ANEXO 1

MATERIAL RECOPILADO DURANTE LA ACTIVIDAD DE FORMACIÓN

AVOCADO BREEDING AND SELECTION

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So far, successful avocado breeding has been primitive (2, 4). The leading cultivars around the world originated as chance seedlings-not even the female parent is known. This seems likely to change in the years ahead since procedures involving the known parentage of both sex cells are now available and are considerably more efficient. The use of such procedures is likely to markedly increase the chances for obtaining superior new cultivars by breeding.

The Flower and Its Behavior

The avocado flower is rather typical, but it functions in a peculiar manner. Intelligent breeding requires a clear knowledge of the unusual functioning, which can only be understood in terms of the structure (Fig. 1) (2, 3, 4).

Flower Structure

The perianth includes both sepals and petals. This grouping is especially appropriate in the case of the avocado, since its sepals (often green in other plants) and petals (often brightly colored) are almost identical. There are 3 of each, alternating, about 5 mm long, pale or greenish-yellow.

The stamens are 9 in number, arranged in an outer circle of 6 and an inner circle of 3. The inner 3 have a basal pair of nectar-secreting "nectaries" and alternate with 3 nectar-secreting "staminodes". Each stamen has 4 pollen sacs which release the mature pollen through valves hinged at the top. The pistil is in the center of the flower. It has an enlarged tip (stigma) on which the pollen germinates, then grows down through the long, slender style to reach and fertilize the egg inside the enlarged base (ovary). The fertilized ovary grows into the distinctive, delicately-flavored fruit prized around the world as the avocado.

The flowers are grouped in compound inflorescences of a few to several hundred flowers each. Unlike some plants, the avocado cannot possibly produce a fruit from each flower. In fact, if as few as 1% of the flowers mature fruit, the crop may still be too heavy for the tree.

Flower Function

Each normal avocado flower has both male and female organs. Most such plant species readily self-pollinate, *i.e.*, pollen from a given flower can fertilize the egg of that flower. However, the avocado flower performs in such a way that self-pollination is highly unlikely within a given flower and is difficult within a given tree or even a given cultivar. This is because each cultivar is functionally male one part of the day and functionally female another part of the day.

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Female flower. The first time an avocado flower opens, the pistil is alone in the center, with the stamens and other flower parts close together at an angle of 45° or more away from the pistil (Fig. 1). The stigma is then receptive to pollen so that the egg can be fertilized. The flower is female in function but it is not functionally male since the stamen valves remain tightly shut and no pollen is or can be shed. The flower remains open in this female stage for perhaps a couple of hours, then closes for the rest of the day and that night.

Male flower. The flower opens for the second and last time on the next day, but it is then functionally male, as the stamen valves open and pollen is released (Fig. 1). The stigma is commonly discolored or withered and is no longer receptive, so that the flower can no longer function as female. The flower remains open in this male stage for several hours, then closes again, permanently.

A and B flower types. Nearly all avocado cultivars (and seedlings) fall clearly into 1 of 2 contrasted categories conventionally designated A and B (16). A-type cultivars have their first or female opening in the morning, perhaps about 9 AM to noon. The second or male opening is the afternoon of the following day, perhaps noon to 6 PM. So, for a particular flower, the total time span from first opening to final closing is about 34 hours. B-type cultivars first open in the afternoon, perhaps 1 to 4 PM. The second opening is the following morning, perhaps 8 AM to 1 PM, so the total time span is about 24 hours.

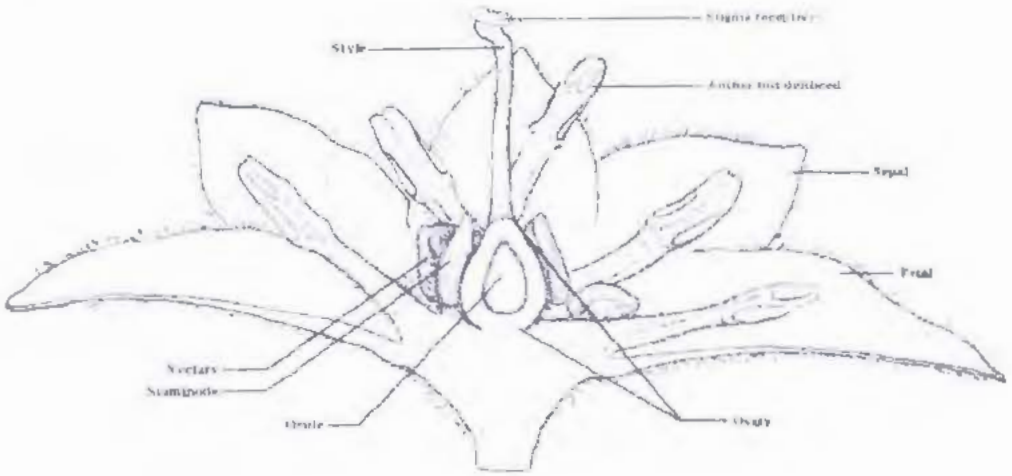
Daily synchronization. 'Hass' is an example of an A flower type, 'Fuerte' of a B. There may be a thousand 'Hass' trees, with perhaps a million flowers opening (first, female stage) each morning in the blooming season in a given climatic area. All of them will open at about the same time and close at about the same time-like a million reasonably accurate clocks. Similarly, each afternoon perhaps a million flowers will have their second or male opening, and will do so again about synchronously. Hence, opportunity for self-pollination will be very limited (but see tin-article by Gazit on pollination, p. 88). A thousand adjoining 'Fuerte' trees would behave the same way, but with the times of the male and female stages reversed.

Consequences of Avocado Flower Behavior

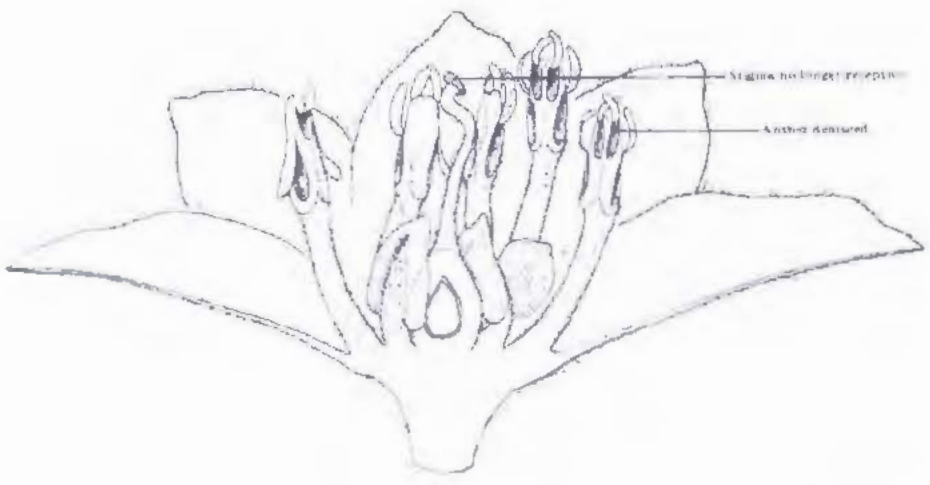
Cross-pollination is the inevitable result of the flower functioning described above. In our examples, 'Fuerte' is functionally male, *i.e.*, is shedding pollen over the entire period that 'Hass' is functionally female, *i.e.*, its pistils are receptive and must be pollinated if fruit is to set. The converse is true of 'Fuerte' set. Thus, A and B trees provide complementary cross-pollination.

Genetic variability within the individual is the inevitable result of cross-pollination. This means that any individual seedling or cultivar has different hereditary options at many different gene locations and can be expected to produce an almost unlimited assortment of sex cells for the next generation. Hence, avocados are more like humans than they are like tomatoes or other plants in which pure breeding lines produce any number of seedlings genetically identical to the parent. This means that in avocado breeding, we do not have to hybridize cultivars in order to obtain segregating variability from which to select—each cultivar has immense genetic variability.

Self-pollination. Our analysis of flower functioning showed self-pollination to be difficult, but it is by no means impossible. Although self-pollination within a flower practically never occurs, pollination among flowers of a single cultivar occurs more readily. There is a little variability in time of flower opening or closing due to differences in location on tree (in terms of sun or wind exposure *etc.)*, or other causes of differences in internal physiology. Adjoining trees of the same cultivar would be expected to have this variability accentuated, plus possible effects from rootstock differences. Finally, weather changes can affect the timing of some flowers more than others. It is usually possible to obtain ample breeding progenies from self-fertilization.



A



B

Fig. 1. Schematic longitudinal sections of avocado flower. A, Female stage, with stigma receptive, but stamens bent outward and anthers not dehisced. B, Male stage, with stigma no longer receptive, but stamens upright and anthers dehisced. (Reproduced from Macgregor, S. E. 1976. Insect pollination of cultivated crop plants. U.S.D.A. Agr. Handb. 496. p. 93.)

Breeding Techniques

The technique to be used will depend on whether the breeder wishes to hybridize or self. Hybridization is the only way to obtain progeny with 2 or more desirable traits that are not present in an available breeding line. This is also true when one needs a trait intermediate between available superior lines, such as season of maturity or ecological adaptation. Indeed, the major cultivars being grown in Florida, Hawaii and some other areas with similar climates are first or later generations from hybridization of Guatemalan and West Indian races. The major cultivars being grown in California, Israel, South Africa, Australia and similar less tropical climates are hybrids of the Guatemalan and Mexican races. These latter 2 races are also the hybrid source of promising new cold-hardy selections for central and northern Florida (13).

Hybridization has 2 major disadvantages when compared with selfing: 1) obtaining positive hybrids is far more difficult and expensive and 2) the breeding worth of each parent tends to be obscured by the contribution from the other parent. Nevertheless, a technique is described later for obtaining at low cost a considerable proportion of hybrid seedlings for situations where hybridization is indicated.

The avocado breeding program of the University of California at Riverside is using both approaches. Selfing has proven to be generally much more efficient, as seedlings can be obtained from isolated or buffered trees at a cost no higher than that of the fruits.

Breeding worth has proven to be only poorly correlated with commercial worth. For example, 'Fuerte' was used as the chief parent in extensive hybridizing during the early years of the California program. Not 1 commercial cultivar has resulted from all this work. Had the wrong lines been chosen as the other parent? Several hundred 'Fuerte' selfs quickly showed the true situation: they were a remarkably poor lot (7), indicating that 'Fuerte' is a highly undesirable breeding parent for California and similar regions.

'Hass', on the other hand, has proven to be an exceptionally good parent (6). Its selfed seedlings have averaged as superior in terms of productivity and quality as the 'Fume' selfs were inferior on both counts. From about 400 trees of each, there were no 'Fuerte' selections and 18 'Hass' selections.

Further selfing of 'Hass' selections has further increased the proportion of seedlings worth selecting. More than a quarter of the seedlings in one line were actually considered to merit commercial testing by the third selfed generation—a remarkable 6-fold increase over the impressive first generation of 'Hass' selfs. Unfortunately, while larger fruit size and green color have been obtained, none of the selections so far has appeared to achieve the outstanding 'Hass' standards of flavor, long season and unblemished surface.

Hybridization by Hand

This approach may be prohibitively expensive in terms of human labor (2), since about 99% of the flowers will fail to mature fruit and there is currently no way to differentiate those that are going to make it. However, methods for increasing the

success proportion will be suggested in a later section. Avocado pollen is not collectable by the usual suction methods. Flowers in the male stage may be picked off and the pollen clumps daubed onto female-stage stigmas directly or a fingernail can be used to remove the pollen and transport it. Emasculation of the female flower is unnecessary (2).

In a greenhouse. A few dozen avocados have matured on such trees growing in large containers or directly in the soil. Tree care costs are greater, but conditions can be made much more conducive to fruit set. Since bloom is usually considerably earlier indoors, one can hybridize lines with discrete blooming periods by establishing the later-blooming parent under glass.

In sleeves. Sleeves made of plastic (usually) 1 x 0.5 m are useful to enclose a flowering branch. The material should let in as much light as possible. Sleeves can also be used to protect the male parent's pollen from bees.

In field cages. Instead of enclosing a flowering branch, field cages enclose the whole tree. Plastic screening on wooden frames of convenient size are bolted or otherwise fastened together to form the walls and a loose screen is put over the top and tied down. A zippered or hinged entrance-way should be provided.

Hybridization by Bees (or Other Pollinating Insects)

Seedlings produced here will probably be both selfs and hybrids in a varying and unknown mix. The majority should be hybrids with the preferred parental choice of 1 A and 1 B flower type. Also, the very different parental types that are usually present when hybridization is desirable should make it possible to segregate selfs from hybrids—at least in the fruiting stage. Hybrids produced by insects cost a tiny fraction of those produced by hand. The honeybee accounts for the majority of avocados set in California but other insects apparently predominate in more tropical areas.

In field cages. These can be made longer in order to include 2 adjoining trees that one wishes to hybridize or there may be only 1 tree with branches of the second parent worked into it. A hive of bees is placed inside the cage with a source of water after both parents have started to bloom.

In isolation. This approach is not at all expensive. Indeed, it can be as simple as harvesting the fruits from adjoining branches of 2 desired parents in the field. They may be from contiguous trees, or grafts of the complementary parent may be worked into established trees in a solid grove. No third parent should be closer than perhaps 100 m, although considerably closer distances appear safe under California conditions.

Selfing Techniques by Hand

Situations where selfs would be worth the cost in human labor must be exceedingly rare—no such situation has arisen in the University of California program. They could be obtained if field selfs were impractical and if selfs were that valuable or labor that cheap. One would want to increase the likelihood of male-female overlap by treating part of the tree or a second tree differently, such as by shading or by reflected light or heat. In the absence of bees, pollen would be more likely to hang on the valves into the next female-flower opening. The flower could be

protected in greenhouse, sleeve or cage, as in the case of hybridizing.

Selfing Techniques by Insects

In a cage. A tree is caged and a hive of bees placed inside, as described for hybridizing. Fruit set has been variable to heavy. Evidently the strict sex alternation has had appreciable exceptions under these conditions, or possibly the closely contained bees have forced open flowers that were near enough to maturity to function sexually.

Isolated trees. This is perhaps the most desirable method for most avocado breeding because of its ready availability and low cost. The source can be lone avocado trees or trees that are isolated from other genetic lines by buffer trees of the line to be selfed. Out-crossing should be practically nil with 100 m or more isolating distance.

Maximizing the Breeding Set

General Procedures to Increase Seed Yield

There are various means to increase the number of breeding fruits obtained from selfing and hybridizing.

Heavy-setting cultivars should be selected as the seed parent in hybridizing. Desirable traits may thus be efficiently introduced into highly productive lines by using their light-bearing carriers as the male parent. In selfing, it may be necessary to use lines with less fruit set in hope that better setters will segregate out with other virtues intact or that more genetically uniform lines for use as male parents as described above will be obtained.

The productive ("on") year can make a light bearer set well and a heavy bearer set very heavily. Nearly all avocado lines are subject to alternate bearing. If the natural pattern of alternation does not fit in with the breeder's convenience, he can usually create an upcoming "on" year by removing all set fruits by midsummer or earlier.

An optimum location in terms of climate and other factors may make the difference between a breeding program with plenty of material from which to select and one that suffers from the fatal flaw of insufficient controlled-parentage seedlings. Breeding avocados is indeed a long-range program. It may be best to delay its start if to do so makes possible a location that ensures an adequate number of progeny.

Optimum care is important to permit the realization of the above potentialities. The breeder should understand and apply sound principles and practices of horticulture, soil science, irrigation, fertilization, wind and frost protection, disease and insect control or have his trees looked after by someone else. Fruit set is poor when trees arc in stress situations.

Maximum light availability is important to maximize avocado fruit set. Southern exposure is very helpful in more-northerly avocado regions and the reverse is true south of the equator. The breeder can often select his particular breeding trees with this in mind. Adjoining avocado or other less valuable trees can be cut back

as needed. The materials used for sleeves and cages should transmit as much light as is consistent with safety from pollinating insects. Increased cooling capacity in the greenhouse may be a good investment to permit less roof shading.

Girdling often increases fruit set, and it increases fruit number more than total weight (14). Hence, it offers more of an advantage to the breeder than to the commercial grower. Girdling may be an excellent investment, especially with light-setting lines or with a major cost undertaking such as in hand hybridization.

Specific Means to Increase Seed Yield in Hybridization

Different large-scale hand-hybridizing programs have-had success rates (in terms of pollinated flowers that mature fruit) ranging from less than 0.04% to about 5% (3). The later remarkably high order of magnitude is made-possible in part by the general procedures described above. Further increases in percentage take are attributable to techniques involving the hybridization process itself.

Optimum weather is worth waiting for. Hot and dry conditions can cause desiccation of both the pollen and the pistil. Cold weather may inhibit proper sex cell functioning. A climate (like California's) that is generally cooler than optimum during the blooming period delays most fruit set until the latter part of the blooming season so the breeder should concentrate his efforts accordingly.

Pollinate few flowers per cluster. Each cluster can mature only a very small proportion of its flowers. Thus, excess hybridization is not only time-wasting, but can actually lead to reduced set via excessive fruit drop resulting from competition among the developing fruits. However, one should hybridize more than the number of flowers estimated to be the maximum that the cluster can mature, as defective ovules (18) and other problems will reduce the theoretical set. It has been suggested that the excess flowers should be removed a few days prior to hybridizing to increase chances of set by reducing flower competition. Statistical advantage has, to my knowledge, never been shown and it would seem uncertain that the limited gain would be worth the labor involved.

Pollinate at the first opening only. Occasional stigmas look fresh and receptive at the second (male) flower opening, but no second-day pollination has proved successful, to my knowledge—the stigma may be less receptive than it looks, or the internal egg apparatus may be degenerating.

Never pollinate an abnormal pistil. A considerable proportion of the female organs are deformed or otherwise aberrant to varying degrees. Some stigmas are darkened or withered or otherwise unhealthy looking, even at the first flower opening. The odds against successful hybridization are high enough for the best of flowers without wasting time on inferior ones.

Pollinate by mid-afternoon. This applies especially to a climate like that of California where the temperature, especially toward evening, is well below optimum for avocado flowers. Later pollination will cause temperature-induced slower pollen tube growth. Instead of reaching the egg in about 3 hours (Shmuel Gazit, personal communication), the sperm may not arrive until the egg has broken down. Other parts of either sex cell may degenerate in the meantime.

Breeding Objectives

Avocado breeding objectives (Table 1) vary somewhat on some points among the different producing regions and there is not full agreement on some points, even among the avocado people of a given region.

Table 1. Avocado breeding objectives.

Fruit quality	
Medium size	Thick ovate shape
Uniformity	Pulp
Skin	Proper softening
Medium thickness	Appetizing color
Readily peelable	Absence of fibers
Insect, disease resistance	Pleasing flavor
Free from blemishes	Long shelf life
Attractive color	Slow oxidation
Long tree storage	Chilling tolerance
Seed	High oil content
Small	High nutritional value
Tight in its cavity	

Shoot qualities	
Spreading habit	Tolerant of chlorosis
Easy to propagate	Tolerant of other stresses
Strong grower	Short fruit maturation period
Tolerant of pests and diseases	Precocious
Tolerant of wind	Regular bearing
Tolerant of cold	Wide adaptability
Tolerant of heat	Heavy bearer
Tolerant of salinity	

Rootstock qualities	
Conducive to high quality fruit	Easily grafted
Conducive to healthy, productive trees	Tolerant to *Phytophthora* and other organisms
Free from sun-blotch	Tolerant of salinity
Dwarfing or semi-dwarfing	Tolerant of chlorosis
Genetically uniform	Tolerant of drought
Hardy and vigorous	Tolerant of other adverse soil conditions
Easily propagated	

Fruit Qualities

Medium size. The markets in which most California avocados are sold prefer a size about 260 g. The optimum range might be 200-300 g (Jack Shepherd, private communication). Israel's European markets like a slightly larger fruit (5). The markets for fruits grown in more tropical regions where the West Indian race is

adapted have come to favor considerably larger fruit size. The breeder will need to give careful consideration to present and potential size preferences of the markets for which he is breeding.

Thick ovate shape. While a round shape may be more efficient, most avocado markets have come to associate the fruit with a somewhat pear or at least ovate form. New selections would have to have outstanding compensating traits to be able to disregard this established market preference.

Uniform size and shape. Variation in both traits among available cultivars may help to meet individual consumer preferences and identify for the consumer individual differences in flavor and other qualities. Variation within a given cultivar should be minimized. Moreover, fruit variation increases marketing problems. Seedlings and cultivars vary in the degree to which shape is altered by climatic differences and to which size is altered by fruit clustering differences.

Medium skin thickness. The skin should be thick enough to provide good protection under normal shipping and handling and to permit good peeling. Too thick a skin is undesirable (11) as it may make the detection of ripeness difficult and represents more wastage.

Skin peelability. The importance of peelability depends on how the fruit is eaten. Peeling doesn't matter when the flesh is scooped out of the skin—for example, eaten in the half-shell or smaller segments. However, avocados are eaten in various ways that involve pre-peeling (11). Thus, peeling reduces flesh attractiveness, as in salads, and always means more wastage.

Resistance to pests and diseases. These problems vary greatly in severity among the world avocado-growing regions, so the breeder's concerns will vary accordingly. A thicker fruit skin is desirable where fruit flies are a problem. I do not know of reported genetic differences in resistance to other fruit insects. Fungal diseases are of little concern in California but they are a serious consideration in more tropical areas (16) and potential breeding lines differ markedly in resistance.

Free from blemishes. Appearance may be much more important than flavor in determining consumer acceptance. The detrimental effect of surface flaws on retail purchases of avocados has been clearly shown (19). Tendency to skin russetting varies widely at different locations and in different breeding lines (17).

Attractive skin color. Color preferences depend largely on prior familiarity. The green color of 'Fuerte' has been the "right" avocado color in the extensive markets long dominated by that variety and its relative smoothness has made a rough skin less desirable in the same markets. These are 2 reasons that 'Hass' is less acceptable in the French market served by Israel (Shimon Zackai, private communication). In some markets, dark fruit is preferred. Apart from actual color, or skin blemishes, general attractiveness varies considerably and is significant (15).

Long tree storage. The longer edible fruits can be left on the tree without dropping or deteriorating, the more favorable is the marketing potential. The more quickly fruit mature, the shorter the tree storage period usually is. 'Fuerte' is an early-maturing cultivar with an exceptionally long storage period; unfortunately, for other reasons,

it is an inferior parent. 'Hass' passes its long storability on to a high proportion of its seedlings.

Small seed. Cost per edible portion is a frequent criticism of the avocado (11, 15). Seed proportion of total fruit weight varies markedly, but has high heritability in some lines (my unpublished data). Guatemalan race genes can add this desirable trait to the less favored backgrounds of the other 2 races.

Tight seed. The seed should completely fill its cavity. Moreover, the outer coat of loose seeds will often adhere to the flesh, thereby wasting edible flesh and the consumer's time.

Proper flesh softening. The entire flesh should soften simultaneously. In some regions, especially late in the season, 'Fuerte' and other partly Mexican-race cultivars have problems with this (Shmuel Gazit, private communication). A seedling with a marked degree of this weakness should probably be discarded.

Appetizing flesh color. Consumer preferences vary, but most people find less appeal in pulp that is nearly white, or mostly green, or has a dull appearance, or has discoloration under the skin.

Minimal fibers. Flesh is also less appetizing when it is traversed by fibers that are distinct because of a dark or reddish color. Indistinct fibers can be objectionable because of toughness when eaten.

Pleasing flavor. Nothing is more subjective than flavor. Prior familiarity influences present avocado preference, as with size, shape and color preferences. Still, most consumers agree that certain lines have too bland, or too strong, or otherwise too objectionable a taste.

Long shelf life. Once picked, the avocado is much more difficult to store or ship than such fruits as the apple. Some lines ripen (soften) in 3 or 4 days while others at a comparable stage of maturity may take 2 weeks to ripen. Ripe avocados deteriorate much more rapidly at room temperature. Some lines will remain edible only about a day, but others have the advantage of remaining in reasonably good condition for as long as 3 or 4 days.

Slow oxidation. This is another aspect of keeping quality. Cut surfaces of most avocados discolor rapidly— often within minutes. The Israeli cultivar 'Horshim' maintains a fresh appearance much longer. I have seedlings that do not discolor noticeably for several hours.

Chilling tolerance. A final aspect of keeping ability, this refers to proper softening, without flesh browning, following prolonged cold storage. 'Hass' is outstanding and 'Nabal' nearly as good; most West Indian lines and many lines derived from the other 2 races tolerate chilling poorly. This trait is especially important in the avocado because of its generally short shelf life.

High oil content. This is important in California because of a legal 8% minimum limit on fruit grown and marketed within the state. Oil content affects flavor and there are personal preferences as to whether or not high levels are pleasing.

High nutritional values. Higher oil content usually means higher calorie count—which

is desirable or undesirable depending on whether one's problem is obtaining adequate nourishment or avoiding overweight. Higher oil content is correlated with higher content of some vitamins. Other cultivar differences in vitamin content are independent of oil level (10).

Shoot (Tree) Qualities

Spreading tree habit. Tall trees are more difficult and more expensive to pick and to spray and are more susceptible to wind injury.

Easy to propagate. This is a composite of good bud formation, compatibility with the proper rootstocks and good graft-uniting ability. Propagation difficulty may make an otherwise good selection prohibitively expensive (8).

Strong grower. Grafts may take readily enough and yet the resulting trees grow too poorly for commercial success. Several cultivars derived from the Guatemalan race have had this serious drawback.

Tolerant of pests and diseases. These tolerances have so far been a minor consideration in California, but are important in some avocado-growing regions.

Tolerant of wind. Wind resistance is advanced by stronger wood and wider crotches in addition to a low, spreading habit as noted above. Strong crotches also minimize limb breakage from heavy fruit set.

Tolerant of cold. This virtue enables the avocado to be grown in colder areas and provides a safety factor against occasional freezes in less rigorous areas. There is some variation in cold tolerance within each horticultural race and wide variation among the races. West Indians may be injured even above freezing while Mexicans may tolerate negative 9°F or more of frost. Knight (12) has used artificial freezing tests in avocado breeding, as have other Florida researchers.

Tolerant of heat. In California, Mexican-race lines have appeared somewhat more hardy to heat than most Guatemalan lines. The West Indian race tolerates much heat where the humidity is consistently high.

Tolerant of salinity. The rootstock is more important limn the scion in this regard, but the superiority of the West Indian race as stock has been observed also in racial comparisons of the lops (9).

Tolerant of chlorosis. The rootstock is the major determinant, but, differences in susceptibility have been observed among California cultivars.

Tolerant of other stresses. Differences have been noted among potential breeding lines in terms of resistance to nutritional deficiencies and to miscellaneous environmental stresses.

Short, fruit maturation period. It is desirable for all fruits on a tree to mature about simultaneously in order to permit the picking of all fruits at one time.

Precocious. This aspect of productiveness is important in order to permit an early return on investment. Moreover, earliness and heaviness of fruit yield are partly correlated.

Regular bearing. Perhaps all California cultivars tend to alternate in the amount of fruit set year after year, but they do so to markedly different degrees. Alternation on a regional basis *(e.g.,* 'Fuerte') is especially serious because of the added marketing problems that result from sharply fluctuating crops. Alternation that is mainly on an individual tree basis *(e.g.,* 'Hass') largely avoids this difficulty. Even so, alternate bearing has the weaknesses of added limb breakage, sunburned fruits and branches and overall set that is below the physiological maximum.

Wide adaptability. While factors like tolerance of climatic extremes and other stresses may limit some cultivars to certain areas, there is an additional factor of differing adaptability. Some lines perform and bear well in more diverse locations than do other lines. The more limited is adaptability, the larger the number of cultivars required and so the greater the marketing problems.

Heavy bearer. If this virtue is present, many other weaknesses may be tolerable. If it is absent, all other virtues may be futile.

Rootstock Qualities

Conducive to high-quality fruit. Differential rootstock effects are known in other fruits. Careful study may identify such in the avocado.

Conducive to healthy, productive trees. Major root-stock effects on scion performance have been identified by Ben-Ya'acov in Israel and complex rootstock-scion interactions also exist (1). Cultivars in California have made up to twice the growth on Guatemalan as on Mexican rootstocks (my unpublished data). Rootstocks from seeds of the West Indian 'Waldin' cultivar are proving inferior for California's relatively cool winter soils.

Free from sun-blotch. Genetic resistance to this virus disease is unknown. Hidden carriers of it must be avoided as female parents in rootstock breeding.

Dwarfing (or Semi-Dwarfing). Mexican-race lines have a somewhat dwarfing effect which has not been observed to increase the tree precocity or productivity but has produced trees somewhat less subject to wind injury and easier to pick. Stocks that cause true dwarfing could be a tremendous boon to the industry.

Genetically uniform. Rootstock segregation contributes to variable scion performance to an unknown but probably substantial degree. Complete uniformity can be achieved only by asexual propagation or, theoretically, by doubling the chromosomes of a haploid. Helpful approaches to uniformity should be possible by repeated self-fertilization.

Hardy and vigorous. West Indian rootstocks have major advantages in terms of resistance to salinity and chlorosis, but they are more difficult to grow in the less tropical California climate. Even Guatemalan lines are tender under some conditions, in comparison with the Mexicans. Quite apart from hardiness, some lines produce a much higher proportion of weak, slow-growing seedlings than other lines.

Easily propagated. This refers to the ease with which the rootstock itself can be multiplied. A conflict arises with the desired quality of genetic uniformity noted above, as clonal stocks are the only way to achieve uniformity within a reasonable time, but

they are far more expensive to propagate. There are wide differences in fruit setting ability even among seed-propagated stocks, *e.g.*, 'Duke' selections for superior *Phytophthora* resistance have been shy bearers.

Easily grafted. Some progeny sets grow to graftable size more quickly than others. Some, for unknown physiological reasons, take more readily when grafted. Some Guatemalan cultivars have a failure rate many times as great on healthy, strong Mexican seedlings as on comparable-appearing Guatemalan seedlings.

Tolerant of Phytophthora *and other organisms.* For California and some other avocado regions, *Phytophthora* resistance is the paramount rootstock *desideratum*. Several lines with some resistance are known, but none has enough resistance to be a commercial solution. Resistance to nematodes and other harmful soil organisms would be advantageous.

Tolerant of salinity. Harmfully high salt concentrations can be present in the soil or brought in with irrigation water. Rootstocks that translocate less salts to the tree top are desirable in California, Texas, Israel and other areas with salinity problems (5). More and more California avocados have been grafted on West Indian seedlings for this reason—but with subsequent problems as noted above. Guatemalan stocks average superior to Mexican, but there is variation within both races.

Tolerant of chlorosis. Iron chlorosis can injure or even kill trees (5) under conditions of high lime content, or of other soil conditions that provide sub-optimal rootstock functioning. Again, West Indian stocks are most resistant; Guatemalans are most susceptible.

Tolerant of drought. Any advantage here would lower production costs, reduce the need for critical cultural care and permit wider distribution of avocado production. Trees have been reported to suffer less water stress on Guatemalan than on Mexican stocks (private communication from 2 growers).

Tolerant of other adverse soil conditions. 'Irving' seedlings have proven especially inefficient in extracting nitrogen from the soil (my unpublished data). Differences in tolerance of diverse soil limitations may prove to be common when critical tests are made. Superior rootstocks could reduce production costs and permit higher yields.

Summary

The avocado flower has both male and female organs, but the female organ functions only on 1 specific day and the male organ of that flower functions only on the following day. Moreover, either all female-functioning flowers of a cultivar are open only in the morning and all male-functioning flowers in the afternoon (A type) or, conversely, the open flowers are female-receptive in the afternoon and shedding pollen in the morning (B type). This makes cultivar self-pollination usually rare. Cross-pollination of 2 A or 2 B cultivars is also difficult, although often to a lesser degree. An A and a B cultivar together provide ideal complementary cross-pollination. Therefore, each avocado cultivar is highly variable (highly heterozygous) genetically. This means that a specific cultivar may itself provide all

of the seedling variability needed for selection of superior cultivars. Self-fertilization is the indicated avocado breeding approach, since it provides the least expensive seedlings and it effectively tests the breeding worth of a parent. Nevertheless, for some breeding and especially for subsequent breeding, hybridization may be desirable.

Hybridization may be prohibitively costly in terms of hand labor, but it can be done in a greenhouse or outside in plastic mesh sleeves or cages. Bees or other pollinating insects will do the hybridizing at a tiny fraction of the cost per hybrid, with the proper setup. However, they will probably simultaneously produce a smaller proportion of self-pollinations, to be differentiated only in the later seedling stage. Hybrids can be obtained from adjoining trees of the 2 parents, or branches of 1 worked into the other. The tree(s) may be isolated, or may be protected from nearby third parents by a cage with bees placed inside.

Self-fertilization by hand will only rarely be desirable, but it can be carried out in a greenhouse, sleeve or cage. Self-fertilization by insects is the heart of an efficient avocado breeding program. As with hybridization, the tree(s) may be isolated or enclosed with bees in a cage.

For any breeding procedure, seedling numbers can be increased by using heavy-yielding parent(s), working in the "on" year in the case of alternate bearing, growing trees in the best location and giving them the best care. The tree should receive maximum light. Girdling may markedly increase the number of fruits. Some additional ways to increase the yield of hybrid seedlings are to pollinate during optimum weather, pollinate few flowers per cluster, pollinate the first or female opening only, avoid any abnormal pistil and avoid late afternoon pollination.

Breeding objectives for the fruit include: medium size (about 260 g for the California markers); thick, ovate shape; uniform size and shape; skin of medium thickness, peelable, resistant to pests and diseases, free from blemishes, with attractive color; long tree storage life; small, tight seed; flesh that softens uniformly, has an appetizing color, a minimum of objectionable fibers, long shelf life, slow discoloration when cut, tolerance of chilling in storage, a high oil content in certain circumstances, high vitamin content and other nutritional values.

Desirable shoot qualities include: spreading tree habit; easy propagation; strong growth; tolerance of pests, disease, wind, cold, heat, salinity, chlorosis, and other stresses; maturing its fruits over a short period; precocity; annual productivity; wide adaptability; heavy yielder.

Rootstock qualities to aim for include: promoting quality fruit on productive trees; freedom from sun-blotch; dwarfing; uniformity; hardiness and vigor; easy propagation and graftability; tolerance of *Phytophthora,* salinity, chlorosis, drought and other adverse conditions.

Literature Cited

1. Ben-Ya'acov, A. 1976. Avocado rootstock-scion relationships: a long-term, large-scale research project. V. Final report on some orchards planted during the years

1960-1964. *Calif. Avoc. Soc. Yrbk.* 59:122-133.

2. Bergh, B. O. 1969. Avocado. *In:* Outlines of perennial crop breeding in the tropics, F. P. Ferwerda and F. Wit, ed. Wageningen, Netherlands, p. 23-48.
3. _____. 1974. The remarkable avocado flower. *Calif. Avoc. Soc. Yrbk.* 57:40-41.
4. _____. 1975. Avocados. *In:* Advances in fruit breeding, J. Janick and J. N. Moore, ed. Purdue Univ. Press, West Lafayette, IN. p. 541-567.
5. _____. 1975. Avocado research in Israel. *Calif. Avoc. Soc. Yrbk.* 58:103-126.
6. _____ and R. H. Whitsell. 1974. Self-pollinated 'Hass' seedlings. *Calif. Avoc. Soc. Yrbk.* 57:118-126.
7. _____ and _____. 1975. Self-pollinated 'Fuerte' seedlings. *Calif. Avoc. Soc. Yrbk.* 58:128-134.
8. Coit, J. E. 1957. Avocado varieties. *Calif. Avoc. Soc. Yrbk.* 41:37-42.
9. Cooper, W. C., A. Peynado, N. Maxwell and G. Otey. 1957. Salt tolerance and cold-hardiness tests on avocado trees. *J. Rio Grande Valley Hort. Soc.* 11:67-74.
10. Hall, P., J. G. Moore and A. F. Morgan. 1956. B vitamin content of avocados. *Calif. Agr.* 10(11): 13-14.
11. Hochstim, E. S. 1958. Homemakers appraise citrus products, avocados, dates and raisins. U.S.D.A. Agr. Res. Rept. 243. 33p.
12. Knight, R. J., Jr. 1974. The potential of cold-tolerant avocado introductions in breeding for enhanced winter hardiness. *Proc. Fla. State Hort. Soc.* 87:348-353.
13. Krezdorn, A. H. 1975. An expanded variety improvement program. *Proc. Fla. State Hort. Soc.* 88:460-462.
14. Lahav, E., B. Gefen and D Zamet. 1972. The effect of girdling on fruit quality, phenology, and mineral analysis of the avocado tree. *Calif. Avoc. Soc: Yrbk.* 55:162-168.
15. Manley, W.T. and M. R. Godwin. 1962. The consumer market for Florida avocados. Fla. Agr. Expt. Sta. Bul. 625. 54p.
16. Ruehle, G. D. 1963. The Florida avocado industry. Fla. Agr. Expt, Sta. Bul. 602. 102p.
17. Storey, W. B., B. O. Bergh and R. H. Whitsell. 1974. Factors affecting the marketability of avocado fruit. *Calif. Avoc. Soc. Yrbk.* 57:33-39.
18. Tomer, E., M. Gottreich and S. Gazit. 1976. Defective ovules in avocado cultivars. *J. Amer. Soc. Hort. Sci.* 101:620-623.
19. Williams, F. W., D. L. Brooke and W. B. Riggan. 1962. The effect of price variation, skin blemish and firmness on retail sales of Florida avocados. Fla. Agr. Expt. Sta. Bul. 645. 38p.

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Evaluation of Avocado Germplasm Using Microsatellite Markers

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Abstract. **Three horticultural races of avocado (*Persea americana* Mill.) are known: Guatemalan, Mexican, and West Indian. Each race has unique characteristics and current commercial varieties have been selected from within the races or from interracial hybrids. Using 14 microsatellite loci we investigated the genetic variation among 224 accessions (394 plants) maintained at the National Germplasm Repository (NGR) in Miami, Fla., and a set of 34 clones from the University of California South Coast Field Station (SCFS) located in Irvine, Calif. The 14 microsatellite loci had an average of 18.8 alleles per locus and average unbiased genetic diversity was 0.83. The total propagation error in the collection, i.e., plants that had been incorrectly labeled or grafted, was estimated to be 7.0%. Although many unique alleles did exist, no useful race-specific markers were found. A general concordance between the horticultural race and the clusters obtained from molecular data was observed. Principal Coordinate Analysis (PCA) grouped the Guatemalan and Mexican races into two distinct clusters. The West Indian also grouped into a unique major cluster but with an outlying group. Using the PCA a change in the racial designation or interracial hybrid status for 50 accessions (19.7%) is proposed. The unbiased gene diversity estimate was highest in the Mexican and Guatemalan races and lower in the West Indian group. This demonstrates the need to collect more of the West Indian germplasm to broaden the genetic diversity and to emphasize the identification of individuals conferring resistance to Phytophthora Root Rot (PRR).**

The avocado (*Persea americana* Mill.) is an evergreen subtropical tree that is native from Mexico to northern South America and produces a fruit that is unique and nutritious. This fruit was known by the Aztecs as ahuacacuauhitl, which was later shortened by the Spaniards to aguacate. In the United States avocado was introduced into Florida in 1833, California in 1848 and to Hawaii by 1855 (Nakasone and Paull, 1998). Major commercial production of avocado in the United States is limited to California and Florida. In 2000, global production exceeded 2.4 MMT and the major producers were Mexico, Indonesia, South Africa and the United States (Anonymous, 2001).

P. americana has been subdivided into three horticultural groups: Mexican [*P. americana* var. *drymifolia* (Schect. & Cham.) Blake], Guatemalan (*P. americana* var. *guatemalensis* Wms.) and West Indian (*P. americana* var. *americana* Mill.) races. The West Indian race is known to be from the lowland areas of the Pacific coast of Central America and not the West Indies, while the Guatemalan and Mexican races are native to specific highland areas in each country (Scora and Bergh, 1992). The collection at the NGR-Miami contains 224 accessions with all three races represented, as well as hybrids between them. The earliest introductions were collected by Wilson Popenoe in Guatemala in the 1920s, and the newest introductions were collected by Avraham Ben-Ya'acov throughout Central and South America during the 1990s (Ben-Ya'acov, 1995; Popenoe, 1920). The University of California collection at the South Coast Field Station (SCFS) in Irvine contains a large number of accessions and breeding lines mostly of the Mexican and Guatemalan races and mixed interracial hybrids. These two collections contain a comprehensive representation of the genetic diversity currently in avocado germplasm collections.

The three racial groups can be distinguished by the percentage oil content in the fruit with the West Indian cultivars ranging from 2.5% to 8.0%, Guatemalan accessions from 10% to 13%, and the Mexican accessions ranging from 15% to 20% (Knight, 2002). The racial classes also vary phenotypically for characters such as fruit size and shape, skin thickness, skin color, seed size, and fruit ripening (Lahav and Lavi, 2002). Avocado is a diploid with 2n = 24 (Garcia, 1975) and sterility barriers do not exist between or among the three racial types (Lahav and Lavi, 2002). Avocado has a distinct flowering habit known as protogynous, diurnally synchronous dichogamy (Bergh, 1969). This type of reproductive behavior promotes outcrossing; however, significant amounts of self-pollination are known to occur in commercial plantings (Davenport et al., 1994). Named cultivars often originate from open-pollinated seedlings. The pollen parent is unknown but has often been estimated based on the flower types of available donor trees. Many of the cultivars grown in Florida are interracial hybrids between Guatemalan and West Indian types while those grown in California are hybrids between Mexican and Guatemalan types (R. Knight, personal communication). Morphological characters have been used to infer parentage, although these characters are influenced by environmental factors and may not unambiguously distinguish closely related genotypes or interracial hybrids.

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Table 1. Listing of the 254 Avocado accessions evaluated with microsatellite markers by racial lineages, interracial hybrid groups, and changes in lineages based on the microsatellite analysis.

Cultivar	Race	MIA	Source
Akbal	Guatemalan	654	SHRS
Anaheim	Guatemalan		SCFS
Brooks Late	Guatemalan	22618	SHRS
Carlsbad	Guatemalan		SCFS
Choquette	Guatemalan	34962	SHRS
Gordo	Guatemalan		SCFS
Green Gold	Guatemalan		SCFS
64x23	Guatemalan		SCFS
Ithom	Guatemalan	657	SHRS
3241	Guatemalan		SCFS
3112	Guatemalan		SCFS
Julio	Guatemalan		SCFS
Kavish	Guatemalan	660	SHRS
L228	Guatemalan		SCFS
L339	Guatemalan		SCFS
Lamar	Guatemalan	646	SHRS
M59	Guatemalan		SCFS
NA251	Guatemalan		SCFS
Nabal	Guatemalan	658	SHRS
Nabal Seedling 1	Guatemalan	20713	SHRS
Nabal Seedling 2	Guatemalan		SHRS
Northrich a	Guatemalan	32160	SHRS
Simmons b	Guatemalan		SCFS
SN10	Guatemalan		SCFS
PI [illegible]	Guatemalan	35008	SHRS
PI37	Guatemalan		SCFS
Queen 3	Guatemalan	20722	SHRS
Reed	Guatemalan		SCFS
Taylor	Guatemalan	19262	SHRS
Williams	Guatemalan		SCFS
YX3	Guatemalan		SCFS
X311	Mexican		SCFS
(Sue X Kansas)	Mexican	21110	SHRS
Aduco No. 1	Mexican	4043	SHRS
Bacon	Mexican	20709	SHRS/SCFS
Bacon Seedling	Mexican	24429	SHRS
Brooksville Seedling 1	Mexican	25239	SHRS
Capac	Mexican	681	SHRS
Carchi	Mexican	635	SHRS
Celaya-25	Mexican	25875	SHRS
Chota	Mexican	639	SHRS
Del Oro #2	Mexican	18109	SHRS
Duke	Mexican	17461	SHRS
Duke Seedling	Mexican	17468	SHRS
Egas	Mexican	640	SHRS
Esperita	Mexican	34966	SHRS
Gainesville	Mexican	18431	SHRS
Gainesville Seedling	Mexican	17459	SHRS
Guayabarroba	Mexican	24544	SHRS
Hoyta	Mexican	32203	SHRS
Jessup	Mexican		SCFS
Irgamma seedling 2	Mexican		SHRS
Jim	Mexican		SCFS
J. P. Young	Mexican	32157	SHRS
Mayo	Mexican		SCFS
Mexicola	Mexican	32159	SHRS/SCFS
Miramar de Monte de Oro	Mexican	34957	SHRS
Morocco No. 42	Mexican	19327	SHRS
Morocco No. 43	Mexican	19328	SHRS
Naranjo No. 1	Mexican	24867	SHRS
Naranjo No. 2	Mexican	24868	SHRS
Pacific	Mexican	34964	SHRS
Provoco	Mexican	24535	SHRS
Puebla	Mexican		SCFS
Reina Victoria	Mexican	24535	SHRS
Salcedo No. 1	Mexican	19329	SHRS
San Rafael	Mexican	34898	SHRS

Cultivar	Race	MIA	Source
Seedless Mexican	Mexican	2980	SHRS
Stewart	Mexican		SCFS
Tamayo	Mexican	438	SHRS
Toltec	Mexican	14766	SHRS
Yopa Topa	Mexican		SCFS
Young No 1	Mexican	20728	SHRS
Young No 2	Mexican	20729	SHRS
Young Shipkin	Mexican	21330	SHRS
Young Special	Mexican	21331	SHRS
Zutano	Mexican		SCFS
Antigua	West Indian	14858	SHRS
Argenta	West Indian	21893	SHRS
Argui 3	West Indian	34894	SHRS
Arue Seedling 1	West Indian	23442	SHRS
Avocadosa 3	West Indian	34868	SHRS
Ayeock Red No. 3	West Indian	20022	SHRS
Aycock Red No 19	West Indian	20021	SHRS
Belize	West Indian	20023	SHRS
Bernecker	West Indian	24244	SHRS
Biscayne Seedling 1	West Indian	3353	SHRS
Biscayne Seedling 2	West Indian		SHRS
Biscayne Seedling 3	West Indian		SHRS
Biscayne Seedling 4	West Indian		SHRS
Biscayne Seedling 5	West Indian		SHRS
Biscayne Seedling 6	West Indian		SHRS
Biscayne Seedling 7	West Indian		SHRS
Biscayne Seedling 8	West Indian		SHRS
Biscayne Seedling 9	West Indian		SHRS
Catalina	West Indian	17248	SHRS
Catlin's Hawaii Seedling	West Indian	6896	SHRS
Dade	West Indian	673	SHRS
Donaldson	West Indian	20024	SHRS
Dora	West Indian	20025	SHRS
Dr Dupuis No. 2	West Indian	25071	SHRS
Family	West Indian	3895	SHRS
Fuchs	West Indian	31946	SHRS
Galvan	West Indian	34867	SHRS
General Bureau	West Indian	19321	SHRS
Hornfindo 1	West Indian	34872	SHRS
Jose Antonio	West Indian	17252	SHRS
Kilo 4 Ceiba	West Indian	20026	SHRS
Maxeras	West Indian	23271	SHRS
Orizaba 4	West Indian	34856	SHRS
Pollock	West Indian	19846	SHRS
Progreso 1	West Indian	34854	SHRS
Raul Arango	West Indian	20029	SHRS
Ruehle	West Indian	19848	SHRS
Seedless Avocado	West Indian	8485	SHRS
Simmonds	West Indian	679	SHRS
Simmonds Seedling	West Indian	3900	SHRS
Tantoyo 2	West Indian	34870	SHRS
Tela	West Indian	34853	SHRS
Tino Perla	West Indian	20031	SHRS
Town 2	West Indian	27195	SHRS
Trapp	West Indian	19949	SHRS
MIA 35729	West Indian	35729	SHRS
MIA 35734	West Indian	35734	SHRS
MIA 35732	West Indian	35732	SHRS
Waldin	West Indian	19850	SHRS
Wessel	West Indian	675	SHRS
Wilson Popenoe	West Indian	3357	SHRS
68366	GxM	20708	SHRS
68366	GxM		SHRS
Antigua Market	GxM	13959	SHRS
Catalina	GxM	17872	SHRS
Elmhurst	GxM	25993	SHRS
Estanzia	GxM	14304	SHRS/SCFS

Cultivar	Race	MIA	Source
Pierce Popenoe	GxM	34542	SHRS
Pingtom	GxM	23692	SHRS
Nemeth	GxM	24808	SHRS
Nordehuen	GxM	23093	SHRS
Pinkerton	GxM	31153	SHRS
Sharwil	GxM	21332	SHRS
Tenerife	GxM	17690	SHRS
Winter mexican	GxM	19260	SHRS
Agnes	GxWI	21878	SHRS
Borrego	GxWI	13250	SHRS
Choquette	GxWI	22683	SHRS
Collinred Seedling 10	GxWI	6889	SHRS
Collinred Seedling 82	GxWI		SHRS
Collinred Seedling 83	GxWI		SHRS
Collinson	GxWI	492	SHRS
Dunedin	GxWI	3896	SHRS
Gripina 12a	GxWI	24273	SHRS
Gripina 12b	GxWI		SHRS
Hanwasee	GxWI	28063	SHRS
Lula	GxWI	7928	SHRS
Melendez	GxWI	19770	SHRS
Melendez 2	GxWI	34005	SHRS
Monroe	GxWI	30852	SHRS
Semil 34	GxWI	24224	SHRS
Semil 43	GxWI	24225	SHRS
Somon	GxWI	19853	SHRS
Suarina	GxWI	17253	SHRS
Tormaje	GxWI	19847	SHRS
Urbano	GxWI	19768	SHRS
Winslowson	GxWI	3889	SHRS
Winslowson Seedling	GxWI	3888	SHRS
Yon	GxWI	19261	SHRS
Courreight	WIxM	32154	SHRS
Brooksville Seedling 4	Complex hybrid	25864	SHRS
Brooksville Seedling 5	Complex hybrid	25865	SHRS
Brooksville Seedling 6	Complex hybrid	25867	SHRS
Brooksville Seedling 7	Complex hybrid	25868	SHRS
Brooksville Seedling 8	Complex hybrid	25870	SHRS
Brooksville Seedling 9	Complex hybrid	26924	SHRS
Brooksville Seedling 10	Complex hybrid	30506	SHRS
Brooksville Seedling 11	Complex hybrid	30507	SHRS
Brooksville Seedling 12	Complex hybrid	30508	SHRS
Brooksville Seedling 13	Complex hybrid	30509	SHRS
Brooksville Seedling 14	Complex hybrid		SHRS
Brooksville Seedling 15	Complex hybrid		SHRS
Brooksville Seedling 16	Complex hybrid		SHRS
Brooksville Seedling 17	Complex hybrid		SHRS
Irving 120	Complex hybrid	20718	SHRS
Vero Beach (G.R.D.)	Complex hybrid	20636	SHRS
Vero Beach No. 01	Complex hybrid	18435	SHRS
Vero Beach No. 15	Complex hybrid	20638	SHRS
Vero Beach S.E.-7	Complex hybrid	20635	SHRS
P. subgigena		34889	SHRS
P. subgigena 2		34891	SHRS
P. schiedeana		34875	SHRS

Cultivar	Race	MIA	Source
Previously labeled as MxW			
Gottfried Seedling 1	West Indian		SHRS
Gottfried Seedling 2	West Indian		SHRS
Gottfried Seedling 3	West Indian		SHRS
Gottfried Seedling 4	West Indian		SHRS
Knorr	West Indian	19247	SHRS
Previously labeled as GxM			
68369	West Indian		SHRS
Tonsan	West Indian	17114	SHRS
Previously labeled as Mexican			
Ittamma Seedling 1	GxWI		SHRS
Brooksville Seedling 2	Complex hybrid	24305	SHRS
Brooksville Seedling 3	Complex hybrid	25856	SHRS
Gottfried	Complex hybrid	672	SHRS
Miramar de Monte de Oro a	Complex hybrid		SHRS
Previously labeled as West Indian			
Avocadosa	Guatemalan	34861	SHRS
Orizaba 3	Guatemalan	34871	SHRS
Gen. Francisco Rubles	Mexican	24534	SHRS
Orizaba 5	Mexican	34864	SHRS
Biscayne	GxM	26512	SHRS
Novitero	GxWI	34860	SHRS
Previously labeled as Guatemalan			
Apaka 1	Mexican	35695	SHRS
Key Largo	MxWI	22121	SHRS
La Piscina	Mexican		SHRS
Tertos	West Indian	650	SHRS
Dickenson	GxWI	2354	SHRS
Lima Late	GxWI	20034	SHRS
Collins	MxWI	21139	SHRS
Collins Seedling	MxWI	2897	SHRS
Queen	MxWI	20028	SHRS
MIA 35730a	MxWI	35730a	SHRS
MIA 35730b	MxWI	35730b	SHRS
MIA 35730c	MxWI	35730c	SHRS
Previously labeled as GxWI			
Sitio del Nino #5	Guatemalan	34961	SHRS
D.W.I. Benk	West Indian	19767	SHRS
Duvardin	West Indian	23345	SHRS
Gripina	West Indian	19769	SHRS
Previously labeled as Complex hybrid			
Marshfield	West Indian	20712	SHRS
Trapnon	West Indian	1276	SHRS
CRC 195-36	GxM	25873	SHRS
Hass	GxM		SCFS
Iriet	GxM		SCFS
Irving 59	GxM	20715	SHRS
Irving 65	GxM	20729	SHRS
Irving 78	GxM	20716	SHRS
Irving 96	GxM	20717	SHRS
Irving 134	GxM	20712	SHRS
Lamb Hass	GxM		SCFS
Teague	GxM	20714	SHRS
Yama 003	GxM	20724	SHRS
Yama 381	GxM	20726	SHRS
Yama 423	GxM	20727	SHRS
Hawaa	GxWI	3893	SHRS
Previously labeled as unknown			
"636"	Guatemalan	636	SHRS
MIA 35731	Mexican	35731	SHRS
MIA 35733	West Indian	35733	SHRS
"8456"	GxM	8456	SHRS
San Miguel	GxM	21965	SHRS
Alicia Cordero	GxWI	34965	SHRS
Pope	GxWI	32156	SHRS
Semil 38	GxWI	30455	SHRS
Argui I	Complex hybrid	34890	SHRS
Brogden	Complex hybrid	13303	SHRS
Egami	Complex hybrid	34892	SHRS
El Venado	Complex hybrid	34863	SHRS
Leyad Aro a	Complex hybrid	34893	SHRS
Leyad Aro b	Complex hybrid		SHRS
San Javier 1	Complex hybrid	34887	SHRS

Molecular markers are often used to clarify genetic relationships between individuals. In avocado germplasm evaluated using minisatellite markers, racial pattern differences were observed and fingerprinting of 26 cultivars was accomplished (Lavi et al., 1991). Mhameed et al. (1997) used variable number of tandem repeats (VNTR) markers to evaluate genetic relationships among 24 avocado cultivars that had been classified using morphological traits. The VNTR data supported the racial classification for most of the accessions. In another study, RAPD markers were used to evaluate 16 accessions representing the three races. Race-specific markers were identified and the similarity between races ranged from 53% to 58% (Fiedler et al., 1998). Similarly, Davis et al. (1998) used Restriction Fragment Length Polymorphism (RFLP) to evaluate a set of 36 cultivars from the University of California SCFS and their results were in agreement with the previous studies.

Microsatellite markers have been developed for avocado and utilized to produce a crude linkage map (Sharon et al., 1997). These markers have distinct advantages over the other types of molecular markers used to classify avocado germplasm. Their advantages include their abundance in most genomes, uniform distribution, hypervariability, codominance, and PCR-based protocols. Even though the genetic diversity analysis from the aforementioned studies sampled a small number of accessions, they have adequately demonstrated the usefulness of molecular markers in the classification of avocado germplasm.

Our objectives were to estimate genetic diversity within the large collection maintained at the USDA, NGR in Miami and a subset of the collection maintained at the SCFS by the University of California using microsatellite markers. Genetic diversity within and between populations of avocado was of interest, particularly within the West Indian race that is known to have genes involved with tolerance to phytophthora root rot (PRR). Another objective was to clarify the anecdotal information on parentage of mixed race hybrids and seedling selections from commercial cultivars. Since all plants (clones) of a given accession were genotyped, we also investigated the fidelity of germplasm propagation within accessions, as clonal collections are known to contain identical accessions with different names and mixtures of genotypes with the same name (Schnell et al., 1999).

Materials and Methods

Plant materials. Leaf material was sampled from the *Persea* germplasm collection at the NGR in Miami, Fla., and from the SCFS, University of California, Irvine, Calif. The number of plants genotyped for this study was 428. This included 254 accessions, 224 from the NGR-Miami and 34 from the University of California SCFS at Irvine with four cultivars common to both collections. Of the 224 accessions at the NGR, 104 accessions were represented by multiple plants. These 274 plants include 54 accessions with two, 37 accessions with three, 10 accessions with four, and three accessions with five plants.

In total, individuals of the following backgrounds were studied: 51 Mexican, 35 Guatemalan, and 65 West Indian, 100 interracial hybrids, and three related species. Hybrids were designated as follows: Complex (CH), Guatemalan x West Indian (G x W), Guatemalan x Mexican (G x M), and Mexican x West Indian (M x W). The out-group consisted of two accessions of *P. nubigena* and one accession of *P. schiedeana* (Table 1).

Table 2. Microsatellite loci and primers used in the analysis of the avocado germplasm collections developed by Sharon et al. (1997).

Locus	Repeat	Primers (5'-3')	Annealing temp (°C)	Alleles (no.)	Size range (bp)	Diversity [$\hat{H} \pm V(\hat{H})$]
AVAG05	$(AG)_{10}$	GGATCTTGATGTGTGGGGGAG				
		CCTGTCGGAAAAGACTATGCG	50	19	83–125	0.7906 (0.0394)
AVAG03	$(TC)_{17}$	GCACTTCCTAAACTTGCAGGT				
		CTGAACATCCAATGACAAACATCC	45	14	92–122	0.8414 (0.0551)
AVAG25	$(TC)_{14}$	ATGGTTTTTTCCTGCCCTTT				
		AACAAGCCCCCTAAAAGAA	50	20	96–140	0.8640 (0.0596)
AVAG13	$(CT)_{18}$	CTGCGATAACAACTGGAC				
		AACTAGGACCTGAAACCG	50	29	96–160	0.9150 (0.0912)
AVAG11	$(AG)_{20}$	AGCGATGAACATTACCA				
		ATTTCTTCAACCCATCTGTC	50	15	105–161	0.7553 (0.0388)
AVMIX03	$(TG)_{16}(AG)_{20}$	GATATTCCTGTTGTCACTGC				
		GATATTCCTGTTGTCACTGC	50	23	139–196	0.8912 (0.0718)
AVAG21	$(CT)_{22}$	TGTAAGTTTTAACCCCACAA				
		AATCACTATTAGAGTTTTCAGTCG	50	30	153–219	0.8911 (0.0729)
AVAG07	$(TC)_{15}$	ATCCAAAATGCACAAGGTGAGG				
		TGTCGCTATGTCCAAAATGTGG	50	8	98–114	0.6524 (0.2801)
AVMIX04	$(AG)_{12}(CAA)_5(ACAG)_{10}$	CCGTTTGCTTCCTGTATC				
		GTTATCCCTTCCACTTTC	50	19	158–194	0.8922 (0.0750)
AVAC01	$(TG)_{15}$	CTGGTTGCTCTCTTGTCTACATAATA				
		CGGTTTTGTAAGTTGATAG	40	16	95–185	0.8627 (0.0597)
AVMIX02	$(TC)_6(TCC)_3$	GAGTCACGCTCGTAGGCT				
		TATAAATTCAAATGACAC	40	10	147–135	0.7335 (0.0363)
AVAG06	$(CT)_{18}$	CGACCTCTTCTTATACTC				
		GTACCTCTGATAATGAGCAT	40	15	59–89	0.8625 (0.0629)
AVAG10	$(CT)_{22}$	GAATTACAAAGCACTAGAG				
		GTAGAAAGTGGGCACACAT	45	30	149–234	0.8695 (0.0607)
AVAG22	$(GA)_{15}$	GATCATCAAGTCCTCCTTGG				
		GATCTCATAGTCCAAATAATGC	55	16	96–130	0.8206 (0.0597)

DNA extraction was performed on 200 mg samples of leaf tissue using the Fast DNA kit (BIO 101, Inc.; Carlsbad, Calif.) and a cell disrupter (FastPrep FP 120; Savant Instruments, Inc.; Holbrook, N.Y.). The kit protocol for plant tissue was followed including the optional SPIN protocol. Tissue was homogenized using the Garnet Matrix and two ¼-inch spheres as the Lysing Matrix combination, at speed 5 for 30 s, repeated three times. DNA was quantified on a spectrophotometer (DynaQuant 200; Amersham Pharmacia; Piscataway, Calif.)

Microsatellite markers and PCR amplification. The microsatellite markers used in this study were reported by Sharon et al. (1997). Initially, 39 primer pairs were tested of which 14 were selected based on amplification consistency and level of polymorphism. Microsatellite locus name and primer sequence are listed in Table 2. PCR amplification reactions were carried out in a total volume of 10 μL, or 20 μL for multiplex reactions, containing 0.25 ng·μL^{-1} genomic DNA. All PCR reactions contained 0.025 U/μL Amplitaq (Applied Biosystems, Inc.; Foster City, Calif.), 0.2 mM dNTPs, 0.25 μM each forward and reverse primers, 1× GeneAmp PCR buffer (1.5 mM $MgCl_2$, 10 mM Tris-HCl pH8.3, 50 mM KCl, 0.001% (w/v) gelatin). Thermal cycling profile consisted of the following: 4 min denaturation at 94 °C; followed by 33 cycles of denaturation at 94 °C for 30 s, 1 min at appropriate annealing temperature for each primer (Table 2), 1 min extension at 72 °C; with a final 7 min 72 °C extension. PCR was carried out on a DNA Engine tetrad thermal cycler (MJ Research, Inc.; Watertown, Mass.). The following PCR multiplex reaction combinations were used: AVAG11 and AVMIX03, AVAG21 and AVAG07, and AVMIX02 and AVAG06, all other primer pairs were run individually.

Electrophoresis. Capillary electrophoresis (CE) was performed on an genetic analyzer (ABI Prism 3100; Applied Biosystems, Inc.) using Performance Optimized Polymer 4 (POP 4, Applied Biosystems, Inc.). Samples were prepared immediately before electrophoresis by adding 1 μL of PCR product to 12 μL of deionized formamide and 0.1 μL of GeneScan 500 ROX size standard (Applied Biosystems, Inc.), then denatured at 95 °C for 5 min, and chilled on ice. PCR products and size standards were doubled for preparation of multiplex PCR amplifications. Samples were injected electrokinetically at 3 kV for 10 s and were run at 15 kV and 60 °C for 25 min. Resulting data were analyzed with GeneScan 3.7 (Applied Biosystems, Inc.) for internal standard and fragment size determination. Allelic designations were ascertained using Genotyper 3.7 (Applied Biosystems, Inc.).

Data analysis. Gene diversity values for each locus and averages across all loci for the three races and interracial groups were calculated using Nei's (1987) unbiased estimate $\hat{H} = n(1 - \sum p_i^2)/n - 1$, where n = number of individuals sampled, p_i is the frequency of the ith allele. The variance of this statistic was calculated as $V(\hat{H}) = 2/[(n-2)[\sum p_i^3 - (\sum p_i^2)^2] + \sum p_i^2 - (\sum p_i^2)^2]$.

Unbiased gene diversity (H_{nb}) and observed heterozygosity (H_{obs}) were estimated from the allele frequencies of the Guatemalan, Mexican, and West Indian populations as well as CH, G x M, G x W, M x W, and the *Persea* spp. group, at each locus using GENETIX ver. 4.0 (Montpellier, France).

The principal coordinate analyses (PCA; Sokal and Rohlf, 1998) were performed on subsets of the data using the SAS System for Windows ver. 8.0 (SAS Institute; Cary, N.C.) with modified Rogers' distance (Wright, 1978). Due to the number of taxa and resulting difficulty in visualizing individuals in the 3-D PCA, the Guatemalan, Mexican, and West Indian populations were analyzed together first. This was followed by the analysis of the other interracial hybrid groups, one at a time, to determine where they clustered in relation to the three primary populations.

The relationships between the populations were also represented using a phenetic tree constructed from allele frequencies averaged over populations, using the Cavalli-Sforza and Edwards (1967) chord distance and the neighbor joining method (NJ; Saitou and Nei, 1987). Statistical analysis was accomplished by testing for similarities in allele frequencies using genotypic counts between groups as suggested by Weir (1996). Chi-square analysis of the contingency tables was generated using the Proc. Freq procedure with Monte Carlo simulation for estimates of exact P values and 1000 iterations. The Monte Carlo option was used due to the size of the data set and the computational resources required by the analysis (SAS, 1999).

Results

Level of polymorphism. The 14 microsatellite loci were highly polymorphic. The number of alleles varied from eight (AVAG07) to 30 (AVAG21), with an average of 18.8 alleles per locus and the average gene diversity was 0.83 (0.65 to 0.91) (Table 2). Eleven of the 14 microsatellites were composed of simple dinucleotide repeat motifs. Seven of these gave amplification products differing by either two bases or multiples of two for each allele. An example of the allelic diversity is given in Fig. 1 for locus AVAG06 showing 8 of the 15 alleles detected at this locus in eight accessions including *P. nubigena*. AVAG13 had two of 29 alleles that were not multimers of the repeat unit, while AVAG21 contained nine of 30 alleles that differed by a single base. Additionally, AVAG10 and AVAG11 generated two of 29 and three of 15 alleles, respectively, that were not multimers of the repeat unit. Two of the three microsatellite

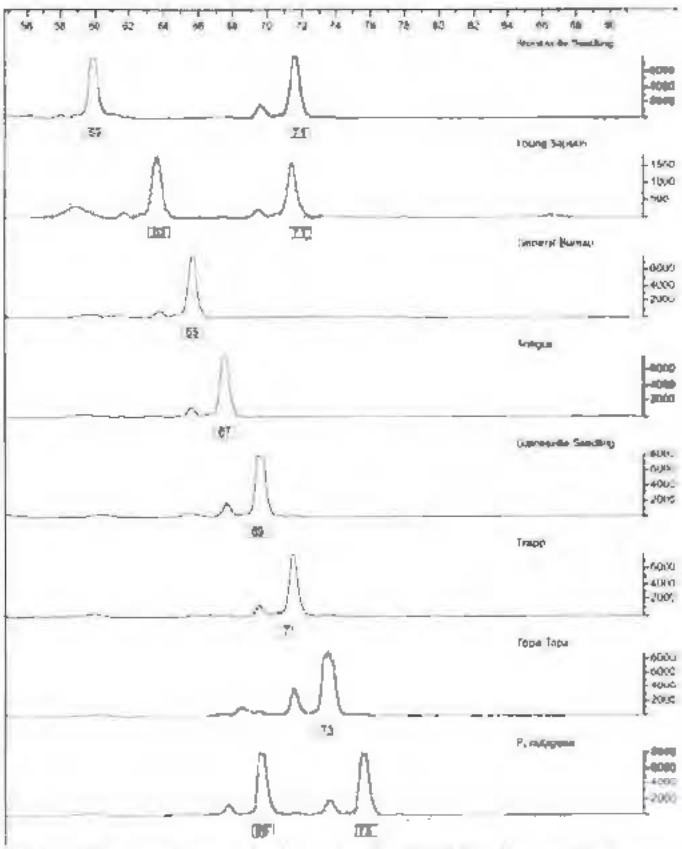

Fig. 1. Allele sizes in base pairs detected at the AVAG-06 microsatellite locus in eight *Persea* accessions illustrating eight alleles using the computer program Genotyper ver 4.0. The major peak in each electropherogram is the PCR amplified allele at this locus.

Table 3. Allele size for each locus and comparison of Ettinger and Pinkerton for allele sizes at the 14 microsatellite loci.

Allele	AVAG05	AVAG03	AVAG25	AVAG13	AVAG11	AVMIX03	AVAG21	AVAG07	AVMIX04	AVACO1	AVMIX02	AVAG06	AVAG10	AVAG22
1	83	92	96	96	105	139	153	—	158	95	147	59	149	96
2	85	94	98	98	107	141	167	98	160	99	149	63	174	98
3	87	96	100	100	109	143	171	100	162	103	153	65	176	100
4	89	98	102	102	113	145	173	102	164	105	155	67	178	102
5	91	100	104	104	115	147	175	104	166	107	157	69	180	104
6	93	102	106	106	116	151	177	106	168	109	159	71	182	106
7	95	104	108	108	118	152	179	108	170	111	163	73	184	108
8	97	106	110	110	125	154	180	112	172	113	165	75	185	110
9	99	108	112	112	129	156	181	114	174	115	167	77	186	112
10	101	110	114	114	131	158	183		176	117	185	79	187	114
11	103	112	118	116	132	160	186		178	119		81	188	116
12	105	114	120	118	133	162	187		180	121		83	190	118
13	107	116	122	120	137	164	188		182	123		85	192	120
14	109	122	124	122	147	166	189		184	127		87	194	122
15	111		128	126	161	168	190		186	131		89	196	124
16	113		130	128		170	191		188	135			298	130
17	119		134	130		172	193		190				200	
18	121		136	132		174	194		192				202	
19	125		138	134		178	195		194				204	
20			140	136		182	196						206	
21				138		190	198						208	
22				141		192	199						210	
23				143		196	201						216	
24				146			202						218	
25				148			203						220	
26				152			204						222	
27				154			207						224	
28				158			209						228	
29				160			215						230	
30							219						234	
Allele size														
Ettinger(Akko Expt. Station)														
	95/99	96	100	134/140	109/115	146/147	172/184	108/120	164/170	113/117	153/171	81/87	153	103/117
Ettinger (NGR-Miami)														
	87/95	94	102	122/128	109/161	143/172	167/190	100/112	162/170	109/113	157/167	77/83	190	100/114
Pinkerton(Akko Exp. Station)														
	95/99	98/106	106/110	130	107	146/170	192/200	110/113	180	117	153/171	77/85	149/153	117
Pinkerton (NGR-Miami)														
	89/91	96/102	104/106	118	107	143/166	181/198	102/106	180	NA[z]	149/167	69/81	149/186	114

[z]NA = no amplification.

loci that contained mixed repeat motifs gave amplification products differing by two bases or multiples of two for each allele.

Accession identification. Of the 104 accessions with duplicate plants, 85 (82%) had identical allelic configurations over all loci. Nonidentical allelic configurations usually resulted when one of three or four trees was not the same genotype. Many of these off-type plants had been previously detected based on phenotypic differences and the molecular data confirmed the misidentification. The 104 duplicated accessions are represented by 274 plants, only 19 of which were genotypically different from their sibling clones. The rate of error associated with propagation of the avocado collection is estimated to be 7.0%. Of the four cultivars common to both the NGR and SCFS, three were identical between collections, 'Bacon', 'Ettinger', and 'Mexicola'. 'Nimlioh' differed at 12 of the 14 loci. The cultivars 'Ettinger' and 'Pinkerton' were used in the study by Sharon et al. (1997) where the microsatellite markers were developed. These two cultivars were also included in our study. The comparison of allele sizes for 'Pinkerton' produced very similar fragment sizes, ours being on average 4.5 bp smaller, with the exception of alleles at two loci. For locus AVAG10 Sharon et al. (1997) reported allele sizes of 149 and 153 bp, whereas our alleles are 149 and 190 bp. Another difference occurred at locus AVACO1 where we obtained no amplification products, whereas Sharon et al. (1997) reported one amplification product at 117 bp. The comparison of allele sizes for 'Ettinger' also produced very similar fragment sizes, ours being on average 4.8 bp smaller, with the exception of alleles at six loci that were on average 20 bp larger. For example, the largest difference occurred at locus AVAG11 where Sharon et al. (1997) reported allele sizes of 109 bp and 115 bp, whereas our amplification products were 109 bp and 161 bp (Table 3).

Genetic differentiation between horticultural races and relatedness of cultivars. The PCA supports the grouping for the West Indian, Guatemalan, and Mexican races as illustrated in Fig. 2A where only the individuals within each race were analyzed. The PCA was able to summarize 29.7% of the total variability onto the three axes shown in this plot (18.3%, 6.4%, and 5.0%, respectively, for Prin1, Prin2, and Prin3). The PCA based on gene frequency suggested that the race of some accessions was incorrectly assigned. Six of the West Indian accessions did not group with the West Indian cluster. Two of these six, 'General Francisco Robles' and 'Orizaba 6' are clearly Mexican; both contained estragole in their leaves. Two others, 'Avocatosa' and 'Orizaba 3' clustered with the Guatemalan group while 'Biscayne' is most likely a M x W hybrid and 'Novillero' is most likely a G x M hybrid. Based on the PCA, 12 of the Guatemalan accessions clustered in problematic areas. 'Tehtoh' is clearly a West Indian accession. 'La Piscina' is Mexican and nine of the other accessions are mixed racial hybrids. 'Collins Seedling 2', 'Collins', 'MIA35730a', 'MIA35730b', 'MIA35730c', and 'Key Largo' all are M x W hybrids while 'Dickinson' and 'Lima Late' are G x W hybrids. 'PIC9651' clustered in the Mexican accessions; however it was listed as a Guatemalan accession (Ben-Ya'acov, 1995) and does not contain estragole in the leaves. Based on the PCA, five of the Mexican accessions clustered in problematic areas. 'Brooksville Seedling 2' and 'Brooksville Seedling 3' seem to be West Indian or West Indians hybrids while 'Gottfried' and 'Miramar de Monte de Oro' seem to be CH. 'Itzamna Seedling 1' clustered as a G x W. These changes are illustrated in Fig. 2A where the original racial designations are indicated with different symbols and the putatively misidentified accessions are labeled. In

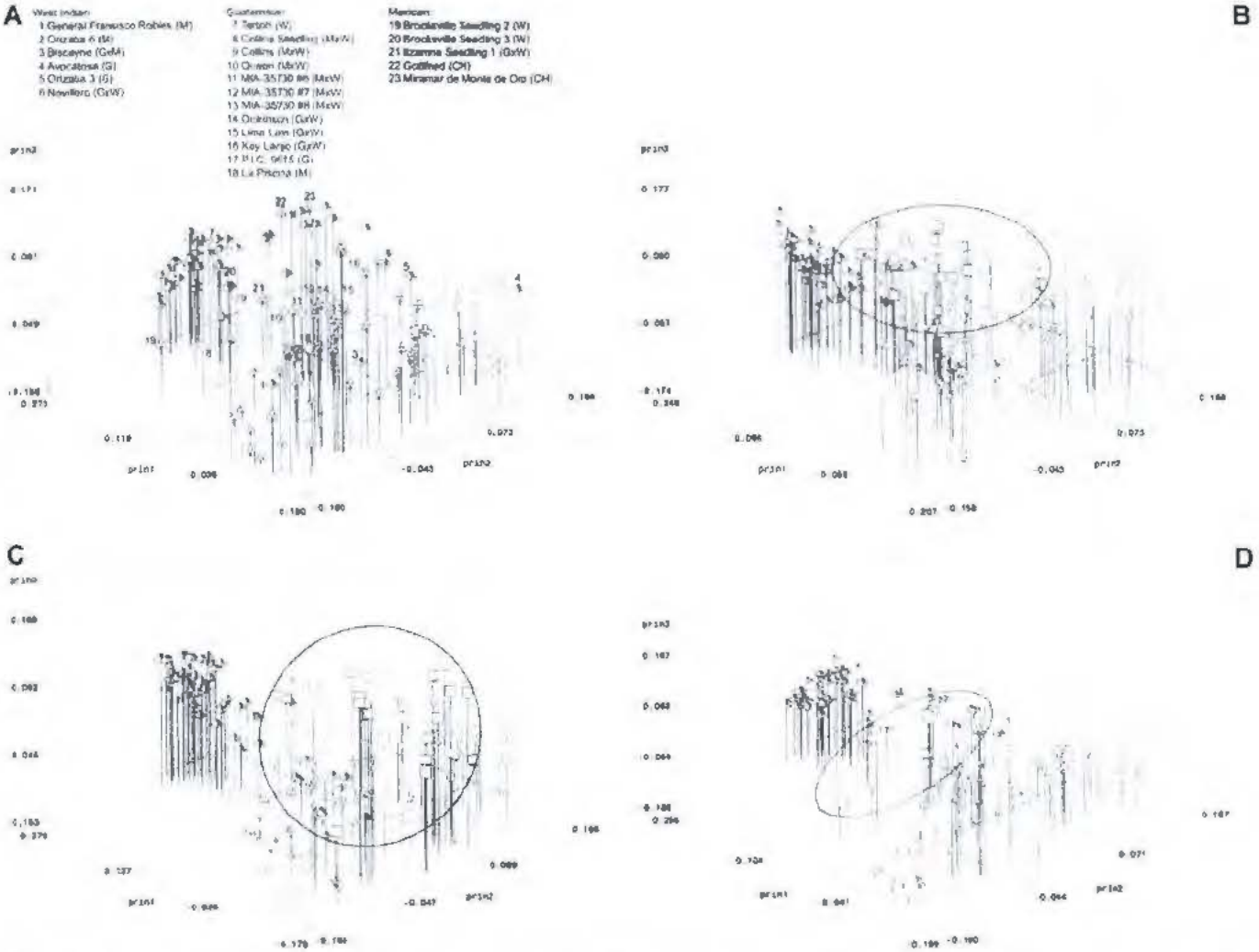

Fig. 2. Principal coordinate analysis (PCA) for the microsatellite evaluation of the avocado germplasm. ♦ Guatemalan accessions, ♣ Mexican accessions, Δ West Indian accessions. (A) PCA for the Guatemalan, Mexican, and West Indian avocado accessions based on 14 microsatellite loci and illustrating incorrect population classification within each racial group, correct classification in parenthesis. This plot contains 29.7% of the total variability, 18.3% for the first principal coordinate axis, 6.4% for the second, and 5.0% for the third. Symbols (♦, ♣, Δ) indicate original classification. (B) PCA for the Guatemalan, Mexican, West Indian races, and the G x W hybrids. Plot contains 27.9% of the total variability, 17.4% for the first principal coordinate axis, 5.9% for the second, and 4.6% for the third; ❑ = G x W. (C) PCA for the Guatemalan, Mexican, West Indian races, and the GxM hybrids. Plot contains 29.5% of the total variability, 20.1% for the first principal coordinate axis, 5.1% for the second, and 3.9% for the third; ❑ = G x M. (D) PCA for the Guatemalan, Mexican, West Indian races, and the MxW hybrids. Plot contains 29.9% of the total variability, 20.4% for the first principal coordinate axis, 5.0 % for the second, and 4.0% for the third; ❑ = M x W.

a similar manner, the other interracial hybrid groups were analyzed and changes made based on the PCA. All of the Unknown accessions (15) could be placed in racial or hybrid groups so they were no longer considered as a group for analysis purposes. A total of 50 accessions (19.7%) were changed based on the PCA and these changes are listed in Table 1. All further analysis, including PCA and phylogenetic, was done using this corrected dataset.

