Gummosis, Die-back, and Fruit Rot Disease of Mango (*Mangifera indica* L.) Caused by *Physalospora rhodina* (B. & C.) Cke. in Puerto Rico

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INTRODUCTION

Mango trees (*Mangifera indica* L.) grow spontaneously and widespread in Puerto Rico on many types of soils and under varying ecoclimates. They produce high yields on the hills and coastal plains of the southern and western areas of the Island whereever a relatively dry environment permits good blossoming and setting of fruit. Like many other orchard plants, however, mangos are subject to attack by cryptogamic parasites which can be of considerable economic importance under certain circumstances.

Mangos grow vigorously in humid areas but young twigs, blossoms, and fruits usually are attacked by the anthracnose fungus, *Colletotrichum gloeosporioides* Penz., which impairs production of heavy crops.

Fawcett (*3*)[2] in 1909 reported *C. gloeosporioides* Penz., causing blossom blight, leaf spot and fruit rot in Puerto Rico, but no other diseases of mangos have been mentioned in the literature or studied under our environment.

Several mango varieties growing at the Fortuna Substation, near Ponce, were found in December 1965 showing symptoms of gummosis and die-back. A batch of grafted mango seedlings succumbed to die-back of both scions and root stocks in January 1966 and new seedlings had to be grafted.

Mangos are becoming an important crop on the South Coast of Puerto Rico. Recognition of this disease are essential for successful production and practical control of its various cultivars.

The following report concerns investigations of a gummosis and a die-back of some varieties of mango, the parasite responsible for the disease and its morphology and physiology, its host relationships, and the possibilities for its control.

DESCRIPTION OF SYMPTOMS

Diseased, grafted mango seedlings in the orchard were characterized by rapid die-back of scions. Primary sysmptoms occurred at the grafted and wounded areas. Necrosis of the affected tissues was evident, spreading lengthwise, up and down the scions and stocks. The soil of grafted plants

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[2] Italic numbers in parentheses refer to Literature Cited, p. 450.

had been sprinkled regularly with water from a hose, to favor union of scions and root-stock tissues.

Several trees, particularly of the varieties Jacqueline and Kent, were showing stem canker, twig blight and die-back of some top branches. New twigs occasionally developed below the infected areas. In some cases apparently new infections took place, causing the new growth to die back. Leaves of affected twigs were chlorotic and eventually abscissed.

Diseased trees produced abundant gum with subsequent formation of necrotic cankers at the sites where the gum was oozing out (fig. 1). Peeling

FIG. 1.—External symptoms of gummosis on mango variety Jacqueline: A, On main branch of a young tree and B, cankerous area at juncture of branch and stem.

the bark at such infected areas revealed discoloration of cambial and adjacent tissues. These tissues sometimes turned brownish or even black. At this stage of infection the lesions were spreading upward and rapidly along some of the branches and stems; the twigs eventually were invaded and killed. The infection progressed primarily forming black streaks up and down the stems with a lengthwise cracking and deterioration of the bark (fig. 2).

The symptoms on stems, branches, and twigs correspond very closely with those observed by Das Gupta and Zacchariah (*2*) in India, and by Batista (*1*) in Brazil, in connection with a die-back disease of mangos in their respective countries.

Fruits on necrotic twigs became mummified and some remained hanging on the trees. Infected, ripe fruits showed dark, spreading necrotic lesions over the skin. Fernando (*4*) reported a spot rot of mango fruits, the infection occurring through the peduncle, also a storage rot of mango; in both instances caused by *Botryodiplodia theobromae* Pat. (*Physalospora rhodina*).

The fruit rot symptoms described can be mistaken for those produced by the fungus *C. gloeosporioides*. However, close examination of infected peduncles and fruits, particularly of those kept at high relative humidity,

FIG. 1

reveal the presence of erumpent pycnidia in contrast with the formation of acervuli with pinkish spores as is the case with *C. gloeosporioides*.

ISOLATION STUDIES

THE PATHOGEN: *Physalospora rhodina* (B. & C.) CKE.

