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COLLECTIONS OF PERSEA IN CENTRAL AMERICA AND MEXICO FOR DISEASE RESISTANCE TESTS

GEORGE A. ZENTMAYER[1]

PHYTOPHTHORA ROOT ROT, a disease caused by the soil-inhabiting fungus *Phytophthora cinnamomi,* in the presence of excess soil moisture, is the most serious problem facing avocado production in California. The disease has resulted in losses of an estimated 2500-3500 acres of valuable avocado land. The same disease occurs in other areas where avocados are grown, including Florida and Texas in the United States, Mexico, Honduras, Costa Rica, Puerto Rico, Peru, Argentina, South Africa, and Australia.

Phytophthora cinnamomi causes a similar root rot, or in some cases a crown rot or a stripe canker, on a number of other plants. The list of hosts includes over 100 species of plants, representing some 58 genera. Some of the more important and common plants affected are: pineapple, pine, rhododendron, camellia, azalea, cinchona, chestnut, heather, and cinnamon.

Phytophthora cinnamomi grows and produces its two spore stages (oospores and zoospores) in soil. The zoospores are swimming spores, produced in the presence of abundant soil moisture; they provide ready dissemination of the

1. Associate Plant Pathalogist, University of California, Citrus Experiment Station, Riverside, California.

fungus and infection of roots under wet soil conditions. Trees may be affected at any stage from seedlings in the nursery to mature trees; the majority of the trees affected in California are 10 years of age or older. Symptoms of the disease include small leaves with poor color, usually a gradual dieback of the top, reduction in fruit size and number (although affected trees in early stages of disease may set a very heavy crop), lack of new growth, and blackened and rotted feeder roots.

Numerous attempts have been made to control the disease in California, using various types of soil amendments, soil fungicides, fumigants, soil conditioners, careful irrigation practices. None of these measures has given adequate control, though the disease can be retarded by carefully irrigating the grove so that moisture does not accumulate around diseased trees, and under at least some soil conditions, by additions of alfalfa meal to the soil. Several fumigants will kill *P. cinnamomi* and are thus useful for treating nursery soil or eliminating the fungus from small isolated spots of infection. These materials are D-D at 100 to 150 gallons per acre, methyl bromide at 2 lbs. per 100 cu. ft. of soil, chloropicrin at 30 gallons per acre. Citrus, macadamia nut, cherimoya, and persimmon trees are immune or highly resistant to this disease, and from current indications can be safely used to replant land where avocados have been removed because of Phytophthora root rot.

One of the most promising approaches for control of a disease of this type is the use of resistant rootstocks; inasmuch as most cultivated avocados are budded or grafted on various rootstocks this approach is entirely feasible. This therefore is one of the primary phases of investigation on control of this disease in California. Preliminary results of testing several species of *Persea* from Mexico and Central America, provided by Dr. C. A. Schroeder of the University of California at Los Angeles, have already been published (6).

The avocado (*Persea americana* Miller and *P. americana* var. *drymifolia* (S. & C.) Blake), and closely related species of *Persea* are indigenous to Central America, Mexico, and possibly portions of South America. With the objective in mind of making collections for disease resistance tests

a trip was made to Honduras, Costa Rica, El Salvador, Guatemala, and Mexico in the summer of 1952. The emphasis on this trip was to make collections from trees growing in swampy locations or in very heavy, wet, poorly drained soils. Also, root cultures were made from all trees from which budwood or seed was collected, as well as from other trees, to determine the distribution of *P. cinnamomi* in Central America and Mexico. The ideal type of material for resistance would be a healthy tree growing in poorly drained soil in the presence of *P. cinnamomi*. Avocado trees growing naturally in very wet locations should have a better chance of resistance to this type of disease than similar specimens adapted to growing in dry or well-drained sites, regardless of the ocurrence of the fungus, however.

Locations for some of the collections were found by examining herbarium collections of Central American and Mexican species of *Persea* in the Chicago Natural History Museum, the Missouri Botanical Garden, and the University of California at Berkeley. From these herbarium collections locations were noted for specimens that had been collected in notably wet or swampy places. Similar data were provided from herbaria at the Smithsonian Institution, Gray Herbarium and the New York Botanical Garden, through the courtesy of the curators of these herbaria.

This paper presents the results of this trip, including information about specimens collected and root cultures made, and observations on avocado diseases as well as some diseases of other plants.

