Fungus Diseases of Papaya in the U.S. Virgin Islands[1]

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INTRODUCTION AND REVIEW OF THE LITERATURE

The Federal Agricultural Experiment Station in St. Croix, U.S. Virgin Islands, has, since 1954, been testing papaya varieties from all parts of the tropical world.

In 1955 a disease was observed attacking several varieties. Water-soaked areas on the upper levels of the stem were observed on all affected papaya plants. These areas often appear greasy rather than wet (fig. 1). The disease has been denominated "greasy spot," and "papaya decline."

In Hawaii, Bembower (*2*)[6] observed water-soaked spots on the stems of papaya, and determined them to be caused by *Phytophthora parasitica*.

After comparing the symptoms found on papaya plants in St. Croix with those described from Hawaii, the writers concluded that the diseases were different. In Hawaii, the fruits become affected (fig. 2) and eventually shrivel and drop to the ground. Such is not the case in St. Croix.

Samples of roots taken from 'Solo' papaya plants displaying the stem lesions were sent by air to the University of California, Riverside, on several occasions, but no *Phytophthora* species were isolated. Papaya decline was one of the major causes of loss of bearing trees during 3 years of field-work in St. Croix (*8*).

In the spring of 1963 Wellman (*12*) reported that there was a leaf spot disease on these 'Solo' plants probably caused by *Cercospora*. The symptoms of disease observed in the Virgin Islands are different, however, from those reported as caused by *Cercospora* in Hawaii (*1*).

Besides the aforementioned water-soaked spots or greasy spots, the following symptoms characterize the St. Croix disease:

The foliage of affected plants appears scant, rigid, and generally chlorotic.

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[6] Italic numbers in parentheses refer to Literature Cited, p. 200.

