Studies on the Pathology of Diplodia Boll Rot of Cotton (Gossypium Hirsutum L.) Caused by the Fungus Diplodia Gossypina Cke.

Jaime Delgado

Louisiana State University and Agricultural & Mechanical College
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STUDIES ON THE PATHOLOGY OF DIPLODIA BOLL ROT OF COTTON (GOSSYPiUM HirsUTUM L.) CAUSED BY THE FungUS DIPLODIA GOSSYPiNA CKE.

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STUDIES ON THE PATHOLOGY OF DIPLODIA BOLL ROT OF COTTON (GOSSYPUM HIRSUTUM L.) CAUSED BY THE FUNGUS DIPLODIA GOSSYPINA CKE.

A Dissertation

Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Botany and Plant Pathology

by
Jaime Delgado
B.S., Escuela Nacional de Agricultura, Mexico, 1955
M.S., Louisiana State University, 1966
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ABSTRACT

In 1967 several studies were made to test the pathogenicity of one isolate of the fungus Diplodia gossypina Cke. on immature bolls of upland cotton Gossypium hirsutum L. Deltapine smooth leaf variety was chosen as representative of the commercial varieties grown in Louisiana. Several hundreds green bolls were inoculated with a conidial suspension of the fungus for the purpose of determining first, how the boll becomes penetrated and infected by the fungus, and second, what factors are involved in predisposing the boll to disease.

Apparently healthy bolls of several ages were inoculated superficially, without wounds, and kept in a humid environment.

The first symptoms of disease were observed at the bract tips as they approached maturity. As soon as the boll reached maturity, it was penetrated by the fungus through natural openings that is, stomatal openings and cracks between the carpels almost simultaneously. It was noticed that under the moist environment the drying out process of the boll tissues, especially the outer layers, slowed and infection developed rapidly inside the tissues.

Boll pedicels were found also to be sites of penetration by the fungus. No evidence was found of fungus penetration from the pedicel to the boll. The infection developed rapidly in the cortex, and, later, in the phloem and pith tissues. The symptoms of disease occurred almost simultaneously with the outer boll symptoms.
These studies indicate that nectar is a suitable source of nutrient substances and favors the growth of fungi on boll and leaf surfaces especially on and around nectaries. The nectaries become infected at maturation time, although no evidence was found of penetration through uninjured tissues.

Under moist field conditions the wide appressed bracts as well as the nectaries are important sources of moisture and nutrients which result in increased growth of Diplodia and other microorganisms that encourage decay of the boll.

Mycetophagous organisms such as the mite Tyrophagous putrescentia Schrank and the fungus gnats Sciara spp. are involved in the biological breakdown of Diplodia invaded tissues. The role of these organisms is discussed.

Arsenic acid, frequently used as a desiccant, at harvest time, was found to increase the incidence of Diplodia boll rot when applied as a harvest-aid chemical in a warm, humid environment.
INTRODUCTION

Pathology is defined as the course of disease. It relates both to the host and to the causal agent. It is therefore necessary, in an investigation of this kind, that the existing knowledge of both the causal agent and the host be brought together in summary review as it relates to this disease.

Diplodia gossypina Cke. is one of many members of the genus Diplodia. It is found widely distributed throughout the tropics, growing as wound parasites or as a saprophyte on numerous tropical and sub-tropical hosts (19, 62, 66). Several members of the genus have been found to be of widespread occurrence in the southern portion of North America (12).

Published reports of surveys of the fungi associated with boll rot of upland cotton (Gossypium hirsutum L.) show that species of Diplodia are some of the most widespread organisms in several cotton areas of Louisiana. Studies made by Neal and Gilbert (48) revealed that species of Diplodia are especially pathogenic on cotton in some areas of the Mississippi valley. Similar observations were made at the beginning of this century by Edgerton (20), who reported Diplodia boll rot to be more prevalent on cotton growing on alluvial soils and bluff lands rather than in hills.

Diplodia rot is a problem wherever cotton is grown in a warm and humid environment.

In a search of the world literature by Pinckard and Chilton (54) in 1966, a total of 75 microorganisms which possibly damage cotton
bolls was found. From this number they concluded about 40 were present in Louisiana.

Losses in Louisiana caused by Diplodia alone have never been recorded because Diplodia is part of a boll rot complex which, according to Pinckard (52), causes an average loss of about 16 percent of the cotton yield over the entire state.

Upland cotton matures and opens its bolls at the end of the summer. Most of the time the opening of the boll occurs in a warm and moist environment. Consequently, the speed of opening under humid conditions slows, and the bolls often become exposed to infection by boll rotting organisms such as Diplodia. The complete explanation of how Diplodia, being a saprophytic fungus, penetrates and destroys the boll tissues is not very clear today. Diplodia seems to require a certain set of physiological conditions peculiar to the host before it can penetrate and cause infection (20).

Excessive amounts of moisture on the lower portions of the plant at maturation time predisposes bolls to infection by the fungus. Diplodia overwinters as a saprophyte in the soil until new bolls develop on the cotton plant. Rain and wind then carry the spores of the fungus to the susceptible bolls, which become infested by the pathogen (20).

The survival of the fungus is apparently favored by some morphological features of the bolls of upland cotton. One of these features is the wide, appressed bracts which, under humid conditions, hold moisture and create a humid microclimate around the boll, thus protecting the fungus from desiccation (39). Some reports have noted
that bracts harbored boll rotting fungi and predispose the boll to disease (38).

Uncertainties still exist with respect to the mode of penetration and spread of the fungus through boll tissues. Several ways of penetration have been found or suggested (7). Once the boll is penetrated, infection and development of disease occurs rapidly. This rapid spread of the fungus inside the boll tissues could be caused by the secretion of some phytotoxic substance by the fungus (20, 45).

The purpose of this study is to learn how Diplodia gains admission into the boll, to investigate the factors predisposing the boll to infection and to relate the knowledge obtained to control of the disease, which is the ultimate purpose of all such investigations.
REVIEW OF LITERATURE

In tracing the distribution and taxonomic status of Diplodia, it is apparent that it has been described or reported under many different names and on various host species. Certain workers have reduced many of these names to synonyms, which are also included under Physalospora rhodina (1, 19, 62, 64, 66).

Cooke (10) was the first to describe Diplodia gossypina on cotton bolls from India in 1879. It is a member of the order Sphaeropsidales. Only two life stages of the fungus are known, mycelial and pycnidial.

In 1892 Patouillard described Botryodiplodia theobromae on fruits of cacao from Ecuador (66). Prilleux and Delacroix described a fungus isolated from roots of cacao from Central America in 1894. They attributed the injury of the plant to this fungus, which they named Macrophoma vestita (66).

Howard (30), in 1901, found Diplodia cacaoicola on cacao and sugarcane in the West Indies. He was the first to investigate the parasitism of the fungus in connection with the dieback disease of cacao.

Petch, in 1906, working in Ceylon, described a fungus on Hevea brasiliensis and Castilloa elastica, which he named Botryodiplodia elasticae, and another on pods of cacao, which he named Chaetodiplodia grisea. After further studies of these forms, he showed that B. elasticae was identical with Diplodia cacaoicola (66).
During this same period, Charles (8) recorded Lasiodiplodia as being parasitic on cacao and *Mangifera indica* in Santo Domingo.

In further studies Petch, in 1910, concluded that Chaetodiplodia, recorded by Van Hall and Drost on cacao pods in Surinam, and Diplodia *rapax*, described by Massee as causing a disease of Para rubber in the Malay states, were the same as *Botryodiplodia theobromae* (66). On the basis of certain characteristics, Petch suggested the abolition of the genera Lasiodiplodia and Chaetodiplodia, the former to be included under *Botryodiplodia* and the latter under *Diplodia* (66).

Ellis and Everhart (66) described *Lasiodiplodia tubericola* on sweet potato in Java in 1886, and Evans (28) described the fungus *Diplodia natalensis* on citrus fruits from South Africa in 1910. Edgerton (20) in a critical review of work on boll rot to 1912 lists *Diplodia gossypina* Cke. among other fungi as one of the most important boll rotting microorganisms in Louisiana.

Taubenhaus (64), in 1915, was one of the first to investigate this group of fungi in this country. In comparing the growth of *Lasiodiplodia theobromae*, *L. tubericola*, *Diplodia gossypina* Cke., and *D. natalensis* Evans, on sweet potatoes in a moist chamber, concluded that these species are congeneric. He also concluded that the four genera, *Diplodia*, *Chaetodiplodia*, *Lasiodiplodia*, and *Botryodiplodia* should be combined to form one genus, Diplodia. Accordingly, the sweet potato fungus is now *Diplodia tubericola* (E. & E.) Taub.

Nowell (49), in 1923, reported many of these forms as they occurred on various crop plants in the lesser Antilles and suggested that the genus *Lasiodiplodia* and the fungus *L. theobromae* are not
based on constant characteristics. Thus, he includes the forms considered above as synonyms of his combination Diplodia theobromae.

Most of the critical work with this fungi was begun by Stevens (61) in 1925. He reported the results of his investigations with certain tropical forms of this group - the pycnidial stages - which are quite similar morphologically. He described Physalospora gossypina from cotton and other hosts in the southern states of North America and considered it to be the perithecial state of Diplodia gossypina Cke. The following year Stevens (62) described Physalospora fusca with brown ascospores along with a hyaline-spored Physalospora on citrus and other hosts. (The conidial stages of these fungi are practically indistinguishable.) He called the latter P. rhodina (B & C) Cke, and included D. natalensis, D. gossypina, and his previous combination, P. gossypina, as synonymous. Stevens (62) also suggested that Diplodia cacaoicola, so generally parasitic on Hevea and cacao, was probably identical to P. rhodina.

More recently, Eddins (18) showed that D. tubericola, D. natalensis, and D. gossypina caused a rot of ears of corn similar to that caused by D. frumenti E. & E. It also caused a similar rot of other crop plants. In a similar manner, Eddins and Voorhees (19) obtained positive infection on 31 species of woody and herbaceous plants representing 24 families with cultures of D. tubericola, Physalospora zeicola, and Physalospora rhodina. They showed that D. frumenti, on corn, was morphologically similar to certain Diplodia species on other crop plants and that it was the conidial stage of Physalospora zeicola E. & E. Other workers (1, 19, 48, 62) also have
demonstrated that many of the Diplodia forms occurring in the southern states of North America can pass readily from one host to another.

According to Voorhees (66) Diplodia and related genera offer one of the greatest taxonomic problems confronting mycologists and plant pathologists. The large number of destructive diseases caused by Diplodia along with its wide distribution, both geographically and in its host relationships, of the many forms considered has led to a multiplicity of names, many of which might well be included under one binomial. The attainment of this end, as he explained, is made somewhat difficult by the large number of taxonomic entities comprising this group and their variability in certain morphological and physiological characteristics as well as by the lack of information on their sexual stages.

Voorhees (66) studied in detail 100 isolates of Physalospora rhodina obtained from the southern United States and other parts of the world. His results indicate that P. rhodina comprises an indefinite number of races which may be similar in appearance but which may differ from one another in one or more characteristics.