PCA for the Guatemalan, Mexican, and West Indian populations and the three interracial groups are illustrated in Fig. 2B–D. The PCA that includes the G x W hybrid grouping accounted for 28% of the total variation and the G x W accessions clustered between the two source populations (Fig. 2B). Likewise, Fig. 2C illustrates the G x M accessions clustering between the two source populations and the PCA accounts for 29.5% of the total variation. Fig. 2D illustrates the M x W accessions again clustering between the two source populations with the PCA accounting for 29.9% of the variation. The PCA for the CH group is not illustrated but these cluster together in a different area that overlaps with the other interracial hybrids.

Significant differences were found for each racial comparison, Guatemalan vs. West Indian, Guatemalan vs. Mexican, and Mexican vs. West Indian for 13 of the 14 loci from the Chi-square analysis. Significant frequency differences were also detected for locus AVAG22 between the Guatemalan vs. West Indian and

Table 4. Chi-square values for tests of similarity of allele frequency by racial background.[z]

Locus	AVAG05	AVAG03	AVAG25	AVAG13	AVAG11	AVMIX03	AVAG21	AVAG07	AVMIX04	AVACO1	AVMIX02	AVAG06	AVAG10	AVAG22
G vs. W	135.83**	84.05**	83.14**	141.18**	97.45**	114.82**	98.33**	76.20**	94.09**	105.42**	151.85**	100.66**	123.13**	20.75*
G vs. M	70.17**	29.12**	32.71**	68.74**	50.34**	68.83**	68.28**	44.15**	81.69**	44.98**	68.24**	45.36**	67.34**	14.42
M vs. W	133.65**	112.53**	120.11**	156.22**	150.15**	144.40**	149.90**	108.77**	155.91**	78.72**	183.45**	144.69**	140.98**	21.47*

[z]G = Guatemalan, M = Mexican, and W = West Indian races.

[y]Significant at $P < 0.05$ or 0.01, respectively.

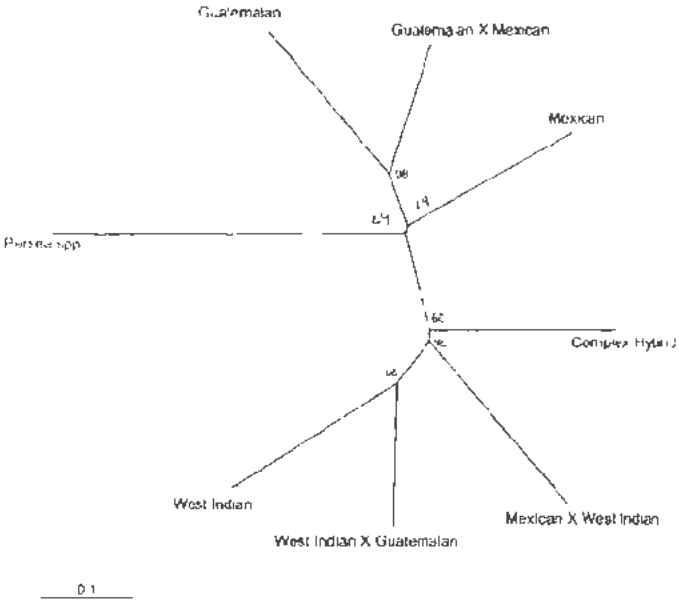

Fig. 3. Neighbor-joining tree of Guatemalan, Mexican, and West Indian races and interracial hybrids based on the Cavalli-Sforza and Edwards chord distance calculated from microsatellite data. Bootstrap percentages were computed using 1000 replications. Bootstrap values below 50% are not shown.

Mexican vs. West Indian but not for the Guatemalan vs. Mexican comparison (Table 4).

Phylogenetic analysis of the microsatellite data for the three populations and the inter-population hybrids was in agreement with the previously reported genetic relationships that separated the Guatemalan, Mexican and West Indian races (Davis et al., 1998; Fiedler et al., 1998; Mhameed et al., 1997). The NJ tree based on Cavalli-Sforza and Edwards (1967) chord distance grouped the three races into distinct clusters. The *Persea* spp. were distinct from any other group. The Guatemalan race and G × M hybrids clustered together with a high bootstrap value and the West Indian and W × G clustered together again with a high bootstrap value, all other groups had little bootstrap support (Fig. 3).

All of the groups had a high average allele number, with the exception of the M × W group that only contained eight individuals (3.57 to 13.35, Table 5). All loci were polymorphic when considered over all groups. H_{eh} was high in the Mexican (0.83) and Guatemalan populations (0.81) and lower for the West Indian group (0.61). The other groups had a narrow range 0.79 to 0.75. H_{obs} did not follow the same pattern being higher in the Guatemalan population (0.75), lower in the Mexican (0.66) and lower still in the West Indian (0.50). The M × W, G × W hybrids and CH had a high H_{obs} (0.87, 0.79 and 0.78 respectively, while the *Persea* spp. and G × M were similar, 0.70 (Table 5).

With the exception of the M × W hybrids, all other racial groupings contained unique alleles, 27 among the Mexican accessions, 16 in the West Indian group, seven among the Guatemalan accessions, and from one to four among the other groups (data not shown). Of these unique alleles, only three were at a frequency greater than 0.05. Two of these were found in both of the *Persea* spp. accessions (0.33 and 0.75 due to the existence of a null allele in *P. schiedeana*) and the other in the CH group (0.13). Only one allele in the Guatemalan population had a frequency greater than 0.50, allele 9 for locus AVMIX02 with a frequency of 0.71. Both the Mexican and West Indian populations contained this allele but at low frequencies of 0.17 and 0.04, respectively. The highest frequency in the Mexican population was 0.44 at locus AVAG11 for allele 2 and 0.46 at locus AVMIX02 for allele 2. The West Indian population had alleles with frequencies greater than 0.50 for eight of the loci, AVAG05 allele 6 (0.75), AVAG03 allele 6 (0.53), AVAG11 allele 2 (0.72), AVAG07 allele 3 (0.92) AVACO1 allele 5 (0.58), AVMIX02 allele 3 (0.90), AVAG06 allele 3 (0.51) and AVAG10 allele 1 (0.62). Allele 3 at AVAG07 was common to most of the West Indian accessions. 58 accessions were homozygous for this allele, six were heterozygous leaving only one accession not having this allele. This allele also occurred in the Guatemalan and Mexican populations but at lower frequencies, 0.34 and 0.28, respectively. Allele 3 at AVMIX02 also occurred in a high frequency with 36 accessions homozygous, 18 heterozygous and 11 not containing this allele. The frequency of this allele was low in the Guatemalan population (0.07) and moderate in the Mexican (0.20).

Discussion

Attributes that make microsatellites desirable as molecular markers include their hypervariablity, abundance and automated experimental procedures for detection. By using an automated high throughput CE system, we were able to analyze a large number of individuals for 14 microsatellite loci. The pattern of the PCR products produced by amplification of the genomic DNA was usually simple. It was possible to distinguish the full size amplification products containing the microsatellite from the stutter products and from the +A product. High levels of microsatellite polymorphism have been attributed to two molecular mechanisms, replication slippage and unequal crossing over (Johnson et al., 1992; Messier et al., 1996). Eleven of the 14 microsatellite loci had 15 or more alleles in this study and loci AVAG21, AVAG13, and AVAG10 could be considered hypervariable with 30, 29, and 30 alleles

Table 5. Genetic variation within the three horticultural races and among hybrid populations across 14 microsatellite loci; No.= sample size; $P_{95\%}$ = proportion of polymorphic loci when most frequent allele does not exceed 95%; A = mean number of alleles per locus; H_{eh} = unbiased gene diversity (Nei, 1978); H_{obs} = observed heterozygosity. Standard deviations are indicated in parentheses.

Population	No.	$P_{95\%}$	A	H_{eh}	H_{obs}
Guatemalan	35	1.0	11.29	0.806 (0.110)	0.746 (0.136)
Mexican	51	1.0	13.35	0.830 (0.065)	0.664 (0.163)
West Indian	65	1.0	11.14	0.607 (0.226)	0.496 (0.242)
G × W[z]	32	1.0	9.93	0.770 (0.099)	0.792 (0.102)
G × M[y]	30	1.0	9.86	0.792 (0.062)	0.702 (0.174)
M × W[x]	08	1.0	6.00	0.788 (0.090)	0.866 (0.115)
Complex hybrids	80	1.0	8.79	0.752 (0.109)	0.779 (0.144)
Persea spp.	03	1.0	3.57	0.793 (0.134)	0.702 (0.294)

[z]Listed in germplasm records at Guatemalan x West Indian Hybrids.
[y]Listed as Guatemalan x Mexican hybrids.
[x]Listed as Mexican x West Indian hybrids.

SPECTRUM®

Material Safety Data Sheet

NFPA	HMIS	Personal Protective Equipment
	Fire Hazard 1 Reactivity 0	 See Section 15.

Section 1. Chemical Product and Company Identification

Common Name/ Trade Name	Indole-3-butyric acid
Manufacturer	SPECTRUM LABORATORY PRODUCTS INC. 14422 S. SAN PEDRO STREET GARDENA, CA 90248
Commercial Name(s)	Hormodin Seradix
Synonym	3-Indolebutyric Acid
Chemical Name	Indole-3-Butyric Acid
Chemical Family	Not available.
Chemical Formula	C12H13NO2
Supplier	SPECTRUM LABORATORY PRODUCTS INC. 14422 S. SAN PEDRO STREET GARDENA, CA 90248

Catalog Number(s).	IN127
CAS#	133-32-4
RTECS	NL5250000
TSCA	TSCA 8(b) inventory: Indole-3-butyric acid
CI#	Not available.

IN CASE OF EMERGENCY
CHEMTREC (24hr) 800-424-9300

CALL (310) 516-8000

Section 2. Composition and Information on Ingredients

		Exposure Limits			
Name	CAS #	TWA (mg/m³)	STEL (mg/m³)	CEIL (mg/m³)	% by Weight
1) Indole-3-butyric acid	133-32-4				100

Toxicological Data on Ingredients

Indole-3-butyric acid:
ORAL (LD50): Acute: 100 mg/kg [Mouse].

Section 3. Hazards Identification

Potential Acute Health Effects

Hazardous in case of skin contact (irritant), of eye contact (irritant), of ingestion, of inhalation. Severe over-exposure can result in death.

Potential Chronic Health Effects

CARCINOGENIC EFFECTS: Not available.
MUTAGENIC EFFECTS: Mutagenic for mammalian somatic cells.
TERATOGENIC EFFECTS: Not available.
DEVELOPMENTAL TOXICITY: Not available.
Repeated exposure to a highly toxic material may produce general deterioration of health by an accumulation in one or many human organs.

Continued on Next Page

Section 4. First Aid Measures

Eye Contact	Check for and remove any contact lenses. In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Get medical attention.
Skin Contact	In case of contact, immediately flush skin with plenty of water. Cover the irritated skin with an emollient. Remove contaminated clothing and shoes. Wash clothing before reuse. Thoroughly clean shoes before reuse. Get medical attention.
Serious Skin Contact	Wash with a disinfectant soap and cover the contaminated skin with an anti-bacterial cream. Seek immediate medical attention.
Inhalation	If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Get medical attention.
Serious Inhalation	Not available.
Ingestion	If swallowed, do not induce vomiting unless directed to do so by medical personnel. Never give anything by mouth to an unconscious person. Loosen tight clothing such as a collar, tie, belt or waistband. Get medical attention immediately.
Serious Ingestion	Not available.

Section 5. Fire and Explosion Data

Flammability of the Product	May be combustible at high temperature.
Auto-Ignition Temperature	Not available.
Flash Points	Not available.
Flammable Limits	Not available.
Products of Combustion	These products are carbon oxides (CO, CO2), nitrogen oxides (NO, NO2...).
Fire Hazards in Presence of Various Substances	Slightly flammable to flammable in presence of heat. Non-flammable in presence of shocks.
Explosion Hazards in Presence of Various Substances	Slightly explosive in presence of open flames and sparks. Non-explosive in presence of shocks.
Fire Fighting Media and Instructions	SMALL FIRE: Use DRY chemical powder. LARGE FIRE: Use water spray, fog or foam. Do not use water jet.
Special Remarks on Fire Hazards	As with most organic solids, fire is possible at elevated temperatures
Special Remarks on Explosion Hazards	Fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.

Section 6. Accidental Release Measures

Small Spill	Use appropriate tools to put the spilled solid in a convenient waste disposal container. Finish cleaning by spreading water on the contaminated surface and dispose of according to local and regional authority requirements.
Large Spill	Poisonous solid. Stop leak if without risk. Do not get water inside container. Do not touch spilled material. Use water spray to reduce vapors. Prevent entry into sewers, basements or confined areas; dike if needed. Eliminate all ignition sources. Call for assistance on disposal. Finish cleaning by spreading water on the contaminated surface and allow to evacuate through the sanitary system.

Continued on Next Page

Section 7. Handling and Storage

Precautions	Keep away from heat. Keep away from sources of ignition. Ground all equipment containing material. Do not ingest. Do not breathe dust. Wear suitable protective clothing. In case of insufficient ventilation, wear suitable respiratory equipment. If ingested, seek medical advice immediately and show the container or the label. Avoid contact with skin and eyes. Keep away from incompatibles such as oxidizing agents, alkalis.
Storage	Keep container tightly closed. Keep container in a cool, well-ventilated area.

Section 8. Exposure Controls/Personal Protection

Engineering Controls	Use process enclosures, local exhaust ventilation, or other engineering controls to keep airborne levels below recommended exposure limits. If user operations generate dust, fume or mist, use ventilation to keep exposure to airborne contaminants below the exposure limit.
Personal Protection	Safety glasses. Lab coat. Dust respirator. Be sure to use an approved/certified respirator or equivalent. Gloves (impervious).
Personal Protection in Case of a Large Spill	Splash goggles. Full suit. Dust respirator. Boots. Gloves. A self contained breathing apparatus should be used to avoid inhalation of the product. Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.
Exposure Limits	Not available.

Section 9. Physical and Chemical Properties

Physical state and appearance	Solid. (Crystals solid.)
Molecular Weight	203.23 g/mole
pH (1% soln/water)	Not applicable.

Odor	Odorless.
Taste	Not available.
Color	Off-white. White to yellowish.

Boiling Point	Not available.
Melting Point	124°C (255.2°F) - 126 C
Critical Temperature	Not available.
Specific Gravity	Not available.
Vapor Pressure	Not applicable.
Vapor Density	Not available.
Volatility	Not available.
Odor Threshold	Not available.
Water/Oil Dist. Coeff.	Not available.
Ionicity (in Water)	Not available.
Dispersion Properties	Not available.
Solubility	Insoluble in cold water.

Section 10. Stability and Reactivity Data

Stability	The product is stable.
Instability Temperature	Not available.
Conditions of Instability	Excess heat, incompatible materials
Incompatibility with various substances	Reactive with oxidizing agents, alkalis.
Corrosivity	Non-corrosive in presence of glass.

Continued on Next Page

Special Remarks on Reactivity	Light sensitive.
Special Remarks on Corrosivity	Not available.
Polymerization	Will not occur.

Section 11. Toxicological Information

Routes of Entry	Inhalation. Ingestion.
Toxicity to Animals	Acute oral toxicity (LD50): 100 mg/kg [Mouse].
Chronic Effects on Humans	**MUTAGENIC EFFECTS:** Mutagenic for mammalian somatic cells.
Other Toxic Effects on Humans	Hazardous in case of skin contact (irritant), of ingestion, of inhalation.
Special Remarks on Toxicity to Animals	Lethal Dose/Conc: LD[Rat] - Route: Oral Dose: >500 mg/kg
Special Remarks on Chronic Effects on Humans	May affect genetic material.
Special Remarks on other Toxic Effects on Humans	Acute Potential Health Effects: Skin: Causes skin irritation. Eyes: Causes eye irritation. May cause conjunctivitis Inhalation: Causes respiratory tract irritation. Ingestion: Harmful if swallowed. May cause gastrointestinal tract irritation with nausea, vomitting and diarrhea. The toxicological properties of this substance have not been fully investigated.

Section 12. Ecological Information

Ecotoxicity	Not available.
BOD5 and COD	Not available.
Products of Biodegradation	Possibly hazardous short term degradation products are not likely. However, long term degradation products may arise.
Toxicity of the Products of Biodegradation	The products of degradation are less toxic than the product itself.
Special Remarks on the Products of Biodegradation	Not available.

Section 13. Disposal Considerations

Waste Disposal	Waste must be disposed of in accordance with federal, state and local environmental control regulations.

Section 14. Transport Information

DOT Classification	Not a DOT controlled material (United States).
Identification	Not applicable.
Special Provisions for Transport	Not applicable.

Continued on Next Page

DOT (Pictograms)

Section 15. Other Regulatory Information and Pictograms

Federal and State Regulations

TSCA 8(b) inventory: Indole-3-butyric acid

California Proposition 65 Warnings

California prop. 65: This product contains the following ingredients for which the State of California has found to cause cancer which would require a warning under the statute: No products were found.

California prop. 65: This product contains the following ingredients for which the State of California has found to cause birth defects which would require a warning under the statute: No products were found.

Other Regulations

OSHA: Hazardous by definition of Hazard Communication Standard (29 CFR 1910.1200).
EINECS: This product is on the European Inventory of Existing Commercial Chemical Substances.

Other Classifications

WHMIS (Canada)

Not controlled under WHMIS (Canada).

DSCL (EEC)

R22- Harmful if swallowed.
R36/37/38- Irritating to eyes, respiratory system and skin.

S26- In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39- Wear suitable protective clothing, gloves and eye/face protection.
S45- In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

HMIS (U.S.A.)

[illegible]	2
Fire Hazard	1
Reactivity	0
Personal Protection	E

National Fire Protection Association (U.S.A.)

Flammability
Health
Reactivity
Specific hazard

WHMIS (Canada) (Pictograms)

DSCL (Europe) (Pictograms)

TDG (Canada) (Pictograms)

ADR (Europe) (Pictograms)

Protective Equipment

Gloves (impervious).

Continued on Next Page

Lab coat.

Dust respirator. Be sure to use an approved/certified respirator or equivalent.

Safety glasses.

Section 16. Other Information

MSDS Code	I3070
References	Not available.
Other Special Considerations	Not available.

Validated by Sonia Owen on 4/26/2004.	Verified by Sonia Owen. Printed 8/25/2004.

CALL (310) 516-8000

Notice to Reader

All chemicals may pose unknown hazards and should be used with caution. This Material Safety Data Sheet (MSDS) applies only to the material as packaged. If this product is combined with other materials, deteriorates, or becomes contaminated, it may pose hazards not mentioned in this MSDS. It shall be the user's responsibility to develop proper methods of handling and personal protection based on the actual conditions of use. While this MSDS is based on technical data judged to be reliable, Spectrum Quality Products, Inc. assumes no responsibility for the completeness or accuracy of the information contained herein.

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FERTILIZER DOSAGES FOR DRIP IRRIGATION
for Young Citrus and Avocado Trees

The following are recommended fertilizer rate tables for liquid and dry formulations to be injected into drip irrigation systems for young trees. It is a balanced program designed to produce optimum tree growth during the first three years of an orchard's life.

The rates are generous and represent maximum amounts to be applied under normal or average conditions.

Any particular soil may not require all the nutrients included in both of the tables. It is designed to take care of an orchard with varied soil conditions such as occur in hillside plantings with little topsoil. We think it is a good starting point. For more precise custom evaluations, of course, see your soil advisor.

If growth has been successful during the first three years, the fourth year should be a good bearing year and the dosages may be reduced. Beginning the fourth year and fifth year you should be guided by leaf and soil analyses rather than simplified tables such as these.

FERTILIZER RATE TABLE I

Form	Material	PPM	Vol./1000 gal. Of Irrigation Water	Wt./1000 gal. Of Irrigation Water
1. Liquid	20-0-0 (Ammonium Nitrate)	93N	.37 gal.	2.22 lbs.
2. Dry form (mix as a slurry before adding to injector)	Supplementary Iron Chelate (Sprint 138) 8% Fe			
	a)One shot quick corrective	9.6 Fe	3 cups	1 lb.
	b) Constant feed	1.36 Fe	6 Tbs.	2.28 oz.
2. Dry form (mix as a slurry before adding to injector)	Supplementary Zinc Chelate (14.2% Zn)			
	a)One shot quick corrective	4.26 Zn	3/4 cups	4 oz.
	b) Constant feed	0.36 Zn	1 Tbs.	1/3 oz.

Example:
Let's suppose that you start out by applying only the liquid Ammonium Nitrate (20-0-0) through your drip system by means of a small injector tank. If you have, as an example, 674 trees, each with one (1) gallon per hour emitter, and you plan to irrigate for six hours, then your total amount of

irrigation water would equal 674 trees (times) 6 hours = 4044 gallons of water. The ratio of Ammonium Nitrate that you would use would be expressed as:

gal. of water used / 1000 gal. x .37 = (or) 4044 / 1000 x .37 = 1.5 gal. of Ammonium Nitrate (20-0-0)

that you would put into your injector tank to be dispensed during the six hour irrigation period at the slowest rate possible.

Should it be inconvenient to use liquid Ammonium Nitrate you could substitute 9 lb. of granular Ammonium Nitrate thusly:

gal. of water used / 1000 gal. x 2.22 = (or) 4044 / 1000 x 2.22 = 9 lb.

CAUTION: Be sure that your lines are charged with pure irrigation water before you begin injecting. Otherwise the fertilizer may rush to specific areas of your orchard causing severe damage to some of your trees.

Lines 2 and 3 in FERTILIZER RATE TABLE I suggest two dosages each of Iron Chelate and Zinc Chelate. The dosage listed in each case as "One shot quick corrective" is for cases of pronounced chlorosis and/or other deficiency symptoms. the other dosage is, as noted, a constant feed dosage to be used at each irrigation along with the Ammonium Nitrate.

FERTILIZER RATE TABLE II

Form	Material	PPM	Vol./1000 gal. Of Irrigation Water	Wt./1000 gal. Of Irrigation Water
1. Liquid	75% Phosphoric Acid (food grade or technical)	16.8	6 fl. Oz.	---
2. Solids dissolved in 500 gals. Of solution	750 lbs. KNO_4 (Potassium Nitrate) 10 lbs. $CuSO_4 5H_2O$ (Copper Sulfate)	19N, 52K,0.5Cu	.79 gal.	1.2 lb. KNO_4, .25 oz. $CuSO_4$

FERTILIZER RATE TABLE II is included as an optional suggestion for the grower who may want to provide these additional nutrients. If Phosphoric Acid is mixed with Iron Chelate it may form a precipitate which could clog emitters. For this reason it may be wise to inject these two at different intervals during the watering cycle.

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Mounding Avocado Trees

Here is some information that may be helpful in your preparations for trees. Watering is of the utmost importance. Be sure to review the *Care of Citrus and Avocado Trees* pamphlet on the subject.

Mounds can provide enhanced aeration for avocado roots and encourage a quick start for young trees. You should use native soil, free from pre-emergent herbicides. The mound should be incorporated or mixed into the floor of the orchard to eliminate any possibility of an interface that could inhibit water percolation. Build the mound 1 to 1-1/2 feet high, at a 4:1 slope. The mound should be settled with water and allowed to dry out somewhat so you are not planting in muck. Dig a hole 18" in diameter and about 15" deep. Place the nursery tree in the hole to check planting height. The soil of the nursery ball should be about 1" above the top of the mound to keep the ball exposed to irrigation water. Cut and remove the bottomless container only after checking and adjusting the planting height. Backfill the tree little by little lightly tamping out air spaces. Do not overly compact the soil. Water the tree in with about 10 to 20 gallons of water to assure no air spaces remain around the root ball.

We have seen good results from capping off the mound with 15 to 35 lbs. of gypsum, then cover the mound with a coarse mulch about 4" to 6" thick. Be careful to keep the mulch from piling up around the trunk, it's best left exposed. The calcium in the gypsum inhibits the Phytophthora fungus and the mulch provides an ideal environment for shallow aerated roots to develop.

Trees should be treated every three months with a fungicide to control root rot until the trees are well established.

We hope this is helpful please do not hesitate to call if you have any questions.

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SOME COMMON PROBLEMS WITH YOUNG, ESTABLISHED AVOCADO TREES

Faulty Watering

Almost all difficulties with young avocado trees can be traced to watering practices, or soil conditions and of the two causes, watering practices is the most common. A grower should use a soil probe to test the moisture in and around the tree ball. The soil in both places should always be adequately moist and never soggy for an extended period of time. Common results from faulty watering are salt damage and suffocation as listed below:

A. Salt Damage. This is normally detected by "tip burn" of the leaves. This is a drying out and dying of the leaves, and especially the leaf tips. Semi-burned leaves are rather small, and often yellowed. They contrast with thick, large dark green leaves of a well watered and nourished tree. The tree bark is often sunburned. The condition often shows up in the winter, but is usually a result of damage done during the previous summer or fall.

All soils and waters contain salt, and when soil dries out because of evaporation of moisture, the salts become more and more concentrated. The plant, thirsty for water, sucks up the concentrated solution and the salts concentrate in the borders of the leaves. Since they have no efficient way to escape from the leaves, they poison the tissue. Hence, while the normal remedy of salt-laden soil is to leach it thoroughly and wash out the salts, it is usually too late by the time the tree shows the above-described symptoms. The best that you can do is to try to avoid a recurrence of the condition.

B. Suffocation. It is very easy to overwater a young avocado plant and suffocate the roots. The roots seem to need oxygen-laden air in their environment. If it is not present, they simply cease to suck water from the surrounding soil, even though it is thoroughly saturated. The leaves of the plant then wilt; many of them fall off, leaving a weak, sickly, sunburned stem with perhaps a few small leaves on the tips of the branches. Such a tree is not worth saving. Dispose of it immediately.

Chlorosis

Chlorosis is another common problem and is most commonly associated with a deficiency of available iron. Some general paleness may be due to nitrogen deficiency, but is normally of a milder form. Although iron is an extremely common element in the universe, and most of our soils contain quantities of it, under certain alkaline conditions it is unavailable for plant use. When this occurs, avocado trees become pale yellow, and in severe cases, their leaves begin to burn. The leaves may drop. This condition is detected in its early stages because the new, normally maroon or rich red growth is orange-ish or yellow-ish in hue. If the condition is not corrected, the tree will be weak, lose its leaves, and be unproductive. There are two common countermeasures to iron chlorosis, when it is correctable -- and it usually is.

Countermeasure number one is to apply readily available iron. This may be accomplished by the addition of Spring 138 iron chelate. If you have a chlorotic condition, we recommend as a starter for corrective measure, a one-shot application of one pound of the chelate per 1,000 gallons of irrigation water. For constant feed, use two ounces of the chelate per thousand gallons of irrigation water.

There may be other chelates which will serve as well. However, if your tree is chlorotic, we recommend immediate corrective action according to the above formula. Fool around with other brands and types later.

Counter measure number two is to stop irrigating so often, and to let the root zone try out more. Often, too soggy soil will cause a deficiency to available iron in leaves. The leaves may contain plenty of iron when analyzed, but for some reason, it is unavailable. Of course, this treatment invites the danger of exeessive salt accumulation, as described above in A., but one must simply work around two problems instead of one. Water less often and leach periodically.

The over concentration of soil water may be your whole problem or none of it. In any case, apply countermeasure number one, and then consider the probability of too wet a soil environment.

Zinc Deficiency

Zinc deficiency is sometimes detectable by a blotchy kind of yellowness between the veins of the leaves, but is difficult to definitely diagnose in this way. It is positively diagnosed by the presence of short leaf inter-nodes and long, narrow, small leaves concentrated at the tips of branches. Fruits are small and abnormally spheroidal. The condition may be corrected by an application of a spray of one pound of zinc sulfate per 100 gallons of water, with spreader added, when the new leaves just become physically expanded. This is usually in June or late summer. It may be corrected, also, by soil applications of zinc chelate but these must be applied carefully, as it is easy to kill trees with the material. Apply at the rate of :

Corrective action: 4 oz. zinc chelate per 1,000 gallons irrigation water, one time only.

Constant feed maintenance: 1/3 oz. zinc chelate per 1,000 gallons irrigation water.

Characteristics of Avocado Rootstocks

	Toro Canyon	Dusa Merensky 2	Latas Merensky 1 (h)	Duke 7	Borchard	Thomas	Zentmyer PP4 (h)	Uzi PP15 (h)	Mexican Seedling	G-6
Normal Propagation Method	Clonal	Clonal	Clonal	Clonal	Clonal	Clonal	Clonal	Clonal	Seed	Seed
Productivity "Clean" Soil - a	3	4	?	4	4	2	2	3.5	3	3
Productivity "Root Rot" Soil - b	3.5	4	4	3	2	3	5	5	1	2.5
Tree Size "Clean" Soil - a	4	5	5	5	5	5	5	5	5	5
Tree Size "Root Rot" Soil - b	3	4	4	2	0.5	4	4	5	0.5	1
Tolerance to P. cinnamomi - c	3.5	5	4.5	3	0.5	4.5	5	5	0	2
Tolerance to P. citricola - d	5	?	?	4	3	2	3	4	3	3
Salt Tolerance - e	3.5	4	5	3	4.5	1	1	4	2	2
Tolerance to Lime-Induced Chlorisis	3	?	?	4	5	3	?	?	2	2
Frost Tolerance - f	4.5	4.5	4.5	4	4.5	4.5	5	?	4.5	4.5
Tolerance to Dothierella - g	5	5	5	5	5	2	4	3	5	2
Horticultural Race	Mexican	Mex X Guat.	Mex X Guat.	Mexican	Mexican	Mexican	Mexican	Mexican	Mexican	Mexican
Parentage	Escape Seedling	Escape Seedling	Escape Seedling	Duke	Escape Seedling	Escape Seedling	Breeding Plot	Breeding Plot		Collection
Geographic Origin	Carpinteria	South Africa	South Africa	Riverside	Oxnard	Escondido	Riverside	Riverside	Mexico	Guatemala

Legend: 0 = Poor, 5 = Best

Ratings by J. Menge, G. Bender, and M.L. Arpaia, 2002

Footnotes

a. Yield and canopy volume expressed as percentage in comparison to Topa Topa (Mexican seedling), based on 7 years of data (6 years for Thomas) at South Coast Field Station (Arpaia et. Al 1993)

b. Yield and canopy volume expressed as percentage in comparison to Thomas (consolidated data from J. Menge, 2002)

c. Consolidation of performance of young replant trials, ratings by J. Menge

d. Results from greenhouse trials by A. Alizadah and J. Menge (unpublished)

e. Rootstock trial in sand tanks treated with three levels of saline water (Oster and Arpaia, 1991)

f. Observations of ungrafted rootstocks by G. Bender and J. Menge after freezes in 1988-1991

g. Results from greenhouse trials by A. Alizadah and J. Menge (unpublished)

h. Not commercially available as of 11/12/02. Many horticultural characteristics are preliminary or unknown

No rootstocks withstand prolonged soil saturation

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Care of Citrus and Avocado Trees

by

W.H. Brokaw

The Sleeve-Grown, Fully-Leafed Tree:

A fully-leafed, sub-tropical evergreen must be treated differently than the standard, deciduous, temperate plant. Normally, it is planted somewhat later in the year so as to capitalize on the warming spring soil, and special allowance must be made for the plant's high transpiration rate.

Citrus and avocados have tender succulent roots so their earthen balls may not be as physically stable as those of other plants. Therefore:

- Never lift or carry them by grasping the trunk or stake, and
- Be sure the tree is lowered into, and correctly set in the planting hole before you slit the poly container.

Water the Tree Right After Planting:

The planting of citrus and avocado trees is different from deciduous trees. Your new tree has a large number of active, working leaves which must be kept well supplied with water at all times so as to function and not wilt. Since the ball of the tree contains all of the tree roots it must be kept moist to serve as reservoir for the water. When you remove the plastic sleeve from the ball, you will find that many of the roots are concentrated at the outside vertical surface. It is, therefore, very important that the tree be watered immediately after planting since these surface roots will otherwise be unable to function properly.

Leave the upper surface of the ball exposed:

The soil in the ball has been specially formulated -- it contains special nutrients and is designed so the ball will readily absorb water that is added directly to its upper surface.

Specific Planting Steps:

- Dig a hole much wider than the ball of your tree. 18 inches wide is an ideal hole. If your soil is good you need not add any soil amendment to the hole. Avoid adding more than 50% (by volume) commercial compost planting mix.

- Adjust the depth of the hole so that the upper surface of the tree ball will be 1 inch above the surrounding ground when the tree is lowered into it.

- Lower the tree into the hole, slice the container open vertically on one side, and backfill with 6" to 8" of loose soil or soil/compost mixture to stabilize the tree before removing the slit container. It is important that the rootball is not moved after the container is slit.

- Pull the plastic tube container out of the hole and away from the tree to discard it. The poly container is not degradable but may be recycled. This will leave the roots exposed on the surface of the ball.

- Gently tamp the loose soil around the ball immediately. Promptly fill the rest of the hole with loose soil, gently tamping as you fill. Fill it up to the top, but leave the upper surface of the original ball exposed.

- It is important that the loose soil you put back in the hole be free of large clods, as these do not dissolve easily with water and will cause air spaces which are injurious.

- The upper surface of the ball is left exposed so that you may add water directly to the ball, even after the tree is planted. If you cover this surface with anything, do not let it be soil; use sand, loose sawdust, coarse gravel, or anything through which water will pass very rapidly.

- Build a basin with a three foot diameter around the tree, sloping the bottom of it so that all water drains to the exposed surface of the ball. The basin should have a capacity of about five gallons.

- Fill the basin with water once. If it drains rapidly, fill again. If it requires two minutes or more to drain, do not refill.

- Reform the bottom of the basin, as the dirt in the hole should now have settled somewhat. Be sure that the top of the ball is still exposed.

- It is a good idea, once the basin has stabilized, to cover the bottom with straw, sawdust or some other mulching medium.

- If you plan to use drip irrigation, be sure that the emitter is fastened to the exposed ball of the tree with a "U" shaped piece of wire or hook. This prevents the dripper from creeping away from the root ball as the hose expands and contracts. Check your emitters frequently to see that each tree is getting watered; clogged emitters are a common problem.

- Once your tree becomes established and the roots start reaching out into the sur-rounding soil (usually about 1 to 2 months after planting), the emitter should be moved away from the top of the ball to a distance of about 6" to 8".

- As the roots extend further outward and downward, you will want to add more emitters and move them further away from the trunk of the tree. A fully mature citrus or avocado (six years old) will often have four to five emitters spaced in a ring around the tree near the drip line.

- Under normal circumstances, water the young tree every 5 to 10 days for a period of 6 to 10 weeks. Two to five gallons of water per irrigation will be sufficient provided the ball itself receives water each time and remains damp inside. Do not allow the soil to remain soggy; a happy medium is mandatory.

- If you plan to plant these trees in areas where trees have died or avocado root rot has occurred, chemical control of this disease may be necessary to assist the establishment of the trees. Ridomil® and Aliette® are suitable systemic fungicides registered for use on citrus and avocados.

Watering:

Do not allow the ball - ever - to dry out. For instance, avocados are native to areas which, unlike California, have almost daily rains during the summer. Their favored soils under these conditions are often acid, sandy and weak, characterized by good internal drainage which doesn't allow them to remain soggy. Therefore, once your tree has begun to establish a root system, keep the soil damp but not soggy. Water deeply. We recommend the use of a soil core probe, slanted toward the side of the ball so that it penetrates the ball about 12" below the soil surface, in order to determine soil moisture. Apply water according to the needs of the tree.

The tree may be watered by basin for a full year. However, the basin should be broken down during the wet season if water has any tendency to stand in it. After a year you should consider the use of sprinklers or drippers.

Warning: Avocado roots are very easily suffocated by excessive water. This problem is most severe between planting and the period when the roots reach out into the surrounding soil. This means that the trees are particularly vulnerable when planted in the fall. Therefore, under no circumstances allow water from rains or other sources to stand around the tree ball or run over the ball for extended periods of time. Such treatment will almost certainly result in rotting roots, and probably, in an unsatisfactory tree. If the tree is planted on a slope you may consider placing a diverting trough above the tree in such a way as to deflect any water currents away from it.

Mounding: In heavier soils it has been shown to be helpful to plant the trees on 12" to 18" mounds sloping to 5' to 8' bases or plant the trees in raised beds. This allows optimum aeration for the root and assures the proper planting height.

Fertilization:

During the first two or three years, your tree should be fairly heavily fertilized so as to make maximum early growth. Heavy fertilizer to one person often means entirely something else to another, so we suggest that the following rates be used during the pre-bearing years:

Sprinkle a tablespoon of nitrogen-bearing fertilizer (ammonium nitrate, urea or such) over the root area and water it in thoroughly. Repeat every three or four weeks. Take care not to concentrate it in one area. Increase the dosage gradually according to the increasing size of your tree. Apply the plant food around the drip line, or in the path of irrigation water.

A fully bearing, average sized citrus or avocado tree (one that has a foliage diameter of about 15 to 20 feet) is usually fertilized at the rate of 1 to 1 1/2 lbs. of actual nitrogen per year (ammonium nitrate is 32% actual nitrogen). This can be taken care of by sprinkling 1 to 1 1/2 lbs. of dry ammonium nitrate on the ground around and beneath the skirts of the tree two or three times a year. Wash the fertilizer into the ground with a good soaking (2 inches of water). Early spring, summer and fall are good times for fertilizer application, as the roots will be active then. You may, as a special precaution, add also, a half pound of Ciba-Geigy 138® iron chelate and a zinc chelate to the soil at the same time that you are adding the nitrogen. The chelates will correct many cases of leaf yellowing. For smaller or dwarf trees fertilizer dosages should be reduced proportionally according to the area of tree canopy.

Frost Protection:

Young citrus and avocado trees are very vulnerable to prolonged frost conditions. However, there are certain precautions you may follow during the first year or so which will often save a tree.

Wrap the trunk of your new tree with heavy paper, corn stalks or the special thermal wraps. If this is done to a point above the bud union, the chances are that you will have a complete budded tree when winter is over even though the exposed parts of your tree are killed. At the onset of spring, you will be able to unwrap the damaged tree and select a shoot or shoots, above the bud union, so as to renew your tree. Do not remove dead tree parts until new shoots are growing well.

An even more effective insulation to preserve the bud union is a collar filled with sawdust to a point 6 to 12 inches above the union. The collar may be 5 or 6 inches in diameter. It is almost impossible to freeze tissue within this mass of sawdust.

Foliage is more difficult to save under severe frost conditions. Any wrapping around and through it will help. Sometimes bunches of straw are intertwined with the foliage and matted around the branches to serve as an insulating mass. A suspended canvas and wood canopy above the tree will help. Under very extreme conditions, people have erected tents and placed lighted electric bulbs within the structure. Remember two things:

- A complete enclosed covering of polyethylene or other non-breathing plastic is often worse than nothing; especially where it touches the tree.
- Trees do not survive well in darkness, so the tree must be allowed to see sunlight during the day.

All in all, we recommend the thermal wraps mentioned first above or the sawdust filled-collar.

Weeds And Pests:

If you want good growth, it is imperative that weeds are not allowed to develop near your trees. Keep the space clean for a full six feet from the base of your tree. Allow no weeds nor grass to grow in this area and apply no systemic weed killers that may be absorbed by the tree roots.

Insect pests rarely damage avocado fruit trees. Allow a bit of nibbling. Citrus pests are often aggravated by ants. Low branches on citrus trees should be clipped so they do not droop to the ground allowing ants access to the tree. If ants can only go up the trunk they can be easily controlled and eliminate many pests.

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AVOCADO SOIL AND WATER REQUIREMENTS

SOIL pH

It is generally advisable to plant in soil which is neither too strongly acid or too basic in reaction. pH values in the range of 5.5 to 8.0 have been associated with good growth. Extreme pH values usually indicate some other problem from either toxic mineral or deficiency.

SOIL SALINITY

Avocados are fairly sensitive to soluble salts whether derived from natural sources or due to excess fertilizer. The standard system of testing soils for salinity is known as the saturation extract method wherein the conductivity of the saturation extract at 25° is measured in millimhos per centimeter (Ex X 103). For avocados, this value should never exceed 3.0 and should preferably be in the range of 2.0 or lower. Since salts in the soil solution become more concentrated as soil moisture is removed by evaporation, or plant consumption, a marginal salinity level may be quite injurious if soil becomes too dry.

SODIUM

This element can be specifically toxic to avocados if present in high proportion to other minerals. Evaluation of the sodium status is usually reported as sodium adsorption ratio (SAR) or exchangable sodium percentage (ESP). SAR values should not exceed 5 and ESP values should not exceed 6.

WATER SALINITY

This feature is measured in several ways, one of which is conductivity usually expressed as micromhos per centimeter (EC X 106) and also as total dissolved solids (TDS) determined by an evaporation technique. A rule of thumb to convert from conductivity to total dissolved solids is to multiply the conductivity figure by 0.7. There is probably no single maximum figure that can be established as a tolerant level for avocados since much depends upon the composition of the minerals in the water. However, a conductivity in the range of 1,000 to 1,200 might be considered the upper tolerant range and this would coincide with a TDS of 700 to 850 ppm.

SODIUM STATUS OF WATER

This is usually expressed as SAR which takes into account the proportion of sodium to calcium and magnesium. Values in excess of 5 should be avoided. Ideally, the sodium concentration in the water expressed as milliequivalents per liter (meq/1) should not exceed 3.

WATER CHLORIDE CONTENT

Avocados are quite sensitive to excess chloride particularly under conditions of poor irrigation management where excessive drying is permitted. Chloride concentrations in excess of 3 milliequivalents per liter (107 ppm) would be considered dangerously high.

WATER BORON CONTENT

Avocados are moderately sensitive to excess of boron and concentrations in excess of 0.7 ppm in the irrigation water would be considered dangerous.

To evaluate the effect of water, soil and fertilizer practices it is helpful to carry out leaf analyses for the elements of interest and concern. This fairly critical method of determining what the plant is actually absorbing is commonly used in commercial avocado production.

PHYTOPHTHORA DISEASES

An Introduction to Avocado Root Rot and Research on Integrated Approach to its Control

By Michael Coffey, Ph.D.

Phytophthora cinnamomi is the cause of this extremely destructive disease of the feeder roots of avocado. The infective spore of P. cinnamomi is the zoospore, small motile spores which can be moved passively in run-off and irrigation water and remain motile up to 24 hours. Within about six hours after contact with avocado feeder roots, zoospores have infected, penetrated and killed feeder roots. After root food reserves are used up, sporangia can be formed which can proliferate under warm, moist conditions producing many more zoospores. In addition, soil already contaminated with P. cinnamomi contains decaying roots which hold mycelium and chlamydospores (thicker walled, survival spores) and these structures are highly infective giving rise to sporangia and zoospores. Physical movement of wet soil is a common method of spread of the disease, both within a grove and from property to property.

P. cinnamomi is known to be parasitic on over 1000 different host plants, including ornamentals and many fruit trees. Since P. cinnamomi is widespread in most countries where avocados are grown, and as it can survive in soil apart from a host, there is no ornamental planting which does not pose a threat to avocado production.

An outline for Integrated Disease Control

There are five principal factors to consider:

1. Nursery practice
2. Cultural practice
3. Clonal rootstocks
4. Biological control
5. Chemical control

1. **Nursery Practice**. In California, the avocado certification program outlines conditions to minimize the possibility of P. cinnamomi infection of nursery trees but does not however, cover P. citricola or other Phytophthora species.

Nursery practice should include:

a. The use of steamed or fumigated mixes.

b. The propagation of all trees on benches

c. The provision of adequate drainage within the nursery to minimize the risk of spread of P. cinnamomi.

d. The periodic sampling of tree roots for P. cinnamomi during the 2-year production period.

e. The avoidance of the use of fungicides such as Ridomil^{R} and Aliette^{R} to prevent suppression of P. cinnamomi thereby hampering early detection.

2. **Cultural Practice**. Since P. cinnamomi is favored by wet conditions, irrigation and cultural practices should aim to minimize the effects of excessive watering including:

a. Provision of a well drained soil, especially in heavier soil by planting on mounds or ridges.

b. Provision of adequate irrigation and correct use of drip irrigation facilities.

3. **Clonal Rootstocks.** Development of clonal rootstocks Duke 7, G6 and G755 have revolutionized concepts about avocado planting. These rootstocks have moderated field tolerance to P. cinnamomi and therefore require special care in the provision of proper planting conditions including well drained soild and adequate drainage conditions (ie. planting on mounds).

4. **Biological and Cultural Control.** Some scientists conclude that the main effect of biological control is primarily cultural, by providing a good physical and chemical environment for root growth, and a generally suppressive biological soil environment for P. cinnamomi. An exciting possibility for the near future may be the incorporation of specific antagonistic microbes with clonal rootstocks to reduce root rot in the establishment years, possibly in conjuntion with a fungicide such as Ridomil^{R} or Aliette^{R}.

5. **Chemical control.** There is no good evidence with avocados that fumigation (methyl-bromide, Vapam^{R}, or Mylone^{R}) is necessary. With the development of chemicals such as Ridomil^{R} and Aliette^{R} which are highly active specifically against P. cinnamomi, new possilbilities of disease control have recently emerged.

Chemical control research continues with:

a. Preventative nursery treatment of clonal rootstocks at planting time.

b. Post-plant alternation of Aliette^{R} and Ridomil^{R} for stable disease control.

c. New methods of post-plant treatment including tree injections.

Summary of disease control. The critical factors for adequate control of avocado root rot are the planting of a good clonal rootstock, careful cultural practice and intelligent use of fungicides. This is an integrated control approach and does not rely excessively on any single method for reducing the impact of root rot.

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Avocado Clonal Rootstock Characteristics

Rootstock	Tolerances/Advantages	Suceptibilities/Disadvantages
Toro Canyon	root rot, 2 citricola crown rot, 1 salinity, 1 production, 2	wet feet
Duke 7	root rot 3 citricola crown rot, 2 tree color salinity, 2 production, 1	wet feet
Thomas	root rot, 1 production, 2	citricola crown rot, ? salinity wet feet
Borchard	lime-induced chlorosis production, 1	root rot citricola crown rot, ? wet feet
Barr Duke	root rot, 2 production, ?	wet feet
D9	root rot, 2? small tree size,? production, ?	wet feet
Dusa (Merensky 2)	root rot tolerance, 1? Production, 1?	wet feet

- The numerical rating following some characteristics indicates a retative relationship with other rootstocks so marked. 1 is best.
- Question marks are assumptions based on inconclusive, ongoing research.
- Research summaries are published in the California Avocado Society Yearbooks.
- No avocado rootstocks are able to tolerate prolonged periods of soil saturation.

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DRY NITROGEN FERTILIZER DOSAGE RATES
For Young Avocado Orchards

The following table is a recommended fertilizer rate sheet for hand application of dry nitrogen-bearing fertilizer for young avocado trees. It is a kind of compromise between conventionally recommended rates and the dosages we at Brokaw Nursery believe to be suitable. Our idea is that copious quantities of fertilizer should be added during the first three years for fast tree growth. We believe that in nearly all cases the fourth year will be a good bearing year and the dosages will thereafter be more conservative.

Beginning with the fourth or fifth year you should be guided by leaf analysis rather than by a simplified table such as this.

With this table we are recommending nitrogen rates only and the formulations include only two sources of nitrogen. Under special soil conditions you may find it more useful to use other nitrogen carriers. Also, you may want to utilize bearers of micro-nutrient elements such as Zinc and Iron. All this will depend on your water and soil composition. All computations assume approximately 110 trees per acre.

Year	Frequency of Applications	Total Weight/Tree/Year			Total Weight/Acre/Year			Approx. Amount per Tree per Application	
		N*	Urea 45%N	Amm.Nit 35%N	N*	Urea	Ammon Nitrate	Urea 46%	Ammonium Nitrate
1st	1/mo	1/8 lb	4.4 oz	5.7 oz	12.5 lb	28 lb	36 lb	3/4 Tbs.	1 Tbs.
2nd	6x/year (Feb, Ap, Jn, Jl, Aug, Sep)	1/4 lb	8.9 oz	11.4 oz	25 lb	55 lb	71 lb	3 Tbs.	4 Tbs.
3rd	4x/year (Feb, May, July, Sept)	1/2 lb	18 oz	23 oz	50 lb	111 lb	143 lb	.6 cups or 9.5 Tbs.	.8 cups or 12 Tbs.
4th	2x/year (Feb, June)	1/2 lb	18 oz	23 oz	50 lb	111 lb	143 lb	1.3 cups	1.6 cups

*N signifies elemental Nitrogen

Be sure that you add the fertilizers with sufficient water to dilute them. Place them in areas where they will soon be carried into the soil by rain or irrigation water. When the water is spread over a large area such as by rain or by sprinklers, broadcast the material over the root zone so that it is not concentrated in one spot.

Avocado Tree Physiology – Understanding the Basis of Productivity

New Project: Year 1 of 5

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Benefits to the Industry

A grower's margin of profit is the difference between the input costs to produce a marketable crop and the output, or the production itself. Any influence that affects one or both of these two items can make the difference between profit and loss. The management of the avocado tree under southern California conditions, which can experience rapid changes in temperature and relative humidity, provides a challenge under the best of conditions. 'Hass' productivity in California tends to be less than in other countries such as Mexico, Chile, New Zealand and South Africa where environmental conditions are less stressful. Additionally, increasing market competition from other countries is pressuring the California grower to become increasingly ingenious in orchard management practices so that profits can be made. . These practices include changes in how irrigation of orchards and management of tree size. Increasing numbers of growers are pruning older trees or considering high-density plantings. Canopy management strategies hinge on effective light management to increase fruit size and production. Unfortunately, the science behind the current strategies used to manage tree canopies and tree water status are poorly understood. We do not understand how the 'Hass' avocado responds to either light or water stress. This project will examine in detail the response of the avocado leaf to light, temperature, and changes in light and temperature according to carbon assimilation (which fuels both tree and fruit growth) and changes in evaporative demand (which governs the amount of water the tree requires). The outcome of this project will be a better understanding of the tree's response to environmental stress. This in turn will allow us to develop a canopy model of total carbon assimilation that will predict the effects of changes in relative humidity and temperature upon the assimilation. This research will provide the framework for predicting tree and canopy management strategies to optimize productivity.

Project Objectives:

1. An understanding of the effects of environmental variables (light, temperature, and relative humidity) on avocado leaf gas-exchange and carbon assimilation, essential for plant growth and fruit production.
2. An understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular how many layers of leaves within the canopy will support a positive carbon balance to the plant and how the duration of light flecks through the canopy can induce a positive carbon balance.
3. Development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on horticultural practices and will aid researchers in developing future research endeavors.

Summary

Assimilation as Affected by Relative Humidity

This year our work centered upon air relative humidity and how changes in it could alter efficient photosynthesis. When the humidity is very low, much more water evaporates out of the leaf through the stomata (transpiration). Under the best of conditions water from the soil flows through the xylem and wall spaces of cells to replace this lost water by transpiration. Unfortunately, often soil water potential[1] is low and that soil's water cannot replace the transpirational loss. Furthermore, resistance to flow within the tree through the xylem system causes a delay in the water movement, and thus the water lost through transpiration cannot be replaced rapidly enough to maintain the water potential of the leaf. The leaf's water potential governs the stomata gas conductance[2] and many metabolic processes so it is critical for the leaf to keep the potential as high as possible. It is our hypothesis that the avocado leaf can support some transpiration loss but as the relativity humidity falls, the tree (and the soil) cannot supply enough water and the water potential of the leaf falls. The immediate consequence is that the stomata close to prevent the high transpirational loss. Stomata closure will limit CO_2 entry, which is the critical metabolite of photosynthesis. These relationships are shown in the next two figures.

Figure 1 shows the water potential of a leaf, when it is held under a constant transpiration stream held for either 1 or 3 hours in cotton. After one hour the minimum leaf water potential is about -520,000 Pascals (about – 5 bars). Even for relatively high transpiration (1.5 mmol/m^2 sec) under these conditions, the water delivery system can maintain the water potential at a reasonable level. However after 3 hours of the high transpiration, the water delivery system (from soil through the xylem system) begins to fail and the leaf water potential begins to decline (reaching nearly –8 bars). Long-term transpiration shows the limitations of the system and the level of the water potential reaches a critical state.

The actual dependence of the leaf stomatal conductance upon the leaf water potential is shown in Figure 2 for Oleander. There seems to be a threshold from 0 to about -6 bars where the conductance remains high. After the leaf falls below this threshold, the stomata begin to close reaching only 10% of full opening at –18 bars (generally, marked as a wilted leaf). This is true for many other plants but the actual levels of threshold and amount of conductance will vary with species. We don't yet have good numbers for these types of measurements for avocado.

Of course, the stomatal conductance[3] (or aperture) directly affects the photosynthetic productivity since the movement of carbon dioxide into the leaf is directly proportional to the stomatal conductivity as:

$$\text{Assimilation} = \text{conductance} \times (\text{difference in } CO_2 \text{ from outside to the inside})$$

The amount of light, which is absorbed and converted into energy to drive photosynthesis, alters the CO_2 level inside the leaf and so affects assimilation, but, with all else being equal, the conductance directly affects the assimilation rate.

Leaf Chamber System

Our leaf chamber system functions by measuring the photosynthetic responses of discs cut from fully expanded leaves. Water surrounds the cut edge of the leaf to provide high water potential such that the leaf should suffer only minimum water potential deficits. These measurements should indicate the maximum efficiency of photosynthesis when water potential is near maximum.

Using the continuous monitoring capability of the leaf chamber, we have been studying the time course for partial closure of the stomata. We have changed the maintenance of our trees from which we collect the leaves for the leaf chamber experiments. These data were taken from leaves from small trees kept within green houses (with

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[1] The concept of water potential is a powerful one in plant physiology since it defines the driving force behind water movement. Water potential is a measure of energy with pure liquid water defined as 0 bars or Pascals. Nearly anything done to water (evaporation or the addition of salts) will lower the water potential so that typically soils are –3 bars or for very dry soil, -25 bars. This means that water will tend to flow into that soil and the plant must expend considerable energy to extract water from that soil.

[2] Stomatal conductance is a measure of the flow of gases through the stomata and is generally in units of moles of the gas flowing through a square meter of leaf surface per second. Conductance is low when the stomata are partially closed and when they are fully open, conductance is maximum.

[3] Often stomatal conductance is abbreviated as g_s.

controlled temperature and no injury nor pests). These leaves had stomata conductance and assimilation rates as indicated in Table 1. Before the light illuminates the leaves to drive photosynthesis, respiration can be measured (expressed as a negative number in Table 1). Both the respiration and assimilation are reasonable compared with other tree crops. The stomata are not completely closed even after long periods of darkness, which allows the determination of respiration by measuring the amount of CO_2 released into the gas stream surrounding the leaf disc. We expected to be able to see a difference between younger and older leaves, but the data scatter and the apparent small differences made it impossible to statistically demonstrate any dissimilarity (see Table 1 Section B).

Within the leaf chamber a low relativity humidity rarely affects the stomatal conductance. Once conductance reaches a steady state level, it remains at that level for hours (see Table 2). The conductance rarely declines, even when the relative humidity of the air stream is lowered from the normal of 37% to a very dry 14%. On the other hand the temperature of the leaf rarely rises above 25 C, which is unlike a warm summer day where the air temperature can easily reach 35 C (95 F). So though the relative humidity is very low, the water vapor gradient from the leaf to the air is small because the temperature is low[4].

These are not unexpected results, as water should rarely become a problem in this system. The cells within the leaf disc are not very far from a source of water at the cut edge, and so the leaf water potential should not fall to a low value.

Light Intensity

Light intensity is critical for high levels of photosynthesis to provide metabolites (initially carbohydrates) to the plant and developing fruit. We suspected that in avocado the light dependence of photosynthesis was lower, but needed to demonstrate it.

The methodology to probe photosynthetic light dependence was made available using the leaf chamber. After the leaf within the chamber reached a steady state level of gas exchange after about 50 –60 minutes[5], the light intensity was decreased with neutral density filters[6]. Each level of intensity was held for about 5 minutes, which is long enough such that the assimilation rate can be accurately measured yet short enough to prevent the stomata from closing to limit CO_2 exchange. A typical curve is shown in Figure 3A. This cycle can be repeated if the light intensity is returned to 100% for about 45 minutes in order for the steady state of assimilation to be re-established. Typical curves for young and old leaves are shown in figure 3B. Interestingly the maximum assimilation (A_{max}) is higher for the younger leaf, while the half-saturation (K_1) is lower.

These data are taken under conditions where the stomata do not limit CO_2 exchange. These are the maximum extrapolated values for photosynthetic assimilation and may not be reached when the stomatal limitation is factored in. In fact, under our conditions after the light illuminates the leaf disc, the assimilation rate tracks the opening of the stomata, and under most conditions the stomata remain the limiting step for assimilation. This may explain why we did not see any statistical difference in stomatal conductance between younger and older leaves when the leaf disc was under high light (see the last section). The stomata conductance was altering the possible higher photosynthetic rate and thus both types of leaves responded similarly.

Relative Humidity

We have had considerable success in our measurements of stomatal conductance of leaves which remain on trees kept in the growth chambers (where the relative humidity can be precisely controlled). We measure the conductance on a series of leaves in the morning and in the late afternoon. After examination of the data, we found that relationships are more easily seen if we examine the change or difference in the conductance from the afternoon to the morning, as {g_s-PM} – {g_s-AM} as Δg. This difference is correlated to the conductance in the morning. If the morning conductance is high, the subsequent water loss generally leads to a closure of the stomata and so the

[4] The amount of water vapor that the air can contain rises exponentially with air temperature and so a low relative humidity at high temperature places a much greater force to remove water from the leaf than the same relative humidity at a lower temperature.

[5] Under these conditions the assimilation rate is often limited by stomatal conductance and, more importantly, any decline in light intensity will be ultimately balanced by a partial closure of the stomata to re-establish that balance.

[6] Neutral density filters are constructed such that the light quality or dependence upon wavelength or color of light is not altered as the intensity is uniformly decreased. The intensity of the light was lowered but the general color of the light was not altered.

afternoon conductance is quite a bit lower. However, if the morning conductance is low, the lower water loss leads to maintenance or even an increase of the conductance in the afternoon. Typical data are plotted in Figure 4. The relationship described above seems to hold very well for the varied experiments.

We have developed a model for this and are currently validating it (see Figure 5). More importantly, one would predict that a higher water loss (that is, for a given conductance, a lower relative humidity of the ambient air) would lead to a lower conductance in the afternoon, which would increase the slope of the lines in Figure 4. The parameters presented above are the least squares regression of the lines[7] in Figure 4. Generally these are for 12-18 leaves in each trial. In most case, the constant of the regression declines slightly, while the slope increases up to nearly two-fold. This means that for a drier atmosphere in the afternoon, the conductance (compared with the morning value) falls further than for a wetter atmosphere, leading to a larger impairment of the assimilation capacity of the leaf. Since it appears that assimilation is limited by the stomatal conductance under most cases, a drier atmosphere in the afternoon would have striking effect on productivity.

Other Areas

We are beginning the work that will allow us to measure total leaf conductance of a branch. We have purchased and are using on trees in green houses a sap flow monitoring system. It is a highly sophisticated system, which requires a great deal of understanding to make it work properly. Yet the data thus far gathered suggests that this measurement of sap flow will allow us to test our concepts in the field with changes in relative humidity induced by spraying water into the air around trees. The data will be incorporated into a model that will allow us to predict what we expect the stomata to be doing from simple micrometerological data, such as air temperature, relative humidity, light intensity and wind speed. Once we can predict stomatal conductance, we should be able to predict photosynthetic assimilation and productivity.

References

Bunce, J.A. (1978) *Effects of Shoot Environment on Apparent Root Resistance to Water Flow in Whole Soybean and Cotton Plants.* Journal of Experimental Botany, **29**: 595-601.

Brinckmann, E., et al., *Effects of Atmospheric and Soil Drought on Leaf Water Status and Stomatal Response.* pp 135-140. Cram,WJ, K Janácek, R Rybová, K Sigler[502.], 1984.

[7] Regression is for: [{g_s-PM} - g_s-AM] = constant + slope x {g_s-AM}, as is plotted in Figure 3.

Table 1 Part A. The Response of Gas Exchange to Illumination. The data were collected in the Leaf Chamber, before and after the leaf disc was illuminated by 1550 μmole light/m^2 sec (about ¾ of sunlight). The assimilation before illumination was actually a measure of respiration. The data are for a total of 5 older leaves and 6 younger leaves.

	No Light (Respiration)		Light (Steady Photosynthesis)	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Average	7.13	-0.13	15.43	1.43
Std. Dev.	8.88	0.23	4.52	0.58

Part B. The Response of Gas Exchange to Illumination for Older and Younger Leaves. The data were collected as above, but the analysis was on the basis of younger (but fully expanded) and older leaves, as judged by the collector of the leaves. There is no difference (to within 5%) by the Student t-test of older and younger leaves, in respiration or photosynthesis.

	No Light (Respiration)		Light (Steady Photosynthesis)	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Older Leaves				
Average	2.92	-0.02	16.04	1.69
Std. Dev.	2.12	0.31	3.41	0.61
Younger Leaves				
Average	9.93	-0.20	14.93	1.21
Std. Dev.	10.43	0.12	5.22	0.46

Table 2. The Stability of Leaf Discs to Longer Periods of Illumination under Varied Relative Humidity. Data were collected as described in the text and in Table 1. These data are for leaves that had reached a constant transpiration and were exposed to light for 3 more hours, with relative humidity either at 37% (no change) or dropped to 14% (indicated as shift in relative humidity). There was no change in either the conductance or in the assimilation observed from the rate data throughout the test or as tested by Students t-test to 5% significance.

	No change in relative humidity		Shift in relative humidity	
	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)	Conductance (mmol/m^2 sec)	Assimilation (μmol/m^2 sec)
Average	14.9	1.32	16.6	1.86
Std. Dev.	4.8	0.75	6.0	1.13

Table 3: Dependence of assimilation rate upon the light intensity. The experiments were performed as described in the text and Figure 3, where the stomata were not limiting. The assimilation-intensity relation was used to determine the kinetic parameters, in which: assimilation rate followed Michaelis-Menten Kinetics or rate = maximum assimilation x intensity / (intensity + half-saturation_intensity).

Leaf	Maximum Assimilation (umol/m^2 sec)	Half-Saturation Intensity (μmoles of photons /m^2 sec)
Young	2.07 ± 0.17	270 ± 20
Old	1.70 ± 0.59	560 ± 160

Figure 1. Changes in the leaf water potential with varied amount of transpirational water loss. (shown to the right)

The data are taken from Bunce (1979) for cotton. The stomatal conductance is measured for the plant as transpiration rate, which is the actual amonnt of water being lost by the plant. The leaf water potential is then measured for each rate. There are two experiments here: open circles, 1 hour after transpiration is constant and darkened square, 3 hours after the indicated transpiration has been constant.

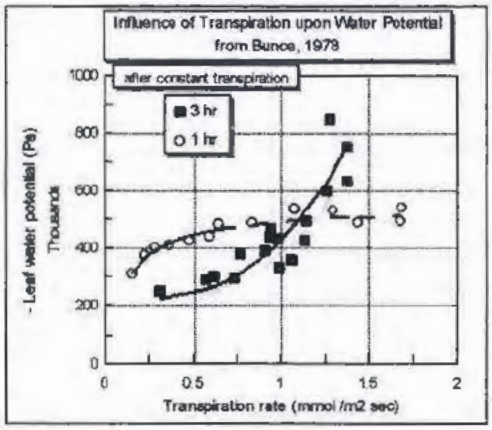


Figure 2. Dependence of Leaf Stomatal Conductance Upon the Leaf Water Potential. (shown to the right)

Data are taken from Brinckmann et al., 1984, for *Nerium oleander*. The leaf water potential was altered by allowing the soil to dry out naturally over 7 to 8 days. The water vapor deficit gradient (dependent upon the relative humidity of the air) was held constant at 10 mBar / Bar air pressure during the measurement of the leaf conductance.

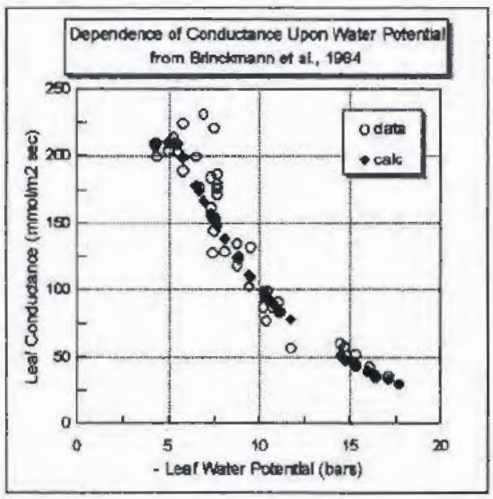
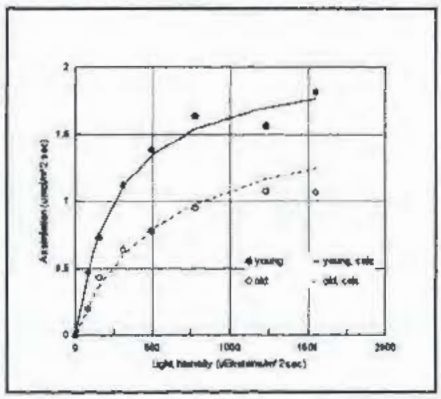


Figure 3. Typical Experiment for the Determination of the Assimilation Dependence upon Light Intensity.

After steady state was reached (under full illumination, generally about 1500 μEinsteins/m^2 sec of white light, from a tungsten filament source), neutral density filters were placed in the light path as indicated on the right hand figure [at the top figure]. These values are in optical density (OD) and are converted to intensity (% of full) by 10^{-OD}. The figure below shows the converted assimilation and intensity relationship for younger and older leaves.



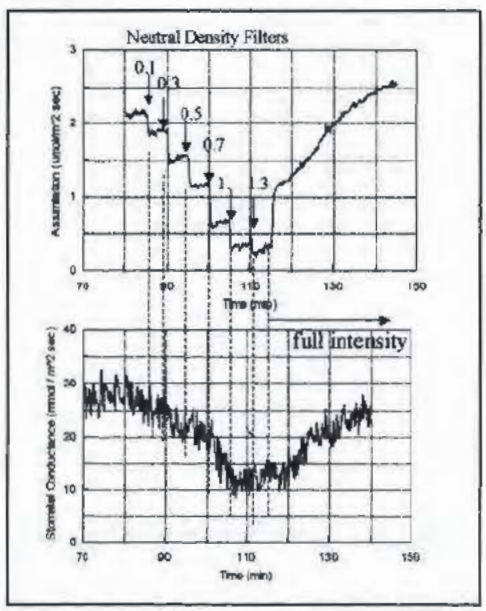
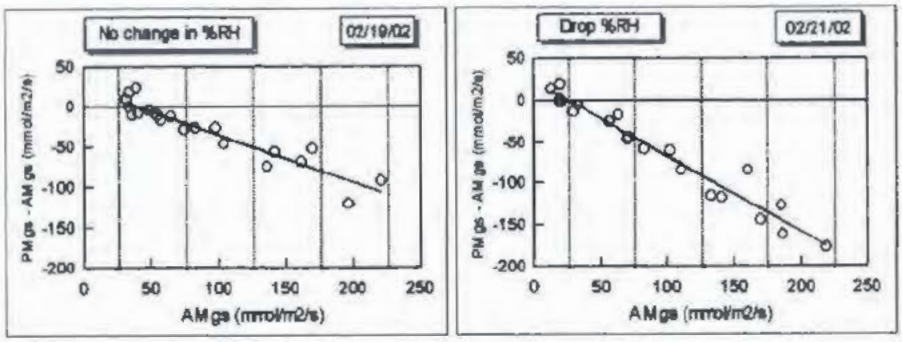


Figure 4. Part A. Conductance Changes Between Morning (AM) and Afternoon (PM) Induced by Relative Humidity of Chamber.

The conductance was measured using the LICOR 1600 Porometer in the morning or in the afternoon over a 2 hour period. Between the measurements (just before noon) the relative humidity within the Growth Chamber was either not changed (morning RH was about 45%) or lowered to approximately 20%. This plot emphasizes the change in the conductance in the afternoon by subtracting the morning conductance from it. This is a typical curve found during one week experiment. The slopes of the line are indicative of how greatly the afternoon conductance depends on the morning conductance.



Part B. Summary

The experiments were done as above. Fifteen weeks of measurements on 8 different trees generally gave the results as above, but experimental scatter makes it difficult to express. Here we took averages for each week for several trees and many leaves and then plotted the results. The data are similar to above; the slope of the line is greater under a shift in RH from AM to PM.

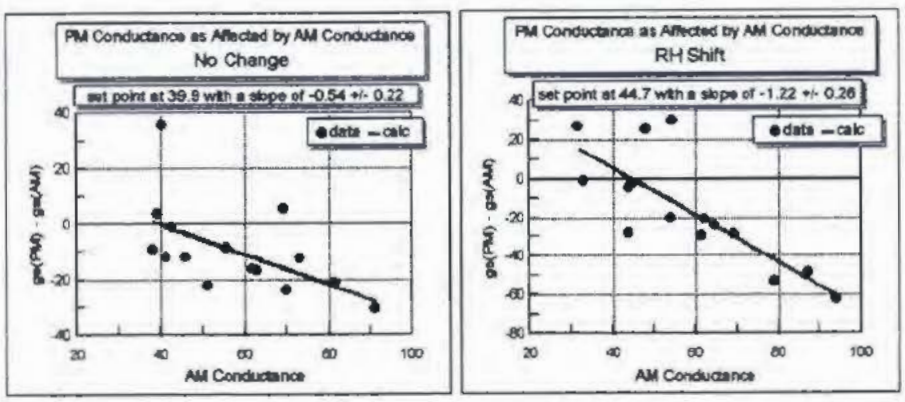
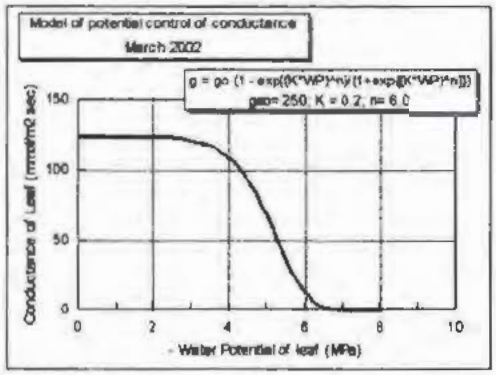
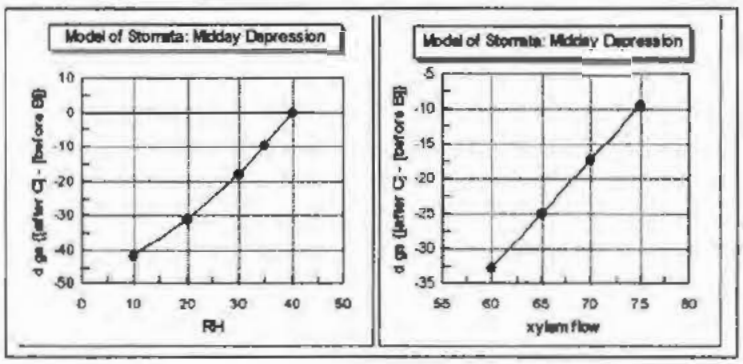


Figure 5. Model of water flow.

A model of water flow in Avocado was constructed in which the conductance was governed by a curve that looked much like the one of Figure 2 and is shown to the right. The loss of water was calculated over a 4-hour period (from noon to 4 PM) with a resistance to water flow from the soil was integrated into the total water picture (called xylem flow). If the soil and xylem couldn't deliver the water to the leaf, the leaf's water potential would fall and that would change the conductance of the leaf (and thus slow water loss). Important in this model is that the lowering of the conductance will lower assimilation and productivity. The curves for the change of conductance from morning to afternoon are shown for the final PM relative humidity (left side, below) and xylem "resistance" to water flow (right side, below).