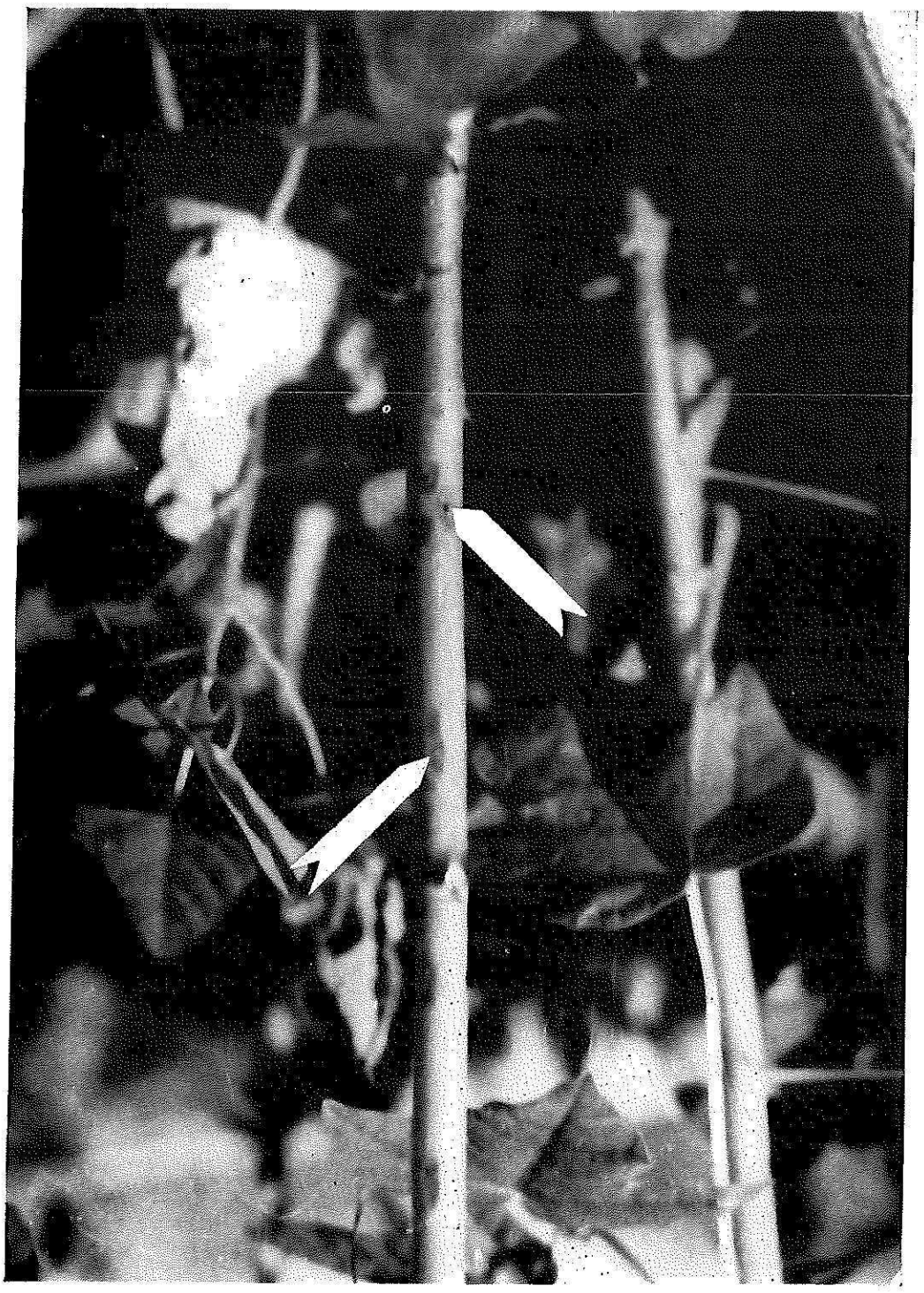

FIG. 1. Water soaked areas on the stems of papaya plants affected by the St. Croix disease.

Diseased plants develop an umbrellalike shape (fig. 3) which resembles that associated with papaya bunchy top (*3,4*), but close observation shows that this disease is not related to bunchy top since:

1. Latex issues from the tissues of plants affected by the St. Croix disease.

2. The leafhopper vector of bunchy top, *Empoasca papayae*, has not been found in the papaya fields of St. Croix. (In 1946 Martorell and Adsuar (*9*)

FIG. 2—Fruit of *Carica papaya* affected by *Phytophthora parasitica*.

did collect an apparently new species of *Empoasca* breeding on papaya in St. Croix.)

3. Symptoms of the two diseases differ in many other respects.

In the field the lower leaves of affected plants are dotted with yellow spots (fig. 4) which range in size from just visible to as large as a silver dollar. The centers of these spots are generally necrotic. Leaves of affected plants are often found dried and hanging alongside the stem. In some instances, if the petiole is detached from the stem, moist, cankerous lesions are exposed.

This suggests that the leaf-spotting agent gains access to and infects

FIG. 3.—*Carica papaya* plant affected by the St. Croix decline disease. This is an advanced case of the malady with typical "pencil-point" effect.

stem tissue. In many of the plants with the cankerous lesions, the lower part of the stem is distorted by bumpy areas (fig. 5). We refer to this condition as "bumpy stem." Bumpy sections are not found on the stems of all the plants with a reduced canopy and other symptoms of the disease.

FIG. 4.—Necrotic lesions induced by *Corynespora cassiicola* on the leaves of papaya plants of the variety 'Solo'.

A possibly different ring spotting, as well as curling of the leaves, was observed in several plantations. None of these symptoms could be associated with those of any of the virus diseases that occur in the Caribbean area.

Some of the native (Cruzan) papaya plants are relatively free of leaf spots, but in some individual cases the malady is severe. The native papayas