Budwood was collected in Costa Rica, El Salvador, Guatemala, Honduras, and Mexico from 42 trees of six species of *Persea* and of *Phoebe mexicana,* a related member of the Lauraceae. The species of *Persea* collected were: *P. americana, P. Donnell-Smithii, P. gigantea, P. Popenoei, P. Schiedeana,* and *P. Skutchii.* The summary of information on collections is presented in Table 1, at the end of this paper.

Root cultures were made from 106 trees, of the same species of *Persea,* of *Phoebe mexicana,* and of *Nectandra sinuata,* all genera of the Lauraceae. The fungus *Phytophthora cinnamomi* was recovered from 18 of these trees; the

summary of root collections and cultures is given in Table 2, at the end of this paper.

HONDURAS

Budwood was collected from 12 trees in Honduras representing five species of *Persea* (*P. gigantea, P. Donnell-Smithii, P. americana, P. Popenoei,* and *P. Skutchii*). Seeds were collected from *P. Skutchii,* fruits were not present on the other species in July or August.

Persea americana Miller — Specimens of this species were collected from small, young trees growing near the pass in the San Juancito mountains, Dept. Morazán, along the road to the San Juancito mine, at an elevation of approximately 2,000 m. The surface soil was fairly light in this region, but in some places it was underlain by heavy yellow clay.

Persea Donnell-Smithii Mez — Specimens of this species were collected in a small, swampy area in the mountains between Tegucigalpa and El Zamorano, west of Mt. Uyuca, Dept. Morazán. This same species was seen again in a swampy near Tactic, Guatemala; *Persea Donnell-Smithii* is one of the few species of Persea that grows in swamps, which is of significance with regard to resistance to the Phytophthora root rot disease. Trees in the Honduras location were restricted to the immediate vicinity of the swamp, and were growing in areas of standing water or in slowly running water. Specimens were all small, with the largest approximately 25 feet in height.

Persea gigantea Williams — This species was collected on top of Mt. Uyuca, Dept. Morazán. A number of large, old specimens of this species grow at or near the top of this mountain which is 2,000 m. in elevation. Inasmuch as this is a cloud forest habitat the trees are growing under wet conditions; however, there is a thick layer of humus in which root development is abundant, and the soil beneath the leaf mulch is light in texture.

Persea Popenoei Williams — This species was collected in the San Juancito mountains, Dept. Morazán. A number of large old trees of this species are found scattered through

the forest at an elevation of from 1,800 to 2,000 m. in this area; in some places the trees are growing in soil underlain by heavy yellow clay. The trees were in flower in early July; no fruits were present.

Persea Skutchii Allen — Collections of budwood and fruit of this species were made from a tree growing along the banks of the Río de la Orilla, Dept. Morazán, approximately one mile southwest of the Escuela Agrícola Panamericana. This species has beautiful shiny green foliage, and the small bluish-purple fruits (about the size of a pea) were abundant in July.

Budwood of another tree belonging in the *Persea americana* group was collected at the Lancetilla Experiment Station, near Tela on the north coast of Honduras, Dept. Atlantida. This is a type with very strong anise odor in the leaves, which grows wild in the hills near Lancetilla. This is a region of very high rainfall, averaging approximately 125 inches per year. This collection is thus of interest from the standpoint of adaptation to wet soils.

Materials for making laboratory cultures of roots were taken on the trip, in order to obtain more information on the distribution of the avocado root rot fungus (*Phytophthora cinnamomi*) in tropical America, and to provide a sound basis for selecting material with potential resistance to Phytophthora root rot. *Phytophthora cinnamomi* has been reported on avocados from Mexico and Costa Rica by Zentmyer (3), and on avocados in Honduras by Zentmyer and Popenoe (5). The only other host on which the fungus has been reported from Central America is cinchona.

Determinations of *P. cinnamomi* on avocado are made by planting small pieces of feeder roots on cornmeal agar. Roots are collected with a small quantity of soil to keep them from drying out before culturing, then are washed briefly in water, dipped briefly in 70 percent alcohol, blotted and placed on cornmeal agar in petri dishes. Within two to three days the fungus may be detected growing out from affected root, and then may be identified under a microscope.