He did not find evidence concerning the possibility of new races arising from sectors or mutations, but it was quite evident that many races arise in the sexual stage in nature. Practically all of the races are differentiated by the aversion reaction between them in culture. The stability of this reaction was maintained through one or more mycelial transfers in culture and through inoculated host material.
Voorhees (66) reported a synonymy of *Physalospora rhodina* which includes *Botryodiplodia theobromae* recorded by Petch, *Lasiodiplodia theobromae* by Bancroft, *Diplodia tubericola* by Tubenhaus (64), and *Diplodia theobromae* by Nowell (49). The fungus *Lasiodiplodia triflorae* Higgins is also included (66). This synonymy, listed below, of *P. rhodina* is an extension of that initiated by Stevens (62) in 1926, but it is not yet completed.

*Physalospora rhodina* (Berk. and Court.) Cke. Grevillea 17:92, 1889.


Following is a description of *P. rhodina*:

Pycnidia simple or compound, single or grouped, stroma present or absent, immersed to erumpent or almost superficial, hairy or glabrous, varying in size and complexity with substratum, frequently papillate with prominent ostiole, 0.5-6 mm. in diameter; conidia borne on short conidiophores, nonseptate, hyaline and granular when young, becoming brown and uniseptate at maturity, and usually with longitudinal striations, 17-34 x 10-18 microns; paraphyses present or absent.

Perithecia single or gregarious to closely crowded, scattered or in semi-parallel rows, immersed to erumpent, papillate, opening by a black rounded ostiole, externally carbonous, globose, 225-300 microns in diameter; ascī clavate-cylindried, stalked, sessil, double walled, the inner wall remaining to enclose the mature spores, typically,
eight-spored with spores crowded-biseriate, 75-110 x 18-25 microns, spores hyaline to dilute olivaceous, one-celled elliptical to ellipsoidal often tapering to narrow, rounded ends, 27-38 x 9-17 microns.

The following synonyms are reported to be based on examination of type or authentic materials:


Synonyms based on descriptions and various host collections:


The Disease:

Diplodia boll rot is a disease which damages and destroys the bolls and fiber of cotton in many areas of the southeastern part of the United States. It is mainly a disease of the bolls, although under certain conditions it may be found on seedlings and mature stems of the plant.

The disease is more prevalent under conditions of high rainfall and temperature during the late summer. It is also more prevalent when plants grow tall, thus shading the lower parts and keeping them moist.

Diplodia is not the only organism which causes boll rot. In the review made by Edgerton (20) in 1912, he found Glomerella gossypii (Southworth) Edg., Bacterium malvacearum E. F. Sm., Diplodia gossypii Cke., Fusarium sp., Rhinotrichum tenellum B. & C., and Olpitrichum carphophylum A. & K. to be the principal boll rotting agents in Louisiana. He lists other fungi, such as Botryosphaeria feluginosa (M. & N.) E. & E., Sclerotium rolfsii Sacc., Volulella sp., Nectria
sp., *Corticium vagum* var. *Solani* Burt and *Rhizopus Nigricans* Ehr. as being boll rot organisms but the latter appear to be less important than the former.

In a search of world literature made by Pinckard and Chilton (54), they found a total of 75 microorganisms which possibly damage cotton bolls. From this number they concluded about 40 to be present in Louisiana. As a result of thousands of isolations made by Pinckard (52), a collection of about 250 isolates from diseased bolls has been made. These isolates are under study.

Guidroz and Pinckard (28) in 1967 made a survey directed toward cataloging the kinds and relative numbers of potential phytopathogenic microorganisms present in and on three-fourths grown cotton bolls. They collected boll samples from several cotton areas of Louisiana and made isolations from bracts, nectaries, scrapings from boll surfaces, and from the interior of apparently healthy bolls. The frequency of organisms found indicates that *Fusarium*, *Alternaria*, and *Penicillium* were the most prevalent fungi on the boll surface. Other organisms found were *Rhizoctonia*, *Cephalosporium*, *Helminthosporium*, *Verticillum*, and other kinds of fungi and bacteria not identified. The results obtained by them did not indicate the presence of *Diplodia* on the material collected, but they emphasize that green, apparently healthy, bolls approaching maturity carry a large number of fungi and bacteria on their surfaces that are potential boll decaying organisms.

A survey made by Neal and Gilbert (48) in 1955, showed that *Diplodia* in the Mississippi valley causes the greatest damage to the lower bolls and bolls in contact with the soil in the lower fertile areas where rank vegetative growth occurs.
Marsh et al (41), in 1965, found Fusarium spp., Diplodia gossypina Cke., and Colletotrichum gossypii to be the most important boll rotting organisms which damage the cotton fiber in South Carolina.

Symptoms of the disease caused by Diplodia:

According to Edgerton (20), Diplodia rot is principally a disease of the bolls, but the fungus may also attack the lower part of the stem of the cotton plant. He noticed that the inner portion is first attacked, the first evidence of the presence of the fungus being the dark, water-soaked, appearance of the regions around the line of dehiscence of the carpels. After several days the whole boll becomes black, with a smutty mass of spores exuded from the pycnidia. Aycock (1) noticed that on bolls which had developed in dry weather the boll appeared as a dark brown, dry, and somewhat shrivelled mummy covered with pycnidia. Under these conditions, if the boll dries out very rapidly, it may become more or less brittle and remain closed. Also if the boll becomes diseased before it is half grown, it will not open.

Edgerton (20) also noticed that the fungus invades the lint and seed once infection takes place. In such instances it is able to reduce the lint to a black mass. These black masses are very seldom picked. If they are, they are worthless, as the lint is weak and is torn to pieces in the gin. The seed is of low quality and usually does not germinate.

Diseased bolls which remain closed cannot be harvested by the picking machine. However great numbers of diseased half-open bolls
may be picked by the harvesting machine and thus lower the grade or quality of the crop as a whole.

Diseased bolls do not open easily and the fiber remains non-fluffed. This condition is referred to by Brown and Ware (5) as "tight lock" bolls. Another kind of damage may occur when half open bolls become humid and are attacked by cellulose-destroying organisms. Such damage is referred to by Brown and Ware (5) as "weathering." Marsh et al (41) found on open bolls several rot-causing organisms which produce these effects. Some of them were: *Alternaria* spp., *Fusarium* spp., *Diplodia* spp., *Glomerella* spp., and various yeasts and bacteria.

**Methods of boll penetration by Diplodia:**

In the review of literature no reports were found which indicate specifically the means of penetration of the cotton boll by *Diplodia*. This is explained by the assumption that *Diplodia* is a part of a boll rot complex which involves a great number of fungi, some bacteria, insects, and the plant itself, all of which predispose the boll to penetration by the fungus. Of the different organisms found on the boll and generally accepted as the causes of the boll rot complex, according to Cauquil and Ranney (7), few are parasites. They assumed that *Xanthomonas malvacearum* (E. F. Sm) Dows and *Glomerella gossypii* Edg., either by themselves or in conjunction with *X. malvacearum* and possibly *Diplodia gossypina* Cke. and *Bacillus subtilis* Cohn, are able to penetrate the pericarp of the boll. They made a collection of thousands of apparently healthy bolls from different varieties and different locations. They allowed the bolls
to incubate for several days then opened the bolls to determine possible sites of origin and cause of internal rot. Between 5 to 15 per cent of the bolls were found with some symptoms of initial infection. From these studies they classified the different symptoms into three categories related to the mode of penetration of rot organisms:

1) Parietal, or penetration between the valves, 2) Nectarial, or penetration through the bracts or nectaries in the receptacle,
3) Pedicellar, or vascular or subcortical penetration from the pedicel to the placenta, 4) Insect injury, which produces damage to the boll, the site of which becomes ports of entry for the rotting fungi.

Inoculation studies made by Luke and Pinckard (39), in 1967, indicated that bracts are sites of penetration by Diplodia and several other fungi. They noticed that the bract tips are first penetrated by the fungi, and, later, the whole bract becomes diseased. In this way the bract harbors the fungus until some opening develops, allowing the fungus to penetrate into the boll. Luke (38), in further studies, found that wide, somewhat appressed bracts hold moisture which is important for the survival and progress of infection in the bracts.

**Progress of the disease:**

According to Edgerton (20) the infection first starts on the inside of the boll. The threads of the fungus grow rapidly in all directions in the immature lint, seed and seed cavity until they come to the line of dehiscence between the carpels. At this place there is a break in the hard internal lining of the boll, and there is a thin fold of parenchymatous tissue which extends for a short distance
inwards. The fungus mycelium is not able to penetrate the boll lining, but it very readily attacks the fold of parenchymatous tissue. Edgerton (20) was of the opinion that some secretion of the fungus killed the plant cells in advance and that this was the reason the fungus spreads from the point of infection. Once the fungus enters the carpel tissue that has been killed by the toxic secretion, it spreads very rapidly.

The cycle of disease:

According to Edgerton (20) the fungus remains in diseased bolls and plant refuse in the soil during the winter. The spores are well protected inside the pycnidia. It is possible for the fungus to grow saprophytically on dead parts of other plants. In this way, the fungus is able to live until the following year, when bolls begin to form. The spores are long-lived. They are able to germinate and grow in the following spring.

Wind and rain are some of the factors which spread the inoculum from the soil to the lower bolls of the plant. These again start the disease and spread it through the cotton fields. There is some possibility that insects which feed on diseased bolls spread the inoculum (2, 20, 24, 40, 44, 63, 65).

Losses due to Diplodia:

Losses caused by Diplodia are quite variable from one season to another, and from one place to another. Edgerton (20), in 1912, found the Diplodia disease to be more prevalent in the alluvial soils and bluff lands rather than in the hills.
Neal and Gilbert (48), in 1955, indicated that losses in the Mississippi Valley, particularly Louisiana and Mississippi, sometimes reached 10 per cent, and the losses in the latter state average about 2 per cent.

At the 1968 conference of the Cotton Disease Council in Arkansas, workers reported low boll rot counts during 1967. Crawford (11), in 1968, stated that Diplodia spp. was the most prevalent boll rottng organism associated with early maturing cotton in southern Georgia.

**Losses in Louisiana:**

Boll rot losses caused exclusively by *Diplodia gossypina* Cke. have never been recorded. In Louisiana there are several *Diplodia* species present in abundance on sweet potatoes, corn, and cotton. Other organisms besides *Diplodia* are involved acting together on the same boll.

Estimations made by Pinckard (53), in 1966, indicate that losses over a period of 15 years, due to boll rot organisms, varied from 1.0 to 30 per cent, the latter figure being the average for 1964. Causal organisms found were *Fusarium*, *Diplodia*, *Myrothecium* and *Curvularia* with species of *Fusarium* predominating.

**Chemical control of Diplodia:**

Several attempts to control boll rot diseases by using chemical fungicides were made by Pinckard et al (55) in 1964 and 1965. Tests with 9 applications of 5 fungicides at 4 to 5 day intervals failed to give measurable control in a field which showed 36 per cent loss from boll rot.
Ranney (57), in 1964, reported results obtained with the use of several fungicides and bottom defoliants. In both instances indications were that a boll rot reduction trend was present. However, physical damage to the plants caused by the equipment reduced the yield.

Chen and Yu (9), in 1967, reported that *Diplodia gossypina* Cke. is one of the most troublesome diseases of cotton in Formosa. They made attempts to control the disease with chemical fungicides. They found Difolatan (0.1%) was the most effective fungicide against *Diplodia* boll rot.

