SEEDBED ANTHRACNOSE (*COLLETOTRICHUM GLOEOSPORIOIDES* PENZ. CACAO STRAIN) OF CACAO (*THEOBROMA CACAO* L.) IN COSTA RICA.

by

ARTHUR B. LEIBOVIT

INTER-AMERICAN INSTITUTE OF AGRICULTURAL SCIENCES

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APPROVED: Frederick L. Wellman
Advisor

[signature]
Committee

L. R. Holdridge
Committee

Date:____________________

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BIOGRAPHY OF THE AUTHOR

Arthur B. Leibovit was born in the city of West Palm Beach, Florida. He attended Palm Beach High School there and was graduated in the summer of 1943. Thereupon he entered the University of Florida, Gainesville, Florida to major in tropical horticulture. After completion of one semester he was called into the service of the Army of the United States for a period of about 3 years. Upon his return from foreign service in the Pacific he re-entered the University of Florida, from which seat of higher learning he was duly graduated in February, 1949, and received the degree of Bachelor of Science in Agriculture with honors. Shortly thereafter he was employed by the University of Miami, Coral Gables, Florida as a research assistant in the Tropical Plant and Food Research Center, before commencing work on a Cacao fellowship awarded by the Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica, in October, 1949 for post-graduate studies in tropical horticulture and pathology.

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INTRODUCTION

Theobroma cacao L. is a tropical evergreen tree native of the New World, which produces a pod-like fruit containing numerous seeds or beans that are the source of commercial cocoa or cacao, and chocolate. The terms "cocoa" and "cacao" (89)[1] are often used interchangeably, leading to much confusion. The correct name of the raw product is cacao as used in French, Dutch, German, Spanish and Portuguese. Only the English used the word cocoa because of an early erroneous conception that the fruit came from the coconut tree (Cocos nucifera). The British adopted the rule of applying cacao for the tree and the raw product, and cocoa for the processed and finished products of the tree. However, this rule is not generally observed in the United States and cacao and cocoa are often used interchangeably. As one of the reforms that would affect the standardization of the secondary tools of research (73) it is indicated that the majority of scientists throughout the world now working in the interests of the industry appear to prefer the word "cacao" to describe the tree and the bean. After roasting and comminution at the factory, "cocoa" is rightly the

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[1] Numbers in parenthesis refer to list of Literature Cited.

preferable term. Before such processing and comminution it is "cacao".

Although the total Costa Rican cacao production is small (production for the crop year 1949-50 was 4,406,989 kilos, and was worth $1,891,910.) (20) in respect to world production figures, it nevertheless is an important product in the economy of the country.

This study was commenced in October, 1949 and terminated in February, 1951 and was conducted both at the Inter-American Institute of Agricultural Sciences in Turrialba, Costa Rica, and at La Lola Farm, a cacao plantation leased to the Institute and located near the town of Madre de Dios. It was at the latter location that attention was first focused on a condition of young seedling cacao plants in which all or part of the foliage of the terminal part of the stem was attacked, so that they were defoliated repeatedly and developed leaf-free or bare terminals. Such a condition was first encountered in a fertilizer experiment and later in seedlings to be used for fungicidal screening tests.

Since the above mentioned disease was somewhat of a limiting factor in the production of necessarily large numbers of normal cacao seedlings, an investigation was made to determine the causal organism and complete symptomatology of the disease, its life cycle as well as other pertinent studies to formulate control measures or recommendations for control.

HISTORY, GEOGRAPHICAL RANGE, AND ECONOMIC IMPORTANCE

Two important anthracnose (39) genera have been mentioned as occurring on Cacao, namely Gloeosporium and Colletotrichum, and were reported either from the fruit, foliage, or branches, or from all of these combined.

Apparently Gloeosporium affine Sacc. was the first of the above-mentioned parasites on cacao, having been found first by Hennings (18) in 1895 on cacao leaves in the Botanical Gardens of Berlin. Also branches were attacked and showed dark spots of an irregular form, and a part of the branch above the diseased place withered and died. Whitish spots were formed on the upper side of the leaves.

Small (70) stated that Zimmerman described C. incarnatum from cacao pods in 1901.[2]

In 1903, C. theobromae was described from pods by Appel and Strunk (2) based on a collection made by Strunk from the botanical gardens at Victoria, Cameroons, in West Africa.

Two more species, namely C. theobromicolum and C. brachytrichum were both described in 1905 by Delacroix (25) the former on cacao pods in the Antilles, and the latter on

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[2] In literature up to and including the year 1911, the writer has been unable to find mention of C. incarnatum Zimm. as a cacao parasite. Saccardo (94) reported it was originally described by Zimmerman from branches of Coffea liberica in Buitenzorg and Java in 1901.

foliage of cacao in Trinidad.

Van Hall and Drost (83) described *C. luxificum* (*luxifilum* in Saccardo) from branches, buds, and fruits of cacao in Surinam and in Demerara in the West Indies in 1908. According to Nowell (55) they (in 1907) made an elaborate study of the Surinam witches' broom disease and concluded it was due to *C. luxificum*, although inoculations failed. Nowell also indicated that Rorer (1913) found *C. luxificum* was common in Trinidad but that the disease was absent. G. Stahel (1915) confirmed Rorer's findings and described the causal fungus of witches' broom as a new species under the name *Marasmius perniciosus* .

In 1910 Bancroft (9) described *C. cradwickii* found on cacao branches in Jamaica. The following year, in 1911,(8) he described and illustrated *C. cradwickii* on two separate samples of diseased cacao pods received at the Royal Botanic Garden, Kew, from Jamaica,and the fungus was present on all the pods. He also stated that five species of *Colletotrichum* had been previously recorded on the cacao plant, and that they were distributed over the West Indies, Cameroons, and Surinam and had been named *C. luxificum* Van Hall and Drost; *C. theobromae* Appel and Strunk; *C. theobromicolum* Delacroix; *C. brachytrichum* Delacroix; and *C. incarnatum* Zimmerman.

In 1913, Cook (21) stated that among other fungus

pests of more or less importance might be mentioned C. cradwickii Bancroft (Jamaica); C. theobromae Appel and Strunk (America); C. brachytrichum Delacroix (Trinidad); C. theobromae Delacroix (West Indies); and C. incarnatum Zimmerman. The same year (1913), Shear and Wood (65) reported finding perithecia and mature asci and ascospores of Glomerella cingulata (Stonem) S. and v. S. on cacao leaves in moist chambers taken from greenhouse plants in Washington, D. C. (?). They associated the conidial form C. theobromicolum Dela. with it.

Another Gloeosporium, G. theobromicolum was described by Vincens (85) in 1918 from pods collected in Pará, Brazil.

Small (67, 69), who worked in Uganda in 1921, demonstrated that C. coffeanum Noack originally described from Coffea arabica in Brazil in 1901 and an organism that occurred regularly on Uganda coffee in association with what was termed "die-back," derived from coffee berries, was capable of infecting artificially wounded cacao pods and twigs. However, he was unable to obtain infection on unwounded pod and twig surfaces. Also, he inoculated coffee leaves with cultures obtained from conidia on rotted cacao pods (Small stated that Kew regarded the form on hardened pod shells as C. theobromicolum Del; but what appeared to be exactly the same form on rotted parts of pods had been regarded by Small as C. incarnatum Zimm.) and obtained positive results.

In an annual report of the Uganda Department of Agriculture published in 1921, and covering the period April 1 to 31 December 1920, Snowden (72)reiterated the results obtained by Small above, and also added that "rot of pods" caused by *Phytophthora faberi* Maubl. (?) and *Colletotrichum* spp. was fairly common, as was also hardening of pods caused by *C. incarnatum* Zimm; while *C. theobromicola* (*Colum* ?) Del. was the cause of a "die-back" of twigs.

Nowell (55) stated, in 1923, that at least one definite anthracnose of cacao pods occurred in the West Indies, and existed quite apart from other types of pod diseases. His later observations of the disease in St. Vincent were in agreement with a description which he quoted from F. W. South. He also stated that a disease which closely resembled the West Indian anthracnose in its characters had been recorded by Busse from cacao in the Cameroons and was attributed to *C. incarnatum* Zimm. (*C. theobromae* App. and Strunk), originally described from coffee in Java. The same fungus occurred on cacao pods in Ceylon. Nowell also acknowledged that *C. theobromicolum* Delacroix, *C.luxificum* Van Hall and Drost, and *C. cradwickii* Bancroft had been described from cacao pods in the West Indies, and speculated as to whether they were really separate from each other and from the Old World species.

In 1925 Small (68) reported from Uganda the presence

of die-back of cacao branches with which were associated species of *Colletotrichum* and *Botryodiplodia theobromae* Pat. He further stated that cacao had not been a success in Uganda, and its cultivation had been abandoned more or less completely. The most characteristic field feature of the ill-health of the crop was the above-mentioned die-back.

Also in the year 1925, Da Camara and Coutinho (22) continued to associate *C. luxificum* with witches' broom of twigs and fruit in Saint Thomas Island (Sao Tomé) in West Africa. They also stated that Patouillard observed *C. theobromae* App. and Str. there in 1919 which they also stated was reported by Amando de Seabra.

In 1926 Small (70) reported from Uganda that a *Colletotrichum* indistinguishable morphologically from the form on coffee had been obtained on several occasions from rotted and hardened cacao pods and from apical twigs which had died back. Strains of the fungus infected coffee leaves and they developed the same *Glomerella* stage as the coffee strains and therefore could have been safely named *C. coffeanum.*

The same year (1926), Stevenson (76) very briefly mentioned some of the species of *Colletotrichums* and one specie of *Gloeosporium* occurring on cacao, as well as brief notes on their distribution and symptoms. The species

listed were as follows: *C. brachytrichum*, leaf spotting (Trinidad and Ceylon); *C. cradwickii*, pod anthracnose (Jamaica and Puerto Rico); *C. incarnatum*, pod anthracnose (Ceylon, Java, Belgian Congo, and Uganda); *C. luxificum*; *C. theobromae*, pods (Central Africa); *C. theobromicolum*, pod anthracnose (West Indies and Uganda); *Gloeosporium theobromicolum*, leaf and pod anthracnose (Brazil).

Dade, in the Gold Coast, reported in 1927 (23) that a *Colletotrichum* sp, probably *C. cradwickii* appeared to account for a larger proportion of cacao pod diseases than had been previously supposed.

In 1927 it was reported from the Philippines (56) that in the provinces of Cavite and Laguna cacao suffered from witches' broom (*C. luxificum*).

In his "Host Index of the Fungi of North America" Seymour (64), in 1929, mentioned cacao as the host for the following species of *Colletotrichum*: *C. brachytrichum*, *C. cradwickii*, *C. incarnatum*, and *C. luxificum*. He associated *C. theobromicola* with *Glomerella cingulata* (Stonem.) Spaulding and V. Schrenk.

A severe and widespread die-back disease was reported from the Philippines in 1929 (57) which rendered the cultivation of cacao unprofitable in many parts of the Islands, and was stated to have been caused by a species of *Colletotrichum*.

Toro (81) stated in 1930 that a Colletotrichum sp. was among the three important fungi that attacked cacao in the Zarzal Caldas region of Colombia.

In 1931 Dade (24) reported that in the Gold Coast a much larger proportion of cacao was damaged by pod disease fungi, among which were included C. cradwickii, in the early output rather than in the late.

An unidentified species of Colletotrichum that caused the development on cacao pods of sunken, dark brown spots was reported by Steyaert (78) in 1932 in the Stanleyville District of the Belgian Congo.

Verwoerd and Du Plessis (84), in 1933, described C. brachytrichum as a species not previously recorded from South Africa. The collection was made from cacao leaves in the University Botanic Gardens at Stellenbosch.

Also in 1933, Kaden (41) reported C. luxificum as a secondary parasite on affected trees in St. Thomas and Princes Islands.

During the course of studies to determine the true cause of cacao black pod disease in 1936 Baker (5) reported that, in Trinidad, out of 663 diseased pods examined, 31 were infected with C. gloeosporioides. He also reported an unidentified species of Colletotrichum. It was stated that 2 of the pod-rotting fungi, especially Dothiorella ribis and the common anthracnose fungus C. gloeosporioides, were more

noticeable in the dry season.

In a summary of world cacao disease literature and observations made in the cacao zone of Bahia, Brazil, in 1937 Rombouts (59) mentioned the following species of Colletotrichum and Gloeosporium: C. brachytrichum (Brazil); C. cradwickii (West Indies); C. gloeosporioides Penz. (Brazil); C. incarnatum (Cameroons and Ceylon); C. luxificum; C. theobromae (Cameroons); C. theobromicolum (Antilles); and Gloeosporium affine (Berlin).

The above two citations, one by Baker and the latter by Rombouts are believed to have been the first reports, insofar as the writer is aware, of C. gloeosporioides Penz. as a cacao parasite and Rombouts stated that it was encountered as much on the foliage as the fruit. According to him, Mueller (48) indicated it as a conidial form of Glomerella cingulata. He also believed Mueller made an error because according to his statement, the "Central Bureau voor Schimmelcultures" proved by pure cultures that a conidial form of that Glomerella was C. nigrum Ell. and Halst.

The subject of latent infections caused by C. gloeosporioides and allied fungi was reviewed in 1940 by Baker, Crowdy, and Mc Kee (3) in Trinidad and they reported that isolations indistinguishable from C. gloeosporioides had been made from cacao, and stated that the cacao

anthracnose species (C. incarnatum, C. theobromae, C. theobromicolum, C. luxificum, and C. cradwickii) required reinvestigation.

Garces (34) reported, in 1941, that a pod anthracnose was encountered with some frequency in the cacao plantations of the Departments of Valle and Cauca in Colombia and was caused by a species of Colletotrichum.

In a summary of cacao disease investigations in Costa Rica during the period October, 1947, to April 1, 1948, Newhall (51) reported that C. theobromicolum was found attacking pods of all sizes, causing anthracnose pod spot. He also stated it was the cause of a great deal of spotting and dry marginal burning of cacao leaves. The fungus was repeatedly isolated from fruits, leaves, and twigs and pathogenicity was demonstrated on cacao seedlings. It was also stated that it might have been the same fungus as the one that commonly attacked coffee.