In Honduras, cultures were made from roots of 38 wild and cultivated avocado trees and other species of *Persea,* and from *Nectandra sinuata,* a related tree of the family Lauraceae. Cultures were made from trees of the five species

of *Persea* listed under budwood collections in Honduras, as well as from a number of cultivated avocados in the plantings at the Escuela Agrícola Panamericana and at the Lancetilla Experiment Station. *Phytophthora cinnamomi* was recovered from 10 of the 38 trees; all were cultivated specimens, the fungus was not found on native species of *Persea* in Honduras. The fungus was recovered from one seedling of *Nectandra sinuata* in one of the plantings at the Escuela Agrícola Panamericana.

The avocado root rot disease was found to be causing serious damage in several plantings at the Escuela Agrícola Panamericana, involving nursery trees as well as those up to five years of age. The fungus *Phytophthora cinnamomi* was readily isolated from rotted small feeder roots and from lesions on larger roots of affected trees. Symptoms of the disease were similar to those seen on trees in California. The disease was found to be more prevalent in the heavier soils, which retain moisture for a longer period of time than the more well-drained sites, and thus favor development of the fungus. Seedlings as well as budded trees were affected.

Root rot was also causing much damage to the avocado planting at the Lancetilla Experiment Station. A number of trees, including the Pancho, Gottfried, and Lula varieties budded on seedlings presumably of the "West Indian" race were severely affected with the disease; *P. cinnamomi* was also recovered in cultures from this area.

OTHER AVOCADO DISEASES IN HONDURAS

At the Escuela Agrícola Panamericana a new and destructive seedling blight of avocados was observed, with A. S. Muller, in August, 1952 (4). According to Dr. Muller this same type of disease killed approximately 80 percent of the seedlings in the seed beds in 1951. This disease was found to be caused by another species of *Phytophthora,* probably *P. palmivora,* which Conover (1) has described as the causal agent of a seedling blight of avocados in Florida.

Symptoms depend on the stage of growth at which seedlings are affected. When seedlings were invaded at the time or shortly after the young shoots emerged from the sawdust, in which the seeds were planted, the shoots turned

brown and were killed within a few days. When invasion took place some distance up the stems, after the seedlings had reached a height of 6 to 12 inches, the obvious symptoms were a wilting of the terminal leaves, a bending over of the top from the point of invasion and eventual death of the seedling as the fungus progressed down the stem. The stems were invaded at any location, from the base to the terminal bud. Close inspection revealed definite cankers on the stems, with sporangia abundantly present on the cankered areas during periods of high humidity. Conditions were favorable during rainy periods for dispersal of sporangia in wind-driven rain. Sporangia on the avocado stems were papillate and ranged in size from 20 to 30 microns by 27 to 40 microns.

A black leaf spot, caused by the fungus *Meliola* was observed on *Persea Donnell-Smithii* in the swamp near Mt. Uyuca. Powdery mildew (*Oidium sp.*) was observed on leaves and small branches of large avocado seedlings growing near the Escuela Agrícola Panamericana. A smaller, less conspicuous leaf spot as yet unidentified was found on *Persea gigantea* and *P. Popenoei*. Scab (*Sphaceloma perseae*) was common on leaves and fruit of *Persea americana* (Guatemalan and West Indian races) at the Lancetilla Experiment Station, where it disfigured the fruits considerably.

Most of the laboratory work and examination of specimens collected in Honduras was done at the Escuela Agrícola Panamericana, and much use was made of the fine library and herbarium.

COSTA RICA

Budwood was collected from seven trees in Costa Rica, representing three species of *Persea* (*P. Schiedeana, P. Skutchii,* and *P. americana*), and from a tree of a related genus, *Phoebe mexicana*. Seed was collected from four trees, of *Persea americana, P. Skutchii,* and *Phoebe mexicana*. Budwood and seed collections were from the following species:

Persea americana Miller — Budwood and seed were collected from a healthy, vigorous tree at San Mateo (near Orotina, west of San José). Fruits were thin-skinned, green

but turning dark when mature, and 4 to 5 inches in length with a short neck; no anise odor in the leaves. This tree was in an area where many surrounding trees were affected with root rot; root cultures from this tree showed that *Phytophthora cinnamomi* had not yet invaded the root zone, however.

Persea Schiedeana Nees — This species is known locally as "Yás" in Costa Rica. Budwood collections were made from trees in the Cerro de la Carpintera, Prov. of Cartago, near the Finca Quirazú in the mountains between Tres Rios and Cartago. The species is abundant in this area, with some huge old trees observed. One old specimen had a trunk approximately four feet in diameter. One tree was growing in a good site for root rot resistance, in very heavy, wet soil, in a small swampy area. No mature fruits were found in July, a few small fruits were seen on some of the trees.

