees in the field. Both mature and "juvenile-like" scions can be used even during August, the worst time of the year to graft.

Table 4-1: Juvenile Interstocks for Topworking Mamey Sapote

Date.	Stage ^z	Cult.	Gfts ^y	% Take
March	Seedling	-----	45	92
Aug.	"juv"	'No. 2'	10	100
	"juv"	'Magana'	10	80
	Mature	'No. 2'	10	80
	Mature	'Magana'	10	80

^z
"juv" ("juvenile-like")

^y
Gfts (number of grafts per scion maturity)

CHAPTER V

CARBOHYDRATE ACCUMULATION RELATIVE TO GRAFT TAKE

Introduction

It is a general practice with several plant species to girdle the scionwood some weeks prior to grafting (22, 51). This is done to increase stored carbohydrate accumulations, thereby ensuring ample food reserves for the scion while the graft union is forming (22, 51, 84).

Mamey sapote is girdled by some propagators (117, 123), but the wood is so brittle that the prepared shoots may snap off in gusts of wind. The girdled areas may be braced with wooden splints and wrapped with tape to prevent breakage (C. W. Campbell, pers. comm.). There have been reports that girdling does increase graft take (117) but it may be only as much as 5% (68).

Experimental objectives were to determine if graft take is influenced by a natural rise and fall of carbohydrates, and to determine if levels of total soluble sugars or stored carbohydrates (starch) have effects upon graft take.

Materials and Methods

Samples of graftwood were randomly collected for the carbohydrate analysis at the same time as those in Chapter III for the juvenility experiments. Mature, "juvenile-like", and seedling scions were analyzed. Cultivars used were 'No. 2' and 'Magana'.

All samples were separated according to tissue maturation and analyzed separately. Samples were chopped and mixed. Three replications of 2-gm each were weighed out from each sample. The samples were analyzed using a phenol-sulfuric acid assay to deter total soluble sugars (44).

Samples were ground in a tissue grinder with 10 ml of 100% ethanol, centrifuged, and the supernatant filtered off. The pellet was stored for further analysis of starch and the liquid was analyzed for total soluble sugars. The supernatant was diluted 100 fold with distilled water. An aliquot of 0.5 ml of the sample was put in a test tube with 0.5 ml of 5% phenol and 2.5 ml of concentrated sulfuric acid. An intense reaction took place with the tubes becoming very hot and the sample turning a brown to red color. After the test tubes cooled for 30 minutes, the results were determined colorometrically on a spectrophotometer at 490 nm using glucose standards for reference.

For starch analysis, the stored pellets were incubated in an enzyme solution for 12 hours at 34°C. The enzyme solution consisted of: 40 ml of a 0.04 M phosphate buffer solution pH 6.8 (8), 20 mg alpha amylase, 20 mg amyloglucosidase, and 17.6 mg calcium chloride (44).

The starch is converted to maltose by the alpha amylase, and the maltose is in turn converted to glucose by the amyloglucosidase. After the incubation period, the pellet/enzyme solution is centrifuged until a new pellet is formed. The clear supernatant is poured off and then diluted with distilled water 200 fold. The diluted supernatant was then analyzed as previously described for total soluble sugars.

Results and Discussion

Because of the similarity in concentrations of soluble sugars and starch for the two cultivars, the results were averaged together according to scion maturity and reported as mg/ml glucose. On a monthly basis the results of the total soluble sugars measurements were erratic when compared to graft take percentages (Table 5-1).

On a seasonal basis, correlation coefficients for soluble sugars were positive for the months of December and November, and significant at the 1% level.

For the months of February, April, and July, the results were negatively correlated and not significant (Table 5-2).

Results were compared by scion maturation and seasons for sugar in relation to percentage graft take. Correlation coefficients for the overall comparisons of total soluble sugars relative to scion maturity and grafting season were low and not significant at the 5% level (Table 5-3). These comparisons show that total soluble sugars are not a strong mediating factor in relation to graft take.

Starch accumulation was compared to graft take (Table 5-4). As with sugars, the starch content within cultivars was similar and the results were averaged together and reported as glucose in mg/ml.

A statistical analysis shows that on a seasonal basis, starch content had a positive correlation coefficient. In the months of February and April the correlation coefficients were significant at the 1% level, and December, April, and November were significant at the 5% level. (Table 5-5).

Variability of the graft takes was not explained upon scion maturity alone relative to starch accumulation. In an overall comparison of starch content with seasons and scion maturation, the variability of graft take and maturity of the scionwood had a positive significant correlation coefficient at the 1% level (Table 5-6). This comparison indicates an interaction between seasons and scionwood maturity in starch accumulation.

As the starch content rises within a given season, the scions with the highest starch content had the highest graft take. Starch content was higher in the seasons prior to the beginning of an active growth period (February and April) for the seedling tops and the "juvenile-like" scions. During the periods of active growth (July) and dormancy (November), starch content was lower for mature and "juvenile-like" scions and the graft take was lower.

Starch content, especially in mature scions within each month, appears to have an active role in the percent graft take. Total soluble sugar measurements do not appear to be a reliable indication of the amounts measured relative to percentage graft take (Tables 5-1 and 5-4).

Table 5-1: Carbohydrate Accumulation--Total Soluble Sugars Relative to Graft Take

Date	Stage ^z	Gfts ^y	% Take	mg/ml
Dec.	mature	25	11	13.3
	"juv"	25	36	14.6
	seedling	25	52	16.9
Feb.	mature	15	47	19.7
	"juv"	15	80	18.1
	seedling	15	60	14.1
April	mature	15	24	21.0
	"juv"	15	72	19.6
	seedling	15	68	12.6
July	mature	15	0	19.9
	"juv"	15	26	13.0
	seedling	15	66	27.0
Nov.	mature	15	20	21.3
	"juv"	15	46	22.7
	seedling	15	80	31.0

^z "juv" ("juvenile-like")

^y Gfts (number of grafts per scion maturity)

Table 5-2: Statistical Seasonal and Sugar Comparisons

Date	R value	DF ^z	Sig
Dec.	.910	2	**
Feb.	-.164	2	NS
April	-.040	2	NS
July	-.611	2	NS
Nov.	.951	2	**

^z Significance (** 1%; NS, Non significant)

Table 5-3: Stastical Comparisons of Combined Scion Maturity and Sugar Accumulation

R-value	DF	Sig. ^z
0.297	14	NS

^z Significance (NS, non significant)

Table 5-4: Carbohydrate Accumulation--Stored Starch relative to graft take.

Date	Stage ^z	y Gfts	Take	mg/ml
Dec.	mature	25	11	30.6
	"juv"	25	36	33.5
	seedling	25	52	51.0
Feb.	mature	15	47	29.5
	"juv"	15	80	51.0
	seedling	15	60	45.0
April	mature	15	24	24.3
	"juv"	15	72	37.5
	seedling	15	68	34.3
July	mature	15	00	24.3
	"juv"	15	26	38.6
	seedling	15	66	47.9
Nov.	mature	15	20	20.1
	"juv"	15	46	38.6
	seedling	15	80	43.1

^z "juv" ("juvenile-like")

^y Gfts (number of grafts per scion maturity)

Table 5-5: Stastical Seasonal Starch Comparisons

Date	R-value	DF	^z Sig.
Dec.	0.869	2	*
Feb.	0.932	2	**
April	0.987	2	**
July	0.883	2	*
Nov.	0.915	2	*

^z
Significance (*, 5%; **, 1%)

Table 5-6: Stastical Comparisons of Combined Scion Maturity and Starch Accumulation

R-value	DF	^z Sig.
0.738	14	**

^z
Significance (**, 1%)

CHAPTER VI

SUGAR PULSING

Introduction

Sugar pulsing is a method of increasing the shelf life of cut flowers (26,42). Placing cut flowers in a sugar-water solution, with a low pH (3.0-4.0), extends shipping and vase life for roses and carnations (42). The effect is attributed mainly to reduction of microbial population with lowered pH of the water, and retardation of stem blockage which prevents a decline in flow rate of water through rose stems and increases uptake of energy in the form of sugar (76).

It was shown in Chapter V that mature scionwood had a lower stored carbohydrate content (and poorer graft take) than "juvenile-like" scions and seedling tops. The objectives of this experiment were to determine if sugar pulsing would increase available carbohydrates to mature scionwood while graft unions are forming.

Materials and Methods

Two grafting periods were chosen. The first was June, 1982, when the summer heat is intense and the rainfall and

the relative humidity are high. The second period was early February, 1983, when there are cool day and cool nights.

The pulsing solution consisted of 5% sucrose in distilled water with the pH adjusted to 4.0 with hydrochloric acid.

There were 2 treatments: distilled water with a pH of 4.0 as a control, and the pulsing solution. Two cultivars were used ('No. 2' and 'Magana') and 2 scion maturities (mature and "juvenile-like"). The graftwood was collected in the field and recut when placed in the 2 solutions. The scions were left in the solutions for 24 hours at 26° C.

There was a total of 80 grafts: 10 of each scion maturity for each of the solutions. The rootstocks were grafted with a modified veneer graft and wrapped with polyethylene tape as described in Chapter III.

In June, 1982, the weather conditions for the day of grafting were an overcast sky and very hot. In February, 1983, the weather was cool and the sky clear. Data were collected in July and in April, respectively.

Results and Discussion

In June, there was 0% graft take for all treatments. After the graftwood was soaked and before grafting, the petiole stumps were abscising on many of the individual buds sticks of the "juvenile-like" cultivars. None of the budsticks from the water treatment had abscising petiole stumps and neither did the mature budwood from the sucrose treatment.