Genetical means of controlling *Diplodia* boll rot:

Luke (38), in 1968, has reviewed some of the most important literature on boll rot resistance in cotton. His review indicates that most of the work on the subject has been related to the causes and only recently have attempts been made to seek a control of the disease. He mentioned that several studies had been made which show a relationship between the morphological features of the boll and their possible role in the incidence of the disease.

Luke and Pinckard (39), in 1967, found that the bract acts as a predisposing factor to boll rot. Studies made in both greenhouse and the open field showed a consistent reduction in boll rot percentage when bracts were removed by hand after anthesis.

Luke and Pinckard (39) also found that in comparing upland cotton varieties having wide, appressed bracts with varieties having narrow, somewhat reduced bracts, the upland cotton varieties support
fungal growth by affording the fungus moisture and protection under the bracts.

In further studies Luke (38) concludes that the presence of the cotton bracts in a humid atmosphere predisposes the boll to decay, and that the development of a bractless cotton variety would be of material assistance in the control of boll rots.

Some evidence concerning the susceptibility of several genetic lines or varieties of cotton to Diplodia gossypina Cke. were found by Ware and Pinckard (67) in 1967. Of 90 varieties tested none showed resistance to D. gossypina infection when inoculated by means of deep wounds through the carpel wall and scrape wounds of the epidermis. Seven varieties escaped infection when the inoculation was made by dipping the blossom half of the boll into a spore suspension. These varieties will be studied further for resistance to Diplodia.

Jones and Andries (34), in 1967, tested three varieties into which the frego bract had been bred for boll weevil and other insect resistance. They found that the frego bract character had a significant effect on boll rot reduction, amounting to a 50 per cent overall decrease for three locations. Further investigations were made by Jones et al (35), in 1968, whereby characters other than the frego bract were studied. These are qualitative characters which differ from those of normal cotton by a single genetic factor pair and are either known to be or suspected of being important in reducing the cost of insect control. The five characters under investigation were: Okra leaf, Super okra leaf, frego bract, redleaf, and akdjura red.
The results obtained indicate promising reduction in the incidence of boll rot.

Other possible means of controlling Diplodia boll rot:

Delgado and Pinckard (16), in 1966, and Delgado (15), in 1967, found some possibilities of biological control of Diplodia boll rot and other boll diseases by using antagonistic actinomycetes isolated from some bolls and Louisiana soils. In vitro studies indicated 40 of the isolates were able to inhibit the growth of Diplodia spp., Fusarium spp. and Pellicularia filamentosa Pat. Rogers. Antibiotic substances produced by some of these isolates, when tested in vitro, resulted in the inhibition of the same boll rotting organisms.

Goyette (26), in 1962, found that chemical defoliants could be useful in the control of boll rot diseases. He made applications of phosphatic defoliants to the bottoms of the cotton plants. The results he obtained were on the reduction of the incidence of boll rot through the reduction of moisture at the lower part of the plant.

Other means of control of boll rot diseases are cultural practices to avoid excessively rank cotton and moisture on the lower portion of the plants near harvest time.
GROWTH AND DEVELOPMENT OF THE COTTON PLANT

According to Tharp (63), the cotton plant develops in a consistent characteristic sequence depending on local climate, variety, and methods of culture. Development differs considerably from one production area to the other. Roughly, the time sequence is as follows:

Seed germination and emergence - under favorable conditions, as early as 4 days after planting; under unfavorable conditions, as late as 3 to 4 weeks after planting. Under Louisiana conditions, emergence varies from 7 to 10 days after planting (Table 1).

Appearance of first floral bud - usually about 40 days after seedling emergence. (Earlier if temperatures are favorably high, later if they are below average.) Under Louisiana conditions, the first floral bud appears 32 to 38 days after seedling emergence (Table 1).

Development of the floral bud into an open bloom - usually requires 25 days or less. According to Tharp (63) this period is somewhat more constant than that between emergence and appearance of the first floral bud. Under Louisiana conditions, the development of the floral bud into an open bloom requires 23 to 25 days (Table 1).

Appearance of subsequent blooms after the first - with reference to a given node of one fruiting branch and the same node
of the next fruiting branch, the interval between blooms is about three days. With reference to successive nodes of an individual fruiting branch, the interval is about six days.

Shedding - squares may be shed at almost any age, but most of those that are shed, according to Tharp (63), fall well before bloom. The time of square shedding is effected by several factors, including drought, extreme temperatures, cloudy weather, insect injuries, and disease. Shedding as a result of injury may occur as soon as 36 hours or as late as 10 days after the injury. Boll shedding, regardless of cause, occurs most commonly about 7 days after blooming; the interval ranging approximately from 3 to 10 days. Bolls more than 10 days old are seldom shed, except when the plant is subjected to extreme shock such as that caused by chemical treatment, severe water stress, or frost.

Boll, seed, and fiber properties - in a boll which requires 50 days for maturity (from open bloom to open boll), the periods required for full development of individual properties according to Tharp (63) are as follow:

<table>
<thead>
<tr>
<th>Property</th>
<th>Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boll size (length)</td>
<td>18 days</td>
</tr>
<tr>
<td>Boll weight (dry)</td>
<td>45 days</td>
</tr>
<tr>
<td>Seed size (length)</td>
<td>18 days</td>
</tr>
<tr>
<td>Seed weight (dry)</td>
<td>45 days</td>
</tr>
<tr>
<td>Oil percentage of seed</td>
<td>42 days</td>
</tr>
<tr>
<td>Protein percentage of seed</td>
<td>45 days</td>
</tr>
<tr>
<td>Fiber length</td>
<td>18 days</td>
</tr>
<tr>
<td>Fiber strength</td>
<td>45 days</td>
</tr>
<tr>
<td>Fiber thickness (weight)</td>
<td>45 days</td>
</tr>
</tbody>
</table>
Some facts about the rate of development of the cotton plant in the State of Louisiana are given in Table 1.

Table 1. Facts about the Louisiana cotton plant

<table>
<thead>
<tr>
<th>Event</th>
<th>Number of days Average</th>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>From planting to emergence</td>
<td>7</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>From emergence to square</td>
<td>32</td>
<td>27</td>
<td>38</td>
</tr>
<tr>
<td>From emergence to white bloom</td>
<td>23</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>From white bloom to open boll</td>
<td>55</td>
<td>45</td>
<td>66</td>
</tr>
<tr>
<td>June blooms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July blooms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rich soil 4 to 5 days later than thin soils</td>
<td></td>
<td>45</td>
<td>55</td>
</tr>
<tr>
<td>Rich soil 4 to 5 days later than thin soils</td>
<td></td>
<td>55</td>
<td>65</td>
</tr>
<tr>
<td>From bloom to full grown boll</td>
<td>21</td>
<td>20</td>
<td>25</td>
</tr>
<tr>
<td>Average number of days from planting to peak blooming</td>
<td>90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average number of days from first bloom to peak blooming</td>
<td>35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time required to produce normal crop</td>
<td>150</td>
<td>130</td>
<td>170</td>
</tr>
<tr>
<td>From first white bloom until 60 per cent of crop is open</td>
<td>85</td>
<td>75</td>
<td>95</td>
</tr>
</tbody>
</table>

Planting dates between April 15 and May 15.

There is a critical period in fiber length which depends on the availability of soil moisture. It varies according to the variety of each location. This period usually varies from 13 to 20 days. The fiber gradually increases in strength after the maximum length is reached until 3 to 4 days before boll opening. (A deficiency of moisture during this period results in increase in strength.)

\textsuperscript{a}/Supplied by the Louisiana Extension Service
Development of the fruit:

According to Brown and Ware (5), and Hayward (29), the boll or the capsule containing the seed, lint, and fuzz, is the end result of the development of the ovary as a whole. The ovary, or young boll, enlarges rapidly and reaches full size in a comparatively short time - about 21 days in upland varieties. From 50 to 65 days elapses before the boll is ripe, or ready to open.

The capsule of mature upland cotton is ovate with a pointed tip. The boll size varies from one production area to another and from one variety to another. The size of the boll of Deltapine smooth leaf in Louisiana was found to vary from 4 to 5 cm long and 3 to 4 cm wide. It has a dark-green color. The number of carpels varies from 3 to 5. Nectaries are present on leaves and flowers, although not all the flowers have nectaries on the outside involucre.

According to Rainey (56) and Martin et al (42), growth of the developing ovules and carpels is extremely rapid during the earlier part of the boll development. Dry weight of the ovules was doubled, on the average, every 2 to 4 days during the first 2 weeks of growth. Because of this they assumed that the boll is very vulnerable to temporary interruptions in the supply of nutrients, and it is, in fact, during this period that the boll is particularly liable to physiological shedding. Shedding, even in a good crop, may account for more than half of the total bolls set.

They found that between the 4th and 5th weeks the growth of the boll wall ceased, with no further increase in the fresh or dry weight of the carpels or in boll diameter. The fresh weight of the developing ovules reached the maximum during the same time; however, the dry
weight of the ovules continued to increase steadily with highly significant weekly increases up to the age of 9 weeks.

Rainey (56) found the ovules were rich in reducing sugars during the period of growth in size (approaching 50 per cent dry weight at 1 to 2 weeks) and had a high moisture content (85-90 per cent) but contained very little oil and protein. He mentioned that much of the structural differentiation occurs after the boll reaches full size - hardening of the seed coats, differentiation of the embryo, and thickening of the lint. As the boll matures, sugars and water were almost completely replaced by the constituents of the mature boll protein, oil, and cellulose. They found 88 per cent of the oil and 60 per cent of the nitrogen of the mature seed were present after 5 weeks, when the growth in size had ceased. Similarly, over 40 per cent of the lint cellulose was formed after 6 weeks, although the lint had already reached its full length by this stage. The percentage of cellulose in the developing lint continued to rise steadily up to the time of the boll opening (93 per cent after 10 weeks).

Rainey (56) found reducing sugars were at a maximum during the early phase of rapid growth and not when the formation of cellulose, oil, and protein was most active, which was about a month later. However, the total dry weight of the contents of 4-week-old boll, in which reducing sugars were just beginning to decline, corresponded to little more than a third of the dry matter of the final seed cotton, most of which must therefore have entered the boll in the course of its subsequent development. According to Rainey (56), it is doubtful that the general decline in the proportion of reducing
sugars during boll development is evidence that these sugars were probably the main carbohydrate utilized in the formation of protein, oil, and cellulose.

According to Rainey (56) oligosaccharides were found in higher concentrations after growth in size had ceased. Sucrose has been suggested as the main form in which sugars enter the very young boll, while raffinose is probably primarily a reserve material occurring in cotton seed meal. He showed some indication of the demands made on the plant by the developing boll, underestimating the amount of any constituent lost by respiration.

The weight of water per boll was found highest at 5 weeks and amounted to about twice the weight of the seed-cotton finally produced.

The demand for assimilates was found to vary less with age than the demand for water but tended to be highest at 5 to 7 weeks. Nitrogen was also taken up continuously throughout boll development, with a maximum rate of uptake between 6 to 8 weeks. The fruiting structures of the crop thus demanded a continuous supply of nitrogen over a period of at least 3 months.