AVOCADO TREE PHYSIOLOGY – UNDERSTANDING THE BASIS OF PRODUCTIVITY

New Project: Year 2 of 5

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Benefits to the Industry

A grower's margin of profit is the difference between the input costs to produce a marketable crop and the output, or the production itself. Any influence that affects one or both of these two items can make the difference between profit and loss. The management of the avocado tree under southern California conditions, which can experience rapid changes in temperature and relative humidity, provides a challenge under the best of conditions. 'Hass' productivity in California tends to be less than in other countries such as Mexico, Chile, New Zealand and South Africa where environmental conditions are less stressful. Additionally, increasing market competition from other countries is pressuring the California grower to become increasingly ingenious in orchard management practices so that profits can be made. . These practices include changes in how irrigation of orchards and management of tree size. Increasing numbers of growers are pruning older trees or considering high-density plantings. Canopy management strategies hinge on effective light management to increase fruit size and production. Unfortunately, the science behind the current strategies used to manage tree canopies and tree water status are poorly understood. We do not understand how the 'Hass' avocado responds to either light or water stress. This project will examine in detail the response of the avocado leaf to light, temperature, and changes in light and temperature according to carbon assimilation (which fuels both tree and fruit growth) and changes in evaporative demand (which governs the amount of water the tree requires). The outcome of this project will be a better understanding of the tree's response to environmental stress. This in turn will allow us to develop a canopy model of total carbon assimilation that will predict the effects of changes in relative humidity and temperature upon the assimilation. This research will provide the framework for predicting tree and canopy management strategies to optimize productivity.

Project Objectives:

1. An understanding of the effects of environmental variables (light, temperature, and relative humidity) on avocado leaf gas-exchange and carbon assimilation, essential for plant growth and fruit production.

2. An understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular how many layers of leaves within the canopy will support a positive carbon balance to the plant and how the duration of light flecks through the canopy can induce a positive carbon balance.

3. Development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on horticultural practices and will aid researchers in developing future research endeavors.

Summary of this Year's Progress

- ABA-induced responses seem to be normal within the avocado, which indicates that if water stress induced ABA within the root, the conductance of stomata will be influenced and made lower.
- The high evaporative demand induced by high temperature closes the stomata routinely in the afternoon similar to a shift of relative humidity to a lower value. The conductance in the morning influences greatly the change in the conductance in the afternoon.
- Assimilation is highest from 20-24 C and then declines nearly 80% (from 7 to 1 μmoles/m^2 sec, at medium light levels) as the air temperature rises to 38C. Concurrent with the fall in assimilation, the respiration of the leaf rises nearly 5-fold (from 0.2 to 1.1 μmoles/m^2 sec) over the same temperature range. The stomatal response with temperature indicates that it remains the major control of assimilation under relatively high light.
- A series of experiments indicate that the LiCOR porometer measurement does not take into account conductance of water vapor through the boundary layer, which seems to be limiting for the large avocado leaf at the low wind velocity.
- Wind (6 mph) increases the sap flow (and so the transpiration) by 31% within a green house. If the assimilation (limited by the conductance) is increased by the same amount by raising its boundary layer conduction, then the productivity would increase by 31% over midday.
- While the light reflectance of a leaf is slightly different from the top and bottom surfaces (from 400 to 1100 nm) and also differs for varied ages of leaves, the differences is only about 10-15% of the total.
- During the day only a small change in the sap flow between the east and west branch is found (about 10-15%). The environmental data suggests that diffuse light (that light which is reflected and scattered from the environment and tree) is nearly as good at supporting photosynthesis as direct light in Avocado.

Details

Leaf Chamber System

The production of avocado fruit is the *raison d'être* of growers; increased and more efficient fruit production begins with the ability of the plant to assimilate carbon through a process called photosynthesis. The source of carbon comes from the atmosphere as gaseous carbon dioxide (CO_2). Once it is converted into a stable compound (e.g. carbohydrates), the movement and arrangement of carbon can vary, but for efficient productivity, should be directed into the production and growth of fruit. Carbon dioxide enters the leaf through the stomata, which are analogous to tiny pores that have the ability to open and close in response to light and water stress among other factors. For efficient carbon assimilation the stomata must remain fully open during the day so that photosynthetic conversion of CO_2 is maximized at the prevailing light level; open stomata have maximum gas conductance, governing the speed of the gas movement[1]. However, since the stomata also control water loss from the plant, open stomata can lead to excessive water loss that is, unfortunately, harmful to the plant, especially when soil water is limiting. Stomatal physiology is set such that water loss is minimized under most water stress conditions, often at the expense of carbon assimilation. We wish to understand the mechanisms that control the stomata, a sophisticated control system to balance water loss and assimilation—a normal balance designed to maintain the well being of the plant, but not necessarily to maximize fruit production.

In southern California during the spring and summer months which are critical for fruit set and growth, rapid shifts to high temperatures and low relative humidity can occur. This can result in stomata closure early in the day thereby potentially limiting carbon assimilation and ultimately fruit production. This cause and effect is made worse when the relative humidity changes from relatively high (50%) in the morning to very low (<10%) in the afternoon, due to Santa Ana winds, which bring about rapid warming of the air mass around the canopy with concurrently lower relative humidity. It is our belief that high temperatures in the afternoon also detrimentally affect net assimilation and fruit production. Certainly, as the temperature increases, the relative humidity declines driving an increased water loss from the leaf. This water loss will ultimately cause the closure of the stomata, leading to an assimilation fall. Results from the first year of this project have suggested that another process, respiration, (a process that maintains the leaf by burning stored carbon and releasing CO_2) may be in operation. It has been shown in many plants that at higher temperatures the respiration of the leaf increases much more rapidly than does assimilation. Thus, respiration decreases the amount of fixed carbon (both as a percentage and absolute amount) available for translocation to the fruit, which could impact both fruit set and fruit growth. We have learned that although relative humidity is important, air temperature, as it controls the leaf temperature, may be even more important.

In 2001 – 2002 we began a collaborative effort to examine the effects of various environmental conditions and cultural practices on avocado leaf photosynthesis. The goal of this research is to determine how environmental factors such as light and relative humidity affect photosynthesis so

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[1] The nomenclature is, photosynthesis, photosynthetic CO_2 fixation, and (carbon) assimilation are the same and equal to the rate of or amount per unit time of carbon dioxide which is converted into carbohydrate (of all kinds) on the basis of leaf area. Translocation is the amount of carbohydrate moved out of the leaf to some other part of the plant. Transpiration is the rate of water loss out of the leaf and is governed by the opening of the stomata in leaf, which is related to the conductance of water through the stomata.

that growers and researchers alike can understand how orchard management decisions ultimately influence productivity. This year (2002-2003) we are continuing and expanding those finding to begin to generate a good model of how to manage the avocado trees.

Individual Leaf

Our lab has a leaf chamber system in which a leaf disk can be exposed to a mixture of gases that can be rapidly changed within tens of seconds. Rates of carbon assimilation (uptake of CO_2) and transpiration (production of water vapor by evaporation within the leaf) can be measured with two Infra-Red Gas Analyzers (IRGA). The monitoring of these important components of the gas stream and the leaf is carried out simultaneously with gas exchange alterations and give a good measure of CO_2 assimilation and stomata conductance. The cut edge of the leaf disk is sealed in a separate compartment through which flows a water solution. Thus, the water potential of the leaf is maximized since the cells are no further than 3 cm from plentiful water supply.

We proposed, using this equipment with 'Hass' avocado leaves, to examine the temperature dependence of both respiration (the rate of CO_2 production in the dark) and photosynthesis. Unfortunately we had problems with temperature changes between the production of the relative humidity, the chamber and the measuring system. The lower room temperature induces a lowering of the higher air temperatures, which does not harm our measurements as we monitor air temperature carefully. However, the temperature change from the chamber to the IRGA monitors is very detrimental as the measurement of the water vapor and CO_2 depend absolutely upon the temperature in the monitor, which we can't determine. We suspected that this would be a problem and we are trying on more modification to change the leaf temperature without generating artifacts of temperature changes. The technological problems made it impossible to perform the experiments we wished to, but we determined the temperature dependence of assimilation and respiration using the growth chambers and small trees (see later section).

On another note, conductance of leaves can be changed by exposure of the cells to abscisic acid (ABA). This phytohormone is produced when the tissue water potential drops to a low value due to a loss of water from the tissue; ABA is the signal to the stomata to close and so to conserve water. Typically the roots produce ABA due to the depletion of water within the soil; the ABA then flows to the leaf via the transpiration stream. To ascertain that the avocado stomata responded normally to ABA, we infiltrated the leaf with ABA (at 20-50 μM) in the water solution bathing the cut edges of an avocado leaf (**Figure 1**). ABA caused a drop in the conductance within 5-10 minutes and assimilation followed that drop, again demonstrating that the assimilation under these conditions was governed by the conductance (a finding of last year). The ABA infiltration was stopped after an hour (when the conductance decline leveled off), but the leaf did not recover since the ABA was still within the leaf. It generally requires an hour or two to metabolize the ABA to a form that it does not trigger the closure response; we are investigating that. Thus, ABA-induced responses seem to be normal within the avocado, which indicates that if water stress induced ABA within the root, the conductance of stomata will be influenced and made lower. This is not especially surprising but it is important to demonstrate that our thoughts about avocado's physiology are correct.

Growth Chamber

Our large growth chamber, which has good temperature and humidity control, can hold about 4 small trees. While the light intensity is lower than full sunlight, this chamber can be used to shift the relative humidity and temperature of the air rapidly. We are currently running experiments in

which the air temperature was shifted to a higher level in the afternoon (much like an afternoon in southern California), but it required some work to settle on a temperature/humidity protocol that yielded a reproducible closure in the afternoon. In later experiments we maintained the relative humidity while shifting the temperature. The evaporative demand (which is linked to the actual water vapor pressure in the air and drives water loss from the leaf) is linked to both relative humidity and air temperature, but shifts upwards with a simple air temperature change. We are still evaluating the data but it appears that the curves look similar to those reported last year. Thus far, it appears that the high evaporative demand produced by high air temperature closes the stomata routinely in the afternoon similar to a shift to a low relative humidity. Interestingly, the conductance in the morning greatly influences the change in the conductance in the afternoon. Low conductance in the morning gives rise to a very small amount of lowering in the afternoon, while a high conductance in the morning gives rise to a very large amount of lowering of conductance in the afternoon. The high rate of water loss in the morning makes it critical that the stomata close in the afternoon. As we discussed last year, the concept of a "set-point" for the optimal stomata conductance seems to be the best way to think of how the stomata change. Unfortunately for ease of prediction, the water loss of the day before seems to influence the current day's response. We have no clear picture of how to understand this yet.

We decided to change how we were measuring the leaves' responses and began to use a LICOR 6200 system, which measures both assimilation and conductance, in the growth chambers. The machine is larger and harder to use in the chambers but it gave us the opportunity to determine how assimilation (measured by CO_2 uptake in the light) and respiration (measured by CO_2 release or "negative assimilation" in a dark period) operated at varied temperatures. These data allowed us to by-pass the problems that we face with the individual leaf chamber and allow us to develop a relationship of chamber temperature *verses* respiration (CO_2 use in the dark, obtained by covering the leaf for a few minutes) and photosynthesis (the assimilation). Since we also measured the stomata conductance by changes in relative humidity, we developed a relation of temperature and conductance. These measurements are shown for a typical run in **Figure 2**. The protocol of how the experiment was done is shown at the bottom of the figure. It required about an hour to develop a stable temperature in the growth chamber (after a shift), which is about the same time required for stomata to change conductance to a stable value (see last year's report). We then spent about 15 minutes setting up the system to take the assimilation (or photosynthesis) measurements. A black cloth was draped over the leaf and Licor 6200 and after several minutes, respiration measurements were taken. This was repeated for about 3-4 leaves on 3 branches. Experiments were performed with an increase in chamber temperature over a day and with a decrease in temperature over a day. While there is variation from day to day and leaf to leaf, the general observation shown in the figure hold true for both an increasing and a decreasing air in chamber temperature. Assimilation is highest from 20-24 C and then declines up to 38C (our last temperature). It is noted here that that decline is nearly 80% (from 7 to 1 μmoles/m^2 sec). Concurrent with the fall in assimilation, the respiration of the leaf rises nearly 5-fold (from 0.2 to 1.1μmoles/m^2 sec). Indeed, at the highest temperature measured here, the respiration rate is nearly that of the assimilation rate[2]. There are two points about the stomata conductance: [1] it

[2] There is some question of how to express this because respiration is occurring during the assimilation measurement. For this particular graph, at 24 C photosynthesis is actually measured assimilation + respiration or (6.8 + 3.0) – 9.8 μmoles/m^2 sec while at 38 C photosynthesis is actually (1.3 +1.1) = 2.4 μmoles/m^2 sec or a decline from the maximum of 1 – (2.4 /9.8) = 75.5%

does not change by much (generally about 3-6%) in our short dark period and [2] it appears to be still limiting the photosynthesis as we have observed before (a plot of assimilation *verses* conductance is nearly linear). We have not completed all the evaluations for all the experiments, but point [1] is absolutely true, while some of the limitation indicated in [2] may not be completely true for all cases of branches/leaves.

Branch Water Use With Sap Flow System

The change of water flow through a branch gives a measure of conductance of all the leaves upon that branch. The carry out this measurement we are using a sap flow system on the branch for a continuous measurement of transpiration (**Figure 3A**). This sap flow system is placed upon the bark of the branch and a small amount of heat is applied within a insulated container. The temperature near the point of heating is monitored on both sides. The heat is lost by movement through the bark up and down but a sap flow through the branch increases the heat loss on the upwards side of the branch and so the temperature on that side is higher (**Figure 3B**). With correct calibrations we can measure total transpiration of that branch.

We can set the system up on a branch and let it run for at least two weeks. Once the heating is adjusted so that no injury occurs to the branch, but the heat is enough to be measured by the thermocouples within the system, the system works very well. The data is collected every 15-30 minutes into a data logger. After down-loading the data, the sap flow can be calculated on a spread sheet. A typical experiment is shown in **Figure 4**. It does vary during the day (3-10 grams/hour flow) due to temperature and light. It ceases during the night and takes time to rise to a high level in the morning, much like is measured for individual leaves. Thus, the system works very well and is stable. The leaves of the branch seem to be unaffected by the monitor clamped to the branch. In addition, the branch is smaller and more easily manipulated than a small tree.

Two major experiments upon a tree in the green house resulted in what were thought initially disappointment, as shown in **Table 1**. We found that the sap flow measurement of a branch is much lower than that calculated from the total transpiration of each leaf measured by a LiCOR 1600 porometer, which measures the actual stomatal transpiration. We felt that this may be due to two problems: [1] the LiCOR porometer measurement does not take into account conductance of water vapor through the boundary layer, which may be very high for the large avocado leaf and the low wind velocity in the green house, and/or [2] the portion of the branch measured (it is really measuring only a portion of a small branch) is not feeding water to all leaves on the branch. In other words, the sap flow through the branch is asymmetric. With other data (as discussed below) the concept has developed that the LiCor system does not measure the real movement of gas through the leaf. In other words, the LiCor by stirring the measurement chamber vigorously does measure the actual stomatal conductance[3]. However, the real gas flow is due to a flow through the stomata after moving through the boundary layer, which is the

[3] The actual Licor 1600 system takes the measurements by clamping small chamber on the bottom surface of the leaf. There is a small fan to rapidly circulate the air within the small chamber and the humidity of the chamber is measured. As the humidity of the chamber changes due to transpiration, the rate of change is used to calculate the transpiration rate (as a function of vapor pressure deficit) and the conductance.

unstirred layer surrounding the leaf. That movement is characterized by the boundary layer conductance[4].

Table 1. The branch used had 15 and 16 leaves on it and each leaf was measured by the porometer. The leaf area was estimated by measurements of length and width and the total conductance was calculated Each conductance was summed to provide the total LiCOR transpiration. The sap flow meter was calibrated as described by the methods provided by the distributor.

Trial	LiCOR Transpiration (grams/hour)	Sap Flow (grams/hour)
A	200	1.4
B	143	3.5

Again in Table 1 we test the real flow of the sap flow by measuring the measured stomata conductance with the sap flow. We find that the value is nearly 20-100 times lower. This suggests that the sap flow meter is incorrect or that the LiCor transpiration rate does not measure the actual transpiration stream. If the measurement systems are correct, the only interpretation is that we have not taken into account the boundary layer correctly. Unfortunately, this is suspected to be true by many researchers, but that boundary layer is very difficult to calculate or measure.

Tests of the Boundary Layer Limitation Concept

Our basic concept to date is that the stomata of the avocado provides the major limitation to gas flow into the leaf. With a gas flow limitation, light may not provide the measure of the maximum productivity of the leaf and anything that interferes with the stomata will limit CO_2 fixation, photosynthesis, and so limit productivity. In fact, our concept is that by measuring stomata conductance we can measure the productivity of the tree and our goal would be to maximize stomata conductance, either by manipulation of the microenvironment or by altering the tree's physiology.

Under that concept we re-examined some earlier data obtain under field conditions. In **Figure 5** we plot the data collected by Xuan, Mickelbart and Arpaia (unpublished). While these data were collected under very different conditions in the field, they fit a scatter plot of photosynthetic assimilation verses stomata conductance. The conductance measured in the field was very large under some conditions but so was the assimilation. **Figure 5A** show how well the data fit a enzymatic mechanism. While the data show a scatter (there is a large variability of the microenvironment—light intensity, relative humidity and air temperature—and of the leaf age), it does fit well an enzymatic concept, as given below.

$$A = - R + A_{max}\ g / (g + K_g)$$

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[4] The schematic of the movement of gases is shown in **Figure 4**. CO_2 is higher on the outside of the leaf (since it is being fixed within the leaf and so its concentration is lower there). Thus the flow is from outside to in and is govern by the resistance to flow and the gradient of concentration. Water vapor flow similarly except it is from inside (at 100% RH) to outside (bulk atmosphere). Both conductances are similar, except for the mass of the gas involved, and so CO_2 and H_2O move under similar driving forces There are however two resistances, stomata (r_s) and boundary layer (r_b).

where A = Assimilation, R = respiration, A_{max} = maximum assimilation, g = conductance and K_g = a constant governing the shape of the curve. This indicates that there is a maximum assimilation possible even when the conductance is very large.

The respiration measured here is similar to what is obtained under relatively high air temperature (see Figure 2). The conductance is higher than what was normally obtained under lower light conditions (high light has been correlated with higher conductance). Thus, here the highest assimilation rate would be about 54 μmoles/m^2 sec with a respiration of about 16% of the maximum at a field air temperature of 30-35C. Note that under most conditions, these data could be fit to a linear relation (assimilation varies linearly with conductance), indicating that the stomata limit assimilation.

Interestingly these data also yield a measure of how higher assimilation rate depress the internal CO_2, as expected (**Figure 5B**). The depression of CO_2 due to fixation causes the internal concentration to be lowered to about 200 ppm (with an external concentration of about 380-400 ppm). Thus once the internal CO_2 reaches 200 ppm, maximum assimilation is reached, suggesting the mechanism of feedback control on the avocado. Also shown in **Figure 5C** is the variation of conductance with a water vapor pressure deficit or differential (VPD) between the air and the interior of the leaf (there is a threshold before depression occurs of about 2 kPa and then conductance ceases when the VPD reaches 3.5 kPa).

Another test of the concept that we are not taking into account of the boundary layer conductance correctly can be seen with the sap flow monitor and an artificial wind (use of a fan). In the experiments shown in **Figure 6**, a fan blows air on a portion of a small tree (wind at 3 m/sec = 6.5 mph, onto gauges 1 and 3). On day 1 no wind is used but on day 2 wind is used on the branches that are monitored by gauges 1 and 3 (gauges 2 and 5 are on a portion of the tree not reached by the wind). The rate of sap flow is higher on the branches that are affected by the wind (the wind lowers the boundary layer resistance and thus should allow a faster gas flow and a higher level of transpiration and sap flow). The peak is higher under the wind but a better measurement can be made by integrating the total flow of sap during the period of wind (from 11AM to 5PM). If we add the increase on the wind gauges divided by the sap flow on the control (no wind) branches, we obtain a wind effect of 31%. In other words, the wind increases the sap flow by 31%. If the assimilation (limited by the conductance) is increased by the same amount (by lowering the boundary layer conduction), then the productivity would increase by 31% over the 6 hours of wind. More experiments are in progress but it is clear that measuring the stomata conductance by the LiCor which does not take into account the boundary layer and so does not measure the true gas exchange[5]. Furthermore, rapid mixing of the air above the leaf by wind increases the assimilation, at least under high light conditions.

Light Intensity

Another factor, which can greatly influence productivity, is light. Light provides the energy source that drives carbon assimilation in the leaf. Avocado trees, 'Hass' in particular, can develop a very large umbrella-like canopy that is largely empty inside. There is only a relatively

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[5] Licor claims that boundary layer is considered. It is in the actual measurement as a value is given to the program calculating the conductance which is governed by the rapid movement of air by the fan in the chamber. It is not this that is the problem. It is the lack of measurement of the boundary layer within the field that gives the wrong indications. In fact, it is very difficult to measure this on one leaf. It really takes an integrated measurement of the flow of transpiration stream (sap flow) that we feel is the correct measurement.

thin layer of leaves on the outer part of the canopy. It appears that light can be fully absorbed by this layer of leaves, leaving no light available for interior layers of leaves. This is a typical observation in physiological terms for other plant species, but it is quite striking in avocado. Once the light intensity drops, photosynthesis drops. If the photosynthetic rate is too low, it cannot compensate for respiration.. Under these conditions the leaf has a negative carbon balance; the leaf uses more carbon than it produces and is therefore a net drain on the plant. This can lead to leaf abscission. A large, somewhat empty canopy and leaf abscission is not a problem for the large-canopied tree *per se* but it is a problem for the grower since yield efficiency (the amount of fruit produced per cubic foot of tree) is greatly reduced. At this stage we do not understand the relationship between light intensity over a day and the potential for a leaf to abscise.

Yet an understanding of the developmental physiology of avocado leaves and how this relates to canopy management depends upon how the layers of leaves within the canopy can support a positive carbon balance to the plant and how a longer duration of a light period throughout the canopy can maintain a positive carbon balance. We now believe that light flecks of less than 10-20 minutes do not contribute to the overall productivity of the tree. It is rather the amount of prolonged periods of light that each leaf intercepts which drives the productivity and longevity of the leaf. The question of how long during the day each layer of leaves can be illuminated seems to be the more important point.

Initially we tested the movement of light through a leaf by applying the measurement of light reflected from and transmitted through a leaf using the Licor 1850 spectroradiometer (see **Figure 7A**). The measurements are light intensity at each wavelength into the surface of the leaf (I_{in}), the light intensity reflected from the leaf surface (I_{ref}), and the light transmitted through the leaf (I_{trans}). The difference of the amount in *verses* the amounts through and reflected is the absorption of the pigments within the leaf (see **Figure 7B**). It is that which is absorbed in the visible range (wavelengths of 400-700 nm) that drives photosynthesis and is critical for productivity. On the other hand, that which is absorbed in the IR range (700-1100 nm) alters the temperature of the leaf by heating. While the reflectance is slightly different from the top and bottom surfaces and those same parameters differ between the ages of each leaf, we see only about 10-15% total differences. These differences are too small to affect any productivity differences between different aged leaves or orientation and to affect greatly the leaf temperature. To be sure, under certain conditions of modeling they should be taken into account, but they are small shifts in light absorption and use and should not greatly affect productivity. It would be interesting to see certain differences between varieties as there are differences in wax composition/structure over the varieties and that might indicate how waxes affect the light absorbed by the leaf and so affect productivity. However, at this stage we feel that the effects are small (5-10%).

We are concerned about the amount of light which is intercepted by the leaves. There are two types of light: direct (that falling directly on the leaf from the sun) and diffused or indirect (that which is reflected from the sky and other surfaces). Direct is most intense and drives photosynthesis but diffuse can give rise to many effects of physiology and does provide enough energy to generate some photosynthetic products. In particular, avocado seem to carry out photosynthesis under relatively low light intensity, which is that of diffuse light. We wished to tested the notion that diffuse light maintained the stomata in an open conditions. Using the sap flow monitor, but applying separate monitors to two branches on opposite sides of the tree, east

and west facing, we followed sap flow during the course of the day. We expected that the full sunlight on the east side in the morning would support more open stomata on the leaves on that branch with a higher sap flow, while the opposite should occur in the afternoon—the sap flow of the branch on the east side should be lowered while the sap flow on the west side branch should increase. Unfortunately that was not exactly the case (see **Figure 8**). There was a small change in the sap flow between the east and west branch during the day but it was small (about 10-15%). In part we feel that is true because conditions change during the day as to the condition of the tree and the environment near the branches. But we also suspect that diffuse light (that light which is reflected and scattered from the environment and tree) is nearly as good at supporting photosynthesis as direct light. This has profound impact on how we model any canopy—it is not simply the direct light that causes productivity and so the side of the tree that is illuminated may not be very important in productivity calculations.

We tried to obtain an idea of the amount of light intensity that we have to be concerned with by measuring the light intensity in different directions at 8 AM in the morning (**Figure 9**). Naturally the sun was low in the east and the maximum intensity was measured in that direction (1650 µmol of photons /m^2 sec). Yet because of atmospheric scattering light was observed in every direction with about 200 µmol of photons /m^2 sec towards the west. This may be important since the dependence of photosynthesis in avocado seems to be saturated as relatively low light intensity, or at least that intensity is where the stomata limitation is the greatest and so higher light may not add much to photosynthesis. We are beginning a series of more controlled experiment in which the local environment on both sides of the tree are heavily monitor and more branches are monitored (as we have bought more branch sap flow monitors) to see how great this effect is.

An understanding of the developmental physiology of avocado leaves and how this relates to canopy management depends upon how the layers of leaves within the canopy can support a positive carbon balance to the plant and how a longer duration of a light period throughout the canopy can maintain a positive carbon balance. While we continue to be concerned with light flecks of less than 10-20 minutes which do not contribute to the overall productivity of the tree, we don't understand how these light flecks interact with the tree to allow it to use diffuse light more effectively. **Figure 10** illustrates a typical light exposure experiment conducted by the Mickelbart's group at Lincoln University. One-year-old potted 'Hass' avocado trees are maintained in a growth room with light levels around 1000 µmol of photons/m^2/s (about half of full sunlight on a cloudless day). Individual leaves are exposed to about 200 µmol of photons/m^2/s for a period of time before increasing the light level to close to 2000 µmol of photons/m^2/s (roughly full sunlight). We see variable photosynthetic responses in the leaves, both during light transitions and after. There are potentially several reasons for this. First, we may not be adapting the leaf to the lower light level for long enough. We are currently testing this theory. Second, we believe that the response will be dependent on leaf age and, more specifically, the flush in which the individual leaf was produced. We are currently setting up a series of experiments to test these theories and hope to have a working model for light response of individual leaves by the end of next year's work. At the moment, we believe it is the amount of prolonged periods of light that each leaf intercepts which drives the productivity and longevity of the leaf, rather than its ability to respond to short periods of intense light exposure. However, this needs to be rigorously tested before any conclusions can be made. The question of how long during the day each layer of leaves can be illuminated seems to be the more important point.

Productivity Model

We are developing a model of productivity based upon how the microenvironment around the individual leaves affect the stomata conductance and how the conductance and the light intensity alter the leaf's carbon productivity. This model will be a simple spread sheet that can be used to predict how the microenvironment in the field as measured by a few simple instruments can affect carbon fixation. However, it seems that the data obtain above are critical to understand which parameters of such measurements are important and how to integrate these measurements into the simple model. It is hoped that an individual small model can be used as a full tree model to yield predictions that will add to the ability of the grower to understand how certain treatments will affect his/her productivity.

Figure 1. Alteration of conductance by application of ABA. The gas flow across a cut leaf was measured as described in the previous report, but the leaf water potential was maintained at normal by solution of water flowing across its cut circumference. The data shown here are from a leaf that has been illuminated for about 1 ½ hours to reach stable state of assimilation and conductance. ABA solution (25 μM) was added at the arrow. Without ABA the assimilation and conductance remained the same (as shown). At the next arrow the ABA solution was replaced by a water solution. The steps down to zero for both traces were due to automatic zeroing of the IRGA used to measure the CO_2 and water vapor and, while necessary, were not part of the actual measurements of the leaf.

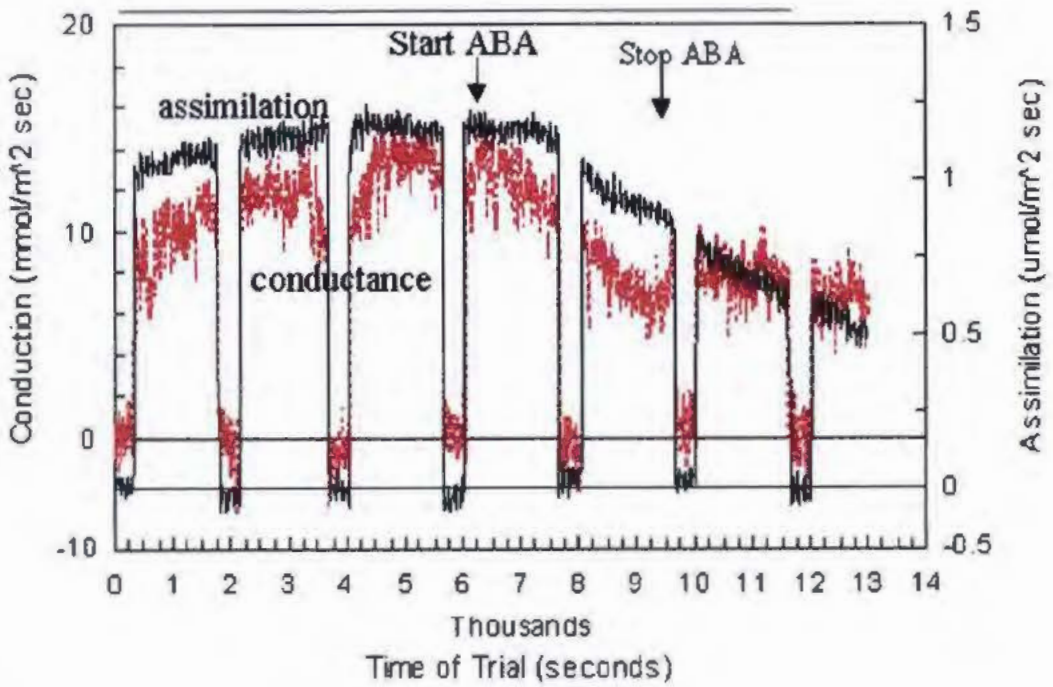
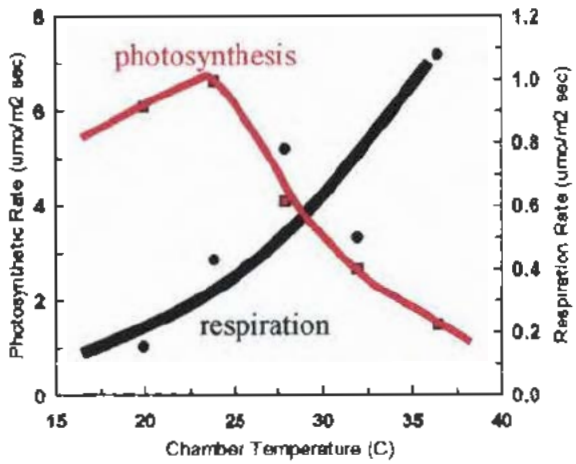
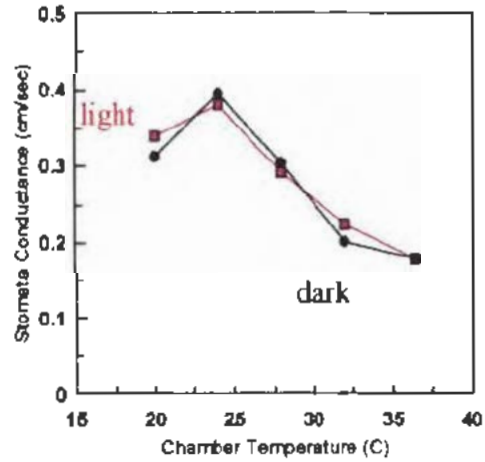


Figure 2. Temperature dependence of assimilation and respiration of leaves on a tree. The growth chambers were as used before (see previous report) and maintained at a constant relative humidity (43%). The air temperature of the chamber was shifted up (from 20 to 36C, as shown here) or down (from 36 to 20C, data not shown). The conductance and CO_2 assimilation of individual leaves (10 on 2-3 different branches of three small trees) were measured by a Licor 6200 system. CO_2 fixation (assimilation) or uptake (respiration) was measured in the light or dark (see Figure 2A) for varied chamber temperatures (average of 10). In 2B, the conductance of each leaf was measured in the light or dark. The sequence of the measurements for each chamber temperature was shown in Figure 2C. In all cases stable chamber temperature and stomata conductance was reached before any measurement were made. During the dark period the stomata begin to close but the measurements were made before any sizable change was measured (less than 10%).





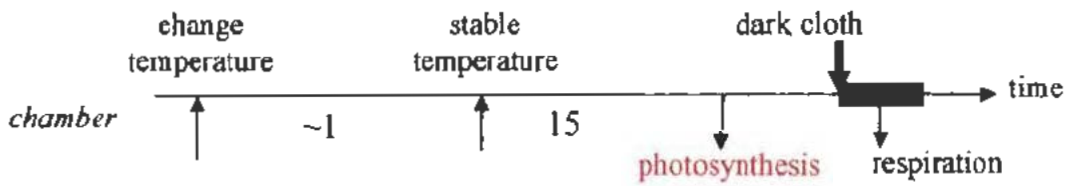


Figure 3. Sap flow measurement system. In figure 3A, the sap flow meters on the branches are shown within the insulation to slow heat flow out of the system. In figure 3B, the schematic of the heat flow within the system is shown and indicates how the measurements were made. A constant amount of heat is put into the branch and thermocouples measure heat flow up and down and outward to the atmosphere. The difference between in and out (up and down) was used to calculate the flow of transpiration upward, since that flow of essentially water carried heat up the branch. In figure 3C, a typical heat flow (dT) and actual sap flow (flow) is shown over the course of a week. The actual flow varied due to the microenvironment of the leaves on each branch. At night the heat flow was small and the calculation induced a large amount of inaccurate variability. Thus, the flow at night was set to zero. The time is 24-hour Pacific Standard Time.

Figure 3A

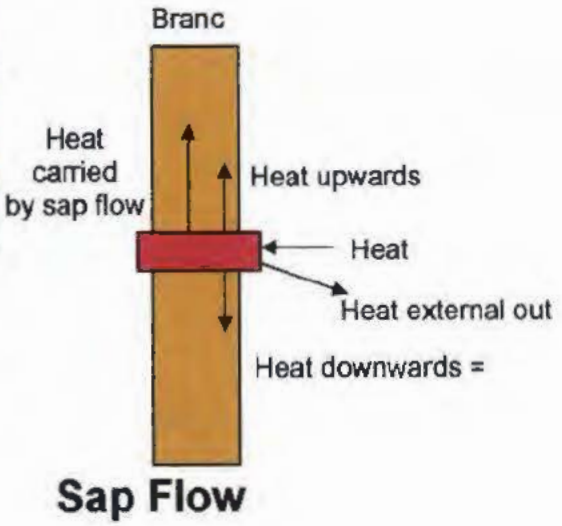


Figure 3B

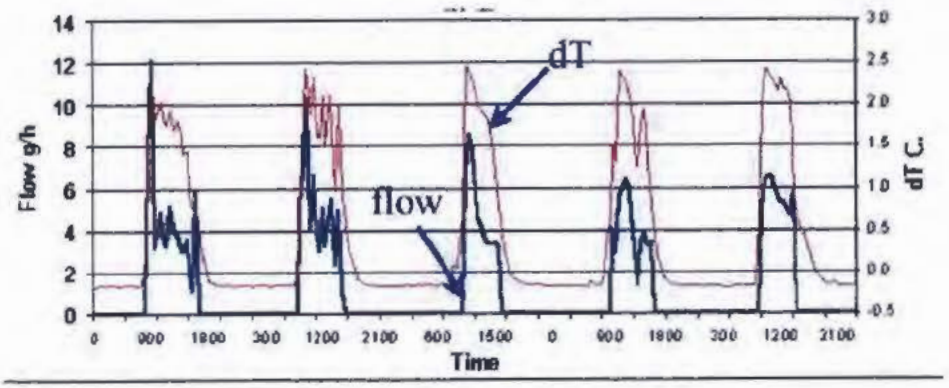


Figure 3 C

Figure 4. The schematic of the gas flow into/out of a leaf. See text for the description but the flux of the gas is proportional to the difference in concentration between inside and out regions and the total conductance of that flow. A better method of thinking about the flow is that the pathway gives a resistance to the flow. The total resistance is due to the sum of a boundary layer (b) and a stomata resistance (s). Then the conductance is just the inverse of the total resistance.

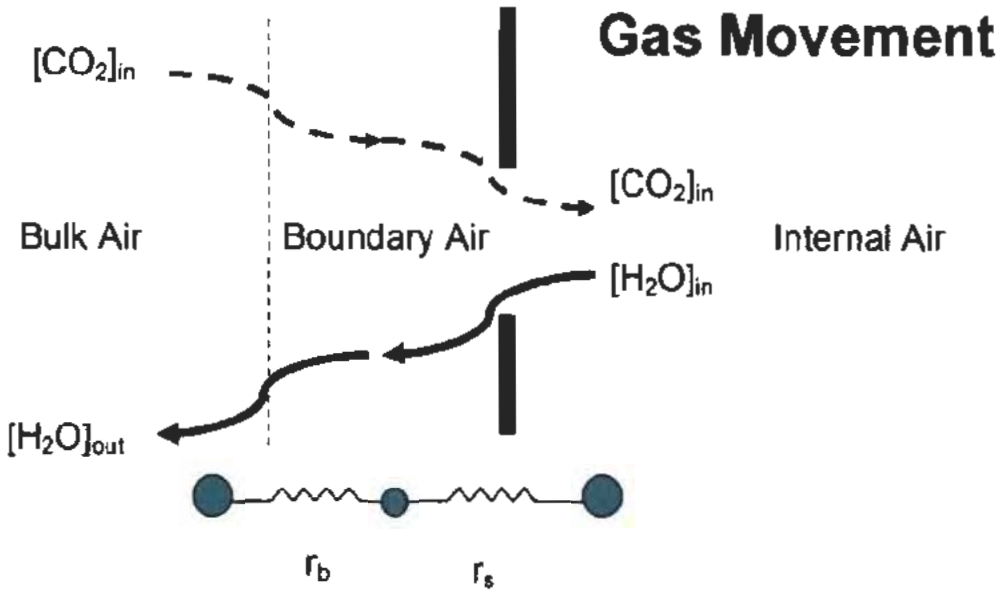


Figure 5. Gas flow from Avocado leaves. **A.** Data were taken from Xuan, Mickelbart and Arpaia, using a LiCor 6200 porometer on Hass Avocado trees. The data of assimilation and conductance is indicative of the field in Riverside over a wide range of microenvironments and leaf age and was fit to the enzymatic formulation of dependence of assimilation on conductance (see equation in text). The scatter of the data fits a Gaussian curve with a standard deviation of 3.5 μmoles/m^2 sec over all the assimilation data points, as an indication of variation. **B.** The dependence of the calculated CO_2 level within the leaf upon assimilation. As the assimilation increases, the internal concentration of CO_2 declines reaching a constant level of about 55-60% of the external level. C. The dependence of the conductance upon the leaf vapor pressure deficit (which is calculated as the difference between the internal water pressure of the leaf at 100% and the external water pressure of the atmosphere below 100%).

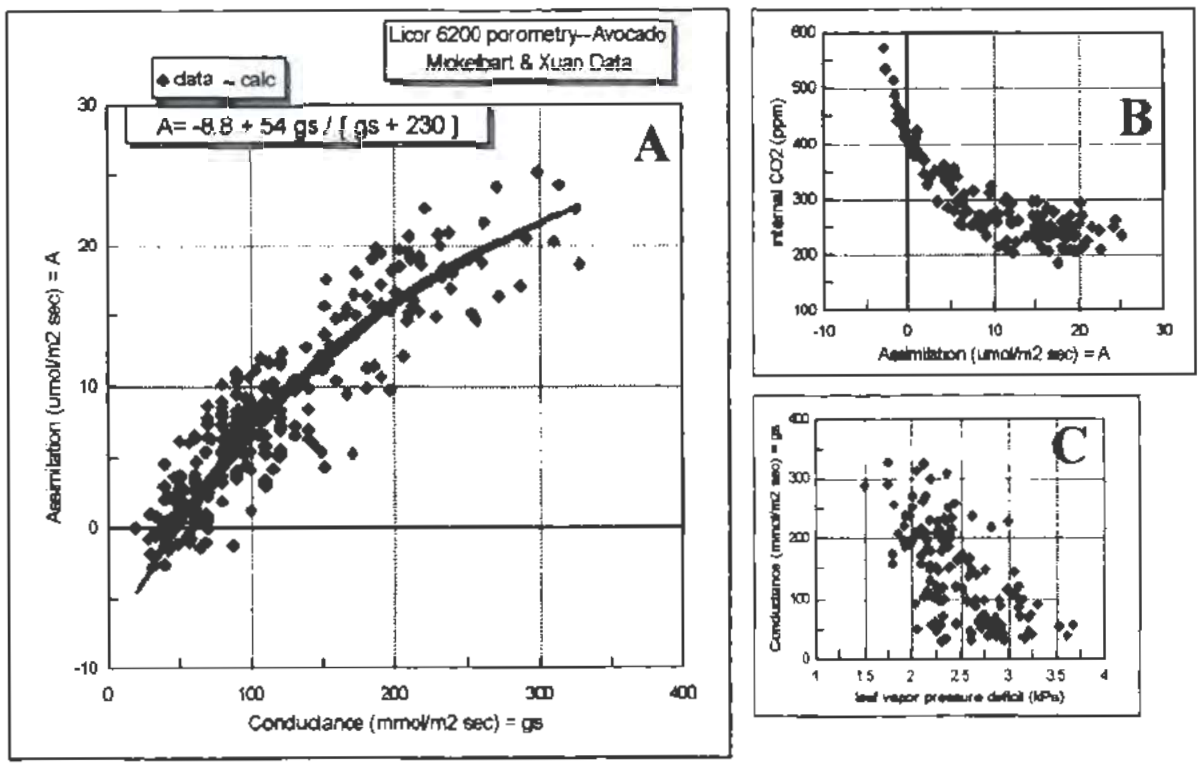


Figure 6. Effect of wind upon the sap flow of branches. Sap flow was measured by the sap flow monitor (see Figure 3). Four gauges were fitted on four separate branches of a single tree. Wind was blown on the one portion of the tree (see Figure 6A) from 11:00AM to 5:00 PM on day 2, while no wind was blown on day 1, see Figure 6B for the data.

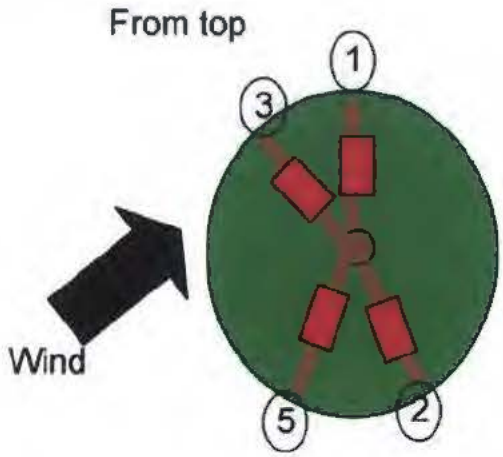


Figure 6A

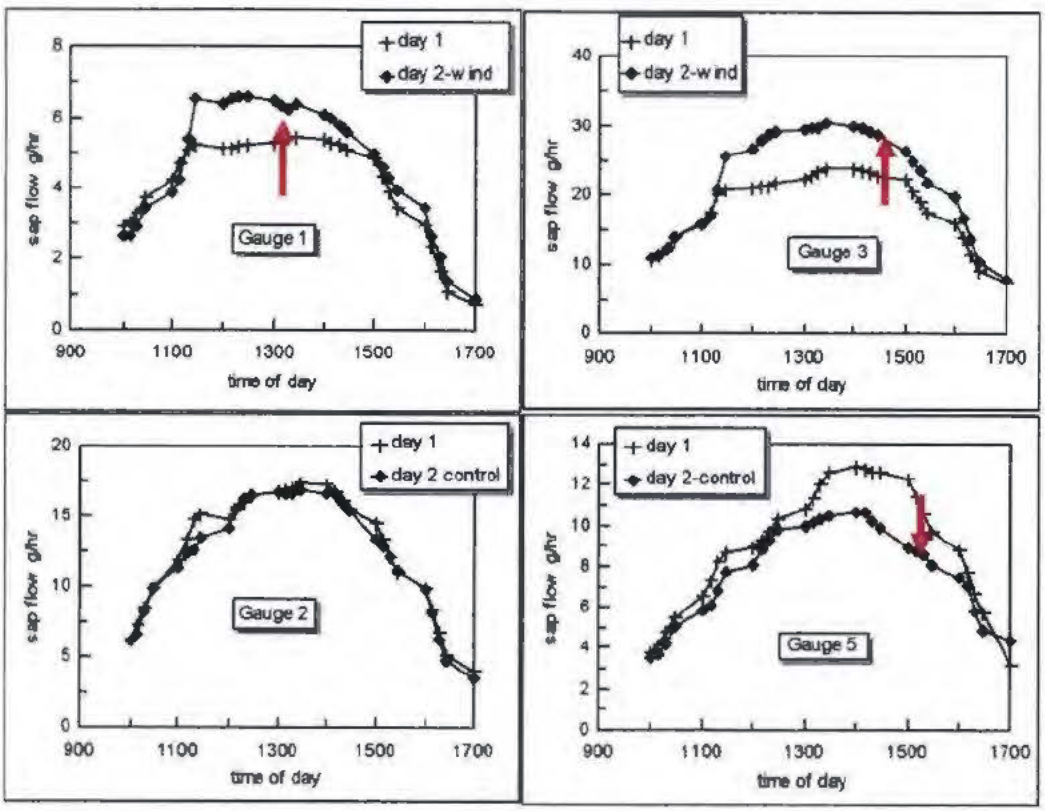


Figure 6B

Figure 7. Light absorption by avocado leaves. **A**. The distribution of light upon a leaf. I represents the intensity of light at a certain wavelength over a given band width of light intensity, where in = incident, ref = reflectance, trans = transmitted, and abs = absorbed calculated as [in – ref – trans]. **B**. Typical light parameters of an avocado leaf from a wavelength of 400 to 1100 n, in 5 nm intervals. The leaf is a mature leaf from a Hass avocado.

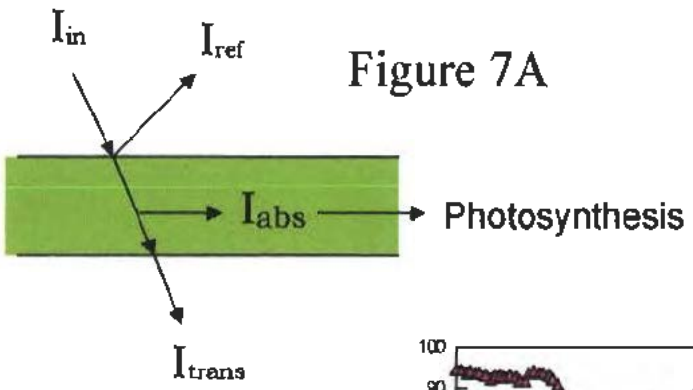


Figure 7A

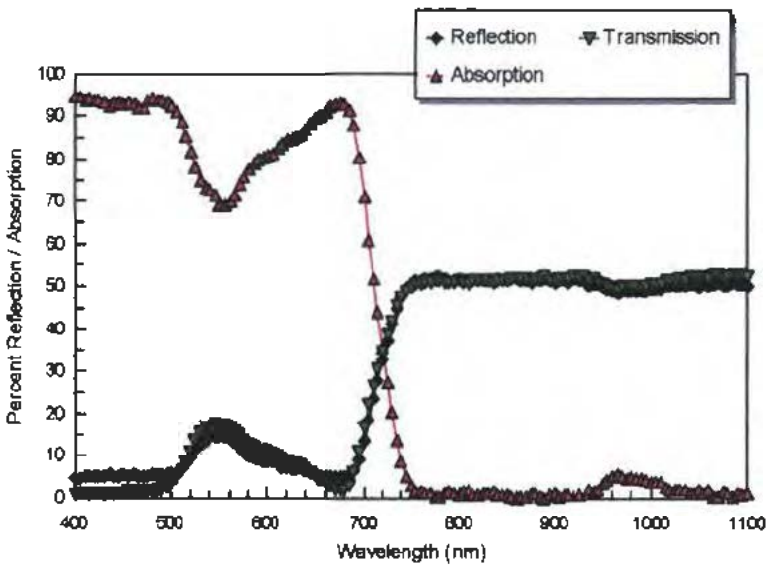


Figure 7B

Figure 8. Sap flow from a tree with sap flow monitors on branches separated by 180°. A comparison of a west and east facing branch on a Hass avocado for two days. Each day varied in its air temperature and light intensity but the sap flow on each branch has been normalized to 100% (while each were similar, they were: maximum flow on the east branch = 6.37 g/hr and on the west branch = 8.86 g/hr).

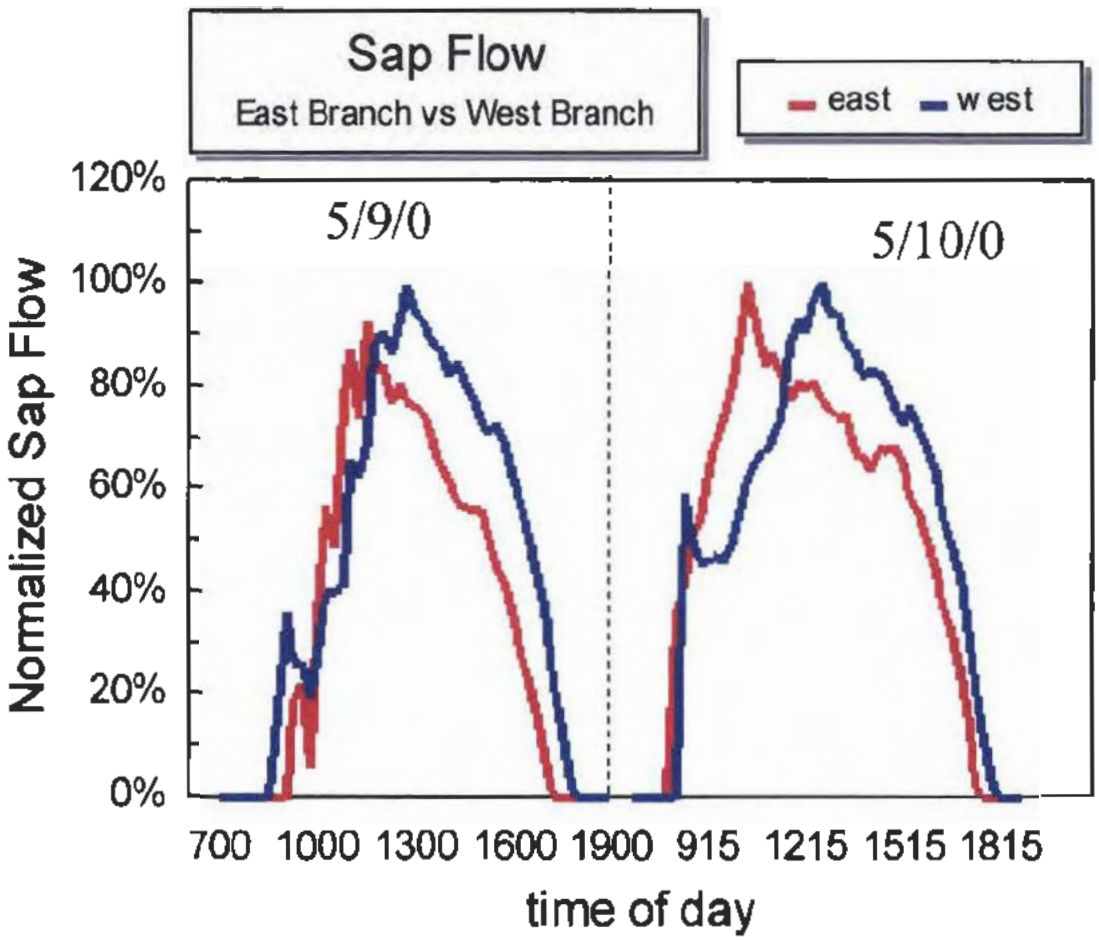


Figure 9. Directionality of sun light. The intensity of sun light in the summer as measured with a Licor 1850 spectroradiometer. The band measured was Photosynthetically Active Radiation from 400-700 nm. The region measured was at 45° from the horizon at 8 AM in the morning.

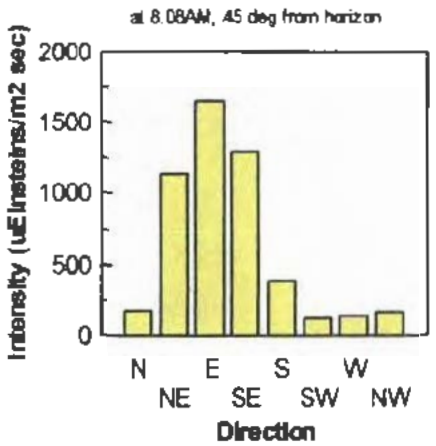
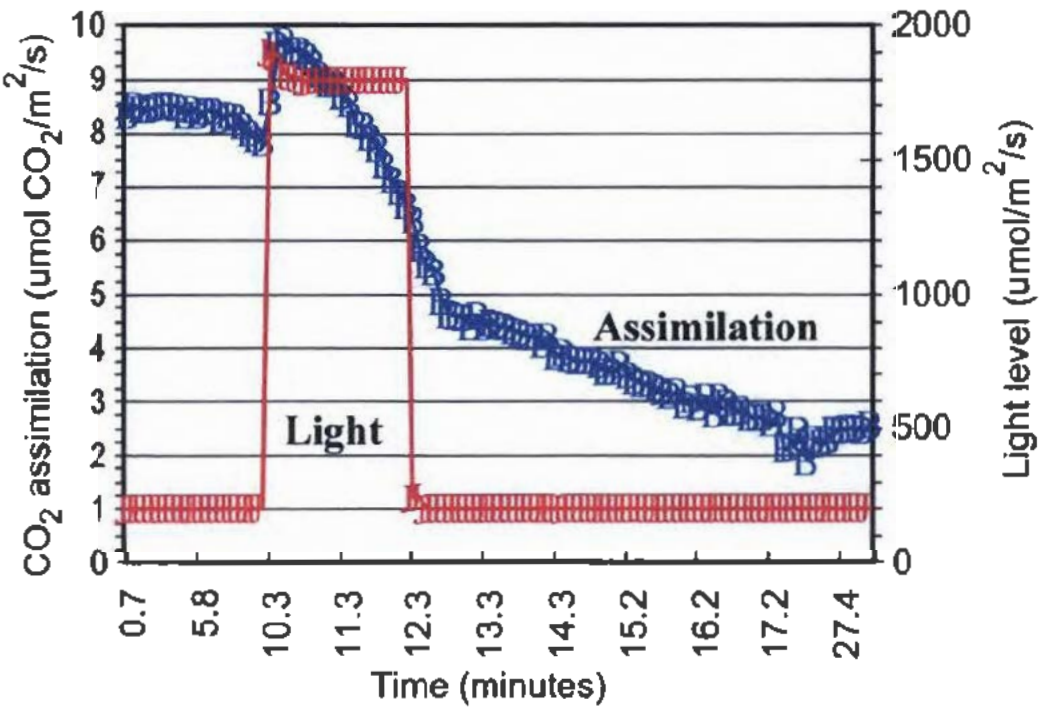


Figure 10. Leaf response to sudden changes in light (simulated "sunflecks"). The data were obtained with a Licor 6400 system to measure the assimilation rate. The gray line (denoted by "light") shows the light level the leaf was exposed to, and the black line (denoted by "assimilation") shows CO_2 assimilation rate. See text for more details.



Proceedings of the California Avocado Research Symposium, October 30, 2004. University of California, Riverside. California Avocado Commission. Pages 65-88.

Avocado Tree Physiology - Understanding the basis of Productivity

New Project: Year 3 of 5

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Benefits to the Industry

A grower's margin of profit is the difference between the input costs to produce a marketable crop and the output, or the production itself. Any influence that affects one or both of these two items can make the difference between profit and loss. The management of the avocado tree under southern California conditions, which can experience rapid changes in temperature and relative humidity, provides a challenge under the best of conditions. 'Hass' productivity in California tends to be less than in other countries such as Mexico, Chile, New Zealand and South Africa where environmental conditions are less stressful. Additionally, increasing market competition from other countries is pressuring the California grower to become increasingly ingenious in orchard management practices so that profits can be made. These practices include changes in irrigation schedule of orchards and management of tree size. Also increasing numbers of growers are pruning older trees or considering high-density plantings. Canopy management strategies hinge on effective light management to increase fruit size and production. Unfortunately, the science behind the current strategies used to manage tree canopies and tree water status are poorly understood. We do not understand how the 'Hass' avocado responds to either light or water stress. This project examines in detail the response of the avocado leaf to light, temperature, and changes in light and temperature according to carbon assimilation (which fuels both tree and fruit growth) and changes in evaporative demand (which govems the amount of water the tree requires). The outcome of this project will be a better understanding of the tree's response to environmental stress. This in turn will allow us to develop a canopy model of total carbon assimilation that will predict the effects of changes in relative humidity and temperature upon the assimilation. This research will provide the framework for predicting tree and canopy management strategies to optimize productivity.

Project Objectives:

1. An understanding of the effects of environmental variables (light, temperature, and relative humidity) on avocado leaf gas-exchange and carbon assimilation, essential for plant growth and fruit production.

2. An understanding of the developmental physiology of avocado leaves and how this relates to canopy management. In particular how many layers of leaves within the canopy will support a positive carbon balance to the plant and how the duration of light flecks through the canopy can induce a positive carbon balance.

3. Development of a model of carbon assimilation and allocation in avocado that will allow growers to make informed decisions on horticultural practices and will aid researchers in developing future research endeavors.

Summary of this Year's Progress

We have made progress on many fronts but two topics—leaf growth and temperature optima—have been largely described, leading to much better understanding of how we can follow flush development and why extreme California afternoons are a problem for production.

We have developed a methodology to measure leaf growth that allows us to place leaves into a coherent physiological development sequence and place branch development into coherent pattern. In other words, a few non-destructive measures on several developing leaves of a branch can give us a time scale of the full branch's development and a measure of the growth rate of that branch. This protocol has been a remarkable development of our measurements and allows for placing each of the flushes into their proper physiological context.

We have a concept of how varied air temperatures affect the rate of photosynthesis, as limited by the stomata conductance, and of dark respiration. It seems that the level of the CO_2 level inside the leaf (internal CO_2) plays an important role in control, in that as the temperature rises, the internal CO_2 becomes closer to the ambient with the assimilation rate leading stomata conductance. This means that photosynthesis *per se* is what higher temperatures are affecting, rather than stomata conductance. This raises two questions: [1] how long of a duration can high temperature be present before damage permanently alters the tissues and [2] does the loss of water and subsequent closure of the stomata, while lowering net productivity, actually help the survival of the tissue. Any field methodology used to change either air temperature or relative humidity, including when to start and how long to maintain, will be set by these findings.

Using a combination of varied measurement technologies, we have made progress in understanding the relative importance of the boundary layer around the leaf and within the canopy to gas exchange. Following of the leaf temperature by an infra-red camera has given us the tool to determine both the boundary layer interaction and the actual leaf temperature with respect to the air temperature. Under many conditions, wind does count in altering the real productive, as controlled by gas exchange.

In the long term, we hope to adapt models of conductance & assimilation to predict in productivity in environments of the field, based upon simple measurements of physical properties of the environment.

Details

Leaf Development

When we began our work, the community did not have a good method for describing how to measure avocado leaf development. There was no understanding of what measurements to make to determine where the leaf was in its normal physiological process from initiation to senescence and how it fit within the productivity of the branch, which contained it. We suspected that changes occurred in its time scale but did not know if that time scale was predictive in the leaf's productivity. Furthermore, it was not obvious of how a leaf's productivity was linked to the full productivity of the branch and each of its flushes. That total productivity would seemingly be linked to the production of fruit, but we had no simple linkage mechanisms. This is especially true in California, which has two flushes per year, where we suspect that one flush feeds the flower production and the other feeds the fruit production.

We suspected that young leaves were dependent upon mature portion of plant but, under some conditions, very old leaves were a drag upon the plant's efficiency and therefore were prime targets for abscission. To follow both the flushes and abscission efficiency, we needed to be able to determine a leaf's age with a few simple measurements. Then we could follow that age as we measure the efficiency of the leaf and how it relates to each flush on a branch.

A major accomplishment this past year has been to [1] determine a correlation between width and length, and area of the leaf, although it is variety dependent, [2] show that the area can be found accurately by using only length measurement, and [3] find that area can be correlated with leaf age. The leaf position along the flush (leaf number) can be correlated with leaf age and therefore flush age, according to each branch. Surprisingly, once a flush initiates the leaf production, its growth is surprisingly constant. Each leaf develops according to the flush and branch, rather than randomly growing. Most importantly, we have found that we do not have to take many measurements on individual leaves for a single branch. One measurement for every other leaf along the branch once a week is more than adequate to describe the leaf growth and the full flush growth characteristics—including the date of flush initiation and specific growth rate of each leaf.

Leaf Growth

In order to determine how photosynthetic assimilation (in the leaf) changes during leaf and fruit growth, it is important to understand the relationship between leaf physiological and chronological ages. Our major obstacle was the determination of the physiological age of a leaf. It was not possible to do this using a "single" measurement; rather we have resorted to developing a model which estimates an accurate growth curve for all leaves. The literature describes several types of growth functions, many of which only fit over one portion of the growth; however, the most reasonable model was the Logistic function since it duplicates most of the phases of leaf growth. The Logistic function possesses a rapid increase phase when the leaf is very small, followed by a shift to an exponential rise phase to a maximum size. The Logistic function is very similar to the Gompertzian function, which has been previously used for growth of animals and populations. While it seems that those differences are relatively trivial when both are compared, most series of measurements can show that the Logistics curves fit the

real growth more precisely[1]. The Logistics function has been often used in plant research and so we follow that tradition.

Briefly the Logistic function is described by leaf area (A) as follows, in which the growth rate is limited by the maximum size (A_{max}) but has a characteristic rate (κ):

$$dA / dt = \kappa A [(A_{max} - A) / A_{max}] \quad [1]$$

In integrating this equation we obtain a more useful equation ([2]) in which the symbols are given by t = time, A_{max} = maximum size of leaf, with α being an integration constant. If this equation is solved for leaf area, we obtain:

$$A = A_{max} / [1 + \exp(\alpha - \kappa t)] \quad [2]$$

This can be converted into a linear plot easily by some simple algebra and becomes:

$$\text{Ln}[(A_{max} - A) / A] = \alpha - \kappa t = \psi(A) \quad [3]$$

Thus, a plot of $\psi(A)$ verses time (t) yields a straight line from which we can obtain the value of κ (the slope of the plot) and α {$= \kappa t'$, where t' is the value of time when $\text{Ln}[(A_{max} - A) / A] = 0$ or when $A = A_{max}/2$, the half-grown leaf}. Thus, if we have several data points of A during the growth phase and the final size of the area (A_{max}), we can find α and κ for the leaf. The value of "α"gives a measure of when (in time) the leaf is half-grown. If there is a sequence of leaves growing under the same conditions (in which the growth rate, κ, is constant), then the value of "α" increases with leaf number[2].

We have found that under relatively constant conditions (in a growth room, data described in the figures):

[1] the "κ" values for a branch are virtually the same but that the value for each leaf does decreases somewhat with more recently formed leaves on that branch;

[2] the "α" values increase uniformly with leaf number, signifying a set-period between each leaf initiation act;

[3] the maximum size of leaves rises nearly 50% over the first 4 to 5 leaves but that size does vary somewhat over all the following leaves[3];

[4] while each branch has approximately the same "κ" value, the "starting-α" value (the time of initiation of the first leaf) varies between branches and trees.

This analysis can be used in a green house situation in which variability of growth is much greater for measurements that are made only twice a week. In essence, we have found our method for age analysis—a simple method of occasional measurements which gives us the leaf size for any time point and fixes one point of the growth in time (the half-grown size) from which all other events can be measured.

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[1] In the field the growth conditions is not constant due to variation in the ecological conditions and frequent measurements can show this. Unfortunately that then makes the fitting (described below) of the area to a logistics curve more difficult. Strangely we found that fewer measurements actually leads to the ability to fit a curve which is an average growth rate (in turn an average of the environment). Under these conditions it is difficult to determine the difference in these two curves.

[2] Leaf number is an important factor in this analysis. The numbering system is not arbitrary, but rather leaf 1 must be the first leaf to appear on the branch and the highest leaf number is the last leaf to appear with all others being sequential (see Figure 1).

[3] Later we will show that the morphology of the leaf (length & width) likewise changes from first initiated leaves to the later ones.

Theoretical Analysis

Our analysis of what the Logistic function signifies mechanistically is based upon Thornley's discussion (1990) in which the foundation of the use of the Logistic plot to describe leaf growth is laid. Thornley uses two interrelated equations to formulate how two variables interact to allow non-linear growth. Equation [4] is based upon productivity of photosynthesis (the "y" variable), which allows the area of the leaf (the "x" variable) to control its net productivity rate. Equation [5] is based upon decreasing the amount of photosynthetic capacity, used for the individual leaf growth; the decrease is due to the development of export capacity of the leaf to other sink regions of the plant[4].

$$dy / dt = \mu_1 x \qquad [4]$$

$$dx /dt = \mu_2 x e^{-b y} \qquad [5]$$

The two constants (μ_1 and μ_2) govern the growth of the leaf and the production of carbohydrate, both based upon the leaf area. The combination of these two equations allows for the defining equation of the logistic equation, as shown in equation [1].

$$d \{(1/x) (dx /dt) + b \mu_1 x\} / dt = 0 \qquad [6]$$

The solution of this equation is:

$$(1/x) dx /dt + b \mu_1 x = \text{constant} \qquad [7]$$

in which the constant should be defined as:

$$\text{constant} = \mu_2 + b \mu_1 x_i \qquad [7']$$

We will return to the x_i definition later. Here when $dx /dt = 0$ or no net change in the area (x), the x (at that point, x_f) is the maximum area of the leaf, and so using equation [7]:

$$(b \mu_1) x_f = (b \mu_1) x_i + \mu_2 \qquad [8]$$

$$x_f = x_i + \mu_2 /(b \mu_1) \qquad [8']$$

Combining all these relationships we obtain:

$$(1/x) dx /dt + b \mu_1 x = b \mu_1 x_f \qquad [7'']$$

$$(1/x) dx /dt = \mu_1 b (x_f - x) \qquad [9]$$

$$dx /dt = \mu_1 b x [x_f - x] = \mu_1 b x_f x [(x_f - x)/ x_f] \qquad [9']$$

which is directly related to the defining equation of the Logistic equation, where:

$$dA / dt = k A [(A_{max} - A) / A_{max}] \qquad [1]$$

Here with A = x, then $x_f = A_{max}$ and $\kappa = \mu_1 b x_f = \mu_1 b A_{max}$.

Results

One of the most important goals is to maintain a labeling sequence for the leaves on a branch. The two (or more) flushes must be separated as shown in **Figure 1**. Furthermore, the leaves

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[4] Both x and y are in units of g-DW but x stands for total weight with area as the surrogate for weight and y is for assimilation as carbon. Equation [4] represents total assimilation with y in units of g-DW /sec and so μ_1 is in units of sec^{-1}, and is proportional to κ. Equation [5] represents units of g-DW or m^2-area /sec and as such the units of μ_2 is likewise in sec^{-1}. Additionally "b" must be in units of $g\text{-}DW^{-1}$ in order for the exponential to be unit-less.

should be numbered from the first leaf of a flush to the last leaf (larger number, often 12 to 18 in most cases). The point of this exercise is to be able to easily monitor non-destructively the growth of each leaf and relate that to a physiological process. Further this monitoring must be such that missing a few days of observation does not handicap the analysis process.

Firstly we built upon our previous observations that the length and width of a leaf were related. Using a series of leaves from 'Hass' trees (on clonal Duke 7 rootstock) maintained in a green house, we measured the length, width and area[5]. **Figure 2A** demonstrates that the length times the width of a leaf is proportional to the area; this means that length *times* width *times* a constant factor (L x W x f) is equal to the area of a leaf. Thus, we do not have to scan the full area of the leaf but can use only length and width to obtain a measure of the area. Furthermore, with some small amount of error, only the length can be used to obtain a measure of the area if that length is squared (see **Figure 2B**). Again a nearly constant factor (g) times the length^2 can be used as the area. The error is due to the variation of width divided by length with leaf number. The first leaves tend to be broader than the later leaves, but there is some variability (see **Figure 3**). Thus, for our determinations we used [L x W x g] as the area.

Typical leaf growth curves are shown in **Figure 4**. These data are from Mickelbart (for growth of branches during 2003 in New Zealand in a controlled growth chamber) for 'Hass' avocado. The area has been determined as above for the first four leaves of Tree 1 & Branch 1. We determined full leaf growth for at least three branches on nine trees. While there is variation in area, the general trend is that each leaf slowly increases its rate of growth until it is about half its maximum size and then the growth rate slows ultimately ceasing when the maximum size of the leaf is reached. The period required for a leaf to reach maturity (maximum size) is about 26-32 days.

If the area data for one leaf is transformed into a Logistic expression $\{\text{Ln}\,[(A_{max} - A)/ A]\}$ is plotted versus the chronological time scale (starting a fixed date as time = 0), we obtain a straight line with a declining in value with time. For each leaf on a branch, the measured area is fit to a logistic curve giving a measure of two parameters (α and κ). In some cases due to insufficient observations we have to estimate the maximum size of the leaf. From a least squares regression fit, an estimate of the time (in terms of days of observation) that the leaf required to reach a 50% size (α / κ, in days) is calculated. That value of time to reach a 50% size is subtracted from the days of observation and denoted as the plastochron day; all leaves reach 50% of their maximum size at zero platochron day. The leaf size is likewise scaled to a percentage of the maximum size (by dividing the maximum area into the observed area). Those calculated data are then plotted for all leaves on a given branch (see **Figure 5A** for one shoot/branch). The data are uniform and seem to follow a single curve with the variation in data (denoted by crosses) being quite small. That total data (all points) are then fit to a branch/shoot Logistic curve (see **Figure 5B** for the linearly transformed data). The constant (α) and slope (κ) for each branch are relatively constant for each branch of the same tree. Further under uniform growth conditions those values are nearly the same for several trees (e.g., the nine that we measured here), although the date of initiation for the first leaf varies with each branch[6].

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[5] The leaves were scanned and then the area was determined from the number of pixels that the scanned image had. The calibration was with a known area of paper.

[6] The date of initiation of the first leaf of the branch is somewhat arbitrarily denoted as the zero plastochron day of a non-existent zero leaf.

For three separate branches on one tree (#2), the data for these parameters are uniform. In **Figure 6A** the leaf area (given as square dimension) becomes larger with leaf number, reaching a large size after the emergence of about 5-8 leaves. There is some evidence that the maximum size of each leaf varies somewhat for the higher numbers and may even vary between every other leaf from very large to somewhat large. This variation makes mere guessing the final size difficult and thus each leaf should be measured until it reaches maximum size (A_{max}). In **Figure 6B** the number of days to reach 50% of the maximum size of the leaf (zero plastochron day) seems to vary linearly with leaf age. The time between each leaf (the slope of this plot) is constant at 2.3 days, the time between leaf initiations. While we expect the value of κ to be constant, it seems to decline by about 20-30% from the first to the last leaf. The scatter of the data is large and often an average of all the κ values is adequate (see **Figure 6C**). The values of α ultimately rise, which is expected since it should nearly linearly track the increased value of the initiation (to 50% of the maximum size) **(Figure 6D)**. We have summarized all the data from this set of experiments (27 branches in all) and the variations of the growth rate and initiation rate of each leaf and branch are very small (less than 15%) under uniform growth conditions, which indicate how well the data fits to the theory.

Based upon this extensive data set we have developed a computer program to carry out the fitting of the data to the Logistic formula easily and have successfully used this technique to obtain growth patterns under several greenhouse conditions. We are currently using it to predict how old (plastochron age) a given leaf, is based upon its measured area (using length times width) in other studies.

This concept was found by studies in a growth chamber in which the amount of light and its directionality is fixed along with the relative humidity and temperature. This, of course, is not a usual situation but allowed for the determination of the relation of leaf growth. We have continued these studies to test their applicability to the "real world" through greenhouse studies (in which the air temperature and relative humidity was held constant) but the light varied normally throughout the day and a retrogressive study of field data collected a few years ago.

The greenhouse data (with constant temperature and variable light) was done with a longer spacing between each time points. Under these conditions, the light variability between days and the assumed difference in growth seems to average out. In other words, the data set can not see day to day variation but the increase in leaf area does fit a logistics curve in which the growth constant is the average over several weeks of growth. The field data collected in Irvine during spring and early summer (*from Xuan and Arpaia, 1998*) was taken every three weeks. Again the number of leaves within a flush and their timing off set gives rises to another possible fit to the logistics curve, but again an average growth rate and initiation time is found. Thus, the system seems to work well regardless of the timing of the data, but under variable environmental conditions, the average values are the best for understanding total productivity and for ease of data collection.

We are continuing these studies for flush development. Here the production of a new flush gives rise to a loss of the older flush, but not completely. We seem to have three cases of leaf loss—full loss of leaves, loss of half the leaves, and no loss from the older flush. We do not understand what conditions lead to what types of leaf loss but our fundamental hypothesis is that a balancing act exists between production and the flushes. The older flush "feeds" the younger flush initially but once the younger flush becomes able to contribute enough carbon to the newly growing leaves of that flush, the older flush is not required. However, the older flush does have the

ability to give more carbon to any developing fruit on that branch. It is our hypothesis based upon other work that one specific branch does not easily transport carbon to other branches. The question remains as to what causes the leaves to fall off of older flushes? It is a light limitation as new flushes on other branches shade the older branch or do plant hormones play a critical role in shifting the older flushes efficiency or need for continuation? We simply do not know at this point; however, evidence points to the role of light, intensity and duration.

Interestingly, the conditions in New Zealand can support up to 4 flushes at a time on a small tree. For our study of 12 trees, leaf loss was proportional to total leaf area. An individual branch seemed maintain a constant total leaf area at the expense of older leaves.

The Effect of Temperature on Photosynthetic Efficiency

We believe that temperature plays a large role with the problems associated with carbon assimilation in the afternoon. Our earlier work with humidity showed that low humidity caused excessive water loss from the leaf, especially when the stomata were highly open in the morning. This excessive water loss seemed to lower the water potential of the leaf therefore inducing an early closure of the stomata. This will consequently limit the assimilation of carbon dioxide into carbohydrates and so influences overall tree productivity. Yet when relative humidity declines during many summer afternoons, most of that decline is due to a rising temperature of the air. From a plant perspective this is expected to increase respiration and to lower assimilation. The question we are asking in these studies is while the stomata limit assimilation in avocado under many conditions, does this limitation also hold for higher temperatures?

In order to answer this question we first needed to observe how air temperature affected the normal assimilation, respiration and water vapor exchange of avocado leaves. We have a growth chamber available in which two trees can be maintained for about three weeks. In that chamber we can maintain both a set temperature (from about 21 to 37 C; 70 to 99 F) and a set relative humidity. Typically the chamber is maintained at a day temperature and relative humidity of 28C (77F) /40% and a night temperature and relative humidity of 15C (59F) /80%, with 12 hours of constant light (200-300 µmoles of light /m^2 sec) for the day. Twice a week (Tuesday and Thursday) we subject the trees to a changing temperature program as shown in **Figure 7**, starting at about 10AM. The chamber temperature is raised in steps from 20C (68F) to 36C (97F). After stabilization of air temperature and stomata conductance (requiring about 40 minutes), we measure the assimilation rate and conductance using a Licor 6200 system. We then cover the leaf and Licor 6200 with a dark cloth and measure the assimilation rate and conductance again (however, the assimilation rate is then negative denoting a production of CO_2, the dark respiration rate). We do this for five leaves, measuring each one in triplicate. We then reset the chamber's temperature, ramping up by 4C (7F) (see **Figure 7**), wait one hour and then repeat the series of measurements. There are two measurements sets in the morning followed by three measurements in the afternoon.