Thompson and Johnston (18), in 1948, stated that the commonest leaf disease of cacao in Malaya, and that occurred on almost all trees, was associated with species of Colletotrichum and Pestalotia. It was also reported that among secondary fungi after Helopeltis injury to pods was a Colletotrichum sp. A Gloeosporium sp. was found on older decaying pods. Cotyledons of seedlings grown from seed imported from Ceylon were rotted by a specie of

Gloeosporium.

Colletotrichum theobromicola was reported on cacao pods by Steyaert (77) in a contribution to the study of the plant parasites of the Belgian Congo in 1948.

Wellman (90), on a trip to consult on cacao problems in Ecuador from July to August 1949, stated there were six different pods rots that attacked cacao in Pichilingue, a hacienda near the town of Quevedo, and that among them was Colletotrichum which caused much pod decay.

In March, 1940, Vivero (86) reported an abnormality of young seedbed cacao plants in Costa Rica in connection with a fertilizer experiment. He indicated it was of a pathological nature involving a Colletotrichum sp. and Phytophthora palmivora. He also stated that Müller[3] found C. gloeosporioides in Brazil and Venezuela that occasioned the dropping of leaves and the death of terminal buds of cacao seedbed plants.

In a report based on a 19 day visit to Ecuador in March and April, 1950, Mc Laughlin (46) stated that Colletotrichum pod rot or anthracnose was generally present in all the cacao examined and to a greater extent than it was found in Costa Rica. He also stated that it must be

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[3], Dr. Albert S. Müller, Pathologist; Escuela Agricola Panamericana, Tegucigalpa, Honduras. In seminar titled "Enfermedades del Cacao" presented in December, 1949, for students of Cacao Center, Inter-American Institute of Agricultural Sciences, Turrialba, Costa Rica.

classified as a serious pod disease in Ecuador. He reported the disease on abandoned cacao trees not far from Tingo María, Perú, in June, 1950 (47).

Desrosiers (26) stated, "Colletotrichum blight is, in my humble opinion, also a serious factor in our cutting bins (Estación Experimental Agrícola del Ecuador) where it affects leaves and the tips of the cuttings, and is apt to destroy them by eliminating all of the superstructure while the base of the cutting remains in good condition." He also reported leaf and pod spottings caused by Colletotrichum, and secured perithecia from cultures of the leaf spot organism.

In Table 1, below is presented a summarized version of the above material, in chronological order for each of the several species cited.

TABLE 1. CHRONOLOGICAL ARRANGEMENT OF HISTORY AND GEOGRAPHICAL DISTRIBUTION OF SPECIES OF COLLETOTRICHUM AND GLOEOSPORIUM DESCRIBED FROM CACAO AND HOST PARTS AFFECTED.

Genus and species	Host parts affected	Year and Source	Geographical distribution
G. affine	Leaves, branches	1895, Hennings	Botanical Gardens, Berlin

Table 1. (Continued).

Genus and species	Host parts affected	Years and source	Geographical distribution
G. theobromicolum	Pods	1918, Vincens	Pará, Brazil.
G. theobromicolum	Leaves, pods	1926, Stevenson	Brazil.
Gloeosporium sp.	Pods, Cotyledons	1948, Thompson and Johnston	Malaya.
C. brachytrichum	Leaves	1905, Delacroix	Trinidad, Ceylon.
C. brachytrichum	Leaves	1933, Verwoerd and Du Plessis	South Africa.
C. brachytrichum	General	1937, Rombouts	Brazil.
C. coffeanum	Pods, twigs	1921, Small	Uganda.
C. coffeanum	Pods, twigs	1926, Small	Uganda.
C. cradwickii	Branches	1910, Bancroft	Jamaica.
C. cradwickii	Pods	1911, Bancroft	Jamaica.
C. cradwickii	Pods	1926, Stevenson	Porto Rico.
C. cradwickii (?)	Pods	1927, Dade	Gold Coast.
C. cradwickii (?)	Pods	1931, Dade	Gold Coast.
C. gloeosporioides	Pods	1936, Baker	Trinidad.
C. gloeosporioides	Pods, leaves	1937, Rombouts	Brazil.
C. gloeosporioides	Pods	1938, Baker	Trinidad.
C. gloeosporioides (?)	Pods	1940, Baker, Crowdy, Mc Kee.	Trinidad.

Table 1. (Continued).

Genus and species	Host parts affected	Year and source	Geographical distribution
C. gloeosporioides	Leaves, leaf buds	1950, Müller	Brazil, Venezuela.
C. incarnatum	Pods	1901, Zimmerman	--
C. incarnatum	Pods	1921, Snowden	Uganda.
C. incarnatum	Pods	1923, Busse	Cameroons.
C. incarnatum	Pods	1923, --	Ceylon.
C. incarnatum	Pods	1926, Stevenson	Ceylon, Java, Belgian Congo, Uganda.
C. luxificum	Buds, branches, pods	1907-8, Van Hall and Drost	Surinam, Demerara.
C. luxificum	Twigs, pods	1925, Da Camara and Coutinho	St. Thomas Island, West Africa.
C. luxificum	Twigs	1927, --	Philippines.
C. luxificum	General	1933, Kaden	St. Thomas and Prince Islands.
C. theobromae	Pods	1903, Appel and Strunk	Victoria, Cameroons.
C. theobromae	--	1919, Patouillard	St. Thomas Island, West Africa.
C. theobromae	Pods	1926, Stevenson	Central Africa.
C. theobromicolum	Pods	1905, Delacroix	Antilles.
C. theobromicolum	Leaves	1913, Shear, Wood	Washington, D.C. (?).

Table 1 (Continued)

Genus and species	Host parts affected	Years and source	Geographical distribution
C. theobromicola	Twigs	1921, Snowden	Uganda.
C. theobromicolum	Pods, leaves, twigs	1947-8 Newhall	Costa Rica.
C. theobromicola	Pods	1948, Steyaert	Belgian Congo.
Colletotrichum sp.	Pods	1921, Snowden	Uganda.
C. sp.	Twigs	1925, Small	Uganda.
C. sp.	Twigs	1929, --	Philippines.
C. sp.	--	1930, Toro	Colombia.
C. sp.	Pods	1932, Steyaert	Belgian Congo.
C. sp.	Pods	1936, Baker	Trinidad.
C. sp.	Pods	1941, Garcés	Colombia.
C. sp.	Leaves, pods	1948, Thompson and Johnston	Malaya.
C. sp.	Pods	1949, Wellman	Ecuador.
C. sp.	Seedlings, general	1950, Vivero	Costa Rica.
C. sp.	Pods	1950, Mc Laughlin	Ecuador.
C. sp.	General	1950, Mc Laughlin	Perú.
C. sp.	Cuttings, leaves, pods	1950, Desrosiers	Ecuador.

The above table was specifically designed to demonstrate the large number of species described from the

single host, cacao, and also to illustrate how widely distributed the different species are from a geographical standpoint.

MATERIALS AND METHODS

Cultures of the organism were obtained from various parts of naturally infected cacao seedlings that demonstrated the characteristic disease lesions described below. Cultures were also made from diseased pods of various sizes, as well as from fruit pedicels, flowers, and seeds. Diseased material was collected at La Lola farm, the Institute in Turrialba, and at La Hulera (United States Department of Agriculture Rubber Station, Turrialba) and was brought to the pathological laboratory of the Institute for the cultural work.

The culture media used were ready-made and fresh potato-dextrose agar, water agar, unhulled rice medium (made with 1 part, by volume, of rice to a little more than 2 parts of tap water), and maltose-peptone agar which was prepared on the basis 5 grams of plain agar and 2½ grams each of maltose and of peptone, added to 250 cc of tap water. Such preparations were generally autoclaved for 20 minutes at 15 to 20 pounds pressure. To avoid bacterial contamination, all of the above media, except rice, were acidified with 1 to 2 drops of 25 per cent lactic acid added after autoclaving, but before the agar had solidified.

The general procedure used for preparing diseased tissues for culture was as follows: diseased portions were first washed well in running tap water, followed by cut-

ting out the advanced or outer areas of the lesions with a sharp razor blade, after which they were put in a small beaker or flask. When a sufficient number had thus been prepared, the top of the container was covered with a single thickness of cheesecloth held in place with a rubber band. Then, tap water was allowed to flow over the tissues for 15 to 20 minutes, after which the flask was partially filled with a solution of bichloride of mercury (1:1000) or sodium hypochlorite ("Purex", 14 cc/1,892 cc water), and allowed to stand for a period of 3 to 5 minutes for the purpose of surface sterilization. The solution was then poured off, the tissues were washed 3 times with sterile tap water, and were then retained on the cheesecloth after the final wash. The cloth was later removed from the flask, the flask inverted, and the cloth with enclosed tissues was placed on the inverted flask. These were brought to a hood where the tissues were removed one by one with alcohol-flamed forceps and transferred to the culture media in standard Petri dishes.

For symptom studies of the disease, most of the work was conducted in the laboratory and greenhouse at Turrialba, with material brought from La Lola farm. Such studies were correlated with many observations, notes, and sketches of natural infection under field conditions, and then a series of artificial inoculations were made. The results of these inoculations were again compared with the progress of the field

disease symptoms. For seed inoculation experiments, pods were obtained from mature field trees, surface sterilized, the seeds aseptically removed, and 3 to 5 were placed in a large pyrex test tube which had been previously plugged with non-absorbent cotton and autoclaved. Some seeds were also surface sterilized, washed with sterile tap water, and placed in the tubes. Such seeds were inoculated either with spore masses placed on the surface of each seed with a flamed needle, or spores in water suspension were sprayed over the surfaces with a De Vilbiss atomizer. An equal number of seeds were prepared in the same manner, but without the addition of spores which served as the checks. These tubes were then placed horizontally in a series of moist chambers.

For the preliminary phases of the inoculation studies with seedlings, moist chamber bowls were surface sterilized and filled with fine wood shavings. Then 10 "Arasan" treated cacao seeds were planted in each of several bowls, the seeds having been peeled to remove the pulp and seed coat previous to planting. Later work was conducted using autoclaved pots of screened soil and either "Arasan", bichloride of mercury, or sodium hypochlorite disinfection of the seeds. The inoculum for such experiments consisted of either mycelium or tap water suspensions of spores from 5 to 10 day old cultures, prepared by pure culture techniques. The mycelium was applied directly to leaf surfaces with a sterile

needle, while spores were applied in water suspension to the upper and lower sides of leaves with a small hand atomizer. After inoculation, small beakers of water or wet paper towelling were placed at the base of the plants to maintain high humidity, and then, the plants were covered with tall cylindrical glass jars.

Spore germination studies were conducted with cultures of the fungus growing on maltose-peptone agar either in standard Petri dishes or on agar slants in test tubes. Spores were removed from such 4 to 5 day old cultures with a sterile needle and suspended in 10 cc of tap water in a small beaker, which was then thoroughly stirred to separate the conidia from the matrix. A single drop or two of the suspension was then placed on a clean glass slide, and the procedure was repeated depending on the number of replications needed, generally, 3 to 6. Such slides were placed singly in a Petri dish on a piece of moist paper towelling with a hole cut in the center corresponding to the spore suspension. Thus, microscopic observation was possible without removing the slide from the dish. In some tests, the standard hanging-drop type mount was made. Once prepared, the dishes were each numbered to correspond to the particular replicate and were placed on a shelf in the laboratory or in a controlled temperature oven at either 25 or 29°C. Per cent germination was determined by simply counting 100 to 200 spores per replicate at random, and recording the number germinated and non-germinated.

Cultural studies were conducted using a single 8 day old pure culture of the organism that was growing on maltose-peptone agar. From it, 10 1 mm discs were cut from the rapidly developing outer edge with a sterilized cork borer. Each disc was then aseptically transferred to a 300 ml. cotton sealed flask containing 25 ml. of standard potato-dextrose agar acidified with 2 drops of 25 per cent lactic acid. Such inoculated flasks were placed on a table not far from a window and at the end of 56 hours, measurements of diameter increase were commenced using a small centimeter ruler, and also observations were recorded on growth characteristics of the mycelium. Such observations and measurements were made every 48 hours after the first 56 hours had elapsed.

For the cross-inoculation experiment conducted with Miss Hastings, the cacao organism and the coffee fungus, C. Coffeanum were used. Young seedlings of coffee and cacao in 5 inch pots were used in the experiment, and they were placed in paired groups in 4 different concrete tanks provided with frame-glass tops. The tanks were so regulated that 2 had a high moisture content, while the other 2 had a low content. Each of them were roughly regulated to produce high temperature and low temperature by regulating light and humidity. Tap water spore suspensions of the coffee and cacao organisms were prepared and applied to the appropriate coffee and cacao plants with separate atomizers, after which the plants were returned to the chambers. The cacao seedlings had by then

matured the first flush of leaves, and the coffee foliage was also in a mature condition.

In the apple inoculation experiment, apples were first surface sterilized 5 minutes in a sodium hypochlorite solution in a large glass preserving jar, then rinsed 3 times in sterile tap water. The apples were transferred to small Petri dish tops placed in moist chambers, both of which had been previously disinfected with the same type solution. Four apples were placed in each chamber. After having added a small amount of sterile water to the bottom of each chamber to maintain high humidity, the chambers were closed by placing sterilized glass plates over the top. Half of the apples in each chamber were wounded by making 6 punctures in each apple with a sterile needle. Pieces of sterile absorbent cotton were then dipped in plain tap water and placed on the upper surface of all the check apples. The same procedure was repeated for the other 2 groups of apples, but also a spore suspension from the appropriate organism was used. The chambers were placed on a table in the laboratory to observe the results.