Diplodia theobromae (Pat.) Now. and its immature stage *Macrophoma* were found associated with the die-back disease of mango. The *Diplodia* stage was reported previously on sugarcane trash and cacao in Puerto Rico by Seaver and Chardón (*10*).

During the course of our investigations concerning die-back and fruit rot of mango, the *Diplodia* and *Chaetodiplodia* pycnidial forms frequently were obtained in culture from infected material (fig. 3).

FIG. 2. A, Exudate from an initial lesion on a stem and B, internal necrotic streaks on an area of heavy gumming.

The polymorphic nature of the organism probably accounts for the various names under which it has been described in the literature. Various authors have presented evidence showing that many of the imperfect forms reported are stages of one and the same organism, i.e., *Physalospora rhodina* (B. & C.) Cke. which is the perfect stage of *Diplodia theobromae* (Pat.) Now.

D. theobromae has been reported attacking: *Theobroma cacao* L., *Hevea*

FIG. 3.—Mature, 2-cell, dark pycnospores of *Diplodia* stage of *Physalospora rhodina*.

brasiliensis Muell., *Vanilla planifolia* Andr., *Ananas comosus* (L.) Merr., *Carica papaya* L., *Coffea arabica* L., *Cajanus cajan* (L.) Millsp., *Cattionella elastica* Cerv., *Cocos nucifera* L., *Manihot esculenta* Crants., *Nicotiana tabacum* L., *Saccharum officinarum* L., *Citrus* spp., *Musa paradisiaca* L., *Lycopersicon esculentum* Mill., *Mangifera indica* L., and many other plants. It has been shown that some *Diplodia* spp. can pass from one host to another. In Puerto Rico we recently have found *D. theobromae* on *Nicotiana tabacum* L., *Cajanus cajan* (L.) Millsp., *Carica papaya* L., *Cypripedium* spp., *Chrysophyllum cainito* L., *Medicago sativa* L., *Citrus* (chironja) sp., and *Catteleya spp.*

MORPHOLOGY

MYCELIUM

On Potato-Dextrose-Agar (PDA), pH 6.5

Hyphae were septate and granular, later guttulate, turning grayish and eventually black, the substrate slate gray. Growth was rather superficial, verrucose and lumpy, with the formation of pseudoesclerotial, carbonaceous, lumpy structures.

On V-8 Dextrose-Agar

Growth was similar to that observed in PDA, through not so profuse. The substrate turned dark. Hyphae were brownish and formed numerous thick-walled, brown gnarls.

On Corn Meal Agar

Growth, color, and nature of the colony was similar in its characteristics with those shown by the fungus cultivated in PDA.

On Caimito (Star-apple) Fruits

The mycelium was septate, white in recently infected fruit, growth intracellular and intercellular generally circumscribed to the rind and skin tissues of the fruit. However, when ripe fruits were opened and exposed to high relative humidities, the mycelium developed profusely in the white, meaty, sugary flesh of the fruit. The mycelium usually was grayish in color during the first days of growth, but gradually turned black, and formed verrucose, lumpy pseudoesclerotial, carbonaceous bodies as when it was cultured in caimito fruits (fig. 4).

On Mango Fruits

The skin of inoculated mango fruits became completely invaded by the fungus, forming dark areas which spread rapidly from the point of infection. The fruits turned black, necrotic, and soft. When placed in a dry environment they mummified without decay.

On Mango Twigs

When inoculated and kept under high relative humidity for extended periods, mango twigs became covered with a grayish mycelium. If the humidity was moderate, the organism developed subepidermally and the twigs turned black.

On Papaya Stems and Petioles

When inoculated and kept in a humid environment, watersoaking was common-place at the site of inoculation. Such areas were covered with a

gray mycelial growth. Stems and petioles kept in a moderately humid environment stayed firm, and the mycelium spread subepidermally.