We have run four series of these experiments (each for about 3 weeks) using different trees. The low light in the chamber causes a problem to develop with the trees leading to early abscission after about 4-5 weeks. We started with two experiments were run per week, one following a ramping up and the other, a ramping down in temperature. We are in the process of summarizing all of these runs. The description below is for a typical run with a ramping up in temperature.

The direction of the ramping is critical as we do not obtain exactly the same shape of curve for both directions. The ramping down in temperature leads to lower inhibition of assimilation. We suspect that higher temperatures in the morning (starting at 36C) do not lead to a greater water stress in the afternoon when the temperature and water loss is less (at 20C) when compared with the protocol that leads to a higher temperature in the afternoon. Also the ramping down (starting at 99F and lowering the temperature) created problems with the health of the tree; avocado seem not to do well if the morning temperature is high. For California growers, the ramping up with a high afternoon temperature is more natural and so we are adapting that protocol for further experiments designed to find a recovery threshold for higher temperatures.

Assimilation seems to be highest at about 20-22C (68-72F; see **Figure 8A**); however, the scatter of the data makes it difficult to see a clear temperature peak for many trials. Unfortunately the growth chamber often cannot stabilize below 18C (64F), making many of the lower temperatures unreachable and so we begin our trials at near the optimum temperature for assimilation. Under most conditions and in **Figure 8A**, the assimilation falls to near zero by 36C, certainly the assimilation rate is less than 20% that of the maximum assimilation seen at 21C (70F).

The stomata conductance seems to follow the same trend as assimilation but the relationship is not totally clear (**Figure 8B**). Certainly the conductance is lower at 36C compared with 20C, but there is much variability in the data. We can conclude that conductance does not seem to be the limiting factor at the higher temperatures (see later). While a linear relation between assimilation and conductance occurs as has been previously discussed, the variation of the data makes the relationship unclear. From these data the question remains, at higher temperatures is the stomata conductance forcing the declining assimilation or is the decline in assimilation inducing stomata closure?

Leaf respiration rate increases with increased temperature (**Figure 8C**) and that increase can be fit by a linear curve or an exponential curve. Both work well from 20 to 36 C, except that the linear curve generates a positive respiration rate below 18C, which is not reasonable. The exponential curve possesses a negative rate at all values of temperature above 0C, but rises rapidly from 20 to 36 C. The scatter of the data points makes it impossible to judge which curve is best but from the literature one would suspect that the exponential curve is more realistic. Clearly the respiration rate at 36C is much higher (5-10x) than at 20C.

One concern was that the light intensity was not uniform across the leaves within the growth chamber for our measurements[7]. This seems to be the case (see dotted line in **Figure 9**). Some leaves are closer to the light source than others and their positions cannot be changed. In order to try to correct for any light intensity problems, we decided to use internal CO_2 concentration as a measure of effective photosynthesis (the internal concentration should be lower at more effective photosynthesis). For a more complete understanding, we have added a discussion of this parameter in the following section.

The Effect of Temperature upon Carbon Dioxide Use

The movement of water vapor from inside the leaf to the outside atmosphere (transpiration) is best described by an electrical conductance analog in which the water vapor moves from a

[7] When we perform experiments of changing relative humidity or temperature, we select leaves at the same height within the growth chamber so that the illumination is the same.

highest (chemical) potential down a diffusion gradient from mesophyll tissue to a region of the lowest chemical potential (the atmosphere surrounding the leaf) as illustrated in **Figure 10**. There are three principle conductances of movement (g^8) in the total pathway: (1) the boundary layer, (2) the stomata pore, and (3) the tissue and leaf air space itself. In the pathway for H_2O, the sites of evaporation for water vapor are cell surfaces very near the stomata pore via either epidermal or mesophyll cells near the guard cells. This means that there are only two conductance pathways for water vapor (number 1 and 2 above).

The stomata govern the rate of gas flow into the mesophyll cells within the leaf, where photosynthesis occurs. Two gases—water vapor and CO_2—are critical to the plant. The water vapor flow out of the leaf is responsible for the movement of inorganic nutrients up from the root zone. However, if water is evaporated from the leaf too rapidly, the water potential of the leaf falls and that alters many processes of metabolism including the ability of the stomata to open fully. Thus, there is a balance between water vapor loss and the conductance through the stomata.

On the other hand, CO_2 movement into the leaf is critical for assimilation and production of carbohydrates. If the light intensity is high enough, the assimilation may still be limited due to the flow of CO_2 into the leaf. The level of CO_2 within the leaf (called the internal CO_2 concentration) is due to a balance between gas flow into the interior and CO_2 assimilation (or uptake) via photosynthesis. In general, the internal CO_2 level is lower than the external concentration by a relatively small amount. The leaf tries to balance the flow in (via conductance) to the use inside (via photosynthesis) to maintain a relatively constant internal concentration. As light intensity rises and photosynthesis increases, the stomata open to allow more gas flow into the interior. Unfortunately, this allows the loss of more water vapor into the exterior, altering the water potential of the leaf.

We now understand that the assimilation rate of avocado leaves is largely governed by the conductance of gas flow, in that assimilation is linearly dependent upon conductance. It must be remembered that conductance is not exactly water vapor flow (or transpiration rate). That is governed by conductance and the gradient of water vapor from the leaf interior to the exterior.

While the water potential difference drives the movement, the conductance provides a measure of the resistance to flow. This formula is linear and follows an equation of flux = conductance times force (for water vapor, wv, and for CO_2, c).

$$j_{wv} = g_T \times \Delta(\text{force})_{wv} \qquad [10]$$

If we define the flux inwards as positive, then the $\Delta(\text{force})_{wv}$ must likewise be defined to the positive when the outside force is higher than the inside force. We will find that this is not the case for water vapor as the water vapor "force" is higher inside, thus this "difference" term is negative and so the flux is negative (from the inside towards the outside). With those definitions, the sign in equation [10] is correct as positive.

This means that the $\Delta(\text{force})_{wv}$ is due to the gradient of water vapor from inside, where it is nearly 100% relative humidity, to the outside, where it is governed largely by the relative humidity of the air. Thus equation [10] can be written (in general terms) as:

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[8] Although we speak of conductance to flow, the past mathematical formalism used a resistance to flow similar to Ohm's law for electricity in which the linear relation between a gradient of concentration or of electrochemical potential and flux or flow of material is given by r.

$j_{wv} = g_T \times \Delta$relative humidity $= g_T \{RH_{inside} - RH_{outside}\} = g_T \{100\% - RH_a\}$ [10']

There is another way of looking at the relation of assimilation to conductance. It begins with understanding that the flow of CO_2 is governed by the same relationship as water vapor, but in the opposite sense.

$j_{CO2} = g_C \times \Delta(force)_{CO2}$ [11]

The $\Delta(force)_{CO2}$ is the gradient of CO_2 concentration from outside to the inside and is given by the same relationship:

$j_{CO2} = g_C \times \Delta[CO_2] = g_C \{[CO_2]_{out} - [CO_2]_{in}\}$ [11']

The beauty of these relationships is that the conductances are related to each other. The conductance of water vapor is higher due to the lower molecular weight of H_2O, relative to CO_2. The relation is through the Lewis coefficients, so that $g_c = 0.958/1.346\ g_{wv} = 0.712\ g_{wv}$.

The flow or flux of CO_2 is the assimilation rate (A) and so equation 11' becomes:

$A = 0.712\ g_{wv}\ \Delta[CO_2]$ or $A = 0.712\ g_{wv}\ \{[CO_2]_{out} - [CO_2]_{in}\}$

$\Delta[CO_2] = \{A / (0.712\ g_{wv})\}$ [12]

Thus, if the gradient between outside and inside CO_2 (or for a constant external CO_2, the inside CO_2) is held constant, a plot of A against g_{wv} should be a straight line with a zero intercept or a plot of $\{A / g_{wv}\}$ against nearly anything (e.g., conductance, light intensity or temperature) should be constant. That is true only if the "anything" is not altering the basis relation that internal CO_2 is held constant.

Measurement of Internal CO_2 Concentration

Returning to the light intensity problem we calculated (for all temperatures) the average value of A / g_{wv} from the data set (see **Figure 9**). We found that there seemed to be little, if any, light dependence (shown as leaf position). The average value of A / g_{wv} was 1458 ± 262 $\mu mol/m^3$, which for this particular trial translates into an internal CO_2 concentration of 345.0 ± 9.0 ppm (for an external concentration of 395.0 ± 1.1 ppm). Thus, we felt that we could use this measure effectively for determination of the effect of higher temperatures.

From the data set shown in **Figure 8**, the difference in CO_2 concentration (effectively according to equation [12]) can be plotted against an increasing temperature. Under those conditions, we can easily see that the assimilation rate/stomata conductance declines as temperature increases (**Figure 11**). The falling gradient means that the internal CO_2 concentration is becoming closer to the ambient level (since that is constant with temperature, data not shown). It seems that the rise in internal CO_2 is nearly linear with temperature, going from nearly 300 ppm at 20C to nearly 400 ppm (ambient) at 36C (see **Figure 11**). If the stomata were closing prematurely (e.g., a more rapid closure than a lowering of assimilation would support), then the internal CO_2 should fall since the assimilation was using the CO_2 at a more rapid rate than the conductance could support. The rising internal CO_2 suggests that assimilation is being inhibited more completely than the conductance closure, leading to a closer equilibrium between the internal and external CO_2 levels. In many of the experiments, assimilation at 36C was close to zero while a sizable, non-zero stomata conductance remained.

These data suggest that high temperature presents a triple problem for avocado trees. The assimilation rate is becoming inhibited while the stomata remain partially open. That leads to water loss, with little assimilation. Furthermore, the respiration rate is dramatically increasing, leading to a loss of the carbohydrate made earlier in the day. The net carbohydrate within the leaf must be falling due to a decline in assimilation and an increased use of carbohydrate, and a water potential problem is develops within the leaf.

There was another interesting observation from the data set. Under most conditions, the dark period to measure respiration was only a few minutes and the stomata conductance did not change. This was expected from the light pulse experiments on the leaf disks; sun flecks of a few minutes duration do not cause much opening of the stomata. However, when stomata conductance was relatively high in the light, even a few minutes of dark would close the stomata and lower the conductance (**Figure 8B**). This effect was only observed for the higher conductance and suggested that the lack of assimilation (in the dark) would induce a rapid stomata closure. Thus, we would expect a faster closure in the dark if the conductance were high. We are re-examining our earlier data to determine if this is true. This effect has an important consequence on how conductance and assimilation responds when the leaf is shaded after a long period of direct sunlight.

Boundary Layer as Detected by Leaf Temperature

Last year we suspected that boundary layer conductance was influencing how the stomata behaved. For the most part, we measure only stomata conductance by porometry and hope that the boundary layer conductance is very high so that it does not matter. The effect of the boundary layer was suggested by the sap flow measurements, which were lower than that expected by the porometer measurements. Another test of this concept that we are not taking into account of the boundary layer conductance correctly can be seen with an infrared camera, which detects long infra red radiation due to black body emission. This emission is proportional to the temperature of the body and for a leaf, can detect the surface temperature of the leaf. If a leaf is maintained in the light at an air temperature of 28C, its surface temperature reaches about 31.0C (**Figure 12**). This temperature is a steady-state temperature which is the balance between the influx radiation (sun light) and losses of radiation due to a sensible heat loss or convection due to hot air rising and latent heat of evaporation (due to the loss of water through the stomata, see left side of **Figure 12**). The other heat loss is conduction due to air movements or wind. The principle balance to incoming radiation is heat of evaporation through the stomata. If a wind is applied to the leaf, it cools rapidly (ca. 3.0F) due to the loss of heat through conduction and this is directly a measure of the boundary layer. If the wind ceases, the temperature of the leaf returns to the previous value rapidly. The speed of this change is within tens of seconds. The actual calculation of boundary layer conduction is somewhat difficult (see Monteith and Unsworth, 1990), but can be done. Furthermore, if the air flow due to the wind is lamellar, the conduction can be calculated according to theory involving wind speed and leaf dimensions (see Schlichting & Gersten, 2000).

We are currently using this technology with the sap flow experiments to determine if we can show that sap flow measures only the flow of water through the stomata and that the LICOR value of stomata conductance is only part of the story of water flow (the other part is boundary layer conduction).

Canopy Structure

We continue to investigate the role of the canopy in allowing light to penetrate into the internal leaves. This penetration is critical to understanding sun flecks and the full canopy's productivity. Leaves on the outside of the canopy are exposed to at least half of the day length of full sunlight and thus can be as productive as the physiology and other environmental parameters will allow. This is the Layer I of leaves. Leaves deeper within the canopy are not so productivity since the Layer I can shade them for at least part of the day. The fundamental question is how many Layers of leaves can be supported by the normal distribution of leaves.

Experiments along the "sun fleck" line (described in the last years' reports) are continuing. Since the placement of the sensors within the canopy is critical, it was decided to do some modeling with more simplified systems to understand what types of events may be happening. **Figure 13** shows such a model. Here each leaf has the same dimension and orientation (they are "facing" upwards). Each layer of leaves has equal spacing from the one above and the leaves overlay directly the opening in the layers below and the next leaf in the layer two down. This structure allows for a simple model in which the illumination "rises" from the right and "sets" in the left side of the model. The angles (θ) can be related to the time of day and the durations of the illumination of full light on a given layer can be calculated during the day. For a given spacing and leaf size we can obtain the amount of and area illuminated by light for angle (or time of day). There are two points of interest: [1] the amount of light that the third layer receives increases as the spacing of the layers increases (concurrently with a decline in light for the second layer, see **Figure 14A**) and [2] this duration of light is probably the key to understanding whether or not the leaf is a net producer[9].

Of more interest is the actual light intensity falling on the leaf surface and how that intensity drives photosynthesis. If a leaf is not illuminated directly "face on", it does not receive as much intensity. As the angle varies from the normal (angle goes from 0 to 90°), the intensity falls as the cosine of the angle; as the intensity falls so does the photosynthetic rate. **Figure 14B** shows actual calculated productivity (per leaf) as the angle varies from 90° (sunrise) to 0° (at noon). Layer I receives all the illumination and reaches a maximum at noon. Layer II, partially shaded by Layer I, receives illumination later in the day but reaches maximum at noon (see Figure 13 for the geometry). Its total production will be less than Layer I. Layer III receives some illumination but never is the full leaf illuminated at any time and shading becomes severe at noon. It never becomes fully productive.

Although these are very artificial situations, they do give insight on the orientations that are most critical for leaves and how they may arrange themselves into "correct" layers for efficient productivity.

Continuing Experiments

We continue to test our sap flow measurements against both actual water use from a pot and the total transpiration rate through the leaves as measured by the Licor 1600 steady state porometer. These data sets have yet to be fully evaluated and thus we cannot say as yet what problems may

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[9] The total productivity of a leaf is equal to its photosynthesis over the entire area, when the leaf is illuminated less the respiration, which occurs throughout the day regardless of illumination. Thus, there is a minimum duration of light, which can provide enough photosynthetic carbohydrate to balance the loss through respiration.

exist. However, with small trees that have been pruned to a single branch, the water loss by sap flow measurements are within 20% of the water loss measured by water addition. However, it seems that the Licor porometer does not measure the actual water loss very well.

We are continuing our experiments with wind to lower the boundary layer but have not yet obtained reproducible results that are statistically significant. We have traced the problem to the changes in stomata conductance due to high water loss in the afternoon and are modifying the protocol.

We are continuing measurement of the assimilation with varied light intensity and duration by the use of trees within a controlled green house environment in order to better define the rate of response of the stomata to relatively brief illumination time of the leaves.

We continue to develop models of productivity based upon how the microenvironment around the individual leaves affect the stomata conductance and how the conductance and the light intensity alter the leaf's carbon productivity. This model will be a simple spread sheet that can be used to predict how the microenvironment in the field as measured by a few simple instruments can affect carbon fixation. It is hoped that an individual small model can be used as a full tree model to yield predictions that will add to the ability of the grower to understand how certain treatments will affect his/her productivity.

Reference

Monteith, J.L. & M. H. Unsworth (1990) Principles of Environmental Physics, 2nd edition, Edward Arnold Publishers, London.

Schlichting, H. & K. Gersten (2000) Boundary Layer Theory. 8th edition. Springer-Verlag, Berlin.

Thornely J. H. M. (1990) A new formulation of the logistic growth equation and its application to leaf area growth. Annals of Botany, 66, 309-311.

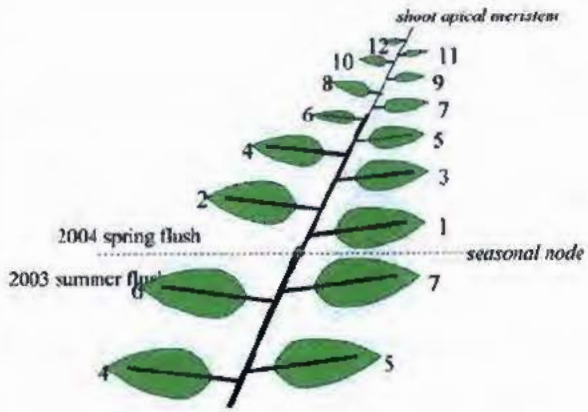


Figure 1. The Numbering Sequence for a Branch. The oldest leaf has the lowest number while the youngest leaf has the highest number. The physiology of leaves, which we wish to investigate, depends upon when the flush begins (denoted as when the first leaf appears) and the time between the initiation points of each leaf.

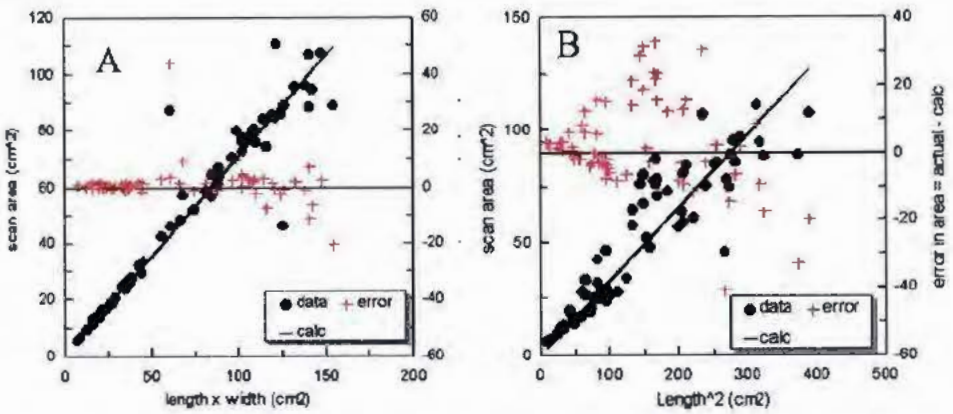


Figure 2. Calibration of Leaf Area by the Linear Dimensions of the Leaf. **A**, Calibration by Length and Width. **B**, Calibration by Length Alone. The scan area was that derived from the use of a Leaf Area meter, which measures the real area of the leaf by passing at a constant speed or by scanning, a leaf over a line of photodiodes, some of which are eclipsed by the leaf. The number of dark photodiodes over the scanned time represents the area. A ruler measured the lengths and widths. The error points (shown in red) are the difference between the actual area and the calculated area. The lines represent (for A) the actual area in cm^2 equal to (for A) f x length x width or (for B) g x length^2, with both length and width in cm. Both f and g are scale factors and vary with variety of avocado.

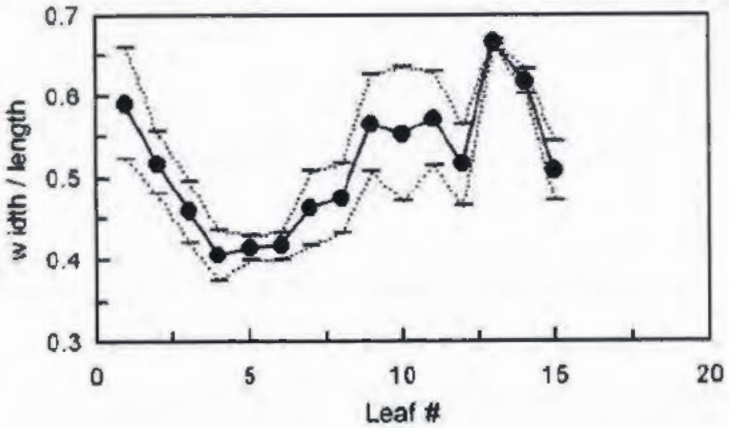


Figure 3. The Variation in the Width/Length Ratio of Avocado Leaves. Leaf number was measured as shown in Figure 1. The bars represent an error measurement (2 *x* standard error of the data, which was the leaves from three branches.)

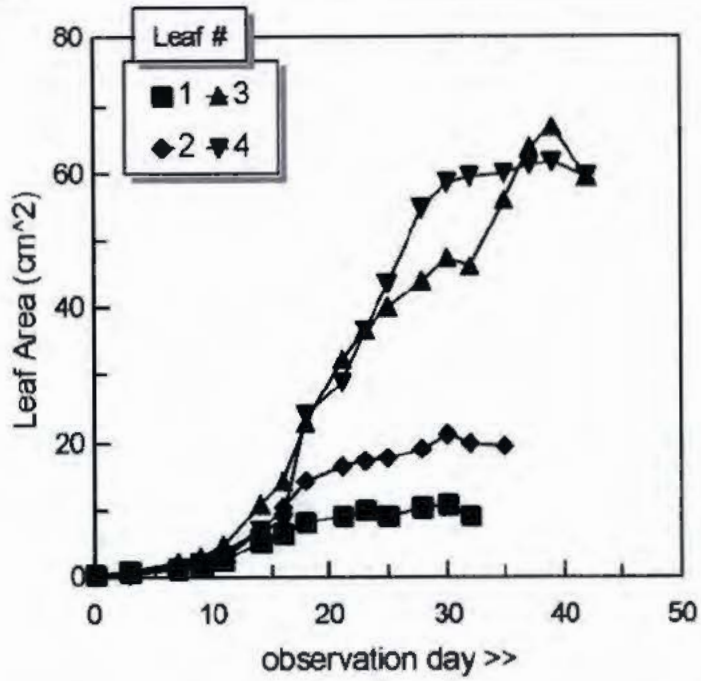


Figure 4. Growth Curve for a Few Typical Avocado Leaves. The Leaf Area was calculated from the length and width of the individual leaf. The "zero observation day" was Nov 13, 2003, when the experiment was started, and is arbitrary. One of the critical elements of the growth curve is the maximum size that the leaf reaches, as it is not absolutely the same for each leaf.

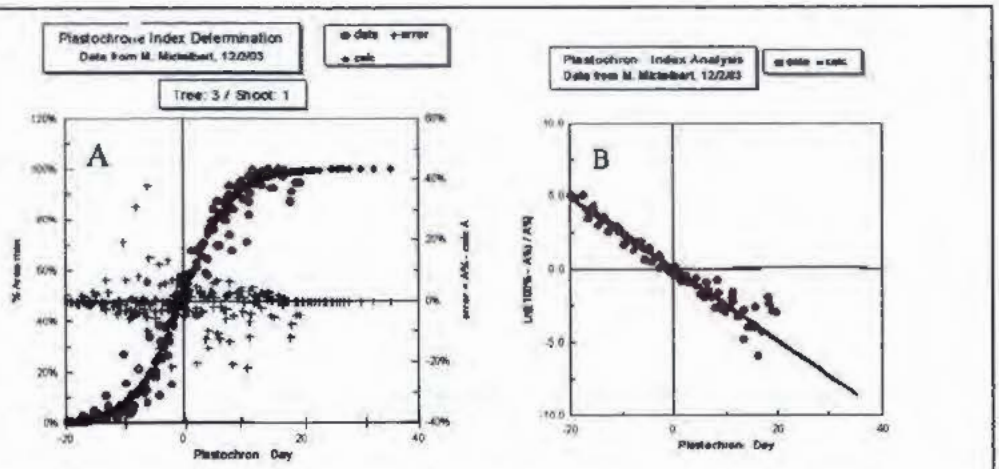


Figure 5A. Combined Leaf Measurements from One Shoot/branch of one Tree. The leaf area was scaled by the maximum size of the leaf and the zero plastochron day was set when the area was 50% of the maximum area (see text for more details.). Here 15 leaves from one branch were used, each leaf had a different time shift (which was equivalent to when the zero plastochron day occurred. **Fig. 5B**. The Logistic linear transformation of the leaf growth curve. Some of the data was taken from Figure 4 and transformed by Equation [3], see text.

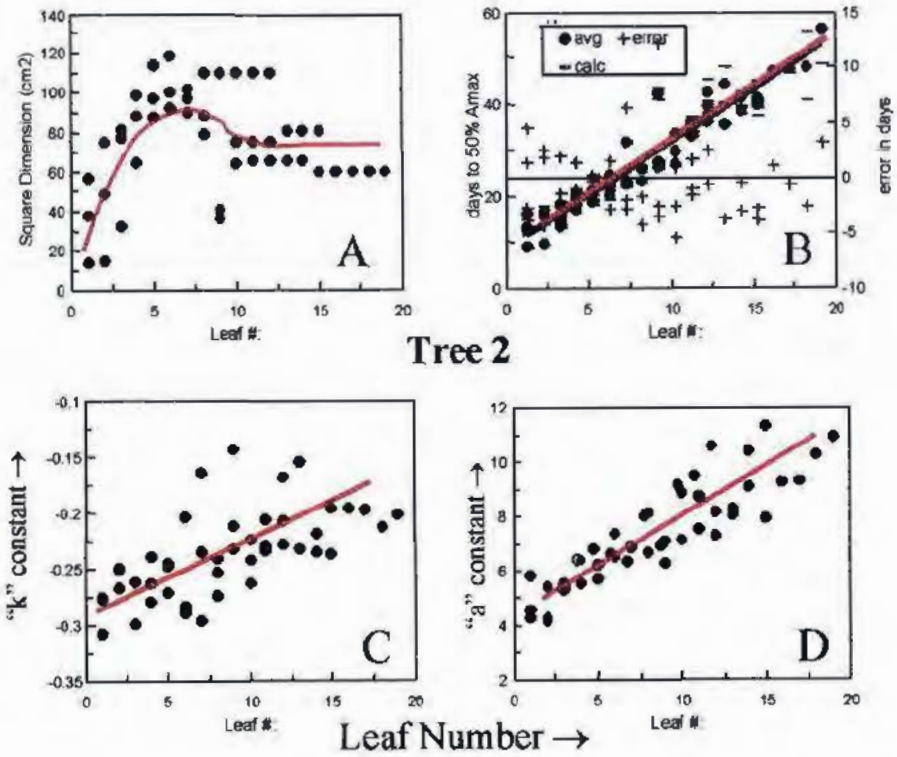


Figure 6. Logistic Parameters from One Tree. The various parameters for the Logistic formulation are shown in the above panels. They have been determined as described in Equation [3] and Figure 5B for three branches of one tree. **A**. The maximum size of each leaf. **B**. The time required to reach 50% of the maximum size, as measure of the plastochron day. The slope of the line represents the average time between initiation of each leaf and for this tree the time is 2.3 days between the initiation of each leaf on the three branches. However, each branch begins its initiation at a slightly different day (about 3-6 days variation). **C & D**. The two constant parameters of the Logistic growth curve—growth rate (κ) and point of initiation (proportional to α).

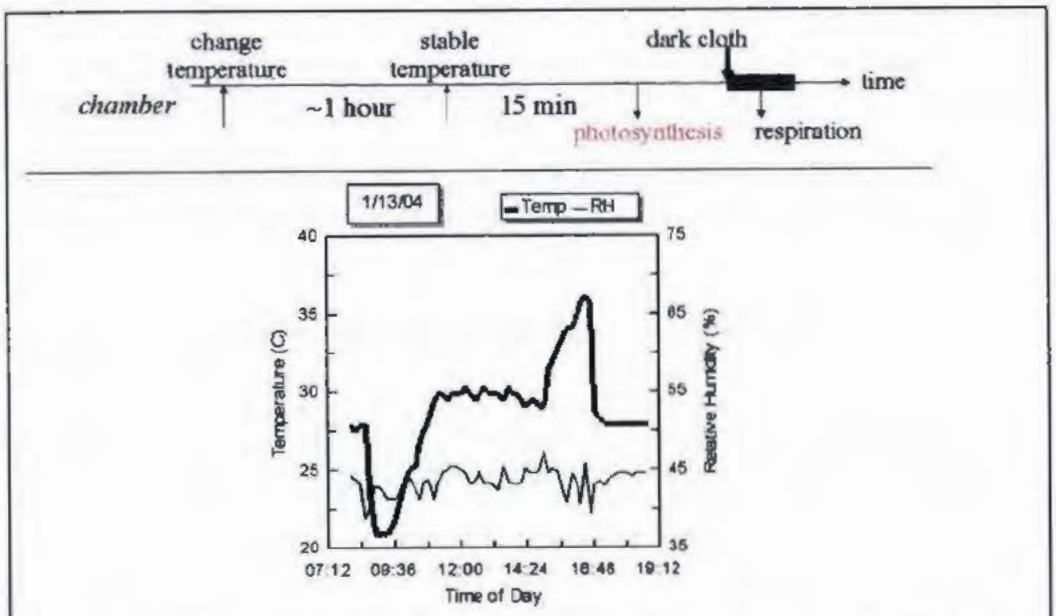


Figure 7. The Protocol for Growth Chamber Shifts in Temperature. These data were taken from a HOBO automatic sensing device placed within the growth chamber at the leaf level. It represents temperature and relative humidity change during the course of a day-experiment when the temperature was being ramped up from 20C (68F) to 37C (97F). Note the lack of change of relative humidity. Respiration is measured within a few minutes of the leaf begin place in the dark (under a black cloth), see Figure 6.

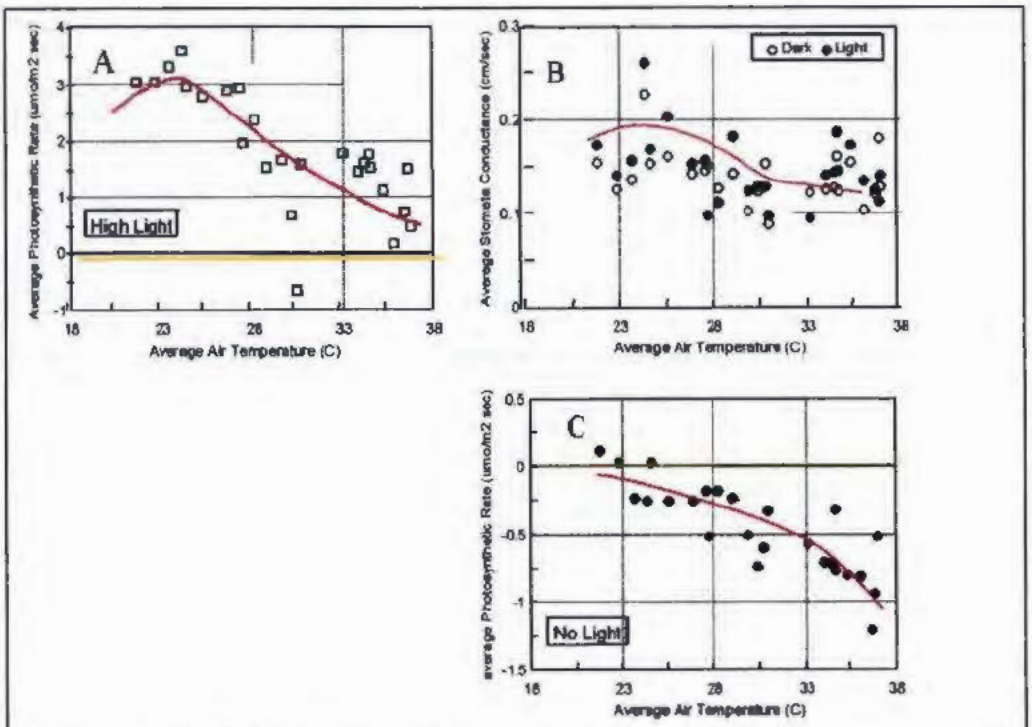


Figure 8. Effect of Temperature upon the Photosynthetic Rate, Stomatal Conductance and Respiration Rate of avocado leaves. This is an example of a typical experiment in which 5 leaves are measured on two trees. The measurements were by a Licor 6200 system. The data points are an average of three measurements on one leaf for each temperature. The yellow line represents zero (which is off-set in some plots) and the red line represents a "best-guess" line for the average of the data. **A**, Average Photosynthetic rate. **B**, Average Conductance Rate. **C**, Average Respiration Rate.

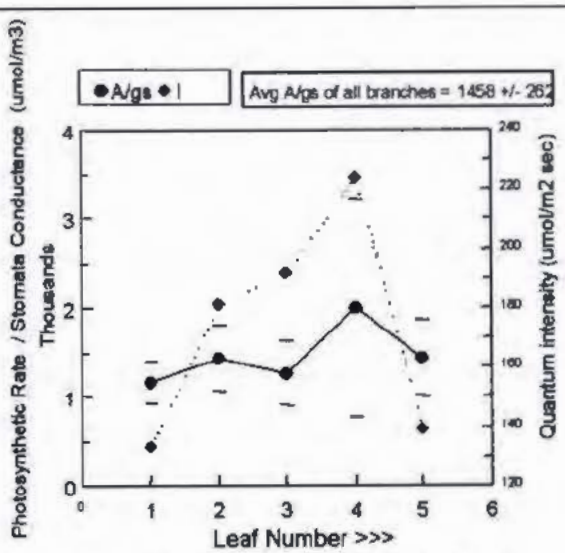
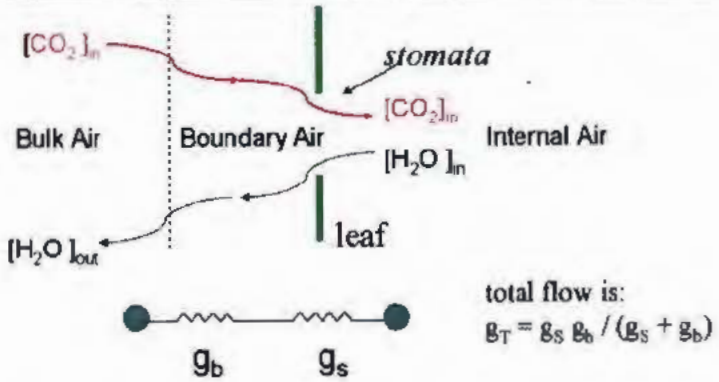


Figure 9. Light Intensity at Each Leaf and Internal CO_2 Concentration for Each Leaf in a Typical Experiment. Here the leaf number is arbitrary and represents merely a different leaf. The internal CO_2 concentration (as given by assimilation divided by the conductance, vertical axis) is averaged over all temperatures for these experiments. These measurements are done within a growth chamber and thus each leaf is at a slightly different level (distance from lights) and so experience different light intensities (as given by the dotted line).



Photosynthesis rate $= g'_T \{[CO_2]_{out} - [CO_2]_{in}\}$

Transpiration rate $= g_T \{[H_2O]_{in} - [H_2O]_{out}\}$

Figure 10. Movement of water vapor from inside to outside the leaf. The varied transition zones are indicated by name and conductance (g_i) as the top two lines, while the regions of "constant" gas concentration are shown as the third line. The bottom line indicates a possible concentration of the gas within each region. CO_2 movement is shown by the red line and equation while H_2O movement is shown by the green line and equation.

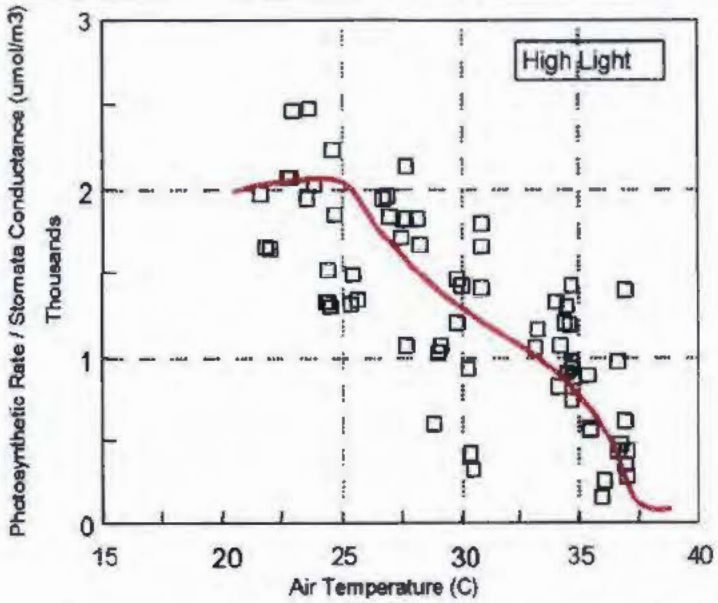


Figure 11. The Dependence of Internal CO_2 Concentration Ipon the Air Temperature. The internal CO_2 is calculated as described in Figure 10 and by Equation [12] in the text. The air temperature is that temperature measured next to the leaf that is being measured by the Licor. Here the zero point of the vertical axis (photosynthetic rate / stomata conductance) is equal to the ambient concentration of CO_2 (generally near 380 ppm). This value is the lowering of the ambient CO_2 due to the uptake of CO_2 due to photosynthesis. Note that by 37 C (98F) there is little or no photosynthesis occurring.

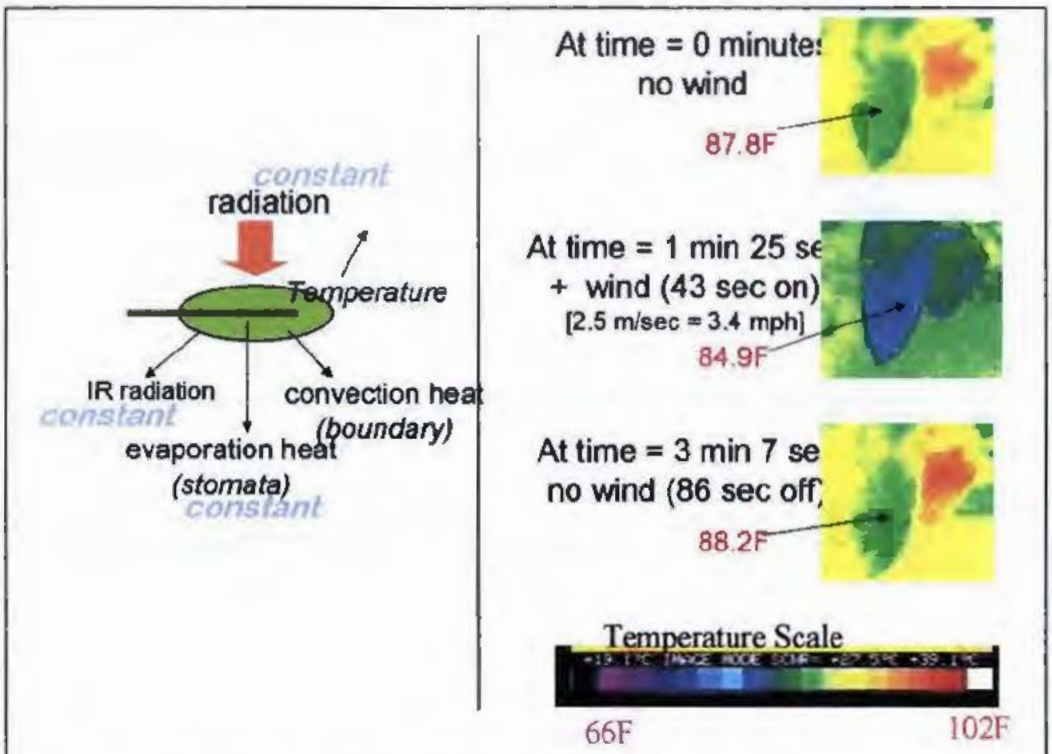


Figure 12. Leaf Surface Temperature as Measured by an Infra-Red Camera. An Inframetrics IR camera was used to record the surface temperature with the image being captured with a VCR. The image was captured using Dazzle Digital Video Creator Image capture interface and software. A Hass avocado leaf was used in a green house at an air temperature of about 28 C at 10AM.

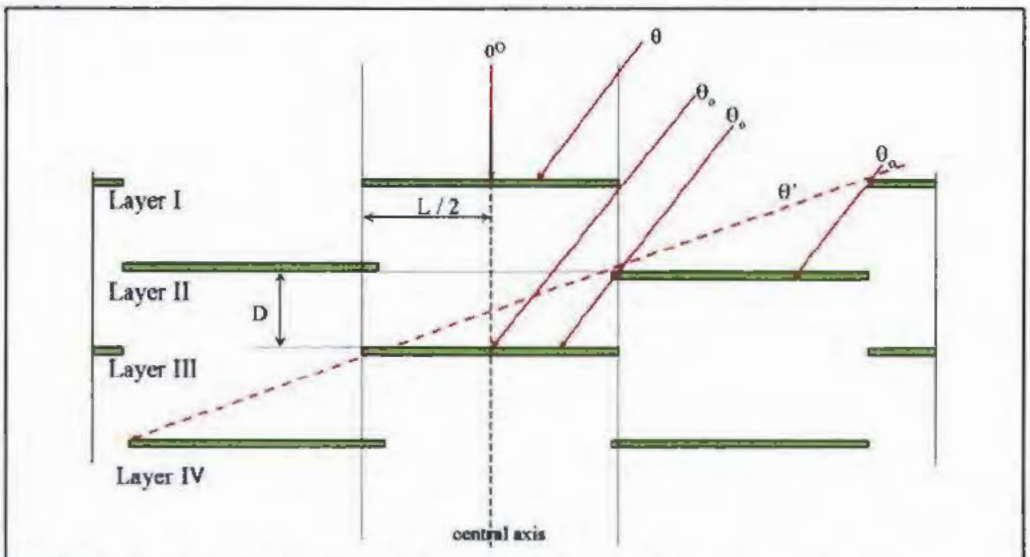


Figure 13. Two Dimensional Model of a Canopy Structure θ_o = arctan (2D / L) θ' = arctan(D/L) with D. The intensity of light (I) is equal to the intensity at the maximum (from the top) *times* the Cos(θ). For more details see text.

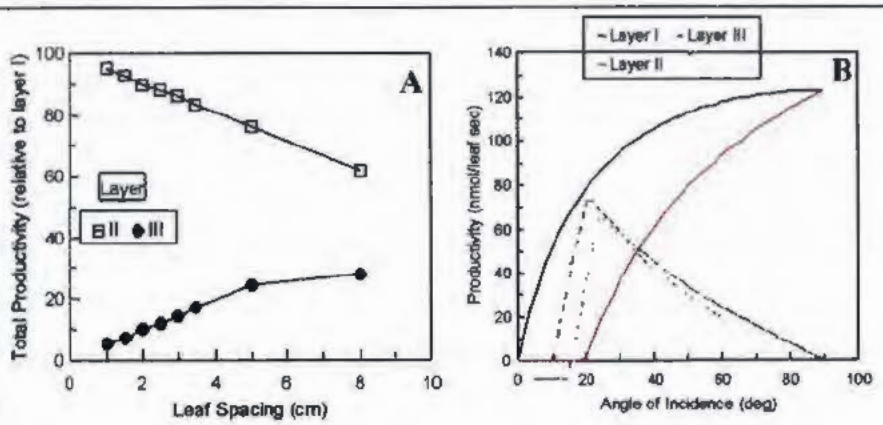


Figure 14. Data from the Model of Figure 13. **A**. Dependence of Productivity Upon Leaf Layer Spacing. Amount of illumination is with respect to layer I. The size of the leaf was constant at 10 cm. **B**. Dependence of Productivity Upon Illumination Angle. The leaf was taken as 10 cm on the side (L) with a spacing (D) of 3 cm. The photosynthetic productivity was calculated as $A = A_{max}\,[I] / \{K + [I]\}$, where A = assimilation rate and A_{max} = maximum rate at 20 μmoles/cm^2 area sec. The surface intensity (I) is in units of μmoles /m^2 sec with its maximum being full sunlight and K is a half-saturation coefficient of about 20% full sunlight.

1998 California Avocado Research Symposium pages 1-2
California Avocado Society and University of California, Riverside

Enhancement of Avocado Productivity. I. Plant improvement - selection and evaluation of improved varieties and rootstocks

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollenizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance. Collaborate with Dr. Menge and Dr. Thorp on identification and evaluation of

dwarfing material.

D. To assist Dr. Mike Clegg on coordination of pollinizer research plots.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.

G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar.

This is the primary objective of the breeding program. To this end, during the last year we contacted all cooperators on record who have test material. These initial contacts are being followed up this current year with field visits and site evaluations. In Spring 1998 we are initiating further cooperator trials at the following sites: San Luis Obispo, Santa Paula, Moorpark, Fallbrook and Highland Valley. These trials will have the most promising selections topworked onto existing field trees in replicated trials and include BL667 (Nobel), BL516 (Marvel), RT5176, BL1058, 3-29-5 (Gem), N4 (-) 5 (Harvest), OA184. The Moorpark site will be designed as a pollinizer site to test the usefulness of some of the new selections as pollinizer varieties for 'Hass' and 'Lamb Hass'. We are also expanding the plantings at the UC South Coast Research and Extension Center (SCREC) in Irvine. These expansion plantings will allow us to evaluate a number of the new selections similar to the new cooperator trials. In October 1997 we began collecting dry weight information at 2 to 3 week intervals on the following varieties: 'Marvel', 'Nobel', 'Gem', 'Harvest', 'Hass', 'Lamb Hass', 'Pinkerton', 'Regal', OA184 and 'Sir Prize'.

With the assistance of Dr. Allan Dodds (Dept. of Plant Pathology, UCR) we have initiated a sunblotch testing protocol on trees at the SCREC. Samples from all fields assigned to this program have been tested. Priority is being given to trees that are used as a budwood source. A second priority is the testing of trees that were suspect due to growth habit. Forty-nine trees have been tested so far and only 2 trees have tested positive (suspect trees). In another field which we are using for budwood, we have tested 18 trees (all negative). We have tested at least 3 trees for each unreleased variety represented in Field 4 at South Coast and will continue to test until all the trees have been cleared.

A major effort during this last year has been the establishment of a database that includes all trees at SCREC and UCR. Each tree has been given its own unique identification number and a way to track budwood from individual trees has been included in the database. The notes and observations which Mr. Martin left upon his

departure have also been incorporated into the database as well as the notes taken by the Volunteers assisting the program.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

We have initiated discussions with other avocado selection programs regarding receiving interesting and promising material.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance. Collaborate with Dr. Menge and Dr. Thorp on identification and evaluation of dwarfing material.

In Spring 1998, we will be topworking trees of various clonal rootstocks to the 'Lamb Hass' variety. This will allow us to assess its performance on selected rootstocks. We are planning a new clonal rootstock trial (to be planted in 1999) that will be planted at SCREC with Dr. Menge. The 'Hass' and the 'Lamb Hass' will be included in this trial on selected clonal rootstocks. A clonal rootstock trial will be planted in Spring 1998 in the San Joaquin Valley. This trial will use the 'Sir Prize' as the scion variety. Three sites will be used, UC Lindcove Research and Extension Center in Exeter, Cutler-Orosi and Porterville. We continue to collaborate with Dr. Crowley in his salinity research. An interstock trial using Colin V-33 will be planted in Fallbrook in Spring 1998. The trees will not be grafted to a scion variety until Spring 1999. We are considering at this time to use the 'Lamb Hass' when the trees are grafted.

D. To assist Dr. Mike Clegg on coordination of pollinizer research plots.

As noted above, one of the new experimental sites being established this spring will be a pollinizer study. We continue to discuss with Dr. Clegg ways to incorporate the B flower type selections into an organized research program to evaluate the value of outcrossing and which pollinizers to utilize.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

We have not initiated any activities with this objective. Dr. Xuan Liu, however, has initiated preliminary studies to compare the photosynthetic activity of the 'Lamb Hass' (tolerant to Persea mite) to the 'Hass' variety.

F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block. We have purchased a pole pruner and plan to begin a block rejuvenation by topping all trees to approximately 15 feet. This will allow us to manage the block more efficiently. Additionally we have decided to maintain only 2 trees of each variety. This will allow us to continue to expand the collection within the allotted space. We are also planning, in conjunction with Dr. Menge, to include representative trees of major rootstock varieties. This over time will serve as a source of budwood and identification material for growers. The volunteers have been instrumental in maintaining this block.

We are continuing our efforts to expand and improve the Persea germplasm block. Part

of this effort includes the repropagation of material collected by Dr. Zentmyer which is planted elsewhere at SCREC.

G. To insure the timely and effective dissemination of information developed from this research program.

Mr. Stottlemyer is working on the development of a web site for the breeding program. This web site will include information on varieties and rootstocks. We have requested a number of avocado growers to preview the web site to provide suggestions prior to releasing the address for general use.

Enhancement of Avocado Productivity

I. Plant Improvement - Selection and Evaluation of Improved Varieties and Rootstocks

Continuing Project; Year 3 of 20

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar. Index trees for distribution for sunblotch viroid with assistance of Drs. Allan Dodds, Jim Heick and Deb Matthews.
B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material. Provide material to Drs. Richard Litz and Witjaksono from the University of Florida upon request.
C. To collaborate with Dr. Menge and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.
D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Drs. Ben Faber and Betty Fetscher; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.
E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips as requested.
F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.
G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round.

Field Trials. This is the primary objective of the breeding program. Since 1998 we have established the following cooperator trials:

Topworked trials at Non-UC Sites
Santa Paula (Ventura County) - 1998
'GEM', 'Harvest', 'SirPrize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates

De Luz Canyon (San Diego County) - 1998
'Lamb Hass', 'SirPrize', 'GEM', 'OA184', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest'; 10 replicates.
Approximately 80 'GEM' trees divided roughly into 3 groups at the cooperator site.

San Luis Obispo (San Luis Obispo County) - 1998 (Trees suffered from freeze in 12/98 necessitating re-grafting of some selections in 1999.
'RT5176', 'Hass', 'SirPrize', 'GEM', 'Harvest', 'OA184'; 9 replicates

Rainbow (San Diego County)
1997 Trial: 2 'GEM', 2 'Nobel', 2 'Harvest', 3 'Marvel', 2 'BL312'
1998 Trial: Provided budwood for 40 trees each of 'GEM' and 'Harvest' and 20 trees of 'Lamb Hass' with the plan of having 10 replicates. Actual field grafting was not done according to UC request.

'Nobel' trees at UC South Coast REC - 1998
20 clonal trees: 8 planted in Field 4; 12 planted in Field 46. Purpose of trees is a) budwood and b) fruit source.

Topworked trees at UC, Riverside Campus - ongoing
Replacement trees in Field 10

San Joaquin Valley Variety Trial - 1999 at two sites (Porterville, Lindcove) with "new trees"
All on Thomas Roostock; 'SirPrize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'

Yield data from unreleased material. We have collected some yield data from Field 4 at the UC SCREC (UC South Coast Research and Extension Center) (Figure 1). The 2000 yield data is still being collected and may change slightly from what is presented. In 1998 - 1999 'Harvest' had the highest yield followed by 'Marvel' (BL516) and 'GEM'. 'Sir Prize' and 'OA184' had the poorest yield. In this current season preliminary data indicate that 'Harvest' has very little fruit (indicating alternate bearing) whereas 'GEM' appears to have good crop.

Dry Weight Tracking. Figure 2 presents the trends in dry weight for the 'GEM' at the 4 testing sites (UC SCREC, UCR, De Luz, San Luis Obispo). This data shows similar trends at all sites throughout the season. The comparison of dry weight changes for the 'GEM' as compared to the 'Hass' for the UCR and UC SCREC sites are presented in

Figure 3. One can see that the two cultivars track very closely with each other. Figure 4 presents data similar to Figure 3 for the 'Marvel' (BL516) selection compared to 'Hass'. There appears to be some difference at the UCR site between the 'Marvel' and 'Hass'. Figures 5 and 6 presents data for the 'Nobel' (BL667) at 3 sties and as compared to 'Hass', respectively. We have also collected dry weight data for the 'Harvest' selection which is presented in Figure 7. Note that as compared to the other selections, that the 'Harvest' has lower overall dry weight even in the late season. Finally, Figure 8 shows a comparison of 6 varieties from the ACW Ranch in De Luz Canyon from March through September 2000.

New Material for the Breeding Program. We have planted approximately 220 seedlings from mixed maternal sources to provide material for the "next generation" of avocado selections. This was accomplished following consultation with Drs. Bob Bergh (UCR) and Uri Lavi (Volcani Institute, Israel). The trees were planted in Spring 2000. We are collecting additional seed material this year and hope to plant a similar number of seedlings in 2001. Additionally we have established a series of "isolation" blocks at UCR for generating additional seed material for the future (parents selected following consultation with Dr. Bob Bergh).

Sunblotch indexing. A section of Field 46 at UC SCREC tested positive for the sunblotch viroid. These trees were removed in March 2000. We have conducted a preliminary indexing of the 225 seedlings due to be planted at UC South Coast REC. One group of 15 trees tested as a weak possible positive and Dr. Dodd's group hopes to do further testing with this group of trees. The remainder of these seedlings will be planted out in Spring 2000 as outlined above.

Table 1. Listing of rootstock material acquisition from Israel.

	Obtained	
VC#	*May '99*	*March '00*
6		X
7		X
15		X
26		X
28		X
31		X
40	X	X
49	X	X
51		X
65	X	X
66	X	X
75		X
803		X
804		X
817	X	X
828		X

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

Introduction of new germplasm. In May 1999 D. Stottlemyer, T. Chao and M. L. Arpaia visited Israel. One of our objectives was to visit with Dr. A. Ben-Ya'acov to review the status of the various rootstock selections which he had made over the years. In May 1999 M. L. Arpaia brought material from 5 selections plus budwood of the 'Ardith' and the 'Gil'. M. L. Arpaia revisited Dr. Ben-Ya'acov in March 2000 and obtained additional material as listed in Table 1. Budwood from the 5 selections obtained in May 1999 was also once again acquired. This material will be in quarantine for 2 years before it can be released for field testing. We have tested this material for the presence or absence of sunblotch. The VC49 selection (introduced in 3/00) has tested positive and we are currently waiting for indexing results from Israel.

We have continued to supply material to Dr. Richard Litz's program in Florida on an on-going basis during early fruit development. We are also supplying fruit and plant material to other researchers when requested.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

In Spring 1998 we topworked trees in the old 'Gwen' rootstock trial to the 'Lamb Hass' variety. This will allow us to assess its performance on the following roostocks: G755A, G755B, G755C, Toro Canyon, Borchard, Duke 7, D9, Thomas, Topa Topa. The "take" in this trial has been mixed but we have successfully established sufficient trees for evaluation. We planted a new clonal rootstock trial at UC SCREC with Dr. Menge in Spring 1999. The 'Hass' and the 'Lamb Hass' are included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates).

A clonal rootstock trial was planted in Spring 1998 in the San Joaquin Valley. This trial used the 'Sir Prize' as the scion variety. Three sites were planted: UC Lindcove Research and Extension Center in Exeter, Cutler-Orosi and Porterville. Unfortunately these research sites were greatly affected by the December 1998 freeze. Although the trees are still in the ground, we anticipate that we will terminate the Cutler-Orosi site and possibly the Lindcove REC site. The trees in Porterville appear to have survived and we hope to collect reasonable information from this site.

We continue to collaborate with Dr. Crowley in his salinity research.

D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Drs. Ben Faber and Betty Fetscher; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.

Pollinizer Trials. In conjunction with Ben Faber we established a pollinizer site in Ventura County (Oxnard) in Spring 1999. The varieties included in this trial are 'Ettinger', 'Fuerte', 'Bacon', 'Zutano', 'Harvest', 'SirPrize', 'Nobel' and 'Marvel'. There are 60 trees of each variety divided into 6 replicates of 10 trees each. The trees in this trial

have been incorporated into the Avocado Pollination and Bee Biology project headed by Drs. N. Waser and B. Fetscher. Ben Faber and M. L. Arpaia also established a site in Ventura County (Somis) looking at the distance from the pollinizer row vs. yield and now have 3 years of yield data (no significant trends observed). Finally we established a pollinizer trial in San Luis Obispo County using 'Bacon', 'Nobel' (BL667), and 'Marvel' (BL516) with 7 replicates of each in Spring 1998. This trial was affected by the 12/98 freeze and required somre-working of the trial trees. We hope to begin collecting data from this trial in 2001.

We continue to discuss with Dr. Clegg ways to incorporate the B flower type selections into an organized research program to evaluate the value of outcrossing and which pollinizers to utilize and to discuss future directions for the breeding program.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

We have not initiated any activities with this objective. Mr. Stottlemyer has coordinated some activities with Dr. Hoddle, namely providing plant material for Dr. Hoddle's laboratory testing.

F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block. Any changes to the planting are being recorded in the master data base maintained by David Stottlemyer. The volunteers have been instrumental in maintaining this block. Several new and/or historical varieties are being grafted on an on-going basis by the volunteers. We are also in the process of establishing the capability of growing clonal trees on a small-scale for the breeding program. This is being done using the greenhouse facilities at UC SCREC.

G. To insure the timely and effective dissemination of information developed from this research program.

The avocado website at www.ucavo.ucr.edu has been on-line since June 1998. The site continues to be updated with new information and photographs of different varieties. Questions sent via e-mail are answered on an ongoing basis.

Figure 1. Field 4 Variety Trial at UC SCREC in Irvine, CA. Average number of fruit per tree for 1999 and 2000.

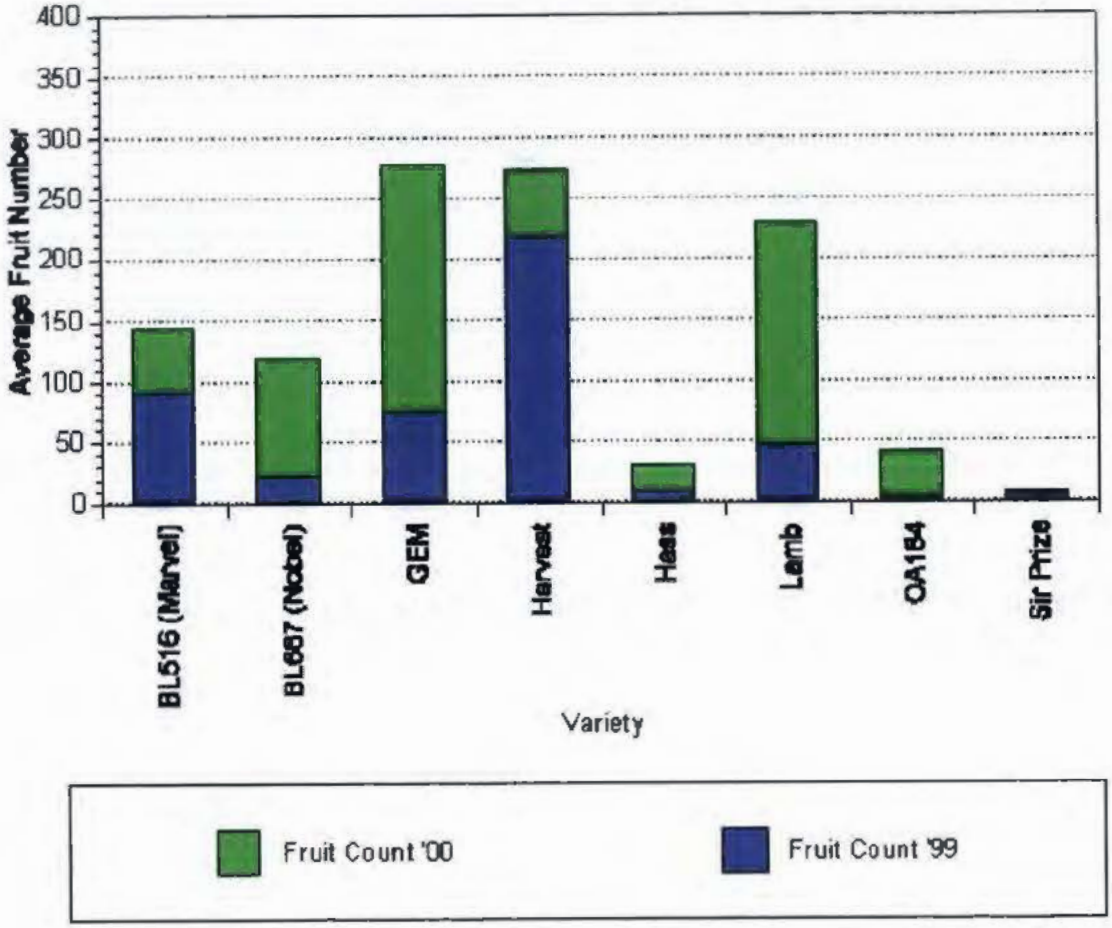


Figure 2. The average percent dry matter for 'GEM' for four sites from 12/99 to 9/00.

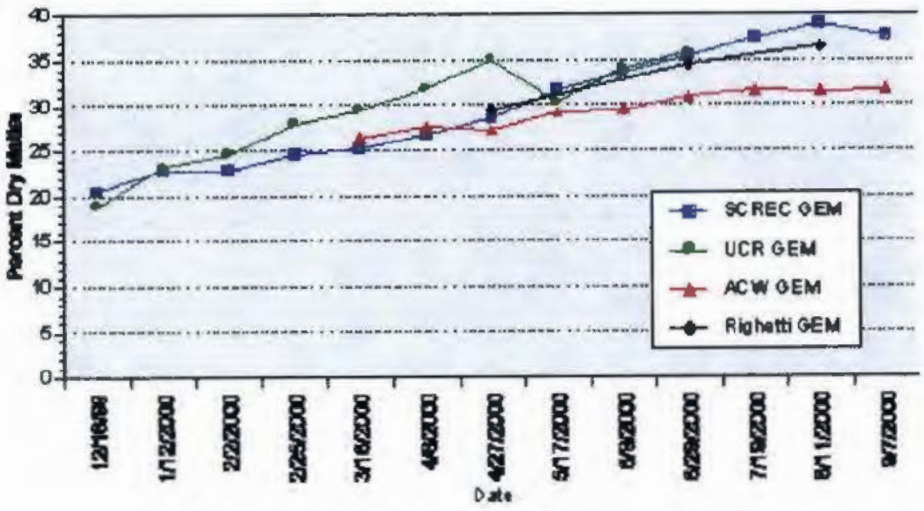


Figure 3. The changes in dry weight for 'Hass' and 'Gem' from UC SCREC and UCR.

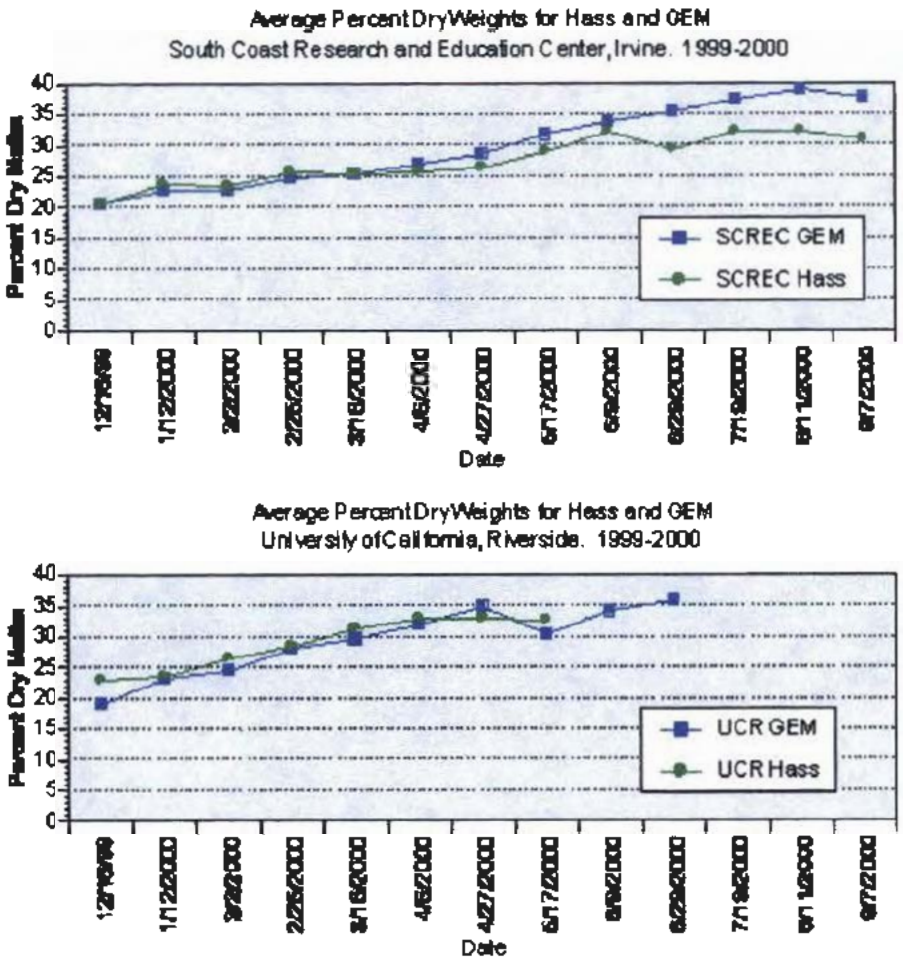


Figure 4. The changes in dry weight for 'Hass' and 'Marvel' from UC SCREC and UCR.

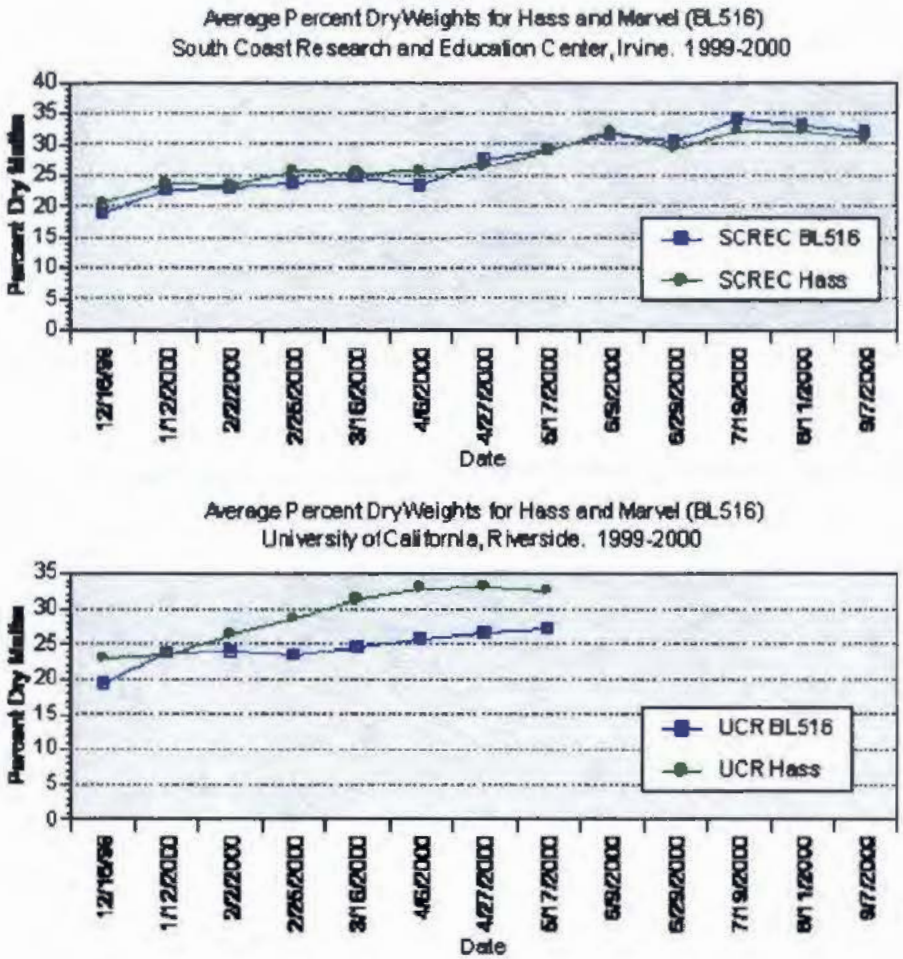


Figure 5. The average percent dry matter for 'Nobel' for three sites from 12/99 to 9/00.

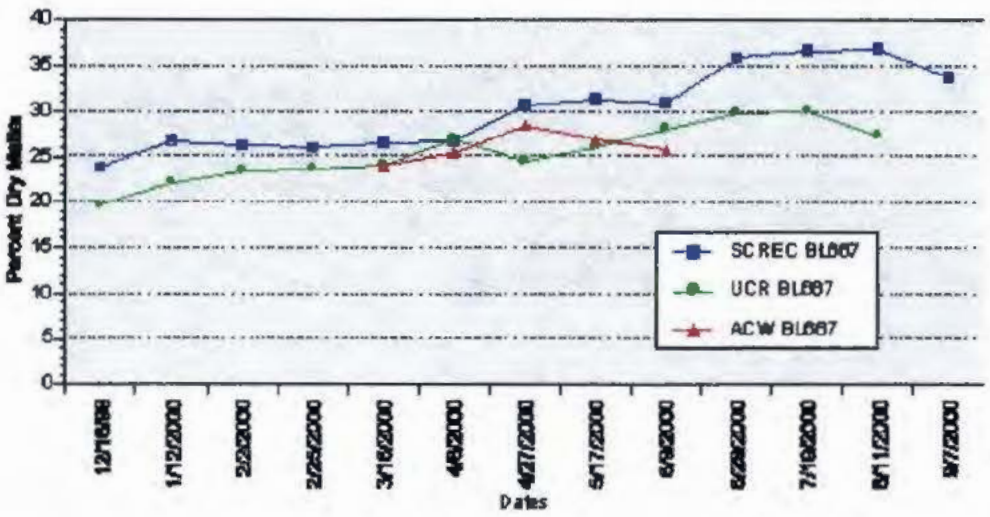


Figure 6. The changes in dry weight for 'Hass' and 'Nobel' from UC SCREC and UCR.

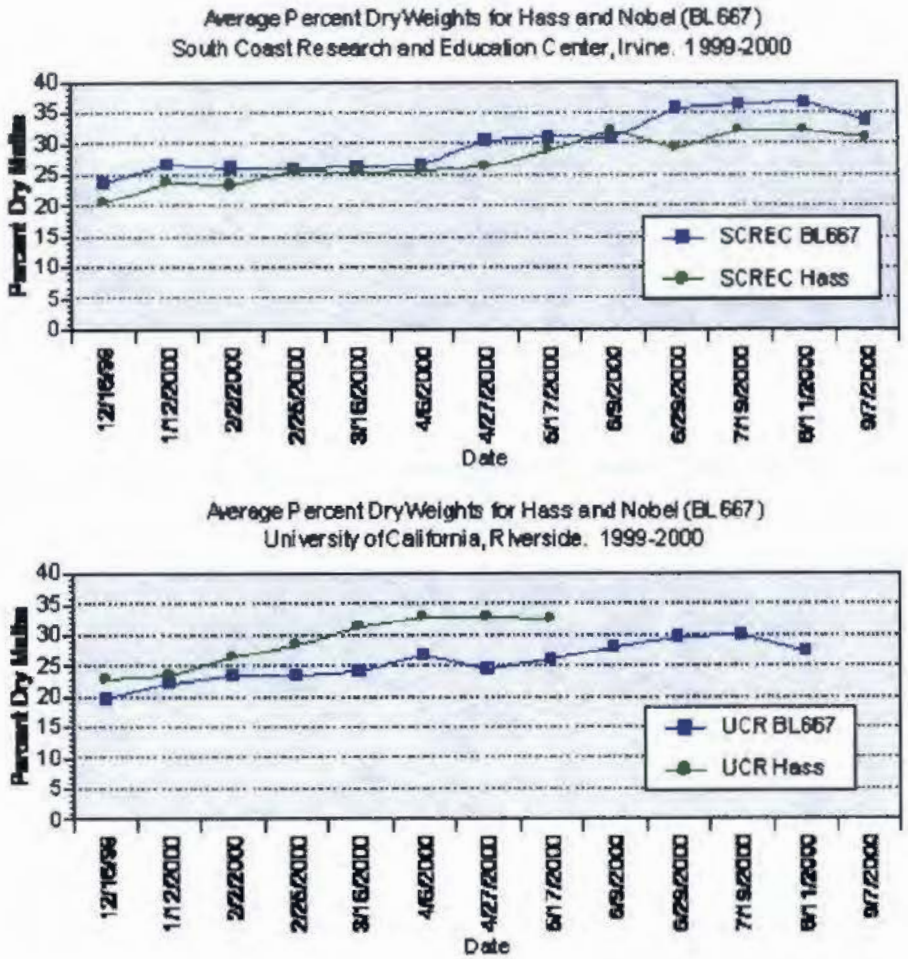


Figure 7. The average percent dry matter for 'Harvest' for three sites from 12/99 to 9/00.

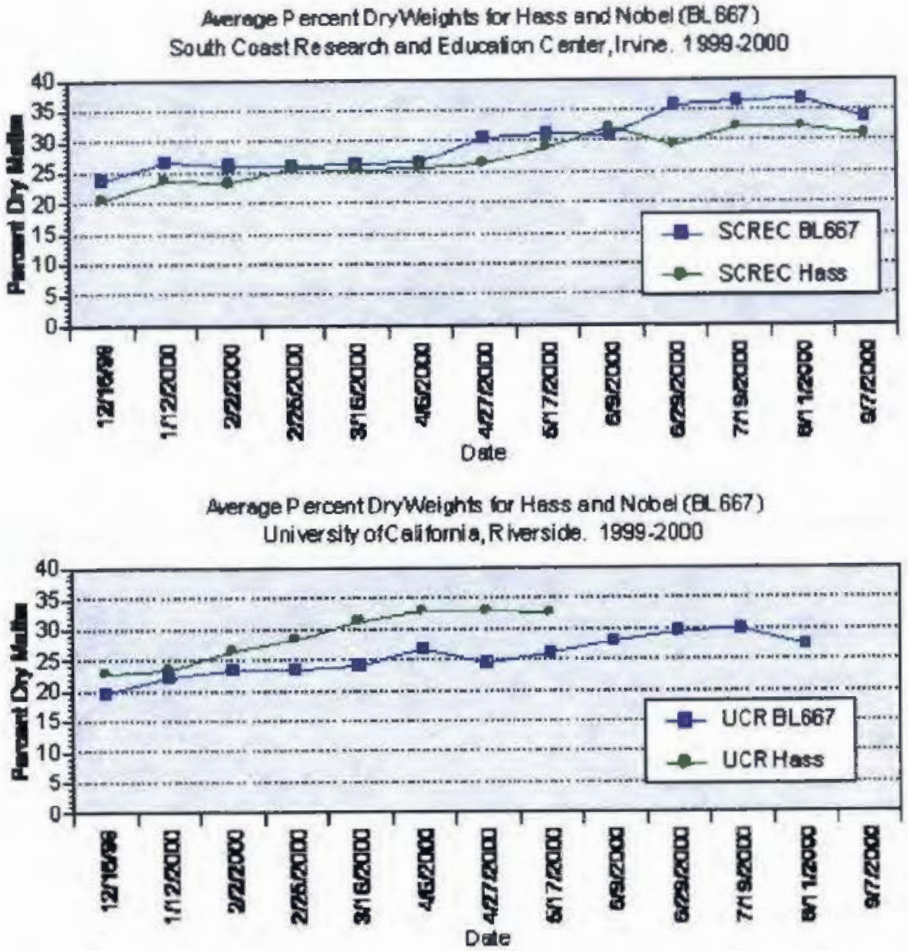
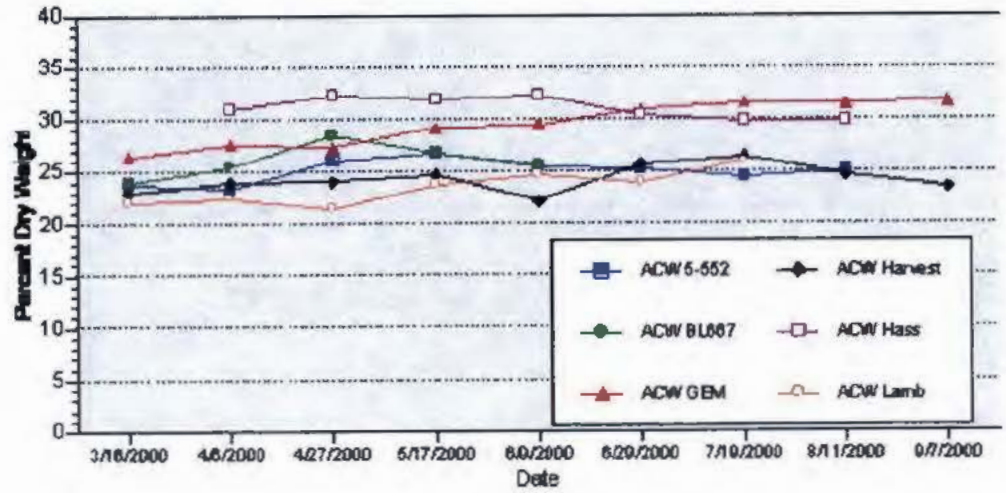


Figure 8. The average percent dry weight for various selections at the ACW Ranch in De Luz, CA from 3/00 through 9/00.