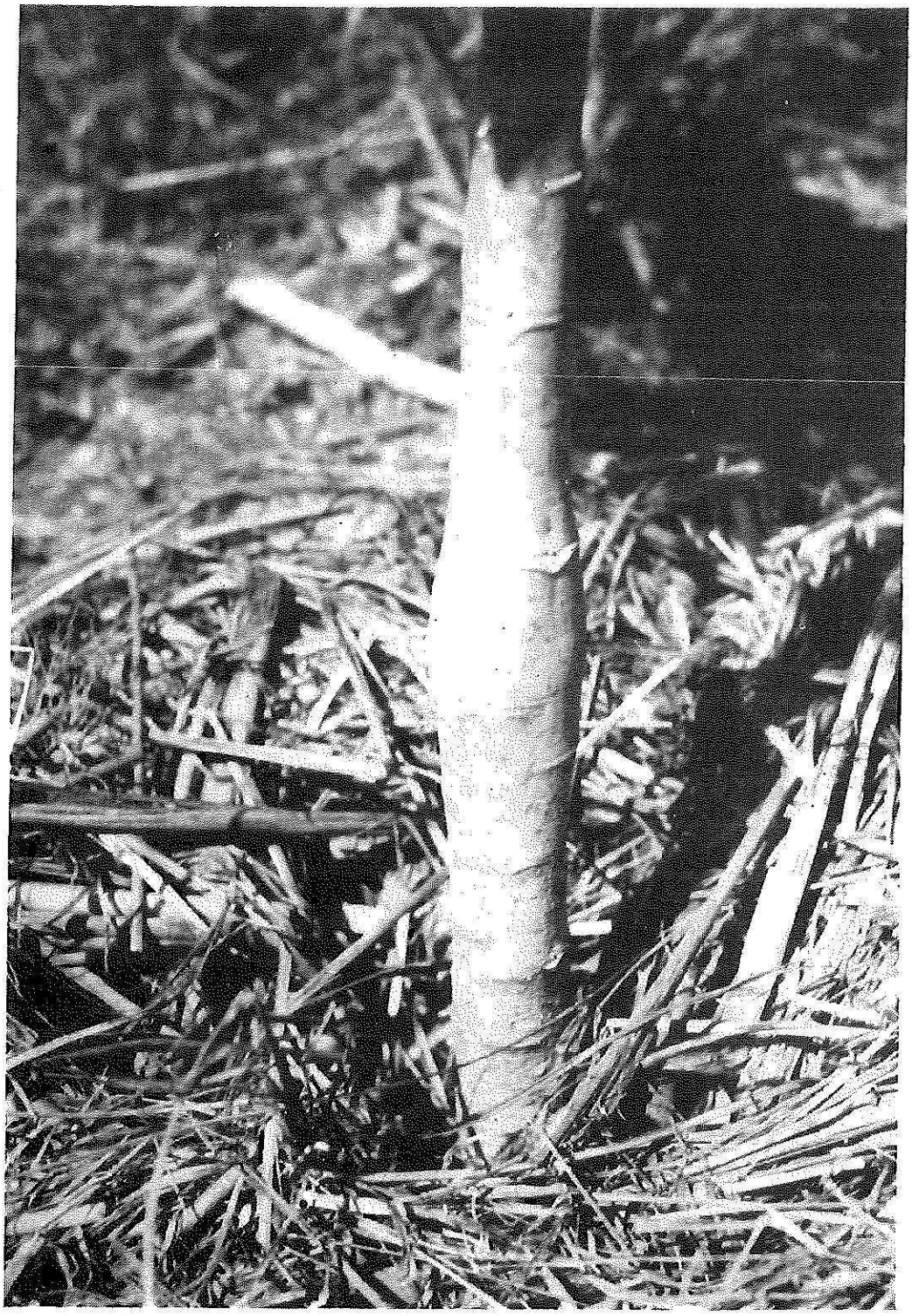

FIG. 5. Swelling of the lower stem ("bumpy stem") is often found in association with the decline disease in the field. Similar swellings developed on plants that were artificially inoculated with *Corynespora cassiicola*.

also become affected by the bumpy stem condition but seem in general not to be greatly injured by it.

OBJECTIVES

Papaya fruits of the 'Solo' type are in great demand in the Miami, New York, and San Juan markets. However, certain virus and fungus diseases limit the production of 'Solo', as well as other papaya varieties, in the West Indies. Papaya decline is a very serious disease in some of these islands. It was decided to undertake work on this malady in an attempt to elucidate its etiology, and also to develop means for its control.

ISOLATION AND INOCULATION STUDIES

ISOLATION STUDIES

Specimens from lesioned areas of the stems and from leaf spot-affected, curled, and ring spotted leaves were collected from various fields in St. Croix, washed with tapwater and placed in polyethylene bags. The material was divided, and part was plated on acidified PDA after surface disinfection with Clorox[7] ($\frac{1}{20}$). Another batch was used for isolation of bacteria, and a third—with curled and ring-spotted leaves, was ground in order to secure sap for mechanical inoculation of various plant hosts. No bacteria could be isolated from affected stem or foliage tissues. A series of test plants belonging to several families and including such species as: *Carica papaya* ('Solo', Cruzan, and Puerto Rican varieties), *Phytolacca decandra*, *Chenopodium amaranticolor*, *Capsicum annum*, *Nicotiana tabacum*, *Nicotiana glutinosa*, and *Cucumis sativus* were dusted with carborundum and mechanically inoculated with the sap expressed from curled and ring-spotted leaves. Ten plants of each of the aforementioned species and varieties were inoculated in each of two treatments and observed daily for a period of 3 months. None of these plants developed symptoms of disease, thus indicating that no mechanically transmissible virus is associated with the St. Croix disease.