For studies of the host-parasite relationship, both green mature and young wine-colored leaves of seedlings were used. They were first washed in running tap water and then surface disinfected in sodium hypochlorite or in bichloride of mercury (1:1000, 3 to 5 minutes) solution, after which they were placed in moist chambers disinfected with one or the other of the same solutions. Such leaves were placed in

equal numbers, some with the upper side up, and others with the lower side up, on beakers in the moist chambers. Then, small rectangular or square areas on the upper and lower leaf surfaces were marked lightly with a wax crayon. Within the marked areas, several drops of a spore suspension were deposited with a glass eye-dropper. On part of the leaves, only drops of plain water were applied and served as checks. The moist chambers were then covered and placed in a controlled temperature oven at 25°C.

After a lapse of 7, 26, 48, 168 (14 days), and 336 hours (28 days), such leaves were fixed in acetic-alcohol by first cutting out the inoculated areas with a sterile razor blade, then subdividing them, and placing the smaller pieces in the solution. In this solution, the tissues were killed and it also extracted the chlorophyll if left in the solution for about 24 to 48 hours. After killing and extraction, such pieces were removed from the solution and stained in either safranin in chloral hydrate followed by fast green, acid fuchsin; or acetocarmine. After varying periods of time, determined by periodic test observations, the tissues were removed and mounted for direct observation in a drop or two of 50 per cent glycerine on a glass slide and covered with a cover slip. To observe infection phenomena,the same killing and fixing process was used, but after extraction, the tissues were mounted 3 to 4 at a time between 2 pieces of soft pith, bound with a rubber band, and

mounted for making microtome sections. Such sections were transferred to 70 per cent alcohol in a Petri dish with a camel's hair brush. From this alcohol sections were removed as needed and placed in acetocarmine for 10 to 15 minutes, and then, were transferred to a slide for observation as described above.

The soil used in the studies leading to control measures was of a dark organic type, which was screened previously to remove extraneous material. It was placed on a concrete floor where it was inoculated by sprinkling it, layer by layer, with a water spore suspension, prepared from cultures about 10 days old that were growing on maltose-peptone agar. Afterwards, the soil was thoroughly mixed with a shovel, then placed in three large wooden boxes as the chemical treatments were administered. The treatments were as follows: the formaldehyde drench treatment as recommended by Newhall and Lear (50) which was prepared at the rate of 1 gallon of formalin (40 per cent formaldehyde) to 50 gallons of water; ½ gallon of this solution was applied per square foot of soil surface. Chlorpierin was applied at the rate of 8 ml. per cubic foot of soil. After application, the boxes were covered with several thicknesses of wet newspaper to maintain the vapors. Two days afterwards, the soil was dumped out of the boxes into 3 piles, each separated by a wooden board. It was stirred 2 to 3 times daily to obtain a workable condition,

and 10 days afterwards, seedflats were filled with the treated soil. The flats were arranged side by side on a table covered by glass and an artificial shade of about 50 per cent.

Seeds were obtained from La Lola farm, and the pods were surface washed as previously mentioned prior to opening. In total 2700 seeds were to be planted, half of which had the pulp and seed coats removed. Five seed treatments were applied as follows: 1) Check - inoculated, 2) Check - non-inoculated, 3) Thiosan - 2 grams per liter of water, 4) Fermate - 2 grams per liter water, and 5) Mercuric chloride - 1 gram per liter water. All seeds except those of the non-inoculated treatment were dipped in a spore suspension for 5 minutes prior to applying the treatments. Then, the respective treatments were given as 5 minute liquid dips. The seeds were then placed on separate pieces of paper over night, and were planted the following day. Each of the flats was subdivided into 5 sections with wooden insert walls. Data were recorded on: 1) number of seeds germinated, 2) incidence infection of outside of cotyledons, 3) incidence of stem infection, 4) incidence infection of young leaves, 5) incidence of general cotyledon infection, and 6) measurements of height.

THE DISEASE

Symptoms of the disease have been observed in Costa Rica on all above-ground parts of cacao seedlings, ez; stems, cotyledons, leaves, and stipules as well as on seeds. Isolations of the fungus were made from flowers and also pods of various sizes but the present work was limited primarily to the seedling phase of the disease.

Symptom Studies

The symptoms of the disease on cacao are described under the following headings: 1) seeds, 2) tender stems, 3) cotyledons and 4) leaves. A striking similarity is notable between the symptoms expressed on some parts of cacao and those of other hosts. This is particularly true with the symptoms on bean seedlings (29, 91, 49), tender leaves and stems of mango (63, 53), and tender leaves and stems of citrus (30, 27). It is of interest to note that parasite damages of the latter 2 hosts is caused by _C. gloeosporioides_. The symptoms here described were based on a combined study of the progress of the disease under natural conditions in the field as well as under artificial conditions of the laboratory and greenhouse.

Seeds

Cotyledon surfaces of seeds in seedbeds prior to germination from which the seed coat had been removed demonstrated lesions that varied in color from dark brown to

black. Generally they were slightly sunken and varied in shape from somewhat circular to very irregular elongate type lesions. In size, the range was from mere pinpoints to large lesions that involved half or more of the seed. When such seeds were opened by pulling the 2 cotyledons apart it was often noted that there were also 1 or 2 to several of the same type lesions within. The latter type lesions were often noted very close to or, actually attacking, parts of the embryonic plumule and radicle. The embryonic parts later rotted and were reduced to a watery mass.

On artificially inoculated seeds in large test tubes very much the same type of symptoms were produced, and a very heavy growth of mycelium was produced on the pulp surrounding the seeds only 2 to 3 days after inoculation. After 6 days such seeds were completely covered by a grayish-white to dark green mycelium (Figure 1). On some of them the typical flesh colored spore masses were observed. The attack of the fungus caused a brownish discoloration of the pulp, that later became slightly sunken. From such attacked areas dark brown or black spots developed that penetrated through the seed coat to the cotyledon surfaces inside, where lesions were also produced.

Tender stems.

Lesions on the tender green part of seedling stems

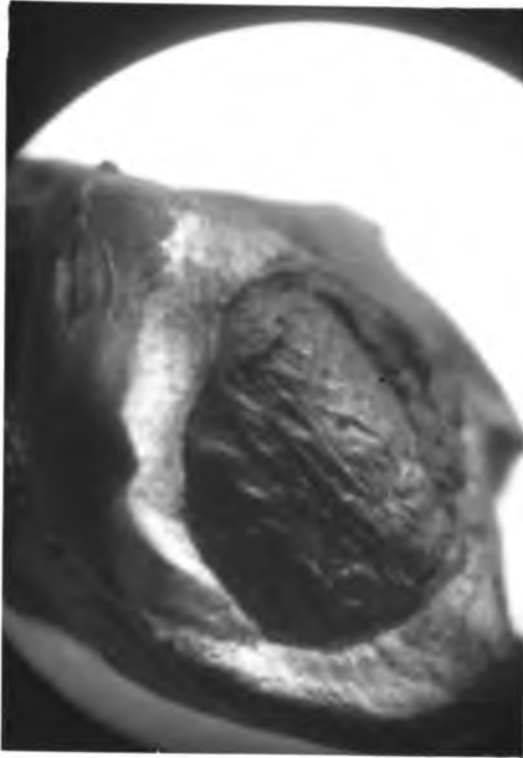


Fig. 1. Mycelial cover on cacao seed 6 days after artificial inoculation with spore suspension. Approx. 4 x.

were quite noticeable due to the contrasting color of the lesions with the stem color. They were generally more extensive on portions of the stem below the cotyledons. The lesions were irregular in shape and size but generally tended to be vertically elongated in the direction of the stem and gave a somewhat tearstained effect. The lesions ranged from small circular dots to elongated streaks, generally very narrow, and ranged from dark brown to black in color (Fig. 2). At times the lesions on the green parts of stems were slightly raised.

Cotyledons

Lesions were observed and produced on the outer and inside surfaces of the cotyledons and there is no doubt that such infections serve as inoculum sources for the propagation of the disease. These spots were circular to irregular in shape and were variable in size, tending to be dark brown to black in color, although sometimes the center was of a grayish color. During humid, moist periods in the seedbeds or under artificial inoculation conditions in the laboratory and greenhouse, such lesions readily formed pinkish spore masses. Such masses of inoculum, under field conditions, were washed down the stem below the cotyledons and produced the type of stem lesions mentioned above. On artificially inoculated seedlings such lesions first began to appear about 9 days afterward.

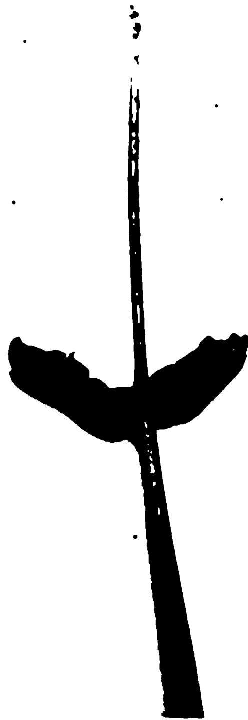


Fig. 2. Stem lesions artificially produced by atomization of spores in water suspension. (D. Fiester).

Leaves

The symptoms on leaves, particularly those on young ones, were the most variable of those encountered in the study. Furthermore such attacks, under favorable environmental conditions, produced the most serious type of damage; that is, the "bare-tip" of seedlings. Numerous artificial inoculations demonstrated that the fungus did not produce lesions on uninjured mature leaves, whether the leaves were detached from the plant in moist chambers or on growing plants under bell jars. By this it is not intended to convey the impression that the fungus did not obtain entrance in such cases but, quite the contrary, for it apparently did penetrate, however without producing symptomatic lesions. This will be discussed in a later section of this paper.

On the other hand young leaves, from the embryonic stage to those about 2 inches long, if still in a wine-colored condition are very susceptible provided that environmental conditions are favorable. With the very young leaves lesions were produced on the upper and lower sides of petioles, midribs, secondary veins and areas between the secondary veins, as well as the production of marginal necrosis. The lesions on the petioles, midribs, and veins followed the pattern of the parts affected, ez. they were narrow and elongated. However, lesions between the veins

may be quite variable in shape and size, although some tended to be somewhat circular or oblong (Fig. 3). The marginal necrosis was very irregular in appearance, and several such spots often coalesced to produce larger lesions.

On very young leaves about 1/2 to 2 inches long, attacks of the fungus produced a curled or crinkled effect on the leaves (Fig. 4) and that, combined with petiole infection caused such leaves to absciss. When the emerging first flush of young leaves of seedling cacao plants were thus attacked the result was the production of a large number of so-called "bare-tip" plants. However, when the leaves escaped infection at this early age, but were infected as they gradually lost the typical "wine" color, they tended to be considerably restricted, somewhat circular in shape, and rarely coalesced. Consequently such leaves were not abscissed and when they reached maturity a yellow halo was noted surrounding the spots. As the leaves grew older some of the necrotic areas fell out, giving them somewhat of a shot-hole appearance. In seedbeds in the field, plants affected with "bare-tip" are very obvious. They present either a bare stem with two cotyledons or, in plants a little older there may be no cotyledons, but a few spotted, mature leaves, followed by a terminal or apical section of stem without leaves but with a closely grouped mass of stipules (Fig. 5) that represent the position where successive flushes were produced and abscissed, but only the stipules remain.

Fig. 3. Typical type of lesions on young seedling cacao leaves. (D. Fiester).

Fig. 4. Curvature of leaves induced by marginal and apical lesions. (D. Fiester).

Fig. 5. Typical field symptom of "bare-tip". Photographed at La Lola farm, Madre de Dios, Costa Rica. (Leibovit).

Common Names

A bewildering confusion of common names was encountered for the various manifestations of disease produced by the *Colletotrichum* parasites of cacao. For the purpose of reducing this confusion the principal types of damage may be designated as follows: 1) pods, 2) branches, 3) leaves, 4) cotyledons, and 5) seedling stems. Apparently descriptions of diesease symptoms on the 2 latter host parts have not been previously reported, but were found to be quite important in the current study. Symptomatic descriptions have already been given for them so no further treatment is included below.

Pods

Names which have been commonly applied to disease symptoms produced by various *Colletotrichums* on pods are as follows: " anthracnose of cacao pods," "*Colletotrichum* pod disease," "rot of pods," "hardening of pods," "*Colletotrichum* pod rot," "anthracnose," "pink rot or anthracnose," and "anthracnose pod spot." It is readily apparent that such a large number of common names may easily cause great confusion in the literature and consequently it has been suggested by Mc Laughlin (46) that the generic name of the fungus involved in the pod rot be used as, "*Diplodia*" pod rot, "*Monilia*" pod rot, or as in the present

case it seems preferable to use the term "Colletotrichum pod rot."

Branches

Manifestations of symptoms of the disease on branches have been variously referred to as "die-back of twigs," and "die-back of branches". In this case there apparently has not been much confusion in respect to common names but several fungi have been previously implicated in the so-called "die-back," so that again it seems advisable to specify "Colletotrichum die-back," or "die-back caused by a species of Colletotrichum, thus avoiding any doubt as to the organism involved.

However in the work with seedlings in Costa Rica, a quite prevalent condition was encountered that did not correspond to the current type of die-back and for that reason it was referred to as "bare-tip". Preference was shown for that term rather than die-back because the apical growth of the seedlings continued forming flushes of new leaves and stipules which were rapidly abscissed by renewed attacks of the fungus. Thus the terminal portion of the stem remained alive but was devoid of leaves, the so-called "bare-tip".

Leaves

Cacao leaves attacked by Colletotrichum or Gloeosporium spp. are said to have been affected with "leaf spot,"

"anthracnose of leaves," "anthracnose," and "leaf anthracnose." The word "anthracnose" seems preferable in this instance since, as pointed out by Jenkins (39):

> It is suggested that, in the future, where this term (anthracnose) and that of scab, may be used in naming diseases caused by fungi of the genera named (Sphaceloma, Elsinoë, Gloeosporium, and Collētotrichum), they be applied in a symptomatic sense, anthracnose for necrotic and hypoplastic diseases with somewhat restricted lesions, as in bean anthracnose, and scab for hyperplastic diseases with scab-like lesions, as in citrus scab.