PYCNIDIA

On Papaya Petioles and Mango Twigs

Upon inoculation with pycnospores, pycnidia developed either singly or in groups; pycnidia were papillate or shortbeaked, globose or subglobose, very variable in size, from very small to 250 μ, membraneous, leathery, or carbonaceous; glabrous or hairy; immature pycnospores were hyaline, subguttulate, one-cell, granular, measuring from 17 μ to 25.8 μ by 8.5–14 μ,

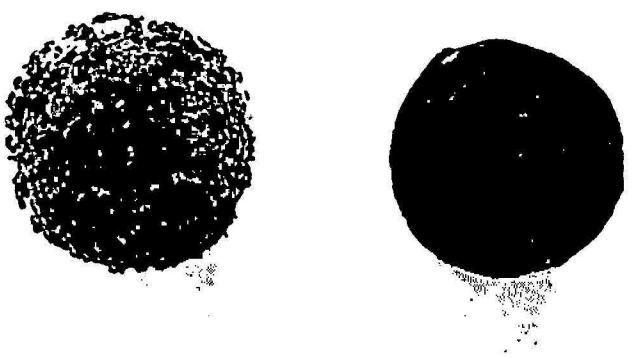

FIG. 4.—Typical lumpy mycelium of *Physalospora rhodina* grown on caimito (*Chrysophyllum cainito* L.) fruits.

subcyclindric or oblong, oozing out in coils through the ostioli; the pycnidia wall was dark, and the inner cells whitish, pseudoparenchymatous, filling the pycnidia when young, and later displaced by the formation of pycnospores; pycnospores produced by abstriction of tip of simple conidiophore cells; cells hyaline 18–20 μ by 3–3.5 μ; pycnidia without stroma; paraphyses present; mature pycnospores, 2-cell, dark, striated, not constricted and darker at the septum (fig. 3); and when extruded, forming lumps when tangled in the hyphal tufts, which under humid condition are developed around the papilla (fig. 5).

PERITHECIA

On Caimito Fruits

While studying the characteristics of the fungus *D. theobromae*, fruits of a green variety of caimito (*Chrysophyllum cainito* L.) showing incipient

FIG. 5.—A, *Chaetodiplodia* pycnidial stage of *Physalospora rhodina* and B, *Diplodia* pycnidial stage of *Physalospora rhodina*.

symptoms of blossom-end rot were brought to the laboratory. A fungus was isolated from the affected area identical with the *Macrophoma* obtained from diseased mango twigs and grafted seedlings. Healthy caimito fruits when inoculated with the above mentioned mango isolate and placed in