The lesioned tissues from the upper parts of the stem, as well as spotted areas from the leaves yielded several fungi on acidified (pH 5) potato-dextrose agar. This included species of *Fusarium* which were discarded as contaminants since they represented a very minor percentage of the isolates in contrast with a fungus that arose from about 90 percent of the plated tissue bits. This last fungus was characterized by a cottony, gray mycelial growth, which changed the color of the substratum from light caramel to dark brown and even to jet black. Microscopic examination of bits of affected upper stem, leaf, and petiolar tissues frequently disclosed slender, cylindrical conidia (fig. 6), suggestive of *Cercospora*, or occasionally of a

[7] Trade names are used only to provide specific information. Their mention implies no guarantee, warranty, or endorsement by the Station.

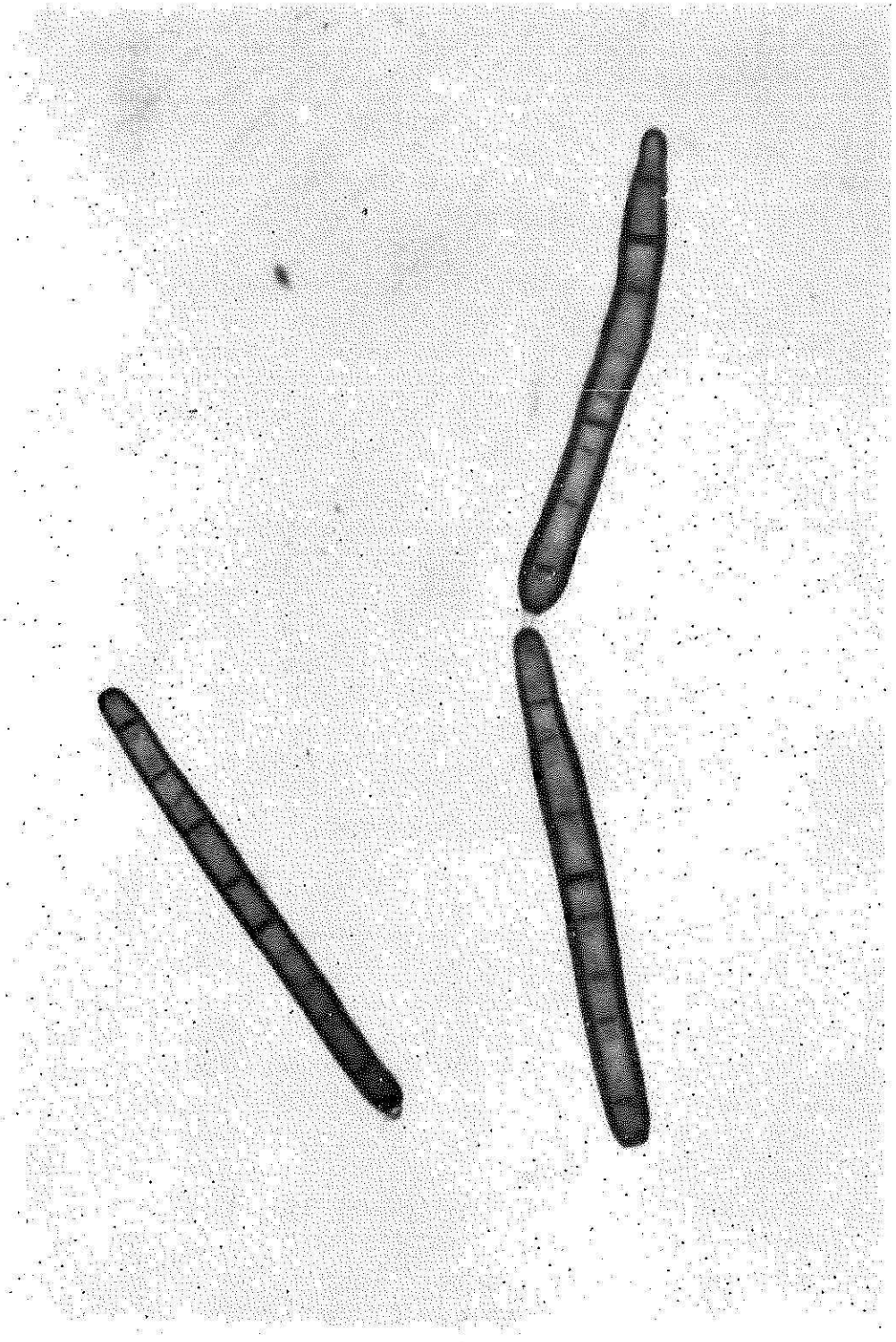

FIG. 6. Conidia of *Corynespora cassiicola* obtained from isolates grown on potato-dextrose agar.

Helminthosporium. Subcultures of the gray fungus from the plated tissues formed conidia, always of the same type, and very similar to those detected on the affected papaya tissues.

Cultures of this fungus were forwarded to M. B. Ellis, Commonwealth Mycological Institute, Kew, Surrey, Great Britain, and to Charles Chupp, Cornell University, Ithaca, N.Y. for identification. Chupp (*5*) stated that the fungus did not belong in the genus *Cercospora*. Ellis (*7*) identified the fungus as *Corynespora cassiicola* (Berk. & Curt.) Wei.

In 1950 Wei (*11*) stated that *Cercospora melonis* and *C. vignicola* (or *Helminthosporium vignae*), were species of *Corynespora*. He found that 16 collections on 11 hosts from the Tropics, previously identified as *Helminthosporium cassiicola* also belonged to *Corynespora*. Wei believed *Corynespora* to be a valid genus, probably most closely related to *Helminthosporium* which has the same type of conidial structure. The writers agree, although they have observed that the shape and length of conidia of *C. cassiicola* was somewhat variable. Conidia formed on papaya plants are generally large, slender, and light olive in color, and at times almost hyaline. Those produced on acidified PDA by the same fungus are generally cylindrical and thicker, their color ranging from light-brown to dark-brown. In the first case the conidia look rather like those of *Cercospora*, while in the second they are more suggestive of *Helminthosporium*. There is no question, however, as to the validity of Ellis' identification of our cultures. Descriptions of the fungus are given by Wei (*11*) and Ellis (*6*).