THE CAUSAL ORGANISM

In the review of the literature which has been presented it is apparent that two different genera and numerous species have been considered as involved in the anthracnose disease of cacao. It is believed that much of this nomenclature may be reduced to synonomy in the future. However for the purpose of this paper the causal organism of the disease has been designated as *Colletotrichum gloeosporioides* Penzig cacao strain.

Taxonomy

The genera *Colletotrichum* and *Gloeosporium* have usually been placed in the Fungi Imperfecti and, as applied by the name, the group consists of propagative or secondary stages of other fungi. As has been pointed out by Clements and Shear (19):

> In consequence, they do not constitute a natural class, but form an artificial group kept together for convenience. Many of them are found in association with the perfect form in nature, while the number of those linked up by means of experimental cultures is steadily increasing.

Various systems of classification of the Fungi Imperfecti have been proposed and have been mentioned by earlier writers (12, 19, 74) but, according to Wolf and Wolf (92) the oldest one, used by Saccardo in Sylloge Fungorum is serviceable and does not appear to have been materially improved by the several modifications proposed. Under this

classification the *Deuteromycetes* (Fungi Imperfecti) consist of 4 orders, the 1) *Phomales* (*Sphaeropsidales*, *Phyllostictales*), 2) *Melanconiales*, 3) *Moniliales* (*Hyphomycetes*), and 4) *Mycelia sterilia*. The genera *Colletotrichum* and *Gloeosporium* pertain to the *Melanconiales*, which consists of the single family *Melanconiaceae*. The authors cite Bender (1931) as having stated that the family contains 92 genera, including the two mentioned above, and more than 600 North American species.

Insofar as the perfect or sexual state of the various species mentioned is concerned, the only ones having a proven connection in available literature are *C. gloeosporioides*, *C. coffeanum*, and *C. theobromicolum*. The perfect stage in all of the mentioned cases has been referred to *Glomerella cingulata* (Stonem.) S. and v. S. So far as is known the perfect stage of *C. gloeosporioides* has not been reported on cacao. Shear and Wood (65) found mature asci and ascospores of *G. cingulata* on leaves of cacao taken from greenhouse plants in 1913 and identified the conidial stage as *C. theobromicolum*, but were unable to produce the perfect stage in artificial cultures. The *Glomerella* stage of *C. coffeanum* was obtained by Small in 1921 and 1926 (67, 69) and according to him, was identified by Kew as *G. cingulata*. He inoculated conidia from the coffee organism into wounded cacao pods and twigs and

obtained the same perfect stage. Desrosiers (26) in December, 1950 reported finding perithecia of Glomerella on leaf spots of cacao in Ecuador.

In the present study the sexual stage has never been encountered on leaves or other host parts nor in artificial cultures. However in the latter, very commonly there were formed dark bodies resembling immature perithecia. Upon examination under the microscope they were found to have been dark green to blackish heavily matted masses of mycelium, and contained numerous conidia.

Glomerella cingulata (Stonem.) S. and v. S. is one of the Ascomycetous fungi and, according to the classification as given by Wolf and Wolf (92), this group is composed of two subclasses, the Hemiascomycetes and the Euascomycetes, of which Glomerella pertains to the latter. For convenience the Euascomycetes have been arbitrarily subdivided into the Plectomycetes, Pyrenomycetes, and Discomycetes. The Pyrenomycetes are usually regarded as comprising 4 orders known as the Dothideales, Hypocreales, Sphaeriales, and Laboulbeniales. The Sphaeriales comprise the largest order of the Pyrenomycetes, and it has been variously estimated that it contains between 6,000 and 10,000 species. The authors distinguished 12 different families, and Glomerella cingulata was placed in the family Gnomoniaceae.

Spore Measurements

Measurements of conidia of the fungus were made from naturally infected seedling leaves obtained from seedbeds in La Lola farm. They were first thoroughly washed and later placed in moist chambers at room temperature (about 24° C) for 3 to 4 days to promote spore production. The spore masses were then placed in drops of water on a series of glass slides and were covered with a glass cover-slip. The high-power lens was used in all the work, and measurements were made by use of an ocular micrometer. A total of 200 were thus measured, and the length and width of each was recorded. The average length and width were then obtained by division of the two individual totals by 200. Afterward they were separately multiplied by 1.75, the calibration correction factor. In Table 2, below, is presented an enumeration of data regarding spore length and width in the form of a frequency distribution.

TABLE 2. FREQUENCY DISTRIBUTION OF MEASUREMENTS OF LENGTH AND WIDTH OF 200 SPORES (MICRONS).

Length	No. spores	Width	No. spores
7.0	1	2.0	4
7.5	4	2.5	28
8.0	19	3.0	131
8.5	24	3.5	34

Length	No. spores	Width	No. spores
9.0	68	4.0	3
9.5	40		
10.0	29		
10.5	6		
11.0	9		

From the above-mentioned data the range in length and width were determined, as well as the averages, and were as follows:

Length:

Range --------- 12.3 - 19.3 u

Average -------- 16.07 u

Width:

Range ---------- 3.5 - 7.0 u

Average -------- 5.3 u

Physiology

Spore germination: temperature effects.

Numerous experiments have been conducted by various workers to determine the temperature relations requisite for spore germination of species of *Colletotrichum* and *Gloeosporium*, but they were almost exclusively from hosts other than cacao. Walker (87) found that the minimum for conidia of onion smudge (*C. circinnans*) was 4° C, while the maximum was 32° C and the optimum was about 20° C.

Sattar and Malik (63) determined that for mango (Mangifera indica L.) anthracnose (C. gloeosporioides), the minimum temperature was between 10 to 15° C, the optimum 25°, and the maximum between 35 to 40° C. The minimum temperature for conidial germination of the watermelon anthracnose organism (C. lagenarium) was stated by Wolf and Wolf (92) to be 7°, while its optimum was 22 to 27° C.

Some investigators reported difficulties in obtaining germination in water beyond a certain percentage under various temperature conditions, while others indicated unusually low percentage germination under constant temperatures for a given time interval. Jensen and Stewart (40) stated that germination of the conidia of C. schizanthi, in sterile water on sterile slides, at no time exceeded 30 per cent. Leach (44) reported difficulty in securing a high percentage of germination of conidia of the bean anthracnose organism (C. lindemuthianum)and also that some previous investigators (Atkinson, Edgerton, Dey,Barrus, and others) also had encountered the problem especially in distilled or tap water. He also stated that Webb was forced to discard the organism from the fungi selected for study of spore germination as affected by hydrogen ion concentration due to his inability to secure germination in control solutions. Leach took spores from sterilized bean pods and placed them in hanging drops of distilled water. Less than

2 per cent of the spores germinated. He also removed the matrix by centrifuging conidia 2 minutes at 1800 revolutions per minute, and that was repeated 5 times, taking a sample each time to place on a hanging drop mount. Such washing did not appreciably increase germination, although a lower percentage of the unwashed spores germinated. He concluded that apparently the matrix was not responsible for the poor germination in distilled water.

Conidia of the croton anthracnose organism (*Gloeosporium sorauerianum*), as reported by Gigante (35), only germinate in water to the extent of 20 to 40 per cent at 25° C. in 24 hours, and 40 to 60 per cent in 48 hours, while 95 per cent germinated in 72 hours.

Kendrick and Walker (42) worked with conidia of 14 day old cultures of 2 strains (CP 4 and CP 5) of the tomato anthracnose fungus (*C. phomoides*). The conidia were suspended in distilled water at concentrations of about 200 spores per low power field on glass slides in Petri dishes. The percentages were derived from an average of 6 replicates. At a temperature of 28° C. they found that at the end of 22 hours germination did not exceed 30 per cent. Strain CP 4 germinated to the extent of only 1/2 per cent and CP 5 only 29 per cent. At the end of 27 hours the former was 9.1 per cent and the latter 73.5 per cent, while after 46 hours CP 4 had germinated 43 per cent and CP 5 germinated 90 per cent.

Similar difficulties were encountered in the present study of spore germination of the cacao organism. Single or double drop conidial suspensions were placed on clean glass slides supported either on wads of moist cotton or on damp paper towelling in standard Petri dishes and were placed in a controlled temperature oven at 25° C (variation .5 to 1° C). Such spores were taken from 4 to 5 days old sub-cultures of the fungus growing on maltose-peptone agar. It should be noted that the experiments were not conducted for periods of time greater than 14 hours, which was due to the fact that the electricity was cut off at about 11:30 in the evening during that period of the study. About 8 to 9 experiments were conducted in total, some at room temperature, others at 25° C, and one at 29° C. Figure 6 illustrates the stages in the germination of a conidium of the cacao organism, while Figure 7 shows a sample microscopic field of germinating conidia in water at room temperature after a period of about 12 hours.

In Tables 3, 4, and 5 that follow are presented data from 3 such germination experiments, thus clearly indicating the nature of the difficulty encountered. From 3 to 6 replications were used in each experiment and the percentage germination was determined by counting 100 to 200 spores per replicate at hourly intervals, recording the number germinated and non-germinated.

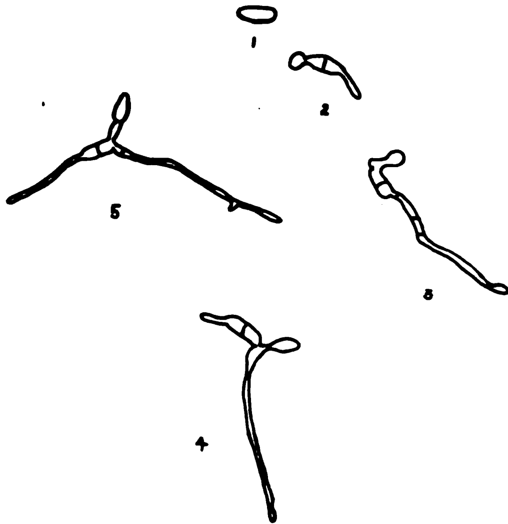


Fig. 6. Stages in germination of conidium of cacao organism: 1) conidium, 2) and 3) appressorium and germ tube, 4) and 5) new conidium. (Camera lucida - Leibovit).

Fig. 7. Conidia germinating in water, room temperature, after 12 hours. (R. Quesada).

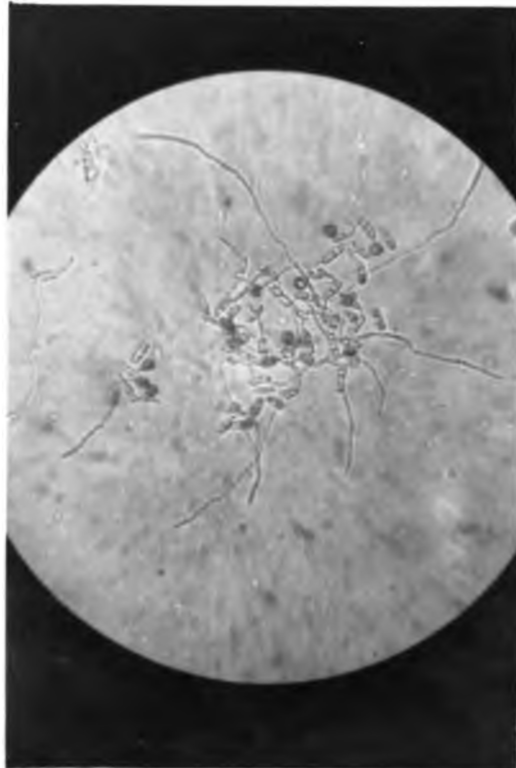


TABLE 3. EFFECT OF TEMPERATURE ON SPORE GERMINATION OF THE CACAO ORGANISM: 25° C (PER CENT PER 200 SPORES COUNTED).

Time	Replicates No. 1	No. 2	No. 3
10:00 A.M.	0.0	0.0	0.0
11:00	0.0	0.0	0.0
12:00	0.0	0.0	0.0
1:00	6.5	0.0	4.5
2:00	5.0	1.0	8.0
3:00	6.5	3.0	4.5
4:00	15.5	7.0	5.0
5:00	12.5	4.5	5.5
6:00	16.0	6.5	10.5
7:00	22.0	4.5	12.0
8:00	22.5	4.5	10.5
9:00	63.0	9.5	44.0
10:00	63.5	10.0	67.5

From the above data it may be noted that no germination occurred during the first 3 to 4 hours, and that after that period there was a rather slow increase in per cent germination until after the twelfth hour, when there was a large increase in germination with the exception of replicate number 2. Thus there was an indication that the conditions on the slide of that particular replicate and

those of the others were different. Consequently a second experiment was designed with 6 replicates, but with 2 spore suspension drops on each slide ez; replicates 1 and 2 were on a single slide, 3 and 4 were on another, and drops 5 and 6 were on a third slide. The results are shown in Table 4 below.

TABLE 4. EFFECT OF TEMPERATURE ON SPORE GERMINATION OF THE CACAO ORGANISM: 25° C. (PER CENT PER 100 SPORES COUNTED).

Time	Replicates					
	No. 1	No.2	No. 3	No. 4	No. 5	No. 6
9:00 A.M.	0.0	0.0	0.0	0.0	0.0	0.0
10:00	0.0	0.0	0.0	0.0	0.0	0.0
11:00	0.0	0.0	0.0	2.0	0.0	0.0
12:00	3.0	3.0	0.0	5.0	9.0	4.0
1:00	5.0	5.0	1.0	7.0	51.0	6.0
2:00	4.0	4.0	3.0	5.0	79.0	17.0
3:00	1.0	3.0	3.0	8.0	84.0	16.0
4:00	2.0	8.0	10.0	6.0	91.0	11.0
5:00	2.0	6.0	13.0	23.0	85.0	23.0
6:00	8.0	14.0	30.0	49.0	87.0	20.0
7:00	11.0	11.0	48.0	51.0	89.0	28.0
8:00	19.0	25.0	67.0	52.0	84.0	40.0
9:00	13.0	41.0	65.0	68.0	95.0	66.0
10:00	13.0	22.0	52.0	70.0	--	--

Again it is noted that, in general, germination did not begin until after 3 hours with the exception of replicate 3 and 4. Then followed a period of about 7 to 9 hours in which the germination was rather slow and very few conidia germinated except in the cases of replicates 1 and 5 which showed extreme variations. From that period on there was a rather rapid increase in germination.