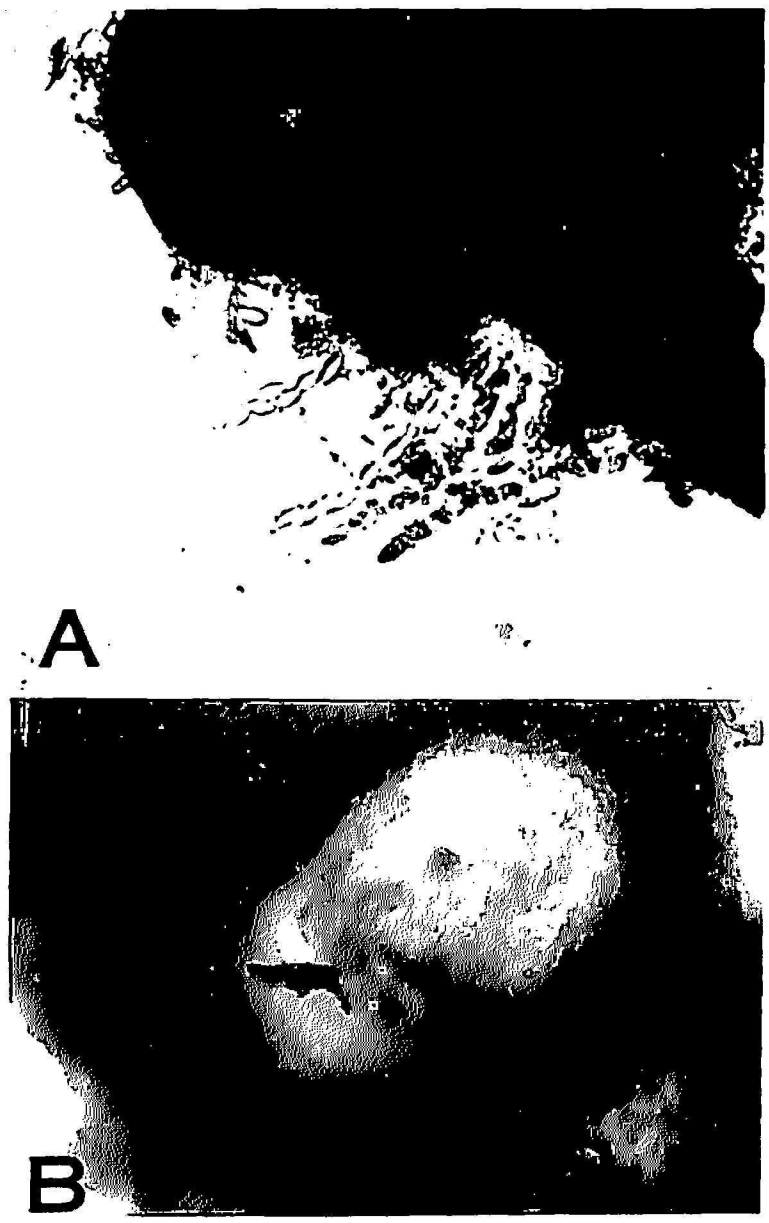

FIG. 6.—A, Asci squeezed out from perithecia and B, immature perithecia showing characteristic white inner pseudoparenchyma.

moist chambers, produced abundant pycnidia which pierced the skin or pericarp within 3 to 4 weeks. By this time the fruits had turned brown in color.

Some of the inoculated fruits were allowed to dry gradually in a 24° C. atmosphere. When reexamined, not only were pycnidia present, but also a perithecial stage with the attributes of the fungus *Physalospora rhodina* (fig. 6).

The perithecia were globose or subglobose, leathery or membranous, nonstromatic, produced subepidermally, later erumpent; glabrous, ostiolate, measuring from 200–350 μ with subcylindric to subclavate; double-walled asci; walls about 3.0 μ in thickness; tip of asci thick, about 5.1 μ; no pore visible; asci sessile varying in lengths, measuring from 61–134 μ by 17.5–20.0 μ; hyaline, subelliptical, slightly arched, arranged distichiously; as an average measuring 17.5–24 μ by 8.5–10 μ. Various workers have reported asci measuring 75–100 μ by 18–25 μ; 65–85 μ by 20–25 μ, and ascospores of 21–20 μ by 9–12.5 μ, 24–42 μ by 7–12 μ, 27–38 μ 9–17 μ.

These variations might be explained on the basis of influence of the media in which the perithecia had developed, on racial characters, or on the stages of development and maturity of asci and ascospores at the time when the measurements were taken.

On Papaya Petioles and Young Stems

Papaya stems and petioles were inoculated separately at the cut ends with mycelial disks obtained from pure cultures of *Macrophoma*, *Diplodia*, and *Chaetodiplodia*, respectively, and placed immediately in large, sterile petri dishes. The petioles and young stems a month later were covered with erumpent fruiting bodies. Macroscopically they resembled the pycnidial forms already obtained in PDA, in caimito fruits and mango twigs. However, a close examination revealed not only pycnidia but also perithecia identical with those seen previously on caimito fruits.

The perithecial stage was obtained, irrespective of the imperfect form of *P. rhodina* inoculated. Perithecia in every instance were intermingled with pycnidia. Hingorami *et al.* (*7,8*) expressed possibility of homothalism.

PHYSIOLOGY

THE AVERSION REACTION

Various *Diplodia* isolated from decaying sugarcane stools and from infected pigeon peas, alfalfa and papaya plants, were compared with the mango isolate. The following experiments were conducted to determine whether these isolates belonged to a single or different entities. Monoconidial cultures of each of the *Diplodia* isolates were prepared in separate plates containing Potato Dextrose Agar (Difco, pH 6.5). The cultures were incubated at 28° C. for 4 days, then used for inoculation purposes.

Mycelial disks were cut from each such culture to seed 6-inch plates containing PDA. A mycelial disk, one from each respective culture, was seeded separately in each of five plates and following a radial arrangement. Therefore, there was one specific isolate in each sector, and one duplicate

isolate in the center of the plate; the latter against itself and against all the other isolates.