The development of the cotton boll involves well marked changes in a number of its major chemical constituents. Rainey (56) and Martin et al (42) found that two main phases of boll development of roughly equal duration may be distinguished. The first is characterized by rapid growth in size, high water content, and an abundance of reducing sugars, while, during the second phase, most of the cellulose, oil, and protein of the mature seed-cotton is produced with no further growth in the external dimensions of the boll.
Morphology of the cotton capsule:

The cotton boll is a dehiscent capsule that splits along the dorsal sutures. It has a dark-green color. The number of carpels varies from 3 to 5. The boll is thick and leathery at maturity and is usually subglobose in shape. Each locule has 8 to 10 ovules which arise in two parallel rows and ultimately form the seeds the coats of which produce the fiber.

According to Baranov and Maltzev (3), and Gubanob (27), a 2 or 3 day old capsule possesses all the principal characteristics of the mature capsule. Its mesocarp consists of many cell layers which are rich in tannins. Within the mesocarp, procambial bundles run in different directions. Near the mesocarp periphery oil glands are found.

The outer layer of the mesocarp is formed from comparatively small, round cells, homogeneous in shape. This layer is several times thicker than the inner layer. This tissue consists only of several rows of cells greatly elongated in one direction. This layer is transformed into sclerenchymatic tissue consisting of several layers with its cells drawn out in tangential direction. The inner layer of the tissue lines the cavity of the boll in which seeds with fibers developing on the surface of their membrane (hull) are formed.

During the development of the capsule, the stomata are formed on the outer layer of the mesocarp. The latter is covered with a wrinkled cuticle. Procambial bundles develop in the mesocarp as a result of differentiation. Later they are transformed into conducting bundles which form a net. The bundles have free ends which are radially arranged and terminate in the peripheral layers of the
mesocarp. These are the radial bundles which differ from the rest of the bundles caused by the large quantity of fibers which they contain. This large amount of radial bundles can be seen in a tangential section of the carpels. According to Cubanob (27), it is assumed that the number and arrangement of the bundles has something to do with the dehiscence of the boll. As the number and anastomoses of the bundles increase, the force and speed of outward shrinkage is increased.

Along the outer and inner sutures, thick sclerenchymatic bundles develop, but the tissue of the sutures themselves remain thin-walled (27).

Baranov and Maltzev (3) found evidence that the arrangement of the radial bundles permits shrinkage in only one direction - radially. This effect leads to progressive contraction of the cells at the periphery. As a result, the turning out of the valves takes place. The endocarp dries out, passively, bends outward, and liberates the fiber portions from the valves.

The partitions in the flower and young capsule are rather fleshy and are characterized by the presence of a large group of cells containing large amounts of mucous substances. These substances swell immensely with water.

These groups of cells are present at one side of the ovary, at the base of the valve, and near the base of the placentas. As the boll matures, these cavities remain in the same position in the boll but become almost flat.

The mucous substances are present more or less in the dry state up to maturity. Once dehiscence takes place, the mucous substances dry entirely and remain embedded in the endocarp.
Development of the embryo:

According to Baranov and Maltzev (3), the first division of the fertilized egg cell takes place 12 to 36 hours following pollination. Once double fertilization occurs, the embryo development begins. Of the two cells formed, the upper one, i.e., the one which points toward the micropyle, is transformed into the suspensor. The lower one, which points toward the center of the embryo sac, develops into the embryo.

Tharp (63) found that the development of the embryo within the seed proceeds at a very slow rate during the first week, when the processes of cell division and differentiation are getting started. Between the 10th and 20th days the embryo grows rapidly. It reaches its full length between the 20th and 22nd day. About the 32nd day, it attains its full volume. At this time it has become differentiated into sprout and seedling leaves and completely fills the enlarged seed envelope, or hull. Further development proceeds rather slowly. Maximum dry weight is attained only a few days before the boll splits.

According to Tharp (63), oil begins to accumulate in the embryo about the 15th day after fertilization. This accumulation proceeds slowly for the next week or 10 days and then rapidly between the 25th and 42nd days, the period within which protein is formed most rapidly. In commercial varieties pigment glands, sometimes called resin glands, appear over the entire surface of the embryo, particularly on cotyledons, during the rapid accumulation of oil and protein.

According to Tharp (63) and Baranov and Maltzev (3), between the 15th and 20th days the differentiation of the axial organs of the
cotyledons are set. At this time the formation of root caps on the root tips takes place.

The central cylinder and cortex are differentiated at this time also. It is possible to distinguish the three main ribs in the cotyledons. Later a small bud, the epicotyl will appear between the cotyledons.

As the cotyledons grow, they become more or less flat, leafy organs, their edges at first bending around the endosperm later becoming folded.

Within 30 to 40 days the embryo fills the whole cavity of the embryo sac. At this time the cotyledons develop the spongy parenchyma, and the procambial bundles are formed and connected to the procambial bundles of the axial organs.

Numerous spherical glands develop in the hypocotyl and in the tissue of the cotyledons. Glandular hairs can be seen on the surface of cotyledons and hypocotyls.

After 30 days the embryo resembles the mature plant. Some differences are the size and the number of folds of the cotyledons.

The mature embryo has a great amount of nutritive materials including protein grains, aleuron grains, oil and some starch. The source of these nutrients is the tissues of the nucellus and endosperm. Later the source of nutrients will be parenchyma cells of the integuments.

**Development of seed coat:**

According to Baranov and Maltzev (3), the seed coat develops from the integuments of the ovule, which grow quite rapidly after
fertilization. The final thickness of the seed coat is reached after approximately 4 weeks. At this time the seed coat is about 6 to 6.5 times the initial thickness. Later this size gradually decreases. Near maturity, its thickness is only about 2 to 2.5 times the initial thickness of the integuments.

The growth of cells of different tissues of the seed coat is different. The cells of the outer epidermis of the seed coat after 10 days begin to stretch and are transformed into the columnar tissue of the seed coat. The cells of the columnar layer increase in thickness, and, simultaneously, a lignification of the lower portion of the layer takes place. This growth stops after about 35 days. There is no columnar layer at the chalazal end of the seed.

Lamellate thickening may be observed on the cell walls of the inner epidermis at maturity. Sometimes two layers of cells may be noticed in this epidermis.

These two epidermal layers - the inner epidermis of the outer integument and the outer epidermis of the inner integument - constitute the protective tissues of the seed coat.

The epidermis of the seed increases in thickness at the end of the active period of growth. It becomes about 4 to 6 times the original thickness. Its cells have thick lamellate walls composed of cellulose impregnated with tannic substances. The same composition is noticed in the remainder of the cells of the parenchyma of the seed coat.

The parenchyma of the two integuments and the lower epidermis of the inner integument gradually become contracted while losing nutritive
materials. Towards maturity they are transformed into the so called pigment layers.

**Development of fiber:**

According to Brown and Ware (5) and Baranov and Maltzev (3), fibers develop on the surface of the seed. These are composed of cells from the epidermis of the external integument. The fiber cells start to grow in volume at the time of anthesis. Not all the cells from the external integuments become fiber cells. The growth of the fiber cells takes place rapidly. The nuclei of the fiber cells increase in volume and later migrate near the center. Fiber development starts at the chalazal end, where the cells are more abundant, and is slower at the micropylar end.

After 6 to 10 days some of the epidermal cells start to grow and these cells produce the fuzz. The fuzz cells grow slowly and will not reach the size in length or diameter of the normal fiber. They are about 2 to 5 mm long while the fiber of the commercial varieties of *G. hirsutum* is about 20-30 mm long.

The elongation of the fiber takes place in 25 to 30 days, but increase in diameter ceases after about 15 days. At the end of the 10th day, it is possible to observe the increase in thickening of its walls. From this age addition of successive layers of cellulose takes place continually until the end of the maturation period.

The lamellar structure of the fiber may be seen in transverse section. When observed from the surface, a criss-cross pattern of the cellulose layers becomes evident. In the center of the cell there is a small cavity which is filled with cell sap surrounded by a thin
coat. This sap represents the cytoplasm. The nucleus is found inside the cytoplasmic coat near the middle of the cell (3).

As a result of progressive thickening, the inner cavity becomes smaller. As the boll nears maturity, it becomes narrow and oval in shape. At maturity the cavity is a flat oval.

The membrane of the fibers is mainly cellulose, and at maturity the fibers are covered with a cuticule. When the fibers start to dry, they may twist together lengthwise (5).

Nectaries:

According to Tyler (65), Mound (47), and Brown and Ware (5), cotton plants have two kinds of glands - external and internal. The external glands are known as nectaries. These are structures specialized in the production of sweet liquids at some stage of the plant's development. There are four sets of nectaries - one inside the flower and three extrafloral. The number and development of the extrafloral nectaries vary considerably in different species.

The floral nectary consists of a ring of papilliform cells at the base of the inner side of the calyx. These nectaries are protected by a layer of vertical lignified hairs. They serve as a sort of cushion between the calyx and the petals, which leaves a certain free space for the nectarium to develop.

A second set of nectaries is found on the outside of the calyx near its base. This set consists, as a rule, of three triangular-shaped nectaries.

A third set, consisting of three nectaries is situated on the pedicel of the flower, one being near to the point of attachment of
each bractlet. (A part of extrafloral nectaries may be lacking on early flowers.) These are longer than the leaf glands and form rounded or pear-shaped shallow pits with rounded secreting cells. These glands resemble shallow round dishes with the bottoms covered by a layer of large buds.

The fourth set of nectaries is located on the under side of the main ribs of the leaf. There is usually one on the mid point of the largest vein. Frequently, two others are in similar positions on the other two large veins of the leaf.

Nectaries may not be apparent on the cotyledons, or even on the next three leaves, but, subsequently, each leaf bears at least one. It is to be found on the abaxial surface of the main vein, about one-third of the length of the leaf from the petiole. Later formed leaves frequently have a nectary on the major vein on either side of the midrib, and sometimes a pair is also developed on the major veins of the lateral lobes of a leaf.

The glandular tissue is dark green, especially on plants grown in pots. The secretory tissue is composed of many closely packed multicellular papillae, the cells of which are small with a large nucleus and dense cytoplasm. The sub-glandular tissue is similar, but some cells contain large crystals. No evidence could be found of a direct vascular supply to the nectary.

Unipapillate nectaries mentioned by Mound (47) occur on the young petioles and peduncles. These structures are a major source of nectar in some cotton varieties. Their exudate is sufficiently fluid to run down the surface of the petioles, contrasting with the more viscous exudate from the other nectaries. These nectaries
consist of single septate papillae similar in structure to the papillae which compose the larger nectaries. They are found in large numbers on the cultivated varieties of *G. barbadense* but are poorly developed on certain hairy varieties of *G. barbadense* and *G. hirsutum* (Figs. 1, 2).

**Composition of the nectar:**

According to Mound (47) the exudates from nectaries on young plants were found to be clear and watery with a solid content of 20 per cent, or less, in weight. Such secretions usually evaporated during the day on which they were produced, leaving a crystalline deposit on the nectary surface. Apparently as a result of this deposit the tissue gradually degenerated or frequently was eaten by visiting insects. The exudate from mature plants did not dry out as easily. This may have been because of the higher concentration of solids in such exudates, 70 per cent or more, which retained the water hygroscopically. Mound (47) did not find evidence of any leaf exudate falling from one leaf to another. Even which produced in large quantities, the secretions were always observed to remain hanging from their nectaries in large sticky drops. These secretions should not be confused with aphid honeydew which is frequently visible dripping from leaf to leaf.