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California Avocado Society and University of California, Riverside

Enhancement of Avocado Productivity
I. Plant Improvement - Selection and Evaluation of Improved Varieties and Rootstocks

Continuing Project; Year 2 of 20

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

Collaborate with Dr. Menge and Dr. Thorp on identification and evaluation of dwarfing material.

D. To assist Dr. Mike Clegg on coordination of pollinizer research plots.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.

G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar.

This is the primary objective of the breeding program. To this end, during 1998 we contacted all cooperators on record who have test material. These initial contacts are being followed up this current year with field visits and site evaluations. In Spring 1998 we initiated further cooperator trials at the following sites:

Topworked trials at Non-UC Sites:

Santa Paula (Ventura County)
'GEM', 'Harvest', 'SirPrize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates

De Luz Canyon (San Diego County)
'Lamb Hass', 'SirPrize', 'GEM', 'OA184', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest'; 10 replicates

San Luis Obispo (San Luis Obispo County)
'RT5176', 'Hass', 'SirPrize', 'GEM', 'Harvest', 'OA184'; 9 replicates Pollenizer trial using 'Bacon', 'Nobel', 'Marvel'; 7 replicates

Rainbow (San Diego County)
1997 Trials: 2 'GEM', 2 'Nobel', 2 'Harvest', 3 'Marvel', 2 'BL312'
1998 Trial: Provided budwood for 40 trees each of 'GEM' and 'Harvest' and 20 trees of 'Lamb Hass' with the plan of having 10 replicates. Actual field grafting was not done according to UC request.

Topworked trials at UC South Coast REC

'Lamb Hass' trial; used 'Gwen' rootstock trial; G755A, G755B, G755C, Toro

Canyon, Borchard, Duke 7, D9, Thomas, Topa Topa; 12 replicates

Nobel' trees at South Coast REC

20 clonal trees: 8 planted in Field 4; 12 planted in Field 46. Purpose of trees is a) budwood and b) fruit source.

Topworked trees at UC, Riverside Campus

Replacement trees in Field 10

Our plans for 1998 - 1999 include:

De Luz Canyon (San Diego County)
100 GEM trees
Pollinizer Trials

Oxnard (Ventura County)
New trees; 'Ettinger', 'Fuerte', 'Bacon', 'Zutano', 'Harvest', 'SirPrize', 'Nobel' and 'Marvel'; 60 trees of each, design not established

San Joaquin Valley Variety Trial
All on Thomas Rootstock; 'SirPrize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'; 3 sites, will approximate same design as trials established in 1998

UC South Coast REC Field 46
Field graft Duke 7 trees with unreleased varieties in Spring 1999

With the assistance of Dr. Allan Dodds (Dept. of Plant Pathology, UCR) we initiated a sunblotch testing protocol on trees at the SCREC in 1997. Samples from all fields assigned to this program have been tested. Priority is being given to trees that are used as a budwood sources. A second priority is the testing of trees that were suspect due to growth habit. A section of field 46 suspected of having sunblotch has tested positive. Several trees unique to this section have tested negative and budwood will be removed in order to save these varieties for further variety evaluations. As soon as this is done, the trees in this section of field 46 will be removed. In field 4, we have checked 73 trees and all have tested negative for the sunblotch viroid.

We have also continued to supply material to Dr. Richard Litz's program in Florida on an on-going basis during early fruit development. We are also supplying fruit and plant material to other researchers when requested.

B. To collaborate with other researchers worldwide in evaluating and exchanging

promising plant material.

We have initiated discussions with other avocado selection programs regarding receiving interesting and promising material. We are planning visits to Israel in Spring 1999 and to South Africa in Summer 1999.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance. Collaborate with Dr. Menge and Dr. Thorp on identification and evaluation of dwarfing material.

In Spring 1999 we topworked trees of various clonal rootstocks to the 'Lamb Hass' variety. This will allow us to assess its performance on selected rootstocks. We are planning a new clonal rootstock trial (to be planted in Spring 1999) that will be planted at SCREC with Dr. Menge. The 'Hass' and the 'Lamb Hass' will be included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates).

A clonal rootstock trial was planted in Spring 1999 in the San Joaquin Valley. This trial used the 'Sir Prize' as the scion variety. Three sites were planted: UC Lindcove Research and Extension Center in Exeter, Cutler-Orosi and Porterville. Unfortunately these research sites were greatly affected by the December 1998 freeze. Although the trees are still in the ground, we anticipate that we will terminate the Cutler-Orosi site and possibly the Lindcove REC site. The trees in Porterville appear to have survived and we hope to collect reasonable information from this site.

We continue to collaborate with Dr. Crowley in his salinity research. An interstock trial using Colin V-33 was planted in Fallbrook in Spring 1998. The trees will be grafted to a scion variety ('Hass') in Spring 1999.

D. To assist Dr. Mike Clegg on coordination of pollinizer research plots.

We in conjunction with Ben Faber are establishing a pollinizer site in Ventura County (Oxnard) hi Spring 1999. We have also established a site in Ventura County (Somis) looking at the distance from the pollinizer row vs. yield. We hope to have fruitlets analyzed from this site (pending Year 2000 funding from the Hansen Trust). We continue to discuss with Dr. Clegg ways to incorporate the B flower type selections into an organized research program to evaluate the value of outcrossing and which pollinizers to utilize and to discuss future directions for the breeding program.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

We have not initiated any activities with this objective. Mr. Stottlemyer has coordinated some activities with Dr. Hoddle, namely providing plant material for Dr. Hoddle's laboratory testing.

D. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block. Any changes to the planting are being recorded in the master data base maintained by David Stottlemyer. Several new and/or historical varieties have been grafted onto rootstock material. We purchased a pole pruner in 1998 and are beginning to top trees in both Field 44 (CAS Variety block) and Field 46 (Breeding Block) to approximately 15 feet. This will allow us to manage the block more efficiently. We are still planning, in conjunction with Dr. Menge, to include representative trees of major rootstock varieties. This over time will serve as a source of budwood and identification material for growers. The volunteers have been instrumental in maintaining this block.

We are continuing our efforts to expand and improve the *Persea* germplasm block. Part of this effort includes the repropagation of material collected by Dr. Zentmyer that is planted elsewhere at SCREC. We are also maintaining some plant material at UCR in the greenhouse. Some of this material will be planted at SCREC, others will be maintained on campus.

G. To insure the timely and effective dissemination of information developed from this research program.

The avocado web site at: www.ucavo.ucr.edu has been on-line since June 1998. The site continues to be updated with new information and photographs of different varieties. Questions sent via e-mail are answered on an ongoing basis.

Enhancement of Avocado Productivity. I. Plant Improvement – Selection and Evaluation of Improved Varieties and Rootstocks

Continuing Project; Year 5 of 20

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar. Index trees for distribution for sunblotch viroid with assistance of Drs. Allan Dodds, Jim Heick and Deb Mathews.
B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material. Provide material to Drs. Richard Litz and Witjaksono from the University of Florida upon request.
C. To collaborate with Dr. Menge and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.
D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Drs. Ben Faber and Betty Fetscher; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.
E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips as requested.
F. To maintain and improve the CAS variety block and the Persea germplasm block located at the UC South Coast Research and Extension Center.
G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round.

Field Trials. This is the primary objective of the breeding program. The following are the cooperator trials:

Topworked trials at Non-UC Sites

Santa Paula (Ventura County) - 1998

'GEM', 'Harvest', 'SirPrize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates

De Luz Canyon (San Diego County) - 1998

'Lamb Hass', 'SirPrize', 'GEM', 'OA184', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest', 10 replicates. Approximately 80 'GEM' trees divided roughly into 3 groups at the cooperator site.

San Luis Obispo (San Luis Obispo County) – 1998 (Trees suffered from freeze in 12/98 necessitating re-grafting of some selections in 1999.

'RT5176', 'Hass', 'SirPrize', 'GEM', 'Harvest', 'OA184'; 9 replicates

Rainbow (San Diego County)

1997 Trial: 2 'GEM', 2 'Nobel', 2 'Harvest', 3 'Marvel', 2 'BL312'

1998 Trial: Provided budwood for 40 trees each of 'GEM' and 'Harvest' and 20 trees of 'Lamb Hass' with the plan of having 10 replicates. Actual field grafting was not done according to UC request.

Clonal trials at Non-UC Sites

Oxnard (Ventura County) – 1996 (originally known as the Newman Ranch site. This trial was flooded in 1997 and many trees died due to this, however we are now working with the new owners to collect data from the trees which survived after the winter of 1997): 'Lamb Hass', 'SirPrize', 'GEM', 'OA184', 'Marvel', 'Nobel', 'Hass', 'Harvest'

'Nobel' trees at UC South Coast REC - 1998

20 clonal trees: 8 planted in Field 4; 12 planted in Field 46. Purpose of trees is a) budwood and b) fruit source.

Topworked trees at UC, Riverside Campus - ongoing

Replacement trees in Field 10

San Joaquin Valley Variety Trial – 1999 at two sites (Porterville, Lindcove) with "new trees"

All on Thomas Rootstock; 'SirPrize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'

Yield data from unreleased material. We have collected yield data for the third year from Field 4 at UC-SCREC (UC South Coast Research and Extension Center). Data collection for 2001 is still incomplete but preliminary data is presented (Figure 1). The 'Harvest' at this point has the largest cumulative yield over the 3-year period, however this variety also appears to exhibit severe alternate bearing. We have also collected the first year of yield data from the Santa Paula and Oxnard sites in Ventura County and the De Luz site in San Diego county (data not presented).

Fruit Characteristics. As an on-going process we are collecting fruit samples from all sites approximately every 3 to 4 weeks from fall through late summer. These fruit are evaluated using standard protocols for such characteristics as fruit shape, peel texture, peel color, flesh color, the percent seed, flesh and skin and skin thickness. Figure 2 shows the average percent seed for the various varieties. These are the averages for all fruit from all sites. Note that there is considerable variation between the different varieties in terms of the relative proportion of the fruit occupied by the seed ranging from a low of 7.1% for the 'OA184' to nearly 18% for 'Bacon'. Figure 3 presents a comparison of the 'GEM' and 'Hass' seed size from each sites averaged over all sampling dates. Note that the 'GEM' seed occupies a slightly higher percentage of the fruit as compared to the 'Hass' in all but one site.

Dry Weight Tracking. Figure 4 presents the trends in dry weight for the 'GEM' at the De Luz site. Similar trends were observed at the other 6 sampling sites (Irvine, Riverside, Santa Paula, Oxnard and San Luis Obispo). Figure 5 presents similar data comparing 'Hass' to 'Nobel'. Similar trends, with the 'Hass' having slightly higher dry weights than the 'Nobel' was observed at the other 4 sampling sites (Irvine, Riverside, Oxnard and San Luis Obispo). Figure 6 illustrates the comparison of 'Hass' to 'Marvel' from the De Luz sampling site. The 'Marvel' tends to lag behind the 'Hass' in dry weight accumulation throughout the season. This trend was also observed at the other 5 sampling sites (Irvine, Riverside, Santa Paula, Oxnard and San Luis Obispo). Figure 7 shows the same data for 'Hass' and 'Harvest'. The 'Harvest' tends to be substantially slower in dry weight accumulation as compared to the 'Hass'.

These large differences were also evident at the other 4 sampling sites (Irvine, Santa Paula, Oxnard and San Luis Obispo). The changes in % dry weight for 'GEM' for the 7 sampling sites are illustrated in Figure 8. The same general pattern for dry weight accumulation appears to occur at all sites. A comparison between dry weight accumulations between two maturity seasons for the 'GEM' variety is presented in Figure 9. This data is from the De Luz site in San Diego County. Both seasons show the same general trends.

New Material for the Breeding Program. In Spring 2000 we planted approximately 220 seedlings from mixed maternal sources to provide material for the "next generation" of avocado selections. An additional 270 open pollinated seedlings are currently being transplanted into sleeves at SCREC to be planted in the field in spring 2002. We are collecting additional seed material this year and hope to plant a similar number of seedlings in 2003. We have also established a series of "isolation" blocks at UCR and the Nakamura Ranch for generating seed material for the future (parents selected following consultation with Dr. Bob Bergh).

Sunblotch Viroid indexing. A group of 8 trees in Field 46 at UC SCREC tested positive for the sunblotch viroid. These trees were removed in Spring 2001. All the trees in field 4 at UC SCREC have been sampled for sunblotch. Results are still out on the last 7 trees but all other trees have tested negative for the sunblotch viroid.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

Introduction of new germplasm. In May 1999 D. Stottlemyer, T. Chao and M. L. Arpaia visited Israel. One of our objectives was to visit with Dr. A. Ben-Ya'acov to review the status of the various rootstock selections, which he had made over the years. In May 1999 M. L. Arpaia brought material from 5 selections plus budwood of the 'Ardith' and the 'Gil'. M. L. Arpaia revisited Dr. Ben-Ya'acov in March 2000 and obtained additional material. Budwood from the 5 selections obtained in May 1999 was also once again acquired. We have tested this material for the presence or absence of sunblotch. The VC49 selection (introduced in 3/00) has tested positive and was destroyed. This remaining material is currently in quarantine at UC, Riverside and is scheduled to be released for propagation and subsequent testing during the next year.

We have continued to supply material to Dr. Richard Litz's program in Florida on an on-going basis during early fruit development. We are also supplying fruit and plant material to other researchers when requested.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

In Spring 1998 we topworked trees in the old 'Gwen' rootstock trial to the 'Lamb Hass' variety. This allows us to assess its performance on the following rootstocks: G755A, G755B, G755C, Toro Canyon, Orchard, Duke 7, D9, Thomas, Topa Topa. The first yield data from this trial has been collected in 2001. The "take" in this trial has been mixed but we have successfully established sufficient trees for evaluation. We planted a new clonal rootstock trial at UC SCREC with Dr. Menge in spring 1999. The 'Hass' and the 'Lamb Hass' are included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates). The trees have set fruit for the 2001/2002 season and will be harvested sometime in Spring 2002.

We continue to collaborate with Dr. Crowley in his salinity research.

D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Drs. Ben Faber and Betty Fetscher; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.

Pollinizer Trials. In conjunction with Ben Faber we established a pollinizer site in Ventura County (Oxnard) in spring 1999. The varieties included in this trial are 'Ettinger', 'Fuerte', 'Bacon', 'Zutano', 'Harvest', 'SirPrize', 'Nobel' and 'Marvel'. There are 60 trees of each variety divided into 6 replicates of 10 trees each. The trees in this trial have been incorporated into the Avocado Pollination and Bee Biology project headed by Drs. N. Waser and B. Fetscher. The first year of differential yield data is presented in Dr. Waser's report. Ben Faber and M. L. Arpaia also established a site in Ventura County (Somis) looking at the distance from the pollinizer row vs. yield and now

have 3 years of yield data (no significant trends observed). Finally we established a pollinizer trial in San Luis Obispo County using 'Bacon', 'Nobel' (BL667), and 'Marvel' (BL516) with 7 replicates of each in spring 1998. This trial was affected by the 12/98 freeze and required re-topworking of the trial trees. We had our first harvest from this plot in 2001.

We continue to discuss with Dr. Clegg ways to incorporate the B flower type selections into an organized research program to evaluate the value of outcrossing and which pollinizers to utilize and to discuss future directions for the breeding program.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

We have initiated a cooperative project with Dr. Hoddle looking at growth flushes and relative susceptibility to persea mite. Dr. Hoddle will report on preliminary results of this effort.

F. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block. Any changes to the planting are being recorded in the master database maintained by David Stottlemyer. The volunteers have been instrumental in maintaining this block. The volunteers graft several new and/or historical varieties on an on-going basis. We have also established the capability of growing clonal trees on a small-scale for the breeding program. This is being done using the greenhouse facilities at UC SCREC.

G. To insure the timely and effective dissemination of information developed from this research program.

The current avocado web site at: www.ucavo.ucr.edu has been on-line since June 1998. The site is being revised and updated with new information and photographs of different varieties and should be online by this fall. Questions sent via e-mail are answered on an ongoing basis.

Figure 1. Yield data (average fruit count per tree) from Field 4 variety trial at the UC South Coast Research and Extension Center in Irvine, CA from 1999 - 2001.

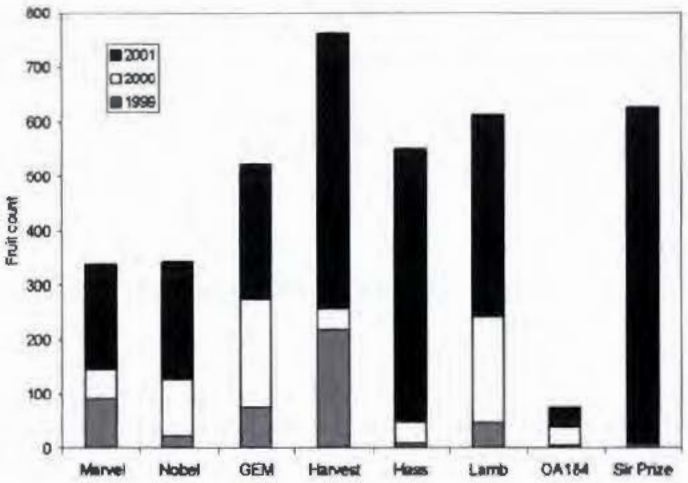


Figure 2. Average seed size (% of total fruit weight) for all sampling sites. Fruit sampled from November 2000 through August 2001.

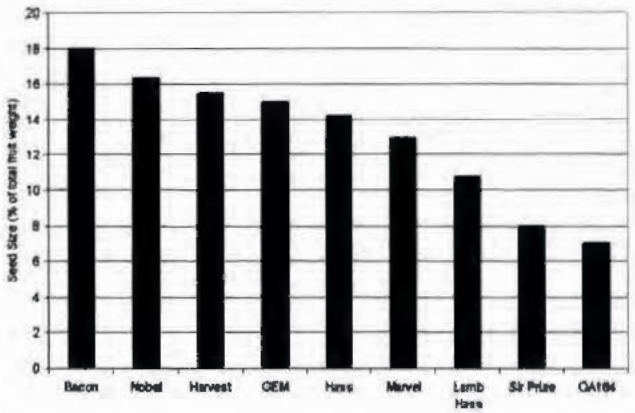


Figure 3. Average seed size (% of total fruit weight) for 'GEM' and 'Hass' from the various sampling sites. Values averaged over all sampling dates.

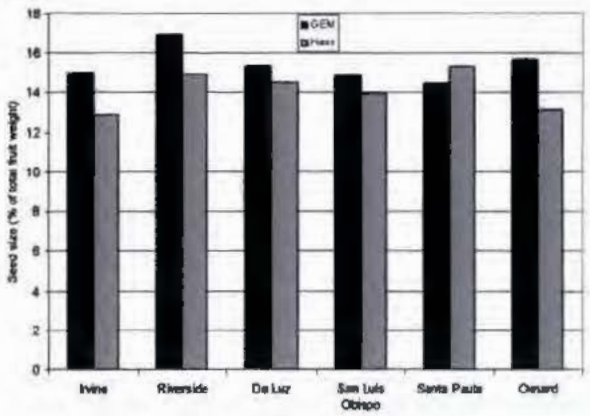


Figure 4. Comparison of changes in % dry weight for 'Hass' and 'Gem' harvested from November 2000 through August 2001 from the De Luz site in San Diego County.

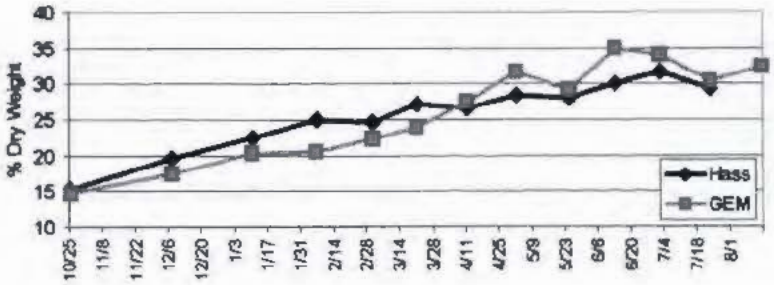


Figure 5. Comparison of changes in % dry weight for 'Hass' and 'Nobel' harvested from November 2000 through August 2001 from the De Luz site in San Diego County.

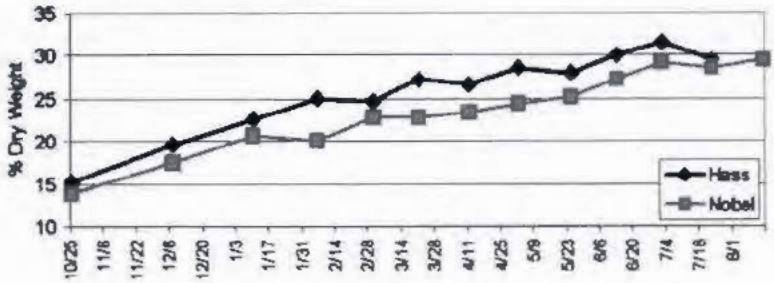


Figure 6. Comparison of changes in % dry weight for 'Hass' and 'Marvel' harvested from November 2000 through August 2001 from the De Luz site in San Diego County.

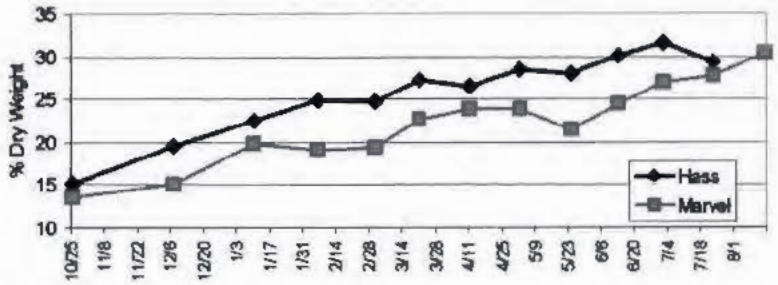


Figure 7. Comparison of changes in % dry weight for 'Hass' and 'Harvest' harvested from November 2000 through August 2001 from De Luz site in San Diego County.

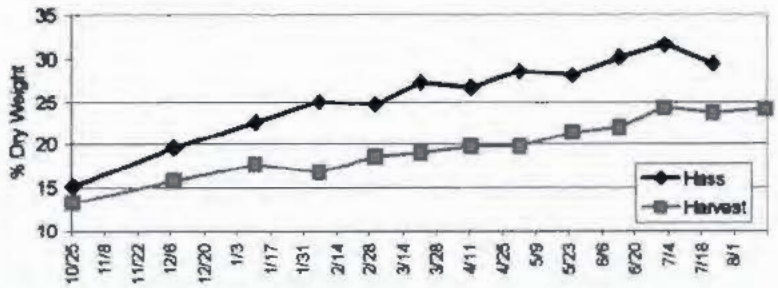


Figure 8. Changes in dry weight percentage for 'GEM' from November 2000 through August 2001 from sampling sites (De Luz, Rainbow, Riverside, Irvine, Santa Paula, Oxnard, San Luis Obispo).

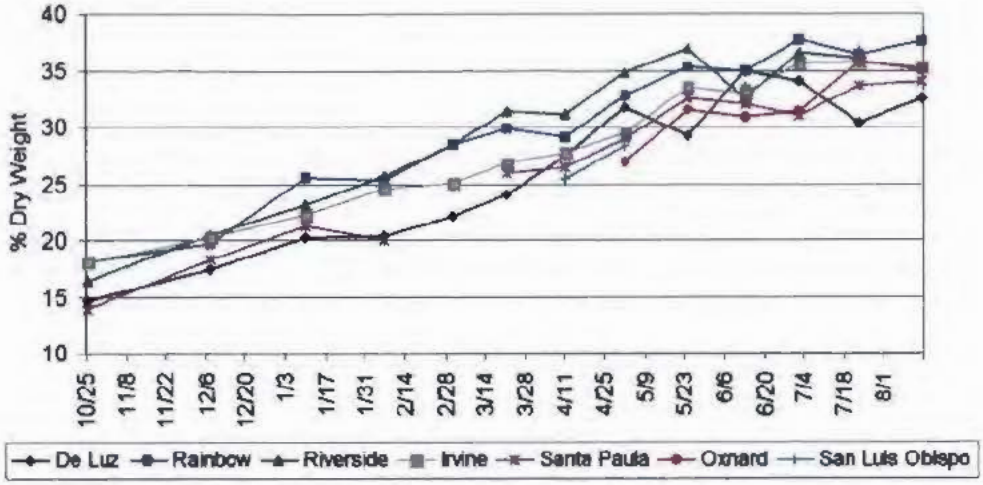
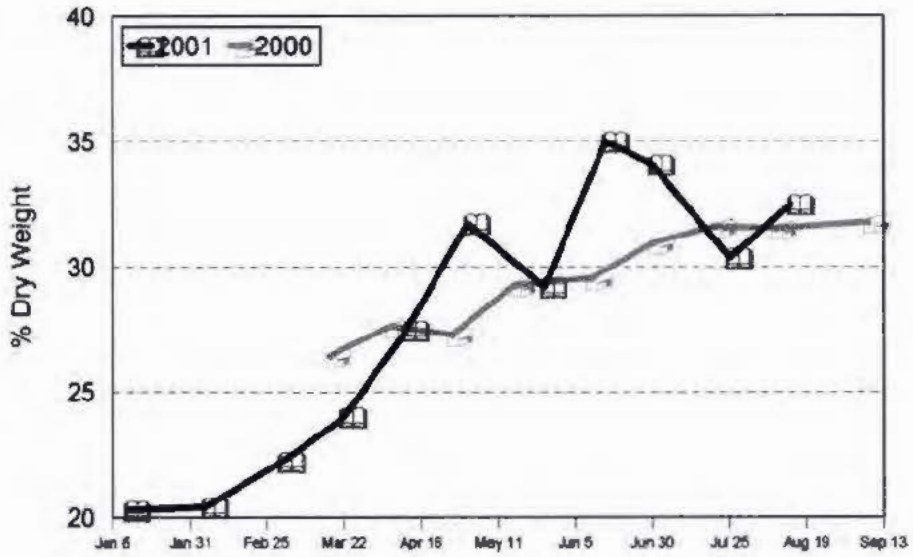


Figure 9. A comparison of dry weight changes for 'GEM' between the 1999/2000 and 2000/2001 maturity seasons. Data collected from the De Luz site in San Diego County.



Enhancement of Avocado Productivity. Plant Improvement: Selection and Evaluation of Improved Varieties and Rootstocks

Continuing Project: Year 6 of 20

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar. Index trees for distribution for sunblotch viroid with assistance of Drs. Allan Dodds and Deb Mathews.
B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.
C. To collaborate with Dr. Menge and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.
D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Dr. Ben Faber; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.
E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips as requested.
F. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.
G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round.

There are 2 components of this objective. The first is the continued monitoring of varieties from the Dr. B. Bergh/Gray Martin selection program. The second component is the new phase of scion selection. Activities for both components are summarized below.

Component 1. Continued monitoring of Bergh/Martin selections

Various field trials have been established to monitor the performance of a number of the Bergh/Martin selections. The following is a list of the cooperator trials we are maintaining. In 2002 we installed data loggers to monitor air and soil temperature and relative humidity at all sites. We plan to use this data to help us assess the selection's response to low/high temperature when these events occur.

There are also additional plantings of the Bergh/Martin selections scattered throughout southern California. We periodically visit these sites to evaluate trees and discuss tree performance with the cooperators.

Topworked trials at Non-UC Sites

Santa Paula (Ventura County); topworked in 1998; 'GEM', 'Harvest', 'Sir Prize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates.

De Luz (San Diego County); topworked in 1998; 'Lamb Hass', 'Sir Prize', 'GEM', 'OA184', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest'; 10 replicates.

De Luz (San Diego County); topworked in 1998; approximately 80 'GEM' trees divided roughly into 3 groups at the cooperator site.

San Luis Obispo (San Luis Obispo County); topworked in 1998 (Trees suffered from freeze in 12/98 necessitating re-grafting of some selections in 1999; 'RT5176', 'Hass', 'Sir Prize', 'GEM', 'Harvest', 'OA184'; 9 replicates.

Clonal trials at Non-UC Sites

Oxnard (Ventura County); planted in 1996; 'Lamb Hass', 'Sir Prize', 'GEM', 'OA184', 'Marvel', 'Nobel', 'Hass', 'Harvest'. (This trial was flooded in 1997 and many trees died due to this, however we are now working with the current owners to collect data from the trees which survived after the winter of 1997)

HTH Ranch (Ventura County); 'Lamb Hass', 'Marvel', 'GEM' and 'Hass' A non-replicated trial used for dry weights and fruit evaluation only.

Topworked trees at UC, Riverside Campus – ongoing; Replacement trees in Field 10.

Topworked trees at UC, South Coast Research and Extension Center (SCREC): Field 4 at the Center has topworked trees (variable number of replicates) from which we collect data. These trees were topworked onto seedling rootstock trees in 1994 – 1996.

San Joaquin Valley Variety Trial – 1999 at two sites (Porterville, Lindcove) with clonal trees (Thomas rootstock): 'Sir Prize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'; 20 replicates per scion variety at each site. We had trouble with tree

establishment for certain varieties, therefore surviving tree numbers varies with site and variety. We have also had problems with certain varieties dropping fruit prior to harvest; however we do have 1 season's worth of dry matter testing and plan to collect further data this upcoming year.

Yield data from Bergh/Martin selections. *We have collected yield data for the fifth year from Field 4 at UC-SCREC (UC South Coast Research and Extension Center). Data collection for 2003 shows that for most varieties, this was an 'on' year (Figure 1). The 'GEM' at this point has the largest cumulative yield over the five year period. Comparing the coefficient of variation shows that there is tendency toward less extreme alternate bearing in 'GEM' (Figure 2; this is calculated by dividing the standard deviation by the mean and gives one an idea of the relative variation of the data for a particular variety).*

We have also collected the third year of yield data from the Oxnard and Santa Paula sites in Ventura County (Figures 3 and 4), the Righetti site near San Luis Obispo (Figure 5), and the De Luz site in San Diego County (Figure 6).

Fruit characteristics of Bergh/Martin selections. As an on-going process we are collecting fruit samples from all sites approximately every 4 to 5 weeks from winter through late fall. These fruit are evaluated using standard protocols for such characteristics as fruit shape, peel texture, peel color, flesh color, the percent seed, flesh and skin and skin thickness.

Seasonal dry matter content of Bergh/Martin selections. Figure 7 presents the trends in dry weight for all varieties at the UC-SCREC site for 2003. Similar trends were observed at the other 6 sampling sites (San Luis Obispo, Riverside, Santa Paula, Oxnard and De Luz). The general pattern for dry weight accumulations for each variety in 2003 is consistent with the 2000, 2001, and 2002 data presented last year. A comparison between dry weight accumulations between three maturity seasons for the 'GEM' variety is presented in Figure 8. This data is from the UC-SCREC site. All three seasons show the same general trends. The same can be seen for the 'Harvest' variety in Figure 9.

Bloom evaluation of Bergh/Martin selections. The bloom of spring 2003 was evaluated on the trees in the unreleased variety block at UC-SCREC. This is the second year of this type of data collection. Bloom was rated for intensity, and an estimate of the number of open flowers was made for each tree. This was done weekly throughout the bloom season. Figure 10illustrates the relative timing of each variety over the 2 year period. Figures 11 and 12 show bloom intensity of the 'GEM' and 'Harvest' material as compared to 'Hass'. We observed in these first two years that 'Nobel' and 'Marvel' have somewhat earlier bloom timing as compared to 'Hass' (Figure 10). 'GEM' (Figure 11) is almost identical with 'Hass' and 'Harvest' (Figure 12) has a slightly later bloom period than 'Hass'.

Release of Bergh/Martin selections. The UC Office of Technology Transfer has obtained patents for two of the Bergh/Martin selections, 'GEM' (U.S. Plant Patent No. 14,239) and 'Harvest' (U.S. Plant Patent No. 14,238) effective October 14, 2003. The decision to move forward with patenting for these 2 selections differed for each. We believe that 'GEM' has

commercial potential for the California industry and wish to make this selection more widely available to growers. The 'Harvest', on the other hand, had been given by G. Martin to researchers in Spain, Israel and South Africa where there is interest in the variety from a commercial perspective. While we are not certain about the selection's commercial potential for California, we were advised by UC-OTT to move ahead with patenting of the selection. The UC Office of Technology Transfer is currently working on patents in various foreign countries that are interested in this material.

Component 2. New Material for the Breeding Program

We are taking 2 approaches towards generating new material for the California industry. These approaches are the outcome of discussions with B. O. Bergh, U. Lavi (Avocado breeder, Volcani Institute, Israel) and A. W. Whiley (Australia). The first approach is to plant out seedlings from interesting maternal sources; this is done without any effort to control paternity. This approach was suggested by U. Lavi. In spring 2000, we planted the first 217 seedlings from mixed maternal sources to provide material for the "next generation" of avocado selections using this approach. An additional 237 seedlings were planted out in 2002. In 2003, 186 seedlings were planted out and changes were made to the greenhouse that would allow an expansion of the seedling germination program. As a result, over 1,000 seeds were collected in 2003 and are currently being transplanted into sleeves to be planted in the field in spring 2004. Table 1 shows the maternal parents of the current seedling population planted at UC-SCREC. Interestingly, we had 1 seedling flower and set fruit in 2001, only 1 year from planting. The maternal source of this fruit was 'Nobel' (BL667). Although the fruit quality is not acceptable (extremely large seed), these results are encouraging since we know that we have germplasm for the breeding program that is very precocious. Although there was some fruit set in 2002, most of the fruit was lost due to an irrigation problem that has since been resolved. In October 2003, Dr. Grant Thorp from HortResearch, New Zealand helped us to evaluate tree architecture of the oldest seedling population. Dr. Thorp is currently working with the dataset to help us define the "ideal" tree type. This will help us in the selection process as well in efforts to evaluate the performance of new selections on clonal rootstocks. In this process we identified one tree in the planting that has a sympodial growth habit which is not normally associated with avocado.

Table 1. Open pollinated seedlings from varying maternal sources planted at the UC South Coast Research and Extension Center from 2000 to 2003.

Year Planted	Maternal Source							Total Planted
	5-552	Marvel	Nobel	GEM	Gwen	Lamb	Thille x GEM	
2000	32	90	37	39	14	5		217
2002		75	51	91		20		237
2003		50	25	41	55		15	186
Totals	32	215	113	171	69	25	15	640

In the second approach we have taken the more traditional approach of Dr. Bergh by establishing isolation plots in various locations. Table 2 lists the location, year established and selections in each isolation block. The potential parents were selected under consultation with Dr. Bergh. The first seeds from an isolation block (GEM x Thille) at UCR were collected in 2002 and planted out this year (as indicated in Table 1 above: Thille x GEM). This year the isolation blocks produced 651 fruit which are currently being propagated for field planting in spring 2004.

Table 2. Isolation blocks established in 1999 – 2001.

Parents	Year established	Location
GEM x Marvel	1999 (topwork)	UC, Riverside
GEM x Thille	1999 (topwork)	UC, Riverside
Gwen x Sir Prize	2000 (topwork)	UC, Riverside
Gwen x Gwen	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x GEM	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Nobel	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Thille	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Reed	2001 (clonal tree)	Nakamura, Ventura Co.
Stewart x Reed	2001 (clonal tree)	Nakamura, Ventura Co.

Sunblotch Viroid indexing. One hundred twenty-seven trees at the UC-SCREC were tested for the sunblotch viroid between October 1, 2002 and September 30, 2003. Of these trees, 13 tested positive for the Sunblotch Viroid and have been removed. All the positive trees were located in one area near the end of Field 46. Two trees at the Pine Tree variety trial tested positive for the sunblotch viroid. These trees have been scheduled to be removed. All the trees in field 4 at UC SCREC have been sampled and have tested negative for the sunblotch viroid.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

Introduction of new germplasm. In May 1999 D. Stottlemyer, T. Chao and M. L. Arpaia visited Israel. One of our objectives was to visit with Dr. A. Ben-Ya'acov to review the status of the various rootstock selections, which he had made over the years. In May 1999 M. L. Arpaia brought material from 5 selections plus budwood of the 'Ardith' and the 'Galil'. The 'Ardith' is already in CA and is actually a selection from Dr. Bergh which has been commercialized in Israel. The purpose of this introduction was to confirm trait characteristics of the tree currently in the avocado variety collection at UC SCREC. M. L. Arpaia revisited Dr. Ben-Ya'aeov in March 2000 and obtained additional material. Budwood from the 5 selections obtained in May 1999 was also once again acquired. The majority of this material is rootstock selections. Some of the material is known to be tolerant to root rot. This material will also be evaluated by Dr. John Menge's program. A number of the rootstocks, while having no known root rot tolerance, were selected based on Dr. Ben Ya'acov's recommendation due to salinity tolerance, poor drainage tolerance or other characteristics. We tested this material for the presence or absence of sunblotch when the material entered quarantine. Unfortunately, one selection, VC49 (introduced in 3/00), tested positive and was subsequently destroyed. The remaining material is currently in

quarantine at UC, Riverside and is scheduled to be released for propagation and subsequent testing during 2003 year. Table 3 lists the material brought to California in 1999 and 2000. These trees were released from quarantine in 2003 and in September 2003 we planted 2 trees of each variety in the Variety Collection at UC-SCREC. These trees will serve as budwood source trees for future evaluations.

Table 3. Material introduced into California from Israel in 1999 and 2000. Material is from the rootstock selection program of Dr. A. Ben Ya'acov and the scion selection program of Drs. E. Lahav and U. Lavi. The material obtained in 1999 was given to John Menge in June 1999.

VC#	Date of collection
6	April '00
7	April '00
15	April '00
26	April '00
28	April '00
31	April '00
40	June '99, April '00
49	June '99, April '00 (discarded due to sunblotch)
51	April '00
65	June '99, April '00
66	June '99, April '00
75	April '00
802	April '00
803	April '00
804	April '00
817	June '99, April '00
Ardith	June '99
Galil	June '99

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

As of July 2003 we discontinued the two clonal rootstock trials planted in 1986 and 1988 respectively. We planted a new clonal rootstock trial at UC SCREC with Dr. Menge in spring 1999 and collected a second year of yield data from this plot in spring 2003. The 'Hass' and the 'Lamb Hass' are included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates).

We continue to collaborate with Dr. Crowley in his salinity research and identified a research plot in Santa Barbara for testing of 'Hass' and 'Lamb Hass' on selected rootstocks from Dr. Menge's program, South Africa and Israel.

D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Dr. Ben Faber; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.

In conjunction with Ben Faber we established a pollinizer site in Ventura County (Oxnard) in spring 1999. The varieties included in this trial are 'Ettinger', 'Fuerte', 'Bacon', 'Zutano', 'Harvest', 'Sir Prize', 'Nobel' and 'Marvel'. There are 60 trees of each variety divided into 6 replicates of 10 trees each. We have reported the yield data from the trial each year. The data from the 2003 harvest is shown in Figure 13. This was an "off" year in the plot as shown by the fruit counts. The set in 2004 is very interesting since it is clear that fruit set is moderate to very high near the pollinizer trees but drops off quickly as the distance increases. The cumulative data for this trial is presented in Figure 14 and shows that there are distinct trends emerging from this trial. Most of the activities for this plot are being subsidized from a grant from the BARD. We have this project as a collaborative effort with Drs. Arnon Dag and Sharoni Shafir (Israel) and Dr. Tom Davenport (University of Florida). .

We have continued our discussions with Dr. Clegg on ways to incorporate the B flower type selections into an organized research program to evaluate the value of outcrossing and which pollinizers to utilize and to discuss future directions for the breeding program. A boost to this effort came through the collaboration with Dr. Davenport who is working with Dr. Raymond Schnell (USDA-ARS, Miami, FL) on a more economical way to determine paternity. We assisted Dr. Davenport in October 2003 by collecting fruit samples from one of the replicates in the trial (48 samples of 20 fruit each). We are awaiting the results of their testing.

E. To assist Drs. Morse and Hoddle on identifying plant material tolerant to Persea mite and the avocado thrips.

We continue to discuss periodically with Dr. Hoddle the influence of tree phenology and variety on relative susceptibility to persea mite. We assisted Drs. Hoddle and Morse on their thrips studies in 2003.

F. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block at UC-SCREC. Any changes to the planting are being recorded in the master database maintained by David Stottlemyer. The UC-SCREC avocado volunteers have been instrumental in maintaining this block. The volunteers graft several new and/or historical varieties on an on-going basis. In December 2002 we were able to obtain budwood of the 'Puebla' variety from the Catholic University of Valparaiso in Chile. This budwood is currently in quarantine. Once released, this material will be planted in the Variety collection.

G. To insure the timely and effective dissemination of information developed from this research program.

The current avocado web site at: www.ucavo.ucr.edu has been on-line since June 1998. The site is periodically revised and updated with new information and photographs of different varieties. Questions sent via e-mail or forwarded from the California Avocado Commission are answered on an ongoing basis.

Figure 1. Variety trial yield data (average fruit count per tree) collected from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 - 2003. Trees were topworked onto seedling rootstock in 1994 – 1996.

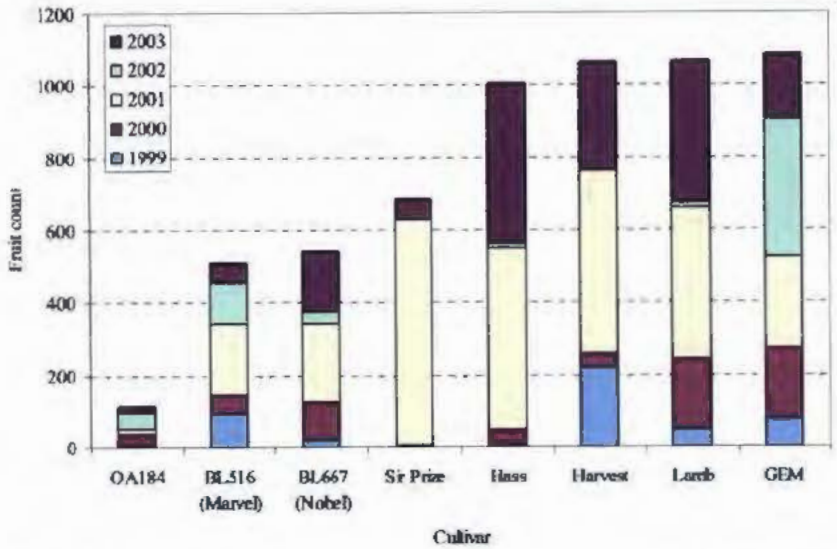


Figure 2. The Coefficient of Variation (%) in yield (fruit number) for each variety from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 – 2003.

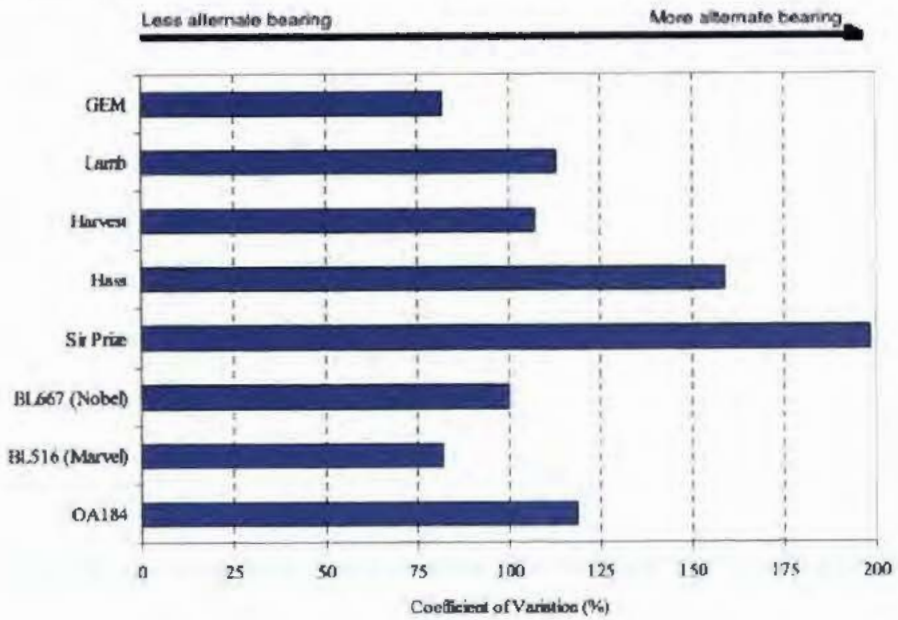


Figure 3. Variety trial yield data (average fruit count per tree) collected from DeBusschere Ranch, Oxnard, CA for 2001 – 2003. Trees were planted on clonal Duke 7 rootstock in 1996.

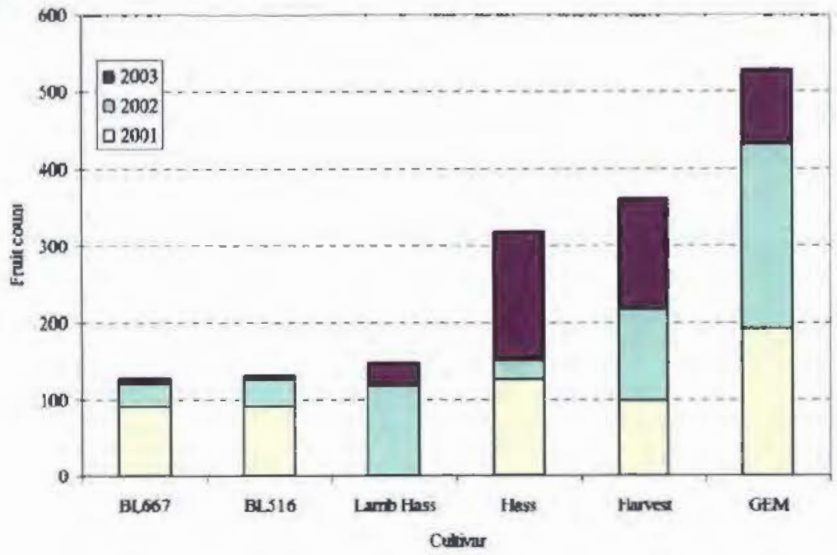


Figure 4. Variety trial yield data (average fruit count per tree) collected from Pine Tree Ranch in Santa Paula, CA for 2001 – 2003. Trees were topworked onto seedling rootstock in 1998.

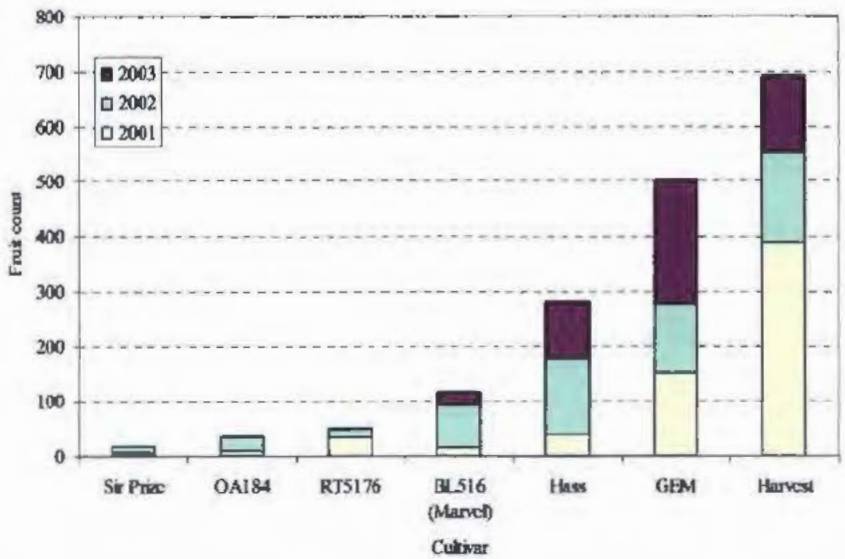


Figure 5. Variety trial yield data (average fruit count per tree) collected from Righetti Ranch in San Luis Obispo, CA for 2001 – 2003. Trees were topworked onto seedling rootstock in 1998 - 99.

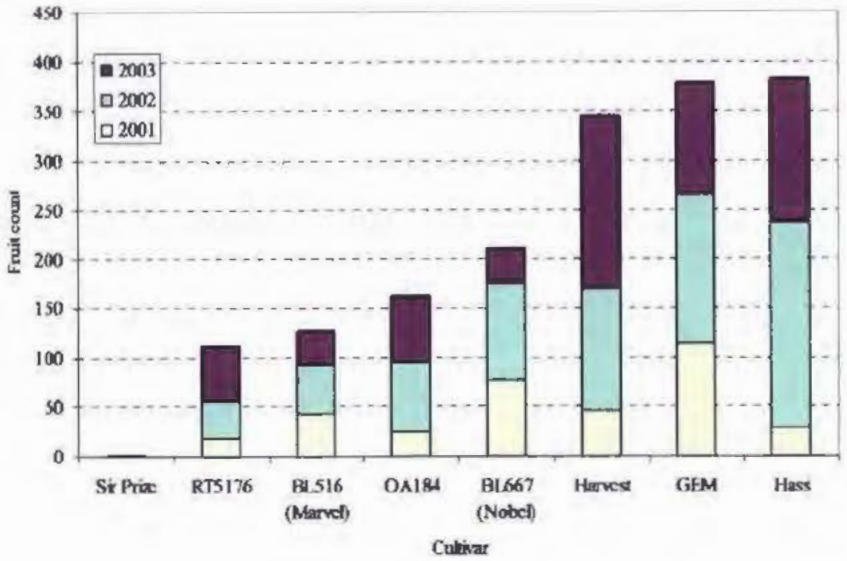


Figure 6. Variety trial yield data (average fruit count per tree) collected from ACW Ranch in De Luz, CA for 2001 – 2003. Trees were topworked onto seedling rootstock in 1998 - 99.

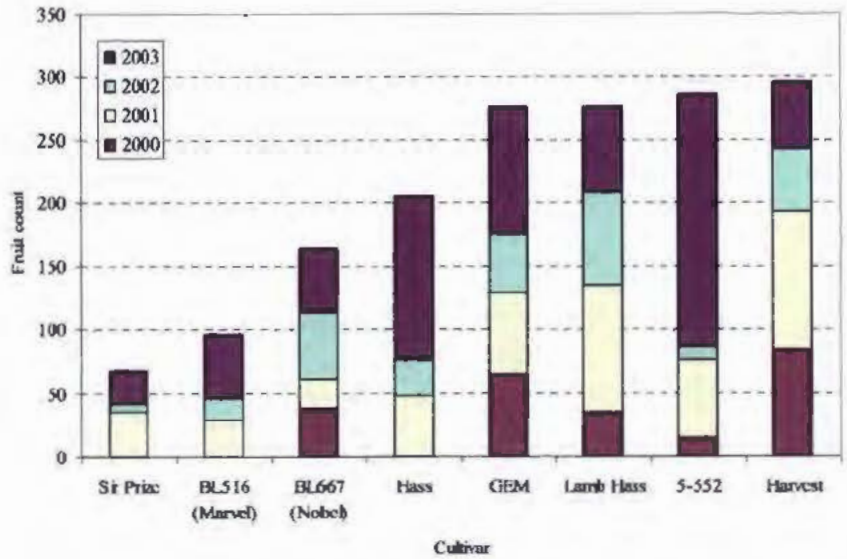


Figure 7. Comparison of changes in dry matter content (%) for all varieties harvested from January 2003 through September 2003 from the SCREC site, Irvine, CA.

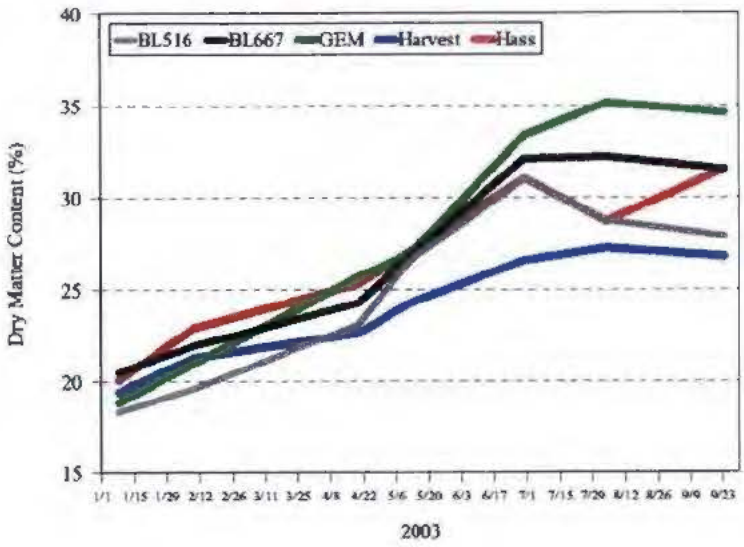


Figure 8. Comparison of changes in dry matter (%) for 'GEM' harvested during the 2000 - 2003 maturity seasons. Data collected from the SCREC site, Irvine, CA.

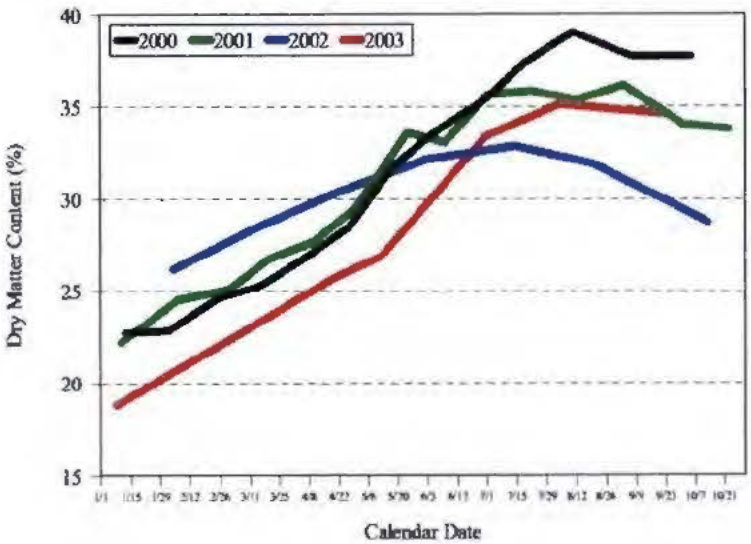


Figure 9. Comparison of changes in dry matter (%) for 'Harvest' harvested during the 2000 - 2003 maturity seasons. Data collected from the SCREC site, Irvine, CA.

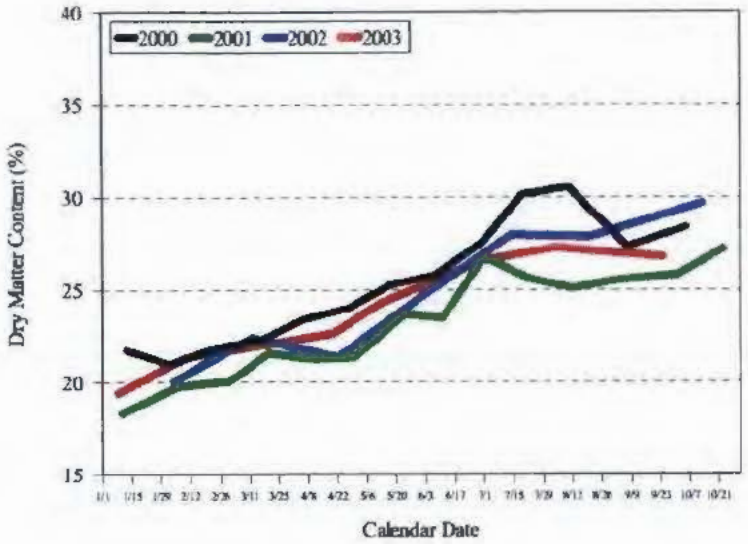


Figure 10. Comparison of average bloom dates for two years for all varieties from February through May at the UC South Coast Research and Extension Center, Irvine, CA.

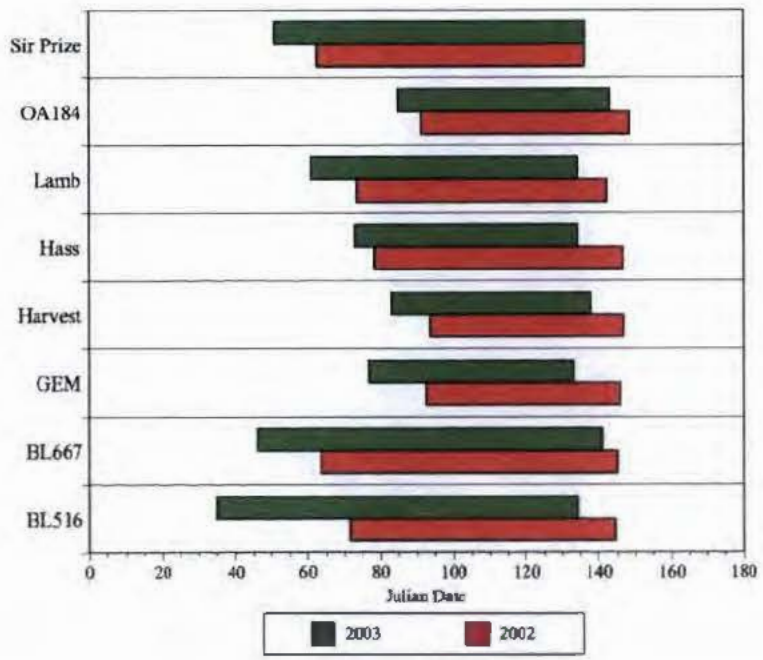


Figure 11. Comparison of percent bloom for 'Hass' and 'GEM' from February through May 2003 at the UC South Coast Research and Extension Center, Irvine, Ca.

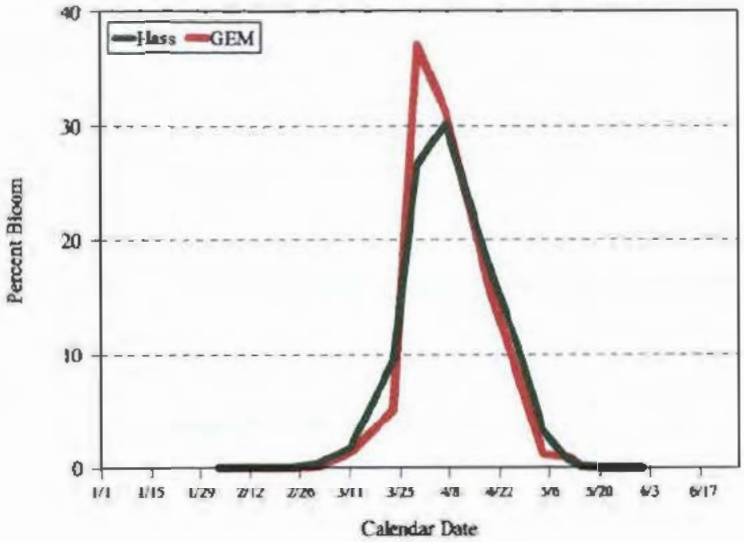


Figure 12. Comparison of percent bloom for 'Hass' and 'Harvest' from February through May 2003 at the South Coast Research and Extension Center, Irvine, Ca.

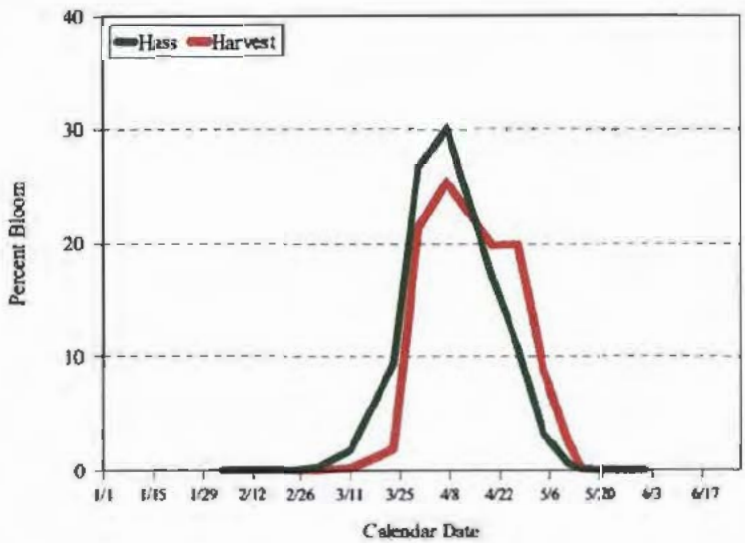


Figure 13. 'Hass' yield (fruit count) from the DeBusschere pollinizer trial in Oxnard in 2003 as influenced by pollinizer variety and distance from the pollinizer.

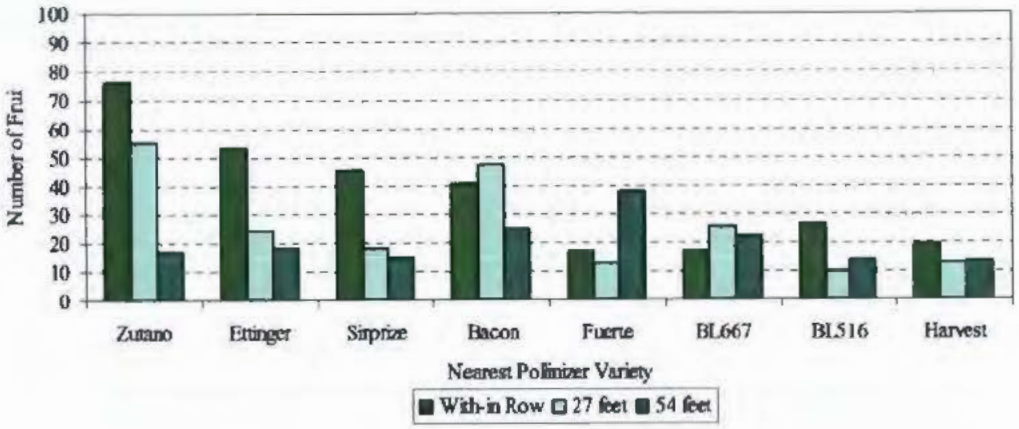
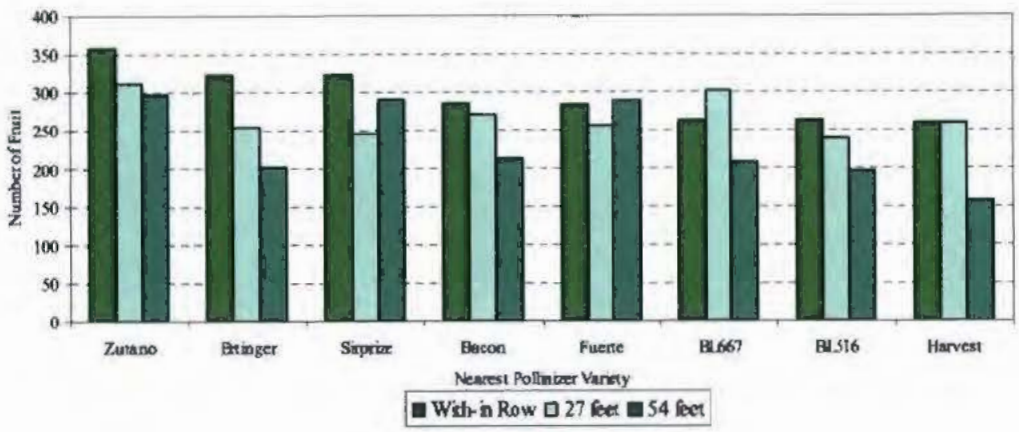


Figure 14. Cumulative 'Hass' fruit counts (2001 – 2003) from the DeBusschere pollinizer trial in Oxnard as influenced by pollinizer variety and distance from the pollinizer.



Proceedings of the California Avocado Research Symposium, October 30, 2004. University of California, Riverside. California Avocado Commission. Pages 9-23.

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Continuing Project: Year 8 of 20

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G. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round.

There are 2 components of this objective. The first is the continued monitoring of varieties from the Dr. B. Bergh/Gray Martin selection program. The second component is the new phase of scion selection. Activities for both components are summarized below.

Component 1. Continued monitoring of Bergh/Martin selections

Various field trials have been established to monitor the performance of a number of the Bergh/Martin selections. The following is a list of the cooperator trials we are maintaining. In 2002 we installed data loggers to monitor air and soil temperature and relative humidity at all sites. We plan to use this data to help us assess the selection's response to low/high temperature when these events occur.

There are also additional plantings of the Bergh/Martin selections scattered throughout southern California. We periodically visit these sites to evaluate trees and discuss tree performance with the cooperators.

Topworked trials at Non-UC Sites

Santa Paula (Ventura County); topworked in 1998; 'GEM', 'Harvest', 'Sir Prize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates.

De Luz (San Diego County); topworked in 1998; 'Lamb Hass', 'Sir Prize', 'GEM', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest'; 10 replicates.

De Luz (San Diego County); topworked in 1998; approximately 80 'GEM' trees divided roughly into 3 groups at the cooperator site.

San Luis Obispo (San Luis Obispo County); topworked in 1998 (Trees suffered from freeze in 12/98 necessitating re-grafting of some selections in 1999; 'RT5176', 'Hass', 'Sir Prize', 'GEM', 'Harvest', 'OA184'; 9 replicates.

Clonal trials at Non-UC Sites

Oxnard (Ventura County); planted in 1996; 'Lamb Hass', 'Sir Prize', 'GEM', 'OA184', 'Marvel', 'Nobel', 'Hass', 'Harvest'. (This trial was flooded in 1997 and many trees died due to this, however we are now working with the current owners to collect data from the trees which survived after the winter of 1997)

HTH Ranch (Ventura County); 'Lamb Hass', 'Marvel', 'GEM' and 'Hass' A non-replicated trial used for dry weights and fruit evaluation only.

Topworked trees at UC, Riverside Campus – ongoing; Replacement trees in Field 10.

Topworked trees at UC, South Coast Research and Extension Center (SCREC): Field 4 at the Center has topworked trees (variable number of replicates) from which we collect data. These trees were topworked onto seedling rootstock trees in 1994 – 1996.

San Joaquin Valley Variety Trial – 1999 at two sites (Porterville, Lindcove) with clonal trees (Thomas rootstock): 'Sir Prize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'; 20 replicates per scion variety at each site. We had trouble with tree establishment for certain varieties, therefore surviving tree numbers varies with site and variety.

We have also had problems with certain varieties dropping fruit prior to harvest; however we do have 1 season's worth of dry matter testing and plan to collect further data this upcoming year.

Yield data from Bergh/Martin selections. We have collected yield data for the sixth year from Field 4 at UC-SCREC (UC South Coast Research and Extension Center). Data collection for 2004 shows that for most varieties, this was an 'on' year (Figure 1). The 'Lamb Hass' at this point has the largest cumulative yield over the six year period. Note also the extreme alternate bearing of the 'Sir Prize' during this time. The fruit load was so heavy in 2004 that the trees had virtually no flowers during the spring 2004 flowering season. Comparing the coefficient of variation shows that there is tendency toward less extreme alternate bearing in 'GEM' (Figure 2; this is calculated by dividing the standard deviation by the mean and gives one an idea of the relative variation of the data for a particular variety).

We have also collected the fourth year of yield data from the Oxnard site and are in the process of finishing the harvesting at the Santa Paula site in Ventura County (Figures 3 and 4), the Righetti site near San Luis Obispo (Figure 5). Data from the De Luz site is incomplete at this time.

Fruit characteristics of Bergh/Martin selections. As an on-going process we are collecting fruit samples from all sites approximately every 4 to 5 weeks from winter through late fall. These fruit are evaluated using standard protocols for such characteristics as fruit shape, peel texture, peel color, flesh color, the percent seed, flesh and skin and skin thickness.

Seasonal dry matter content of Bergh/Martin selections. We collected dry matter data as in previous years. The general pattern for dry weight accumulations for each variety in 2004 is consistent with the 2000 – 2003 data as previously reported.

Bloom evaluation of Bergh/Martin selections. The bloom of spring 2004 was evaluated on the trees in the unreleased variety block at UC-SCREC. This is the third year of this type of data collection. Bloom was rated for intensity, and an estimate of the number of open flowers was made for each tree. This was done weekly throughout the bloom season. Figure 6 illustrates the relative timing of each variety over the 3 year period. Note that the bloom in 2004 was very much abbreviated. This could be due to a number of reasons including the unseasonable hot and dry weather we had in late April and May.

Release of Bergh/Martin selections. The UC Office of Technology Transfer has obtained patents for two of the Bergh/Martin selections, 'GEM' (U.S. Plant Patent No. 14,239) and 'Harvest' (U.S. Plant Patent No. 14,238) effective October 14, 2003. Budwood for this variety was collected by various nurseries in the spring of 2004. Growers interested in these varieties can either contact M. L. Arpaia, D. Stottlemyer or Dr. William Tucker at the UC Office of Technology Transfer for more information.

Component 2. New Material for the Breeding Program

We are taking 2 approaches towards generating new material for the California industry. These approaches are the outcome of discussions with B. O. Bergh, U. Lavi (Avocado breeder, Volcani Institute, Israel) and A. W. Whiley (Australia). The first approach is to plant out seedlings from interesting maternal sources; this is done without any effort to control paternity. This approach was suggested by U. Lavi. In spring 2000, we planted the first 217 seedlings from mixed maternal sources to provide material for the "next generation" of avocado selections using this

approach. An additional 237 seedlings were planted out in 2002 and 186 seedlings in 2003. So far, 242 seedlings have been planted out in 2004 with another 350 seedlings to be planted this fall. We anticipate an additional 350 seedlings will be planted out in Spring 2005. Table 1 shows the maternal parents of the current seedling population planted at UC-SCREC.

Table 1. Open pollinated seedlings from varying maternal sources planted at the UC South Coast Research and Extension Center from 2000 to 2004.

Year Planted	Maternal Source								Total Planted
	5-552	Marvel	Nobel	GEM	Gwen	Lamb Hass	Thille x GEM	GEM x BL516	
2000	32	90	37	39	14	5			217
2002		75	51	91		20			237
2003		50	25	41	55		15		186
2004	**30**	**61**	**48**	**42**	**55**			**6**	**242**
Totals	62	276	121	213	124	25	15	6	882

Of the 217 trees planted in 2000, 73 produced fruit and were evaluated this year. Three seedlings show some promise and have been grafted onto Duke7 rootstock for further evaluation. A fourth seedling tested very high in percent dry weight at 35. 12% on 1/5/04. Thinking this was a mistake, it was retested on 3/1/04 and came in at 42.64%! This may well be a very early maturing variety an as such is being watched closely for the upcoming season.

In the second approach we have taken the more traditional approach of Dr. Bergh by establishing isolation plots in various locations. Table 2 lists the location, year established and selections in each isolation block. The potential parents were selected under consultation with Dr. Bergh. A total of 433 seed were collected for germination from the isolation blocks including the first group of seeds from the Nakamura site in 2004.

Table 2. Isolation blocks established in 1999 – 2001.

Parents	**Year established**	**Location**
GEM x Marvel	1999 (topwork)	UC, Riverside
GEM x Thille	1999 (topwork)	UC, Riverside
Gwen x Sir Prize	2000 (topwork)	UC, Riverside
Gwen x Gwen	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x GEM	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Nobel	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Thille	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Reed	2001 (clonal tree)	Nakamura, Ventura Co.
Stewart x Reed	2001 (clonal tree)	Nakamura, Ventura Co.

Sunblotch Viroid indexing. One hundred forty six trees at the UC-SCREC were tested for the sunblotch viroid between October 1, 2003 and September 30, 2004. Of these trees, 3 tested positive for the Sunblotch Viroid and have been removed. Two of the positive trees were in field 46 and represent our continuing effort to eliminate sunblotch from that field. One positive tree was found in field 44. This is the first positive tree in that field and it was removed immediately.

All surrounding trees were tested but all were negative. Current plans are to retest the adjacent trees again in 2005 to make sure the viroid has been eliminated from this field.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

Introduction of new germplasm. We have continued to plant out new varieties as they come out of quarantine. Last year's release of Ardith (re-introduced from Israel) and the VC series of Israeli rootstocks has gone well as all of the trees are established in the field at the UC South Coast REC. Trees from the VC series were provided to Dr. Menge for field testing.

The next scheduled release of quarantine trees will be in the spring of '05 when the two Andes selections and Puebla will be available for planting out. The Andes selections are believed to be seedlings or budsports of Hass and were selected in Chile by the Andes Nursery Association. This material came to California under a test agreement. Once the trees are released from quarantine we plan to plant out a limited number of trees for evaluation at South Coast Research and Extension Center. The Puebla, which is a heritage variety originating in California was brought back to California in 2002 from the germplasm collection of the Catholic University of Valpariso, Chile. With the aid of Dr. Ben Ya'acov, who confirmed the identity of the variety in Chile, we elected to bring this variety back to California for placement in the variety collection. Other Puebla trees in California are of uncertain identity and this introduction will aid us in identifying Puebla trees growing throughout southern California. The planting of this material may need to take place in fall of '05 as they will most likely require a hardening off period in the UCR lathhouses before they can be planted into field 44.

Finally, two additional varieties of interest have been brought in from Chile from the Andes Nursery Association (A.N.A.). This material arrived in September of 2004 and is now in quarantine for two years.

C. To collaborate with Dr. Menge (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

As of July 2003 we discontinued the two clonal rootstock trials planted in 1986 and 1988 respectively. We planted a new clonal rootstock trial at UC SCREC with Dr. Menge in spring 1999 and collected a third year of yield data from this plot in spring 2004. The 'Hass' and the 'Lamb Hass' are included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates)

We continue to collaborate with Dr. Crowley in his salinity research whenever possible and have assisted in the evaluation of a salinity/*Phytophthora* rootstock trial established in Santa Barbara using rootstocks from Dr. Menge's program, South Africa and Israel.

D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Dr. Ben Faber; assist Dr. Mike Clegg on coordination of pollinizer research plots as requested.

In conjunction with Ben Faber we established a pollinizer site in Ventura County (Oxnard) in spring 1999. We are using funding from BARD (a collaborative effort with Drs. Arnon Dag and Sharoni Shafir (Israel) and Dr. Tom Davenport (University of Florida)) to collect floral data as well at this site as well as 2 other sites. Below we have included a progress report of both our CAC and BARD funded efforts. Dr. Loretta Bates oversaw the field research efforts for the 2004 season. In late March 2004, Dr. Gad Ish-Am visited the research sites and discussed avocado flowering with Arpaia, Davenport and Bates.