Persea Skutchii Allen — This species is common also in parts of the Cerro de la Carpintera, Prov. of Cartago. Budwood and fruits were collected from several specimens on the Finca Quirazú, from La Suiza and from a tree along the highway near Cartago. Large, spreading trees up to 24 inches in diameter were seen. In Costa Rica the habitat was not strictly streamside, but the trees were growing in a region of high rainfall. Fruits were abundant on some of the trees of *P. Skutchii* in mid-July.

Phoebe mexicana Meissn. — A small tree of this genus was observed along the highway west of Cartago. Striking features are the red leaf petioles, red fruit pedicels, and the cup-like calyx at the base of the fruit. Budwood and seeds were collected; fruits were abundant in mid-July.

Root cultures were made at the excellent laboratories at La Hulera, the U. S. D. A. Cooperative Rubber Plant Field Station at Turrialba, Costa Rica through the courtesy of Dr. E. P. Imle. Cultures were made from 19 wild and cultivated avocado trees of *Persea* spp., and of *Phoebe mexicana*. *Phytophthora cinnamomi* was recovered only from three seedling avocado trees (*Persea americana*) growing under semi-cultivated conditions at San Mateo, near Orotina. In this area Phytophthora root rot was an important problem. Several acres of large seedling avocado trees were

either dead or in various stages of the disease, with typical symptoms and ready isolation of *P. cinnamomi* from the dying roots. Avocado scab was common on fruit seen in the markets at Siquirres and Orotina.

EL SALVADOR

Collections of budwood and seeds were made from *Persea Schiedeana* in El Salvador, with two different types of this species represented. *Persea Schiedeana* is known as "Chupte" in El Salvador; the two types recognized are "Chupte negro" and "Chupte blanco". One collection was made on the grounds of the Centro Nacional de Agronomia, Santa Tecla, the other near the town of Santa Ana. The season for fruits of *P. Schiedeana* was nearly over in the latter part of July. Some very large trees of this species were observed.

No cases of avocado root rot were observed in El Salvador. Root cultures were made from six trees of *Persea americana* and *P. Schiedeana;* no *Phyphthora cinnamomi* was recovered in these cultures.

The fine laboratory facilities of the Centro Nacional de Agronomía, Santa Tecla were made available through the courtesy of the Director, Dr. J. Guiscafré-Arrillaga.

GUATEMALA

Budwood was collected from 16 trees in Guatemala, representing the species *Persea americana, P. Schiedeana,* and *P. Donnell-Smithii.* Seeds were collected from two specimens of *P. Schiedeana,* near Tactic and near Cobán; early August was the latter part of the season for fruits of this species in these localities.

Persea americana Miller — Collections of this species were made near the vicinity of San José Pinula, southeast of Guatemala City; and near San Pedro Jacopilas, north of Lake Atitlán, in the Departament of El Quiché. The collections made near San José Pinula were from large old trees of the "typical" Guatemalan race, with large oval fruits; fruits were not mature the latter part of July. The budwood collections from the area just north of San Pedro

Jacopilas were from trees growing in exceptionally heavy, wet, poorly-drained soil. These specimens were healthy but had not made rapid growth under the unfavorable soil conditions. Fruits were present on these trees but were immature in early August. These collections should be very promising from the standpoint of root rot resistance.

Persea Donnell-Smithii Mez—This species was collected in a large swamp near the village of Tactic. Numerous small trees of this species are scattered through the swamp, growing under exceedingly wet conditions. No fruits were seen on any of these trees in early August. This collection would seem to have good possibilities of root rot resistance.

Persea Schiedeana Nees—Budwood and seeds were collected from trees of this species near Tactic and Cobán, and budwood collections were made also from *P. Schiedeana* growing at Finca Chocolá. The collections in the vicinity of Tactic were in heavy, wet soil on the margin of the swamp in which *P. Donnell-Smithii* was collected; those from Cobán were also in heavy, wet soil, and those from Finca Chocolá from an area of high rainfall. This species is known as the "coyó" in Guatemala; it is particularly abundant in the hills near Cobán, with considerable variation evident in fruit and foliage characteristics.