INOCULATION STUDIES

Inoculations with C. cassiicola

Mycelial mats of cultures from the same isolates that were forwarded to Ellis, were suspended in sterile distilled water. This material was homogenized in a sterile Waring Blendor, and the supernatant liquid was transferred to a sterile 250-cc. Erlenmeyer flask. For inoculation the supernatant liquor was stirred and transferred to a DeVilbliss No. 151 atomizer. The suspension was impelled by air at 20 pounds p.s.i. and directed from a distance of approximately 1 foot to the foliage and stems of the test plants. Six hundred healthy 1-foot 'Solo' papaya plants growing in six different flats were inoculated in the described manner. After the plants were inoculated the flats were transferred to a moist chamber and kept at 90- to 100-percent relative humidity. A set of 100 control plants growing under the same conditions were sprayed with sterile distilled water, and then transferred to a chamber that was also kept at the same relative humidity and temperature (±83° F.).

At the end of about 4 days the leaves of most of the inoculated plants

developed small, round, yellowish water-soaked areas. These spots were about one-eighth inch in diameter and their centers were slightly grayish in color, suggesting tissue breakdown. The succulent green parts of the upper stems developed greaselike spots in places. These more or less lenticular spots were of the same general size as the leaf spots. Their centers were observed to be breaking down also. After a week the leaf lesions on the inoculated plants became visibly larger and, in some cases, coalescence of lesions resulted in the breakdown of entire leaves. Necrosis was also evident on the petioles and lower parts of the stems of some inoculated plants. The older upper-stem lesions were gummy, and many of them were eroded at this time. The control plants remained healthy throughout this test.

At the end of 8 days the control and test plants were taken out of the moist chambers and into the greenhouse. Dropping of infected leaves was common. At times these hung along the stem without becoming fully abscissed. The spots on the leaves and the water-soaked areas of the stem were like those encountered in the field. At the end of about 1 month most of the inoculated plants had lost their lower leaves with only four or five of the youngest remaining. A number of plants were completely defoliated, and apical necrosis resulted in several instances.