The 2004 research was conducted at three sites in Ventura County California from April through June 2004. Two sites are located on the flat coastal plain south of Camarillo, California and the third site is northeast of the city of Santa Paula, California on the lower south-facing slopes of the coastal mountains. The two coastal sites were in commercial groves of Mr. Paul DeBusschere. The southern site is the location of the pollinizer trial and the site 3 miles north is the location of the variety trial (yield reported in Figure 3). The pollinizer site was designed as a complete randomized block design to examine proximity effects of various pollinizers on 'Hass' productivity under commercial conditions. There are six replicated blocks. Each block consists of 25 rows of 20 trees. Each row is split into 2 subsamples of 10 trees. There is a pollinizer variety interplanted with the Hass every 6^{th} row. Each subsample has a different pollinizer variety interplanted with 'Hass'. The 'Hass' trees are spaced in 2 blocks, 25 ft x 17 ft. In the remaining 4 blocks the trees are spaced 25 ft x 12.5 ft. We have 10 pollinizer trees per subsample. The variety site was originally planted as a completely randomized block design to evaluate new cultivar varieties from the UC Scion Breeding Program. The trees were planted in 1996. All trees are grafted on clonal Duke 7 rootstock. Due to flooding shortly after the planting of this trial, there are a mixed number of replications. There are 5 to 6 replications per variety. The inland site is in the commercial groves of Mr. Logan Hardison (~25 miles inland). The inland site is a high density commercial planting with pollinizer rows intermixed with 'Hass'.

Temperature and humidity data were collected continuously through the months of April and May by remote sensors in weather stations at each site. Tree flowering phases and flowering stages of individual flowers were observed in the following avocado varieties, Hass, Lamb Hass, Ettinger, Fuerte, Zutano, Bacon, Harvest, Gem, SirPrize, BL516 (Marvel) and BL667 (Nobel).

Observations of tree flowering phases were made April 13-15, April 21-23 and April 28-30, 2004 at the DeBusschere south site and the Hardison site. Observations of flowering stages of individual flowers were recorded May 5-7, May 12-14, and May 19-21, 2004 at both of the coastal sites.

For tree flowering phases, observations were made approximately every hour on three randomly selected trees of each variety. On each tree, a minimum of fifteen flowers on each of three inflorescences on the north side and on the south side were examined. The flower stages were recorded according to the method of Ish-Am and Eisikowitch (1991; New insight into avocado flowering in relation to its pollination, Calif. Avocado Soc. Yrbk. 75:125-137).

To characterize the daily sequence of flowering stages of individual flowers, inflorescences were labeled and flagged on randomly selected trees of each variety. As flowers opened as female, they were marked with colored ink indicating the order of initial opening. Observations were made approximately once each hour for each flower on each measurement day. For each measurement period of 2-3 days, observations were made on 15 to 25 flowers per cultivar.

One goal of the 2004 study was to collect data at a wide range of temperatures, both day and night. Except for two abnormally hot periods, the temperatures at all sites were cool (less than 25°C maximum) on most of the measurement days during April and May of 2004. The coastal plain sites were especially cool and humid, characterized by a daily pattern of morning fog or heavy dew followed by late morning sun. The upland Hardison site was somewhat warmer and drier. Both sites experienced strong on-shore winds commencing by mid-day and continuing until near sundown on most days.

As a result of the cool humid weather, the flowers typically remained open in the morning from the previous day or night or opened after fog lifted, usually by mid-morning. In the early morning, Type A flowers such as Hass, Harvest and Lamb-Hass were often completing male floral stages from the previous day. Flowers opened as females in the afternoon and frequently, they had not yet opened as males by sundown. In Type B flowers (SirPrize, Fuerte, Zutano, Ettinger, BL516, BL667, and Bacon) it was common to see flowers open only as males during the day hours, with flowers opening as females near sundown (or later).

For type B flowers tagged as females on day 1 of a measurement period, opening as males did not occur on day 2 but rather in the morning of day 3 of the measurement period. Type A flowers tagged as females did open as males the following day, although sometimes very late and as mentioned previously, sometimes completing the male stages on the morning of day 3.

The time of opening of Type A flowers as females and Type B flowers as males may be correlated with the minimum temperature of the previous night (correlation coefficient -0.7553 for pooled Type A and Type B data). Data analysis is continuing which should allow a more definitive statement of that correlation.

Table 3 gives examples of tree phases observed on a cool day (April 23; maximum temp, 20.2°C; minimum temp, 7.4°C) at the south DeBusschere site and on the warmest day (April 30; maximum temp, 29.9°C; minimum temp, 11.7°C) at the Hardison site for the varieties Harvest (Type A) and BL516 (Type B).

Table 3. Flowering behavior of 'Harvest' (A floral type) and 'BL516' (B floral type) under

Harvest			BL516	
Cool	*Warm*		*Cool*	*Warm*
D4*	D1*, D2*, D3*, D4		B1*	B3*, C, D1, D2
E	B1, B2		B1*, B2*, B3*, D1	D1, D2
B1	B2, B3		D1, D2	D1, D2, D3
B1, B2	B3, C, D1, D2		D1, D2, D3	D2, D3
B1, B2, B3	D1, D2, D3, D4		D1, D2, D3, D4	D4
B3, C, D1	D1, D2, D3, D4		D2, D3, D4	B1, D4
B3, D1, D2	D2, D3, D4		B1, D4	B1, B2, D4
D1, D2, D3, D4				B1, B2, B3
				B2, B3
				B3, C

*Stages from previous day.

Analysis of the floral data is continuing and will include additional analyses of the correlations between floral stages and temperature averages, maxima and minima. In addition to the analysis of floral biology observations for 2004, HPLC analysis of sugars in honey collected during the 2003 flowering season is continuing.

We harvested the DeBusschere Pollinizer plot April 13-15, 2004 for yield. Figure 7 presents the data for 2004. The yield this year was very heavy from the plot, averaging 220 fruit per tree. The 4-year cumulative fruit count for the trial is presented in Figure 8. As both figures demonstrate, proximity to a pollinizer at this site continues to influence yield. This effect was especially dramatic this year (Figure 7).

The 'SirPrize', a variety released by UC in the mid 1990's has surpassed the other pollinizers in terms of yield. We are monitoring the SirPrize more closely this year to collect better data on its bloom phenology in relation to the 'Hass'. As part of Dr. Gad Ish-Am's March 2004 visit, we spent time in the field examining the flowering phases of the 'SirPrize' as well as the other varieties. We noted in March, that this variety had abnormal pistils on the majority of the flowers. We are monitoring this further. This may account for the "erratic" bearing habit of the 'SirPrize'.

The DeBusschere plot is bordered by a hedge of eucalyptus trees on the east and west sides of the field and between rows 24 and 26 there is a Lombardi poplar wind break. These windbreaks have a negative impact on tree yield and vigor. Figure 9 shows the impact of these windbreaks on average yield of 'Hass' trees. In order to calculate this data we calculated the average fruit count for each row (12 means per row for non-pollinizer rows and 24 means per row for pollinizer rows).

Figure 10 illustrates the relative size of the pollinizer trees to their companion 'Hass' trees in April 2004. Many of the pollinizer trees were as tall as or taller than the nearby 'Hass' trees so in Fall 2003, these trees were topped to approximately 6 to 8 feet.

Table 4. Average 'Hass' dry weight, fruit length/width ratio and seed length/width ratio harvested from pollinizer rows. Mean separation by LSD, $P < 0.05$.

Pollinizer	Dry weight (%)	Fruit length/width ratio	Seed length/width ratio
Bacon	25.34 ab	1.39	1.13 bc
BL516 (Marvel)	24.45 c	1.40	1.14 bc
BL667 (Nobel)	26.20 a	1.43	1.15 abc
Ettinger	25.30 abc	1.37	1.11 c
Fuerte	24.80 bc	1.39	1.19 a
Harvest	24.95 bc	1.45	1.18 ab
Sirprize	24.94 bc	1.37	1.15 bc
Zutano	26.08 a	1.37	1.11 c
Significance	0.05	ns	0.05

At the time of the fruit harvest, we collected 8 'Hass' fruit (6.77 oz average size) from each pollinizer row (6 replications). Dry weight was determined on each fruit. We also measured the length and width of fruit from 3 of the replications. Table 4 presents these results. Note that there were significant differences in both dry weight and the seed length/width ratio due to pollinizer. The 'Hass' fruit that was harvested from the 'BL667' and 'Zutano' rows had significantly higher dry matter than fruit harvested from the 'Harvest', 'Sinprize', 'Fuerte' and 'BL516' rows. We were not able to detect any significant effects on fruit shape as measured by the length/width ratio due to the pollinizer variety. We found, however, that there were slight but significant differences due to pollinizer variety on the length/width ratio of the seeds. 'Hass' fruit from the 'Fuerte' and 'Harvest' rows had slightly more elongated seeds as compared to seeds from the 'Zutano' and 'Ettinger' rows. We plan to do more in-depth sampling this coming fruit season to verify these observations.

E. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block at UC-SCREC. Any changes to the planting are being recorded in the master database maintained by David Stottlemyer. The UC-SCREC avocado volunteers have been instrumental in maintaining this block. The volunteers graft several new and/or historical varieties on an on-going basis. Fields 44 and 46 have been maintained and kept in order through regular pruning and constant observation by both the lab personnel and the volunteer staff. In addition, the sprinkler lines in field 46 have been replaced and updated in coordination w/the SCREC personnel.

F. To insure the timely and effective dissemination of information developed from this research program.

The current avocado web site at: www.ucavo.ucr.edu has been on-line since June 1998. The site is periodically revised and updated with new information and photographs of different varieties. Questions sent via e-mail or forwarded from the California Avocado Commission are answered on an ongoing basis.

Figure 1. Variety trial yield data (average fruit count per tree) collected from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 – 2004. Trees were topworked onto seedling rootstock in 1994 – 1996. Harvest for the selections denoted with a "*" is incomplete as of September.

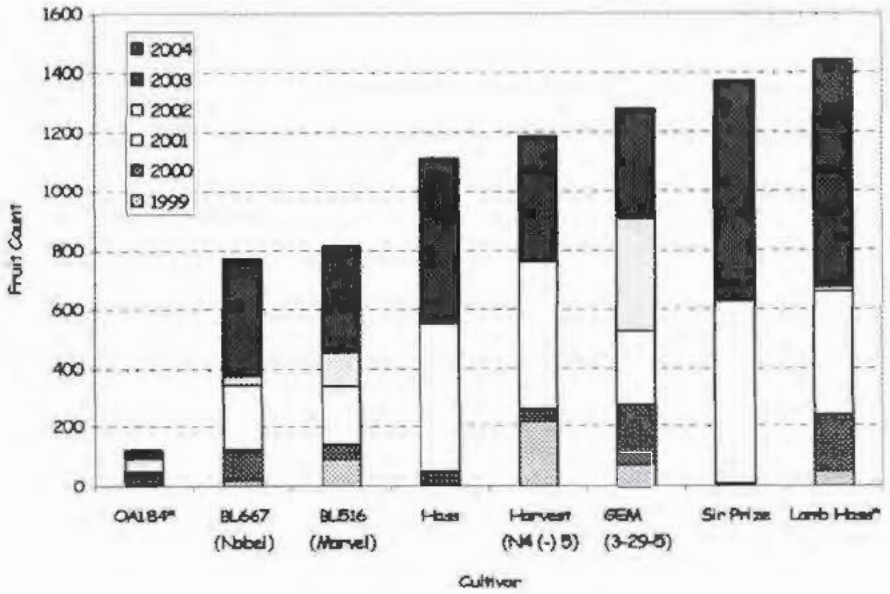


Figure 2. The Coefficient of Variation (%) in yield (fruit number) for each variety from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 – 2004.

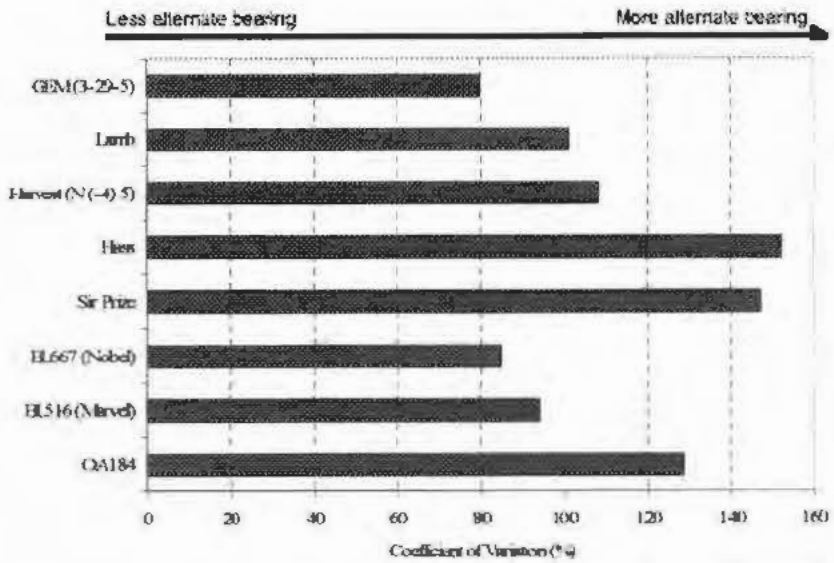


Figure 3. Variety trial yield data (average fruit count per tree) collected from DeBusschere Ranch, Oxnard, CA for 2001 – 2004. Trees were planted on clonal Duke 7 rootstock in 1996.

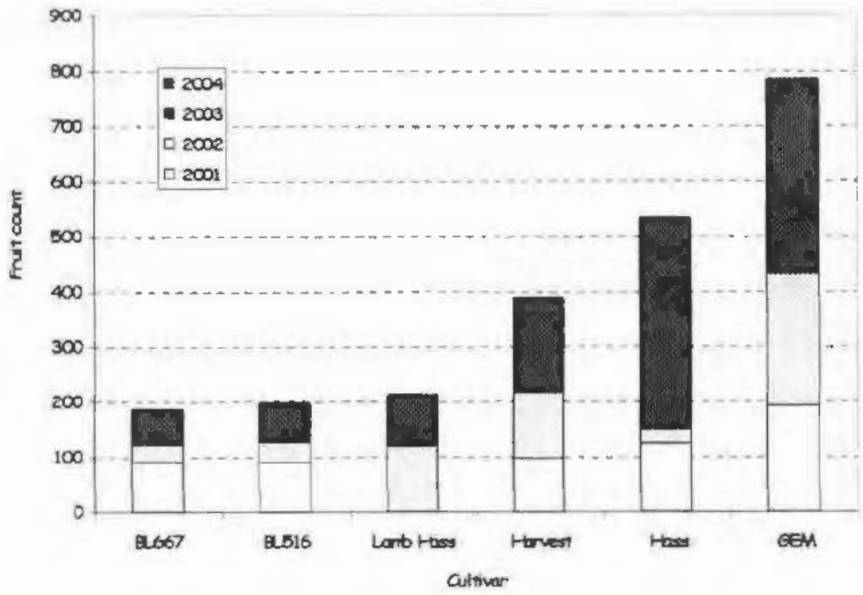


Figure 4. Variety trial yield data (average fruit count per tree) collected from Pine Tree Ranch in Santa Paula, CA for 2001 – 2004. Trees were topworked onto seedling rootstock in 1998.

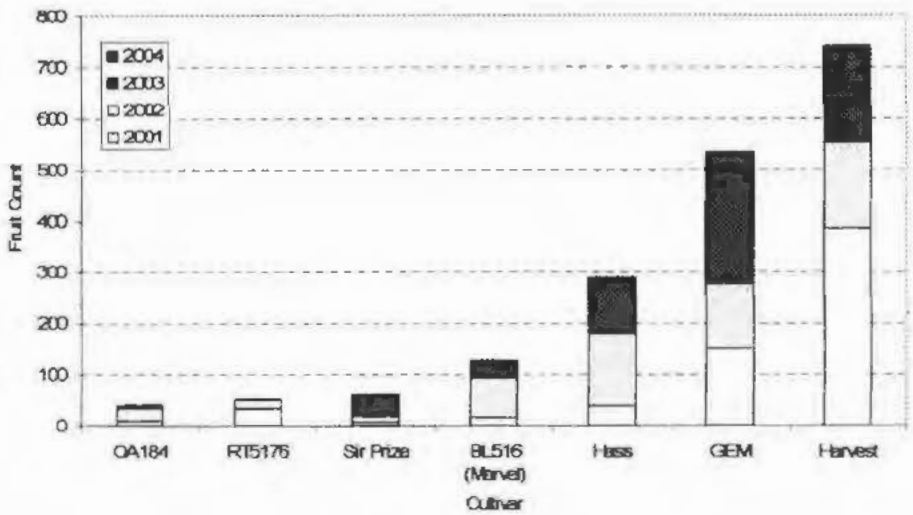


Figure 5. Variety trial yield data (average fruit count per tree) collected from Righetti Ranch in San Luis Obispo, CA for 2001 – 2004. Trees were topworked onto seedling rootstock in 1998 - 99.

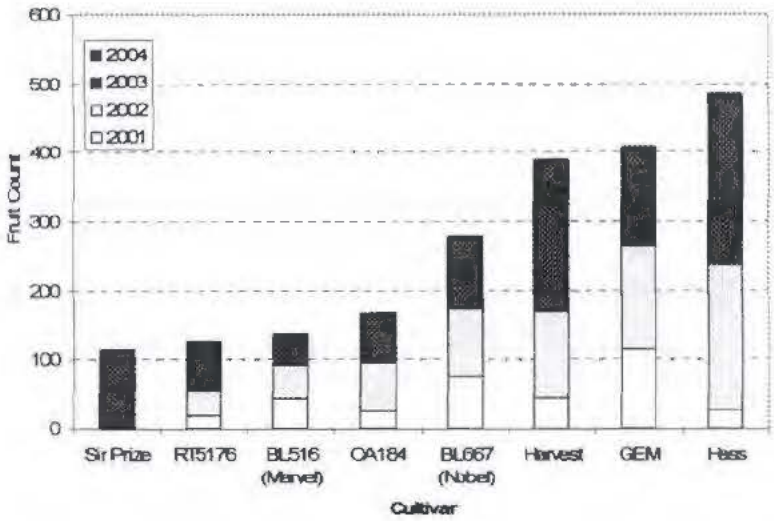


Figure 6. Comparison of average bloom dates for two years for all varieties from February through May at the UC South Coast Research and Extension Center, Irvine, CA.

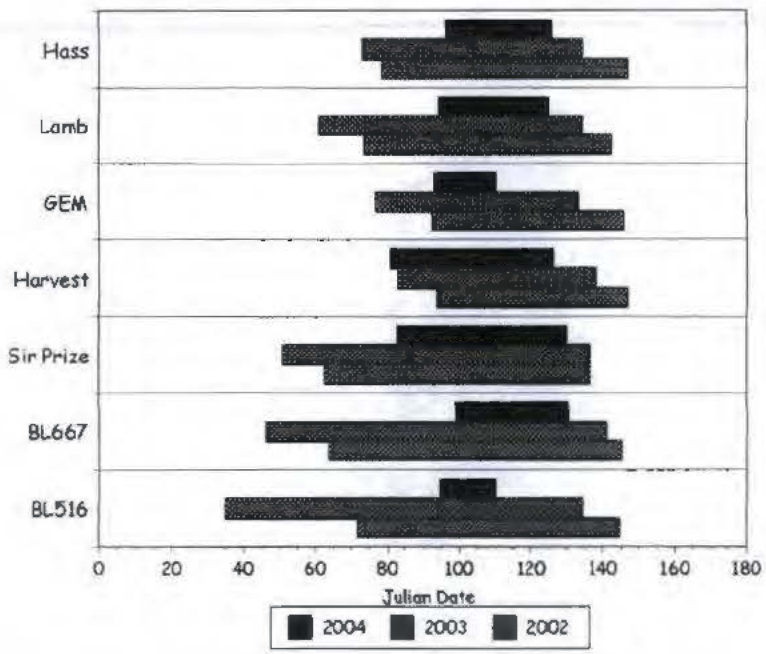


Figure 7. 'Hass' yield (fruit count) from the DeBusschere pollinizer trial in Oxnard in 2004 as influenced by pollinizer variety and distance from the pollinizer.

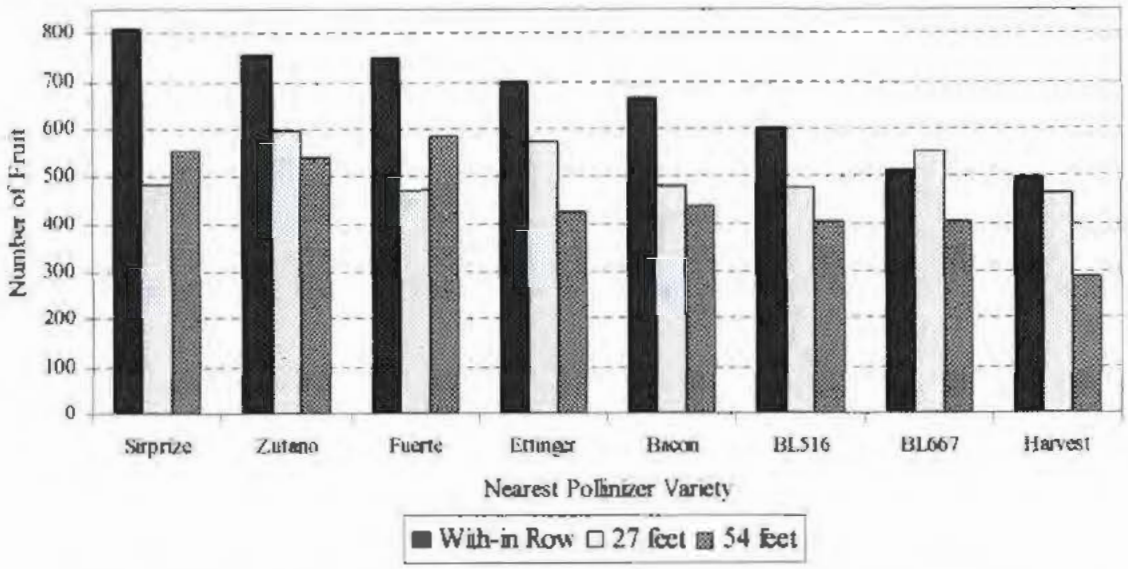


Figure 8. Cumulative 'Hass' fruit counts (2001 – 2004) from the DeBusschere pollinizer trial in Oxnard as influenced by pollinizer variety and distance from the pollinizer.

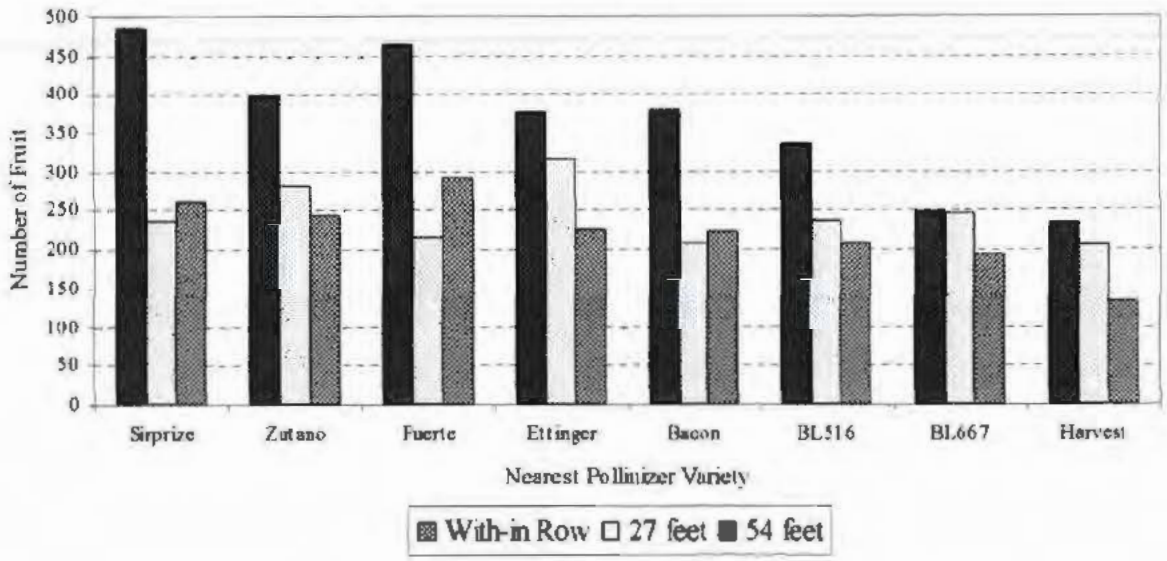
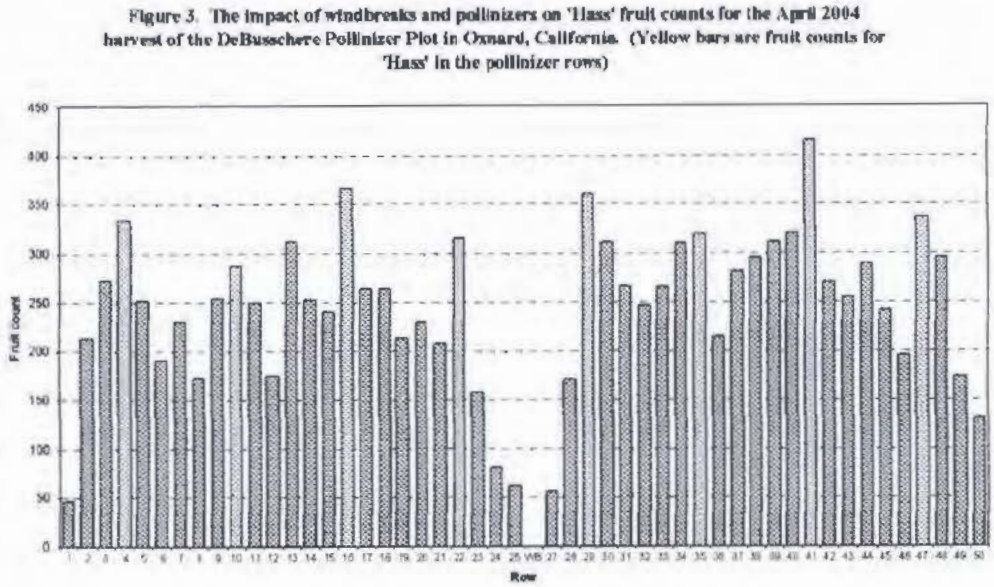


Figure 9. The impact of windbreaks and pollinizers on 'Hass' fruit counts for the April 2004 harvest at the DeBusschere Pollinizer Plot in Oxnard, CA. (Yellow bars are fruit counts for 'Hass' in the pollinizer rows and are the means for 6 replications.)



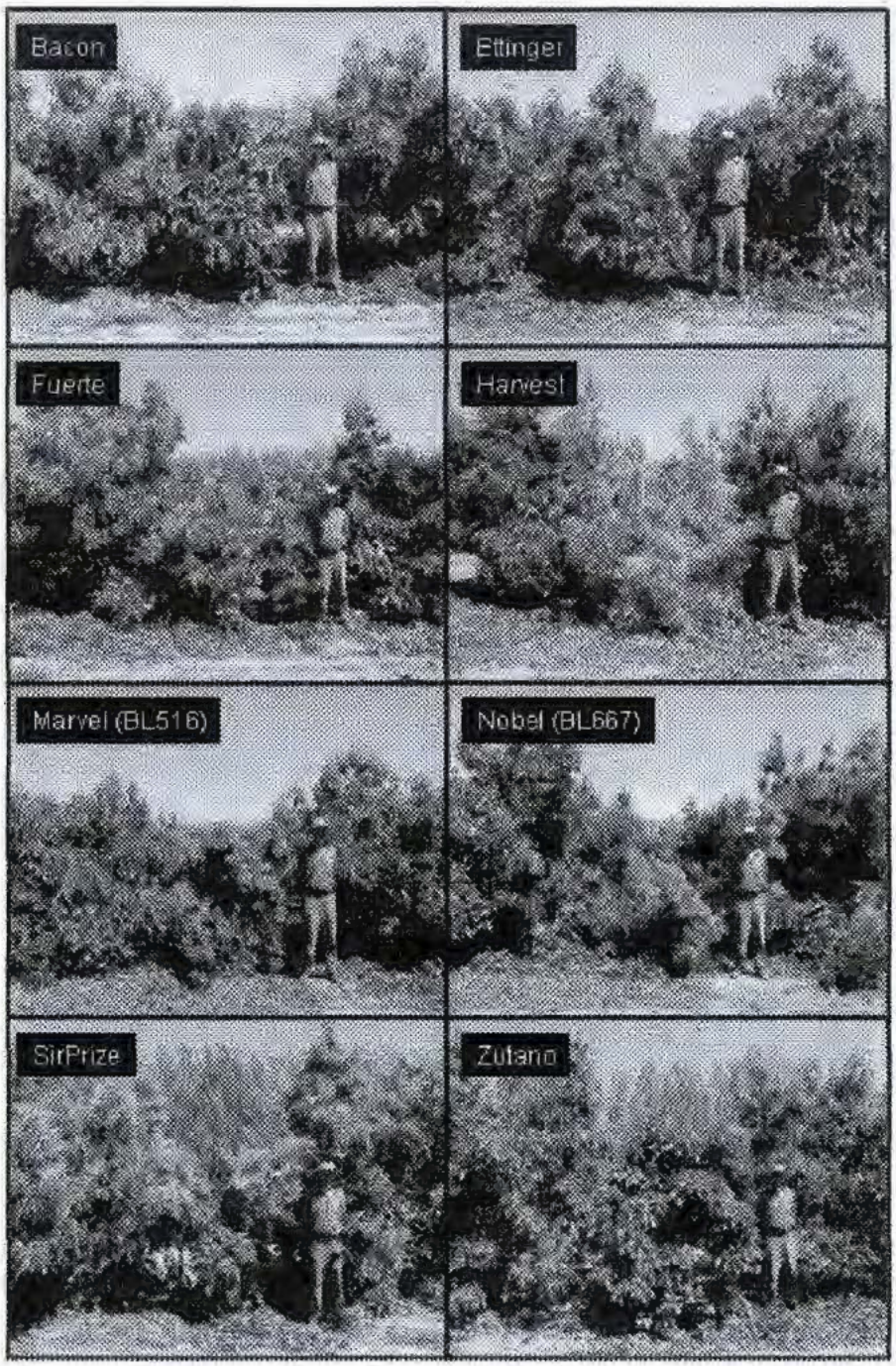


Figure 10. Relative size of pollinizer varieties to 'Hass' trees at the DeBusschere Pollinizer Plot in Oxnard, CA. Photos taken in April 2004. Trees of all pollinizers, with the exception of 'Marvel' and 'Nobel' were topped in Fall 2003.

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Enhancement of Avocado Productivity. Plant Improvement: Selection and Evaluation of Improved Varieties and Rootstocks

Continuing Project: Year 9 of 20

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Benefit to the Industry

This project will help to maintain and enhance the California avocado industry by introducing consistently heavier producing, high-quality avocado varieties, better pollinizer varieties, and improved rootstock hybrids. Increasing the genetic diversity of varieties will decrease the risk of major pest and disease invasions on a susceptible monoculture.

Objectives

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round. This includes determining the different cultural needs of each cultivar. Index trees for distribution for sunblotch viroid with assistance of Drs. Allan Dodds, and Deb Mathews.
B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.
C. To collaborate with Dr. Douhan and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.
D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Dr. Ben Faber and others as requested.
E. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.
F. To insure the timely and effective dissemination of information developed from this research program.

Summary

A. To produce new avocado varieties, superior to 'Hass' in consistent productivity and postharvest fruit quality and marketability, with fruit of optimum maturity and size year-round.

There are 2 components of this objective. The first is the continued monitoring of varieties from the Dr. B. Bergh/Gray Martin selection program. The second component is the new phase of scion selection. Activities for both components are summarized below.

Component 1. Continued monitoring of Bergh/Martin selections

Various field trials have been established to monitor the performance of a number of the Bergh/Martin selections. Several of the sites are now at the end of their allotted time period and after the final data are collected, those sites will be terminated. The following is a list of the cooperator trials we are maintaining and those sites to be terminated. In 2002 we installed data loggers to monitor air and soil temperature and relative humidity at all sites. We plan to use this data to help us assess the selection's response to low/high temperature when these events occur.

There are also additional plantings of the Bergh/Martin selections scattered throughout southern California. We periodically visit these sites to evaluate trees and discuss tree performance with the cooperators.

Topworked trials at Non-UC Sites

Santa Paula (Ventura County); topworked in 1998; 'GEM', 'Harvest', 'Sir Prize', 'RT5176', 'OA184', 'Marvel', 'Hass'; 10 replicates. Ending 2005.

De Luz (San Diego County); topworked in 1998; 'Lamb Hass', 'Sir Prize', 'GEM', 'Marvel', '5-552', 'Nobel', 'Hass', 'Harvest'; 10 replicates. Ending 2005.

De Luz (San Diego County); topworked in 1998; approximately 80 'GEM' trees divided roughly into 3 groups at the cooperator site. Ending 2005.

San Luis Obispo (San Luis Obispo County); topworked in 1998 (Trees suffered from freeze in 12/98 necessitating re-grafting of some selections in 1999; 'RT5176', 'Hass', 'Sir Prize', 'GEM', 'Harvest', 'OA184'; 9 replicates. Ending 2005.

Clonal trials at Non-UC Sites

Oxnard (Ventura County); planted in 1996; 'Lamb Hass', 'Sir Prize', 'GEM', 'OA184', 'Marvel', 'Nobel', 'Hass', 'Harvest'. (This trial was flooded in 1997 and many trees died due to this, however we are now working with the current owners to collect data from the trees which survived after the winter of 1997)

HTH Ranch (Ventura County); 'Lamb Hass', 'Marvel', 'GEM' and 'Hass' A non-replicated trial used for dry weights and fruit evaluation only.

Topworked trees at UC, Riverside Campus ongoing; Replacement trees in Field 10.

Topworked trees at UC, South Coast Research and Extension Center (SCREC); Field 4 at the Center has topworked trees (variable number of replicates) from which we collect data. These trees were topworked onto seedling rootstock trees in 1994 – 1996.

San Joaquin Valley Variety Trial - 1999 at two sites (Porterville, Lindcove) with clonal trees (Thomas rootstock); 'Sir Prize' 'Lamb Hass', 'Harvest', 'GEM', 'Nobel', 'Marvel', 'Pinkerton', 'Fuerte', and 'Zutano'; 20 replicates per scion variety at each site. We had trouble with tree establishment for certain varieties, therefore surviving tree numbers varies with site and variety. In spring 2005, several trees at the Lindcove site collapsed. Subsequent testing revealed that collapse and subsequent tree death was due to *Phytophthora cinnamomi*. We have also had problems with certain varieties dropping fruit prior to harvest and this continued to be a problem in 2004-2005.; however we were able to collect a second season's

data on dry matter. We plan to collect a third and final year of dry matter data this upcoming year.

Yield data from Bergh/Martin selections. We have collected yield data for the seventh year from Field 4 at UC-SCREC (UC South Coast Research and Extension Center). Data collection for 2005 shows that for most varieties, this was an 'off' year (Figure 1). The '(variety as indicated by data) at this point has the largest cumulative yield over the seven year period. Comparing the coefficient of variation shows that there is tendency toward less extreme alternate bearing in 'GEM' (Figure 2; this is calculated by dividing the standard deviation by the mean and gives one an idea of the relative variation of the data for a particular variety).

We have not yet completed the fifth year of yield data from the Santa Paula site in Ventura County, the Righetti site near San Luis Obispo, nor the De Luz site in San Diego County. There is no yield information from the Oxnard site as the grove was inadvertently harvested before the data could be collected.

Fruit characteristics of Bergh/Martin selections. As an on-going process we are collecting fruit samples from all sites approximately every 4 to 5 weeks from winter through late fall. These fruit are evaluated using standard protocols for such characteristics as fruit shape, peel texture, peel color, flesh color, the percent seed, flesh and skin and skin thickness.

Seasonal dry matter content of Bergh/Martin selections. The trends in dry weight accumulation were similar to the trends observed in previous years. The general pattern for dry weight accumulations for each variety in 2005 is consistent with the 2000 – 2004 data presented previously. A comparison between dry weight accumulations between six maturity seasons for the 'GEM' variety is presented in Figure 3. This data is from the UC-SCREC site.

Bloom evaluation of Bergh/Martin selections. The bloom of spring 2005 was evaluated on the trees in the unreleased variety block at UC-SCREC. This is the fourth year of this type of data collection. Bloom was rated for intensity, and an estimate of the number of open flowers was made for each tree. This was done weekly throughout the bloom season. Figure 4 illustrates the relative timing of each variety over the 4 year period.

Release of Bergh/Martio selections. The UC Office of Technology Transfer obtained patents for two of the Bergh/Martin selections, 'GEM' (U.S. Plant Patent No. 14,239) and 'Harvest' (U.S. Plant Patent No. 14,238) effective October 14, 2003. We believe that 'GEM' has commercial potential for the California industry and wish to make this selection more widely available to growers. The 'Harvest', on the other hand, had been given by G. Martin to researchers in Spain, Israel and South Africa where there is interest in the variety from a commercial perspective. The UC Office of Technology Transfer is currently working on patents in various foreign countries that are interested in this material. Growers interested in these varieties can either contact M. L. Arpaia, D. Stottlemyer or Dr. William Tucker at the UC Office of Technology Transfer for more information.

Component 2. New Material for the Breeding Program

We are taking 2 approaches towards generating new material for the California industry. These approaches are the outcome of discussions with B. O. Bergh, U. Lavi (Avocado breeder, Volcani Institute, Israel) and A. W. Whiley (Australia). The first approach is to plant out seedlings from interesting maternal sources; this is done without any effort to control paternity. This approach

was suggested by U. Lavi. In spring 2000, we planted the first 217 seedlings from mixed maternal sources to provide material for the "next generation" of avocado selections using this approach. An additional 237 seedlings were planted out in 2002, 186 seedlings in 2003 and 244 seedlings in 2004. So far, 645 seedlings have been planted out in 2005 with another 300+ seedlings to be planted this fall. We anticipate an additional 350 seedlings will be planted out in spring 2005. Table 1 shows the maternal parents of the current seedling population planted at UC-SCREC.

Table 1. Open pollinated seedlings from varying maternal sources planted at the UC South Coast Research and Extension Center from 2000 to 2005.

Year Planted	Maternal Source												Total Planted
	5-552	Marvel	Nobel	GEM	Gwen	Lamb Hass	Bacon	SirPrize	Thille x GEM	GEM x Thille	Marvel x GEM	GEM x Marvel	
2000	32	90	37	39	14	5							217
2002		75	51	91		20							237
2003		50	25	41	55				15				186
2004	30	61	48	42	55							6	244
2005		60	73	99	23	60	3	17		179	12	113	645
Totals	**62**	**336**	**234**	**312**	**147**	**85**	**3**	**17**	**15**	**179**	**12**	**119**	**1531**

Note: The "Total Planted" per year may not always add up due to some trees with lost labels where the parents are "unknown."

Of the 217 trees planted in 2000, 86 have produced fruit and have been evaluated. Seven seedlings have been selected for further evaluation and have been topworked onto Duke7 rootstock at SCREC, and are also being propagated onto clonal rootstock material for further field evaluations. After the grafting was done, one additional seedling was flagged for further evaluation bringing the total number of interesting selections to 8. Two of these selections were selected for their sympodial growth habit; the other 6 were selected mainly on the basis of flavor.

Table 2. Isolation blocks established in 1999 – 2001.

Parents	Year established	Location
GEM x Marvel	1999 (topwork)	UC, Riverside
GEM x Thille	1999 (topwork)	UC, Riverside
Gwen x Gwen	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x GEM	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Nobel	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Thille	2001 (clonal tree)	Nakamura, Ventura Co.
Lamb x Reed	2001 (clonal tree)	Nakamura, Ventura Co.
Stewart x Reed	2001 (clonal tree)	Nakamura, Ventura Co.

In the second approach we have taken the more traditional approach of Dr. Bergh by establishing isolation plots in various locations. Table 2 lists the location, year established and selections in each isolation block. The potential parents were selected under consultation with Dr. Bergh. A

total of 305 seed were collected for germination from the isolation blocks and 864 from open-pollinated sources for a total of 1169 seeds to be germinated for the 2005-2006 season.

In June 2005, we asked Dr. Uri Lavi (fruit breeder including avocado from the Volcani Institute in Israel) and Dr. Jose Chaparro (citrus and stone fruit breeder from the University of Florida, Gainesville) to review our progress over the last 6 years. They made many useful suggestions for improvement of the program and helped us in developing strategies for the future. Their comments are available upon request to M. L. Arpaia.

Sunblotch Viroid indexing. One hundred twenty seven trees at the UC-SCREC were tested for the sunblotch viroid between October 1, 2004 and September 30, 2005. Of these trees, 11 tested positive for the Sunblotch Viroid and have been removed. All of the positive trees were in field 46 and represent our continuing effort to eliminate sunblotch from that field. In 2004, one positive tree was found in field 44. This was the first positive tree in that field and it was removed immediately. All surrounding trees were tested but found to be negative. Adjacent trees were tested again this year with no sign of the sunblotch viroid.

B. To collaborate with other researchers worldwide in evaluating and exchanging promising plant material.

Introduction of new germplasm. We have continued to plant out new varieties as they come out of quarantine. In August 2005, two Andes selections and Puebla were officially released from quarantine. The Andes selections are believed to be seedlings or bud sports of Hass and were selected in Chile by the Andes Nursery Association. This material came to California under a test agreement. We will plant these trees at UC South Coast REC and evaluating their potential for California. The Puebla, which is a heritage variety originating in California, was brought back to California in 2002 from the germplasm collection of the Catholic University of Valparaiso, Chile. With the aid of Dr. Ben Ya'acov, who confirmed the identity of the variety in Chile, we elected to bring this variety back to California for placement in the variety collection. Other Puebla trees in California are of uncertain identity and this introduction will aid us in identifying Puebla trees growing throughout southern California. Finally, two additional varieties of interest were brought in from Chile from the Andes Nursery Association (A.N.A.) in September 2004. This material is currently in quarantine. We plan to receive additional material from Chile in collaboration with Monica Castro and Claudia Fassio (Catholic University of Valparaiso, Chile) in spring 2006. This material will include the 'Isabel' a promising new selection which they believe is cold-hardy.

C. To collaborate with Dr. Douhan (Dept. of Plant Pathology, UCR), and Dr. Crowley on rootstock selection and evaluation for both root rot resistance and salinity tolerance.

We planted a new clonal rootstock trial at UC SCREC with Dr. Menge in spring 1999 and collected a fourth year of yield data from this plot in 2005. The 'Hass' and the 'Lamb Hass' are included in this trial on selected clonal rootstocks ('Hass' on Day, Duke7, Dusa, Evstro, G755A, Parida, PP4, Spencer, Thomas, Toro Canyon; 20 replicates 'Lamb Hass' on Day, Duke 7, Evstro, Thomas, Toro Canyon; 20 replicates).

We continue to collaborate with Dr. Crowley in his salinity research whenever possible and have assisted in the evaluation of a salinity/*Phytophthora* rootstock trial established in Santa Barbara using rootstocks from Dr. Menge's program, South Africa and Israel.

D. Evaluate the potential of new and established cultivars (B flower types) for use as pollinizers in collaboration with Dr. Ben Faber and others as requested.

In conjunction with Ben Faber we established a pollinizer site in Ventura County (Oxnard) in spring 1999. We are using funding from BARD (a collaborative effort with Drs. Arnon Dag and Sharoni Shafir (Israel) and Dr. Tom Davenport (University of Florida)) to collect floral data as well at this site as well as 2 other sites. Below is a preliminary discussion of our 4 years of yield data. We hope to collect 2 additional years of yield data before completing this portion of the project.

Fruit Number and Proximity to Pollinizers. There is an overall statistical difference in cumulative fruit numbers harvested from the experimental site (Figure 5, Table 3). The highest cumulative fruit numbers were obtained from the 'Hass' trees in the pollinizer rows. Trees one row away (7.6 m, 25 ft) had the second highest yields. There was no significant difference detected between the second or third row (15.2 and 22.9 m (50 and 75 ft) away, respectively). The significance in fruit numbers harvested is related to the high yield obtained in 2004. In this year, significant differences due to distance from pollinizer were also detected (Table 3). These results differ slightly from Bergh et al (1966). In that study the authors report it was only when 'Fuerte' trees were adjacent to 'Topa Topa' did one see a significant increase in yield. The results from this study suggest that proximity to the pollinizer variety can influence yield. A difference between the two studies could be related to the presence of honeybees in the present study and differences in environmental conditions during bloom. The present study site tends to be cooler during flowering than the site used by Bergh et al (1966).

Table 3. Fruit count per tree as a function of distance from a pollinizer. Mean separation using Student-Newman-Keuls Test, $P<0.05$ (n.s. = not significant).

Distance from Pollinizer (meters)	Year				Cumulative Number
	2002	2003	2004	2005	
0.0	179 n.s.	30 a	342 a	45 n.s.	595 a
7.6	180	22 ab	248 b	52	503 b
15.2	153	16 b	213 c	55	438 c
22.9	151	21 ab	197 c	46	415 c

Fruit Number and Pollinizer Variety. Figure 6 and Table 4 present the cumulative fruit count results by pollinizer variety. Pollinizer variety had a significant impact on the fruit number within row, or when the pollinizer was adjacent to 'Hass', however there were no differences due to pollinizer variety as distance from the pollinizers increased. Even though there was no difference between pollinizer varieties 1 or 2 rows from the pollinizer trees, if all data across rows is combined, a statistical difference is detected. In this study, proximity to 'Fuerte' resulted

in the overall highest 'Hass' yields followed closely by 'Zutano' and 'SirPrize'. Kobayashi et al (2000) using genetic markers also reported enhanced 'Hass' yield when 'Fuerte' was in close proximity as compared to other B-Flower type avocado. They also report a proximity influence on fruit yield, higher yields closer to pollinizers.

Table 4. 'Hass' fruit count per tree as influenced by pollinizer variety and distance from the pollinizer. Mean separation using Student-Newman-Keuls Test, P<0.05 (n.s. = not significant).

Closest Pollinizer	Distance from Pollinizer (meters)			
	0	7.6	15.2	All Rows
Bacon	547 ab	432 n.s.	373 n.s.	451 bc
Ettinger	619 ab	544	429	531 ab
Fuerte	675 a	492	523	563 a
Harvest	480 b	444	304	409 c
Marvel	554 ab	495	423	492 abc
Nobel	540 ab	574	456	523 ab
SirPrize	668 a	514	473	552 a
Zutano	616 ab	553	511	560 a

Fruit Characteristics as influenced by Pollinizer Variety. Average fruit weight was calculated by dividing the total number of fruit harvest by the total weight per tree. In 2002 and 2004 (high production years) there were significant differences in average fruit weight related to pollinizer variety. Not surprisingly, in the treatments which had higher fruit numbers, average fruit weight was smaller. Average fruit weight across all pollinizers and distances ranged from 205 to 228 g in 2002, 222-263 g in 2003, 207 to 230 g in 2004 and 282 – 296 g in 2005.

Table 5 presents the results of the dry weight measurements and compares the 2005 data with the data collected in a similar manner to 2004. Note that in both years 'Hass' fruit from the 'Nobel' pollinizer rows had the highest dry matter whereas the 'Hass' from the 'Marvel' pollinizer rows had the lowest. A difference between the 2 years of sampling is seen with 'Hass' fruit from the 'Zutano' pollinizer rows. In 2004 this sample had the second highest dry matter whereas in 2005 the dry weight is the second lowest. The 'Hass' trees in this experiment bloom for an extended period and these apparent differences in dry weight may be related to the timing of fruit set and synchrony with the pollinizer in terms of flowering and fruit set.

Table 6 presents data collected both years for the fruit length/width ratio and the seed length/width ratio as well as the seed percentage for 2005. Note that in 2005 the 'Hass' fruit from the 'Fuerte' pollinizer row had a slightly more elongated fruit as compared to the 'Hass' fruit coming from the 'SirPrize' pollinizer row. There were no significant differences detected in 2004. The seed length/width varied between both years, however in both years 'Hass' fruit from the 'Fuerte' and 'Harvest' pollinizer rows had slightly more elongated seed.

In 2005 we were able to also ascertain the seed percentage of the total fruit weight. In this case, 'Hass' fruit from the 'Fuerte' pollinizer row had the smallest seeds and fruit from either the 'Bacon' or 'SirPrize' pollinizer rows had the largest percent seed. These data suggest that out-

crossing may be occurring and that pollen parent is influencing fruit shape and seed size. The occurrence of metaxenia has been previously reported for avocado (Degani et al, 1990; Gafni, 1984). A weakness of this study has been our inability to test for parentage of the 'Hass' fruit. In a companion project, Dr. T. L. Davenport is collecting paternity data using microsatellite markers. This data should help us to interpret these results.

Table 5. Average 'Hass' dry weight for 2004 and 2005. Fruit harvested both years in April from pollinizer rows. Mean separation by LSD, P<0.05.

Pollinizer	2004	2005
Bacon	25.34 ab	27.42 abc
Ettinger	25.30 abc	26.44 bc
Fuerte	24.80 bc	27.30 abc
Harvest	24.95 bc	28.06 ab
Marvel	24.45 c	25.88 c
Nobel	26.20 a	28.72 a
SirPrize	24.94 bc	26.90 abc
Zutano	26.08 a	25.92 c

Table 6. Average 'Hass' fruit length/width ratio, seed length/width ratio and percentage seed per fruit. Fruit harvested from pollinizer rows in April 2004 or 2005. Mean separation by LSD, P<0.05 (n.s. – not significant)

Closest Pollinizer	Fruit length/width ratio 2004	Fruit length/width ratio 2005	Seed length/width ratio 2004	Seed length/width ratio 2005	2005 Seed % of fruit weight
Bacon	1.29 n.s.	1.45 ab	1.13 bc	1.13 b	12.47 a
Marvel	1.40	1.47 ab	1.14 bc	1.16 ab	12.01 ab
Nobel	1.43	1.50 ab	1.15 abc	1.17 ab	12.09 ab
Ettinger	1.37	1.47 ab	1.11 c	1.15 ab	10.55 bc
Fuerte	1.39	1.52 a	1.19 a	1.20 a	9.91 c
Harvest	1.45	1.49 ab	1.18 ab	1.19 a	11.99 ab
SirPrize	1.37	1.42 b	1.15 bc	1.10 b	12.39 a
Zutano	1.37	1.46 ab	1.11 c	1.13 b	11.70 ab

The preliminary results from this study confirm the observations of Bergh et al (1966) that the use of pollinizers can enhance yield of avocado. These data also suggest that the choice of pollinizer variety may also be important.

E. To maintain and improve the CAS variety block and the *Persea* germplasm block located at the UC South Coast Research and Extension Center.

An accurate plot map has been generated for the CAS Variety Block at UC-SCREC. Any changes to the planting are being recorded in the master database maintained by David Stottlemyer. The UC-SCREC avocado volunteers have been instrumental in maintaining this

block. The volunteers graft several new and/or historical varieties on an on-going basis. Fields 44 and 46 have been maintained and kept in order through regular pruning and constant observation by both the lab personnel and the volunteer staff. In addition, the sprinkler lines in field 46 have been replaced and updated in coordination w/the SCREC personnel.

F. To insure the timely and effective dissemination of information developed from this research program.

The current avocado web site at: www.ucavo.ucr.edu has been on-line since June 1998. The site is periodically revised and updated with new information and photographs of different varieties. Leaf photographs showing both flush and mature leaves are currently being added to the web site with plans to add tree photographs as well. Questions sent via e-mail or forwarded from the California Avocado Commission are answered on an ongoing basis.

Figure 1. Variety trial yield data (average fruit count per tree) collected from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 – 2005. Trees were topworked onto seedling rootstock in 1994 – 1996.

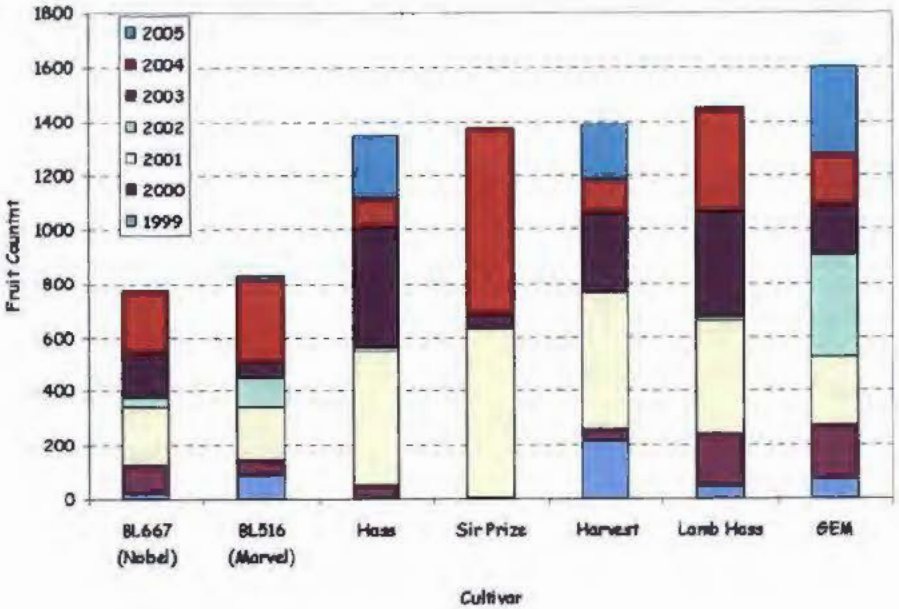


Figure 2. The Coefficient of Variation (%) in yield (fruit number) for each variety from Field 4 at the UC South Coast Research and Extension Center in Irvine, CA from 1999 – 2005. Yield data for 'Harvest' incomplete as of September 2005.

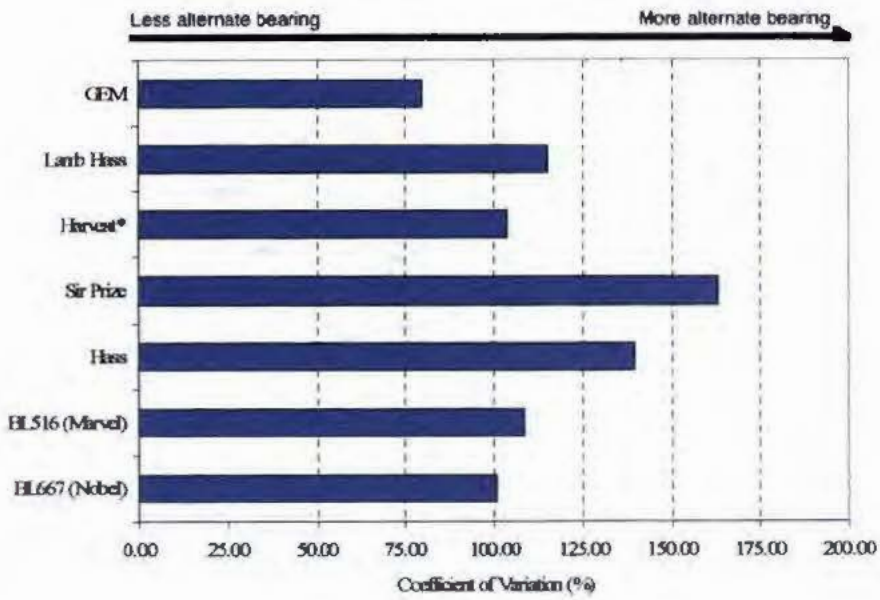


Figure 3. Seasonal trends (2000 – 2005) in dry weight accumulation for GEM from January through October at the UC South Coast Research and Extension Center, Irvine, CA.

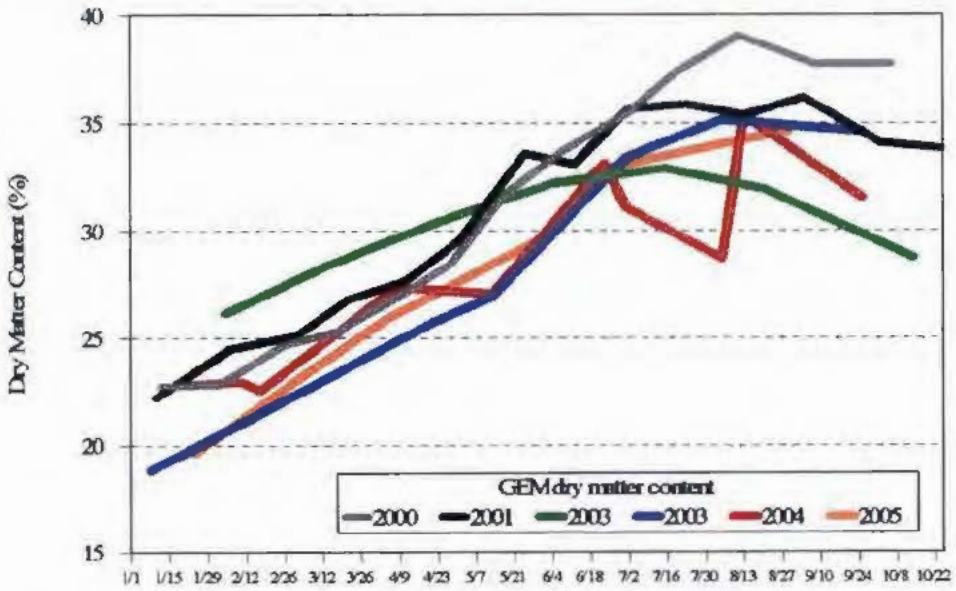


Figure 4. Comparison of average bloom dates for two years for all varieties from February through May at the UC South Coast Research and Extension Center, Irvine, CA.

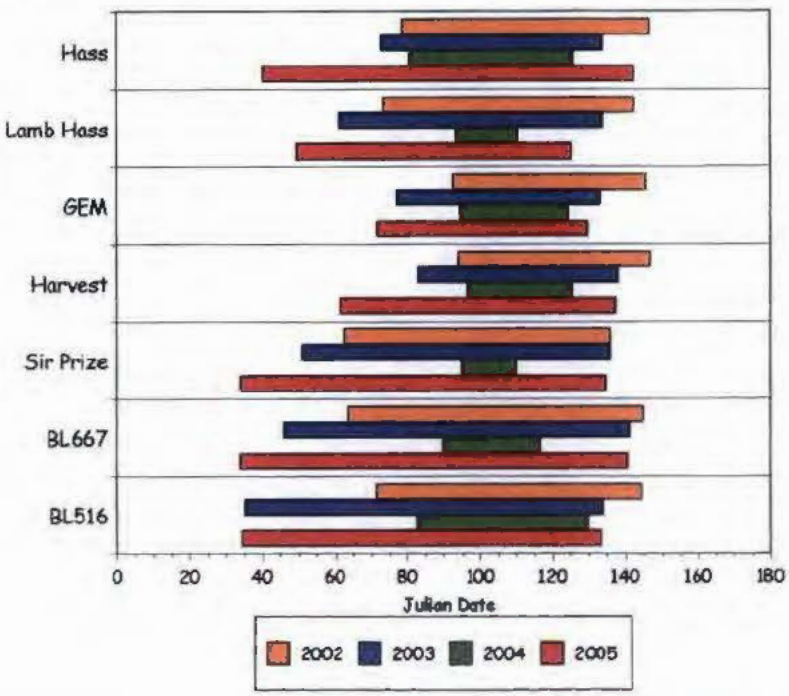


Figure 5. Distance from pollinizers influences the cumulative yield of 'Hass'. Data collected from 2002-2005 From the DeBusschere pollinizer trial near Oxnard.

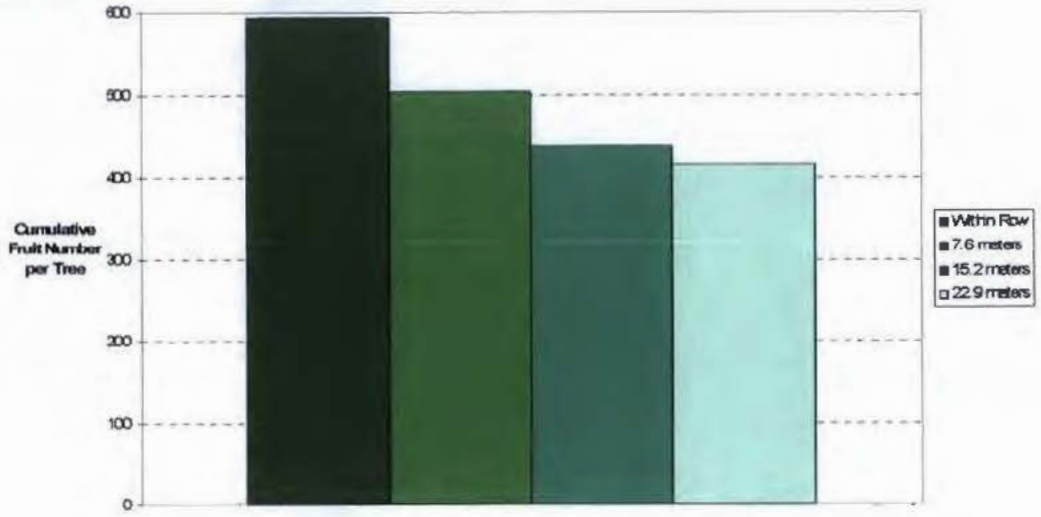
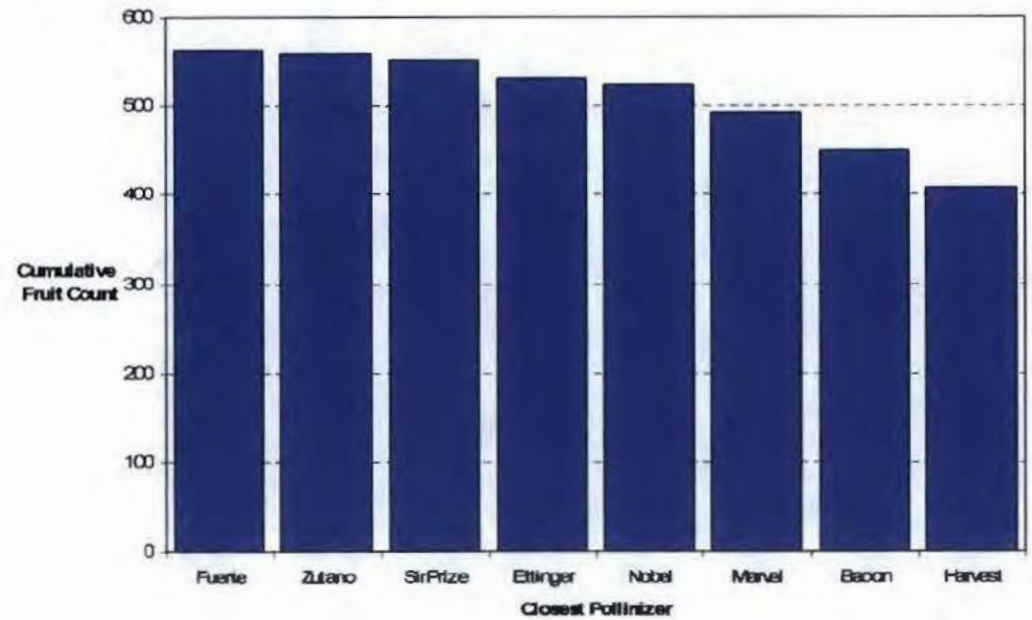


Figure 6. Cumulative 'Hass' fruit number (2002-2005) as a function of pollinizer variety. Data pooled across rows 0 – 2 (0 – 15.2 m). Data collected from the DeBusschere pollinizer trial near Oxnard, CA.



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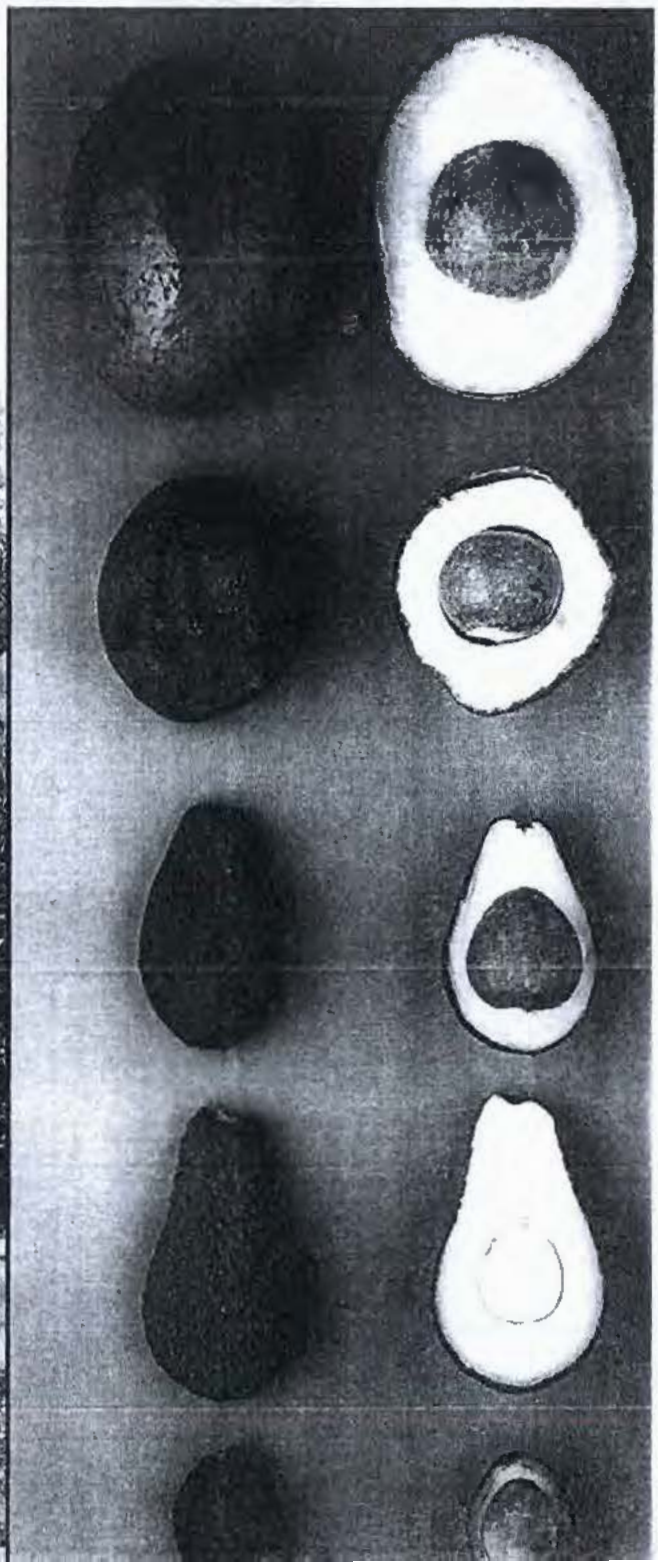

Genealogical Relationships Among Cultivated Avocado as Revealed Through RFLP Analyses

J. Davis, D. Henderson, M. Kobayashi, Matthew T. Clegg, and Michael T. Clegg

Anonymous DNA fragments from the genome of cultivated avocado (*Persea americana* Mill.) were cloned into a plasmid vector and used to screen a total of 36 cultivars. There is a high level of polymorphism among cultivars allowing all cultivars to be assigned a unique genotype based on 14 genetic loci. A cluster analysis of genetic similarities among cultivars revealed three major clusters that correspond to the three major racial groupings of cultivated avocado. Additional clusters appear to reflect cultivars derived through interracial hybridization.

The cultivated avocado (*Persea americana*) is comprised of three botanical races: *P. americana* var. *americana* (West Indian race), *P. americana* var. *drymifolia* (Schect. & Cham) Blake (Mexican race), and *P. americana* var. *guatemalensis* Wms (Guatemalan race) (Bergh et al. 1973; Popenoe 1941). The origins and domestication of the various races is obscure, and in fact the appellation West Indian is a misnomer because avocado was introduced to the West Indies from Central America by the early Spanish settlers (Popenoe W, Zentmeyer G, and Scheiber G, unpublished manuscript). Many of the named cultivars of avocado are selections from open pollinated progenies and the pollen parent is often unknown or a matter of speculation. In some cases, the open pollinated selections that yielded successful cultivars are believed to have arisen from interracial crosses. Thus, for example, the cultivar Hass, the predominant commercial cultivar in California was first obtained as an open pollinated seedling of unknown parentage (Seelye 1994). Current speculation among avocado breeders (Bergh R and Martin G, personal communication) is that cv. Hass is derived from a Guatemalan × Mexican hybrid that was then backcrossed to a Guatemalan pollen parent. It is now possible to test this and similar speculations regarding genealogical relationships among avocado cultivars using molecular markers.

The traditional bases for inferring parentage have been morphological or were based on the proximity of a potential pollen source. It is very difficult to produce fruit where the pollen parent is known with certainty because the mature avocado tree produces in excess of 1,000,000 flowers but fewer than 1,000 are destined to yield mature fruit. Most fruit drop prior to maturity, leaving a small residual of successful pollinations.

Genetic markers provide a way to establish both the parentage of individual trees, and through retrospective analyses, to infer the likely genealogical histories of particular cultivars (Mhameed et al. 1997). In an initial attempt to explore avocado relationships using genetic markers, we investigated a number of cultivars and members of other closely related *Persea* species (*P. floccosa* Mez, *P. nubigena* L. Wms, *P. steyermarkii* C. K. Allen, and *P. shiedeana* Nees) using rDNA, nuclear gene, and chloroplast DNA probes (Furnier et al. 1990). These data revealed that all three races are distinguished by unique mutations and the data provided support for the hypothesis that the Guatemalan race originated through hybridization between *P. nubigena* and *P. steyermarkii*. Furnier et al. (1990) concluded that *P. americana* could be expanded to include *P. floccosa*, *P. nubigena*, and *P. steyermarkii* as one large species which concurs with the earlier recommendations of Bergh et al. (1973) based on the analysis of terpene patterns.

In this article we expand our analyses of genealogical relationships within cultivated avocado through the use of anonymous RFLP markers. Knowledge of genealogical relationships is of more than academic interest; accurate information on genetic relationships is fundamental to the design of

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Table 1. The genotype for each cultivar based on the *Eco*RI (denoted as RI), *Eco*RV (RV), and *Hin*dIII (III) digest patterns

	26RI	50RI	53RI	64ARI	64BRI	73RI	74RI	80RI	95RI	110RI	117RI	121RI	364RI	366RI	26RV	50RV	53RV	64ARV	64BRV	73RV
Arue	22	11	22	22	44	77	11	22	13	11	22	22	11	33	33	11	34	11	22	33
Biondo	22	11	22	22	44	77	11	22	11	11	22	22	11	33	33	11	34	11	22	33
Collinred	22	11	22	22	22	46	11	22	11	13	22	22	11	33	23	11	—	11	22	23
Pollock	22	11	22	22	22	77	11	22	11	11	22	22	11	33	33	11	34	11	22	33
Cellons	22	11	22	22	23	66	11	22	11	11	22	22	11	33	33	—	44	11	12	23
Anaheim	22	11	22	22	23	44	11	23	11	33	23	22	12	34	33	22	33	11	22	22
Esther	22	11	22	22	33	44	11	23	13	33	23	22	11	33	33	12	33	11	22	22
Nabal	22	11	22	22	33	44	11	22	13	33	—	22	11	34	33	22	34	11	22	22
Reed	22	11	22	22	23	34	11	22	15	33	22	22	12	34	12	22	34	11	22	23
Daily 11	22	11	22	22	22	46	11	22	14	33	23	22	11	33	12	22	34	11	22	33
Linda	22	11	22	22	22	44	11	22	—	33	23	22	11	33	11	22	33	11	22	23
Nimlioh	22	11	22	22	22	44	11	22	14	33	33	22	11	33	11	22	33	11	22	23
Fuerte	12	11	22	22	22	44	11	22	13	33	12	22	11	33	23	12	23	11	22	33
Noga	12	11	22	22	22	44	13	22	33	33	12	22	11	33	23	22	33	11	22	23
HX48	22	11	22	22	23	24	11	22	33	33	22	22	11	33	33	22	34	11	22	34
Gwen	22	11	22	22	22	44	11	22	13	33	33	22	11	33	33	22	33	11	22	33
Hass	22	11	22	22	22	44	11	22	13	33	23	22	11	33	33	22	34	11	22	23
H670	22	11	22	22	23	44	11	22	13	33	23	22	11	33	33	22	34	11	22	23
H287	22	11	22	22	23	24	11	22	11	33	33	22	11	33	33	12	34	11	22	23
Bacon	23	11	12	22	23	25	13	23	13	33	33	22	11	33	23	22	23	11	22	14
Zutano	23	11	12	22	33	48	11	23	13	33	33	22	11	33	12	12	23	11	23	34
Lyon	22	11	22	22	23	24	11	23	11	33	33	22	11	33	13	12	33	11	22	34
Thille	22	11	22	22	23	44	11	23	13	33	23	22	11	33	33	12	33	11	22	23
Pinkerton	23	11	22	22	22	44	11	23	11	23	23	22	11	23	33	22	23	11	22	23
Whitsell	12	11	22	22	23	24	11	23	13	33	33	22	11	23	33	22	34	11	22	34
Rincon	13	11	22	22	23	34	11	23	13	33	13	22	11	23	23	12	22	11	22	33
Teague	33	11	22	22	22	44	11	22	33	33	13	22	11	33	22	12	23	11	22	33
Duke 7	13	11	13	22	22	45	11	22	33	33	33	22	11	23	22	12	23	11	22	13
Thomas	33	11	12	22	24	58	11	23	33	33	33	22	11	23	22	12	22	11	23	13
Ignacio	13	11	12	22	24	44	11	22	33	23	33	22	11	23	22	11	22	11	23	22
Ganter	33	11	12	22	23	45	11	22	33	33	13	22	11	33	22	12	23	11	22	13
Yama	33	11	22	22	24	48	11	22	33	33	13	22	11	23	22	11	34	11	23	34
Mexicola	13	11	13	22	24	48	11	22	—	33	13	22	11	23	22	11	34	11	22	33
Duke 6	33	11	33	22	23	44	11	22	33	23	33	22	11	23	22	11	23	11	22	33
Topa Topa	33	11	22	22	23	88	11	22	33	22	33	22	11	33	22	12	44	11	22	33
Khan	33	11	12	23	22	48	11	22	33	23	33	22	11	33	22	12	24	11	22	33
P. schiedeana	33	11	33	11	22	15	11	12	56	33	24	11	—	—	23	22	—	11	22	33
G755C	22	11	22	22	23	13	11	22	45	33	24	12	11	33	13	22	13	11	22	22
G755A	22	11	22	22	12	14	12	22	25	33	24	12	11	33	33	22	13	11	12	23

Each column contains the data for a specific RFLP probe.

efficient plant improvement strategies and such knowledge is essential for effective germplasm conservation. The data from this study reveal a high degree of polymorphism for anonymous nuclear DNA fragments, confirming the impression that cultivated avocado possesses a rich and diverse gene pool. Cluster analysis among a set of 36 cultivars, based on a measure of genetic similarity, reveal groupings that conform with racial designations and that appear to distinguish cultivars derived from interracial hybrids. Thus RFLP analyses appear to provide a powerful basis for inferring genetic relationships among cultivated avocados.

Materials and Methods

Plant Materials

Collections of avocado germplasm are maintained at the University of California South Coast Field Station and on the campus of the University of California, Riverside. Because the method of avocado propagation is clonal, based on bud grafting, each cultivar represents a single genotype. Table 1 provides a list of the cultivars surveyed and their source. In addition, *P. schiedeana* was included as an outgroup in the cluster analyses.