The seeded plates were placed in an incubator at 28° C. and inspected daily. After 3 days, the substrate in the plates was covered with the mycelia of the various isolates.

The isolates showed a typical reciprocal aversion phenomenon, with the exception of the central isolate paired with itself; thus indicating that the

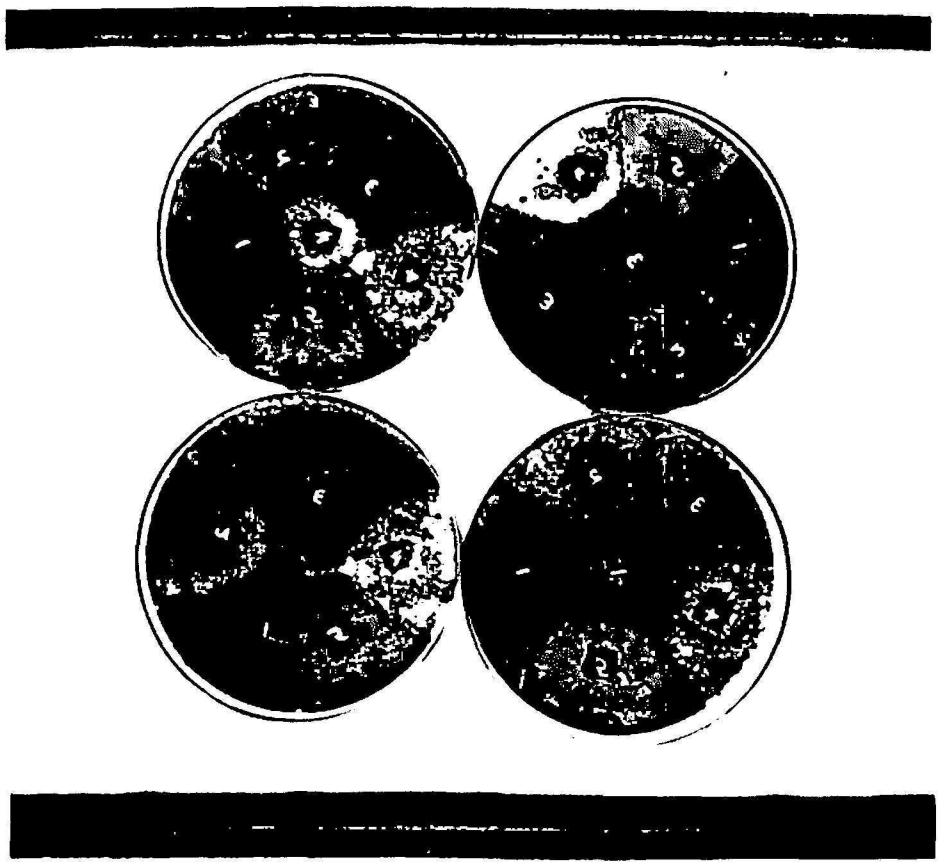

FIG. 7.—Aversion reaction of monopycnospore cultures obtained from various host plants: 1, *Mangifera indica* L., 2, *Medicago sativus* L., 3, *Cajanus cajan* (L.) Millsp., 4, *Saccharum officinarum* L., and 5, *Carica papaya*, St. Croix.

radially arranged, monosporic cultures belong to different physiologic races (fig. 7).

The dependability of the aversion reaction, as a criterion to separate physiologic races of *Diplodia* (*Physalospora rhodina*), has been shown by Hoppe (*6*) and Voorhees (*11*). These investigators found that the aversion between different interspecific ascosporic or monoconidial mycelia, as well as between monoconidial vs. ascosporial mycelium, are indistinguishable.

Pairing of the *Diplodia* isolates in culture media failed in all instances to produce perithecia. Voorhees (*11*) suggested possibility of hybridization among *Diplodia* in nature with the production of new races, that *P. rhodina*

comprises many races differing in one or more characters, and that morphological differences of many conidial stages do not justify specific distinctions.

TEMPERATURE AND GROWTH

The same procedure was followed in seeding plates containing a Difco, pH 6.50, Potato Dextrose Agar substrate. In this series, however, one mycelial disk of each of the various isolates was placed independently in the center of a plate. A set of five plates was used for each isolate.