The 'Magana' budwood, both mature and "juvenile-like", had a gelatinous precipitate on the wood where it had been immersed in the water and sugar solution. There was some precipitate on the 'No. 2' cultivars in both solutions but not as much as on the 'Magana' cultivars.

The level of the solutions in the beakers remained the same before and after soaking, and the scions did not appear to take up any noticeable amount of the solutions. Both the sucrose solution and the water treatments had a milky cast after the budwood soaks. Presumably this was caused by the latex exudate.

The February treatments had similar results with only the 'Magana' "juvenile-like" scions out of all cultivars and scion maturities having 4 takes out of a total of 80 grafts. A statistical analysis using chi-square contingency tables shows that sugar pulsing is not significant at any level and does not have any appreciable effects upon graft take. Out of the two experiments, only 4 grafts lived out of 160 plants.

It is possible that the pulsing is also deleterious, since it has been shown that mamey does not graft well when there is high humidity (88, 91). The budsticks were wet when they were grafted, and the water could have caused the latex to congeal and inhibit the graft union by sealing off the vascular system. The plant usually breaks down the latex when grafting conditions are good and in general there

is no latex effect (68) These artificial conditions could have stimulated the latex to inhibit graft take.

CHAPTER VII

GROWTH REGULATORS APPLIED TO THE GRAFT WOUND

Introduction

Some nurseries in south Florida routinely apply growth regulators (both auxins and cytokinins) to the graft wound of mamey sapote and other plants (S. P. Lara, pers. comm.). Cytex, a seaweed extract, is purported to enhance the graft take of some tropical fruit trees. The objective of these experiments is to determine if growth regulators applied externally to the graft would enhance graft take.

The first test was designed to determine what effect growth regulators have under ideal environmental conditions, using the best rootstocks and scions. Test 2 was to determine if growth regulators would increase graft take under good environmental conditions, using oversized rootstocks and mature graftwood. The third part of the test was to determine the effects of growth regulators under oversized environmental conditions, using poor rootstocks and mature scions.

Materials and Methods

Growth regulators tested were a cytokinin (Benzyl adenine--BA), auxins (Naphthaleneacetic acid--NAA), and Cytex, a commercial product purported to have cytokinin-like activity. All were in a lanolin base. The controls were grafts with no lanolin or growth regulators, and grafts with lanolin alone. There were 20 replications with 2 cultivars in the first 2 tests and 15 replications with 2 cultivars in the next test. Replications were determined by availability of rootstocks and graftwood. The cultivars were 'No. 2' and 'Magana'.

The first experiment was on 22 February, 1983, and there were 2 controls, 1.0% Cytex in lanolin, 0.1% NAA in lanolin, and 0.1% BA in lanolin. The test was conducted in a slathouse with 50% shade and the best rootstocks and scions were chosen. All tests used a modified veneer graft and polyethylene grafting tape as described in Chapter III.

Experiment 2 was on 6 April, 1983 (an ideal time to graft mamey), and conducted at a local nursery. Rootstocks were larger than usually used for grafting and the scions were mature graftwood. A combination of 0.01% NAA and 0.01% NAA + 0.1% BA was added to the previous treatments. These treatments were added to see if a lower concentration of NAA would be more effective and to see if there was an interaction between NAA and BA.

The third test was July, 1983, which was considered to be the worst season to graft mamey. The same controls, concentrations, cultivars, were used. There was a total of 30 grafts per treatment, using 15 of each cultivar.

Results and Discussion

In the February test, none of the growth regulators increased graft take over control I (Table 7-1), and only Cytex had increased graft take over control II but was non significant within treatment variability. Both NAA and BA had lower graft takes than the controls. A chi-square analysis with contingency tables showed that there was a statistical significance of variables with the treatments at the 5% level.

In April, only control I (2.75%), control II (7.50%) and the NAA/BA combination (2.75%) had any graft take. A chi-square analysis showed no significant level of treatment effect. In July, there was 0.0% graft take for the whole experiment.

The data from these experiments do not indicate that the growth regulators at the concentrations used applied to the wound, will overcome adverse weather conditions or stimulate a graft union when using oversized rootstocks and mature scions. The data suggest that the growth regulators used (NAA and BA) could have a negative effect upon graft take.

Table 7-1: Applications of Growth regulators to Graft Wounds

Date	Gr. Reg. ^z	Reps. ^y	% Take	Sig. ^x
Feb.	control I	40	80.0	NS
	control II	40	40.0	
	1.0% Cytex	40	60.0	
	0.1% NAA	40	20.0	
	0.1% BA	40	33.0	
April	control I	40	2.75	NS
	control II	40	7.50	
	1.0% Cytex	40	0.00	
	0.1% NAA	40	0.00	
	0.01% NAA	40	0.00	
	0.1% BA	40	0.00	
	0.01% NAA/0.1% BA	40	2.75	
July	control I	30	0.00	NS
	control II	30	0.00	
	1.0% Cytex	30	0.00	
	0.1% NAA	30	0.00	
	0.01% NAA	30	0.00	
	0.1% BA	30	0.00	
	0.01% NAA/0.1% BA	30	0.00	

^z
control I (without growth regulators or lanolin)
control II (lanolin only)

^y
Gfts (number of grafts per scion maturity)

^x
Significance (NS, not significant)

CHAPTER VIII

ROOTSTOCK APICAL DOMINANCE RELATIVE TO GRAFT TAKE

Introduction

Apical dominance is strongly exhibited in the mamey sapote under field and nursery conditions. Seedling plants, used as nursery rootstocks, may not develop any side branches or lateral growth until the plant is up to 2 m high. Young mamey plants strongly exhibit apical dominance. They have a very large terminal bud and the axillary buds are so small that it is difficult to discern them in the leaf axils. On branches of mature trees, axillary buds are also small. The axillary buds on the mature trees are not suitable for use as T-buds or chip buds because, upon visual examination, without magnification, it is difficult to tell when they are fully developed and mature enough to be used for grafting. If the terminal bud is removed, the lateral buds swell and begin to grow. At this time the buds are suitable for grafting. This method of in situ preparation is used to facilitate off season annona grafting (89).

Quilantan-Carreón (98) described the removal of the terminal buds on rootstocks prior to grafting as a mechanism to remove excess latex and enhance grafting success. Some

authors consider the latex not to be a factor in graft inhibition (68, 88). The removal of the terminal buds on the rootstocks may break apical dominance. The experimental objectives were to determine if apical dominance in the rootstocks is a factor in graft union failure.

Materials and Methods

The experiments were conducted in July, 1983 (one of the hottest months), November, 1983, (cool days and warm nights), and January, 1984 (one of the coolest months). There were 2 groups of plants used in the experiments: the first had the terminal buds removed 24 hours prior to grafting, and the controls had the terminal buds intact. Each group had 3 scion maturities (seedling top, "juvenile-like" and mature) and 15 replications per scion maturity. They were grafted using a modified veneer graft and wrapped with polyethylene tape as described in Chapter III.

Results and Discussion

In July, mature budwood on the topped rootstocks had a graft take of 26.0% compared to 0.0% on the control rootstocks (Table 8-1). "Juvenile-like" on the topped rootstocks had 33.0% compared to 26.0% on the controls. Seedling tops used as scions had 0.0% on the topped rootstocks and 66.0% on the controls (Table 8-1).

The experiments in November show graft take of the mature on the topped rootstocks at 46.0% and the controls at 26.0%. The "juvenile-like" have a graft take of 40.0% on the topped and 46.0% on the controls. The seedling tops used as scions were 80.0% on the topped and the control rootstocks.

Grafting results in January were the worst during the year. Both mature and "juvenile-like" scions had 0% graft take with both the topped and the control treatments. The seedling tops had 86.0% on the controls and 46.0% on the topped rootstocks.

During periods of active growth, removal of the terminal bud on rootstocks with mature scionwood does increase the graft take percentage, and this is of particular interest because mamey generally cannot be grafted in the summer. In January, the weather is cool enough so that the stocks are dormant and there is little to no growth. The removal of the terminal bud does not have an effect on graft take because the terminals are not actively inhibiting lateral growth on a dormant plant.

Table 8-1: Apical Dominance as a Factor in Graft Take

Date	Treatment ^z	Stage ^y	Gfts ^x	% Take
July	control	mature	15	00
		"juv"	15	26
		seedling	15	66
	topped	mature	15	26
		"juv"	15	33
		seedling	15	00
Nov.	control	mature	15	20
		"juv"	15	46
		seedling	15	80
	topped	mature	15	46
		"juv"	15	40
		seedling	15	80
Jan.	control	mature	15	00
		"juv"	15	00
		seedling	15	86
	topped	mature	15	00
		"juv"	15	00
		seedling	15	40

^z
 control (terminal bud intact)
 topped (terminal bud removed)

^y
 "juv" ("juvenile-like")

^x
 Gfts (number of grafts per scion maturity)

CHAPTER IX

COMPONENTS AND LOCATION OF THE VASCULAR CAMBIUM

Introduction

Positioning of the vascular cambium relative to the rootstock and the scion for a graft union is very important for most species. The close developmental relationship between the cambium in the callus and in the components of the graft explains why proper matching of the cambium of the stock and scion speeds up the formation of cambial connection (28, 53). Improper matching of the cambium does not necessarily prevent a union, but it usually delays it (28, 53, 109).