DNA Preparation

DNA was extracted from 10 g of newly emerged leaf tissue using a modification of the protocol of Rawson et al. (1982). The leaf tissue was homogenized in 50 ml of grinding buffer (100 mM Tris, 25 mM EDTA, 0.35 M sucrose, 50 mM KCl, 5% polyvinylpyrrolidone, 10 mM diethyldithiocarbamic acid, 0.2% mercaptoethanol) using a small Waring blender. The homogenate was filtered through cheesecloth and centrifuged at 12,000 G for 20 min (4°C). The pellet was resuspended in 6 ml of lysing buffer (100 mM EDTA, 50 mM Tris-HCl pH 8.0, 2.5% Triton X-100, 2% sarkosyl, 50 μg/ml Proteinase K) and incubated at 37°C in a shaking incubator for 2 h. The lysate was centrifuged at 15,000 G for 10 min (4°C) and the supernatant was precipitated with 2/3 volume isopropanol at −20°C for 30 min. The precipitate was pelleted at 20,000 G for 15 min. (4°C). The pellet was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.0) and the DNA purified through a single CsCl gradient (ρf = 1.390–1.395) as described by Rawson et al. (1982). The DNA sample was precipitated, washed with 70% ethanol, dried under vacuum, and resuspended in TE buffer at a concentration of 1 μg/μl.

Cloning Anonymous DNA Fragments

Total DNA from cv. Hass was digested with the restriction enzyme *Pst*I and size fractionated on 1% agarose gels. (*Pst*I was used to select against chloroplast DNA because it cuts the chloroplast genome infrequently into relatively large fragments.) The fraction corresponding to approximately 400 bp in size was eluted following standard procedures. The size fractionated DNA was ligated into a pUC18 vector and transformed into *E. coli* strain JM101 following established procedures (Sambrook et al. 1989). Recombinant colonies were selected and minipreps of DNA were screened to verify insert size, and these were further analyzed via Southern transfers to exclude any chloroplast-derived in-

Table 1. Extended

74RV	80RV	95RV	110RV	117RV	121RV	364RV	366RV	26III	50III	53III	64AIII	64BIII	73III	74III	80III	95III	110III	117III	121III	364III	366III
22	11	11	11	11	11	23	11	22	55	22	22	33	33	22	22	22	33	11	11	22	33
22	11	12	11	11	11	23	11	22	55	22	22	33	33	22	22	22	33	11	11	22	33
22	11	—	11	11	11	—	11	22	55	22	22	33	33	22	22	22	33	11	11	22	23
22	11	—	11	11	11	33	11	22	55	22	22	33	33	22	22	11	33	11	11	22	23
22	11	—	11	11	11	33	11	22	55	22	12	33	33	22	22	12	33	11	11	22	33
22	11	22	11	11	34	33	11	22	55	22	22	13	33	22	22	22	33	11	11	22	33
22	11	12	11	11	11	33	11	22	25	22	22	13	33	22	22	22	33	11	11	22	33
22	11	12	11	11	13	33	11	22	55	22	22	33	33	22	22	22	33	11	11	22	33
22	12	11	11	11	13	33	11	22	55	22	22	13	33	22	22	22	33	11	11	22	33
22	11	—	11	11	44	33	11	22	55	22	22	11	33	22	22	22	25	11	11	22	33
22	11	22	11	11	14	35	11	22	55	22	22	11	33	22	22	22	33	11	11	22	33
22	11	22	11	11	44	33	11	22	55	22	22	11	33	22	22	22	33	11	11	22	33
22	11	11	11	11	13	33	11	12	25	23	22	33	33	22	22	22	33	11	11	22	14
22	11	11	11	11	33	33	11	12	55	22	22	33	33	22	22	22	33	11	11	22	13
22	11	11	11	11	13	33	11	22	55	22	22	33	33	22	22	22	33	11	11	22	14
22	11	11	11	11	13	13	11	22	55	22	22	11	33	22	22	22	33	11	11	22	34
22	11	11	11	11	13	33	11	22	55	22	22	13	33	22	22	22	33	11	11	22	34
22	11	11	11	11	13	33	11	22	55	22	22	13	33	22	22	22	33	11	11	22	34
22	11	11	11	11	33	33	11	22	25	22	22	11	33	22	22	22	30	11	11	22	14
22	11	11	11	11	11	13	11	12	55	12	12	12	14	22	22	22	23	11	11	22	33
22	11	11	11	11	14	13	11	12	25	22	12	12	33	22	22	22	33	11	11	22	13
22	11	22	11	11	14	13	11	22	25	22	22	12	33	22	22	22	33	11	11	22	13
22	11	11	11	11	14	13	11	22	25	22	22	13	33	22	22	22	33	11	11	22	13
22	11	12	11	11	14	13	11	22	55	23	22	33	33	22	22	22	33	11	11	22	33
22	11	12	11	11	34	33	11	22	25	22	22	11	33	22	22	—	33	11	11	22	13
22	11	12	11	11	34	13	11	12	25	33	22	22	33	22	22	22	33	11	11	22	13
22	11	11	11	11	13	13	11	11	25	23	22	22	33	22	22	22	33	11	11	22	12
22	11	11	11	11	11	11	11	11	35	23	12	33	13	22	22	12	23	11	11	22	33
22	11	11	11	11	11	11	11	11	25	23	11	33	13	22	22	12	33	11	11	22	34
22	11	11	11	11	11	33	11	11	22	22	12	22	22	22	22	22	33	11	11	22	33
22	11	11	11	11	11	33	11	11	25	22	12	23	13	24	22	22	23	11	11	22	33
22	11	11	11	11	11	33	11	11	22	22	12	33	33	24	22	22	23	11	11	22	33
22	11	22	11	11	11	33	11	11	22	22	12	33	33	24	22	22	23	11	11	22	33
22	11	11	11	11	11	33	11	11	33	33	22	23	33	22	22	22	33	11	11	22	33
22	11	12	11	11	11	44	11	11	25	22	22	33	33	44	22	22	33	11	11	22	33
22	12	11	11	11	11	14	11	11	45	12	22	23	33	44	22	22	33	11	11	22	33
22	—	—	11	11	33	—	—	33	22	33	22	44	33	11	22	11	33	11	12	—	—
22	13	11	11	11	12	33	11	23	25	22	22	14	13	12	22	12	33	11	12	12	33
22	12	—	11	11	23	33	11	23	24	22	22	34	33	33	22	11	33	11	12	12	33

serts. Clones with appropriate inserts were screened against Southern transfers of DNA from each of the three races of avocado to establish whether the resulting RFLPs were both single-copy and polymorphic. Finally, selected clones were used as probes to an F_2 progeny derived from self-fertilized Hass to verify nuclear inheritance.

Southern Transfers

Ten micrograms of total plant DNA was digested with the restriction enzymes *Eco*RI, *Eco*RV, and *Hin*dIII and electorphoresed on 0.8% agarose gels for 4 h at 50 V. The DNA within the gel was depurinated with 0.25 M HCl, denatured with 0.4 M NaOH, and transferred by capillary blot technique to Genescreen nylon membranes under alkaline conditions (as described by the manufacturer; DuPont, Boston). Probe DNA (0.2 μg) was labeled with ^{32}P by the method of Fienberg and Vogelstein (1983) and hybridized to the Southern transfers (Southern 1975).

Data Analyses

A genotype was assigned to each cultivar for each probe. Because each cultivar represents a single genotype there are no sampling considerations and the gene frequency for a given allele within a cultivar is either 0.0, 0.5, or 1.0. We calculated a similarity measure (S) between a pair of cultivars as the proportion of alleles shared in common between cultivars. Suppose for example, that cultivars A and B have genotypes a_1a_2 and a_3a_4, respectively, and suppose that $a_1 = a_3 \neq a_4$ and $a_2 \neq a_3 \neq a_4$, then two of the four genes are identical between A and B, and $S = 0.5$. As a second example, suppose the genotypes of A and B are a_1a_2 and a_3a_4, respectively, and suppose that $a_1 = a_3 = a_4$ and $a_2 \neq a_3,a_4$, then $S = 0.5$, because again two genes are identical between A and B. If $a_1,a_2 \neq a_3,a_4$ then $S = 0.0$, and if $a_1 = a_4$, $a_2 = a_3$, or $a_1 = a_3$ and $a_2 = a_4$, then $S = 1.0$. The average similarity is $\Sigma S/n$, where there are n loci. A UPGMA cluster analysis was then preformed on the matrix of average similarities (Sneath and Sokal 1973).

Results

Polymorphism Among Clones

More than 1000 putative clones were screened for inserts of the appropriate size and 20 clones with the appropriate insert size were tested against a panel of avocado DNAs that represented each of the three major races. Fifteen of the screened clones were "single copy" in that the banding patterns appeared to correspond to those expected from one or two genetic loci. Five of the clones produced complex banding patterns characteristic of repeated gene families.

An example of the banding pattern associated with one probe hybridized to a Southern transfer containing a wide spectrum of cultivars is shown in Figure 1. A subset of 13 probes that resolved a total of 14 genetic loci was used to screen a set of 36 cultivars and *P. shiedeana* (represented by the G755A and G755C entries). Every cultivar was uniquely identified in the sense that each cultivar was distinct from all others for at least one genetic locus (Table 1). The numbers of alleles per locus ranged from one (50RI) to seven (73RI). In general there is a high level of polymorphism associated with the probes, consistent with the complex breeding history and hence the broadly based gene pool of cultivated avocado.

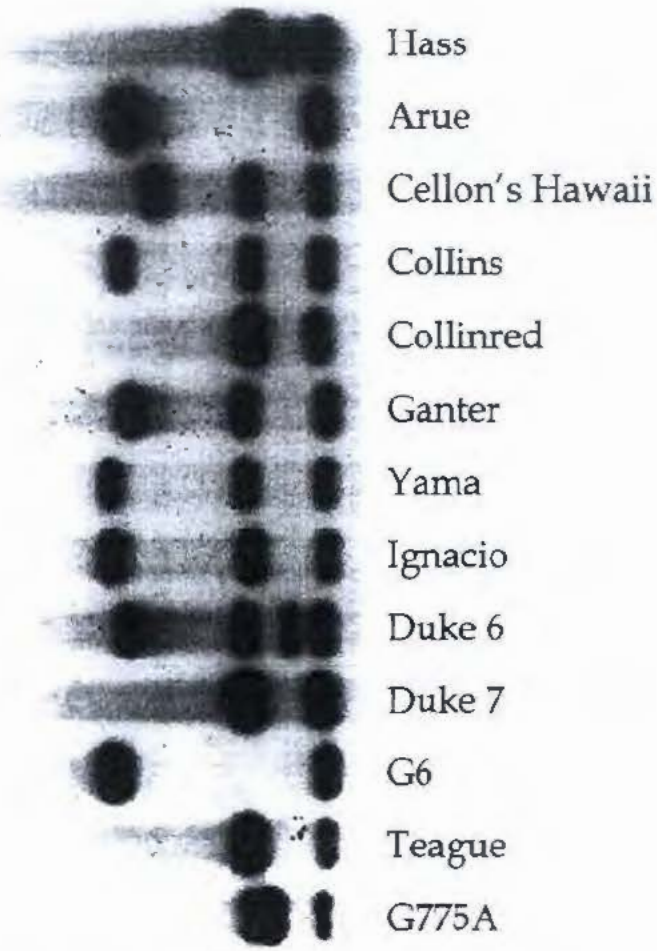

Figure 1. Southern transfer of avocado DNA digested with *Eco*RI and probed with clone 64

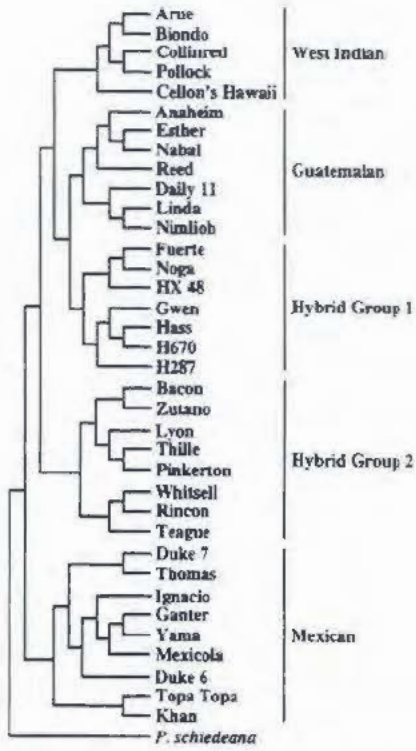

Figure 2. Dendogram of cluster relationships based on a matrix of the average number of genes shared in common between pairs of cultivars. The major clusters are identified by racial group or as of hybrid origin.

Cluster Analysis of Genotypic Data

Figure 2 presents a dendogram that depicts the relationships among the cultivars screened. Also indicated on the figure are the racial designation or the presumed hybrid origin of the various cultivars. Several features of the data are evident from the dendogram: (1) all cultivars are genotypically unique; (2) cultivars cluster within racial groupings so the genetic data conform to the botanical designations; (3) many cultivars appear to be of hybrid origin because they are placed in two clusters that fall between the racial groups; and (4) the Guatemalan and West Indian races are more similar to one another than either is to the Mexican race based on RFLP genotype. This pattern is consistent with the presumed geographic origins of the various races (Popenoe W, Zentmeyer G, and Schieber G, unpublished manuscript).

Discussion

It is important to be able to average over a number of genetic loci because the genetic relationships among cultivated avocados reflect a complex breeding history (Clegg et al. 1993). The transmission path associated with any particular gene may not reflect the average history of the many loci that comprise the genome. Because we are able to average over a number of polymorphic loci, these data resolve the genealogical relationships among avocado cultivars, including patterns of reticulate descent where interracial hybridization was important. The results of this article may be contrasted to those of Mhameed et al. (1997), where minisatellite markers were used to assess relationships among 24 cultivars. Unfortunately many of the cultivars included in the present study differ from those used by Mhameed et al. (1997), so it is not possible to make a comprehensive comparison between the two studies. The cultivars that are common between the two studies do show broadly similar relationships. Thus, for example, the interracial hybrid origin of Hass and Fuetre is also confirmed by Mhameed et al. (1997), however, Hass maps closer to the Mexican representative than to the Guatemalan representative in their Figure 1.

A knowledge of genealogical relationships has already proved useful to the UC Riverside program of avocado improvement. For example, the pollen parent of a semidwarfing cultivar of considerable commercial promise (Gwen) was unknown. The maternal parent was the cultivar Thille, and it was postulated that the pollen parent was Nabal; however, the RFLP data exclude Nabal as a pollen parent and suggest instead that Gwen resulted from a Thille × Hass cross. As a second example, RFLP genotypes have proved useful in the identification and verification of germplasm materials for the commercial nursery industry. Finally, a rich source of molecular markers is essential for genome mapping and to trace genetic transmission patterns. This latter issue has become particularly important in recent years because there is a cross-pollination requirement in avocado that may affect yield. Markers provide an experimental means to establish a relationship between yield and out-pollination by detecting the frequency of outcrossing events among mature fruits.

The long generation times and complex

breeding system of avocado has tended to frustrate genetic analysis in the past. Molecular markers promise to help overcome some of these obstacles and to provide a wealth of new information. As shown in this study, markers provide a basis for inferring genealogical relationships, thereby revealing new information about the process of domestication. Moreover, molecular markers have proved to be useful tools for asking practical questions to guide avocado management strategies. Undoubtedly a host of additional applications will appear as these techniques become better established.

References

Bergh BO, Scora RW, and Storey WB. 1973. A comparison of the leaf terpines in *Persea* subgenus *Persea*. Bot Gaz 134:130–134.

Clegg MT, Gaut BS, Duvall MR, and Davis J. 1993. Inferring plant evolutionary history from molecular data. N Z J Bot 31:307–316.

Fienberg AP and Vogelstein B. 1983. A technique for radiolabeling DNA restriction endonuclase fragments to high specific activity. Anal Biochem 137:266–267.

Furnier GR, Cummings MP, and Clegg MT. 1990. Evolution of the avocados as revealed by DNA restriction fragment variation. J Hered 81:183–188.

Mhameed S, Sharon D, Kaufman D, Lahav E, Hillel J, Degani C, and Lavi U. 1997. Genetic relationships within avocado (*Persea americana* Mill) cultivars and between *Persea* species. Theor Appl Genet 94:279–286.

Popenoe W. 1941. The avocado: a horticultural problem. Trop Agric 18:3–11.

Rawson JRY, Thomas K, and Clegg MT. 1982. Purification of total cellular DNA from a single plant. Biochem Genet 20:209–219.

Sambrook J, Fritsch EF, and Maniatis T. 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press.

Seeyle H. 1994. California roots. Grower 18:28.

Sneath PHA and Sokal RR. 1973. Numerical taxonomy. San Francisco: W. H. Freeman; 230–234.

Southern EM. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517.

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Root Health

Mulching
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Control Root Disease
in
Avocado and Citrus

by

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Root Health...

Mulching to Control Root Disease in Avocado and Citrus

Mulch, as a basic concept, is very broad and includes many materials. Any layer of plant residue or material that occurs naturally or is applied to the soil can be considered to be a mulch. This includes materials such as manure, sludge, sawdust, wood-chips, straw, shredded prunings, plant foliage, paper, plastic, sand, and gravel. Most benefits from the use of mulches in agriculture are derived from improved physical properties of soil related to increased organic matter content[38,11]. The application of thick mulches on the soil surface has the potential to create soil conditions that are beneficial for citrus and avocado growth while at the same time being deleterious to pathogenic soil organisms such as *Phytophthora*[4,7] and nematodes[6,11]. The benefits from organic mulches are well documented in agricultural literature[25,38,42,45].

Benefits

1. Mulching conserves water use by:
- reducing evaporation from the soil,
- reducing run-off and erosion,
- increasing the permeability of the soil surface,
- increasing the water holding capacity of the soil[6,15,25,45].

Jones *et al.*[23] stated that, for citrus production, it is essential to have a high water infiltration rate which will serve two functions: supplying water to the plant and removing salts from the soil. Organic matter increases the number of macropores (0.5-50μm) in the soil that hold the water necessary for the growth of plants[39]. Heavy clay soils, in which root rot is most severe, are composed mostly of micropores with very few macropores. Mulched soils with a high organic matter content will have a greater amount of water available to plants at field capacity. Increasing pore size distribution allows better utilization of the top 12 inches of soil, which is the area that is the most fertile and aerated. It is also the area in which citrus and avocado roots are most active.

2. Mulching can be an effective control of weed growth, thereby reducing the amount of herbicides needed[15,38]. When applied to bare soil, a thick layer of mulch can prevent germination of many annual species[38]. Lord *et al.*[30] found that a hay mulch in combination with simazine was effective in controlling weeds. It was proposed that a hay mulch was also superior in reducing simazine residues and soil leaching compared to no mulch at all, and that the hay absorbed simazine and enhanced its degradation.

3. Addition of organic matter into the soil by mulching improves soil structure and porosity[11,18,45]. Organic mulches cause fine clay particles to aggregate into larger granules the size of sand particles[45]. As organic matter decomposes, compounds are formed that cement soil particles together into stable aggregates[25,38]. This allows greater movement of gases (CO_2 and O_2) into and out of the soil. The maintenance of high levels of organic matter in the soil is a primary factor in soil fertility. The average citrus grove in California has less than

1% organic matter in the soil.

4. Mulching can eliminate or reduce ground water nitrate contamination by providing a continuous slow release of nitrogen, thereby reducing the need for nitrogen applied as chemical fertilizer[33,45]. Legume mulches or leafy plant material provide the most nitrogen, as well as many minor elements. Marked increases in soluble nitrogen, phosphorus, potassium, calcium, magnesium, and boron were observed under a mulch[45]. Parker and Jones[40] attributed larger fruit size in navel orange to increased potassium levels after additions of bulky organic mulches. Weeks, *et al.*[52] found that mulched plots maintained a reserve of nitrogen even after mulch application had been discontinued for nine years. It was also reported that phosphorus levels were eight times higher in the mulched plot compared to the non-mulched control plot. Organic matter increases the cation exchange capacity of soil. By increasing the cation exchange capacity of the soil, the availability of many nutrients to plant roots is increased[25]. Wander and Gourley[52] found that mulching with wheat straw or soybean hay prevented the fixation of potassium at the soil surface and allowed applied potassium to remain mobile and penetrate to a greater depth in the soil.

5. Mulching reduces wide fluctuations in soil temperature by reducing the soil's heat absorption[15,25]. This results in improved root growth, especially in young trees and in areas where summer temperatures are very high.

6. Mulching prevents dispersion of the soil surface by rain droplets[46]. A thick layer of mulch will also reduce compaction due to mechanical activity, such as harvesting or spraying operations[38].

7. There are also indications that mulching can control root rot caused by *Phytophthora* in both citrus and avocado, especially when combined with applications of gypsum[47]. Hoitink, *et al.*[20] speculated that composts prepared from tree bark may release inhibitors of *Phytophthora* spp. Zentmyer[55] found that alfalfa meal mixed with soil at rates of 1-5% gave good control of *Phytophthora* root rot in avocado. Researchers in Mexico have controlled *Phytophthora* in avocado by mulching with alfalfa straw and bovine manure[41]. Intensive mulching and applications of gypsum are used in Australia where they have effectively controlled *Phytophthora* root rot in avocado[7]. Nematodes in citrus may also be reduced by mulching[11].

Disadvantages

There are, of course, problems with the use of mulches.

1. The application of mulches can be very costly on a per tree basis.

2. Mulches can increase the danger of frost damage by insulating the soil and preventing the soil from warming the orchard[30,29]. This can be minimized by applying mulch only under the tree canopy and leaving the soil between rows bare. Also, if mulches are applied in late winter, there may be sufficient time for them to decompose before the next winter, reducing the chance of frost damage.

3. Mulches may introduce weed seed into the orchard if they have not been thoroughly composted.

4. Sludges may contain contaminants such as heavy metals which could accumulate in fruit at concentrations dangerous to consumers[11].

5. Certain mulches with high carbon-to-nitrogen ratios have insufficient nitrogen to support the increased populations of microorganisms that are produced during decomposition. This can cause a short term nitrogen deficiency which would necessitate increased nitrogen fertilization[25].

The History of Mulching for Avocado and Citrus

In the early days of citrus production, mulching was a common practice; but it was largely discontinued in the 1940s when cheap, easy-to-apply chemicals became available. Some growers continue periodically to apply manures to citrus and avocado groves. Craig[8] reported that the use of bean, barley, and alfalfa straw increased navel orange production by 25% and also conserved water. Hodgson[15] recommended that at least half of the nitrogen required for citrus trees should be applied by mulches, especially to young trees. Vaile[50] found that a treatment of cow manure, rock phosphate, and green leguminous manures was far superior to any other fertilizer treatment in trials in Rubidoux on navel and

valencia oranges and on lemons. In trials in Arlington, navel oranges treated with a variety of mulches yielded the same as nitrate-treated trees, 10% more than trees treated with ammonium nitrate, 27% more than manured trees, and 39% more than trees without fertilizer. In experiments in Chaffey and several other locations, Vaile[50] showed that citrus plots mulched with wheat straw were superior to all other treatments. In one experiment, wheat straw-mulched trees fertilized with ammonium sulfate yielded 36% more fruit than trees fertilized with ammonium sulfate only. Vaile[51] concludes that "concentrated inorganic fertilizers used persistently without bulky organic material will not permanently maintain healthy citrus trees under the conditions which prevail in Riverside." McNees[34] and D. C. Lefferts[28] also strongly recommended mulching for improving yields as well as soil conditions. Kelly[24], who provided one of the most learned treatises comparing fertilizers, concluded that alfalfa straw is one of the most valuable additions to citrus orchards, since it both supplies N fertilizer and conditions the soil. Part of the tremendous improvement in yield imparted by mulches in those early days may have been a result of minor elements they provided. Zinc and manganese fertilization was not routinely practiced at the time.

Despite the favorable reports on the use of mulches in citrus groves, mulching was not widely adopted as a standard practice. This was due to the incompatible nature of furrow irrigation with mulch application and the high cost of mulching compared to easy-to-apply inorganic fertilizers. The advent of undertree mini-sprinkler irrigation makes the use of mulches in citrus orchards much more attractive.

Although mulching, especially with green manure, appears to uniformly increase yields of citrus, the practice of growing cover crops between the rows of citrus seems to reduce the yields in many cases. This is probably due to competition between citrus and the cover crops for water and nutrients. These conclusions are reached in spite of Mertz's[35] glowing sentiments when he proved that "green manured" trees were superior in every way (tree size, yield, and fruit size) to trees similarly treated with animal manures.

The Ashburner System of Mulching in Avocado

With the goal of simulating a rain forest environment, Ashburner, from Australia, developed a soil mulching system for avocado that resulted in the creation of a suppressive soil. The following description of the cultural practices used by Ashburner was taken from Broadbent and Baker[7]:

"The grove consisted of 280 Fuerte avocado trees planted in 1940 and subsequent years. For two years after the rain-forest was cleared and before planting the avocados, cover crops were grown. These were disced in at maturity, with fowl manure, dolomite, and superphosphate. While the trees were young, an area of clean cultivation was maintained around them with cut cover crop piled near, but not in contact with, the trees. By the time the trees were 5 years old they supplied sufficient fallen leaves to maintain the organic matter content of the soil under them. In general, the following practices have been pursued subsequently. Fowl manure has been applied twice a year, in March and September, at 2 tons per acre per application. Chemical fertilizer (12.5% N, 12.5% P_2O_5, 20% K_2O) was applied in March-April and October-November at 1 lb per tree per year of growth. Dolomite was applied at 1 ton per acre whenever the pH of the soil fell below 6. Summer cover crops of *Dolichos lablab* and corn were grown and disced in before maturity. Winter cover crops of New Zealand blue lupin were grown and disced in while flowering."

These practices resulted in high levels of exchangeable calcium and magnesium and levels of organic matter similar to those in rain forests. This method is still being practiced in Australia with some modifications. Some growers add mulch of oat, barley straw, or sudan grass together with gypsum to attain the soil suppressive to *Phytophthora*. Others mow weeds and throw them under trees. Still others use tree prunings similar to yard waste as mulch. Regardless of what the materials are, the key appears to be the consistent application of organic mulch together with gypsum. The mulch is applied in large piles sometimes several feet thick. Organic content of the soil is kept in the range of 7%, which is thought to be suppressive to *P. cinnamomi*.

Production and Sources

In the past, mulches used by most growers were produced on the farm as manure or cover crops. However, cover crops are now widely perceived to reduce yields in citrus groves due to competition for water and nutrients[25], and most growers are no longer diversified so as to have access to animal manures. Transportation and application of purchased mulches has not been viewed as cost effective. For these reasons, most California citrus orchards have been in production for 40-50 years without the benefit of added organic material.

It is timely to take a new look at the use of mulches as potentially very large sources are being developed. As much as 40% of the solid waste produced in urban areas is compostable material. The Integrated Waste Management Act of 1989, AB 939, mandates that every county and city sanitation district in California reduce the solid waste stream by 25% by the year 1995 and by 50% by the year 2000. Many landfills throughout the state are implementing programs to separate compostable materials, grind them, and produce mulch or compost. These programs use fee incentives for those who bring in 100% plant residues that are free from non-compostable materials. The tipping fee reduction in Santa Barbara County is half the normal cost per ton. Santa Barbara County is currently producing 1,500 tons of ground plant residue a month. Due to the lack of a developed market, this material is used for the production of electricity in a biomass facility in Madera. A pilot program is being developed to compost these materials and develop a market for the finished product. Los Angeles County produces 50,000 tons of trash per day, of which 6,000 tons are garden waste. Currently, Los Angeles County is implementing a green waste program that includes separate collection of residential green waste, grinding, and composting.

Florida has enacted a similar law, the Solid Waste Management Act of 1988, which mandated a 30% reduction in the amount of solid waste generated by the year 1994. An analysis by Pinellas County, Florida, indicated that 22% of the solid waste generated was yard waste and land-clearing debris which represented approximately 260,000 tons of plant residue per year[5]. Pinellas County initiated a yard waste recycling program in August 1989. The following is a synopsis of their procedure for compost production as described by Bradshaw *et al.*[5]. Yard waste was collected both at curbside and at drop-off centers and then converted to mulch by chipping and shredding in a mobile tub grinder. The ground yard waste was placing in windrows 24 feet wide, 10 feet high, and 120 feet long, which were estimated to contain 300 tons of material per windrow. Windrows were turned and irrigated if temperature readings fell below 110 degrees or rose above 160 degrees Fahrenheit. Windrows also received two rotations at two week intervals. If oxygen fell below 5% or moisture content below 20%, the windrows were turned and irrigated. After 45 days, the finished compost was distributed to the public around the county. Tipping fees in Pinellas County are $32.50 per ton, and the cost of compost production is $22.00 per ton[(Bradshaw, personal communication)]. This has allowed Pinellas County to distribute their finished compost product free to county residents and municipal users such as parks.

Other composting systems have used a variety of methods such as enclosed reactor systems that stir or tumble the composting mass, or static piles using forced aeration[4]. The purpose of these systems is to accelerate the composting process.

Hoitink *et al.*[21] have divided the composting process into three phases. The first phase occurs during the first 24-48 hours, with temperatures rising to 40-50°C. Sugars and other easily biodegradable substrates are destroyed in this first phase. In the second phase, temperatures of 40-60°C occur. Cellulose and other substrates less easily degraded are consumed while lignins break down more slowly. The high temperatures during the second phase kill plant pathogens, weed seeds, and most biocontrol agents. *Bacillus* bacteria survive due to the formation of highly resistant spores that require much higher temperatures to kill. Not all sections of a compost pile or windrow reach high temperatures, and this requires that piles be turned so as to expose all materials to the minimum temperatures that kill plant pathogens and weed seeds. The third phase of composting is a curing phase when degradable components decline along with high temperatures. The mature compost is composed of humic materials and lignins. During the third phase, the compost is quickly

recolonized by a variety of bacteria and fungi.

The United States is in fact far behind the rest of the world in the reclamation of municipal solid waste. There are nearly 90 plants in France processing household refuse into compost with an annual output of 650,000 metric tons of compost from 1,300,000 metric tons of refuse[2]. In the Netherlands, the main producer of municipal waste compost is the VAM Waste Disposal Company which produces 100,000 tons of compost per year[16]. Research has been conducted in the Netherlands on the use of municipal waste compost at the Institute for Soil Fertility since the 1920s.

In addition to municipal solid waste compost, there are already many other sources of mulching materials such as paper and rice hulls, small grain straw, and sludge.

Sludge is a solid by-product of sewage waste, and is being accumulated in the world at the rate of 7.5 million tons per day. Disposal is costly, and yet sludge is a rich source of fertilizer. Fertilizer value of sludge in North America alone is estimated at $674 million per year[5]. Sludge is processed either aerobically or anaerobically to produce compost. It is often mixed with municipal solid waste prior to composting to increase the nutrient content of the finished product[11]. The major limitation for the use of sludge in agriculture is the presence of contaminants such as heavy metals and human pathogens[11].

Disease Control-Inhibition

Mulching has so many effects on the soil it is difficult to pinpoint the exact mechanism by which it can reduce nematode and *Phytophthora* root rot of citrus and avocado. A number of mechanisms are possible, and a combination of them is most likely responsible.

1. Mulching increases the population of soil microorganisms which compete with or inhibit fungal pathogens[25,7]. This mechanism was considered of major importance by Broadbent and Baker[7]. Bacteria living on the root surface can reduce the amount of root exudates available to attract zoospores. It was observed by Gilpatrick[12] that diseased root pieces from soil amended with materials of low carbon:nitrogen ratios did not produce sporangia. Gilpatrick suggested that microbial overgrowth of inoculum could inhibit *Phytophthora*. Malajczuk and McComb[32] found that a loam soil suppressive to *Phytophthora cinnamomi* had higher populations of microflora than a soil conducive to *Phytophthora cinnamomi*. The suppressive nature of the loam soil was lost upon autoclaving, indicating that suppression was due to the living organisms. Nesbitt *et al.*[36] found that the addition of organic matter to conducive soil increased the decomposition of *Phytophthora cinnamomi* hyphae and damaged the ability of *Phytophthora* to produce spores. It was suggested that the increase in microbial population was responsible for the decomposition of *Phytophthora* hyphae. In addition, Nesbitt *et al.*[37] observed extensive bacterial colonization on hyphae and sporangia of *Phytophthora cinnamomi* and correlated bacterial colonization of hyphae with an increase in hyphal decomposition.

2. The production of gases which can inhibit *Phytophthora* is increased by decaying organic matter. Ammonia reached high levels in soil amended with alfalfa meal within one week and was responsible for the elimination of *Phytophthora cinnamomi* in that soil[13]. Tsao and Oster[46] showed that ammonia and nitrite are toxic at low concentrations and inhibit propagule germination for both *Phytophthora cinnamomi* and *Phytophthora parasitica*. Carbon dioxide levels are also higher in soils with high microbial activity, and this may induce dormant survival structures of *Phytophthora* rather than active infection structures[47].

3. Mulch creates a natural litter layer under which roots of both citrus and avocado proliferate. This has been observed in experiments with many different species of trees, with both organic mulch and with plastic mulch[6,15,54]. This interface of soil surface and mulch is a natural microenvironment where roots grow well but where *Phytophthora* cannot survive. Gregoriou and Rajkumar[15] observed that an 8 cm thick mulch under avocado trees promoted vigorous rooting on the soil surface under the mulch. *Phytophthora* needs saturated soil conditions to release its zoospores, which must swim to the roots to cause new infections. Water drains so quickly from a mulch layer that saturated conditions may not exist long enough for zoospores to be released and swim to the root.

4. Soil toxins are produced during the decomposition of organic matter in mulched soil, such as ammonium, nitrite, saponins, and organic acids. Ammonium (NH_4) accumulates

from the decomposition of organic matter[22]. The production of ammonium and nitrite in urea-amended soil greatly reduced the soil population of both *P. cinnamomi* and *P. parasitica*[45]. Zentmyer and Bingham[58] observed that *Phytophthora* was more sensitive to nitrite than was the avocado plant, and that nitrite might retard disease development. Zentmyer and Thompson[57] showed that saponins extracted from alfalfa meal were also toxic to *Phytophthora cinnamomi.*

5. Organic matter can act as a trap for zoospores. The zoospores can encyst on organic matter in the soil rather than the root. It was shown that pectin and high molecular weight polysaccharides induced rapid encystment of zoospores[14]. After encystment, the zoospore can no longer swim to the root. These substances are often present in mulches and may act to intercept zoospores before they reach roots.

6. The actions of toxic gases and compounds due to organic matter decomposition can increase the level of host resistance in the roots by induced phytoalexin production. Roots of *Persea indica* injured by exposure to ammonia had a 50% reduction in infection by *P. cinnamomi* compared to non-injured roots[13]. Avocado roots subjected to certain types of organic matter quickly turned brown. These brown roots functioned well, but were found to be resistant to *Phytophthora cinnamomi* attack unless the brown covering was damaged[(Pinkas and Menge, unpublished)].

7. Lower soil temperatures due to mulching are less favorable to the growth of *Phytophthora* and more favorable for root growth during the summer months.

The Role of Calcium in Inhibition

The incorporation of gypsum ($CaSO_4$) into mulch also appears to play a key role in controlling root rot. The application of calcium in the form of gypsum is already an established soil treatment to increase soil drainage, to prevent crusting, and to remove sodium from the upper soil profile. It is well known that high sodium levels can increase the severity of root rot caused by *Phytophthora* and increase *Phytophthora* soil populations as well[31].

In addition, calcium can increase host resistance to fungal pathogens[1]. *Phytophthora* invades host tissue and dissolves the middle lamella between cells by the action of pectolytic enzymes. Calcium binds to pectic materials in the middle lamella and inhibits the activity of cell wall degrading enzymes produced by *Phytophthora*[1]. Lee and Zentmyer[27] observed that seedlings of *Persea indica*, a close relative of avocado, were more resistant to infection by *Phytophthora cinnamomi* when grown in high concentrations of calcium than when they were grown in low concentrations. Further, it was observed by Falcon *et al.*[10] that soil calcium level was responsible for disease suppression in avocado root rot experiments dealing with the interactions of soil pH, nutrients, and moisture. Snyman[43] has also shown that calcium can reduce root rot in avocado caused by *P. cinnamomi.* Calcium may reduce root exudation from avocado roots since it is known to prevent "leakiness" of roots of a variety of plants[26]. It is known that exudates from avocado roots attract *Phytophthora* zoospores to the root. It is known that calcium enhances the encystment of zoospore[17]. Once encysted, the zoospores lose their motility and are no longer able to cause infection. This phenomenon may be even more prevalent when calcium and organic material are mixed.

Current Mulching Experiments

Currently, there are a number of mulching trials being carried out throughout California. University extension agents Gary Bender, in San Diego County; Guy Witney, in Riverside County; Nick Sakovitch, in Ventura County; and Ben Faber, in Santa Barbara County, have all initiated recent mulching trials on citrus or avocado. Howard Ohr, John Menge, and Diana Freckman have carried out research on one *Phytophthora cinnamomi*-infected avocado plot at the University of California South Coast Field Station for several years. Currently in that plot the two best treatments, measured for total tree canopy, are Aliette®, Aliette®/alfalfa, and the alfalfa/gypsum treatment which are not significantly different from each other, but have roughly twice the canopy size as the non-treated controls. Bredell *et al.*[6] have also observed increased tree volume in mulched citrus.

However, at UCR citrus mulched with spoiled alfalfa or a commercial sewage sludge exhibited poorer growth than non-mulched trees. *Phytophthora parasitica* populations were greatly enhanced by these high-nitrogen

mulch treatments, which led to extensive root damage and reduced tree growth. While the literature predicts that mulches will usually be beneficial for citrus and avocado, these current trials indicate that not *all* mulches will be uniformly beneficial under all soil conditions. Mulches which are beneficial for avocados may not be beneficial for citrus. More research is required to identify mulches which are the most beneficial under California soil conditions.

Conclusions

Benefits from mulching in citrus and avocado can be substantial, especially when organic matter has become low. An obvious beneficial effect is the improvement in soil physical and chemical properties. The reduction in costs for irrigation water, fertilizer, herbicides, and fungicides may offset the costs of mulching orchard fruit trees. Cultural practices that eliminate or reduce environmental pollutants have an inherent value beyond immediate returns, and will probably benefit growers in the long term.

Those soils with poor infiltration and high expendable sodium have the highest incidence of avocado and citrus root rot[3]. Organic mulches used with applications of gypsum are effective treatments that alleviate these soil problems. In so doing, the soil may become less conducive to root rot caused by *Phytophthora* and nematodes.

Mulches are an effective method of improving orchard health when managed properly. Several types of mulch show promise in the control of *Phytophthora* root rot. Whether compost from municipal solid waste or from sludge will also be effective in this regard is as yet unknown. Continued research into the use of mulches and their proper management will increase the benefits derived from them.

Literature Cited

1. Bateman, C. F., and R. D. Lumsden. 1965. Relation of calcium content and nature of the pectic substances in bean hypocotyls of different ages to susceptibility to an isolate of *Rhyzoctonia solani*. Phytopathology 55:734-738.
2. Begnaud, B., and N. Noyon. 1989. Shredding household refuse. BioCycle. March:40-41.
3. Borst, G. 1970. Selection, management of avocado soils. Citrograph 55:263-265.
4. Borst, G. 1983. Lime has four functions in avocado growing soils. Avocado Grower. October:38-39,47.
5. Bradshaw, J., T. Lehmann, and J. Ragsdale. 1991. Reduce/reuse/recycle yard waste in Pinellas County. Proc. Fla. State Hort. Soc.
6. Bredell, G. S., C. J. Barnard, and C. Human. 1976. Deklaagpraktyke: die invloed van deklaagmateriaal op wortelontwikkeling van avokado's en valencias. The Citrus and Subtropical Fruit Journal. November:17-21.
7. Broadbent, P., and K. F. Baker. 1974. Behaviour of *Phytophthora cinnamomi* in soils suppressive and conducive to root rot. Aust. J. Agric. Res. 25:121-137.
8. Craig, V. H. 1916. Experience with mulch on the Ford Ranch. California Citrograph 1:12-22.
9. Fahm, L. 1980. The Waste of Nations: the economic utilization of human waste in agriculture. Allanheld, Osmun & Co., Montclair, New Jersey.
10. Falcon, M. F., R. L. Fox, and E. E. Trujillo. 1984. Interactions of soil pH, nutrients and moisture on Phytophthora root rot of avocado. Plant and Soil 81:165-176.
11. Gallardo-Laro, F., and R. Nogales. 1987. Effect of the application of town refuse compost on the soil-plant system: A review. Biol. Wastes 19:35-62.
12. Gilpatrick, J. D. 1969a. Effect of soil amendments upon inoculum survival and function in phytophthora root rot of avocado. Phytopathology 59:979-985.
13. Gilpatrick, J. D. 1969b. Role of ammonia in the control of avocado root rot with alfalfa meal soil amendment. Phytopathology 59: 973-978.
14. Grant, B. R., H. R. Irving, and M. Radda. 1985. The effect of pectin and related compounds on encystment and germination of *Pytophthora palmivora* zoospores. Journal of General Microbiology 131:669-676.
15. Gregoriou, C., and D. Rajkumar. 1984. Effects of irrigation and mulching on shoot and root growth of avocado (*Persea americana* Mill.) and mango (*Mangifera indica* L.). Journal of Horticultural Science 59(1):109-117.
16. Haan, S. de. Results of municipal waste compost research over more than fifty years at the institute for soil fertility at Haren/Groningen, the Netherlands. Neth. J. Agric. Sci. 29(1):49-61.
17. Hemmes, D. E. 1983. Cytology of Phytophthora. pp. 9-40. In Phytophthora: Its Biology, Taxonomy, Ecology and Pathology. D. C. Erwin, S. Bartnicki Garcia, and P. N. Tsao, editors. American Phytopathology Society.
18. Hernando, S., M. C. Lobo, and A. Polo. 1989. Effect of the application of a municipal refuse compost on the physical and chemical properties of a soil. The Science of Total Environment. 81/82:589-596.
19. Hodgson, R. W. 1925. Fertilizing citrus trees in California. Univ. of Calif. Agric. Exp. Sta. Circular No. 283. Berkeley, CA.
20. Hoitink, H. A. J., W. Chen, M. I. Trillas-Gay, and Y. R. Chung. 1986. Compost for control of plant diseases. In Compost: production, quality and use. pp. 414-419. M. De

Bertoldi, M. P. Ferranti, P. L'hermite, and F. Zucconi, editors. Elsevier Science Publishing Co., New York, NY 10017, USA.

21. Hoitink, H. A. J., Y. Inbar, and M. J. Boehm. 1991. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Disease 75(9):869-873.
22. Huber, D. M., and R. D. Watson. 1974. Nitrogen form and plant disease. Paper No. 5301 of the Journal Series of the Purdue University Agricultural Experiment Station, West Lafayette, Indiana.
23. Jones, W. W., C. B. Cree, and T. W. Embleton. 1961. Some effects of nitrogen sources and cultural practices on water intake by soil in a Washington navel orange orchard and on fruit production, size, and quality. Proc. Amer. Soc. Hort. Sci. 77:146-154.
24. Kelly, W. P. 1922. Our present knowledge concerning fertilization of citrus. Calif. Citrograph 7:186-202.
25. Lanini, W. T., J. M. Shribbs, and C. E. Elmore. 1988. Orchard floor mulching trials in the U.S.A. LeFruit Belg. 56(3): 228-249.
26. Lauchli, A., and E. Epstein. 1970. Transport of potassium and rubidium in plant roots—the significance of Calcium. Plant Physiology. 45:639-641.
27. Lee, B. S., and G. A. Zentmyer. 1982. Influence of calcium nitrate and ammonium sulfate on phytophthora root rot of *Persea indica*. Phytopathology 72:1558-1564.
28. Lefferts, D. C. 1919. Can mulching be recommended as a citrus grove method? Calif. Citrograph 4:160-163.
29. Leyden, R. F., and P. W. Rohrbaugh. 1963. Protection of citrus trees from cold damage. Proc. Amer. Soc. Hort. Sci. 83:344-351.
30. Lord, W. J., R. A. Damon Jr., and B. Gersten. 1968. Effects of simazine alone and in combination with hay or plastic mulch on 'McIntosh' apple trees and accumulation of simazine residues. Proc. Amer. Soc. Hort. Sci. 93:62-70.
31. MacDonald, J. D., T. J. Swiecki, N. S. Blaker, and J. D. Shapiro. 1984. Effects of salinity stress on the development of Phytophthora root rots. Calif. Agriculture, Oct. pp. 23-24.
32. Malajczuk, N., and A. J. McComb. 1979. The microflora of unsuberized roots of *Eucalyptus calophylla* R. Br. and *Eucalyptus marginata* Donn ex Sm. seedlings grown in soil suppressive and conducive to *Phytophthora cinnamomi* Rands. I Rhizophere bacteria, actinomycetes and fungi. Aust. J. Bot. 27:235-254.
33. Maynard, A. A. 1989. Agricultural composts as amendments reduce nitrate leaching from the soil. Frontiers of Plant Sci. Fall: 2-4.
34. McNees, R. 1916. Mulching experiment. Calif. Citrograph 1:10.
35. Mertz, W. M. 1981. The use of green manure crops in southern California. Calif. Agr. Exp. Sta. Bull. 292:1-31.
36. Nesbitt, H. J., N. Malajczuk, and A. R. Glenn. 1979. Effect of organic matter on the survival of Phytophthora cinnamomi Rands in soil. Soil Biol. Biochem. 11:133-136.
37. Nesbitt, H. J., N. Malajczuk, and A. R. Glenn. 19??. Bacterial colonization of *Phytophthora cinnamomi* Rands. (?)
38. Opitz, K. 1974. Mulching citrus and other subtropical tree crops. Coop. Ext. One Sheet Answers No. 271., Berkeley, CA.
39. Pagliai, M., G. Guidi, M. Lamarca, M. Giachetti, and G. Luccaamante. 1981. Effect of sewage sludges and composts on soil porosity and aggregation. J Environ. Qual. 10(4): 556-561.
40. Parker, E. R., and W. W. Jones. 1951. Effects of fertilizers upon the yields, size and quality of orange fruits. Calif. Agr. Exp. Sta. Bull. 722. 58 pp.
41. Romero, M. R., D. T. Ortiz, R. G. Espinosa, and S. S. Garcia. Influencia de estiercio, alfalfa y metalaxil en la dinamica poblacional de *Phytophthora cinnamomi* Rands causante de la tristeza del aguacate (*Persea americana* Mill.). Revista Mexicana de Fitopatologia 4:114-123.
42. Shiralipour, A., D. B. McConnell, and W. H. Smith. 1992. Use and benefits of municipal solid wasted compost: A data base review. Center for Biomass Energy Systems and Dept. of Environmental Horticulture, Univ. of Florida.
43. Snyman, C. P. 1984. The effect of calcium on avocado root growth and avocado root rot caused by *Phytophthora cinnamomi*. South African Avocado Growers Assoc. Yearbook pp. 91-92.
44. Stentiford, E. I. 1987. Recent developments in composting. In Compost: production, quality and use. pp. 52-60. M. De Bertoldi, M. P. Ferranti, P. L'hermite, and F. Zucconi, editors. Elsevier Science Publishing Co., New York, NY 10017, USA.
45. Stephenson, R. E., and C. E. Schuster. 1945. Effect of mulches on soil properties. Soil Sci. 59:219-230.
46. Sudds, R. H., and G. H. Browning. 1941. The effect of soil management methods on certain physical and chemical properties in relation to the infiltration rates in West Virginia orchards. Proc. Amer. Soc. Hort. Sci. 39:38-46.
47. Tsao, P. H. 1969. Studies on the saprophytic behavior of *Phytophthora parasitica* in soil. Proc. First Int. Citrus Sym. Vol. 3.
48. Tsao, P. H., and J. J. Oster. 1981. Relation of ammonia and nitrous acid to suppression of *Phytophthora* in soils amended with nitrogenous or ganic substances. Phytopathology 71:53-59.
49. Tsao, P. H., and G. A. Zentmyer. 1979. Suppression of *Phytophthora cinnamomi* and *Phytophthora parasitica* in urea amended soils. In Soil-borne Plant Pathogens. pp. 191-199, Academic Press, London, 686 pp.
50. Vaile, R. S. 1922. Fertilizer experiments with trees. Univ. of Calif. Agric. Exp. Sta. Bull. 345:467-512.
51. Vaile, R. S. 1924. A survey of orchard practices in the citrus industry of southern California. Calif. Agr. Exp. Sta. Bull. 374:4-40.
52. Wander, I. W., and J. H. Gourley. 1945. Increasing available potassium to greater depths in an orchard by adding potash fertilizer on a mulch. Proc. Amer. Soc. Hort. Sci. 46:21-24.
53. Weeks, W. D., C. T. Smith, and M. Drake. 1950. Residual effects of heavy mulching in a bearing apple orchard on soil nutrients. Proc. Amer. Soc. Hort. Sci. 56:1-4.
54. Whitcomb, C. E. 1980. Effects of black plastic and mulches on growth and survival of landscape plants. Journal Series #3628 of the Oklahoma Agriculture Experiment Station.
55. Zentmyer, G. A. Biological control of Phytophthora root rot of avocado with alfalfa meal. Phytopathology 53:1383-1387.
56. Zentmyer, G. A., and F. T. Bingham. 1956. The influence of nitrite on the development of Phytophthora root rot of avocado. Phytopathology 46:121-124.
57. Zentmyer, G. A., and C. R. Thompson. 1967. The effect of saponins from alfalfa on *Phytophthora cinnamomi* in relation to control of root rot of avocado. Phytopathology 57:1278-1279.

Avocado Diseases

by

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Avocado Diseases

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AVOCADO ROOT ROT

Avocado root rot is the most serious avocado disease in California and most other avocado producing areas of the world. It is caused by the soil fungus, *Phytophthora cinnamomi*, which thrives in areas of excess soil moisture and poor drainage. Trees of any size and age may be affected.

Symptoms

Leaves of infected trees are small, pale green, and often wilted. Foliage is sparse, giving the tree an unthrifty appearance. New growth is usually absent; but if it occurs, new leaves are small and of poor color. Small branches die back in the top of the tree, allowing other branches to become sunburned because of the lack of foliage (*Fig. 1*). Diseased trees frequently set a heavy crop of small fruit.

Fig. 1. *Avocado tree showing symptoms of root rot.*

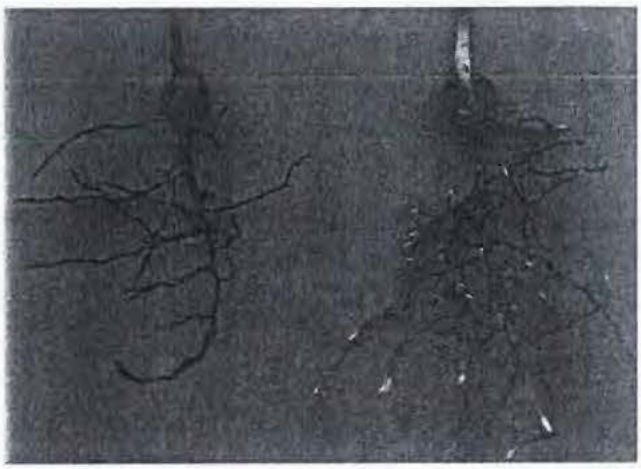

Fig. 2. *Root symptoms of avocado root rot: left, diseased roots; right, healthy roots.*

The small fibrous feeder roots may be absent on diseased trees; if present, they are usually blackened, brittle, and dead (*Fig. 2*). The absence of feeder roots prevents the uptake of moisture, and the soil under diseased trees stays wet even though the tree appears wilted. Roots of pencil size or larger are seldom attacked by the fungus.

Damage

Affected trees will decline and die either rapidly or slowly. The disease can be spread from a few trees to the entire orchard by unaware growers and workers.

Control

The fungus, *Phytophthora cinnamomi*, which causes avocado root rot has over 1,000 hosts and can be spread by moving contami-

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nated nursery stock of avocado and other plants, in water moving over or through soil containing the fungus, on equipment and shoes, in seed from fruit lying on infested soil, or by other types of activity by man or animals in which moist soil is moved from one place to another. Control is best achieved by an integrated approach of prevention, culture, and treatment.

Plant on well drained soil. Root rot develops in soils that have poor internal drainage because accumulated moisture permits the fungus to form its spore stages and to infect the roots. In new plantings, avoid soils favorable to root rot development; in established plantings, manage soils carefully so that moisture does not accumulate in the soil.

Use disease-free nursery stock. Historically, diseased nursery stock has been one of the major causes of the spread of avocado root rot into the avocado-producing areas of California. Avocado trees certified to be free of avocado root rot are available from nurseries that participate in the certification program. It is recommended that disease-free trees be used especially when planting new areas.

Prevent soil or water movement from infested areas. The fungus can be moved by any means by which moist soil is moved, and also can be spread downhill from an infested area by surface or subsurface drainage water. Install water-tight drains to take care of surface runoff if a diseased area lies above your healthy grove. Control gophers, as their runs can provide means of moving the fungus in water.

Irrigate diseased trees and margins of diseased areas carefully. Since high soil moisture favors root rot development, careful irrigation can retard the spread of the disease and often prolong the life of affected trees. Diseased trees have fewer roots to take up water, so do not water soil that is already wet as this increases the disease problem.

Fumigate small spots of disease. If only a few trees are affected, and the disease is detected early, cut off the trees at ground level and fumigate the soil with maximum dosages of fumigant. Check with your local University of California Farm Advisor or a licensed Pest Control Advisor for current availability and use of fumigants.

Establish a barrier. If the disease situation is such that the fungus occurs in only one area and cannot spread downhill in surface runoff or drainage water into the part of the grove to be protected, a physical barrier should retard spread. Establish the barrier at least two tree rows beyond where tests indicate the fungus to be present. The barrier should consist of a fence and/or warning signs to inhibit movement between the root rot area and healthy sections of the grove.

Resistant rootstocks. Some resistance to root rot has been found in several different varieties such as Duke 7, Thomas, and G755. Clones of these and similar varieties are more resistant than seedlings and are in general use in the industry. It is important to remember that these rootstocks are *resistant*—not *immune*. If they are planted or maintained under adverse conditions, they may be killed by the combination of these conditions and the disease.

Fungicides. Fungicides should not be depended upon alone to control root rot, but should be viewed as part of an integrated plan that includes the best cultural and biological controls available. There are currently two fungicides available for use on avocados. You should check with your local Farm Advisor or Pest Control Advisor for the latest information on these fungicides and their use on avocado.

Crop rotation. Replanting the infested soil to resistant crops is one of the best ways to control avocado root rot. The fungus has a wide host range, but there are many plants that are not susceptible, including all varieties of citrus, cherimoya, persimmon, all types of vegetables, most annual flower crops, and many deciduous fruit trees and berries. Macadamia is highly resistant to Phytophthora root rot, although a few cases of Phytophthora trunk canker have been found on macadamia trees in California.

ARMILLARIA ROOT ROT

The Armillaria fungus becomes well established in the roots before any visible effects appear in the top. There may be a gradual deterioration in tree vigor, with the

Fig. 3. *Tree killed by Armillaria root rot.*

foliage yellowing and dropping over part or all of the tree; or there may be a sudden wilting and collapse. Death of the tree usually follows (*Fig.3*).

The most reliable sign of Armillaria root rot is a white, fan-shaped growth of the fungus mycelium under the bark of diseased roots (*Fig. 4*). Purplish-brown cord-like rhizomorphs that resemble feeder roots sometimes grow on the surface of diseased roots.

The Armillaria fungus may produce a mushroom stage around the base of the infected tree during the rainy fall and winter months. The appearance of the mushroom cap is quite variable and may range from a

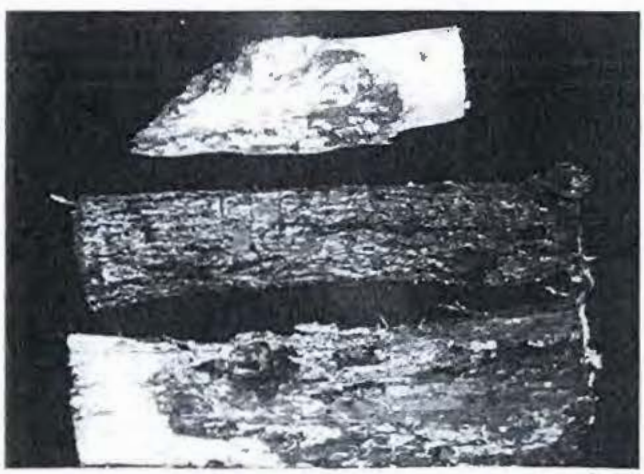

Fig. 4. *Armillaria: Middle root shows cracks and black lines of pseudosclerotium caused by growth of Armillaria in the root. Top root shows the white mycelial fans typical of Armillaria after the bark is removed from the root. Bottom root shows the deterioration of the mycelial fans following fumigation of the soil.*

Fig. 5. *Fruiting structures (mushrooms) of Armillaria.*

cream color to honey-yellow to almost black, and may have a covering of brown scales (*Fig. 5*). A great number of spores are produced, but they do not appear to be an important source of infection in California avocados.

Spread of the Fungus

Armillaria root rot spreads from place to place in infested wood. This wood may be a root fragment or part of an infected nursery tree. It may be carried by flood water, by leaf mulch gathered from under infected trees, by cultivating equipment, by any of man's activities which might move infected wood and soil. Long after the aerial parts of the tree are gone, the fungus remains alive in the roots. When susceptible trees such as citrus, peach, or avocado are planted in soil with Armillaria-infected roots or wood pieces, and the new roots come in contact with the fungus, they are exposed to infection. Infection is accomplished by direct penetration of a rhizomorph into the bark or by root to root grafts. The fungus spreads from tree to tree in diseased areas in the orchard, mainly by growing along diseased roots and infecting the healthy roots of adjacent trees.

Control

Armillaria fungus is very sensitive to drying, and a tree's life may be prolonged by exposing the base of the tree to the air—a technique that works in citrus. Soil fumigation with chemicals has successfully controlled Armillaria root rot under favorable soil conditions by preventing spread of the fungus and permitting replanting of fumigated areas. Check with your Farm Advisor or Pest

Control Advisor for the current availability and use of fumigants for this purpose.

AVOCADO BLACK STREAK

The disease known as avocado black streak (ABS) has been present in California for over 60 years but apparently becomes a problem only under certain poorly-defined environmental conditions. While the disease occurs frequently in California, elsewhere there have been only one observation in Florida and one occurrence in the Canary Islands on trees shipped from California. To date, the disease has only been observed on Guatemalan varieties such as Hass, Reed, and Nabal.

ABS may occur wherever Guatemalan varieties are grown in California. All ages of trees are affected and symptoms have been observed on trees as young as one year to over 35 years old. All groves in an area will not have the disease, and ABS incidence varies considerably within affected groves.

Symptoms

ABS appears after prolonged periods of environmental or cultural stress. An affected tree usually gradually declines and may eventually die, but rapid collapse may occur (*Fig. 6*). Fruit production is usually poor.

Because many of the symptoms are similar to those due to other causes, the canker on the trunk and branches was chosen as the diagnostic symptom (*Fig. 7*). The canker is characterized by the accumulation of a dry, powdery, water soluble sugar that exudes through minute cracks in the bark (*Fig. 8*). In the absence of the powder, the canker is difficult to find. Cankers may range in size from very small to most of the trunk and do not favor any side of the tree.

Scraping of the bark surface over the lesion reveals shallow, reddish-brown areas that rarely extend into the cambium (*Fig. 9*). These areas can often be removed easily by inserting a knife blade under them and prying upwards. Because trees die with very few lesions, the lesions appear to be the result of the disease, and not the cause of tree death. Other symptoms of the disease include chlorosis, early bloom, branch die-back, leaf blotching, zinc deficiency, bunchy growth, wilting of foliage, and rapid death of new growth.

Fig. 6. *Avocado tree with chlorotic foliage and one dead branch caused by Avocado Black Streak.*

Fig. 7. *Trunk lesions associated with Black Streak. Lesions can occur anywhere on the trunk or main branches.*

Fig. 8. *Close-up of Black Streak lesion.*

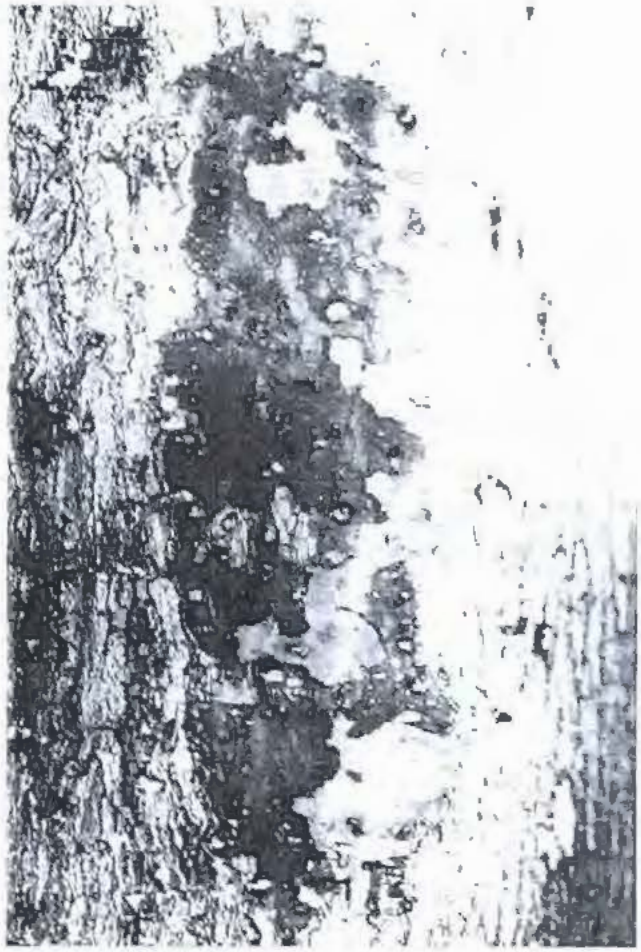

Fig. 9. *Black Streak lesion after bark removal. Lesions are normally shallow.*

Control

Current management of ABS consists of maintaining plant health with good fertilizer and water practices and preventing stress. Unthrifty trees should be removed and the site fumigated before a new tree is planted.

VERTICILLIUM WILT

This disease is caused by the soil fungus, *Verticillium albo-atrum*, quite a different type than the Phytophthora root rot fungus. The fungus enters the roots and invades the water-conducting system, retarding or preventing water movement to the foliage from the roots. Verticillium Wilt is not as serious or as common a disease in California as is Phytophthora root rot.

Symptoms

The leaves suddenly wilt on one part of the tree (*Fig. 10*) or on the entire tree (*Fig. 11*), then turn brown and die, remaining attached to the branches for several months. Brown to grey-brown streaks are seen in the wood of

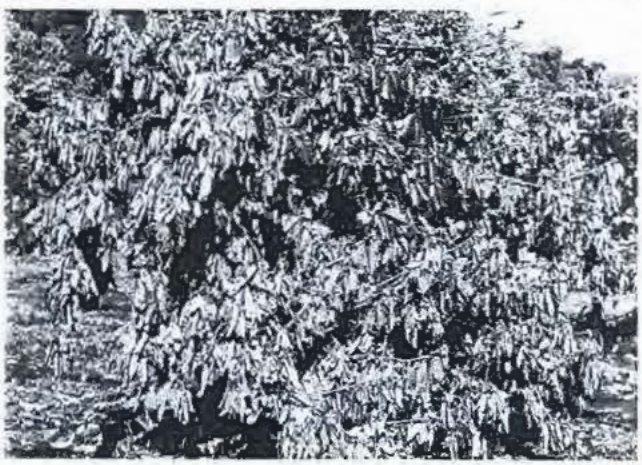

Fig. 10. *Tree partially affected by Verticillium Wilt.*

Fig. 11. *Tree totally affected by Verticillium Wilt*

the branches or roots when the bark is peeled (*Figs. 12,13*). Often, trees affected with Verticillium Wilt send out new, vigorous shoots within a few months after the initial collapse of the tree, and the tree may recover completely.

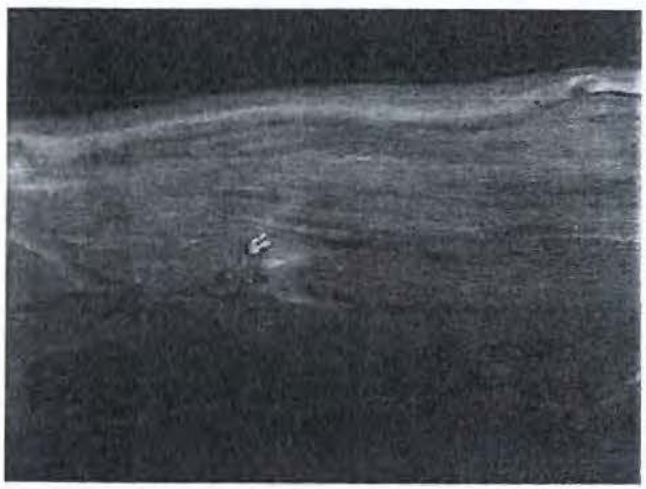

Fig. 12. *Typical brown streaks in a tree branch infected by Verticillium.*

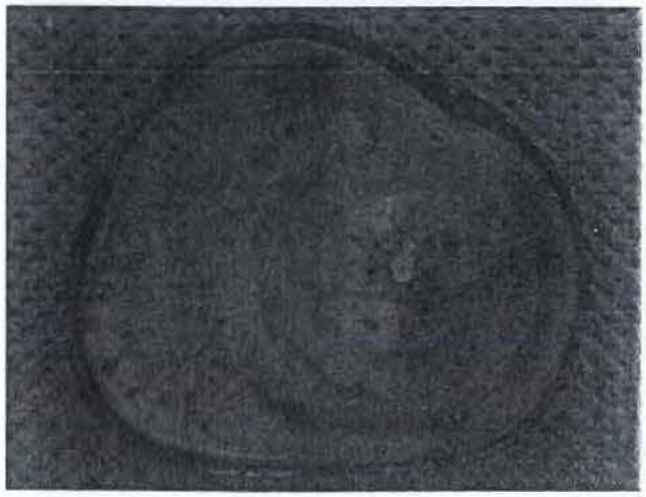

Fig. 13. *Branch cross section from a tree infected by Verticillium from an early age. Note the brown spots indicating infected vascular tissues.*

Prevention

Use Mexican rather than Guatemalan rootstocks; the former appear to be more resistant to this disease.

Do not plant avocados on land that has been used for other crops susceptible to Verticillium Wilt such as tomato, eggplant, pepper, many berries, apricot, potato, and a number of flower crops.

Do not plant susceptible crops in an established avocado grove.

Do not use trees that are or have been affected with Verticillium Wilt as sources of budwood or seeds.

Control

Often, no treatment is necessary as trees recover completely. Dead branches should be removed after die-back has ceased and new growth has begun. In case of severe and recurring disease, fumigate the area following recommendations of your local University of California Farm Advisor or Pest Control Advisor.

PHYTOPHTHORA CANKER OR COLLAR ROT

Phytophthora canker, or collar rot, is caused by the fungus, *Phytophthora citricola*. Occasionally, cankers caused by *P. cinnamomi* are found, but they are rare. Previously uncommon, collar rot has become widespread in California, attacking many trees, and is second only to avocado root rot in severity. *P. citricola* has a wide host range and has been recorded on hosts such as walnut, cherry, cherimoya, and fir trees. As with all Phytophthoras, the disease is favored by excess soil moisture which is essential for dissemination of spores.

Symptoms

Trunk cankers are normally found on the trunk base of older trees, usually originating at or below ground level. The canker appears as a dark region which often gives rise to a red, resinous exudation which on drying turns into a white crystalline deposit *(Fig. 14)*. Cutting away the superficial canker reveals an orange-tan to brown pigmented lesion, instead of the normal white or cream-colored tissues *(Fig. 15)*. The lesion has a fruity odor when exposed. The lesion may progress all the way down to the woody layers, but it is rarely found in these tissues. Lesions can spread in the crown roots and proceed up into the bark of the trunk *(Fig. 16)*. Depending on the local conditions and rootstock, the disease has been found to exist on trees for years, inducing a gradual decline in the tree. In some cases, the disease can progress rapidly, killing trees—whether young or old—in a matter of months, by essentially destroying the phloem (bark), and in effect ring-barking the tree.

Affected trees show a gradual loss of vigor and decline of the top, similar to the symptoms exhibited by trees affected with Phytophthora root rot. Occasionally, in ad-

Fig. 14. *Canker caused by Phytophthora citricola at the base of an avocado tree.*

Fig. 15. *Phytophthora canker with the bark removed, showing the infected tissues underneath.*

Fig. 16. *Cross section of a root infected by* Phytophthora citricola. *Note that the infection has almost girdled the root.*

vanced stages trees will die suddenly, with leaves turning brown within a short space of time. Confirmation of *P. citricola* is achieved by laboratory tissue isolations onto selective media for Phytophthora.

Prevention and Control

All evidence to date indicates that *P. citricola* can easily be spread in or on contaminated nursery material, vehicles, irrigation, and of course by people. The same sanitation procedures should be adhered to as with root rot. Seedling rootstocks are much more sensitive to trunk canker than most of the clonal varieties. In University of California field trials to date, Toro Canyon, Duke 7, Duke 9, and Barr Duke have shown moderate tolerance, as compared to other, more susceptible rootstocks such G1033, G6, and and 755B. Some selections of 755A have shown a much higher level of tolerance to this disease. Without doubt, it is a good practice to consider more than one rootstock when planting a grove with a history of trunk canker and root rot.

In California, these two serious diseases are increasingly found together. Hence, integrated approaches to the control of both need to be followed in orchard disease management strategies. Do not keep the lower trunks wet for long periods, as this increases the chances of infection. Drippers should be placed away from the trunks, and mini-sprinklers should be aimed to avoid wetting the trunks. Avoid wounding the trunks.

If cankers are detected in an early stage, before much of the trunk is invaded, they can sometimes be controlled by cutting out the infected tissue. Although research shows some promise of control, there are, as yet, no chemicals registered for use.

DOTHIORELLA CANKER

Another, less serious, type of canker is caused by the fungus *Dothiorella gregaria*, the same fungus that causes fruit rot in California. This type of canker may appear on branches on various parts of the avocado tree and also may be found on the trunk.

Symptoms

The principal evidence of infection is a white powder that exudes from the bark and a cracking and shedding of the outer bark. Affected trees sometimes gradually die back and look unthrifty; in unusually severe cases, the tree may be killed. Examination of the affected trunk or branches will show brownish discoloration of the bark which is quite shallow; the bark flakes off easily.

Prevention and Control

Mexican varieties are much more resistant to this disease than are Guatemalan rootstocks. The disease is favored by moist conditions. Do not let dead leaves and debris accumulate around the trunks or lower branches, particularly if the tree is on Guatemalan rootstock or if the scion is Guatemalan and the tree is budded low.

Control measures are usually not needed. However, if lesions are abundant on the trunk, scraping the outer bark will remove some of the infection and encourage regeneration of vigorous bark.

ANTHRACNOSE

Anthracnose is not normally a problem in California avocados, but occasionally becomes serious during periods of extended rainfall. During these periods, the disease can cause severe loss of foliage and fruit infections that can be extensive but do not become apparent until the fruit begins to ripen after harvest.

Symptoms

Anthracnose often becomes apparent in avocados when it is noticed that the trees are losing many of their leaves. These fallen leaves and those left on the trees may have large, brown, dead areas appearing in the center and on their margins (*Fig. 17*). These lesions are caused by the fungus, *Colletotrichum gloeosporioides*. This fungus is a natural inhabitant of our avocado and citrus groves, where it grows on dead twigs and leaves and is normally of little importance. With extended periods of wet conditions and mild winter temperatures, a build-up occurs of the fungus growing on the dead twigs and leaves.

This abundance of spores falls not only upon more leaves, where they repeat the cycle, but many also fall upon the fruit.

Fig. 18. *Early symptoms of Anthracnose on avocado fruit.*

After being deposited on the undamaged, green fruit surfaces, the spores germinate and penetrate the fruit, causing small brown to black spots surrounding the lenticels (*Fig. 18*). There is no further development until the fruit starts to ripen after harvest. During ripening, the fungus resumes growth, producing typical visible Anthracnose symptoms (*Fig. 19*). The fungus also enters the fruit via wounds caused by other agents such as insects and infected areas caused by other pathogens.

In time, these spots enlarge, often covering most of the fruit, and become covered with the pink spores of the Anthracnose fungus, *Colletotrichum*. When the fruit is cut in half through one of the spots, it can be seen

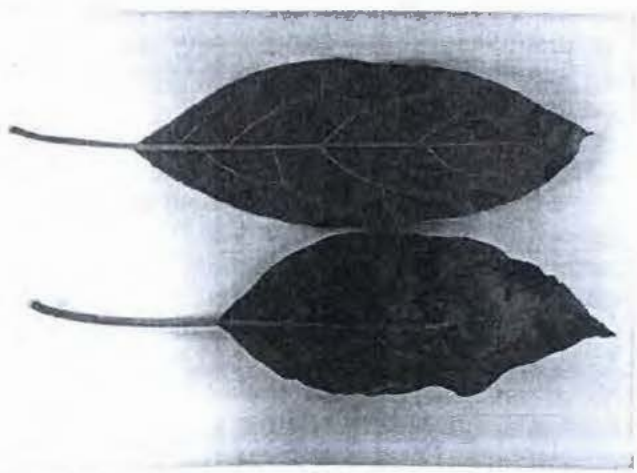

Fig. 17. *Leaf symptoms of Anthracnose caused by Colletotrichum.*

Fig. 19 . *Developing lesions of Anthracnose.*

Fig. 20. *Advanced decay of Anthracnose. Note the sporulation of* Colletotrichum *on fruit at left. Fruit in the middle and on the right show typical hemispherical decay associated with the disease.*

that the rot extends into the flesh in a hemispherical pattern (*Fig. 20*).

Control

Because of the infrequent occurrence of conditions conducive to the development of Anthracnose, nothing has been developed to control the disease. So far as the foliage is concerned, Anthracnose is not disastrous because after a period of dry weather the trees recover. If another wet period occurs, however, symptoms may develop again.

Some suggestions from Australia for field management of the disease include: ensure good ventilation and rapid drying of the foliage by pruning lower limbs so that the canopy is at least 20 inches above the ground; prune out dead twigs and branches before flowering; remove dead leaves entangled in the tree canopy; remove infected fruit which have not fallen from the tree; and control insect pests which damage the fruit. While these are good, general methods to help control the disease, they are likely impractical in California due to the costs involved.

Fig. 21. *Typical lesion caused by* Phytophthora citricola *at the base of an avocado fruit.*

When a disease like Anthracnose occurs, the fruit problems usually persist until the end of the current harvest season. This is due to the ability of the fungus to infect the fruit at all stages and remain dormant until the fruit ripens. There are no chemical controls available, and postharvest handling controls are of limited use. Fruit should be cooled to 41°F as soon as possible after harvest. Temperatures below 41°F should be avoided because internal damage due to chilling injury may occur. Delays of longer than 6 hours before cooling, and higher pulp (air) temperatures during these delays, will result in increased postharvest fruit decay. This is of increasing importance as the season progresses, since fruits ripen faster as they increase in maturity.

Temperature is critical to Anthracnose development. Once fruit starts to ripen, temperatures of 75°F and above will accelerate development of Anthracnose, while temperatures below 59°F will retard development.

PHYTOPHTHORA FRUIT ROT

Phytophthora fruit rot is caused by *Phytophthora citricola*, the same fungus that causes Phytophthora canker or collar rot. The disease is of minor importance in California, causing the most damage during prolonged wet weather, the same conditions that favor Anthracnose. In contrast to Anthracnose, which is primarily a post-harvest problem, Phytophthora fruit rot affects the fruit while it is still hanging on the tree.

Symptoms

Affected fruit are often touching the soil or are hanging on the lower branches. Most damage occurs within one meter of the soil surface. Diseased fruit have a distinct circular black area that usually occurs at the lowest spot on the fruit (*Fig. 21*). While most

Fig. 22. *Less typical lesion on the shoulder of an avocado fruit.*

infections occur at the bottom of the fruit, they can occur anywhere on the surface (*Fig. 22*). Internally, the rot extends into the flesh, darkening it in the same pattern as the affected area on the surface (*Fig. 23*).