Such data demonstrated that there was considerable variation present between 2 drops, even on the same slide, as well as between drops on different slides. Consequently a third experiment was performed using 4 replicates, the results of which are presented below in Table 5.

TABLE 5. EFFECT OF TEMPERATURE ON SPORE GERMINATION OF THE CACAO ORGANISM: 25° C (PER CENT PER 100 SPORES COUNTED).

Time	Replicates			
	No. 1	No. 2	No. 3	No. 4
9:30 A. M.	0.0	0.0	0.0	0.0
10:30	0.0	1.0	0.0	1.0
11:30	7.0	16.0	9.0	20.0
12:30	11.0	19.0	7.0	34.0
1:30	23.0	23.0	18.0	27.0
2:30	19.0	45.0	23.0	46.0
3:30	25.0	36.0	26.0	29.0
4:30	30.0	66.0	28.0	58.0
5:30	28.0	59.0	49.0	47.0

Table 5. (Continued).

Time	Replicates No. 1	No. 2	No. 3	No. 4.
6:30 P.M.	54.0	82.0	62.0	61.0
7:30	65.0	94.0	82.0	84.0
8:30	82.0	97.0	83.0	90.0
9:30	97.0	99.0	94.0	90.0
10:30	96.0	100.0	100.0	95.0

In the latter experiment, germination commenced a little sooner and, at the end of the observation period, was rather different from the previous experiments.

The writer was unable to account for such erratic results as described above since, insofar as known, the conditions of the experiments were the same. However variations in germination were present ranging from 10 to 100 per cent. It is apparent that a preliminary short period, perhaps of water intake, is necessary before the germination process begins. The reason for which, in some cases, it then speeds up very rapidly or remains at a very low figure is not known.

Conidial suspensions were also prepared in the same way as those already described, but at a temperature of 29° C. However after a period of about 3 to 4 hours the conidia began to form large aggregates in the center of the drops of water, and did not germinate. Thus it seemed apparent that the maximum temperature for germination had been passed.

Cultural characteristics and growth rate

A single 8 day old isolate of the cacao organism was selected, which had been previously growing on maltose-peptone agar at room temperature (23-24°C). Ten 1 mm. discs from the outer edge of the culture were cut out with a sterile cork borer and were aseptically placed on acidified standard potato-dextrose agar in 300 ml. flasks, which each contained 25 ml. of the medium. Measurements of increase in diameter and observations of growth characteristics were commenced after about 56 hours and thereafter were repeated approximately every 48 hours until the mycelial extension reached the walls of the flasks. In Table 6 below is indicated the growth rate of each of the 10 replicates, by 48 hour periods, after the first 56 hours.

TABLE 6. GROWTH RATE OF THE CACAO ORGANISM ON POTATO-DEXTROSE AGAR AT ROOM TEMPERATURE, 23 TO 24°C (CENTIMETERS).

Culture Age (hrs.)	Replicates									
	1	2	3	4	5	6	7	8	9	10
56	1.9	2.0	1.2	1.4	1.6	1.8	1.3	1.4	1.3	1.7
104	4.1	4.0	3.3	3.4	3.7	3.9	3.3	3.3	3.3	3.6
152	6.4	6.4	5.3	5.6	5.9	6.4	5.4	5.6	5.5	6.0
200	Reached edge	R. e.	7.3	7.7	R. e.	R. e.	7.5	7.7	7.6	R. e.
248	--	--	R. e.	R. e.	--	--	R. e.	R. e.	R. e.	--

Thus, although the replicates were derived from a single original culture, differences may be noted in the rate of growth between replicates even though the conditions of the experiment were uniform as mentioned above.

Characteristics of the mycelial development were recorded for the same replicates and are described below:

56 hours

The original disc was of a dark green color with some whitish aerial mycelia growing from it. The initial mycelia on the agar was somewhat hyaline and rather closely appressed to the surface. There was a marked clock-wise rotary growth of the mycelia. Some small dark green patches were visible scattered through the mycelia spreading from the base of the disc.

104 hours

A heavy, very coarse aerial mycelia extended from the disc, which was whitish on top but with a dark green circular area below. Beyond the dark green area the mycelia were hyaline and closely appressed to the agar surface. A strong rotary growth was still noted. On the underside of the culture a series of dark green bands were developing, interspersed by whitish-green mycelial growth. The aerial mycelia were so heavy it was not possible to observe if spore production had commenced.

152 hours.

Most of the mycelia were of a characteristic dense, coarse white type, except for a small area immediately surrounding the disc which was grayish to light green as seen from the top, and dark green below. The aerial mycelia extended about 1/4 inch above the substrate. There was still a small outer fringe of hyaline mycelia that closely adhered to the agar. There was no evidence of spore production although isolates from citrus (*Citrus sinensis*) and coffee in another experiment, but the same conditions were producing large numbers of conidia.

200 hours.

The mycelia in the flasks which had reached the edge had commenced growing up the walls of the flasks. The aerial mycelia were whitish-gray in appearance, dense and coarse and somewhat matted. It also produced a number of olive to dark-green bodies dotted on top of and slightly below the mycelial surface. The outer edge of the colonies were streaked with dark green patches which, when seen from the underside, appeared as two circular bands. No spore production was evident.

248 hours.

The only major changes noted were a continued darkening in color of the mycelial mat, both on the upper and lower surfaces. Considerable spore production had commenced on

the surface of the substrate, near the outer edges of the flasks where there was not a heavy covering of mycelia.

When growing on maltose-peptone agar the mycelia of the fungus were mostly characterized by a whitish to hyaline mycelia which were closely appressed to the substrate for the most part, although in some cultures there was a tendency to produce a small amount of aerial mycelia. As the cultures grew older they frequently tended to become dark colored, and commonly produced distinct circular bands of alternating light and dark areas, (Figure 8), and within the former large numbers of conidia were produced. At room temperature spore production was usually prolific, and commenced 3 to 4 days after sub-culturing or from an original isolate.

Host Ranges Reported

As has been previously described, numerous *Colletotrichum* species and several species of *Gloeosporium* have been named from various parts of the cacao tree and, consequently, *Theobroma cacao* L. was considered to be the main host from the standpoint of the present study. However it is necessary to emphasize that some of those species were first described from other hosts, thus *Gloeosporium affine* was first mentioned from foliage of *Hoya* and *Vanilla* by Saccardo (61) in 1884 or slightly

Fig. 8. Typical concentric development of the fungus on maltose-peptone agar at room temperature. Heavy spore production. (D. Fiester).

earlier, in the botanical gardens at Patavino (**Country?**). It was also collected, according to Saccardo, by Magnus on orchid foliage in the botanical gardens at Dresden (**Germany**). Colletotrichum incarnatum was initially described from Coffea liberica. The same applies to C. coffeanum which was first mentioned from Coffea arabica.

However the species which seems to have the widest host range, although the latest mentioned insofar as parasitism of cacao is concerned, is that of C. Gloeosporioides. According to Burger (17) this species was first described by Penzig in 1882 as Vermicularia gloeosporioides. The description, as recorded by Saccardo (62) apparently was from leaves and weak branches of citrus in Padova, Stra, and Cattajo. In 1887 Penzig placed it in the genus Colletotrichum. Since that early period when it was first described as a citrus parasite it has been reported as the causal organism of disease on a wide variety of different species and varieties of citrus, as well as various other hosts. It has also been implicated in the black spot of avocado (Persea americana) fruits (75, 93); withertip" of young shoots and fruit spurs of loquat (Eriobotrya japonica) (16); leaf-spot and die-back of young shoots of nursery seedlings of **Herbea** Hevea brasiliensis (45); fruit rot, blossom blight, wither-tip of tender twigs, and leaf-spot of mango (Mangifera indica)

(4, 54). Thus, from the literature, it is apparent that the latter mentioned species has quite a wide host range.

The remaining species, namely *C. theobromae*, *C. theobromicolum*, *C. brachytrichum*, *C. luxificum*, *C. cradwickii*, and *G. theobromicolum* seem to have been more or less constantly associated as cacao parasites only.

Physiologic Specialization

Ever since the time of Schroeter (1879) who, according to Wolf and Wolf (92) first suggested it, the concept of physiologic specialization in the fungi has been recognized and proven so that today there are very few adherents of the monomorphic hypothesis or the so-called "immutability of species". It has been demonstrated that such varieties, or biologic races or strains, exist within the species of the genera *Colletotrichum* and *Gloeosporium* as well as many other genera of fungi.

The greatest advances of our knowledge in regard to physiologic specialization with these two genera has been with one species of the former genus, namely *C. lindemuthianum* the causal organism of bean anthracnose. Such investigations commenced in 1911 when Barrus (10) reported the discovery of certain strains of the fungus that demonstrated differences in ability to attack certain bean varieties. The same author in 1918 (11) distinguished

2 biologic forms in inoculations with 10 collections on 137 bean varieties. Somewhat later Leach (44) reported that at least 8 distinct biologic forms of the fungus had been found as a result of comparative inoculations made with 15 cultures of C. lindemuthianum on 14 field bean varieties. Heald (37) presented a tabulation of reports concerning physiologic strains as follows: alpha and beta strains (Barrus, 1918); Gamma strain (Burkholder, 1923); 4 strains distinct from American (Muller, Holland, 1926); 9 strains, distinct from American except one (Budde, Germany, 1928); 12 strains (Peuser, Germany, 1931); and 34 strains in 3 groups, each containing one of American strains of Barrus and Burkholder (Schreiber, Germany, 1932).

Taubenhaus (79) proved by many cross-inoculations on the apple and the sweet pea that the anthracnose disease of the latter was due to the same organism, Glomerella rufomaculans (Berk.) Spauld. and von Sch; as causes the bitter rot of the apple. He also showed that Gloeosporium officinale E. and E, G. diospyri E. and E; G. gallarum Ch. Rich; Gloeosporium spp. from May-apple; Colletotrichum nigrum E. and H. and C. phomoides appeared to be identical with G. rufomaculans, since they were able to produce the typical anthracnose disease of the sweet pea and the bitter rot of the apple on the tree.

On the other hand, he stated that C. gloeosporioides, C. lagenarium, G. musarum Cke; and Gloeosporium sp. from Populus deltoides seemed to be distinct species since they were unable to infect the apple or the sweet pea even by puncture inoculation.

As the result of cross-inoculation experiments Nolla (53) concluded that C. gloeosporioides appeared to be the cause of the anthracnose diseases of mango, orange, grapefruit, lemon, avocado, and sometimes of lime. He reported that inoculated small leaves of young buds of the mango were caused to shed comparatively early.

In 1935 Bessey (12) stated that:

> Mr. G. L. Fawcett, then a colleague of the author, grew this fungus (C. gloeosporioides) on over 50 different hosts in Miami, Florida. These had been described in literature under 25 or more species names in the two genera Gloeosporium and Colletotrichum.

A cooperative cross-inoculation test was conducted with Miss Lucy Hastings of the Institute Plant Pathology Department, using isolates from the cacao organism and of the coffee fungus, C. coffeanum that were grown on maltose-peptone agar. Mature seedlings of coffee and cacao in 5 inch pots were placed in 4 separate frame glass covered concrete tanks. Tap-water spore suspensions of the appropriate organisms were applied with small hand atomizers, after which the glass covers were replaced.

Observations of the plants were made periodically thereafter for about 5 weeks. However, insofar as the cacao plants were concerned no typical symptoms appeared, due to the cacao organism or the coffee fungus. However Miss Hastings obtained infection of the coffee with both of the organisms, and the consequent production of the typical anthracnose symptoms. The reason for the apparent lack of infection of the cacao plants is discussed in an ensuing part of the present study.

An inoculation test was conducted with apples in which the cacao and coffee organisms were also used. Both wounded and unwounded apples were used, and after inoculation with a tap water suspension they were maintained at room temperature in moist chambers. After about 10 days lesions were formed on the wounded fruits by both the cacao and the coffee organisms, but none were observed on the unwounded fruits nor any part of the checks. A week later such lesions had expanded considerably and dark acervuli were formed. Three weeks after inoculation small lesions appeared on the non - wounded apples which had been inoculated with the coffee organism, whereas none were produced on the non - wounded apples inoculated with the cacao organism. The experiment was terminated 3 to 4 weeks later but no lesions

were produced on the non-wounded apples with the cacao organism, even though all others previously reported as lesioned exhibited the typical apple ripe rot symptoms. Abundant masses of acervuli and conidia, as well as grayish or greenish mycelia were also produced. The check apples remained free of disease, but the respective fungi were cultured from the inoculated apples.

HOST - PARASITE RELATIONSCHIPS

Spore Dissemination

Investigators seem to be quite generally agreed that inoculum of the genera of Colletotrichum and Gloeosporium, in the form of conidia, is disseminated in a number of different ways. For example, Walker and Weber (88) stated that, in the case of watermelon anthracouse (C. lagenarium), from initial sources of infection the spores of the fungus were spread widely by the wind, rain, cultural implements, animals, and laborers. Edgerton (29) indicated that conidia of the cotton anthracnose organism (C. gossypii) were distributed by insects, rain, and wind. He stated:

> The rain washes the spores out of the acervuli and the wind aids by whipping the upper parts of the plant and bringing them in contact with the ground or the lower part of the stem, where the spores have found lodgment.

In the case of bean anthracnose (C. lindemuthianum), Whetzel (91) showed that drops of rain or dew were effective in dissolving spore masses of the fungus. Very much the same relationship was indicated by Heald (37), but he also emphasized spattering raindrops.

Somewhat similar observations were made in the work in Costa Rica, particularly due to the effects of heavy and, at times, sustained rainfall with the accompanying spattering raindrops. When such raindrops struck previous sources of infection such as lesioned cotyledons, or

infected stems and leaves the exuded spores were disseminated in all directions. Thus new inoculum was transmitted to other susceptible host parts. Such spores were also washed to the ground where, in turn, they were splashed in droplets of soil and water to the host. By gently washing the adhered soil - water drops from tender leaf surfaces after a heavy rain and observing the collected drops under the microscope, numerous Colletotrichum conidia were demonstrated.