There are some species that have an irregular cambium. This may be due to anomalous secondary growth. There are several types of anomalous secondary growth without extra bundles of vascular tissue being split off from the main cambium. One type is that described by Chalk and Chattaway (15), referred to as the lobed stem, where the secondary xylem is laid down unequally at different angles at different points around the stem. The cambium cylinder remains continuous. Venning (119) describes another type of anomalous secondary growth in the lychee where the cambium

grows unequally during the season. Parts of the cambium are actively dividing and growing while other sections of the cambium are inactive at any given time. The result is a continuous cambium with only some sections active and capable of forming a graft union.

The purpose of these experiments was to determine if the cambium of mamey sapote is irregular, and if any tissue stage of scionwood or rootstocks tends to be more regular than another. Stem morphology was also examined to see if juvenility affected internode lengths or numbers of leaf traces per section.

Materials and Methods

Sections for anatomical study were collected in February, April, and July. Seedling stems and terminal branches of the cultivars 'No. 2', and 'Magana' (both "juvenile-like" and mature tissue types) were cut in 30 cm lengths, and were preserved in 70% FAA (formalin-acetic-alcohol) (8) and dehydrated sequentially in 70%, 75%, 80%, 85%, 90%, 95%, 100% ETOH (ethyl alcohol) solutions under vacuum (50, 85).

An attempt was then made to cut the 30 cm specimens into 2-3 cm sections with a single-edged razor blade. When this failed, because the wood was so hard, a sabre saw was used. The sections were soaked in 4% ETD (ethylenediamine) to soften the wood, and 24% HF (hydrofluoric acid) to remove silica and crystalline materials (39, 54). This procedure

is used for woody materials with a specific gravity in excess of 0.75. The chemical ETD is a swelling agent which increases cell wall volume beyond that obtainable by simply saturating the samples in water. The swelling of cell walls induced by ETD is eliminated in later steps. There is no effect by ETD on silica crystals, and other cell inclusions (54).

The specimens were again dehydrated in ETOH and then embedded in GMA (glycol methylacrylate) and molded in 6mm x 12 mm x 5 mm blocks for sectioning on a rotary microtome (7, 14, 33, 85, 103). Because GMA is so hard, a standard metal blade cannot be used, and a 'Ralph' glass knife adapted to the microtome was used instead (104, 107, 108). Sections were cut to a thickness of 12 micrometers.

The plastic sections were floated on a clean slide on a drop of 10% acetone. Slides were then heated on a slide dryer and stained with 0.05% toluidine blue and differential stain (zinc chloride, safarin, orange G, tannic acid orange G, tannic acid, ferric ammonium sulfate) (105) for 30 seconds, rinsed, and dried again on a slide dryer (33). When dry, a drop of permount was applied to the section. The section was covered with a coverslip and dried for 24 hours.

Results and Discussion

The morphological examinations showed that there were 2-8 leaves per cm at the terminals. There were fewer leaves

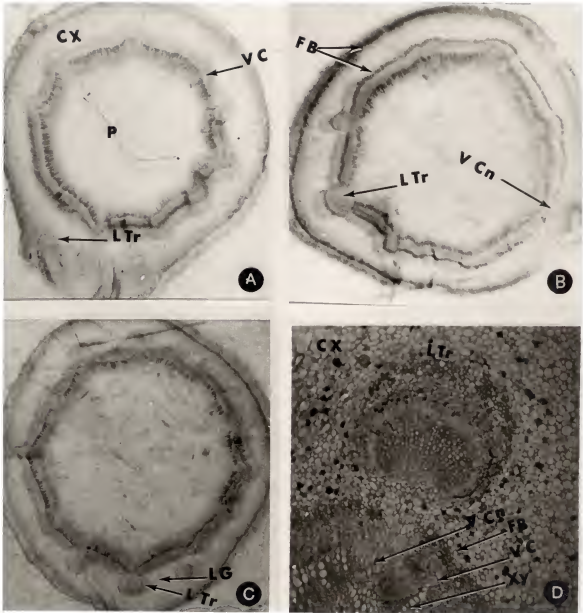
per cm on the mature scions and the rootstock stems than on the seedling subterminals and the "juvenile-like" scions. The leaves of the mamey are alternate but are so closely crowded toward the ends of the branches that they almost appear whorled. There are 3 leaf traces per node (Fig. 9-1 C).

Within any one section, several leaf traces are visible along the cambium (Fig. 9-1 A,B,C), and when the leaf trace breaks away, the cambium cylinder is left with an undulating and looped appearance. An enlarged leaf trace showing the vascular connections between the trace and the cambium is shown in Fig. 9-1 D.

"Juvenile-like" stems (Fig. 9-2 A,B) have more irregular cambium than the mature stems, and the mature stems (Fig. 9-3 A,B) are more irregular than the seedling stems used as rootstocks (Fig. 9-4) (Fig. 9-2,3,4). The cambium irregularities are caused by numerous leaf traces. Whether irregularities in the cambium are caused by anomalous secondary growth or by numerous leaf traces, the effect is the same. It is difficult to find the cambium on scionwood or rootstocks when preparing the wood for grafting if the cambium varies at different levels within the graftwood. Since the cambium is not visible from the outside of the branch, it is difficult to make a precise cut that will smoothly expose the entire cambium along the surface of the wound. Therefore it is difficult to make complete cambial contact with the rootstock and scion and ensure better graft take if the cambium cylinder is erratic.

Figure 9-1: Irregularity of the vascular cambium.

- A. Node with leaf trace (L Tr) and vascular cambium (VC) located between cortex (Cx) pith. 26x. Differential stain.
- B. Fiber bands (FB) under epidermis and outside vascular cambium, with leaf trace (L Tr) and vascular connection (V Cn) to node. 26x. Differential stain.
- C. Node with 3 leaf traces (L Tr) and 2 leaf gaps (LG). 26x. Toluidine blue.
- D. Enlarged leaf trace (L Tr) in cortex (Cx), with fiber bands (FB) outside vascular cambium (VC) with vascular connection (VCn) to xylem (Xy). 70x. Toluidine blue.



- Figure 9-2: Vascular cambium in 'juvenile-like' stems.
- A. 'Magana' "juvenile-like" stem with thick fiber bands, node with 2 leaf gaps (G) and 3 leaf traces. Differential stain. 26x.
 - B. 'No. 2' "juvenile-like" stem at node with vascular cambium (VC) and fiber bands (FB) outside the cambium. Toluidine blue. 26x.

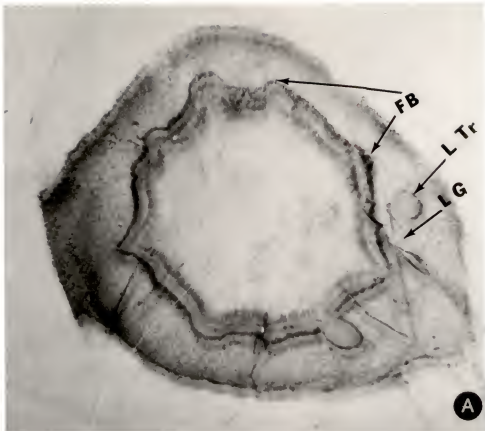
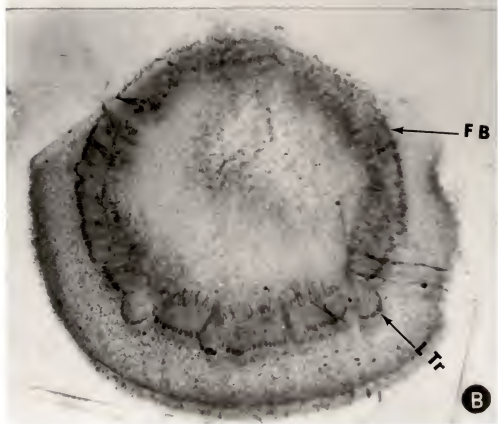
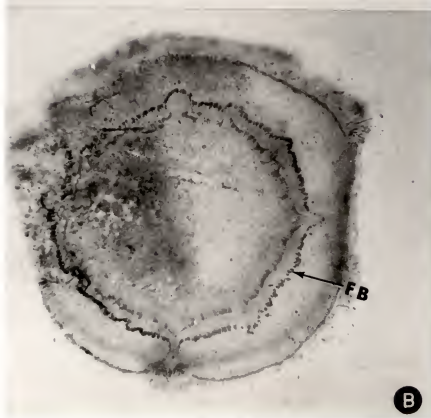
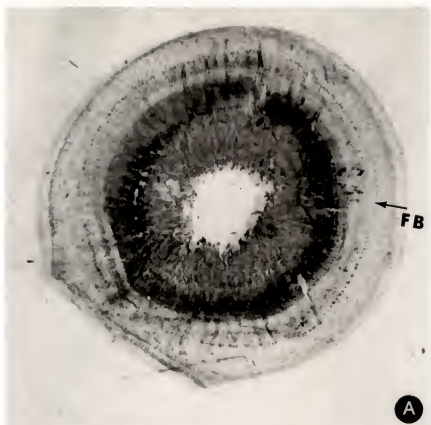


Figure 9-3: Vascular cambium in mature stems.

- A. 'Magana' mature with the vascular cambium (VC) less irregular, some leaf traces (LTr) and leaf gaps (LG) and vascular connection to node (VCn) from the xylem (Xy) and less dense fiber bands (FB). Differential stain. 26X.
- B. 'No. 2' mature with less undulation in the vascular cambium due to leaf traces (LTr) and fiber bands (FB) are less dense. Differential stain. 26 x.