Two months later conditions in the greenhouse became extremely dry, a number of plants markedly improved, and some lost all symptoms of disease. These plants were among those that had retained a good leaf canopy.

One week, two weeks, three weeks, and one and a half months after inoculation, *Corynespora* was recovered from stem, leaves, and petiolar lesions of the artificially inoculated plants. Cultures thus obtained (fig. 7) were found to be identical with the original isolate.

Three months after inoculation some of the "recovered" plants were found to be still vigorous and healthy, while others showed little or no progress. Many of the artificially inoculated plants with lesions on their lower stems had developed, at this stage, sizeable bumps. Similar results were obtained in a second test using subcultures from the original isolates.

Since Olive, *et al.* (*10*) had reported that race 1 of *Helminthosporium vignae* (*C. cassiicola*) lost its pathogenicity after three or four transfers, we repeated the tests described above, using the same isolates after four transfers on acidified PDA. Not a single papaya plant developed symptoms of disease; this beyond doubt shows that virulence was lost on transferring this organism in the medium used.

Inoculations with Botryodiplodia

While the above-reported work was being carried out the third author was attempting to isolate organisms from affected papaya roots and stems

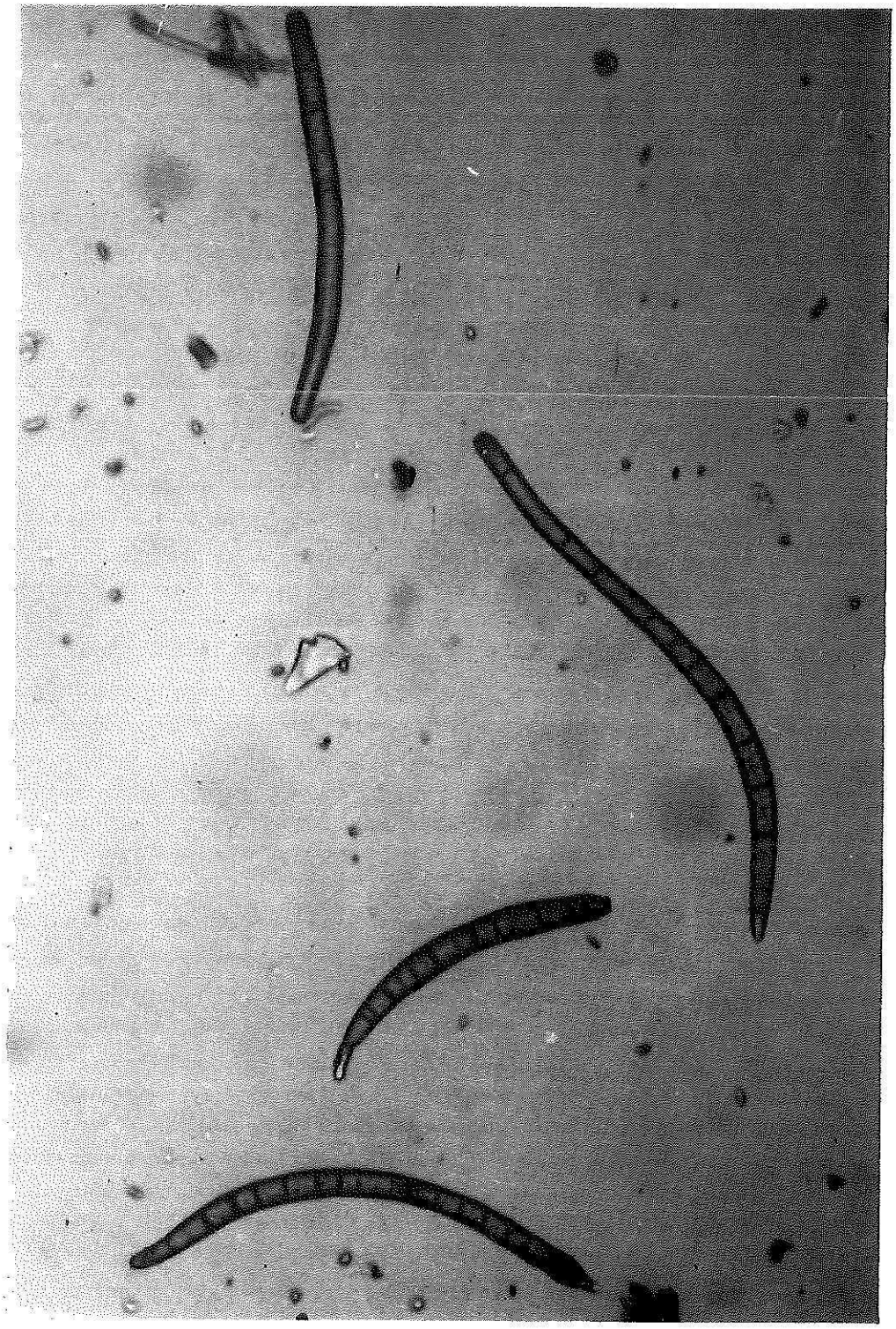

FIG. 7. Slender conidia of *Corynespora cassiicola* recovered from artificially inoculated young *Carica papaya* plants of the variety 'Solo'.

FIG. 8.—A, Seven-month-old papaya seedling of the variety 'Solo' artificially inoculated with PDA disks containing 24-hour-old culture of the isolate (SD4) of *Botryodiplodia* spp. After 5 days water-soaked cankers caused death by girdling. B is an uninoculated control plant.

forwarded by air to California. The first samples yielded several fungi, including a species of *Pythium*. However, none of these organisms was consistently associated with the lesions. Samples received subsequently from St. Croix yielded a fungus with dark mycelium and dark 2-celled

FIG. 9. Closeup of a water-soaked canker developed after inoculation with SC4, *Botryodiplodia*.

conidia. This fungus, a species of *Botryodiplodia*, was consistently isolated from water-soaked stem lesions. It formed numerous pycnidia on the trunks of affected papaya plants.