Small mounds of soil were also built up surrounding the young seedling stems and often extended high enough to embrace the lower part of the cotyledons, and thus inoculum was brought in contact with stem and cotyledon tissues.

Incubation

Germination

There has been a close accord among workers as to the stages in the conidial germination phenomena of the various species of Colletotrichum and Gloeosporium, although most of such descriptions were made based on inoculated drops of water on a slide or in hanging - drop cultures. Sattar and Malik (63) briefly described the germination of conidia of C. gloeosporioides in drops of distilled water at 20° C as follows:

> . . . (The conidia) began to produce germ tubes after about 6 hours and about 7 per cent

> of the spores became one - septate. Germination went on increasing till after about 24 hours it reached a figure of about 70 per cent. Germ tubes generally came out from the ends of spores but occasionally these developed from the middle of the spore length. The germ tubes elongated rapidly and about 12 hours after their formation began to develop appressoria at their ends. The appressoria were knob or club - shaped structures with a dark thick wall. Often more than one appressorium was formed on a single germ tube. In such cases a hyphal branch was given out from the first formed appressorium and this in turn bore another appressorium.

Baker, Crowdy, and Mc Kee (3) reported that no unusual features were observed in the spore germination of _C. gloeosporioides_ or in appressoria formation. They found that conidia in water germinated readily on fruit surfaces of all ages and appressoria were seen regularly 9 hours after inoculation.

Kendrick and Walker (42) studied the germination of the conidia of one strain (CP 5) of the tomato anthracnose organism on the surfaces of ripe and green tomato fruits. On uninjured ripe and green fruit surfaces spores germinated well at 24 hours. Appressoria formed at the ends of about three - fourths of the germ tubes, but no penetration hyphae were evident. No further development was seen even after 70 hours. On injured ripe and green fruit surfaces germination occurred abundantly after 24 hours and the germ tubes grew towards and into the wounds. Appressoria were formed on about 50 per cent of the tubes.

It was stated by Fulton (33) that conidia of tomato

anthracnose germinated readily in droplets of water on the tomato fruit surface. He also noted that fungal hyphae were often associated with epidermal hairs, and that the hyphae were generally tipped with appressoria near the base of hairs.

Dey (28) inoculated bean pods with conidia of _C. lindemuthianum_ and incubated them at 25°C for 24 hours. Thereafter sections were fixed every 4 hours. He reported that appressoria were produced by the germ tubes, and that they appeared held on the surface by means of a mucilaginous sheath. It was stated that the activities of the young germ tube during the period 24 to 40 hours were confined to appressoria production.

According to Taubenhaus (79), who referred to conidia of the sweet pea anthracnose organism (Glomerella rufomaculans):

> In case the spore lodges on a stomate, the germ tube grows away and avoids entrance. The exchange of gases which takes place at the stomates renders them toxic and prohibits the entranc of the germ tube, which often breaks through the epidermal cells as soon as the spore germinates.

In order to study the germination phenomena of the cacao organism the conidia, in water suspension, were placed on the upper and lower surfaces of both very young and mature leaves of seedlings. The leaves were placed in

moist chambers and incubated at 25°C. The inoculated tissues were fixed at time intervals of 7, 26, and 48 hours, stained, and then mounted for direct microscopic observation. For convenience the data on germination are presented below under 4 separate headings, dependent on the maturity condition of the leaf and whether it is the upper or lower side.

<u>Mature</u> - <u>upper</u>

After 7 hours very few spores had germinated. Instances of anastomosis were observed, that involved from 3 to 8 conidia; while others were in groups but showed no signs of germination (Fig. 9). Spores on or adjacent to main veins germinated at the bases of projecting hairs where there apparently were gland - like openings. Only 2 or 3 germinated spores - were noted with appressoria, one of which had germinated and formed a fine infection tube but was still outside of the leaf tissue (Fig. 10), while the other had 2 projections similar to hyphal branches (Fig. 11). After a lapse of 26 hours there was very little change in the amount of germination, although some further anastomosis was observed. Two days (48 hours) after inoculation only 34 spores had germinated of a total of 200 that were counted, or about 17 per cent. All of such spores formed appressoria only, and were developed in close proximity to the individual conidia. No stomata were seen on the mature upper leaf surfaces.

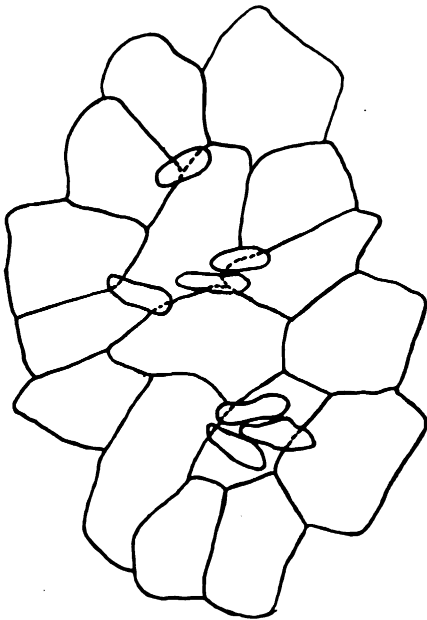
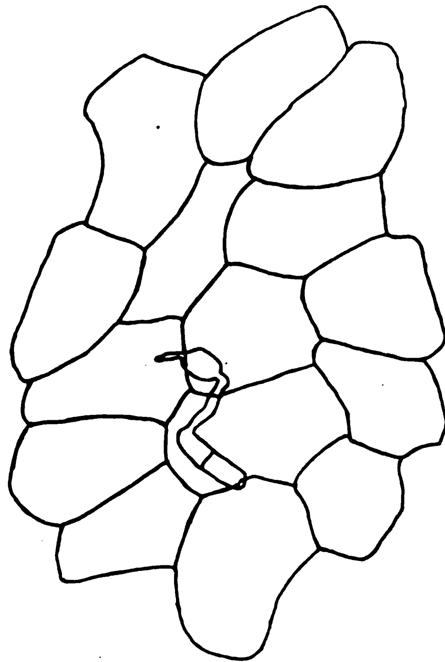
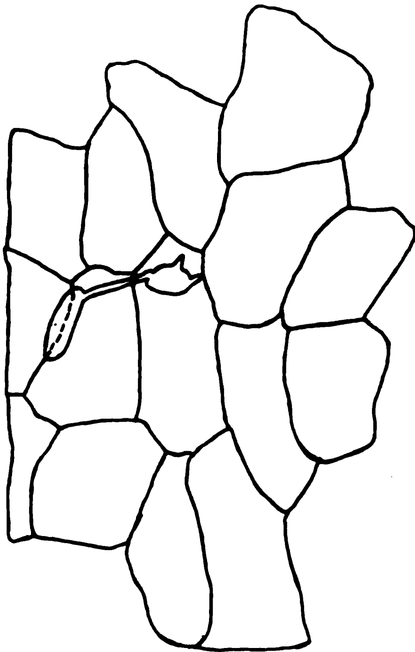


Fig. 9. Reaction of conidia in artificial inoculation of upper-mature leaf surface. (Camera lucida - Leibovit).

Fig. 10. Germinated conidium with appresorium in process of germinating on upper mature leaf surface. No penetration observed. (Camera lucida - Leibovit).

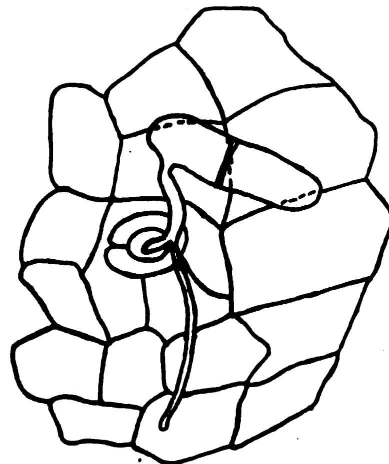




II

Fig. 11. Germinated conidium and appressorium with 2 germ-tube like projections, on upper side mature leaf surface. (Camera lucida - Leibovit).

Fig. 12. Growth of germ tube of conidium of cacao organism toward stomatal opening on underside mature leaf. (Camera lucida - Leibovit).



IV

Mature - lower

Germination phenomena on the under-side of mature leaves were quite different from those on the upper side. At the end of 7 hours germination had commenced and it was apparent that much of it was indirect germination (without appressoria formation). When such spores were in close proximity to stomata there was a marked tendency for the germ tube to grow toward the stomatal opening (Fig. 12). Such observations were not in agreement with those of Taubenhaus (79) mentioned earlier. Germination by appressoria formation was also noted. Two days after inoculation an average of 29.5 percent had germinated of 200 conidia counted. Of that percentage 14 percent were by appressoria only, 14 percent by only one germ tube, and 1.5 percent by 2 germ tubes. Within the group that germinated by means of appressoria, 7.5 per cent formed appressoria over the stomatal openings and the remainder on the epidermal cell tissues. Of the group that formed only a single germ tube, 8 per cent grew toward openings of the stomata or were in the process of entering the stomatal cavity, while the remaining 6 per cent germinated on the epidermal tissues.

Tender - upper

Conidia on the upper sides of tender leaves germinated quite rapidly, and most of them had germinated at the end of 26 hours. Thus, at that time 63 per cent had germinated of which 32 per cent formed 1 germ tube, 15 per cent 2 tubes,

14 per cent a tube and appressorium combined, and 2 per cent formed only appressoria.

Tender - lower

Germination was also rather rapid, and after 7 hours there was a rather high percentage observed. After 26 hours an average of 59.5 per cent had germinated of a total of 200 spores counted. Of that number 17 per cent germinated with 1 tube, 10 per cent 2 tubes, 16 per cent with germ tube and appressorium combined, and 16.5 per cent with appressoria only. Within the group with 1 tube there were 2.5 per cent that grew toward or actually entered stomata (Fig. 13), as well as 2.5 per cent of the spores with 2 germ tubes, 1.5 per cent of those with germ tube and appressorium combined, and 4 per cent of those with appressoria only were formed over the stomatal openings.

Penetration

Direct

The appressoria have been variously referred to as "secondary spores" or "adhesion discs or organs" by different workers. According to Wolf and Wolf (92) Frank first recognized the true nature of appressoria. He stated they were adhesion discs which applied themselves closely to the surface to be penetrated, and thus served to anchor the pathogen while the membranes immediately below were being pierced by the infection hypha. Büsgen (1893) concluded appressoria

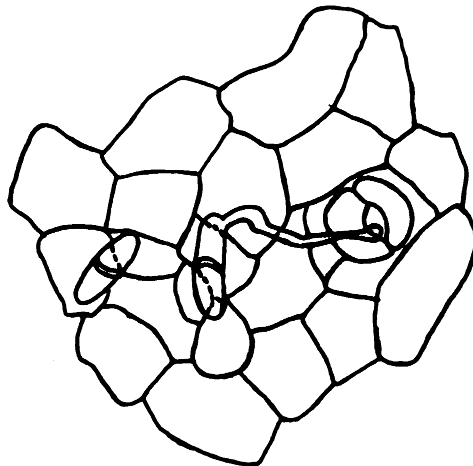


Fig. 13. Germ tube growing toward stomatal opening on underside of tender young cacao leaf. (Camera lucida - Leibovit).

formation resulted in response to contact of germ tube or hyphae with a solid body. Muncie (49) stated they were formed when the germ tube reached a certain length, but Hasselbring (36) who worked with Gloeosporium fructigenum, and Dey (27, 28) who investigated C. lindemuthianum and C. gloeosporioides reported they were formed by the germ tube as the result of stimuli from mechanical contact. Van Burgh (82) who worked with C. phomoides and used formalized gelatin membranes of 5 degrees of hardness, stated that the contact factor was a variable one and that its effect may be modified by that of nutrient materials. The number formed increased with increasing hardness of the membranes, but no typical ones were formed when nutrient materials were present. Dey (28) demonstrated that the young appressorium was sheathed in a mucilaginous coat which attached it to the leaf surface. Such a sheath had been previously described and illustrated for Botrytis cinerea by Blackman and Welsford (13).

In the present work it was often found that appressoria were formed by germ tubes, both in hanging drops and in water on slides, at 25° C. when water around the spores on the outer edges had evaporated considerably, thus leaving the germ tubes in contact with the glass. However, a few cases were noted in which germinated spores with appressoria were floating freely in the water. Such observations appeared contrary to observations of previous workers, and no ex-

planation for such phenomena is known. Observations were in accord with those of Dey and Hasselbring that there is no definite germ tube length prerequisite to appressorium formation. From data presented earlier it is clear that appressoria production was involved to a considerable extent in the initiation of penetration of cacao leaves.

As to the actual method by which conidia of the genera *Colletotrichum* and *Gloeosporium* penetrate host tissue Hasselbring (36) who worked with *G. fructigenum*, suggested it was effected by chemical substances secreted by the penetration hypha. In 1915 Brown (14), with *Botrytis cinerea*, did not find any solution of the cuticle. Blackman and Welsford (13) worked with the same organism and reported piercing of the cuticle was due solely to mechanical pressure exerted by the germ tube as a whole or by a special outgrowth from it. Dey (27, 28) confirmed the same type of penetration for *C. lindemuthianum* and *C. gloeosporioides*. Simmonds (66) worked with *Gloeosporium* and *Colletotrichum* spp. from banana, papaw (*Carica papaya*), and mango and stated the infection thread formed a hyphal structure adjacent to the cellulose wall of the epidermal cell but did not explain if it was a chemical or mechanical action.