Root cultures were made from 33 trees in Guatemala, including the three species of *Persea* mentioned above. *Phytophthora cinnamomi* was not recovered from any of these cultures, nor was there any evidence of avocado root rot in any of the sections of Guatemala visited. This fungus is known to be present in parts of Guatemala, however, having been previously reported on cinchona trees on which it causes a stripe canker (2). There is no report of *P. cinnamomi* on avocados in Guatemala. Cooperative research with Dr. E. F. Darley at Riverside, California, has shown that the isolates of *P. cinnamomi* from cinchona will attack avocado roots, so the absence of the disease on avocados in Guatemala to date is puzzling.

Headquarters in Guatemala were at the Instituto Agropecuario Nacional in Guatemala City, where fine laboratory facilities were made available through the courtesy of the Director, Dr. Roland C. Lorenz.

MEXICO

Budwood was collected from five trees of two species of *Persea* (*P. americana* and *P. Schiedeana*). Seeds were collected from four trees, representing the same two species of *Persea*.

Persea americana Miller—Budwood was collected from three trees of this species in the State of Veracruz, near the towns of Jalapa and Huatusco. The specimens found near Huatusco are particularly promising from the standpoint of Phytophthora root rot resistance; they were growing well in heavy, wet soil underlain with clay and *P. cinnamomi* was present on the roots. Some fruits were still present on these trees on August 11, fruits were small, green to reddish-green, oval, and were generally heavily infested with the avocado seed weevil. The leaves of these trees had no anise odor. The collection near Jalapa was from a tree growing in a very wet site; this tree had small, round, greenish to purple fruits and the leaves had the anise odor.

Persea Schiedeana Nees—Budwood was collected from two trees near Huatusco in the State of Veracruz. One of these had excellent possibilities of root rot resistance as it was growing in a wet location and *Phytophthora cinnamomi* was recovered from the roots of the healthy appearing tree. A few fruits were still remaining on the trees in this area in early August, but they were heavily infested with the seed weevil. This species, known locally as the "Chinini" is quite common in this locality in the State of Veracruz.

Root cultures were made from ten trees of *Persea americana* and *P. Schiedeana* in Mexico. *Phytophthora cinnamomi* was recovered from six of these. Two of these recoveries were from the healthy specimens of *Persea americana* and *P. Schiedeana* in the State of Veracruz, mentioned above in regard to budwood collections. The other four were from seedling *Persea americana* trees showing typical symptoms of Phytophthora root rot near the village of Tenancingo, in the State of Mexico. This latter area was visited with Ingeniero Humberto Rosado, Director de Agricultura of the State of Mexico, following an inquiry on the dying of avocados in this area by Governor Sánchez Colín.

An estimated 20,000 large seedling trees are affected with root rot in heavy, wet soils in this area; top and root symptoms were typical of root rot in California and *P. cinnamomi* was readily recovered from the small feeder roots of all trees sampled. The origin of this infestation is not known; trees have apparently been dying in this locality for a number of years. Trees in this area are not in uniform plantings but are found scattered along fence rows and irrigation ditches.

Headquarters for laboratory work and examination of cultures in Mexico were made at the excellent laboratories of the Rockefeller Foundation in Mexico City.

MATERIAL ESTABLISHED IN CALIFORNIA

Budwood collections made on this trip were sent to California in polyethylene bags via air mail or air express from the various locations in Central America. The Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, in July, 1952, made Los Angeles rather than San Francisco the port of entry for these shipments, thus facilitating importation of the materials. The budwood shipments were all fumigated with methyl bromide (usually 2 lbs. for 2 hours at 75° to 80° F.) by quarantine officials at the San Pedro quarantine station, then rushed to Riverside and the University of California at Los Angeles for budding or grafting onto various avocado stocks. The propagation was done by Robert J. Drake at Riverside and Ted Frolich at U.C.L.A. In spite of this prompt treatment, however, only five of the 41 budwood collections were established in California by March, 1953. Budwood collections which have become established in California are: *Persea americana* from Lancetilla, Honduras; *Persea americana* from near San Mateo, Costa Rica; two collections of *Persea americana* from near San Pedro Jacopilas, Guatemala; and *Persea Schiedeana* from Finca Chocolá, Guatemala.

The lack of establishment of many of the collections is believed the result of: (1) Fumigation injury, (2) Possible poor compatibility with the avocado in the case of some of the other species of *Persea,* such as *P. Skutchii, P.*

Donnell-Smithii, P. Popenoei; and (3) A combination of (1) and (2).

Further evidence is at hand of poor compatibility of *P. Skutchii* and *Phoebe mexicana* with the avocado. In March, 1953, Dr. John Carpenter kindly brought material of these species from Costa Rica to the quarantine station at San Pedro, California. Material was budded and grafted onto avocado seedlings at Riverside and U.C.L.A. but none of the propagations grew.