- Figure 9-4: Vascular cambium in rootstock stem and seedling stem sections.
- A. Rootstock stem vascular cambium more regular than mature or "juvenile-like", fiber bands (FB) are broken interspersed with parenchyma cells. Toluidine blue. 26 x.
 - B. Seedling stems with more irregular vascular cambium and denser fiber bands (FB) than rootstock stem. Differential stain. 26x.



CHAPTER X

GRAFT UNION FORMATION

Introduction

The experimental objectives were to determine the sequence and duration of the graft union formation during Fall, Winter, and Summer; and to determine if wound healing and tissue differentiation differ, or follow the same healing progression throughout the year.

Materials and Methods

Rootstocks were 1/2 to 3/4 m high and were grafted with "juvenile-like" scions using a modified veneer graft and wrapped with polyethylene tape as described in Chapter III. On 21 October, 1982, 60 stocks were grafted, and beginning on day 3 (day 1 being the day of grafting), 3 grafts were collected for samples every 3-4 days. On 17 December, 1982, 30 stocks were grafted, and on 4 August, 60 stocks were grafted. Samples were collected as in October.

Samples consisted of 30 cm sections cut from the mid-section of the grafts. Samples were wrapped tightly with Parafilm prior to collection to prevent graft rupture. Samples were preserved in FAA, and dehydrated in ETOH (as described in Chapter IX).

An attempt was made to cut 1 cm sections from the graft union with a sabre saw, but the graft unions kept splitting apart. The grafts unions were wrapped with another layer of Parafilm and cut on a large band saw. The sections were then treated with ETD, HF, sectioned on a rotary microtome to a thickness of 12 micrometers and stained in toluidine blue as described in Chapter IX.

Results and Discussion

The graft series with the most consistent results was the October series compared to the December and the July series. In October, wound response appeared on day 3 (Fig. 10-1 A), dedifferentiation on days 5 and 7 (Figs. 10-1 B,C,D,E,F) a callus bridge on day 12 (Figs. 10-2 A,B,C), vessels on day 14 (Figs. 10-2 D,E,F), vessel lignification on day 17 (Figs. 10-3 A,B,C), cells lining up laterally on day 21 (Figs. 10-3 D,E,F) increased vessel lignification and on days 24-26 (Figs. 10-4 A,B,C,D), fibers on day 28 (Figs. 10-4 E,F), cambium development on days 31-35 (Figs. 10-4 A,B,C,D,E,F), increasing connection on day 38 (Figs. 10-6 A,B), a complete connection on day 41 (Figs. 10-6 C,D) and an apparent union on days 44-49 (Figs. 10-6 E,F).

In December, initial callusing was slower than in October, with many of the sections breaking apart from lack of connections. Only a few could be sectioned. There was some wound response on day 3 (Figs. 10-7 A,B), with cell dedifferentiation on day 5 (Fig. 10-7 B). In August, many

of the grafts failed, but those that took were slower in development than those of the October series. Wound response was on day 3-5 (Figs. 10-7 C,D), an isolation layer on day 5 (Fig. 10-7 D), and some cell dedifferentiation on day 9 (Figs. 10-7 E,F). Callus was noted on day 12-15 (Figs. 10-8 A,B,C). Fibers in the graft wound were merged from the edge (Fig. 10-8 D). Vessels were noted on day 25 (Figs. 10-8 E,F), and no vascular cambium was detected in this series. Kester (53) indicates 1-2 weeks for vessel differentiation from callus for many species. Hartmann and Kester (43) indicate that parenchyma can differentiate into tracheids with ease.

A graft union is considered complete when there is enough contact from the rootstocks to transfer water to the scion (41, 71), and the buds start to expand and grow (71, 72, 75). In the mamey sapote the terminal buds start to grow in up to 2 months. The primary need of the scion after budding is a sufficient water supply (53, 71). Water supply is first met by the callus bridge, then afterwards the differentiation of the tracheid strands in the callus assures the water supply of the scion. This water movement is first based on lateral water movement from the callus, then longitudinally from the vessels through pit connections, then laterally from the rays (28, 71).

Lin (62) mentions that pit connections are small (5) in the vessel members and the fibers. There is a high percentage of fibers in the wood, and the wood is not

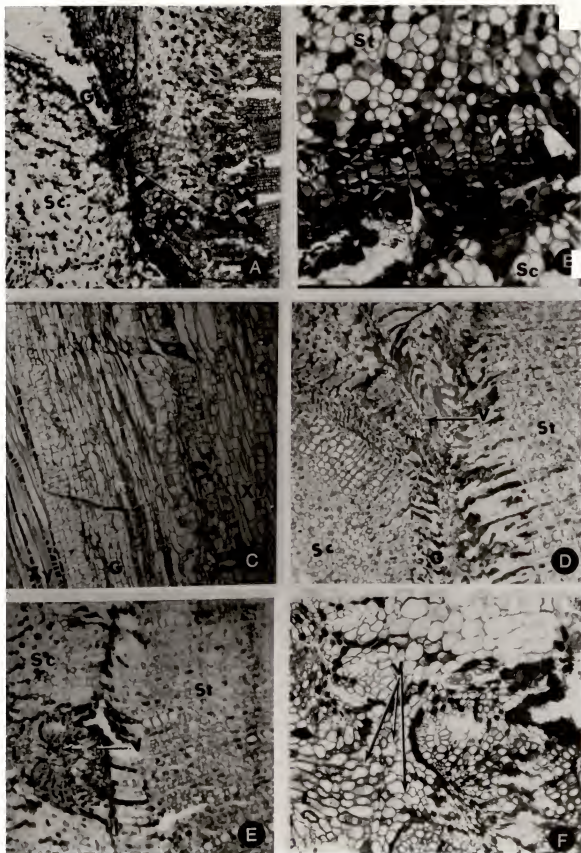
especially adapted for liquid conduction because flow movement between fibers is dependent primarily on simple pits (62). Slow water movement could be a factor in the slow healing rate of the mamey and even lack of unions.

For all time periods tested for the grafting responses of the mamey sapote, there was a general progression of tissue differentiation as reported; however, there were replicates that were erratic in callusing response. The data reported in these experiments were initial observations of the stages, not precise delineations for each sample. From the observations in these experiments, October is a better month for grafting mamey than December or August, because callusing and lignification were shown to be faster in October. In mid-December the graft responses are slowed down because the plant is no longer in active growth. In August, the plant is in active growth, but the environmental effects of high temperature and high humidity slow down the graft take and subsequent healing as noted in Chapter III.

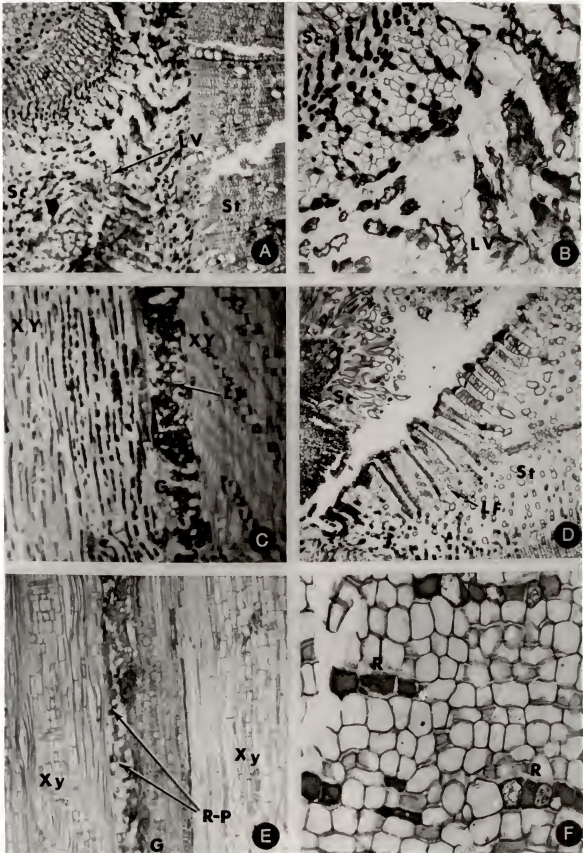
- Figure 10-1: Graft union formation in October, days 3-7.
- A. Day 3, wound response (WR) in graft wound (G) between scion (Sc) and stock (Sk). 70x.
 - B. Day 5, cell dedifferentiation to form callus (Cl) between scion (Sc) and stock (St). 70x.
 - C. Day 5, same site. 200x.
 - D. Day 7, callus (Cl) between scion (Sc) and stock (St). 70x.
 - E. Day 7, same site. 200x.
 - F. Day 7, same site. 500x.
- A-F: Toluidine blue stain.



- Figure 10-2: Graft union formation in October, days 12-14.
- A. Day 12, callus bridge (Cl) at graft wound (G) between scion (Sc) and stocks (St). 70x.
 - B. Same site. (Note rounded isodiametric cells). 100x.
 - C. Day 12, longitudinal section, graft wound (G) between xylem (Xy) of scion and stock. 70x.
 - D. Day 14, vessels (V) in graft wound (G) between scion (Sc) and stock (St). 70x.
 - E. Day 14, vessels (V) with some tissue beginning lateral lining up. 70x.
 - F. Same site 200x.
- A-F: Toluidine blue stain.



- Figure 10-3: Graft union formation in October, days 17-21.
- A. Day 17, healing near graft wound edge, lignification of vessels between scion (Sc) and stock (St). 70x.
 - B. Day 17, same site. 200x.
 - C. Day 17, longitudinal section. Lignified vessels (LV) in graft wound (G) between xylem (Xy) of scion and stock. 70x.
 - D. Day 19, cells forming lateral files (LF) on the stock (St). 70x.
 - E. Day 21, longitudinal section, beginning of ray parenchyma (R-P) in graft wound (G) between xylem (Xy) of scion and stock.
 - F. Day 21, same site with rays (R). 500x.
- A-F: Toluidine blue stain.