Stomatal

Previous investigators have tended to delimit the importance of stomatal penetration in the genera *Colletotrichum* and *Gloeosporium*. Taubenhaus (79) stated that the germ tube

of the anthracnose organism grew away from and avoided stomatal entry. With the bean anthracnose organism, Dey (28) reported only one instance of passage of the infection hypha through a stoma; and even in that case infection was from an appressorium in the usual manner and not directly by the germ tube. Baker, Crowdy and McKee (3) worked with young and mature papaw fruits artificially inoculated with _C. gloeosporioides_ and reported it was probable both stomatal and direct cuticular penetration occurred. They based that idea on the fact that 20 to 50 per cent infection of young fruit took place after 12 hours, which was 18 hours before the heavy infection due to cuticular penetration developed. The early infection was attributed to direct stomatal penetration. They believed stomatal penetration occurred in mature fruit also but could not demonstrate it as natural infection had risen to 20 to 50 per cent. They stated the question of stomatal or cuticular penetration requires elucidation. Simmonds (66) reviewed the work of Baker, Crowdy, and McKee and in regard to stomatal penetration reported that during the process of examining many sections and surface slices from fruits of bean, banana, papaw, and mango he had not observed any signs of stomatal penetration. He obtained definite evidence for cuticular penetration.

From the data which has been presented above it is clear that in the case of the underside of both mature and tender cacao leaves there are two methods of stomatal

entry, namely 1) penetration by germination of appressoria situated over the stomatal cavity, and 2) growth of the germ tube into the stomatal cavity.

Length of incubation period

Young lemon leaves were inoculated with C. gloeosporioides by Dey (27) and were placed in moist chambers at a temperature of 80° F. He reported minute brown spots were detected after 72 hours.

Tender young wine - colored cacao leaves up to 3 inches long were placed in moist chambers, some with the upper side up, and others with the lower side up. Drops of a spore suspension from a 6 day old culture were placed within marked rectangular areas on the leaves which were then incubated at 25° C. Very small dark - brown to black dot - like spots were visible 72 to 96 hours after inoculation, and from which the fungus rapidly spread to the surrounding tissues. Similar results were obtained when very young seedlings were atomized with a spore suspension and covered with bell jars to maintain high humidity.

The same procedure as described above was repeated except that mature seedling leaves were used instead of young, tender ones. However, even though such material was kept at the same temperature and atomized daily, they remained in a green condition and no symptoms developed within the inoculated areas.

Infection

Active

It has been pointed out that the tender leaves were very readily attacked by the cacao organism and thus offered no resistance to infection. In only 4 to 5 days after inoculation such leaves were rather throughly spotted with the typical symptoms previously mentioned. The mycelium quickly ramified throughout the tissue and in 3 to 4 days there was a heavy conidial production; at times on both surfaces of the tender leaves.

Latent

There is yet another type of infection produced by the genera _Colletotrichum_ and _Gloesporium_, known as "latent infection". For some time there existed considerable confusion about the term but Simmonds (66) finally clarified it. He stated:

> It is to this method of survival by initial penetration, as distinct from survival in the free state as a spore, that the term latent infection is applied.

Shear and Wood (65) apparently were the first investigators to encounter the problem on a rather large scale. They reported the development of _C. gloeosporioides_ on leaves and branches of orange and pomelo even though the host parts had previously been disinfected with a corrosive sublimate solution (1 to 500 or 1 to 1,000 for 5 to 15

minutes). Such treatment (1 to 1,000, 3 minutes) was found to kill all spore forms of the fungus. Their explanation was that the conidia germinated, and produced appressoria which in turn sent a germ tube through the epidermis. That tube apparently penetrated at first but a very short distance and did little harm to the host cells, remained in a dormant or inactive condition until the host became weakened or injured or the organ infected died a natural death. However they were unable to identify the fungal hyphae with certainty.

Baker (7) stated Dastur (1916) found Gloeosporium musarum gained entry into plantain fruit in India while they were immature and green, and did not develop further until the onset of final maturation.

Simmonds (66) reported that Bates (1936) put forward several arguments that favored the theory that C. gloeosporioides formed dormant infectionsin organge fruit and later (1937) confirmed that view by isolating the organism from small pieces of skin taken from surface - sterilized fruit. Baker and Wardlaw (6) also demonstrated C. gloesosporioides was involved in latent infection in grapefruit at the time of picking. They also found papaws and mangoes contained latent infection due to Glomerella spp. and C. gloeosporioides. Baker (7) surveyed the organisms that might occur as latent infections during development of citrus, mango, avocado pear, papaw, tomato, and cacao fruits. Young

grapefruits were found to be heavily infected with C. gloeosporioides almost as soon as they were set. The fungus was also recovered from the tissues of petals, sepals, receptacles, and stigmas. It was said to have been the most abundant of latent infections of mangoes. Such infections were also found in various stages of maturity of avocadoes, but in papaws no latent infections were obtained in immature fruit. The same species was found commonly in both immature and ripening cacao pods, while Phytophthora palmivora was not. In green and ripening tomato fruits they found this type infection also. Other fungi found in connection with the work were Phomopsis citri, a Guignardia sp; and Fusarium expansum.

However, it should not be inferred that latent infection occurs only in tropical and subtropical fruits, for quite the contrary has been shown to be true. The same or a similar condition exists with regard to the scab organism (Venturia inaequalis), on apples in storage. Baker (7) stated that although several investigators (A. S. Horne and Colleagues, Fulsom, Bratly, and Wormald) had been working on the problem, they had found the manner in which the lesions of scab developed in storage inexplicable unless it were assumed they were established before the fruit was harvested. Thus he maintained that, while many workers had indicated latent infections must be present prior to harvest, conclusive proof based on isolating the organism from superficially sterilized tissue, had not yet been obtained.

Numerous investigations have also been made regarding latent infections in citrus fruits. Ruehle and Kuntz (60) isolated Phomopsis citri from fruits, young leaf blemishes, buttons, recently killed wood, and dead citrus wood in contact with the soil. In 1932, Kuntz (43) obtained pure cultures of the fungus from buttons of immature fruits. Tisdale and West (80) obtained the same fungus and Diplodia natalensis in pure culture from surface sterilized buttons and stems of oranges and grapefruit. Adam and others (1) investigated latent infection of Washington navel oranges in Australia and found C. gloeosporioides was the principal fungus concerned. They used the tissue plating method and found that significant differences might occur between different parts of the orange, between oranges from different trees in the same grove, and between oranges from different districts. Baker (7) reported that W. T. Horne (1934), from results obtained in storage, inferred that latent infection of Dothiorella ribis was established in avocado while the fruit was on the tree; while Horne and Palmer (1935) showed that the organism entered the young lenticels of immature fruit on the tree and formed mycelia which remained dormant in the air - spaces until the fruit was becoming senescent in storage.

Baker, Crowdy, and Mc Kee (3)artificially inoculated mangoes and papaws with C. gloeosporioides and found young mango fruits were infected as quickly and readily as old papaw fruit, but that older mangoes could not be infected

so easily and their behavior corresponded closely to that of young papaw fruit. They attempted to demonstrate latent infections actually in the tissues of papaw fruits by histological methods. Such attempts were unsuccessful but the presence of infections in inoculated tissues was indicated by the frequent occurrence of secondary cambia in the layers of cells immediately below the epidermis of papaw fruit which gave the appearance of a typical wound reaction.

Simmonds (66) maintained that up to the year 1941 there were two weak points in the evidence brought forward in regard to the latent infection hypothesis; namely 1) length of the period during which the fungus may be expected to survive in the latent condition, and 2) the form or manner in which the fungus persists in the host tissue. He determined that Gloeosporium musarum could infect banana fruit shortly after emergence of the bunch, remain in a dormant condition unrecognizable macroscopically for some 5 months, and then, as the fruit ripened, developed into the typical anthracnose spot. In regard to the second point, he stated that the infection thread after penetrating the cuticle did not enter the lumen of the epidermal cell but broadened out in the vicinity of the cellulose wall to form the fairly well -defined hyphal mass designated as the subcuticular hypha. He concluded that it was in this form of the subcuticular hypha that the fungus maintained its dormant condition throughout the period of latent infection.

He presented a summarized tentative physiological explanation for the change from the latent to the active condition in the case of bananas and mangoes, as follows:

> The infection tube penetrates the cuticle of the green fruit, possibly largely by the exertion of mechanical force developed as a result of contact stimulus, and comes to be against the cellulose layer of the epidermal wall. This presents a mechanical obstruction which the parasite is unable to overcome owing to the chemical nature of the wall or the cell sap at the time. The fungus is therefore forced to enter upon a period of dormancy in the form of the sub - cuticular hypha. The changes occurring in the protopectin of the primary cell wall and middle lamella during the early stages of ripening allow the fungus to extend gradually along this path. Then, later, owing to the withdrawal of toxic substances, or to an increase in enzyme action resulting from better nutrition and growth or alterations in the constitution of the cell sap, an intracellular existence becomes established. Growth then becomes rapid and is accompanied by the destruction of the host tissue and the development of the typical lesion.

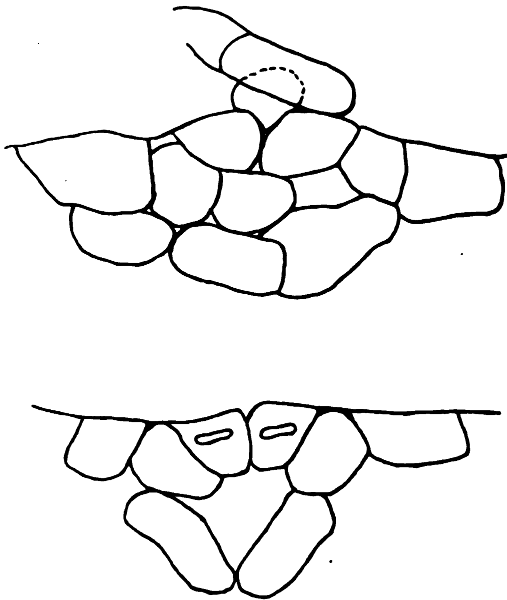
From the evidence cited above it is apparent that previous investigators have almost exclusively tended to limit their work to latent infections in fruits and have not paid much attention to its effects on other host parts. Perhaps such is understandable, since the ripe - rots which these infections are capable of producing are of great economic importance from the standpoint of the chain from the producer to the consumer. However, latent infection may be of considerable importance when vegetative portions of the plant are to be used such as for propagation by cuttings, budding, or grafting.

Shear and Wood (65) demonstrated that C. gloeosporioides was present in all the leaves of the new growth of the orange (Citrus sinensis), as well as the growth of the previous year, and developed more rapidly on the older leaves. They stated that the early and vigorous development of the fungus in the young fruits and the leaves situated at their bases and the rather tardy development in leaves remote from the blossoming shoots seemed to indicate a downward course of development of the fungus and infection by way of the blossoms. Thus Fiester (31) concluded that latent infection by C. coffeanum, mycologically determined by Miss Lucy Hastings, was an important causal agent of losses of as much as 75 percent of leaf-bud cuttings of Coffea arabica, both treated with certain root - inducing chemicals and without such treatments. He also presented data that indicated an apparent positive correlation of chemical hormone concentration and incidence of infection by C. coffeanum.

First interest in latent infection of cacao leaves was aroused when typical lesions and acervuli rather frequently developed on previously disinfected (1 to 1,000 bichloride of mercury, 3 to 5 minutes) leaf surfaces in sterile moist chambers. Further evidence of the latent condition, and a possible way of breaking it was obtained as follows: mature leaves were surface sterilized as above-described and were then inoculated on upper and lower surfaces with a spore suspension placed within

rectangular areas marked on the leaves. Such inoculated tissues were cut out at various time intervals thereafter to observe the development of germination and penetration phenomena. Some were fixed as much as 2 weeks after inoculation, yet the inoculated areas showed no signs of lesions that indicated infection had occurred. However observation of some of the sections, as previously noted, demonstrated that germination and appressoria formation had commenced. Although such sections showed no signs of lesions in the inoculated areas, lesions were developed 3 to 4 days afterward on the periphery of the leaves left in the moist chambers from which the rectangular sections were cut, even though a sterile razor blade was used to cut the tissues.

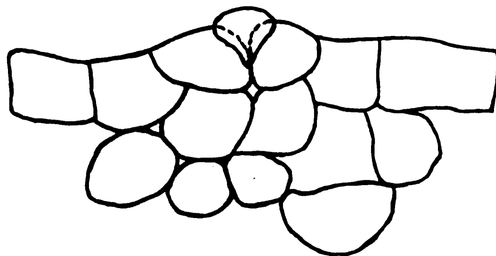
As illustrated in Fig. 14 the appressorium appears partially sunken into a stomatal opening and thus seems considerably depressed below the epidermal cells of the leaf. A normal stoma and accompanying air space below are also presented in the same figure for purposes of comparison. It also may be noted that the wall of the conidium has begun to decompose. In a later stage, as shown in Fig. 15, only the appressorium remains, and it is again evident that it is well below the stomatal cells. The next important phase is the germination or elongation of the appressorium at the basal end to form the very fine infection thread or "peg", as it has been called. This is shown in



IX

Fig. 14. (Top) Germination and appressorium formation of conidium over a stoma. (Below) Normal cells of stoma with air space below. (Camera lucida - Leibovit).

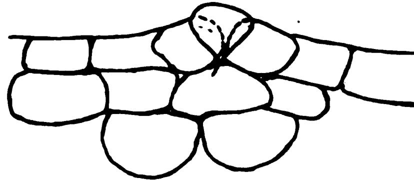
Fig. 15. Later stage of penetration. Conidium has disintegrated. Appressorium considerably depressed below stoma opening. (Camera lucida - Leibovit).



X

Fig. 16, which also emphasizes the minuteness of the infection thread. In the present study no further development of the infection thread in the tissues of mature leaves was observed. Thus, in the case of mature leaves it appears that the infection thread may remain in the latent or dormant condition in the area of the stomatal air spaces.

However, the paramount question which necessarily must be elucidated is in regard to determining the factor or factors controlling the activation of the latent condition. From observations presented earlier it would appear that wounding is one of them.



XI

Fig. 16. Extension of base of appressorium by formation of infection thread. (Camera lucida - Leibovit).