The nectar of cotton is very rich in carbohydrates. Nectar samples from *G. hirsutum* and *G. barbadense* were analyzed by McGregor (43) in Arizona in 1953. He analyzed the nectar qualitatively using paper chromatography and qualitatively by the absorption method. He found in the nectar of the two species a high content of sugars,
especially dextrose, levulose and sucrose, and possibly small traces of maltose.

Mound (47) analyzed nectar samples by standard techniques of descending paper chromatography. He found sucrose, glucose and fructose to be the main sugars present. He was able to identify two amino acids, glutamine and β-alanine.

**Nectar production:**

The production of nectar in upland cotton starts as early as the cotyledonary leaves are found in the seedling stage. The nectaries stop producing nectar a few days before the bolls mature. Nectar on the leaves is produced as soon as the leaves develop on the shoot, and its production ceases before the leaves become yellow with age and fall to the ground.

The production of nectar on the outer involucres of the flower starts 2 to 3 days before the flower opens and lasts for a period of 3 to 4 weeks under greenhouse conditions. The production of nectar in the inner involucres lasts a shorter period of time than production in the outer involucres.

It is assumed that the amount of nectar produced and the length of time for which it is produced are affected by the nutrient balance inside the plant tissue and the physiological age of the nectaries. Mound (47) found that nectar is usually produced at higher rates during the night than during the day.

It is assumed also that the amount of carbohydrates and other substances varies according to the physiological age of the tissues and to the nutrient balance of the plant.
The role of nectaries:

Tyler (65), in 1908, described nectaries of the cotton plant. He found nectaries a stable characteristic which may be useful in the classification of cotton species. He found that the sweet liquids produced by nectaries act as a lure for insects, some of which cause injuries to the bolls. These injuries are ports of entry for rot-causing organisms.

Upland cotton, G. hirsutum L., has leaf, floral, and extrafloral nectaries. Meyer and Meyer (44), in 1961, found that secreted nectar attracts many insects and provides an important food source for them. He found that wild cotton, G. tomentosum Nutell, native to Hawaii, has no leaf and floral nectaries. He made crosses with G. hirsutum and was able to obtain a nectariless cotton plant. He found that the nectariless character is controlled by two pairs of recessive genes. The nectariless cotton he obtained was from second and third back-crosses, incorporating the nectariless character into upland cotton.

Lukefahr and Rhyne (40), in 1960, made studies to determine the influence of nectaries on the incidence of three Lepidopterous insects - cotton leaf worm, Alabama argillacea Hbn., cabbage Looper, Trichoplusia ni Hubner, and pink boll worm, Pectinophora gossypiella Saund. They found that the infestation by cotton leaf worms and cabbage loopers were 7 to 10 times as high on Empire, a cotton with extrafloral nectaries, as on cotton without nectaries. The pink bollworm test indicated very little difference between cottons, both had about the same pink boll worm infestation; however, the average number of tunnels and larvae per boll was less on the nectariless cotton.
Bagga and Laster (2), in 1968, found evidence that the boll weevil, *Anthonomus grandis* Boheman, the boll worm, *Heliothis zea* (Boldie), and the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), initiated boll rot infection. Other insects such as the vinegar fly, *Drosophila melangoster* Meigen, and the cabbage looper moth, *Trichoplusia ni* (Hubner), caused nectary infections which in turn resulted in boll infections. They used isolates of *Alternaria tenuis* Auct. and *Fusarium moniliforme* Sheldon as boll rotting organisms throughout the series of experiments. The explanation they reported was that insects cause injuries to the bolls and may carry the fungus on the mouth parts and transfer it to the boll during the feeding process. Vinegar flies and cabbage looper moths possibly carry boll rot organisms and deposit them in nectaries during the feeding process.

If cotton boll rot was a simple matter of insect transmission the disease could be controlled with insecticides.

**Senescence of the cotton plant:**

According to Leopold (36), in the development of plants the stages of germination, flower initiation, fruit set, and maturation are relatively precise and are subject to relatively simple and concise measurement.

Senescence, on the other hand, is a relatively gross change, or series of changes, leading finally to the death of the plant or plant organ. In plants there are several expressions of senescence, including a variety of patterns, such as morphological deterioration, and characteristic subsidence of growth rates. Deterioration of the
plant results in distinctive changes in the morphology, pigmentation and internal nutrition patterns.

Leopold (36) considers reproductive growth to be a signal of senescence of the plant. Flowering and fruiting brings about a signal of senescence. Cotton is a perennial plant which shows progressive senescence of leaves from the base slowly up the stem. Fruit maturation takes place in two directions - up the stem and from the main stem to the tips of the fruiting branches. He suggests senescence in cotton may serve several functions: (a) as individual leaves become suppressed and shaded, the onset of leaf senescence permits the recovery of the bulk of inorganic and organic nutrients which have been committed to the leaf; (b) with senescence, the fruits crack open and the carpel through abscission layers, dry out, leaving the lint and seed free.

According to Leopold (37), the explanation of the mechanisms which control organ senescence of the plant is still obscure.

Under environmental conditions which prevail in Louisiana, cotton is an annual crop. It is seeded in April, starts to flower in July, and begins to mature in August and September. At this time, the senescence of the fruits marks the ending of the economic importance of the plant. Leopold (37) suggests that deterioration of the auxin regime with age is responsible for organ senescence. For example, the photosynthetic apparatus of a leaf, as it grows old, appears to become markedly less effective as indicated by lowered photosynthetic ratios and depressed net assimilation. Its auxin supply dwindles, and it appears that the cause of this effect is an increase in the enzyme systems which destroy auxins. According to Leopold (37), as the leaf
becomes older, there are changes in the metabolism of carbohydrates and proteins. There is a general respiratory maximum and an associated deterioration of the chlorophyll pigments in favor of carotenoids and anthocyanins. As this metabolic shift takes place, there is a gross export of many of the organic and inorganic nutrients from the leaf until abscission interrupts. He is of the opinion that senescence in leaves may be a correlation effect which is controlled by physiological events occurring in remote parts of the organ. The cotyledon is the first organ which shows senescence. Its senescence may not be only a simple deterioration but may be controlled by the apex of the plant. This condition, according to Curtis and Clark (14), involves mobilization forces which seem to be strongest in flowers and fruits, less strong in growing points, still less strong in lateral buds, and weakest in roots. No explanation for the means by which mobilization takes place was discussed. Oota and Takata (51) suggested that the mobilization future of the meristem is related to its components of RNA. RNA migration from the older tissues into the meristems, is thought to maintain the mobilizing centers therein.

On the other hand Leopold (37) reported that a growth regulator, kinetin, deferred leaf senescence. He mentioned that this chemical could cause leaf mobilization of carbohydrates, amino acids and various inorganic ions from untreated zones.

According to Leopold (37), senescence is not caused by the depletion of nutrients resulting in starvation of a plant organ, but to the tendency of a given organ to hydrolyze and dissipate its nutrients.
Boll maturation:

According to Brian and Presley (4), boll maturation is defined as the interval between the opening of the flower and boll opening. They found the length of this period to be a more suitable indication of maturity than was the date of the beginning of flowering. They found also that varietal differences and date of blooming greatly influenced the length of time from the flower opening to boll opening.

Martin et al (42), in 1923, found differences in the rate of development of cotton plants, especially of the fruiting branches. These differences were related to appearance and growth of the floral buds, the flower sequence, and boll growth under three different environmental conditions and types of cotton. In this year they studied the rate of growth of commercial varieties grown in Arizona, Texas, and South Carolina. They found very sharp differences in the rate of growth between areas, and between varieties of cotton. Under balanced cultural conditions, water is an important factor in the growth of cotton plants. An adequate supply of moisture may result in rapid vegetative growth. On the other hand, Brown and Ware (5) found an over supply of water, when associated with subnormal temperature, a lengthy period of cloudiness, or excessive nitrogen, usually throws the plants into a vegetative cycle that may retard fruiting-branch development for a period, or until excess water subsides and some other condition changes.

He explains that an adequate water supply, with the other factors in balance, usually stimulates fruiting-branch growth along with other plant growth. As a result of the growth of more and longer fruiting branches and more nodes, flowering and fruiting increases.
Morris (46), in 1964, found that the boll maturation period for upland cotton varies between inbred lines, between seasons, between flowering dates, within a given inbred line and trial, and between boll set on one flowering date within one seed line and trial.

At maturation time the cotton plant has bolls of several ages, the opening of which occur at different times and continue for a long period. Usually the bolls close to the peripheral zone of the plant open late.

According to Baranov and Maltzev (3) the opening of the carpels outwardly looks like a simple process. Once the boll matures, the sutures connecting the carpels dehisce and the juicy tissue of the bract rapidly dries out; consequently, the carpels shrink and turn away from the boll as a result of which the latter opens.

According to Gubanob (27), the opening of the carpels of the boll is controlled internally by metabolic functions of the boll and externally by factors which promote the drying out of the carpel tissue. He explained that incipient opening of the cotton boll is regulated by internal processes, as a result of which there is a loss of turgor in the normally functioning juicy tissue of the carpels. At maturation time a disruption of the turgor in the cells takes place, and the carpel tissue starts to dry out rapidly. This drying out process leads to the shrinkage and the opening of the carpels.

On the other hand, Leopold (36) stated that fruit maturation and ripening appear to be triggered by the termination of development of seed structures or by some other metabolic activities in the fruit itself.
Recent discoveries indicate that there are growth regulator substances other than auxins and gibberellins which possibly play an important role in boll maturation and opening. Smith et al (59) and Ohkuma, et al (50), in 1963, were able to extract from young cotton fruit a growth regulator which is called abscisin II. This compound was found able to accelerate abscission and senescence, inhibit flowering, and induce dormancy. It has no activity as does auxin and gibberellin, but it was found to counteract the action of these hormones. They found also that abscisin II is readily translocated within the plant.

Smith and Lyon (60), from previous experiments, found that acidic extracts from young bolls yielded not only abscisin II and several gibberellins but also one or more substances which retard abscission. Upon further purification of one of these substances which retard abscission, he found indications that it has a high molecular weight and is a non-polar compound. He found also that the abscission-retardant interacts with abscisin II and gibberellin GA3 in preventing abscission.

**Boll opening:**

At the initial period of development of the boll, all its components have a relatively high water content (at least 80 per cent). However, as the boll ages, the water content in the seed and in the fiber at first begins to drop slowly and at the cessation of boll growth it drops rapidly. Gubanob (27), in 1966, noticed a gradual decrease of water content in the inner layer of the mesocarp tissue. At the same time the water content of the outer layers remain at the
previous high level, a drop of which is noted only when the boll
begins to open. He found that the cells of the inner layer of bract
tissue die early. When the bolls are about 20 to 30 days old they
become slightly hollow, exhaust the internal contents, and lignify.
As a result of the deposition of lignin, their water content gradually
diminishes.

Gubanob (27) found evidence that the cells of the outer layer of
the bracts remain alive until the opening of the boll. A characteris­
tic feature of the cells of this layer is that they contain large
amounts of proteins, pectin substances, and various free carbohydrates.
All of these substances permit the absorption and retention of large
amounts of water, the bracts thus remain well hydrated during the
entire period of boll development. Only prior to its opening does
dehydration of the bract tissues occur, which is, as emphasized
above, the cause of their opening.