- Figure 10-4: Graft union formation in October, days 24-28.
- A. Day 24, large vessels (V) in scion (Sc) near graft wound, lateral files (LF) of cells well developed in stock (St). 70x.
 - B. Day 24, same site with silica crystal (SC) in callus. 200x.
 - C. Day 26, more vessels and greater lignification in graft wound (G) between scion (Sc) and stock (St). 70x.
 - D. Day 26, same site with lignified vessels. 200x.
 - E. Day 28, longitudinal section with poor union but some fibers forming. 70x.
 - F. Day 28, same site with vessels (V) and fibers (F). 500x.
- A-F: Toluidine blue stain.

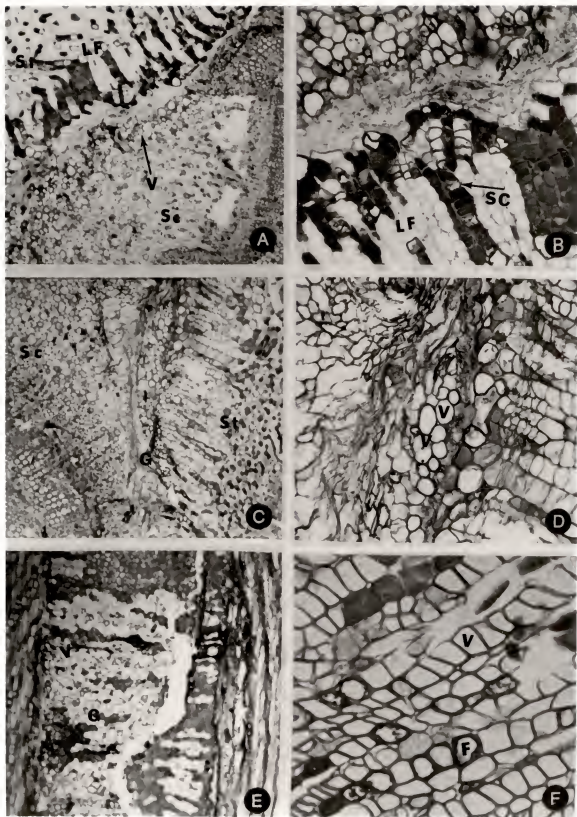
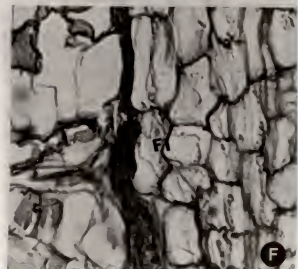
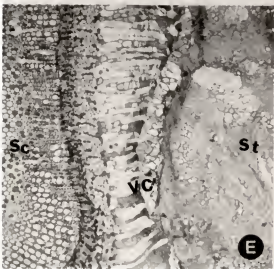
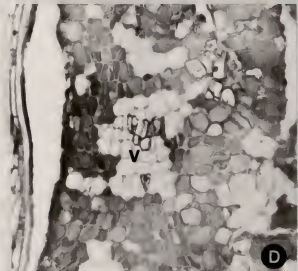
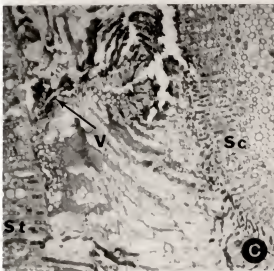
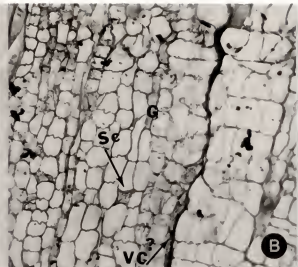
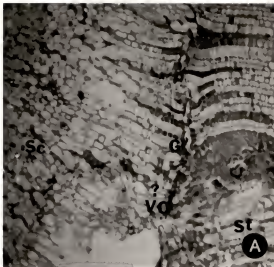


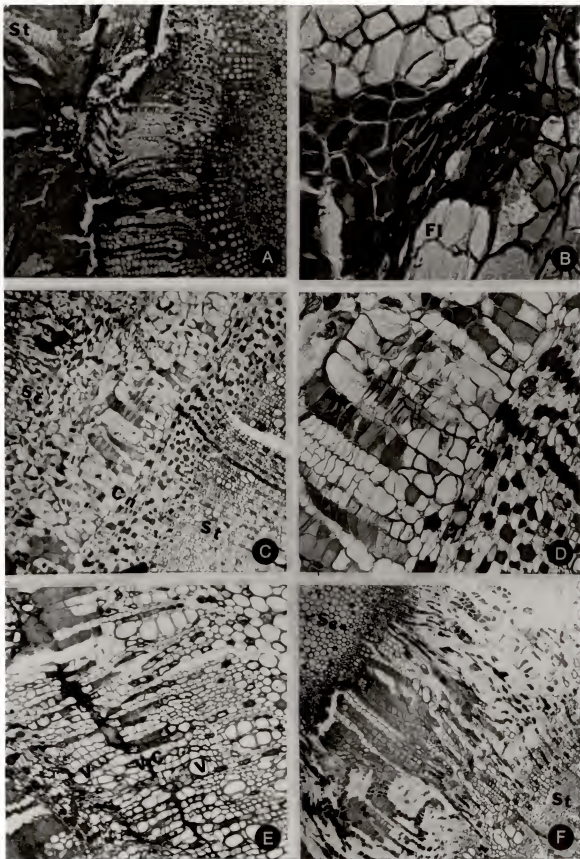
Figure 10-5: Graft union formation in October, days 31-35.

- A. Day 31, First indication of possible vascular cambium (?VC) in graft wound (G) between scion (Sc) and stock (St). 70x.
- B. Day 31, same site, possible vascular cambium (?VC) with silica crystal (Sc) in graft wound (G). 200x
- C. Day 33, poor union with numerous vessels (V), no cambium. 70x.
- D. Day 33, same site with lignified vessels (V). 200x.
- E. Day 35, good healing with many rows of lateral cells outside of vascular cambium (VC) between scion (Sc) and stock (St). 70x.
- F. Day 35, same site with fusiform initials (FI). 2000x

A-F: Toluidine blue stain

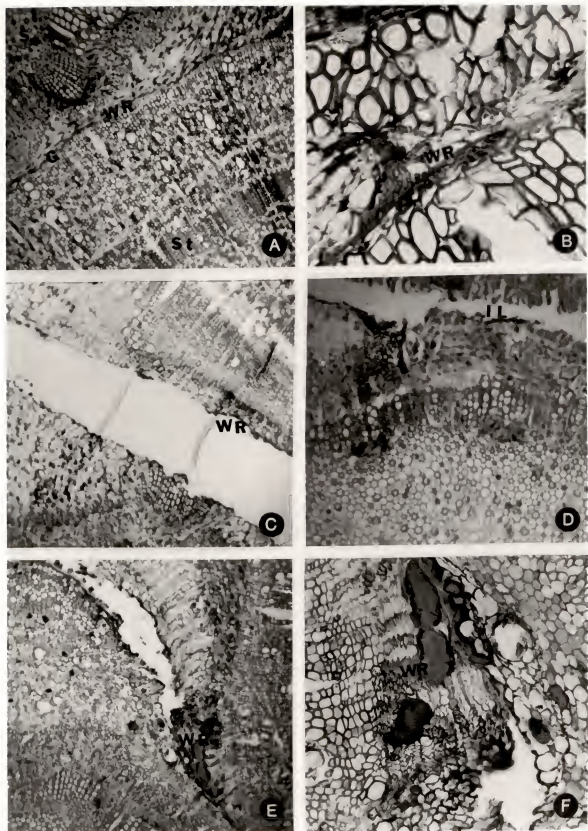


- Figure 10-6: Graft union formation in October, days 38-49.
- A. Day 38, vascular cambium (VC). 70x.
 - B. Day 38, same site, fusiform initials 2000x.
 - C. Day 41, increased lateral cell formation near cambium with good connection (Cn) between scion (Sc) and stock (St). 70x.
 - D. Day 41, same site, connection (Cn) 500x.
 - E. Day 44, apparent water connection across vascular cambium (VC) and large vessels (V). 200x.
 - F. Day 49, completed graft union. 70x.
- A-F: Toluidine blue stain

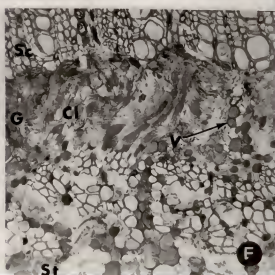
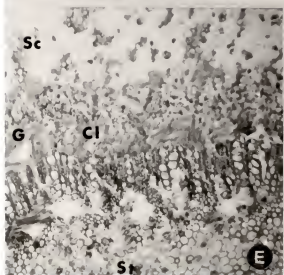
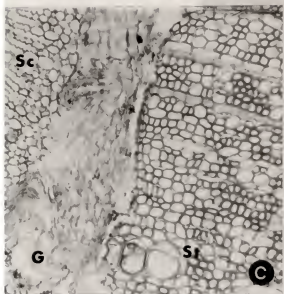
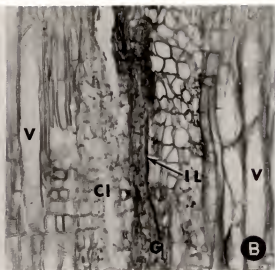
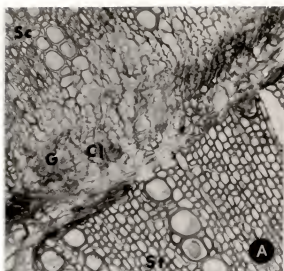


- Figure 10-7. Graft union formations in December and August.
- A. Day 3, December, wound response (WR) in graft wound (G) between scion (Sc) and stock (St). 70x.
 - B. Day 5, initial dedifferentiation and wound response (WR). 500x.
 - C. Day 3, August, wound response (WR). 70x.
 - D. Day 5, isolation layer (IL). 70x.
 - E. Day 9, some dedifferentiation and wound response. 70x.
 - F. Day 9, same site showing wound response (WR). 200x.