Fumigation experiments now under way at Riverside indicate that some of the wild species of *Persea,* notably *P. Schiedeana,* are severely damaged by the standard dosages of methyl bromide. Damage increases with the length of time elapsing after collection of the budwood. Quarantine officials have expressed interest in this work and in finding some means of reducing injury to budwood importations and at the same time preventing the introduction of harmful pests.

Seeds have been successfully germinated and established at Riverside from eight of the 13 seed collections made in Central America and Mexico. Seed collections which germinated successfully are: *Persea Skutchii* from Honduras and Costa Rica; *P. Schiedeana* from Cobán and Tactic, Guatemala and from El Salvador; *Persea americana* from Costa Rica. The Bureau of Entomology and Plant Quarantine has cooperated in facilitating the entry of seed, in a manner which provides no danger to the California avocado industry, by permitting fumigation of seed at El Paso, Texas, and subsequent shipment directly to California. Permission for the modification of past quarantine procedures for avocado seed importations was obtained in August, 1952.

Tests of the best types of packaging material for avocado budwood shipments have shown polyethylene to be effective in preserving budwood viability after a three-month storage period at 25° C. (77° F.). Pliofilm and Saran films were also excellent for from 4 to 6 weeks storage under the same conditions, but did not hold up quite as well at the end of the three-month period as did polyethylene. Cellulose acetate (a moisture-pervious type used to package avocado fruit) was very poor for budwood storage; sphag-

num moss and waxed paper was effective for 3 weeks with no added moisture, but rapidly dried out after longer storage. Figures on percentage of green weight lost by avocado budwood stored in the different materials are given in Table 3.

ACKNOWLEDGEMENTS

Deep appreciation is expressed to many people in Central America and Mexico who were helpful in many ways in facilitating the work. These include particularly Dr. Wilson Popenoe, Dr. L. O. Williams, Dr. A. S. Muller, and Dr. V. C. Dunlap of the United Fruit Company, Escuela Agrícola Panamericana and La Lima Research Station in Honduras; Dr. E. P. Imle, Dr. John Carpenter, and Mr. Robert Worley of the U.S.D.A. Cooperative Rubber Plant Field Station, Turrialba, Costa Rica; Dr. J. Guiscafré-Arrillaga of the Centro Nacional de Agronomía, Santa Tecla, El Salvador; Dr. F. LeBeau and Dr. Roland Lorenz of the Instituto Agropecuario Nacional, Guatemala City, Guatemala; and Dr. John Niederhauser and Dr. Lee Heidrick of the Rockefeller Foundation, Mexico City, Mexico. Also appreciation is due to those who propagated the material that was sent in to California: Robert J. Drake and William A. Thorn at Riverside, and Ted Frolich at U.C.L.A. A contribution by the California Avocado Society to aid in financing the trip is also gratefully acknowledged.

TABLE 1. Avocado collections in Central America and Mexico, 1952.

Country	Budwood	Seed	*PERSEA* *americana* B	S	*Popenoei* B	S	*Skutchii* B	S	*Schiedeana* B	S	*Donnell-Smithii* B	S	*gigantea* B	S
HONDURAS	12	1	3		3		1	1			2		3	
COSTA RICA	7*	4*	1	1			3	2	2					
EL SALVADOR	2	1							2	1				
GUATEMALA	16	2	11						3	2	2			
MEXICO	5	4	3	3					2	1				
Totals	42	12	18	4	3	0	4	3	9	4	4	0	3	0

* Budwood and seed of *Phoebe mexicana* collected also.

TABLE 2. Avocado root cultures from Central America and Mexico, 1952.

Country	Number of trees sampled	Number of trees with *Phytophthora cinnamomi*	Number of trees with root rot symptoms
HONDURAS	38	10	10
COSTA RICA	19	2	2
EL SALVADOR	6	0	0
GUATEMALA	33	0	0
MEXICO	10	6	4
Totals	106	18	16

TABLE 3. Moisture loss of avocado budwood stored at 25° C. in various materials.

Material	Percent of green weight lost after: 27 days	44 days
Saran	19.0	27.8
Pliofilm	10.5	21.4
Polyethylene	9.1	14.4
Cellulose acetate	61.9	71.3
Sphagnum and waxed paper	28.5	77.6

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