Inoculations of small (12 to 18 inches tall) papaya seedlings ('Solo' variety) in the greenhouse at Riverside, Calif., with mycelium of the *Botryodiplodia* isolated, resulted in severe necrosis of stems (figs. 8,9) within a week after inoculation. The same fungus was reisolated from the lesions.

DISCUSSION

It appears to us that most of the symptoms of the papaya-decline syndrome were reproduced under controlled conditions in Puerto Rico by inoculation with *Corynespora cassiicola*.

C. cassiicola is a highly pathogenic organism, although it loses its virulence after series transfers through artificial media. In St. Croix it can be a dangerous pathogen in papaya orchards receiving poor care. Manzate D gives satisfactory control if applied every week or 10 days at the rate of 1¼ pounds per 50 gallons of water. There is suggestive evidence that there may be heritable factors for resistance. A good many wild papaya plants show no symptoms, and old untended 'Solo' orchards usually have a scattering of trees that remain vigorous and fruitful, even though they have light infections of *Corynespora* on the leaves. Selections from such plants are now under observation at the Federal Experiment Station, St. Croix, U.S. Virgin Islands.

It is not known with certainty what role *Botryodiplodia* may be playing in the papaya decline syndrome. It is possible that many of the moist cankers that have been attributed by us to *Corynespora* may actually have been induced by *Botryodiplodia*. Further research is needed to elucidate the etiology of the soft cankers of papaya plants in St. Croix.

SUMMARY

Corynespora cassiicola (Berk. & Curt.) Wei, and a species of *Botryodiplodia* were consistently isolated from various tissues of *Carica papaya* plants affected by the papaya decline disease. Young papaya plants of the 'Solo' variety were successfully inoculated with the aforementioned fungi. Most of the symptoms of the decline disease developed on papaya plants after inoculation with *Corynespora*. Severe stem necrosis of papaya plants resulted from inoculation with *Botryodiplodia*. *C. cassiicola* loses its virulence after series transfers through artificial media.

RESUMEN

Se logró reproducir la mayoría de los síntomas del mal común de la papaya de Santa Cruz después de inocular plantas de papaya de la variedad

'Solo' con el hongo *Corynespora cassiicola* (Berk. & Curt.) Wei. Se produjo una severa necrosis del tallo cuando se inoculó la mencionada variedad con una especie de *Botryodiplodia*. *C. cassiicola* perdió su virulencia al transferirse en serie, en un cultivo artificial.

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