Studies made by Gubanob (27) demonstrated some of the changes in
individual indices of bolls as related to their growth and develop­
ment. He found changes in the state of the cell colloids of the
mesocarp tissues as the boll ages. The viscosity index of the water
extracts he made remained at a relatively high level during almost
the entire period of boll development, and only just before the boll
opens does it drop markedly. The decrease in the viscosity of the
extracts reflects a change in the state of cell biocolloids of the
tissue of the outer layer of the mesocarp. The degree of hydration
of the colloids decreases with a drop in viscosity. He assumed that
the state of the colloids is associated, in this case, with the
character of cell metabolism of the tissues of the outer layer of the
mesocarp.
Gubanob (27) found that at the time the boll began to open the quantity of various free carbohydrates decreases in the outer layer of the mesocarp, and at the same time there is an increase in the content of tannic substances. He assumed that tannic substances of a phenolic nature had formed at the expense of carbohydrates. According to him, the excessive formation of tannic substances in the mesocarp is apparently the factor which disrupts the stability of biocolloids in the tissue cells and the outer layer of the bract. Under the effect of these substances the colloidal hydration and ability to swell decrease, as a result of which the cells are dehydrated. This leads to drying of the boll and subsequent boll opening.

The opening of the boll is preceded by an accumulation in its mesocarp of not only water soluble substances but also water insoluble phenolic compounds, as described by Gubanob (27). He found a great variation in the rate of formation of carbohydrates and phenolic substances in the mesocarp. The rate of formation of phenolic substances was found to vary according to the earliness of the variety. He found phenolic substances to be involved in oxidation-reduction processes, while certain phenolic compounds act as growth inhibitors.

During the opening of the boll, phenolic substances, being accumulated in excess in the cells of the mesocarp tissues, manifest, in his opinion, and in comparison with all other compounds, a dominating influence on biocolloids. Under the effect of these substances, the biocolloids lose stability which leads to loss of water by cells, drying out of the bracts, and, as a result, the opening of the boll.
Gubanob (27) concluded that at boll maturity there is an harmonic biological process. The transformation of carbohydrates and the formation of an excess of phenolic compounds in the outer layer of the mesocarp begins only when processes of the deposition of the reserve substances (proteins and fats) in seeds and the transformation of free carbohydrates to cellulose in the fiber is completed. 1) Before the boll starts to open, the reserve of various carbohydrates disappears in the outer layer, and, simultaneously, an excess of phenolic substances is formed. 2) The excess of formation of tannic substances adversely affects the state of biocolloids which, under the effect of these substances, lose stability and capacity for swelling. 3) A change in the colloidal state leads to marked loss of water by the juicy tissues of the bracts. In this case, the bracts shrink and separate along the sutures of the boll, as a result of which the boll starts to open.
MATERIALS AND METHODS

Experimental studies were initiated in the summer of 1966 with the purpose of determining how Diplodia gossypina Cke., being a saprophyte, is able to penetrate and infect cotton bolls, since the explanation in the literature reviews for the above was found both confusing and unsatisfactory.

One experiment, in which the pathogenicity of Diplodia cultures was tested on green bolls, was conducted at the Essen Lane Experimental Farm. A method of inoculation which assured positive boll infection without unduly injuring the boll or its floral parts was needed. Several methods of boll inoculations were therefore tried; the most important were:

a. Inoculation of the boll epidermis by scraping lightly and introducing the inoculum and spores into the wound.

b. Inoculation by placing a bit of inoculum under the sepals.

These methods of inoculation were not satisfactory, a few bolls became diseased, but hurricane Betsy destroyed the experiment.

In the winter 1966-67 several experiments were made in the greenhouse with potted cotton plants. Apparently healthy bolls, of known age, were inoculated by spraying with a conidial suspension of D. gossypina Cke. The bolls were covered with polyethylene bags having a piece of moist filter paper inside.

Fifty bolls of age 15, 30, 45, and 60 days each were inoculated once by the above described method and were covered for about 20 days.
The temperature inside the house was maintained from 75-90°F, and the relative humidity from 75 to 100 per cent. Observations on the presence and development of disease were made and recorded.

Because of the excessively artificial conditions under which this experiment was made, a third experiment was undertaken during the fall of 1967. Potted plants having bolls of several ages were chosen and arranged on a bench in the greenhouse. The plants were kept under warm, humid conditions. Moisture was provided by water sprinklers which assured a fine artificial mist for 5 hours every day. Shade was provided with a cover of cheese cloth and the temperature was maintained between 75 and 90°F.

All the plants except the control were inoculated twice with a conidial suspension of \textit{D. gossypina}. As soon as one of the bolls showed symptoms of disease, it was removed and the data recorded.

This experiment was carried on for about 3 weeks in a greenhouse with no screens; consequently, the plants were exposed to insects as well as to wind-borne spores, neither of which seemed to be important.

\textbf{Studies of boll inoculation in humid chambers:}

A technique used by Luke (38), in 1967, was tried. Two 13" x 18" porcelain pans were covered with 1/4 inch square mesh of galvanized wire, surmounted by a wire frame. Fifty bolls of about 50 days old were surface sterilized with a 1:1000 solution of mercuric chloride, and, after the pans were filled with distilled water, the bolls were set on the wire netting with a part of the boll pedicels immersed in the water. All the bolls in the two pans were inoculated with a
spore suspension of the fungus to allow it to cover the bolls. After inoculation, the pans were covered with a large polyethylene bag which, when closed tightly, provided an adequate moist chamber.

Pieces of carpel, bract, and pedicels from apparently healthy and from diseased bolls were taken at various intervals. According to the procedures of Johansen (32) and Sass (58) killing, fixing, embedding, sectioning, and specific staining techniques were used to prepare permanent slides for microscope examinations.

The pieces of tissue were fixed in formalin acetic alcohol solution (FAA). Later the plant material was dehydrated with ethyl and butyl alcohol solutions and embedded in paraffin for sectioning.

Sectioning of the plant material was made with a Spencer Microtome model 820. The sections were mounted and stained with safranin and fast-green. Permanent slides were made by covering the stained sections with canada balsam and placing a glass cover slip over it.

Boll print methods for observation of boll surface microflora:

A method of studying the boll surface microflora was developed. Five per cent water-agar at 40°C was used to make prints and thus recover part of the microflora found on the boll surface. Selected pieces of boll were set on the bottom of a petri plate and covered with the water-agar. The plates were kept in the freezer for one hour to solidify the agar. Later the pieces of tissue were separated from the agar and the agar blocks having the prints were trimmed and stained with carbol-fuchsin. Observations were made with the microscope. Photomicrographs of the fungi and other organisms present were taken.
Another method of studying the boll surface microflora was as follows. Pieces of bracts, sepals, and boll surfaces were bleached with a household bleaching agent (Action). This bleaching agent contains potassium-s-triazinetrione and was used by Draft and Leben (17) to study the microflora on cucumber leaves. The pieces of fresh boll tissues were placed 2 cm above the bottom of a glass chamber on a 1/4 inch screen. Once the plant material was set, a 25 per cent bleach solution was added and the chamber was covered for about 2 to 4 hours. After this period of time, the plant material was removed and the microflora present on the plant surface was stained with carbol-fuchsin and observed under the microscope.

Inoculation of nectaries:

In the spring of 1968 an experiment was designed for the purpose of determining the growth response of Diplodia to nectar secreted by the outside involucre nectaries of cotton bolls.

Fifty green bolls about 40 days old were inoculated with a spore suspension of Diplodia in a particular way. One drop of the suspension was placed on the nectaries of the outer involucres. The bolls were covered individually with a small polyethylene bag containing a piece of moist filter paper. Caution was taken to keep the bolls hanging from the fruiting branches and to prevent the bag and moist paper from coming in contact with the nectaries. Observations related to the development of disease were made as well as surface and boll prints at several stages in the boll development until the boll opened.
Nectar studies:

In the summer of 1967 most of the cotton plants in the greenhouse showed an excessive growth of fungi on the outside of the bracts, especially close to the outside nectaries. This fungus was identified by Ivey and Pinckard (31) as one of the sooty mold fungi *Cladosporium cladosporoides*, a saprophytic fungus which does not damage the plant but does damage the quality of the fiber on open bolls under moist conditions.

The presence of *Cladosporium* on the bracts, especially around nectaries, gave an indication that nectaries could possess some nutrient substances which increase the growth of some microorganisms on the green bolls or boll parts. At this time small amounts of nectar from the Tuxtla and Deltapine smooth leaf varieties were collected and diluted to 50 per cent in sterile distilled water. Five milliliters of nectar solution for each one of the varieties was mixed with 5 ml of a conidial suspension of *Diplodia*.

The hanging drop technique, using Van Tingham cells, was the means of observing the germination and growth development of the fungus for 15 days. Twenty replicates for each variety gave enough information of the reaction of the fungus to the nectar influence.

In the summer of 1968 a second experiment with nectar was done. Nectar from Tuxtla and Deltapine smooth leaf varieties was collected in the greenhouses to make 100 ml of 1.0 per cent nectar for each one of the varieties.

The nectar was stored in a refrigerator at 15° F at the time of collection.
The nectar of each variety was filtered by using a sterile Seitz filter with disposable filter discs in order to exclude bacteria, fungi and other foreign material present in the nectar.

Five milliliters of nectar solution was mixed with 5 ml of one per cent sterile water-agar at 40°C and poured into a sterile petri dish. Ten petri plates were used from each nectar solution, and 10 petri plates with water-agar alone were used as controls. The plates were stored at room temperature. Germination and growth behavior was observed under the microscope for a period of 15 days.

No qualitative or quantitative analysis of the nectar was made. The amount of soluble solids present in the nectar was measured with an Atago refractometer.

**Defoliants in relation to Diplodia boll rot:**

In the fall of 1967 the results obtained with the use of fungicides to control boll rot were not satisfactory (55). On the other hand the genetical approach to the control of boll rot has been found promising. Cotton varieties of the okra leaf type with narrow bract characters were found less affected by rot organisms. This observation has been attributed to the reduction of shade and moisture around the bolls on the lower portions of the plants (5, 34).

The above effect seemed possible at maturity by using chemicals which defoliate partially or totally. During the fall of 1968, experiments were conducted in the greenhouses with potted plants having bolls of several ages. Thirty-six pots, each with about 1/3 of the bolls open, were set on a bench and kept under 5 hours of artificial mist every day. All the plants were previously inoculated by spraying
each with a conidial suspension of *D. gossypina*. The inoculations were made two times within a week previous to the application of the defoliants. The chemicals used were Def (s,s,s-tributyl phosphorothioate), a defoliate which accelerates the formation of the abscission layer of the leaves and without affecting the growth of the bolls, and Desicant L.10 (arsenic acid), which acts on the stem leaf and boll surfaces of the plants. Apparently arsenic acid penetrates into the tissues, produces blisters on the boll surface and accelerates the dropping of leaves and the opening of mature and non-mature bolls (Table 2).