Seeded plates were placed in a battery of incubators, set at a 4° C. interval range, starting at 12° C. and up to a maximum of 40° C.

Temperatures for maximum growth in general were from 28° C. to 32° C.

PH AND GROWTH

Four sets of five plates each containing Difco Potato Dextrose Agar and each set adjusted to a different pH value, were seeded at the center of the medium with mycelial disks (one per plate) of each of the various isolates.

The seeded plates were placed in an incubator at 30° C., temperature at which all isolates have shown their maximum growth rate. Readings were taken 24 hours after seeding.

The isolates showed different growth rates at the varying pH values. In general, all the isolates grew well from pH 4.00 to pH 7.00, with their maximum growth at pH 4.00.

INOCULATION STUDIES

ON GRAFTED MANGO SEEDLINGS

Four mango seedlings, cleft-grafted with scions of the variety Jacqueline, were inoculated on the wounded-grafted areas with a suspension of pycnospores. The inoculated seedlings were kept under partial shade and covered with cellophane bags for 24 hours. At the end of 4 weeks, evidence of die-back was noted. In 8 weeks, the scions and part of the root-stock had died. Four controls were treated similarly but were not inoculated and these remained free from die-back.

ON MANGO FRUITS

Fruits from 18 varieties of mango were tested for resistance to infection with *P. rhodina*. Sets of three ripening fruits per variety were used in each trial. The fruits were punctured on one side and inoculated at the site of the puncture with a mycelial, agar disk of the pathogen.

The inoculated fruits were kept at 24° C. in an air-conditioned laboratory. Necrosis was observed around the inoculated area 4 days after inoculation and spread over the entire fruit of some varieties in about 10 days (table 1). Controls remained free from infection and the fruit ripened normally.

NATURAL INFECTION

FORTUNA NURSERY

Grafted mango seedlings of ten varieties were planted at random in five plots containing five plants each in January 1966 in the Station nursery at

TABLE 1.—*Mango fruit inoculated with* **Diplodia** *mycelial disks of the fungus* **Physalospora rhodina** *(B. & C.) Cke. and kept 7 days at 24° C*

Variety	Infection	Variety	Infection
	Degree		*Degree*
1. Bombay Green	++++	10. Toledo	+++
2. Santaella	++++	11. Francisque	++
3. Frorigon	+++	12. Zill	+
4. Manzano Tete-Nene	++	13. Edward	+
5. Stringless Peach	++++	14. Lippens	++++
6. Davis Haden	++++	15. Haden	+
7. Sucidoro	+	16. Colombo Kidney	+++
8. Jacqueline	+	17. Adams	++++
9. Carrie	++++	18. Irwin	++++

\+ Resistant
++ Moderately susceptible
+++ Very susceptible
++++ Extremely susceptible

Fortuna. They were watered regularly to keep them from wilting because it becomes very dry here during this season.

The grafted seedlings were kept in the nursery until September 1966 where they were checked frequently for signs or symptoms of disease. The ubiquitous nature of the pathogen was confirmed because it was consistently reisolated from the diseased seedlings. Data on the incidence of die-back in this nursery is presented in table 2.

Batista (*1*) in Recife, Brazil, reported that the beetle *Xyleborus affinis* Eichh. is an important biological factor in the dissemination of the spores and vegetative parts of the fungus *Diplodia recifensis* Bat., responsible for mango die-back in that country. This beetle is endemic in Puerto Rico but has not been associated thus far with gummosis of mango trees in Fortuna.

CONTROL

IN THE NURSERY OR PLANT BEDS

The disease was controlled satisfactorily in the nursery by various means: 1, Transferring the grafted seedlings to a well ventilated and dry site, 2, keeping water away from the grafted areas because the pathogen is a wound parasite and requires a humid environment for spore germination and penetration, 3, selection of budwood from healthy trees, 4, sterilization of the budding knife with alcohol after each grafting procedure, 5, by transferring the seedlings to full sunlight after the graft has healed completely. In this respect, Muller (*9*) found *Botrydiplodia theobromae* infecting mango trees damaged by sun scorch.