CONTROL MEASURES

According to Heald (37) seed disinfection to prevent bean anthracnose has been shown to be ineffective, and that spraying was of doubtful value from the standpoint of expense. Best control, according to him, was obtainable from the use of disease - free seed and the production and use of resistant varieties. However Walker and Weber (88) stated that the watermelon anthracnose organism was carried over winter in decayed plant material in the field, on the seed, and on other cultivated host plants, such as cantaloupe and cucumber. They recommended a seed sterilization dip in 1: 1000 bichloride of mercury for 10 minutes, followed by a thorough rinse in fresh water. It was also stated that a coating of 4 - 4 - 50 Bordeaux spray mixture should be kept on the vines at all times, and that a thorough application should be made when the first 2 to 3 leaves had developed and another when the vines had just begun to run. The first spray was considered especially important as it prevented the infection of the first leaves and thereby reduced the source of infection later in the season.

Throughout the investigation with the cacao organism there were no indications of the occurrence seed infection prior to the time pods were opened, unless such fruits had previously been damaged mechanically, or otherwise, which would have admitted Colletotrichum as well as other

fungi. However, as mentioned earlier, examination of planted seeds removed from seedbeds at various time intervals prior to the emergence of the seedlings demonstrated typical lesions on the surface of seed coats,as well as on the surface of the coyledons under the softened seed coat. Lesions also were observed along the inside edges of the suture along which the cotyledons parted after emergence.

Thus it was believed infection of the seeds had occurred in one of two different manners, or perhaps a combination of the two. However the two were involved in cultural practices since there was an unintentional 1) transfer of ineculum from secondary infection on the pods to the sticky pulp of the seeds during the process of opening pods and extracting the seeds prior to planting, and 2) incomplete removal of infected plant material, particularly bits of cotyledons, from the seedbeds before a new crop of seedlings was to be produced.

As a consequence, an experiment was designed to treat seeds only for the purpose of surface disinfection, and at the same time to disinfect the soil in which they were to be planted. Also both peeled (removal of the pulp and seedcoat) and unpeeled seeds were employed, although the current method of planting cacao seeds is with the pulp and seedcoats intact. It was thought that by the former treatment more rapid development could be

achieved, and thus a shorter period of exposure to the inoculum in the soil.

For the experiment a split - plot design was used, with 3 replications. There were 3 soil treatments, which consisted of formaldehyde, chlorpicrin, and a check (no treatment); combined with 5 seed treatments in the form of 5 minute liquid dips in Fermate, Thiosan, bichloride of mercury, and inoculated and uninoculated checks. The test was conducted in a series of seedflats arranged on a table in a glass - covered lathhouse to avoid the complicating factor of rainfall. Artificial shade of about 50 percent was provided also. The soil and the seeds were inoculated, except where otherwise indicated, by conidial suspensions in water. Each block consisted of 6 flats, and each flat contained 5 subplots of 30 seeds each. Thus in each replicated block there were 30 such plots, or a total of 90 subplots.

Data were recorded on 6 different phases during the course of the experiment, namely 1) germination, 2) incidence of infection of the outer surfaces of cotyledons of peeled seeds, 3) average incidence of stem infection of peeled seeds, 4) incidence of general cotyledon infection, 5) incidence of infection of young leaves of the first flush of peeled seeds and, finally, 6) measurements of the height of plants from peeled seeds. It should be

noted that no infection resulted on the unpeeled seeds, so that the only data recorded for them was in regard to germination. Data were recorded for a period of about 2 weeks.

The analysis of variance was used to analyze the data in 1), 2), and 3) above, that is data in relation to germination, incidence of infection of the outer surface of cotyledons of peeled seeds, and the general incidence of cotyledon infection. A numerical rating system of 1 to 3 was used to evaluate the relative degree of infection of cotyledons, in which 1 = light, 2 = medium, and 3 = heavy disease incidence. In the case of the incidence of infection of the outside of peeled seeds, each subplot of 30 plants was thus rated on a per plant basis and the total subplot value was divided by the total number of plants germinated per plot to obtain a plot average, after which the plots were totalled for the 3 replicates. The same procedure was repeated for the incidence of general cotyledon infection, except that a 9 plant random sample was used for each subplot rather than a value for all of the plants in each plot. In table 7 that follows is presented data pertinent to the soil and seed treatments:

TABLE 7. EFFECT OF SOIL AND SEED TREATMENTS ON GERMINATION AND DISEASE INCIDENCE WITH PEELED AND UNPEELED CACAO SEEDS.

Sl. trtms.	Seed trtms.	Germination		Cotyl. infection	
		Peeled	Unpeeled	Outside	General
Formaldehyde	Check-inoc.	80	77	6.52	5.86
	Thiosan	77	74	5.79	5.19
	Bi. mercury	82	70	6.92	6.77
	Fermate	84	79	5.60	4.72
	Check-no. in.	87	60	4.10	3.35
	Total	410	360	28.93	25.89
Chlorpicrin	Check-inoc.	76	75	6.73	5.96
	Thiosan	82	70	5.67	5.68
	Bi. mercury	70	55	6.47	6.56
	Fermate	84	80	5.55	4.44
	Check-no. in.	89	64	4.31	3.11
	Total	401	344	28.73	25.75
Check	Check-inoc.	83	62	7.43	6.13
	Thiosan	80	66	6.38	5.39
	Bi. mercury	83	51	6.82	6.21
	Fermate	78	68	6.42	5.37
	Check-no. in.	89	49	5.10	4.00
	Total	413	296	32.15	27.10

The data specifically in regard to the effect of the seed treatments on disease incidence and germination is summarized below in Table 8:

TABLE 8. EFFECT OF SEED TREATMENTS ON GERMINATION AND DISEASE INCIDENCE ON PEELED AND UNPEELED CACAO SEEDS.

Seed treatments	Germination		Disease Incidence	
	Peeled	Unpeeled	Outside	General
Check - Inoc.	239	214	20.68	17.95
Thiosan	239	210	17.84	16.26
Bi. mercury	235	176	20.21	19.54
Fermate	246	227	17.57	14.53
Check-non. in.	265	180	13.51	10.46

An average treatment and germination value per replicate may be obtained by dividing each of the above values by 3, the number of replicates. The analysis of variance for the 3 data factors considered is presented below in Table 9:

TABLE 9. ANALYSIS OF VARIANCE OF THE EFFECTS OF SEED AND SOIL TREATMENTS AND PEELED AND UNPEELED SEEDS ON GERMINATION AND DISEASE INCIDENCE OF CACAO PLANTS.

Source of variation	"F" Values		
		Disease incidence	
	Germ.	Outside	General
Soil trtms.	2.31	4.98	.36
Seed trtms.	5.06**	37.22**	14.99 **
Seed conditions	98.15**	—	—
Soil trtms. X seed trtms.	5.42**	.72	.45
Soil trtms. X seed conds.	7.96**	—	—
Seed trtms. X seed conds.	9.14**	—	—
Soil trtms. X seed trtms. X seed conditions	.56	—	—

It may be noted, as shown in Table 8, that there was a rather high disease incidence in the non - inoculated check which lessened the apparent treatment values. However the table does indicate that the Fermate dip treatment was the better of the 3 chemicals used in reducing the incidence of outer and general cotyledon infection. The same treatment also gave the second highest rate of germination with peeled seeds, and the highest rate with unpeeled seeds. Thiosan appeared to have been the next most successful treatment insofar as reduction of disease

incidence was concerned. The bichloride of mercury treatment apparently increased the incidence of general cotyledon infection over that of the inoculated check and was almost the same in the case of outside infection.

Table 9 indicates the high significance of the seed treatment on disease incidence, but that the soil treatments were apparently of little or no value. Seed conditions, as seen in the same table and as illustrated in Fig. 17 had a very highly significant effect on germination. Sifnificant values were also obvious for seed treatments, as well as for interaction between soil, seed, and seed conditions.

Fig. 17. Highly significant differences in rate of plant development from peeled and non-peeled cacao seeds. Plants with heavy development of foliage were from peeled seeds. (Leibovit).

DISCUSSION AND RECOMMENDATIONS

It is fully realized, and very humbly so, it may be said, that such an investigation may easily raise more questions than it attempts to answer. However, it is the sincere hope of the writer that something of each has been accomplished, thus making man's endless search for truth worthwhile.

Among the foremost of these questions is the strikingly wide distribution of the genus *Colletotrichum* and, may this not be partly the explanation for the many different species mentioned? The very fact that the species *C. gloeosporioides* seems, and apparently is, so adaptable would appear to justify a large scale investigation to determine if it in reality can move from one host to another with little difficulty.

The phenomena concerning germination of the conidia of this fungus is not believed to be very well understood if one judges from the results of previous workers, as well as the unusual pattern of germination in the present work. It may be necessary to develop a method whereby age and physiological maturity may be controlled, since the conidia are produced in a gelatinous matrix in which it is difficult, if not impossible, to determine the age and stage of development of them. Thus, as has been noted in some of the tests, using tap water and a constant temperature of 25°C, spores germinated 100 per cent after about 14 hours, while in others under

presumably the same conditions such high germination was never reached even after 48 hours. In other cases, there actually seemed to have been an inhibitory effect, and little or no germination occurred.

However, without a doubt, the most interesting part of the entire study has revolved around the section dealing with host-parasite relations and, more particularly, the nature, method, and importance of stomatal penetration of the mature and tender leaves of the cacao seedling. The suggestion for such an investigation was made by Baker, Crowdy, and McKee (3) as previously noted, and was believed by the writer to have been a very valuable one. He, in turn, wishes to add emphasis not only to their suggestion in regard to fruits, but also to recommend further investigations of the importance of latent infection in the vegetative parts of plants and the factor or factors necessary for activating it.

Insofar as control investigations are concerned, it has been apparent that strong measures must be taken to greatly reduce the inoculum potential both in regard to infected debris left in the seedbeds prior to planting and also to eliminate seed infection prior to planting. Provided that the two previously mentioned sources of infection can be controlled, it would appear then that protective sprays need only be applied when the plants were in the process of flushing, and a spray cover maintained until the

flush had matured. It is necessary to emphasize the fact that care must be taken to cover both upper and lower surfaces of the foliage during the mentioned susceptible period.

SUMMARY

A disease was found that attacked young cacao seedlings in seedbeds at La Lola farm in the Atlantic lowlands cacao producing zone of Costa Rica. The causal organism was found to pertain to the genus Colletotrichum, and was designated as the cacao strain of Colletotrichum gloeosporioides Penz.

In a rather thorough search of the available literature it was shown that several species of Colletotrichum and Gloeosporium have been described from cacao and that they have been found in the majority of the producing regions of the world. The geographical distribution and importance of the damage done by the various organisms have also been reported.

Complete symptom studies were conducted and it was determined that damage may be caused to all above-ground parts of cacao seedlings. The severest damage was referred to as "bare-tip."

Studies of spore germination at 25° C were made and indicated that, in general, germination commenced after the first 3 hours and in most cases half or more of the spores had germinated after 14 hours. At 29° C there was an inhibitory effect on germination. The growth rate and growth characteristics of the fungus on potato-dextrose and maltose-peptone agar media were also described.

The host ranges and physiologic specialization of the organism were also studied. In the host-parasite relations investigation it was determined that differences existed between the upper and lower surfaces of mature and tender leaves. Attempts to actively infect mature leaves on either the upper or lower surfaces were unsuccessful, whereas both surfaces of tender leaves were readily infected. Insofar as methods of penetration were concerned, it was found that stomatal penetration, both by direct and indirect means, was of greater importance than previously stated. The relation of the above facts were emphasized in regard to the employment of control measures.

Preliminary control studies were initiated that involved the treatment of soil and seeds, as well as the use of peeled and unpeeled seeds. It was found that soil treatments were of very little value, but that seed treatments were rather highly significant.

RESUMEN

Una enfermedad que ataca las plantículas de cacao fué encontrada en almacigales de la finca La Lola, ubicada en las tierras bajas del Atlántico, región productora de cacao en Costa Rica. Se encontró que el organismo causante de la enfermedad pertenece al género Colletotrichum, y la variedad del cacao fué designada como Colletotrichum gloeosporioides Penz.

Haciendo una investigación bastante completa de la literatura disponible, se demostró que varias especies de Colletotrichum y Gloeosporium han sido descritas en el cacao y que han sido encontradas en la mayoría de las regiones productoras de cacao del mundo. La distribución geográfica y la importancia de los daños causados por estos organismos también han sido reportadas.

Estudios completos sobre los síntomas de la enfermedad fueron realizados y se determinó que los daños pueden ser ocasionados a todas las partes, sobre la superficie del suelo, de las plantículas de cacao. El daño más severo se le denominó como "punta desnuda" ("bare-tip).

Estudios sobre la germinación de esporas a 25° C fueron hechos y estos indicaron que, en general, la germinación comienza después de las primeras 3 horas y que en la mayoría de los casos, la mitad o más, de las esporas habían germinado después de 14 horas. A los 29° C hubo

un efecto inhibitorio sobre la germinación. El período de crecimiento y las características del crecimiento de este hongo en agar papa-dextrosa y agar maltosa-peptona se describen en este trabajo.

Las diferentes plantas que sirven de huéspedes al organismo y la especialización fisiológica del organismo también fueron estudiadas. En las investigaciones de las relaciones entre huésped y parásito se determinó que existen diferencias entre las superficies superior e inferior de las hojas tiernas y maduras. Los intentos de causar infecciones activas en hojas maduras, tánto en la superficie superior como inferior, no dieron resultado positivo, mientras que ambas superficies de las hojas tiernas fueron infectadas fácilmente. En lo que se refiere a métodos de penetración, se encontró que la penetración por las estomas, tánto por métodos directos como indirectos, es de mayor importancia que la que se le había dado hasta el momento. Se da énfasis en este trabajo a la relación de los detalles arriba apuntados con respecto al empleo de métodos de control.

Estudios preliminares sobre control de la enfermedad se iniciaron, incluyendo el tratamiento del suelo y semillas, como también el uso de semillas peladas y sin pelar. Se encontró que los tratamientos del suelo son de muy poco valor, pero que los tratamientos de las semillas fueron altamente significativos.

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