Twelve pots were used for each one of the two chemicals used, and twelve pots without any chemical were used as controls. Two more inoculations of the fungus were made with a 3-day interval between the applications of the chemicals. The experiment was continued for 10 days after the application of the defoliants. Observations related to the physiological action of the chemicals and incidence of disease were made and the data recorded.
Table 2. Two harvest-aid chemicals commonly used to defoliate cotton.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Trade Name</th>
<th>Mfr.</th>
<th>Rate/acre</th>
<th>Active Ingredient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Defoliant</td>
<td>Def</td>
<td>Chemagro</td>
<td>4 gal./acre</td>
<td>s,s,s-tributyl phosphorothioate, 75.5 per cent</td>
</tr>
<tr>
<td>Desicant</td>
<td>L 10</td>
<td>Pennsalt</td>
<td>½ gal./acre</td>
<td>Arsenic acid - 75.0 per cent</td>
</tr>
</tbody>
</table>

The application was made at a time when each plant had about 1/3 of the bolls open.
RESULTS

Inoculation studies on green bolls:

Bolls of several ages which were inoculated and covered with polyethylene bags showed symptoms of disease only at boll maturity.

Ten days after inoculation all the 60-day-old bolls began to open, showing symptoms of disease. Twenty days after inoculation 85 per cent of the 45-day-old bolls cracked and began to show symptoms of disease also. Twenty days after inoculation the 15- and 30-day-old bolls had not reached maturity. There were no visible symptoms of disease (Table 3).

Diseased bolls encased in the polyethylene bags cracked along the suture lines but did not open. Five days after development of symptoms of disease they were covered by a mass of gray mycelium, and after 15 days they appeared blackish in color and were covered with a mass of pycnidia. As the moisture around the boll decreased, the pycnidia extruded white coiled strings of one-celled hyaline conidia clustered together with a gum-like substance (Fig. 4).

In the second experiment on uncovered bolls under shade and artificial mist, mature bolls developed disease as the bolls started to open. The percentage of diseased bolls in these cases was lower than when the covered method was used. Some of the bolls showed symptoms of diseases other than Diplodia rot.

A few days after the bolls developed symptoms of disease, they became black and began to form pycnidia partially immersed in the
Table 3. Percentage of bolls of different ages which become diseased 20 days after inoculation with a conidial suspension of Diplodia gossypina Cke.

<table>
<thead>
<tr>
<th>Age of bolls (days)</th>
<th>No. of bolls inoculated</th>
<th>Inoculation date</th>
<th>Per cent of diseased bolls after 5</th>
<th>10</th>
<th>15</th>
<th>20 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>50</td>
<td>Feb. 26, 1967</td>
<td>50</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>45</td>
<td>50</td>
<td>Feb. 26, 1967</td>
<td>none</td>
<td>none</td>
<td>85</td>
<td>100</td>
</tr>
<tr>
<td>30</td>
<td>50</td>
<td>Feb. 26, 1967</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>15</td>
<td>50</td>
<td>Feb. 26, 1967</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
</tbody>
</table>

a/The boll age was known by tagging each flower at the white bloom stage.

b/The plants were kept in the greenhouse with a moist environment at 80-90°F. All the bolls were covered individually with a polyethylene bag.
carpel tissues of the boll. About 10 days later black pycnidia were noticeable covering most of the boll surface (Fig. 5).

When the artificial mist was shut off, some of the bolls opened, showing the locks of fiber more or less fluffed but damaged by the fungus; however, most of the diseased bolls remained partially open with "locked" fiber.

The inoculation studies made with both covered and uncovered bolls showed that symptoms of disease occurred on the bracts before appearing on the bolls. Symptoms on the bracts began with a yellowing of the bract tips. Two or three days later the bracts showed a water soaked appearance all over, but the tips appeared brown in color. Four to five days after inoculation, especially in a moist environment, the bracts became completely brown and were covered with the white mycelium of the fungus.

Observations indicate that penetration and infection of the boll pedicels is possible. Again the symptoms of disease appeared simultaneously with the opening of the boll (Fig. 11).

**Histological studies:**

Stained sections taken from carpel tissues of inoculated bolls in the green stage showed mycelium of Diplodia growing on the wrinkled epidermal surface of the bolls (Fig. 8). The same was observed on the surface of bracts, sepals and pedicels of the green stage bolls.

The evidence obtained indicate that penetration of the boll occurs at maturity, the port of entrance being the natural openings of the boll and boll parts - that is, stomata (Fig. 8, 9).

Another way of penetration was found through the opening in the abscission layers of the carpels at maturity.
In a few stained sections penetration of the boll was observed via injuries produced on the epidermis by insect wounds or by handling. This type of penetration was not frequent because these studies were made in the greenhouse and in closed moist chambers.

Observations on stained sections of diseased bracts (some of them taken a few days previous to the boll opening) indicate that the fungus had penetrated and grown intercellularly in the spongy tissue of the bract. The fungus was able to grow from the tip to the base of the bract at a very rapid rate. Sections taken from the tip and base of the sepals indicated that the fungus grew in a similar pattern in the sepals.

Observations made at the base of the bracts did not show internal invasion of the fungus from the bract to the carpel tissues.

Observations made on inoculated bolls with no symptoms of disease showed the presence of a large amount of Diplodia mycelium on the epidermis. On some sections Diplodia appeared mixed with other microorganisms (Fig. 10).

Sections taken from pedicels of diseased bolls indicated the presence of the fungus on the epidermis but mainly inside the cortex and phloem. Sections taken 5 days after boll infection demonstrated a large number of young pycnidia on the pedicel epidermis and the invasion of the cortex, phloem, and pith by the fungus (Fig. 11).

**Boll print results:**

Stained water-agar prints taken from the surface of inoculated bolls, when examined under the microscope, exhibited the germination
and growth of the Diplodia spores and mycelium over a large part of the boll and bract surfaces (Fig. 10).

The mycelium of the fungus was observed growing on apparently healthy bolls for about 15 days before the boll opened (the time in which the inoculated bolls became diseased). Large masses of mycelium were noticeable on and around the nectaries from the outside involucres. Very often microorganisms other than Diplodia were found in conjunction with the fungus (Fig. 12).

Stained sections taken from boll nectaries inoculated at the green stage indicate the presence of large amounts of mycelium with a few germinating spores of Diplodia on the surface. Very often Diplodia was mixed with other fungi which were identified as Cladosporium sp., Fusarium sp., and Saccharomyces sp. No infection was visible through nectaries of the green, immature bolls. On the other hand, sections taken from nectaries of diseased bolls 5 days after the first symptoms of disease developed indicated infection on the outside involucre nectaries, although no evidence was found which pointed to the invasion of the inner tissues of the boll through nectaries (Fig. 12).

No inoculations were made on leaf nectaries (Fig. 2), but some evidence was found that they can sustain fungal life on and around them.

Other organisms in relation to Diplodia boll rot:

Stained sections from some diseased bolls indicated the presence of injuries on some boll areas. Diplodia was found present in these areas, and sometimes the presence of other organisms was evident. It is assumed that the injuries were produced by insect injuries or by handling of the plant material.
Several diseased bolls showing large numbers of pycnidia on the surface were brought to the laboratory for microscopic examination. Some of them were found to be covered with great numbers of mites and larvae of some insect, all of which were feeding on the pycnidia and rotten boll tissues. After a few days the number of mites increased, and some flies were observed inside the boxes.

The flies were identified as being fungus gnats which, according to Johannsen (33), belong to the order Diptera, family Mycetophylidae, and genus Sciara spp. The larvae of this insect feed on decaying plant material and fungi (13).

The mites were identified by Boudreaux (6) as Tyrophagus putrescentia (Schrank) which, according to him, is a pest of many food products and feeds on rotting vegetable matter.

Influence of two defoliants on the incidence of Diplodia rot:

Striking differences were found in the incidence of Diplodia boll rot when two defoliants were applied to the cotton plants. Application of the desicant L 10 (arsenic acid) at 1/2 gal. per acre resulted in the increase of the boll rot disease under a moist and warm environment. The mature bolls opened, most of them showing symptoms of disease. Green bolls were affected by the disease and remained closed. The areas in which the first symptoms of disease appeared were those previously affected by the chemical. The disease later invaded the remainder of the boll tissues. Symptoms of disease were also visible on branches and stems of some plants. Some of these were covered with pycnidia. These symptoms were visible especially
Table 4. Results obtained on the use of two harvest aid chemicals in relation to Diplodia boll rot infection.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Name of Product</th>
<th>Green stage</th>
<th>Mature stage</th>
<th>Boll rot percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>healthy</td>
<td>diseased</td>
<td>healthy diseased</td>
</tr>
<tr>
<td>1</td>
<td>L 10</td>
<td>7</td>
<td>26</td>
<td>8</td>
</tr>
<tr>
<td>2</td>
<td>Def</td>
<td>27</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>Control</td>
<td>27</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

*Green stage bolls are those which did not reach maturity by the end of the experiment.*

*Mature stage bolls are those which reached maturity within the time of the experiment.*
in the areas affected by the desicant which caused blistering of the epidermis. Figure 13 illustrates the prolific growth of Diplodia on a blistered area of a boll caused by arsenic acid.

The effects of Def (s,s,s-tributyl phosphorotrithioate) at 4 gal. per acre did not increase the incidence of disease. Most of the leaves dropped off within 10 days after the application. Green bolls were not affected by the chemical nor by the pathogen. Some of the mature bolls appeared to be diseased at maturity, but the number of diseased bolls was similar to the number observed on the controls. This number was also similar to that obtained in previous experiments (Table 4).

Studies on growth of Diplodia mycelium on agar containing nectar:

Cultures made on nectar solutions from the varieties Deltapine smooth leaf and Tuxtla were similar. The spores germinated normally and started to develop pycnidia in about 5 days. After 7 to 10 days the plates were covered with fruiting bodies of the fungus which started to extrude white one-celled conidia. The mycelium took on a smokey color.
DISCUSSION

It is well known that upland cotton is a perennial plant which shows progressive senescence of leaves from the base slowly up the stem. Fruit maturation takes place in two directions - up the stem and from the main stem to the top of the fruiting branches.

Under the environmental conditions which prevail in Louisiana, cotton is an annual crop, seeded from about April 15 to May 15. It starts flowering in July, and maturation of the fruit occurs from the end of August until frost in early November.

The explanation of the changes, or series of physiological changes, which lead to maturity of the fruit are not very precise (37). However, they play an important role in the boll rot disease. The opening of the boll is said to be controlled internally by metabolic functions and externally by factors which promote the drying out of the carpel tissue (27).

The drying out process is initiated several days prior to the external yellowing of the boll parts. Observations made indicate that there is a centrifugal drying out process in the boll which leads the boll tissues to separate and liberate the fiber (Fig. 3). These natural openings provide sufficient opportunity for many surface inhabiting microorganisms (Fig. 10) to enter the boll at will.

At maturity there is a change in the physiological functions of the boll tissues. Some of these changes seem to affect the mechanism of opening and closing of the stomata. There is an increasing loss of
water by the boll tissues. Stomata, being the main avenue for the water to escape, remain permanently open. In this way stomata not only permit the loss of water from the carpels but also provide physical openings for the entry of decay fungi and bacteria (Fig. 8 and 9).

Some differences were found in the maturation time of Deltapine smooth leaf in the greenhouse from one season to another. During the winter maturation time was of about 60 days under a temperature range of 70 to 80°F and a relative humidity of 75 to 100 per cent. During the summer the maturation time is about 50 days under an 80 to 95°F temperature range and a relative humidity of 60 to 100 per cent.