TABLE 2.—*Natural infection: Incidence of* **Physalospora rhodina** *(B. & C.) Cke., die-back disease of 1-year-old grafted mango trees, kept in a half-shade nursery at the Fortuna Substation, Ponce, from June 21 to September 21, 1966*

Variety	I	II	III	IV	V	Total	Infection
							Degree
Edward	—	—	—	—	—	0	0
Parvin	1	1	—	—	—	2	8
Jacqueline	1	2	1	—	1	5	20
Haden	—	—	2	—	—	2	8
Kent	—	—	—	—	2	2	8
Keitt	—	—	—	—	—	0	0
Palmer	1	3	—	—	1	5	20
Irwin	—	—	2	—	2	4	16
Eldon	—	—	—	1	—	1	4
Ruby	—	—	—	—	—	0	0

The disease was controlled in the field by spraying the trees periodically with a suspension of copper oxichloride sulphate (3 lbs./100 gal.) and by the frequent painting of the trunk and main branches with a thick paste of this copper fungicide.

SUMMARY

A fairly large number of grafted mango seedlings, kept under partial shade in the Fortuna Substation, were damaged by a serious die-back disease which caused death of the scions and necrosis of the wounded tissues of the root-stocks. In the orchard, trees of the susceptible variety Jacqueline developed very serious die-back symptoms and stem cankers. The disease was prevalent during December 1965 and January 1966.

The high percentage of natural infection can be attributed to the widespread occurrence of the parasite, *Physalospora rhodina*. The imperfect stage

of the organism, *Diplodia*, is endemic in the Island, and was repeatedly isolated from the following hosts: avocado, orchids, pigeon peas, papaya, alfalfa, mango, and sugarcane. Six distinct physiologic races were recognized when using the aversion test. The perfect *Physalospora* form was produced when the physiologic races were cultured independently on caimito fruits or in young papaya stems and petioles. This organism is a wound parasite and capable of causing great damage under certain favorable conditions, as when grafted mango trees are kept in a humid propagation shed.

In the nursery the disease was controlled by practicing such preventive methods as: Selection of scions from healthy trees, sterilization of the budding knife with alcohol, keeping the grafted trees in a relatively dry environment, and gradual exposure of grafted mangos in plant beds to full sunlight.

In the orchard, control was attained by spraying the mango trees periodically with copper oxichloride sulphate (3 lbs./100 gal.) and by paintings of the stems frequently with a thick paste of the above mentioned fungicide.

RESUMEN

En la Subestación Experimental Agrícola de Fortuna se notó que durante los meses de diciembre de 1965 y enero de 1966 murió un buen número de arbolitos de mango injertados y ubicados en un umbráculo.

La enfermedad también apareció en el huerto de esa Subestación, atacando algunas de las variedades exóticas de mango. La variedad Jacqueline fue la más afectada, observándose que en el tronco y en las ramas aparecían unas llagas, que también causaban una muerte retrogresiva en las extremidades de las ramas jóvenes.

Al estudiarse el material enfermo se aisló un hongo del género *Diplodia*. Ensayos posteriores de patogenicidad revelaron que este organismo era el agente causal de la enfermedad. El estado perfecto, *Physalospora rhodina*, se pudo lograr al inocularse tallos tiernos y pecíolos de papaya (*Carica papaya* L.) y también frutas de caimito (*Chrysophyllum cainito* L.) con picnoconidios del hongo.

La enfermedad fue combatida en el umbráculo con bastante éxito al seleccionarse yemas de árboles sanos, al esterilizar frecuentemente las cuchillas de injertar con alcohol, al mantener los arbolitos de mangos injertados en un lugar ventilado y menos húmedo, y al no transferir los del umbráculo al campo raso, hasta tanto no hubiesen cicatrizado los tejidos del injerto.

La enfermedad se combatió en el huerto asperjando los árboles enfermos semanalmente con oxicloruro de cobre sulfatado (3 lbs./100 gal.) y pintando

los troncos y ramas principales de los árboles con una pasta preparada con el compuesto de cobre ya indicado.

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