A-F: Toluidine blue stain.



- Figure 10-8: Graft union formation in August, days 12-25.
- A. Day 12, callus (Cl) in graft wound (G) between scion (Sc) and stock (St). 200x.
 - B. Day 12, longitudinal section with isolation layer (IL) in graft wound (G), vessels (V) with sclereiform pitting in the xylem of the stock and scion. 500x.
 - C. Day 15, more callus in the graft wound (G) between scion (Sc) and stock (St). 200x.
 - D. Day 18, Fibers (F) from wound edge merged into graft wound (G). 70x.
 - E. Day 25, callus bridge (Cl) in graft wound (G) between scion (Sc) and stock (St). 70x.
 - F. Day 25, some non-lignified vessels (V) in callus (Cl) of graft wound (G). 200x.
- A-F: Toluidine blue stain.



CHAPTER XI

FIBER BAND LOCATION WITH RESPECT TO GENERAL ANATOMY AND THEIR EFFECT UPON GRAFT UNION FORMATION

Introduction

Studies in families that are difficult to root and graft reveal bands of fibers in the cortex near the cambium. These bands have been found in the avocado (West Indian race) (37), the lychee (119), and in the Mimosaceae (101). Gomez (37) found that the dense bands of fibers inhibited rooting in the avocado because root initials arise from parenchyma cells in the cortex near the vascular cambium. Presence of fibers, that do not readily dedifferentiate, reduced the chances of rooting. Mamey is also extremely difficult to root from cuttings and to airlayer (1). The experimental objectives were to determine the location and presence of fibers, parenchyma (with or without inclusions) and other associated cell types such as laticifers in the proximity of the vascular cambium.

Materials and Methods

Slides prepared for Chapters IX and X were photographed to document the locations of fiber bands around the cambia,

any bands in the cortex, and determination of any connections or proximal locations of laticifers and fiber bundles. Notations were made concerning the relative abundance of parenchyma cells near the vascular cambium, within the pith and the cortex.

The tissue maturities examined were those of the mature scions, "juvenile-like" scions and rootstock stem sections or material most likely to be used for grafting.

Results and Discussion

There are distinct bands of fibers on the cortical side of the vascular cambium in all tissue types examined. In mature scions, there are bands of fibers around the vascular cambium, and bands within the cortex (Figs. 9-2, 9-4). There is also a single layer of fibers under the epidermis (Figs. 9-1, 9-2). In the "juvenile-like" scions, there are fewer fiber bands within the cortex but more fibers around the loops of the cambium where the leaf traces branch from the cambium (Fig. 9-3). Stem sections have less distinct bands and the bands are interspersed with more parenchyma between the fibers (Fig. 9-4 A).

In the graft union series, the stock and scion may be readily discerned by the more undulating cambium of the scion ("juvenile-like" tissue) and fiber bands within the cortex of the rootstock.

Fibers are specialized cells that rarely dedifferentiate because of their lignified nature (28, 31). Laticifers are also specialized cells that do not dedifferentiate. Because of the composition of the tissue immediately surrounding the vascular cambium, it is necessary to be precise in determining the position for making cuts in the stock and scion preparatory to grafting. Locations of the fiber bands and laticifers would necessitate the cut to be precisely between the secondary xylem and to the inside of the fiber band (the cambium is inside this zone) (Fig. 9-2 B). An alternative location for the cut would be in the midsection of the cortex where there are fewer specialized cells and more of the parenchyma cells that dedifferentiate readily.

The fiber bands surrounding the vascular cambium in mamey would be in the same position of the band of fibers in the cortex-cambium region of the West Indian race of avocados as described by Gomez (37) in his study relative to rooting avocados. Guatemalan and Mexican races lacked these fiber bands in the cortex-cambium region and these races were easier to root.

In the graft union series in Chapter X, the scions that made a stronger graft union were cut in the mid-cortex region (Figs. 10-2 A, 10-3 A) and the ones that broke more easily were cut in the area between the xylem and the fiber bands (Fig. 10-5 C,D). This would support Gomez's (37) contention that fiber bands are located where root initials

arise (next to the vascular cambium in the cortex) and that cell dedifferentiation is difficult because of the numbers of specialized cells in this area. The fiber bands next to the cambium within the cortex would explain why mamey sapote is also difficult to root and airlayer.

CHAPTER XII

SILICA: CONTENT AND LOCATION, AND EFFECT UPON GRAFT TAKE

Introduction

Silica inclusions are common in the family Sapotaceae and are used for identification at the generic level (55, 56, 57, 58). Several tropical families (Lecythidaceae, Proteaceae, Sapotaceae) that contain fruit trees that are difficult to graft, also have a high silica content. Scurfield (106) reports that silica lines cell walls of some species and the lining may be found in septate fibers, vessels, vessel tyloses, and vertical and ray parenchyma. Silica within the cell lumen may be in the form of silica sand, mixed with starch, and silica crystals that fill entire cells (47, 100, 106). Silica inclusions may have a wrinkled or uneven appearance. Some of the silica crystals may be 70 μm in diameter and brown under normal light, glowing under phase contrast (2, 16, 100, 106).

The experimental objectives were to determine if the scion materials (seedling tops, "juvenile-like", and mature) vary in silica content within tissue types and within seasons (December, February, April, and November). Location of silica within tissue was investigated and the tissues

examined were parenchyma of the cortex, pith, and secondary xylem. The types of siliceous materials to be located were silica cell wall deposits, silica crystals, and silica sand.

Materials and Methods

Materials were gathered from the field at the same time scionwood was collected for other grafting experiments (Chapters III,IV) and samples were randomly chosen from the overall collection. Two cultivars ('No. 2' and 'Magana') were used and the tissue maturities were seedling tops, "juvenile-like", and mature scions. Time periods were December, 1982, February, 1983, April, 1983, July, 1983, and November, 1983.

Tissue preparation consisted of chopping, then air-drying the scionwood for 4 days. After the materials were thoroughly dried, samples were then weighed. One gm samples were analyzed for silica by ashing the sample in a muffle furnace, dissolving the white ash residual in 1 N HCl (hydrochloric acid) solution. The solutions were analyzed at the University of Florida Soils Testing Laboratory by an atomic absorption method. Results are reported in parts per million and these are in turn converted to mg/ml.

Materials for microscopic examination were taken from budstick samples collected for silica analysis. The samples in tissue type and cultivar were the same as for the silica analysis. Preparations for these samples consisted of slicing into 1 cm sections on a band saw, and half of each

replicate was soaked in ETD and HF, dehydrated in ETOH and embedded in GMA (as described in Chapter IX). The other half of each sample was only dehydrated in alcohol and then embedded in GMA. Samples were sectioned and mounted, and stained in toluidine blue (as described in Chapter IX).

Results and Discussion

Graft takes in relation to silica content were compared on a tissue and monthly basis (Table 12-1). There was little difference in cultivars and the results were averaged together for the scion maturities.

Data were statistically analyzed using correlation coefficients (Table 12-2). Silica did not positively affect the graft takes and was negatively correlated in the months of December, February, April, and July. November had a slight positive correlation coefficient. Only April had a significant treatment effect at the 5% level.

Combining the effects of tissue maturation and season, the results were negatively correlated and significant at the 5 % level (Table 12-3).

A negative correlation would indicate that as the measurable amount of silica increases, the graft take decreases. A positive correlation would indicate that if silica amounts increased, graft take would increase.

Microscopic examination of tissue sections indicates that silica is found in the cortex in the form of a cell wall deposit within the parenchyma (Fig. 12-1 A,B), mixed

with starch (Fig. 12-1 C), and as crystals. Silica crystals appear as dark globules under normal light, but under phase contrast they glow (Fig. 12-1 F). Idioblasts (containing solitary silica crystals up to $80\ \mu$ in diameter) in the cortex may be found concentrated particularly around the phloem bundles near the vascular cambium associated with laticifers (Figs. 12-1 D,E,F). Laticifers are found scattered randomly in the cortex, and especially around phloem fiber bundles. In longitudinal sections laticifers also show silica deposits on the walls and within the latex (Figs. 12-1 A,B).

The crystals and silica deposits may be seen, even if the tissues are treated for silica removal with HF. When samples of tissue not treated with HF and ETD were sectioned, the glass knives shattered and up to 10 knives were used to obtain one section suitable for mounting. Tissue samples treated with ETD and HF did not shatter the glass knives and 2-3 samples could be sectioned with numerous slides being prepared from the use of one glass knife.

Slides made from samples treated with ETD and HF had very little ripping and tearing in the secondary xylem (Figs. 12-2 A,B) compared to massive tears and cell damage and distortion in untreated tissues (Figs. 12-2 C,D). Phloem fibers also were torn and distorted in the untreated tissues (Figs. 12-2 E) while the treated tissues made smooth cuts (Fig. 12-2 F).