The time from the yellowing of the boll to its opening is probably less than 48 hours. During this short period carpel tissues become quite susceptible to invasion by decay producing microorganisms. The speed of opening is dependent on the temperature and relative humidity surrounding the boll. The time from boll cracking to fluffing is about 48 hours. During this short period of time the change from carpel resistance to susceptibility and from fiber susceptibility to fiber resistance takes place (25).

Some evidence was found here that mycetophagous organisms such as mites Tyrophagous putrescentia (Schrank) and fungus gnats, Sciara spp., which feed on Diplodia and rotten plant material, possibly act as vectors of Diplodia and other decay producing microorganisms.

Once the Diplodia fungus is in contact with the bolls it does not penetrate the unbroken surface but it does grow over the boll surface until a suitable infection site is provided.
Infestation of the bolls is favored by morphological features. Broad leafy bracts cover the boll surface and in a humid environment the bracts hold moisture and create a humid microclimate around the boll which protects the fungus from desication.

Nectaries of upland cotton, *G. hirsutum* L. play an important role as a source of nutrients which support growth of *Diplodia* and other microorganisms on the surface of the boll. Inoculations indicate that *Diplodia* is able to survive and grow on the boll surface for about 20 days prior to the opening of the bolls. The survival and growth of the fungus could be caused by moisture and the presence of carbohydrates, amino acids and other substances not yet identified. Studies made on inoculated bolls at several stages of growth showed the *Diplodia* fungus spread over the entire surfaces of the bolls prior to maturity. Very often other microorganisms than *Diplodia* were involved, some of them which were identified as *Cladosporium* sp., *Saccharomyces* spp. and bacteria.

The differences obtained from inoculation studies with covered and uncovered inoculated bolls indicate that moisture around the boll was an important factor which favored the survival and growth of these microorganisms.

The evidence indicates that the age of the boll and moisture are the most important factors involved in the rotting of the bolls of upland cotton by *Diplodia*. The symptoms of disease were first observed on the bracts of nearly mature bolls. The appearance of the first symptoms of disease and the cracking of the boll were almost simultaneous. At first the diseased bolls develop dull green,
water-soaked spots, and a few hours later these symptoms are visible on the whole boll.

**Histological studies:**

Histological evidence was found that inoculated bolls under moist conditions become infested by the pathogen, and that this fungus could survive for about 20 days previous to the opening of the bolls. On some areas of the boll, such as nectaries and areas surrounding the nectaries, *Diplodia* mycelium was found growing freely, but no penetration of the plant tissue was found (Fig. 12).

Stained sections of diseased bolls and bracts showing first symptoms of disease demonstrated hyphal threads which had penetrated readily both the senescent bract and boll epidermis through the stomata. Evidence indicates that bract tissues, especially the tips, become senescent earlier than do the boll tissues; consequently, they become infected before the maturity of the boll (Fig. 6).

It could be possible that, once the areas around the nectaries (Fig. 1 and 2) become infested and bracts diseased, a medium is provided encouraging microorganisms to form on the outside of the boll. As soon as the boll matures, these organisms can enter and cause decay of the boll tissues.

Stained sections from nearly open bolls showed the fungus penetrating the boll epidermis through the senile stomata (Fig. 7). Once the boll is penetrated, the organism grows intercellularly in the parenchyma tissue of the carpels. It grows rapidly in all directions, and, within 2 to 3 days, it starts to develop pycnidia, initially in the stomatal chambers first penetrated by the fungus (Fig. 5 and 9). A similar situation occurs in the pedicel (Fig. 11).
Within this time openings start to develop at the abscission layers of the carpels, and hyphal threads readily penetrate and infect the internal tissues of the boll. The fungus starts to grow, invading tissues from the inside to the outside. It grows within each carpel separately and infects the still moist fiber. Growth is rapid inside the spaces found between the lining tissues which separate the carpels, possibly caused by the pectic substances which fill these areas. Later it reaches the tissues of the outer wall of the carpels. After five days the bolls appear black in color and are covered by a large mass of white to gray mycelium. At this time the abscission layers separate, but the boll remains partially closed. After 10 days they appear covered by black masses of mycelium and develop large amounts of carbonaceous pycnidia on and inside the bract tissues. These bolls do not open but mummify and remain attached to the fruiting branches.

If there is a decrease in the humidity, once the infection process has taken place, the diseased carpels start to separate, but the fungus begins to develop and the pycnidia reach maturity, showing their black erumpent beaks on the epidermis of the shriveled carpels. The fiber in such instances would become free and fluffing, and it could be picked by the machine. Very often this fiber can be ginned, but it is so damaged that the quality is reduced.

In many cases diseased bolls can open completely, but the fiber is so damaged that it remains "locked" inside the carpels. This fiber can sometimes be picked but is torn into pieces by the harvest machine.

Another means of boll infection was found. The use of the desiccant, arsenic acid, predisposed the plant parts to infection by
its blistering action on the epidermis thereby permitting decay of the underlying tissues (Fig. 4 and 13). Under field conditions almost any decay producing microorganisms present on the plant could cause rot following the use of arsenic acid desiccant if the weather remained warm and moist. Under actual field conditions, however, Diplodia inoculum is seldom sufficiently abundant to be a limiting factor in the use of arsenic acid as a desiccant.
CONCLUSIONS

1. Weather is one of the most important factors which tend to increase the occurrence of *Diplodia* boll rot. Excessively humid conditions and warm weather favor the development of *Diplodia* boll rot on the lower part of the cotton plant.

2. The bolls of commercial varieties start to mature as early as the end of August. In some years excessive rainfall increases the moisture on early maturing bolls, thus increasing the amount of disease.

3. In the absence of surface wounds penetration of the boll by the fungus takes place at maturity, the stomata of the bract, bolls, and the broken sutures of the carpels become the main avenues for entrance. Development of disease is dependent on the speed of the opening of the bolls. Excessive rainfall and humidity decrease the speed of boll opening, and the bolls become diseased by the fungus.

4. Evidence indicates that senescence of the bracts takes place prior to the boll maturation; consequently, bracts may be penetrated and infected before boll opening. No evidence was found that progress of *Diplodia* infection takes place internally from the bract to the boll placenta.

5. Nectaries present on upland cotton, *G. hirsutum* L., are sources of nutrients; especially carbohydrates, amino acids and other substances which have not been identified. Nectarial surfaces were
found infested with Diplodia and other microorganisms prior to boll maturity but no evidence was found they are sites of penetration by the Diplodia fungus.

6. A morphological feature of some upland varieties, that protect the fungus from desiccation, are the wide appressed bracts which cover the boll and create a favorable micro-climate for the fungus.

7. Entomological research has been utilized to obtain nectariless varieties which could result in the reduction of boll damage. Evidence obtained here indicates that the nectariless character could be a factor in the reduction of surface decay microorganisms.

8. Mycetophagous organisms such as the mite, Tyrophagous putrescentia (Schrank), and the fungus gnats, Sciara spp., are involved in the biological breakdown of Diplodia and can possibly act as vectors of the disease.

9. Harvest-aid chemicals have been investigated as a possible means of boll rot control. Use of this method has not been practical because of mechanical damage during application. Evidence was obtained that some specific harvest-aid chemicals, such as Desicant L 10 (arsenic acid), when applied under warm and humid conditions, increase the amount of Diplodia boll rot. More research in this area is suggested.
Figure 1. Position of nectaries on a flower of upland cotton.
a. Nectaries inside of calix.
b. Nectaries outside of calix.
c. Nectaries of the bracts. Slightly reduced.
Figure 2. Leaf nectaries. The number of leaf nectaries may vary among varieties of upland cottons. Enlarged.
Figure 3. Centrifugal drying out of the boll tissues. About ten days before the boll opens the locules begin to separate along their partitions. Age of boll on left, 40 days; right, 50 days from white bloom. 10X.
Figure 4. Surface of a boll heavily infested by Diplodia gossypina Cke. During humid atmospheric conditions young pycnidia extrude chains of one-celled conidia which stick together with a gummy-like substance. As these coiled chains dry the conidia become dark colored and two-celled. 35X
Figure 5. After penetration of the boll the mycelium grows rapidly in a stomatal chamber and formation of pycnidia begin. After 4 to 5 days conidiospores and conidia form inside of the young pycnidia.
Figure 6. Senescent tip of a bract with Diplodia starting to colonize the non-living cells. 430X
Figure 7. Penetration of Diplodia mycelium through the stomata of a bract approaching maturity. Notice the intercellular growth of the fungus inside bract mesophyl. 960X
Figure 8. The mycelium of Diplodia growing on the surface of a green cotton boll. Penetration of the boll takes place at boll maturity after epidermal cells die. Notice the wrinkled surface of the boll in which hyphal threads can be partially protected from drying. 960X
Figure 9. *Diplodia* mycelium penetrating the boll tissues through a mature stomata. Loss of the physiological function of opening and closing processes by the stomata at maturity may account for the ability of the *Diplodia* fungus to penetrate the boll surface only at this time. 430X
Figure 10. Germination of a Diplodia spore and subsequent mycelial growth on surface of immature green boll. The very small spores (lower arrow) are those of a sooty mold fungus Cladosporium cladosporioides. 430X
Figure 11. Pedicel of a mature boll that has become infested with Diplodia at the same time the boll has become susceptible. Pycnidia initials are forming in the epidermis. 35X
Figure 12. Nectaries of outside involucres produce nectar which favor the growth of Diplodia and other microorganisms on floral surfaces. No infection is initiated before maturity of the boll. 100X
Figure 13. Some harvest-aid chemicals such as arsenic acid may (under high temperature and moisture) predispose the boll to rot by Diplodia. The disease appeared first on the areas of the boll covered by the chemical. Natural size.


VITA

Jaime Delgado was born at Guadalupe, Zacatecas State, Mexico on January 27, 1930. He was graduated from Instituto Potosino High School in 1948 and entered the Escuela Nacional de Agricultura in 1949, where his Bachelor Degree was obtained in November, 1955.

In December, 1955 he accepted a position as Research Assistant with the Dirección de Agricultura of the Comisión del Papaloapan, Veracruz State, Mexico. In December, 1956 he resigned to accept a position with the Ingenio San Cristóbal, Veracruz State, Mexico as Assistant Sugarcane Technologist. In September, 1957, while employed in this capacity, he accepted a position as Research Assistant in the Phytopathology Department of the Instituto Tecnológico Azucarero Veracruzano, Veracruz State, Mexico, where he remained until December, 1958.

In January, 1959 he accepted a position as Assistant Sugarcane Technologist with the Instituto Nacional de Investigaciones Agrícolas, Mexico and was commissioned to the Campo Agrícola Experimental de Zacatepec, Morelos State. He was promoted to a Field Manager of the same branch in March, 1963 and, while employed in this capacity, he accepted a position as a Sugarcane Technologist with the Oficina de Expansion de la Industria Azucarera of the Secretaría de Agricultura y Ganadería, Mexico, where he remained until July, 1964.

He entered the Graduate School of L.S.U. in January, 1965, completed the required work for the degree of Master of Sciences, which he received in August, 1966. He immediately continued his graduate work and is a candidate for the degree of Doctor of Philosophy in January, 1969.
Candidate:        Jaime Delgado

Major Field:      Plant Pathology

Title of Thesis:  Studies on the Pathology of Diplodia Boll Rot of Cotton (Gossypium hirsutum L.) Caused by the Fungus Diplodia gossypina Cke.

Approved:        

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:  January 9, 1969