Disease Prevention and Control

Because infection is probably caused by the splashing of Phytophthora propagules from the soil surface to the fruit during heavy rain, prevention is difficult. Any practice that helps reduce splash, such as a layer of leaves, may help. Fruit lying on the ground should be removed, because the fungus can grow and sporulate on them.

There are no chemicals labeled for this disease on avocado.

Fig. 23. *Internal symptoms of fruit rot caused by* Phytophthora citricola.

DOTHIORELLA FRUIT ROT

Dothiorella fruit rot is caused by the canker-inducing fungus, *Dothiorella gregaria*. This disease is an occasional but minor post-harvest problem of avocados in California.

Symptoms

This disease does not appear when the fruit is still on the tree, but develops after the fruit is picked and starts to soften. Small purplish-brown spots may then appear on any part of the fruit, but more often at the stem end. These spots gradually enlarge, and may involve the entire fruit surface (*Fig. 24*). The flesh is invaded by the fungus, becomes discolored, and develops an offensive odor.

Disease Prevention

The fungus commonly grows on dead leaves, dead margins of leaves, and on dead branches. Do not let dead material accumulate in the groves. Also, avoid saline conditions which induce leaf-burning of leaves, because the fungus will live on the dead portions of the leaves.

Control

See the anthracnose control section.

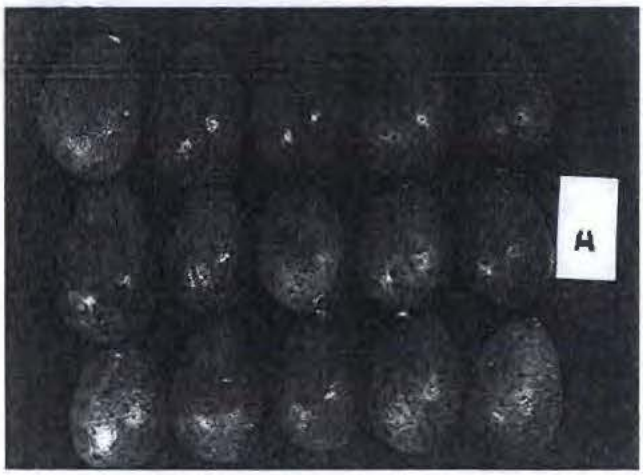

Fig. 24. *Fruit rot caused by* Dothiorella.

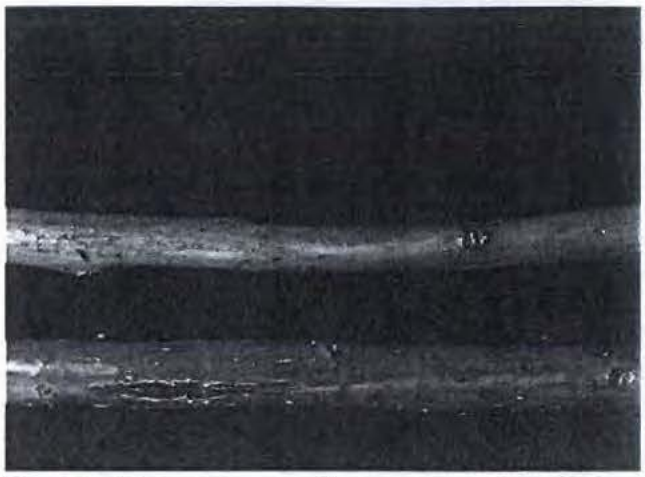

Fig. 25. *Yellow twig streaking caused by Sunblotch.*

SUNBLOTCH

Sunblotch was first described in California in 1928 as a physiological disorder. The disease was shown to be graft-transmitted in the 1940s, and for many years was thought to be caused by a virus. In the 1970s, sunblotch was determined to be caused by a viroid. It is the only known viroid disease of avocado.

Sunblotch can occur anywhere avocados are grown and, while there have been serious outbreaks in the past, it is currently considered to be a minor problem that can be avoided by prevention of the introduction of the disease.

Symptoms

Symptoms on twigs include narrow yellow, red, or necrotic streaks that often are associated with shallow indentations that occur lengthwise along the twig (*Fig. 25*). Fruit may show white or yellow blotches or streaks that may or may not be depressed (*Fig. 26*). Fruit that remains green at maturity usually have white or yellowish areas, while fruit that turns black usually have whitish areas that turn red as the fruit matures. Leaves may have white or yellowish variegated areas, and they often are deformed (*Fig. 27*). Leaf symptoms are uncommon in the field. A fourth symptom is rectangular cracking and checking of the bark on the trunk and larger branches ("alligator bark") (*Fig. 28*). Trees affected by the disease are often stunted with sprawling growth (*Fig. 29*). Trees with visible sunblotch symptoms often have reduced yields.

Causal Agent

Sunblotch is caused by the Avocado Sunblotch Viroid (ASBVD). ASBVD is a small, single-stranded circular RNA molecule of 247 nucleotides with a molecular weight of 0.8×10^5.

Disease Cycle

The viroid is carried within the host tissues. Visual symptoms on the host depend on the host variety, environmental conditions, and viroid strain. Trees that do not show symptoms even though the viroid might be present in high amounts are known as "symptomless carriers." Large reductions in yield of vigorous trees may indicate the presence of ASBVD in the "symptomless carrier" form of the disease. The viroid is transmitted through high numbers of the seed from these infected trees. Although seedlings from such

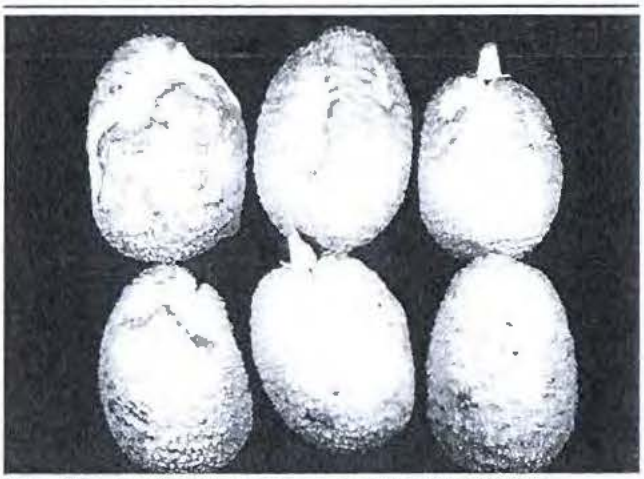

Fig. 26. *Fruit symptoms caused by Sunblotch.*

Fig. 27. *Leaf symptoms of Sunblotch (rare in the field).*

Fig. 28. *Bark cracking on the trunk and major branches due to Sunblotch disease ("alligator bark").*

symptomless carriers do not show symptoms of sunblotch when they are used as root stocks, the disease will often appear on scions grafted to them. Trees with symptoms transmit the viroid to seed at a low frequency, but the resultant infected seedlings normally show symptoms.

Fig. 29. *Stunting of an avocado tree due to Sunblotch.*

Transmission of the viroid most often occurs at grafting by using infected budwood or rootstock seedlings. Other less common methods of transmission are through wounds caused by contaminated cutting tools, root-to- root grafts, and by pollen from an infected tree to the flower ovule of a non-infected plant, resulting in infected seed. No evidence has been observed for transmission of sunblotch to trees whose flowers receive pollen from a diseased tree. There is no evidence of insect transmission.

Control

The primary control measure for this disease is the use of registered trees, which involves careful selection of disease-free scions and seed sources. These sources can be confirmed to be disease free by indexing.

Trees with symptoms may be removed from the orchard, and remaining stumps should be killed. Indexing of suspect orchards can be done to identify positive trees. Pruning tools and harvesting clippers should be sterilized between trees.

In an established orchard, the threat of spread from an infected tree is minor as long as the tree is not used as a scion source or for seed. If the tree is producing good quality fruit, the grower may elect to leave it in place. Often such trees are left in place with no evidence of spread.

BACTERIAL CANKER

Bacterial canker is a disease that is widespread but relatively unimportant. Normal incidence in a grove is a few affected trees. In some groves however, the disease may be severe and affect well over 50 per cent of the trees.

Symptoms

The first visual symptoms on the bark are dark, slightly sunken areas with a watery, necrotic pocket under the surface. As the canker develops, the bark splits, usually at one side of the canker, and the watery fluid oozes out and dries, leaving a white powdery residue around, and sometimes over, the lesion (*Figs. 30, 31, 32*). Typical cankers range from 2-10 cm in diameter. Usually cankers appear at the base of the tree first and often spread upward in a straight line on one side of the trunk or branch (*Fig. 33*). Necrotic streaks extend in the wood from the necrotic areas underneath the cankers both above and below the lesions (*Fig. 34*). Necrotic streaks between the cankers are usually in the xylem, sometimes toward the center of branches or trunks.

Severely affected trees may have from one branch to the entire tree looking unthrifty with thin foliage. Sometimes, on newly planted trees the small tree becomes stunted with many lesions and new branches grow from buds below the affected part. Affected trees often have symptoms of boron deficiency on the leaves.

Cause

The disease in California is caused by the bacterium, *Xanthomonas campestris*, while a similar disease in South Africa has been described as being caused by the bacterium, *Pseudomonas syringae*.

Fig. 30. *Active lesion of Bacterial Canker.*

Fig. 31. *Inactive lesion of Bacterial Canker.*

Control

Normally, the disease appears to be a minor problem with no control necessary. If the disease is severe and yield is affected, the tree should be removed.

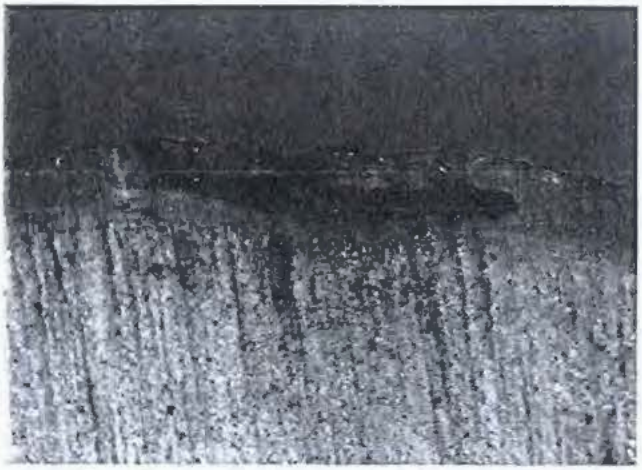

Fig. 32. *Cross section through a Bacterial Canker lesion.*

Fig. 33. *Bacterial Canker. Note the progression up the tree.*

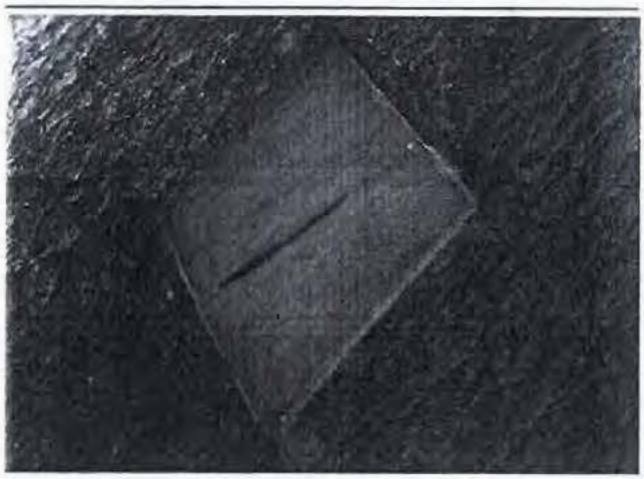

Fig. 34. *Necrotic streaks in the wood between Bacterial Canker lesions.*

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GOOD

AGRICULTURAL

PRACTICES

SELF-AUDIT

Contents

GOOD AGRICULTURAL PRACTICES SELF-AUDIT

This program is intended to assess your efforts to minimize the risk of fruit contamination by microbial pathogens and to ensure optimal quality.

Name: _____

Title: _____

Company Name: _____

Audit Site(s): _____

Main Address: _____

State: _____ Zip: _____ Telephone No: _____

Fax: _____ Email: _____

Date audit conducted: _____

Have you participated in Good Agricultural Practices (GAP) Training? Yes No

Is there a map that accurately represents your farm operations?
Yes No

Are all crop production areas located on this audit site?
Yes No

Other locations to be audited:

_____

_____

Total acres farmed (owned, leased, contracted, etc.):_____

List farm products:_____

Conditions under which an automatic "unsatisfactory" should be assessed:

- Produce is grown or harvested under conditions that promote or cause contamination, presenting an immediate food safety risk.
- The presence or evidence of rodents, or animal or human fecal waste in the orchard or at fruit transportation staging areas.
- Observation of employee practices (personal or hygienic) that jeopardize or may jeopardize the safety of the produce.

Note: **D** in the **DOC** column indicates documentation must be available to receive points for that item.

I. GENERAL QUESTIONS

	Implementation of a Food Safety Program	Yes	No	N/A	DOC
1	A documented fruit quality/safety program incorporating Good Agricultural Practices has been implemented.	15	0		D
2	A specific individual has been designated to implement and oversee the food safety program. Name: _____	15	0		
3	GAP self-audits are performed at least once every six months.	10	0		D
4	A third-party GAP audit is performed at least once per year. Name of auditing firm used: _____ Score and rating of last audit: _____	10	0		
5	Farm personnel are knowledgeable of the proper use of all pre-harvest chemicals.	10	0		
6	A documented employee food safety training program is in place.	10	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

	Worker Health and Hygiene	Yes	No	N/A	DOC
7	Potable (drinkable) water is available to all workers.	10	0		
8	Training on proper sanitation and hygiene is provided to all staff.	15	0		D
9	Readily understandable signs are posted to instruct employees to wash their hands before beginning or returning to work.	10	0		
10	Employees are required to wash their hands before beginning or returning to work.	10	0		
11	All employees and all visitors to the location are required to follow proper sanitation and hygiene practices.	15	0		
12	Employees and visitors are following good hygiene/ sanitation practices.	10	0		
13	All toilet/restroom facilities are clean and properly supplied with single-use towels, toilet paper, hand soap or anti-bacterial soap, and potable water for handwashing.	15	0		
14	Smoking and eating are confined to designated areas separate from where produce is handled.	10	0		
15	Workers with diarrheal disease or symptoms of other infectious disease are prohibited from handling fresh produce and from handling materials and equipment that might come in contact with fresh produce.	15	0		

	Worker Health and Hygiene	Yes	No	N/A	DOC
16	There is a written policy describing procedures for handling/disposition of produce or food contact surfaces that have come into contact with blood or other body fluids.	15	0		D
17	Workers are instructed to seek prompt treatment with clean first aid supplies for cuts, abrasions, and other injuries.	5	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

Comments:

Implementation of food safety program:

Worker Health & Hygiene:

Total points received for GENERAL QUESTIONS: _____

Total Possible = 200
Less "N/A" _____
Adjusted Total: _____
x .7 (70%) _____ **Passing Score**
Your Score: _____

PASS: Y N

II. FARM REVIEW

	Water Usage, Sewage Treatment, and Soils	Yes	No	N/A	DOC
18	What is the source of irrigation water? (Pond, Stream, Well, Municipal, Other/Specify)				
19	How are trees irrigated? (Flood, Drip, Sprinkler, Other/Specify)				
20	Water quality is known to be adequate for irrigation method and/or chemical application.	15	0		
21	If appropriate, water quality is tested annually.	10	0		
22	If necessary, steps are taken to protect irrigation water from potential contamination.	15	0		
23	The farm sewage treatment system is functioning properly and there is no evidence of leaking or runoff.	15	0		
24	There is no municipal/commercial sewage treatment facility adjacent to the farm.	10	0		
25	Groves are not located near or adjacent to dairy or livestock production facilities.	10	0		
26	Measures are taken to restrict access of livestock to the source or delivery system of irrigation water.	5	0		
27	Measures are taken to deter wild or domestic animals from entering avocado groves.	5	0		

	Water Usage, Sewage Treatment, and Soils	Yes	No	N/A	DOC
28	Previous land use history indicates that there is a minimum risk of produce contamination.	5	0		
29	When previous land use history indicates a possibility of produce contamination, soils have been tested for contaminants and land use is commensurate with test results.	5	0		D
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

	Manure and Municipal Biosolids	Yes	No	N/A	DOC
30	Manure lagoons are maintained to prevent leaking or overflowing.	10	0		
31	Raw manure is not used as a soil amendment.	10	0		
32	When raw manure is applied, it is at least at least 120 days prior to harvest.	10	0		
33	Manure or biosolids are properly treated, composted or exposed to environmental conditions that lower the expected level of pathogens.	10	0		
34	Manure or biosolids are properly stored prior to use.	10	0		
35	If composted animal manure or treated biosolids are used, records are maintained showing that they are properly composted, such as certifications or Standard Operating Procedures for composting.	5	0		D

	Manure and Municipal Biosolids	Yes	No	N/A	DOC
36	Measures are taken to minimize recontamination of treated manure or biosolids.	10	0		
37	Records of organic and non-organic fertilizer applications are kept and available for review.	5	0		D
38	Controls are in place to prevent indirect contamination from raw animal manure from adjacent properties.	5	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

	Pesticide Management	Yes	No	N/A	DOC
39	Pesticide applicator number:				
40	Person responsible for permits:				
41	Pesticide application records are on file with county.	15	0		
42	All applicable county, state and federal regulations are followed for pesticide usage.	15	0		
43	Records are maintained for pesticide usage on crops.	10	0		D
44	An independent party is used to test for pesticide compliance.	5	0		
45	Employees are knowledgeable regarding proper use of restricted and/or regulated chemicals, pesticides, fungicides, etc. that are applied pre-harvest production phase.	10	0		

	Pesticide Management	Yes	No	N/A	DOC
46	Pesticides are applied by fully trained applicators.	5	0		D
47	Proper apparel for pesticide application is provided.	5	0		
48	Pesticides are used according to label instructions.	10	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

Comments:

Water Usage, Sewage Treatment, and Soils

Manure and Municipal Biosolids

Pesticide Management

Total points received for FARM REVIEW: _____

Total Possible = 245
Less "N/A" _____
Adjusted Total: _____
x .7 (70%) _____ **Passing Score**
Your Score: _____

PASS: Y N

III. FIELD HARVESTING ACTIVITIES

	Worker Sanitation and Hygiene	Yes	No	N/A	DOC
49	A management program is in place to identify potential contamination risks during the growing and harvesting season.	5	0		D
50	The farm has documented procedures to address sanitation requirements during growing and harvesting.	5	0		D
51	The number, condition, and placement of field sanitation units comply with applicable state and/or federal regulations.	10	0		D
52	Field sanitation units are cleaned and serviced on a scheduled basis and at a location that minimizes the risk for product contamination.	10	0		D
53	Field sanitation units are directly accessible for spills, major leaks and servicing.	10	0		
54	A response plan is in place in the event of a major spill or leak of field sanitation units.	5	0		D
55	Field sanitation units are properly supplied with single use towels, toilet paper, hand soap or anti-bacterial soap and potable water for hand washing.	10	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

	Field Harvesting and Transportation	Yes	No	N/A	DOC
56	If pesticides were used during crop production, treatment records are checked before picking fruit to ensure that all preharvest intervals (chemical withholding periods) required by law have been adhered to.	15	0		D
57	Fruit is picked only when completely dry.	5	0		
58	Fruit that has been in direct contact with the ground (including windfalls and fruit on low-hanging branches) is handled seperately.	15	0		
59	Punctured or rodent-damaged fruit is discarded.	10	0		
60	Harvested fruit is placed directly into bins or laid on tarps. Fruit is never placed directly on the ground.	5	0		
61	If fruit is laid on tarps, tarps are sterilized or replaced frequently.	15	0		
62	Workers are instructed not to stand in field bins.	10	0		
63	Tables, totes, bins, and other harvesting containers are cleaned or sanitized prior to use.	5	0		
64	Damaged or soiled containers are repaired or disposed of.	5	0		

	Field Harvesting and Transportation	Yes	No	N/A	DOC
65	Field bins are high-pressure washed, rinsed, and sanitized before reuse.	5			
66	Clean bins that are not being used immediately are covered to prevent contamination by birds and/or animals.	5			
67	Clippers are treated with alcohol, bleach solution, or quaternary ammonium compounds during breaks.	5			
68	Harvesting equipment which comes into contact with produce is kept as clean as practicable.	5	0		
69	Farm workers are instructed not to use harvesting containers, totes, etc. for carrying or storing non-produce items.	5	0		
70	Water applied to harvested product is potable.	10	0		
71	Efforts are made to remove dirt, mud, twigs, and leaves from fruit and/or containers before sending to the packing facility.	5	0		
72	Transportation equipment used to move produce from field to packing operation or storage and which comes into contact with produce is clean.	10	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

Comments:

Worker Sanitation and Hygiene

Field Harvesting and Transportation

Total points received for FIELD HARVESTING ACTIVITIES:

_____

Total Possible = 190
Less "N/A" _____
Adjusted Total: _____
x .7 (70%) _____ **Passing Score**
Your Score: _____

PASS: Y N

IV. ORCHARD MANAGEMENT

	Rot and Disease Management	Yes	No	N/A	DOC
73	Dead fruit, branches, and leaves are regularly removed from the canopy.	15	0		
74	A mechanical mulcher/chipper is used to speed the breakdown of pruning wood and windfall fruit on the orchard floor.	10	0		
75	Before planting, soil is prepared to allow for good drainage.	15	0		
76	In heavy clay soil, trees are planted on mounds or ridges to allow drainage.	10	0		
77	Only certified disease-free nursery stock is planted.	15	0		
78	Tensiometers or other tools are used to schedule irrigation.	5	0		
79	Irrigation water from reservoirs and canals is treated with chlorine to eliminate inoculum.	5	0		
80	Warning signs are placed between infected and uninfected orchards.	5	0		
81	Boxes containing copper sulfate are placed at grove entrance and all foot traffic is required to dust shoes before entering the grove.	5	0		
82	Shallow chlorinated or copper sulfate-treated water baths are placed at grove entrance for vehicles to drive through when entering the premises.	5	0		

	Rot and Disease Management	Yes	No	N/A	DC
83	After use in a diseased orchard, all equipment (shovels, soil augers, trowels, etc.) is sanitized before reuse.	10	0		
84	Pruning, cutting and injections tools are treated with 15% bleach solution between trees.	10	0		
85	Before grafting, a lab test is run to detect sunblotch viroid in graft wood.	10	0		
86	Fruit on the ground is removed and discarded.	15	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

	Insect, Vertebrate Pest and Snail Control	Yes	No	N/A	DC
87	A qualified Pest Control Advisor or University of California Farm Advisor is consulted before applying pesticides.	15	0		
88	Brush and woodpiles are cleared from in and around orchards.	10	0		
89	Pet food is stored in rodent-proof containers and leftover food is removed from pet dishes.	10	0		
90	Garbage containers are kept tightly covered.	10	0		
91	During first year of growth, tree trunks are protected with 1-inch mesh wire to prevent damage from deer and rabbits.	5	0		

	Insect, Vertebrate Pest and Snail Control	Yes	No	N/A	DOC
92	Groves are fenced in to keep wildlife and pets out.	10	0		
93	Brown garden snails are treated using decollate snails or chemicals (e.g., iron phosphate or metaldahyde).	5	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

Comments:

	Harvesting and Field Handling	Yes	No	N/A	DOC
94	Fruit is picked only when air temperatures are below 90 °F.	15	0		
95	At temperatures near 90 °F, bins are transported to the packing facility as quickly as possible.	10	0		
96	Harvested fruit is covered with leaves or a screen (not burlap) to protect from sunburn.	10	0		
	TOTAL POINTS RECEIVED FOR THIS SECTION:				

Total points for ORCHARD MANAGEMENT:

_____

Total Possible = 235
Less "N/A" _____
Adjusted Total: _____
x .7 (70%) _____ **Passing Score**
Your Score: _____

PASS: **Y** **N**

SECTION	PASS	SCORE	FAIL	SCHEDULED RE-TEST DATE
I. General Questions				
II. Farm Review				
III. Field Harvesting Activities				
IV. Orchard Management				

WORKSHEET 1
PATHOGEN REDUCTION CHECKLIST FOR COMPOST*

Ask your compost supplier for the following information:

1. Percentage and physical make-up of the composted material:
   _____
   _____
2. Date the compost process was started _____
3. Daily temperature readings of 131 degrees Fahrenheit or higher? _____
4. 15 Days or longer at 131 degrees Fahrenheit for windrow composting? _____
5. Windrows turned a minimum of 5 turnings? _____
6. Microbiological testing conducted? (E. coli < 1,000 MPN/gram and Salmonella < MPN/4 grams; "MPN" means "Most Probable Number) _____

*** This form is only a sample and should be modified by the appropriate technical experts and legal advisors to meet the needs of your particular operation.**

WORKSHEET 2
FIELD SANITATION AND WORKER HYGIENE CHECKLIST*

Ranch Name/Location: _____

Field Sanitation	**Yes**	**Description**
Condition of Field Toilets		
A. Correct number of toilet facilities for male and female workers		
B. Close proximity to employees (1/4 mile or 5 minutes)		
C. Clean and sanitary facilities		
D. Documentation of maintenance and sanitation		
1. Average number of employees per week		
2. Number of field toilets in use		
3. Frequency of cleaning		
4. Procedure for maintenance and sanitation		
E. Provisions for regularly checking toilet paper		
Worker Hygiene	**Yes**	**Description**
A. Written training procedures		
1. Frequency and content of training		
B. Document information on hand washing		
1. Daily rinse and clean of wash water tanks		
2. Daily replenishment of water		
3. Source of handwashing water		
4. Sign indicating "For Hand Washing Purposes Only"		

Worker Hygiene	Yes	Description
C. Procedure for providing and replenishing daily		
1. Clean hand washing water		
2. Soap		
3. Single purpose towels		
Drinking Water Policy	**Yes**	**Description**
A. All drinking water potable		
B. Single use cups provided		
C. Drinking water changed daily		
D. Water containers rinsed and cleaned daily		
E. Document source of water		
Medical Leave and Illness	**Yes**	**Description**
A. Written medical leave and illness reporting policy		

*This form is only a sample and should be modified by the appropriate experts and legal advisors to meet the needs of your particular operation.

WORKSHEET 3
FIELD SANITATION MAINTENANCE LOG*

Grower/Ranch
Location: _____

Beginning Date: _____ Through End Date: _____

Week Ending	# of Employees	# of Units	Checked By	Sanitation Frequency

*This form is only a sample and should be modified by the appropriate experts and legal advisors to meet the needs of your particular operation.

WORKSHEET 4
FIELD SANITATION HYGIENE SUPPLIES*

Grower/Ranch
Location: ______________________________

Beginning Date: __________ Through End Date __________

Date	Hand-wash Water	Soap	Paper Towels	Toilet Paper	Checked By
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
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	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	
	Check/ Refill	Check/ Refill	Check/ Refill	Check/ Refill	

*This form is only a sample and should be modified by the appropriate technical experts and legal advisors to meet the needs of your particular operation.

WORKSHEET 5
HARVEST TOOL CLEANING CHECKLIST*

Ranch Location: ____________________ Date: __________

Are tools being maintained so as to remain free of damage such as ragged edges?	Yes	No
Is there a regular repair/inspection program to periodically fix or replace damaged tools?	Yes	No
Are the tools kept clean of extraneous materials such as tape?	Yes	No
Are stations available for the tools to be cleaned and dipped in sanitizing solution periodically during the day?	Yes	No
Is the sanitizer concentration verified and documented in a log?	Yes	No
Gloves are not to be used as a substitute for hand washing. Is there a handwashing program in place? Is it being followed?	Yes	No
Are gloves maintained in a clean and sanitary manner?	Yes	No
Do workers know that gloves and harvesting tools are not to be taken into the toilet facility?	Yes	No
Are gloves cleaned and rinsed periodically during the harvest day?	Yes	No
Is there a program to replace heavily soiled or damaged gloves on a routine basis?	Yes	No

*** This form is only a sample and should be modified by the appropriate technical experts and legal advisors to meet the needs of your particular operation.**

WORKSHEET 6
WATER WORKSHEET*

Grower/Ranch Location:_____

Water Source:	Irrigation Water	Pesticide & Foliar Applica-tion	Hand Washing Water	Drinking Water
Source: Capped well _____ Uncapped well _____	Yes/No	Yes/No	Yes/No	Yes/No
Open Source: canal, reservoir, pond, etc.	Yes/No	Yes/No	Yes/No	Yes/No
Source: Municipal district water	Yes/No	Yes/No	Yes/No	Yes/No
Drip irrigation	Yes/No	Yes/No	Yes/No	Yes/No
Overhead irrigation	Yes/No	Yes/No	Yes/No	Yes/No
Flood irrigation	Yes/No	Yes/No	Yes/No	Yes/No
Filtration system location (attach diagram)	Yes/No	Yes/No	Yes/No	Yes/No
List Applicable Dates for Each Category				
Capped Well Annual Test				
Uncapped water source (well, canal, reservoir) quarterly test				
Municipal District quality Report				
Corrective action & date taken (chlorinate, disinfect, filter, etc.)				
List potential risks from adjacent land (attach additional sheets as necessary)				

*** This form is only a sample and should be modified by the appropriate technical experts and legal advisors to meet the needs of your particular operation.**

WORKSHEET 7
DRINKING WATER CHECKLIST*

Grower/Ranch Name: _____

Beginning Date: _____ **through Year End Date:** _____

Date	Drinking Water	Cups	Water Container	Checked by
	Check/Refill/ Change	Check/Refill	Rinse/Clean	

***This form is only a sample and should be modified by the appropriate technical experts and legal advisors to meet the needs of your particular operation.**

WORKSHEET 8
WORKER TRAINING DOCUMENTATION*

Date:_____

Grower:_____

Topics Discussed:_____

Trainer(s):_____

Attended by:

Attendee's Name	Attendee's Signature

*This form is only a sample and should be modified to meet the needs of your particular operation.

WORKSHEET 9
EMPLOYEE TRAINING DOCUMENTATION*

Employee:_____

Position:_____

Hire Date:_____

Training Date	Topic of Discussion

*This form is only a sample and should be modified to meet the needs of your particular operation.

ANEXO 2

POSTULACION PROYECTO COLABORACIÓN PACIFIC RIM PROJECT

Pacific Rim Pre-Proposal

Applicant Information
Dr. Mary Lu Arpaia
Dept. of Botany and Plant Sciences
University of California, Riverside
CA 92521
559-646-6561 Office
559-646-6563 FAX
arpaia@uckac.edu

Project Title:

International Research Collaboration and Student Training to Manage Avocado Productivity

Amount Requested: $12,300

Duration: 1 year

Co-investigators:

Mary Lu Arpaia (% responsibility: 25%)
Dept. of Botany and Plant Sciences, UC Riverside, CA 92521, 559-646-6561 (office), 559-646-6563 (FAX), arpaia@uckac.edu

Robert L. Heath (% responsibility: 25%)
Dept. of Botany and Plant Sciences, UC Riverside, CA 92521, 951-827-5925 (office), 951-827-4437 (FAX), robert.heath@ucr.edu

Greg W. Douhan (% responsibility: 25%)
Dept. of Plant Pathology, UC Riverside, CA 92521, 951-827-4130 (office), 951-827-4294 (FAX), gdouhan@ucr.edu

Collaborators:

Alejandro Barrientos-Priego (% responsibility: 5%)
Departamento De Fitotecnia, Universidad Autónoma Chapingo. Km 38.5 Carretera México-Chapingo, Chapingo, Edo. De México. C.P. 56230; 011-595-9521569 (office), abarrien@gmail.com

Monica Castro (% responsibility: 5%)
Claudia Fassio Ortiz (% responsibility: 10%)
Department of Fruit Crops, School of Agriculture, Pontificia Universidad Católica de Valparaíso, Casilla 4-D, Quillota, Chile; 011-56-32-274560 (Office); mcastro@ucv.cl, frutales@ucv.cl

Grant Thorp (% responsibility: 5%)
HortResearch, Mt Albert Research Centre,Private Bag 92169, Auckland, New Zealand; 011-64-9-815-4200 ext. 7079 (Office), gthorp@hortresearch.co.nz

First Year Budget

Budget Item	Amount	Justification
TRAVEL		
Airfare	$6,900	A brief internet check of roundtrip Airfare to Chile varied from approximately $900 to $1600. We have estimated that airfare for UC Riverside PIs would be $1300 each; travel for Dr. Barrientos would be $1500 and for Dr. Thorp would be $1500
Food	$1,750	We would need to cover food costs for 3 PIs from Riverside, Dr. Barrientos and Dr. Thorp 5 people x 7 days x $50/day
Lodging	$3,500	We would cover the cost of lodging for 3 PIs from Riverside, Dr. Barrientos and Dr. Thorp 5 people x 7 days x $100/day
Local Transportaion	$0	Our collaborators at UCV would cover local transportation
TOTAL TRAVEL	$12,150	
MEETING/CONFERENCES		
Misc. expenses	$150	Miscellaneous costs associated with preparation of workshop report
TOTAL REQUEST	**$12,300**	

Budget and grants officers' information

Department Contact:
Juliet Cheung, 951-827-4435, juliet.cheung@ucr.edu
Campus Contracts and Grants Officer:
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Other sources of support: None at this time

Abstract

The University of California has been the preeminent research institution in the development of new avocado varieties and rootstocks for the last several decades. Commercial avocado production in the Pacific Rim has been expanding at a rapid rate with the major goal of marketing the fruit in the United States. Mexico, Chile and New Zealand are the major off-shore suppliers of 'Hass' avocados to the US. These countries face the same production problems related to productivity as does the California industry. Overall tree health and vigor, related to rootstock tolerance to disease, salinity and pests are a key to overcoming productivity issues. Additionally, an improved variety that possesses less alternate bearing characteristics and greater tolerance to environmental stresses is desirable.

We propose organizing a planning workshop in Chile that will bring together the researchers involved in avocado research in the Pacific Rim region. Our intent is to discuss mutual research programs, identify potential students in Chile and Mexico and plan a coordinated research effort to further research and graduate student training that will benefit the research institutions involved as well as further knowledge on avocado.

Project Narrative Keywords: avocado, rootstock, research, graduate student training, productivity

Description of Project

Until recently, the major supplier of fresh avocado to the US market has been California. Since the 1970's the preeminent variety has been 'Hass', a variety which originated in California. Beginning in the later 1980's the importation of 'Hass' avocado was allowed from Chile into the United States. This has been followed in recent years by both Mexico and New Zealand. The market presence of avocado fruit from these countries has grown immensely during the last 10 years to the point that California fruit now accounts for less than 50% of the US market. As the market share for these countries has increased and thus the importance of this crop to the overall economy within the Pacific Rim, greater emphasis on research and graduate student training has occurred. Researchers in Mexico and Chile are conducting research that focuses on the factors which influence overall tree productivity. They are examining root health and the avocado's tolerance to disease, poor soil and water quality. This research complements the ongoing research programs conducted at the University of California, Riverside.

The major limiting factors to productivity and tree health in all countries are basically the same. Avocado root rot caused by *Phytophthora cinnamomi* is prevalent in all production areas. In Chile and California, water quality issues such as salinity and poor drainage also limit productivity. A logical approach to addressing these issues is the development of improved rootstocks. The ideal rootstock would be resistant to avocado root rot and other root diseases, tolerant of poor soils and salinity and have a positive influence on the bearing capacity of the 'Hass' avocado which accounts for the majority of commercial production in all countries. Additionally, in all countries there is renewed interest in high density plantings and canopy management to enhance production efficiencies. This means that another goal in rootstock and variety development in the future will be the development of less vigorous material that will be better adapted to these management schemes.

The Riverside campus has had a long standing program emphasizing rootstock development and the study of rootstock:varietal influences. The Plant Pathology department has been the leader in the development of rootstocks tolerant to avocado root rot. This program is considered to be the preeminent program internationally. In recent years, researchers in Chile and Mexico have also initiated research programs to identify and characterize rootstocks for enhanced performance. This research compliments the efforts underway at the Riverside campus.

We are proposing to organize a research planning meeting in Chile with the intent to develop the educational and research capacity of institutions studying the avocado. We would like to hold the planning meeting in Chile since the cooperating institution, Pontificia Universidad Católica de Valparaíso, has several students now focused on avocado improvement. Professor Castro and

Ms. Fassio Ortiz recently received a multi-year grant from the Chilean government that is focused on rootstock selection and improvement for the Chilean industry (see cv of collaborators). A meeting in Chile, therefore, would allow California researchers to assess progress being made in that country in terms of improved rootstocks and would allow us also to interact with students involved in that program.

The overall goal of the meeting would be focused on discussing potential collaborative research as well as developing a graduate student exchange program between the cooperating institutions. Collaboration in this area would help to further our knowledge on avocado, hasten the development of solutions to production problems and enhance graduate student experience. The meeting would include interactions with students working on avocados at the Pontificia Universidad Católica de Valparaíso and field visits to research plots.

The project collaborators all have extensive experience in avocado. Dr. Arpaia has been the Cooperative Extension Specialist for subtropical fruit crops, including avocado since 1983. During this time she has conducted research on the response of avocado to salinity and participated in rootstock screening trials. She also currently oversees the avocado varietal improvement program for UC. Dr. Heath, has been conducting research with Dr. Arpaia that is focused on understanding the response of the 'Hass' avocado to environmental stresses including water relations between the rootstock and scion. Dr. Douhan recently came to Riverside to oversee the avocado rootstock development program. Dr. Barrientos-Priego in Mexico is considered one of the world leading experts in avocado germplasm rescue and also conducts research examining the interaction of rootstock and scion in terms of water relations and tree vigor. Our collaborators in Chile, Professor Castro and Ms. Fassio-Ortiz, oversee the rootstock program underway in Chile. Ms. Fassio-Ortiz recently completed a Master's degree in Chile focused on rootstock development and characterization of water relations between the rootstock and scion. A portion of her research was undertaken on the Riverside campus under the direction of Drs. Heath and Arpaia (Summer 2005). Dr. Thorp from HortResearch in New Zealand has extensive experience in avocado. His research emphasis has been to understand the growth habit of the avocado and the mechanisms of dwarfing. In recent years his research emphasis has shifted to training systems for kiwifruit and apples. His understanding of production systems is crucial for the future development of management systems for avocados under high density planting schemes.

Curriculum Vitae (See attached pages)

CURRICULUM VITAE

Dept. of Botany and Plant Sciences, University of California, Riverside, Riverside, CA 92521
***Mailing address:** UC Kearney Agricultural Center, 2940 S. Riverbend Ave., Parlier, CA 93648, (559) 646-6561; FAX: 559-646-6593, e-mail: arpaia@uckac.edu*

EDUCATION:

B. A.	Botany	1975	University of California, Berkeley, CA
M. S.	Horticulture	1980	University of California, Davis, CA
Ph.D.	Plant Physiology	1985	University of California, Davis, CA

PROFESSIONAL EXPERIENCE:

Dept. of Botany and Plant Sciences, University of California, Riverside, CA 92521

1983-1988 Associate Extension Specialist, Subtropical Horticulture

1988- present Extension Specialist, Subtropical Horticulture

Duties: Development of extension and field research programs dealing with subtropical horticulture. Extension activities include farm advisor training and educational outreach to the subtropical fruit industries of California. Field research includes evaluation of preharvest and postharvest factors on subtropical crop productivity and fruit quality, including rootstock, cultivar, irrigation, pesticide, and nutrition management strategies.

HONORS AND AWARDS:

- Non-Senate Distinguished Research Award for 1991-1992, University of California, Riverside, CA
- Co-Recipient, (with G. Witney, G. Bender, M. Freeman, B. Faber, C. Kallsen, N. Sakovich, N. O'Connell and R. Neja) American Society for Horticultural Science, Extension Education Aids Award, July 1995 for "Subtropical Fruit News"
- Co-Recipient, (with G. Bender, B. Faber, M. Freeman, C. Kallsen, N. Sakovich, N. O'Connell, P. Mauk) American Society of Agronomy, 1996 Educational Materials Contest, Newsletter Category for "Subtropical Fruit News", November 1996
- Co-Recipient (with J. Morse, R. Metcalf and R. Rice), Recognition for Outstanding Service, February 1997 (presented by the California Avocado Commission to the UC Center for Exotic Pest Research)
- Co-Recipient, (with G. Bender, B. Faber, M. Freeman, C. Kallsen, N. Sakovich, N. O'Connell, P. Mauk) American Society of Agronomy, 1998 Educational Materials Contest, Newsletter Category for "Subtropical Fruit News", October 1998
- Recipient, Art Schroeder Memorial Award, October 2001 (presented by the California Avocado Commission, Production Research Committee)
- Recipient, Citrus Research Board commendation for Service, July 2002

SELECTED PUBLICATIONS (LAST 5 YEARS):

Arpaia, M. L. 2000. Enhancement of Avocado Productivity. I. Plant Improvement – Selection and evaluation of improved varieties and rootstocks. Calif. Avocado Research Symposium, Oct. 14, 2000. p. 7-16.

Fetscher, A. E., T. Davenport, S. Shafir, A. Dag, N. Waser, M. L. Arpaia, 2000. A review of avocado pollination and the role of pollinizers. Subtropical Fruit News. 8(1-2): 21-25.

Arpaia, M. L., D. Boreham and R. Hofshi. 2001. Development of a new method for measuring minimum maturity of avocados. California Avocado Society 2001 Yearbook. 85:153-178.

Arpaia, M. L., A. E. Fetscher, R. Hofshi. 2001. Avocado Flowering Basics. AvoResearch 1(2):4-5.
Dodds, J. A., d. Mathews, M. L. Arpaia, G. W. Witney. 2001. Recognizing Avocado Sunblotch Disease. AvoResearch. 1(3): 2 page Insert. Oct. 2001.
Crowley, D. E., W. Smith, B. Faber, M. L. Arpaia. 2001. Trace Metal Nutrition of Avocado. AvoResearch 1(3):6-8, 12. Oct. 2001.
Fetscher, A. E., R. Hofshi, N. Waser, M. L. Arpaia. 2001. Pollinizers and Pollinators – Do they make a difference? AvoResearch 1(2):1-2.
Mickelbart, M. V., R. Miller, S. Parry, M. L. Arpaia and R. Heath. 2001. Avocado leaf surface morphology. California Avocado Society 2000 Yearbook. 84: 139-150.
Oster, J.D., D. Stottlemyer and M.L. Arpaia. 2001. Production function method logy describes the salinity effects on avocado yields and water use. Proc. 2001 California Plant and Soil Conference, California Chapter of American Society of Agronomy and California Plant Health Association, Feb. 7 - 8, 2001. Fresno Ca. p146.
White, A., A. Woolf and M. L. Arpaia. 2001. Long term storage of 'Hass' avocado using 1-MCP. Perishables Handling Quarterly. No. 108 (November 2001): 21 – 23.
Liu, X., J. S. Sievert, M. L. Arpaia, and M. A. Madore. 2002. Postulated physiological roles of seven-carbon (C7) sugars, mannoheptulose and perseitol, in avocado. J. Am. Soc. Hort. Sci. 127(1):108-114.
Mickelbart, M. V. and M. L. Arpaia. 2002. Rootstock influences changes in ion concentrations, growth, and photosynthesis of 'Hass' avocado trees in response to salinity. J. Am. Soc. Hort. Sci. 127(4):649-655.
Oster, J. D. and M. L. Arpaia. 2002. Setting TMDL's for salinity and chloride based on their effects on avocado (Hass) productivity. In: J. C. McGahan (ed.). Proceedings. Helping Irrigated Agriculture Adjust to TMDLs. Sacramento, California, October 23-26, 2002. p. 241-252. US Committee on Irrigation and Drainage, Denver, CO.
Dag, A., A.E. Fetscher, O. Afik, Y. Yeselson, A.A. Schaffer, Y. Kamer, N.M. Waser, M.A. Madore, M.L. Arpaia, R. Hofshi, and S. Shafir. 2003. Honey bee (*Apis mellifera*) strains differ in avocado (*Persea americana*) nectar foraging preferences. Apidologie. 34(3):299-310.
Woolf, A., C. Clark, E. Terander, V. Phetsomphou, R. Hofshi, M. L. Arpaia, D. Boreham, M. Wong, and A. White. 2003. Measuring Avocado Maturity; Ongoing Developments. The Orchardist, May 2003, Vol 76(4): 40-45.
Arpaia, Mary Lu and John A. Menge. 2004. Mejoramiento de la productividad del palto. Mejoramiento de plantas: selección y evaluación de variedades y portaninjertos mejorados (Enhancement of avocado productivity. (Plant improvement: selection and evaluation of improved varieties and rootstocks). In Proceedings of 2nd Seminario Internacional de Paltos, 29 September – 1 October, 2004, Sociedad Gardiazabal y Magdahl Ltda., Quillota, Chile. 10 pages.
Arpaia, M. L., D. Stottlemyer, L. Bates, W. Manor, K. Fjeld, J. Sievert, and E. Focht. 2004. Enhancement of Avocado Productivity. Plant Improvement: Selection and Evaluation of Improved Varieties and Rootstocks. Proceedings of the California Avocado Research Symposium, October 30, 2004. University of California, Riverside. California Avocado Commission p. 9-23.
Heath, R. L., M. L. Arpaia and M. V. Mickelbart. 2004. Avocado Tree Physiology – Understanding the Basis of Productivity. Proceedings of the California Avocado Research Symposium, October 30, 2004. University of California, Riverside. California Avocado Commission p. 65-88.
Woolf A. B., C. L. Requejo, K. A. Cox, R. C. Jackman, A. Gunson, M. L. Arpaia, and A. White. 2005. 1-MCP Reduces Physiological Storage Disorders of 'Hass' Avocados. Postharvest Biology and Technology. 35: 43–60.
Arpaia, M. L., G. S. Bender and G. W. Witney. 200__. Performance of avocado clonal rootstocks under non-Phytophthora conditions. J. Am. Soc. Hort. Sci. (Manuscript in preparation).
Mickelbart, M. V., P. Robinson, X. Liu, G. W. Witney and M. L. Arpaia. 200__. Development of a phenological model for 'Hass' avocado under southern California conditions. J. Am. Soc. Hort. Sci. (Manuscript in preparation).

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Education

B.Sc. California Institute of Technology, Pasadena, California in Physics. (1961)
M.Sc. University of Michigan, Ann Arbor, Michigan in Physics. (1963)
Ph.D. University of California, Berkeley, California in Biophysics. (1967)

Research Experience

Post-doctor Fellow at Brookhaven National Laboratory, Upton, New York with Geoffrey Hind (1967-1969)
Visiting Research Associate at University of California, Davis, California with Al Tappel (1975-76)
Visiting Scientist at University of York, Heslington, York, U.K. with Rachel Leech (1976)
Visiting Professor at Shefield University , Shefield, York, U.K. with David Walker, FRS (1981-82)
Visiting Professor at Lancaster University, Lancaster, Lancs., U.K. with Alan Wellburn (1991-92)

Work Experience

At University of California, Riverside, CA
Assistant Professor; Department of Life Sciences & of Biochemistry; Oct. 1969 to June 1973
Associate Professor; Department of Biology & of Biochemistry; July 1973 to June 1979
Associate Dean, Student Affairs; College of Natural & Agricultural Sciences; July 1981 to September 1988
Executive Associate Dean; College of Natural & Agricultural Sciences; September 1988 to June 1990
Associate Director; Statewide Air Pollution Research Center; July 1990 to Sept. 1992
Professor; Department of Botany & Plant Sciences; July 1979 to present

Selected Publications

Heath, R. L., R. M. S. Hurd, and M. A. Madore. 1990. A generalized photosynthetic model for plant growth within a closed artificial environment. Soc. Automotive Eng. Paper No. 901331. 14 p.
Heath, R. L. 1991. A canopy model for plant growth within a growth chamber: mass and radiation balance for the above ground portion. Soc. Automotive Eng. SAE Paper No. 911494. 14 p. {Also published as: Heath, R. L. 1991. A canopy model for plant growth

within a growth chamber: mass and radiation balance for the above ground portion. Soc. Automotive Eng. Transactions-J. Aerospace. 14 p.}

Guralnick, L. J., R. L. Heath, G. Goldstein, and I. P. Ting. 1992. Fluorescence quenching in the varied photosynthetic modes of Portulacaria afra (L.) Jacq. Plant Physiol. 99: 1309-1313.

Guzy, M. R., and R. L. Heath. 1993. Responses to ozone in common bean varieties. New Phytol. 124: 617-625.

Heath, R. L. 1994. Possible mechanisms for the inhibition of photosynthesis by ozone. Photosyn. Res. 39: 439-451.

Heath, R. L. 1994. Alteration of plant metabolism by ozone exposure, p. 121-146. In Alscher, R. and A. R. Wellburn (eds.), Plant Responses to the Gaseous Environment. Chapman and Hill, London.

Ceccardi, T. L., R. L. Heath, and I. P. Ting. 1995. Low-temperature exotherm measurement using infrared thermography. HortScience 30: 140-142.

Heath, R. L. 1996. Lipid metabolism and oxidant air pollutants, p. 353-374. In Iqbald, M. and M. Yunus (eds.), Plant Response to Air Pollution. John Wiley & Sons, Chichester, UK.

Heath, R. L. 1996. The modification of photosynthetic capacity induced by ozone exposure. In Baker, N. R. (ed.), Photosynthesis and the Environment. Advances in Photosynthesis Series, Kluwer Acad., Berlin, p. 409-433.

Heath, R. L. 1996. Oxidant induced alteration of carbohydrate production and allocation in plants. In Byterowicz, A., M.J. Arbaugh, S.L. Schiling (eds.), Proceedings of the International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems. U.S. Dept. of Agric., Forest Service, Washington, DC. General Technical Report PSW-GTR-166, p. 11-18.

Briggs, W. R., R. L. Heath, R. L. and E. M. Tobin (eds.). 1996. Regulation of Plant Growth and Development by Light. Proc. 18th Ann. Symp. Plant Physiol., January 18-20, 1996. Amer. Soc. Plant Physiol., Rockville, MD. (Dr. Heath organized the meeting; Drs. Heath, Briggs, and Tobin edited the manuscripts.)

Sandermann, H., A. R. Wellburn, and R. L. Heath. 1997. Forest decline and ozone: synopsis. In Sandermann, H., A. R. Wellburn, and R. L. Heath (eds.), Forest Decline and Ozone: A Comparison of Controlled Chamber and Field Experiments. Springer Verlag, Heidelberg, Germany. Pp 369-378.

Heath, R. L. and G. E. Taylor, Jr. 1997. Physiological processes and plant responses to ozone exposure. In Sandermann, H., A. R. Wellburn, and R. L. Heath (eds.), Forest Decline and Ozone: A Comparison of Controlled Chamber and Field Experiments. Springer Verlag, Heidelberg, Germany. P. 317-368.

Sandermann, H., A. R. Wellburn, and R. L. Heath (eds.). 1997. Forest Decline and Ozone: A Comparison of Controlled Chamber and Field Experiments. Ecological Studies No. 127. Springer Verlag, Heidelberg, Germany, 400 pp. (Dr. Heath organized U.S. authors, edited original manuscripts, and helped proof galleys

Heath, R. L. 1999. Biochemical processes in an ecosystem: how should they be measured? Water, Air, and Soil Pollution 116: 279-298.

Mickelbart, M. V., R. Miller, S. Parry, M. L. Arpaia and R. Heath. 2001. Avocado leaf surface morphology. California Avocado Society 2000 Yearbook. 84: 139-150.

Greg W. Douhan
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Education

Ph.D., Plant Pathology, May 2001. Washington State University, Pullman, WA.
M.S., Plant Pathology, May 1998. Washington State University, Pullman, WA.
B.S., Botany and Biology, May 1995. Humboldt State University, Arcata, CA.

Appointments

Assistant Professor, University of California, Riverside, CA (7/2005- present)
Post Doctoral Associate, University of California, Davis, Ca (6/2001-6/2005)

Teaching Experience

Instructor; General Mycology. University of California, Davis, CA (Fall Quarter, 2002)
Teaching Assistant. Washington State University, Pullman, WA. (8/1996-1999)
Foray Leader. University of Idaho Community Enrichment Program (Spring and Fall, 1996-2000)

Conferences

Arthur M. Sackler Colloquia of the National Academy of Sciences, 2004. Irvine, CA
Ecological Society of America, 2004. Portland, OR. Invited speaker
Mycological Society of America (MSA), 2004. Asheville, North Carolina. Oral presentation
International Congress of Mycorrhizae 4, 2003. Montreal, Canada. Oral presentation
MSA, 2003. Alsilomar, CA. Oral presentation/poster
MSA, 2002. Corvallis, OR. Oral presentations/poster
American Phytopathological Society (APS) and MSA, 2001. Salt Lake City, UT. Oral presentation
APS National Meeting, 2000. New Orleans, LA. Oral presentation
APS Society Pacific Division Meeting, 1999. Riverside, CA. Oral presentation
APS Pacific Division Meeting, 1997. Fort Collins, CO. Oral presentation

Honors

First place: student paper competition, APS Pacific Division Meeting, 1999. Riverside, CA.
Second place: student paper competition, APS Pacific Division Meeting, 1997. Fort Collins, CO.
Travel grant awarded from Washington State University Graduate School. Summer 1997.
Scholarship from the Lake Okaboji Environmental Agency to conduct independent undergraduate research at the Iowa Lakeside Laboratory, Summer 1994. University of Iowa, IA.

Publications

Douhan, G. W. and Rizzo, D. M. 2005. Phylogenetic divergence in a local population of the ectomycorrhizal fungus *Cenococcum geophilum*. New Phytologist 166: 263-271

Douhan, G. W., Peterson, C., Bledsoe, C., and Rizzo, D. M. 2005. Contrasting root associated fungi of three common oak-woodland plant species based on molecular identification: host specificity or non-specific amplification? Mycorrhiza 15: 365-372

Bergemann, S. E., Douhan, G. W., Garbelotto, M., and Miller, S. L. 2005. No evidence of population structure across three sub-populations of *Russula brevipes* in an oak/pine woodland in the western Sierra region isolated by distances of 230 - 1090 m. New Phytologist (in press)

Caswell-Chen, E. P., Chen, J., Lewis, E. E., Douhan, G. W., and Carey, J. R. 2005. Revising the standard wisdom of C. elegans natural history: ecology of longevity. SAGE KE 40: 30

Kerrigan, J. L., Smith, M. T., Rogers, J. D. Poot, G. A. and Douhan, G. W. 2003. *Ascobotryozyma cognata*, a new ascomycetous yeast species associated with nematodes from wood-boring beetle galleries. Mycological Research 107:1110-1120

Douhan, G. W. and Rizzo, D. M. 2003. Host-parasite relationships among bolete infecting *Hypomyces* species. Mycological Research 107: 1342-1349

Douhan, G. W. and Rizzo, D. M. 2003. Amplified Fragment Length Microsatellites (AFLM): A method to develop microsatellite markers in organisms with limited amounts of DNA applied to Arbuscular Mycorrhizal (AM) fungi. Mycologia 95: 368-373 (Erratum 96:196)

Douhan, G. W., Murray, T. D., and Dyer, P. S. 2003 Genetic structure of *Tapesia acuformis* in Washington, USA. Phytopathology 93:650-656

Douhan, G. W., Murray, T. D., and Dyer, P. S. 2002. Species and mating-type distribution of *Tapesia yallundae* and *Tapesia acuformis* and occurrence of apothecia in the U.S. Pacific Northwest. Phytopathology 92:703-709

Douhan, G. W., Peever, T. L., and Murray, T. D. 2002. Mulitlocus population structure of *Tapesia yallundae* in Washington State. Molecular Ecology 11: 2229-2239

Douhan, G.W. and Murray, T. D. 2001. Infection of winter wheat by a ß –Glucuronidase – transformed isolate of *Cephalosporium gramineum*. Phytopathology 91:232-239

Dyer, P. S., Furneaux, P. A., Douhan, G. W., and Murray, T. D. 2001. A multiplex PCR test for determination of mating-type applied to the plant pathogens *Tapesia yallundae* and *Tapesia acuformis*. Fungal Genetics and Biology 33:173-180

Curriculum vitae

Alejandro F. Barrientos-Priego

Gender: Male
Status: Married
R.F.C: BAPA-611201UU2
Professional IDE: Num. 1171415
Birth date: December 1, 1961
Maximum studies: Doctorado en Ciencias – Fisiología Vegetal
Home Address: Oyamel 10, San Miguel Tlaixpan, Texcoco, Edo. de México. C.P. 56240. TEL. (595) 952-70-18
Office: TEL . (595) 9521569 and FAX (595) 952-15-69
E-mail.: abarrien@gmail.com and abarrien@correo.chapingo.mx

Description: Professor and Researcher C-2 full time of the DEPARTAMENTO DE FITOTECNIA, UNIVERSIDAD AUTÓNOMA CHAPINGO. Km 38.5 CARRETERA MÉXICO-CHAPINGO, CHAPINGO, EDO. DE MÉXICO. C.P. 56230

Research area: new cultivars and rootstocks of avocado, physiology of fruit crops, avocado genetic resources, plant propagation and general fruit crop management.

Coordination of groups:

Coordinator of the Avocado Network for Avocado Genetic Resources of the National Plant Genetic Resources System (SINAREFI), SNICS-DGVDT-SAGARPA, México, since 2004. Chairman del Technical Working Party for Fruit Crops de la INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS (UPOV), since September 2005.

Coordination of International Documents:

Avocado Descriptor for IPGRI.
Test Guidelines for Avocado of UPOV

Recognition as a National Researcher

Investigador Nacional Nivel I (Level I Researcher); Sistema Nacional de Investigadores (Nacional Researcher System SNI-SEP CONACYT- 2004 - 2008), México.

Participation in Thesis Committees

Bachelor Thesis: 44
Master in Science Thesis: 6
Ph. D. Thesis: 5

Congress Participations: **29**

Papers in Congress Proceedings: **11**

Abstracts in Congress: **40**

Paper in Journals: **55.**

Some Relevant Publications of the Area:

1. BARRIENTOS P., A. F.; M. W. BORYS; AND F. BARRIENTOS P. 1986. ROOTING OF AVOCADO CUTTINGS (*PERSEA AMERICANA* MILL.) CVS. FUERTE AND COLÍN V-33. CALIFORNIA AVOCADO SOCIETY YEARBOOK 70: 157-165.
2. LOPEZ J., A. AND A. BARRIENTOS P. 1987. SELECTION OF DWARFING ROOTSTOCKS OF AVOCADO (*PERSEA AMERICANA* MILL.). I. STUDIES OF BARK:XYLEM RELATIONSHIP IN TRUNKS OF CV. COLÍN V-33 SEEDLINGS. CALIFORNIA AVOCADO SOCIETY YEARBOOK 71: 225-234.
3. BARRIENTOS PRIEGO, A. F.; BARRIENTOS PEREZ, F.; SANCHEZ COLÍN, S.; Y J. J. AGUILAR MELCHOR. 1991. UTILIZACION DEL HUERTO-VIVERO PARA REDUCIR LA FASE JUVENIL DE PLÁNTULAS DE AGUACATE (*PERSEA AMERICANA* MILL.). PROC. INTERAMERICAN SOCIETY FOR TROPICAL HORTICULTURE 35: 18-22.
4. ALEJANDRO F. BARRIENTOS PRIEGO; AVRAHAM D. BEN-YA'ACOV; LUIS LÓPEZ LÓPEZ; GEBHARD BUFLER; MICHAL W. BORYS. 1995 DESCRIPTORS FOR AVOCADO (*PERSEA AMERICANA* MILL.). INTERNACIONAL PLANT GENETIC RESOURCES INSTITUTE (IPGRI-FAO), ROME, ITALY. 52 P.
5. ALEJANDRO F. BARRIENTOS-PRIEGO; LUIS LÓPEZ-LÓPEZ. 2000. HISTORIA Y GENÉTICA DEL AGUACATE., PP. 19-31. *IN*: EL AGUACATE Y SU MANEJO INTEGRADO. D. TÉLIZ, H. GONZÁLEZ, J. RODRÍGUEZ, R. DROMUNDO (EDS.). MUNDI-PRENSA MÉXICO, S.A. DE C.V. D.F., MÉXICO.
6. ALEJANDRO F. BARRIENTOS-PRIEGO; RODOLFO MUÑOZ-PÉREZ; MICHAL W. BORYS; MA. TERESA MARTÍNEZ-DAMIÁN. 2000. CULTIVARES Y PORTAINJERTOS DEL AGUACATE., PP. 35-54. *IN*: EL AGUACATE Y SU MANEJO INTEGRADO. D. TÉLIZ, H. GONZÁLEZ, J. RODRÍGUEZ, R. DROMUNDO (EDS.). MUNDI-PRENSA MÉXICO, S.A. DE C.V. D.F., MÉXICO.
7. REYES-SANTAMARÍA, I.; TERRAZAS, T.; BARRIENTOS-PRIEGO, A.F.; AND TREJO, C. 2002. XYLEM CONDUCTIVITY AND VULNERABILITY IN CULTIVARS AND RACES OF AVOCADO. SCIENTIA HORTICULTURAE 92(2): 97-105.
8. BARRIENTOS-PRIEGO, A. F.; BORYS, M.W.; TREJO, C.; LÓPEZ-LÓPEZ, L. 2003. ÍNDICE Y DENSIDAD ESTOMÁTICA FOLIAR EN PLÁNTULAS DE TRES RAZAS DE AGUACATERO. REVISTA FITOTECNIA MEXICANA 26(4): 285-290.
9. ANDRÉS-AGUSTÍN, J.; NIETO-ÁNGEL, A.; BARRIENTOS-PRIEGO, A. F.; MARTÍNEZ-DAMIÁN, M. T.; GONZÁLEZ-ANDRÉS, F.; SEGURA-LEDESMA, S. D.; CRUZ-CASTILLO, J .G .; GALLEGOS-VÁZQUEZ, C. 2004. MORFOMETRÍA DE HOJA DEL CHIRIMOYO PARA DIFERENCIAR SELECCIONES Y CULTIVARES. REVISTA CHAPINGO SERIE HORTICULTURA 10(2): 103-110.
10. NUNEZ-COLÍN, C. A.; RODRÍGUEZ-PÉREZ, J. E.; NIETO-ÁNGEL, R.; BARRIENTOS-PRIEGO, A. F. 2004. CONSTRUCCIÓN DE DENDOGRAMAS DE TAXONOMÍA NUMÉRICA MEDIANTE COEFICIENTE DE DISTANCIA χ^2; UNA REVISIÓN. REVISTA CHAPINGO SERIE HORTICULTURA 10(2): 229-237.
11. NÚÑEZ-COLÍN, C. A.; BARRIENTOS-PRIEGO, A. F.; RODRÍGUEZ-PÉREZ, J. E.; NIETO-ÁNGEL, R. 2006. VARIABILIDAD ANATÓMICA DE LOS SISTEMAS DE CONDUCCIÓN Y ESTOMÁTICO DENTRO Y ENTRE GENOTIPOS DE *PRUNUS* SPP. DE DIFERENTES ORÍGENES. PESQUISA AGROPECUÁRIA BRASILEIRA (EN PRENSA).

CURRICULUM VITAE

ANTECEDENTES PERSONALES

NOMBRE	MÓNICA BEATRIZ CASTRO VALDEBENITO	
Fecha de Nacimiento	09.09.1962	Nacionalidad: Chilena
Cédula Identidad		

ESTUDIOS SUPERIORES

Título/Grado	Otorgado por	Lugar	Año
INGENIERO AGRÓNOMO	UNIVERSIDAD CATÓLICA DE VALPARAÍSO	CHILE	1987
MAGISTER EN CIENCIAS AGROPECUARIAS, CON MENCIÓN EN PRODUCCIÓN AGRÍCOLA	UNIVERSIDAD DE CHILE	CHILE	1993

ANTECEDENTES ACADEMICOS

Departamento al que pertenece	FRUTICULTURA		
Cargo Actual	JEFE DE DOCENCIA		
Jerarquía Académica	PROFESOR ADJUNTO		
Año de Ingreso U.C.V.	1988	Nº de hrs. Contractuales	JORNADA COMPLETA

INVESTIGACIONES EN QUE HA PARTICIPADO (Últimos 4 años)

Año	Nombre del Proyecto y fuente de financiamiento	Calidad
1997-2001	Introducción y evaluación de nuevas variedades de cítricos para exportación. FIA C97-2-A-047	2
1997-2000	Establecimiento de un Programa de Saneamiento de Cítricos y formación de un Banco de Razas de Virus y Viroides. FONDECYT 1971002.	2
1998-2002	Propagación del Azafrán. FIA C98-1A-051.	1
1998	Micropropagación *in vitro* de *Rhododendron* sp. DGI 242.785/98	1
1999 - 2002	Prospección de razas severas y áfidos vectores del virus de la tristeza en los cítricos presentes entre la I y VII región de Chile. FONDO-SAG V1-15-0199	3
2000-2003	Generación de Tecnologías para la producción intensiva y orgánica de chirimoyo. FONDEF 1056	2
2000 - 2001	Determinación de una nueva raza de viroide de la cachexia que afecta a los cítricos en Chile. DGI 242.792/2000	2
2002-2007	Metodología para mejorar el proceso productivo del níspero japonés y sus posibilidades de exportación en fresco e industrializado incrementando su valor económico y social. FONDEF D01I1053	2
2002-2003	Prospección y selección de portainjertos tolerantes a distintas condiciones de estrés (químico, físico y ambiental) y de variedades de palto con mejores características agronómicas que Hass en distintas zonas agroecológicas de Chile. DI UCV 242.799/2002	1

2002-2005	Programa de introducción, selección y producción de portainjertos y variedades de palto en Chile. FONDEF D01I1054	1
2005	Evaluación agronómica y propagación de nuevos portainjertos y variedades de palto en distintas zonas agroclimáticas de Chile	1

Calidad: 1=Jefe de Proyecto 2=Coinvestigador 3=Colaborador

PUBLICACIONES (Últimos 5 años)

Año	Título y Revista
2001	Potencialidad del azafrán. Empresa y avance agrícola 91: 3-5
2002	Buscando un ancla a tierra. Revista del Campo, El Mercurio, Diciembre.6-8
2002	Situación Nacional de Portainjertos de Palto y su relación con factores de productividad y precocidad. Seminario Internacional "Selección y Uso de Portainjertos y Nuevas variedades de Palto"
2003	Portainjertos de palto: la mitad escondida. Avance agrícola 111:15-16
2003	Programa de introducción, selección y propagación de portainjertos y variedades de paltos en Chile. Avance agrícola, Número especial Día de la palta: 6-7.
2003	Cultivo de tejidos vegetales y su aplicación en frutales. Avance agrícola 116:10-11.
2003	Determinación de rangos de variabilidad en los niveles de producción de palto cv Hass sobre portainjertos de semilla de raza mexicana en Chile. Proceeding V Congreso Mundial del Aguacate. 155-160.
2003	Desarrollo de técnicas para la copia de árboles de palto sobresalientes en Chile. Proceeding V Congreso Mundial del Aguacate. 123-128.
2004	Portainjertos De Palto: Importancia de la Anatomía De Los Vasos Conductores. Revista Empresa y Avance Agrícola.120:18-19
2005	Production variability among Hass avocado trees grafted onto Mexican rootstocks. South African Avocado Growers' Association 2005 Yearbook.Vol 28. 48-50

CURRICULUM VITAE

Claudia Carola Fassio Ortiz

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EDUCATION:

Degree in Science	1998	Pontificia Universidad Católica de Valparaíso, Chile
Agronomist Engineer	1998	Pontificia Universidad Católica de Valparaíso, Chile
M. S.	2005	Pontificia Universidad Católica de Valparaíso, Chile

PROFESSIONAL EXPERIENCE:

1998-1999 Professor Assistant Horticultural. Pontificia Universidad Católica de Valparaíso, Chile
Invited Professor optative course: Special topics in fruit physiology. Pontificia Universidad Católica de Valparaíso. Chile
Professor course: Introduction to Agriculture. Pontificia Universidad Católica de Valparaíso. Chile

1999-2000 Assistant extension office of the Agronomy Faculty, Pontificia Universidad Católica de Valparaíso, Chile.
Project lieder of subtropical fruit Easter Island. CORFO

2000-2003 Assistant research in project: "Generation of technologies for intensive and differentiated cherimoya production" FONDEF D01I1056

2002-2005 Manager and Assistant research in project: "Program of introduction, selection and propagation of avocado rootstocks and varieties". FONDEF D01I1054

SELECTED PUBLICATIONS (LAST 5 YEARS):

Revista del campo: Portainjertos de Paltos: Buscando un ancla a tierra. 2002.6-8
Magazine of the field. Avocado rootstocks: Looking for an anchor to land. 2002.6-8

Articulo "Portainjertos de Paltos: La mitad escondida". Revista Empresa y Avance Agrícola. 2003.111:15-16
Avocado Rootstocks: the hidden half. Journal: Business and Agricultural Advance. 111:15-16

Portainjertos De Palto: Importancia de la Anatomía De Los Vasos Conductores. Revista Empresa y Avance Agrícola. 2004.120:18-19.

Avocado Rootstocks: The importance of the xylem vessel anatomy. Journal : Business and Agricultural Advance. 120:18-19.

Desarrollo de técnicas para la copia de árboles de palto sobresalientes en Chile. Memorias Congreso Mundial del Aguacate. España.2003.123-128

Development of techniques for the copy of outstanding trees in Avocado. Memories World-wide Congress of the Avocado. España.2003.123-128

Programa de introducción, selección y propagación de Portainjertos y Variedades de paltos en Chile. Memorias Congreso Mundial del Aguacate. España.2003.120-121

Program of introduction, selection and propagation of avocado rootstocks and varieties in Chile. Memories World-wide Congress of the Avocado. España.2003.120-121

Determinación de rangos de variabilidad en los niveles de producción de paltos cv. Hass sobre portainjertos de semilla de raza Mexicana en Chile. Memorias Congreso Mundial del Aguacate. España.2003.155-160

Determination of variability in the levels of production in avocados. Memories World-wide Congress of the Avocado. España.2003.155-160

Programa de introducción, selección y propagación de Portainjertos y Variedades de paltos (aguacates) en Chile. Revista RELAFRUT, Cuba. 2004.7

Program of introduction, selection and propagation of avocado rootstocks and varieties in Chile". Magazine RELAFRUT, Cuba. 2004.7

Variabilidad de la producción de Hass sobre portainjertos Mexicanos. Yearbook Asociación de productores de palto de Sud Africa. Vol 28. 48-50.

Production variability among Hass avocado trees grafted onto Mexican rootstocks. South African Avocado Growers' Association 2005 Yearbook.Vol 28. 48-50

Curriculum Vitae

Full name: **Dr Grant Thorp**

Present position: Scientist

Present employer: The Horticultural and Food Research Institute of New Zealand Ltd

Present work address: HortResearch, Mt Albert Research Centre
Private Bag 92169, AUCKLAND

Academic qualifications:

1980	BSc (Auckland) Botany
2003	PhD (Adelaide) Horticultural Science

Years as a practising researcher: 26

Professional positions held:

2003 – present	Scientist, HortResearch, Mt Albert Research Centre, Auckland
1989 – 2002	Undertaking PhD studies in Adelaide (HortResearch Study Leave)
1982 – 1989	Technician, HortResearch, Mt Albert Research Centre, Auckland
1980 – 1982	Technician, HortResearch, Te Puke Research Centre

Honours/distinctions/membership of societies, institutions, committees:

Foundation member, New Zealand Society for Horticultural Science (NZSHS)
Committee Member for Auckland Section NZSHS
Member International Society for Horticultural Science (ISHS)

1985 CIES Scholarship, French Government
1987 Clark/Fletcher Memorial Bursary, New Zealand Fruitgrowers' Federation
1987 Lincoln College Foundation Award, Lincoln College
1989 Queen Elizabeth II Technicians Study Award
1989 DSIR Study Award
1990 Australian Postgraduate Research Award
1993 Travel Award, New Zealand Fruitgrowers' Federation Charitable Trust
1995 NZ/USA Cooperative Science Program Award
1995 New Zealand Horticultural Science Advancement Trust Award
2002 HortResearch Chairman's Award for Outstanding Achievement

Present research/professional speciality:

Orchard and plantation systems. Canopy management and influence of preharvest cultural practices on preharvest and postharvest fruit quality in fruit crops. Rootstock and root system effects on plant growth and productivity. Plant architecture. International aid programmes. Avocado (*Persea americana*) and feijoa (*Acca sellowiana*) germplasm research.

Number of refereed publications: 24

Number of books: 1

Number of patents: 1 ('Opal Star' feijoa)

Number of significant publications not included in the above:

41 Industry Publications, 70 HortResearch client reports.

Research achievements

I have a high profile in New Zealand's horticultural industries for my expertise in plant architecture and the development of pruning systems for fruit trees. My recent research has extended to studies on how changes in plant architecture and canopy management affect the postharvest storage quality of fruits. Several scientific papers have been published on this research, with results presented to conferences and industry groups in Australia, Brazil, California, Chile, Italy, Mexico and Israel. Each year I will prepare at least 3 major commercial reports on projects undertaken on behalf of the New Zealand kiwifruit industry.

Significant outcomes from this research have been the wide scale adoption of new pruning systems for kiwifruit and avocado; development of quantitative models of avocado tree growth to support orchard management decisions; characterisation of plant effects on fruit mineral composition and the susceptibility of kiwifruit and avocado to storage disorders; characterisation of rootstock responses in apple trees, towards the identification of the apple "dwarfing" gene; and the use of reflective ground covers to improve fruit quality and productivity in persimmons and kiwifruit.

Current projects include the development of sustainable production systems for avocados in New Caledonia; establishment of the new yellow-fleshed kiwifruit cultivar 'Hort16A' in California and Italy; and implementation of pruning systems to increase carbohydrate partitioning to developing kiwifruit.

Major Publications (in last five years)

Books

Thorp, T.G. and Bieleski, R.L. 2002: Feijoas: Origins, Cultivation and Uses. David Bateman Ltd., Auckland: 87pp.

Scientific

Thorp, T.G.; Ferguson, I.B.; Boyd, L.M. and Barnett, A.M. 2003 Fruiting position, mineral concentration and incidence of physiological pitting in 'Hayward' kiwifruit. Journal of Horticultural Science and Biotechnology 78: 505-511.

Ferguson, I.B.; Thorp, T.G.; Barnett, A.; Boyd, L.M. and Triggs, C.M. 2003 Inorganic nutrient concentrations and physiological pitting in 'Hayward' kiwifruit. Journal of Horticultural Science and Biotechnology 78: 497-504.

Seleznyova, A.N.; Thorp, T.G.; White, M.; Tustin, S. and Costes, E. 2003: Application of architectural analysis and AMAPmod methodology to study dwarfing phenomenon: the branch structure of 'Royal Gala' apple grafted on dwarfing and non-dwarfing rootstock/interstock combinations. Annals of Botany 91: 665-672.

Thorp, T.G.; Barnett. A.M. and Miller, S.A. 2003: Effects of cane size and pruning system on shoot growth, flowering and productivity of 'Hayward' kiwifruit vines. Journal of Horticultural Science and Biotechnology 78 (2): 219-224.

Seleznyova, A.N.; Thorp, T.G.; Barnett A.M.; Costes, E. 2002: Quantitative analysis of shoot development and branching patterns in Actinidia. Annals of Botany 89: 471-482.

Miller, S.A.; F.D. Broom, T.G. Thorp, A.M. Barnett. 2001: Effects of leader pruning on vine architecture, productivity and fruit quality in kiwifruit (Actinidia deliciosa cv. Hayward). Scientia Horticulturae (91): 189-199.

Thorp, T.G., A. Barnett and J.D. Toye 2000: Harvesting light in persimmon and kiwifruit orchards with reflective ground covers. Acta Horticulturae 557: 363-368

Thorp, T.G. and Stowell, B. 2000: Effect of pruning height and selective limb removal on yield of large 'Hass' avocado trees. HortScience, 36 (4).

Thorp, T.G. and Hallett, I. 1999: Searching for "paradise" in the avocado germplasm. Revista Chapingo Serie Horticultural Vol 5 Nium. Especial: 29-35.