Silica may be found in the secondary xylem as deposits on the walls of fibers and vessel members (Figs. 12-3 A,B,C). Vessel members are easily seen in longitudinal sections because of the scleriform pitting, contrasted to the laticifers in the nearby pith which appear to be full of globules (latex) (Figs. 12-3 A,B). Under phase the same section shows silica deposits along the vessel walls, on the laticifer walls and within the latex (Fig. 12-3 C).

Laticifers are found scattered randomly in the pith, (Figs. 12-3 D,E). Under phase contrast, silica can be found in the cortex, as well as within the secondary xylem rays, in the forms of silica crystals, deposits on vessel walls, within the ray parenchyma mixed with starch (Fig. 12-3 F).

The effect of silica upon graft take is possible in several ways. Parenchyma cells become specialized when they contain large solitary crystals (idioblasts) and specialized cells do not readily dedifferentiate (28, 31). Vessels are coated with silica, and this may inhibit water movement if pit apertures become plugged (pit aperture plugging is common in the Sapotaceae) (62). Cells hardened with silica are subject to ripping and tearing when using a glass knife for sectioning. A metal grafting knife used for making the graft wounds would do a great deal of damage at the graft site, and the tissues would require more healing than if wounds could be cut more smoothly.

Silica content is lower in the species of the Sapotaceae that are easier to graft (Manilkara (0.0%) and Pouteria campechiana (.25%))(6, 57, 58). The species that are more difficult to graft contain more silica (Calocarpum (2.2 to 5%) and Chrysophyllum (.25 to 1.13%)) (6, 55, 56).

For best grafting results, scion materials should be selected that are lower in silica ("juvenile-like") over materials that are higher in silica (mature).

Table 12-1: Silica Content Relative to Graft Take

Take	Stage ^z	Gfts ^y	%Take	mg/ml
Dec.	Mature	25	11	7.2
	"Juv"	25	36	5.0
	Seedling	25	52	5.8
Feb.	Mature	15	47	7.2
	Juv	15	80	3.9
	Seedling	15	60	7.2
April	Mature	15	24	6.5
	"Juv"	15	72	4.1
	Seedling	15	68	4.0
July	Mature	15	00	7.6
	"Juv"	15	26	4.3
	Seedling	15	66	4.9
Nov.	Mature	15	20	5.2
	"Juv"	15	46	3.7
	Seedling	15	80	5.6

^z "Juv" ("juvenile-like")

^y Gfts (numbers of grafts per scion maturity)

Table 12-2: Statistical Seasonal Silica Accumulation

Date	R-value	DF	Sig. ^z
Dec.	-.700	2	NS
Feb.	-.920	2	NS
April	-.990	2	*
July	-.701	2	NS
Nov.	0.299	2	NS

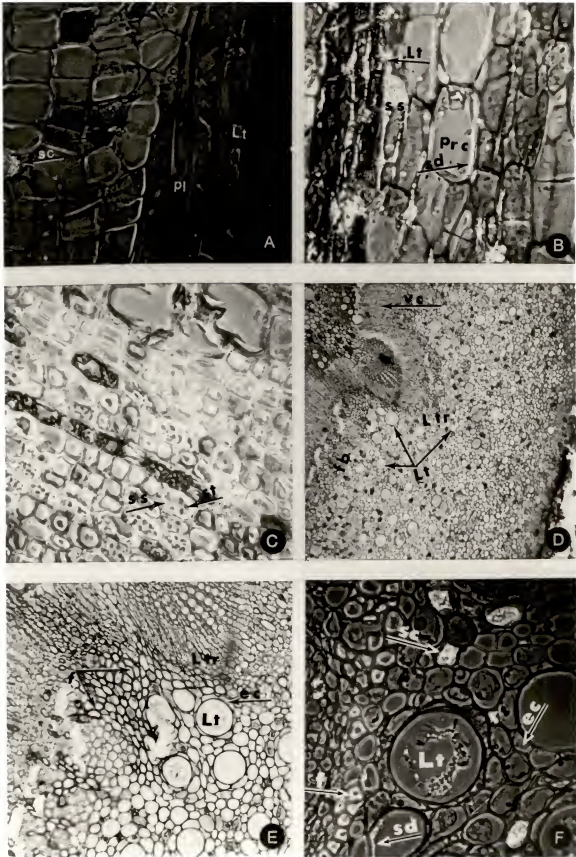
^z Significance (*,5%; NS, non significant)

Table 12-3: Statistical Comparison of Combined Tissue Maturation and Seasonal Silica Accumulation

R-value	DF	^z Sig.
-0.535	14	*

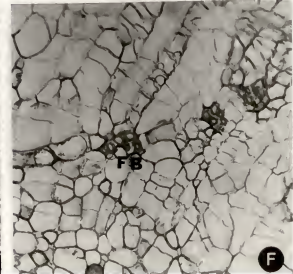
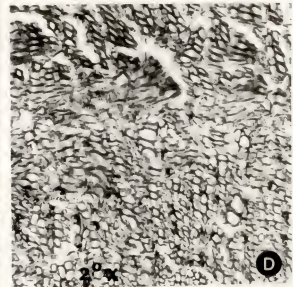
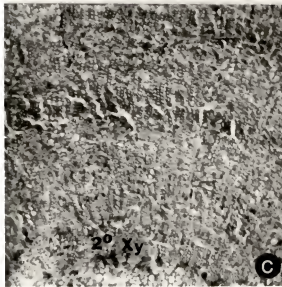
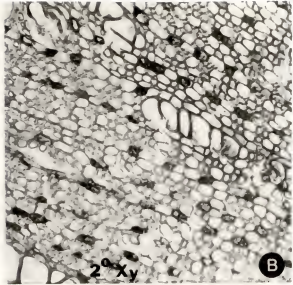
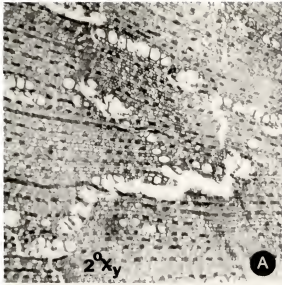
^z
Significance (*, 5%)

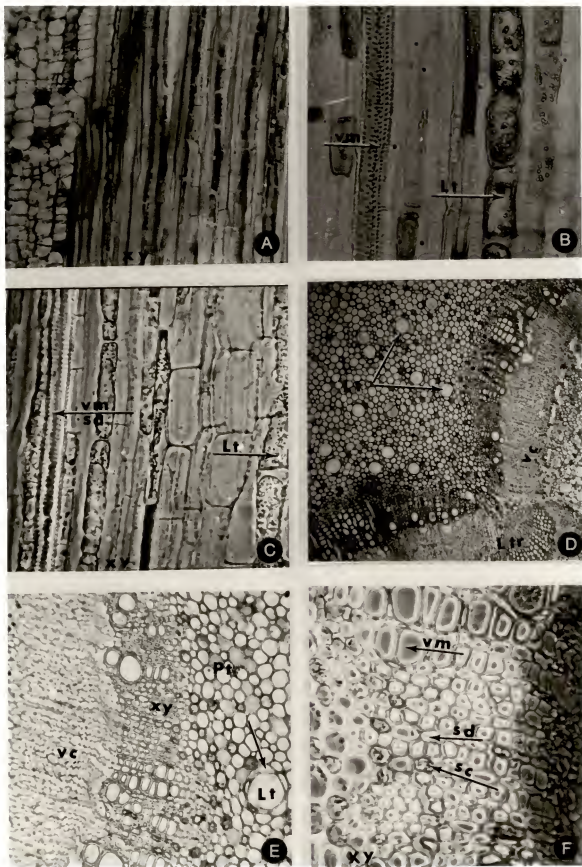
- Figure 12-1: Silica deposits and laticifers within the cortex, phloem, and their association with fiber bundles and leaf traces.
- A. Longitudinal section, silica crystal (SC) in cortex adjacent to phloem (PL), and laticifers (Lt). 500x.
 - B. Longitudinal section, silica found in cortex associated with latex in the laticifers (Lt), silica sand (SS), deposits on cell walls (SD) in parenchyma cells. 500x. Phase.
 - C. Silica sand (SS) with starch (St) in parenchyma cell. 500x. Phase.
 - D. Leaf trace (LTr) with bands of fibers (FB) associated with numerous laticifers (Lt). Dark bodies in cortex are silica crystals. 70x.
 - E. Same site. Laticifers (Lt) with epithelial cells (EC) near leaf trace (LTr). 200x.
 - F. Same site. Silica deposits (SD) found in laticifers (Lt), large silica crystals (SC) intercellular spaces, and latex globules visible epithelial cell (EC) of the laticifers. 500x. Phase.
- A-F: Toluidine blue stain.



- Figure 12-2: Secondary xylem and phloem, with and without ETD and HF treatments.
- A. Secondary xylem (2oXy) with ETD and HF treatment. 200x.
 - B. Same site. 500x.
 - C. Secondary xylem (2oXy) without ETD and HF treatment. More ripping and tissue distortion than treated samples. 70x.
 - D. Same site. 500x. Phase.
 - E. Fiber bundles in cortex with ETD and HF, silica deposits in fibers. 500x. Phase.
 - F. Fiber bundles in cortex with ETD and HF, no tearing and cell distortion. 500x.
- A-F: Toluidine blue stain.

- Figure 12-3: Silica deposits and laticifers in the secondary xylem and pith.
- A. Longitudinal section, vascular cambium on the left with the xylem (Xy) on the right. 200x.
 - B. Longitudinal section, vessel members (VM) with scleriform pitting in the xylem near the laticifers (Lt) in the pith. 500x.
 - C. Longitudinal section, vessel members (VM) with silica deposits (SD) and laticifers (Lt) latex globules and silica deposits. 500x. Phase.
 - D. Laticifers (Lt) in the pith near the secondary xylem and the vascular cambium (VC) adjacent to a leaf trace (LTr). 70x.
 - E. Vascular cambium (VC) adjacent to secondary xylem (Xy) near laticifers (Lt) in the pith. 200x.
 - F. Silica deposits in the secondary xylem (Xy), located on the cell walls of the vessel members (VM), in deposits in fibers (SD), and as silica crystals (SC) in the parenchyma. 500x. Phase.
- A-F: Toluidine blue stain.





CHAPTER XIII

INTERACTIONS OF STARCH, SUGAR, AND SILICA UPON GRAFT TAKE

Data from Chapters V and XII were combined in a multiple linear regression analysis (Table 13-1). The multiple regression model combines the 5 grafting periods, 3 scion maturities, and the 3 quantitative variables. The interactions of starch and silica from the values in Table 13-1 indicate that during the months when starch measurements are high relative to silica, graft take is higher. When silica measurements are high relative to starch, the graft take is lower. Mature scions have consistently higher silica content, lower starch content, and lower graft take percentages than the "juvenile-like" scions.

The regression model (Table 13-2) lists the part of the total variability, or R-square value (60.5%), as sugar (7.4%), silica (26.1%) and starch (44.3%). Starch is the single most important variable according to the model. Interactions of the variables, in order of increasing importance, are sugar and silica (32.3%), sugar and starch (50.2), and starch and silica (55.2%).

Because only 60.5% of the variability within the model is explained by the regression equation, other factors in addition to starch, silica, and sugar affect the graft take of mamey sapote, and these have been detailed in previous chapters.

Table 13-1: Interactions of Starch, Sugar and Silica

Date	Stage ^z	Gfts ^y	% Take	Analysis	mg/gm
Dec.	Mature	25	11	Starch	30.6
				Sugar	13.3
				Silica	7.2
	"Juv"	25	36	Starch	33.5
				Sugar	14.6
				Silica	5.0
	Seedling	25	52	Starch	51.0
				Sugar	16.9
				Silica	5.8
Feb.	Mature	15	47	Starch	29.5
				Sugar	19.7
				Silica	7.2
	"Juv"	15	80	Starch	51.0
				Sugar	18.1
				Silica	3.9
	Seedling	15	60	Starch	45.0
				Sugar	14.1
				Silica	7.2
April	Mature	15	24	Starch	24.3
				Sugar	21.0
				Silica	6.5
	"Juv"	15	72	Starch	37.5
				Sugar	19.6
				Silica	4.1
	Seedling	15	68	Starch	34.3
				Sugar	12.6
				Silica	4.0
July	Mature	15	0	Starch	34.3
				Sugar	19.9
				Silica	7.6
	"Juv"	15	26	Starch	38.6
				Sugar	13.0
				Silica	4.3

Table 13-1 - Extended

	Seedling	15	66	Starch	47.9
				Sugar	27.0
				Silica	4.9
<hr/>					
Nov.	Mature	15	20	Starch	20.1
				Sugar	21.3
				Silica	5.2
	"Juv"	15	46	Starch	38.6
				Sugar	22.6
				Silica	3.7
	Seedling	15	80	Starch	43.1
				Sugar	30.9
				Silica	5.6
<hr/>					
	z "Juv" ("Juvenile-like")				
	y Number of grafts per test				

Table 13-2: Overall Scion Multiple Linear Regression

Multiple linear regression equation:

$$\text{Graft} = .269 + 1.53(\text{starch}) + 1.09(\text{sugar}) - 6.21(\text{silica})$$

R-square value = 60.5

F value: $1837.69/326.6 = 5.62$ with 3,1 DF *5% level

R-square variables in model

Model:

1 7.4% Sugar
 1 26.1% Silica
 1 44.3% Starch

2 32.2% Sugar and silica
 2 50.2% Starch and sugar
 2 55.2% Starch and silica

3 60.6% Starch, silica, and sugar

CHAPTER XIV

SUMMARY AND CONCLUSIONS

Results indicate that several different factors are involved in the formation of the graft union of mamey sapote. These factors are: environment (mediated by seasons that affect temperature, relative humidity, seasonal rainfall, and diurnal effects); juvenility or tissue maturation; seasonal carbohydrate accumulation, in particular starch; apical dominance, perhaps mediated by growth regulators; irregularity of the vascular cambium; proper grafting technique; fiber bands on the cortical side of the vascular cambium that inhibit tissue dedifferentiation in proximity of the cambium; and silica content.

Environmental effects (season, temperatures, rainfall, relative humidity) allow definite grafting seasons. During the summer months in the rainy season when the relative humidity of very high and plant is in active growth, and during the winter months when the plants are dormant, the mamey sapote has poor grafting results. The spring months of February and April, when there are warm days, cool nights, and low relative humidity, are the best grafting times with the highest percentage graft take.

Juvenile scions and scions that have been reverted to a "juvenile-like" morphology are superior to scions collected from mature grafted trees. "Juvenile-like" scions are the scions of choice because they are of known genotype compared to the seedling or juvenile scions of unknown genetic characters.

Carbohydrate content varies according to the season of the year depending upon whether the plant is in active growth or dormant. Starch is a more important quantitative variable compared to sugar relative to graft take percentage. Mature scions have lower starch content and lower graft take compared to the "juvenile-like" and seedling scions.

Sugar pulsing did not enhance graft take, and it almost inhibited graft take completely. The deleterious effect may have been due to intolerance of the tissues to wetting or coagulation of the latex that sealed off the vascular system.

Applications of growth regulators to the graft wound did not enhance graft take and some of the growth regulators may even have inhibited graft take (auxins in particular). Apical dominance tests indicated that removal of terminal buds on the rootstock eliminated an inhibitor (possibly auxins) during rapid growth periods in the summer and partially overcame some of the grafting problems when the season was hot.

Anatomical investigations revealed that the vascular cambium is irregular and has an undulating pattern within the stem. This undulation was found to be caused by numerous leaf traces. Cambium irregularity makes grafting more difficult because it is hard to line up the stock and scion for good cambial contact and subsequent healing.

Other anatomical observations detailed fiber bands and laticifers in the cortex in close proximity to the vascular cambium. These cell types do not readily dedifferentiate into callus for wound healing and differentiation into vascular tissue necessary for a good graft union and conduction of water and food supply.

Proper grafting technique is important because of the irregular cambium and the position of the fiber bands. A modified veneer graft cutting into the cortex and away from the bands of fibers around the cambium is more successful because there is more parenchyma in this area to dedifferentiate into callus. This was shown by sections in a graft union series. The grafts that were made in the cortex were more successful than grafts that were made close to the cambium and the fiber bands.

Silica was noted in the tissue and was found as solitary crystals in idioblasts, lining the cell walls in the xylem, and mixed with starch and with latex. Silica tends to make the wood hard and this results in tissue damage with a lot of tearing and ripping. If a smoother cut could be made, there would be less tissue damage and faster healing of the graft wound.

An analysis of the silica content was made and an interaction of starch, sugar, and silica was indicated. A combination of low silica content relative to a high starch content resulted in increased graft take percentage (as found in the "juvenile-like" and seedling scions). Mature scions with higher silica content and lower starch content had lower graft take percentages.

Taxonomic implications were also noted. Graft take in 4 genera was compared to data published concerning the anatomical characters of tissue type and silica content. Those genera (Pouteria and Manilkara) with more abundant parenchyma (cell types that store starch and dedifferentiate readily to form callus) and lower silica content, are known to graft more readily. The genera Calocarpum and Chrysophyllum have higher silica content and less abundant parenchyma.

The results of this study indicate that optimal grafting conditions are the use of "juvenile-like" scions with a modified veneer graft in late October to early December or late March to early May (periods of warm days, cool nights, low rainfall and low relative humidity).

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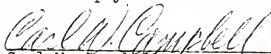
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BIOGRAPHICAL SKETCH

Mary Ann Hollingsworth Ogden was born November 25, 1943, in St. Petersburg, Florida. She attended the Florida State University (1961-63) and received the Bachelor of Science degree in environmental studies from Florida International University in March, 1978. She received a Master of Science degree from the Fruit Crops Department at the University of Florida in August, 1981.

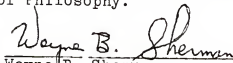
She has been employed as a seed analyst at the Florida Department of Agriculture, in Tallahassee, a park ranger in Everglades National Park, a park naturalist for Dade County Parks and Recreation, a consultant on native plants for the Environmental and Urban Center at the Florida International University and had a private consulting business for the native landscaping and identification of toxic and allergenic plants. She is a member of the following professional organizations: American Society for Horticultural Science, Florida State Horticultural Society, Tropical Region American Society for Horticultural Science, and the Society for Economic Botany.

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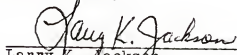
Carl W. Campbell, Chairman
Professor of Horticultural Science

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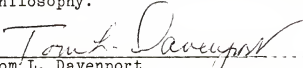
Wayne B. Sherman
Professor of Horticultural Science

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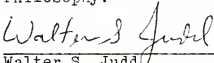
Larry K. Jackson
Professor of Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



Tom L. Davenport
Associate Professor of Horticultural Science

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Walter S. Judd
Associate Professor of Botany

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1984



Dean, College of Agriculture

Dean for Graduate Studies and Research