Thesis submitted by
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in support of
his application for
an Associatehip

1923-24
Notes on a Soft Rot of Cotton Bolls in the West Indies caused by species of Phytophthora.

The Soft Rot of cotton bolls, up to the present only reported from the West Indies, is a disease which, under certain conditions may assume proportions of decided economic importance. It was first reported from Montserrat by Robson in March 1911 on a variety of Sea Island cotton known as Rivers; unopened green bolls with blackened tips were very common but only bolls near the ground were diseased. In 1917, Nowell described a disease of cotton bolls in St. Vincent in which the wall of the boll turned brown and was covered with a glistening powder or white cottony envelope, and both this and the Montserrat disease found to be due to phytophthora. Nowell further remarks that although a spell of dry weather may check the disease, it is manifest again after two or three days of rain, whilst Robson mentions in his report a particularly wet season. It is now known that heavy or prolonged rain is a necessary condition for the spread of the soft rot and it is presumed that infection is brought about by motile zoospores being splashed from the soil on to the lower bolls of the cotton plant. Infection usually takes place at the tip, or at least, the rot is first visible at this end of the boll.

Robson, in 1911, reported considerable resistance to this disease in the Beaton variety of cotton, which is not typically West Indian Sea Island in constitution, having a very heavy boll; large and coarse leaves and, strangely enough, throws vigorous laterals and is not a tall grower thus rendering itself more open to fungus attack. It is said to have been derived from
a cross with Upland cotton and this point is interesting since, as will be seen later, the writer has found high powers of resistance amounting, under field conditions, to immunity in Upland varieties of cotton. Whether this character is genetic or merely environmental it is difficult to say because when grown in St. Vincent, the Heaton cotton was found to be exceedingly susceptible to soft rot; but this cannot be said to be due to change in climatic conditions alone, since the St. Vincent phytophthora is a different species from that reported from Montserrat. The latter was isolated by Miss E.M. Wakefield in 1920 and cultures have produced oospores which are of the same size and development as in \textit{P. parasitica}. Dastur. The St. Vincent strain, which was isolated by Ashby in 1921-22 from St. Vincent bolls, is quite a distinct species and cannot be distinguished in culture from the coconut budrot form \textit{P. palmivora} Butler; \textit{P. faberi} on cacao is apparently a strain or variety of this species. Ashby has also recently isolated a phytophthora from Sea Island cotton bolls in Trinidad, which closely resembles the Montserrat form in growth and asexual reproduction.

The action of these three strains on different varieties of cotton is the subject of the present paper.

It will be convenient to first describe the inoculation experiments and the technique employed and then to discuss some associated physiological phenomena. Advantage was taken of the fact that cotton bolls, if placed in a damp chamber, continue to grow for some days after they have been picked from the plant. Accordingly the following experiments were carried out.

About half grown bolls were chosen and the bracts removed to lessen the possibilities of external contamination. They were then thoroughly washed with soap and water, dipped for 15 seconds
into 95% alcohol, to remove air from the surface, and then placed in a
0.1% aqueous solution of mercuric chloride for 5 minutes, the cut ends
of the stalks were covered with paraffin wax and the bolls were then
washed in three successive jars of water, which had been sterilised in the
autoclave, remaining in each jar for five minutes. They were then suspend
suspended, tip downwards, by means of sterile cotton threads inside
large stoppered bell jars on ground glass plates, both of which had
previously been washed with mercuric chloride solution. Petri dishes,
each containing a suspension of motile zoospores of one strain of
phytophthora in 20 c.c. of sterile water were then placed one in each
jar, and by loosening the stoppers of the bell jars the bolls were lower-
ed, by means of the cotton threads, so that the tips were immersed in
the liquid. The bell jars were then covered with dark brown paper so
that only a small amount of diffused light could enter at top and bottom,
and the bolls were allowed to remain thus for 48 hours. They were then
drawn up to about the centre of the bell jar and examined from day to
day. Control bell jars were in all cases used being identical with
those described except that sterile water was substituted for the zoos-
pore suspension.

In his report on the St. Vincent disease, Nowell mentions that
signs of soft rot were observed after two or three days of heavy rain,
so that under the very favourable conditions in the bell jar, a blacken-
ing of the boll should be observed in the same or a shorter period of
time. This was found to be the case with certain varieties of cotton and
these will be considered as susceptible. Other varieties exhibited a not
after varying periods of time, more or less constant for each variety,
but any boll which shewed no sign of blackening four days after lifting
from the dish was considered to be either highly resistant or totally im-
mune under field conditions. Certain interesting physiological charac-
ters were brought to light by these resistant varieties and will be
dealt with later. In making zoospore suspensions it was found necessary
to adopt the following procedure since the St. Vincent phytophthora pro-
duced sporangia abundantly on culture media on which both the Montserrat
and Trinidad strains bore very few or none at all. It was observed that
that by placing a small piece of aerial mycelium of either
the Montserrat or Trinidad fungus in sterile water in a sterile
petri dish and leaving them overnight, a copious production
of sporangia was obtained in the morning; by draining off the
old water, replacing by more aerated sterile water and leaving
the petri dish near an open window, a good discharge of free
zoosporces took place within ten minutes. Under the same condi-
tions the St. Vincent strain would produce a second crop of
sporangia during the night and a corresponding discharge of
zoosporces could be obtained in the morning.

In describing inoculation experiments the commercial
names under which the various cottons are marketed will be
used in order to avoid confusion which may arise over questions
of taxonomy; the three strains of Phytophthora will be spoken
of as St. Vincent, Montserrat and Trinidad.

In a series of trial experiments it was noticed that
St. Vincent, in all cases, was more virulent than either
of the other two strains. In one experiment in which old
cultures (over a month) were used, no infection was brought
about by Montserrat or Trinidad even on the most susceptible
varieties, whilst St. Vincent not only caused a rot on these
bolts but also infected a somewhat resistant type.

<table>
<thead>
<tr>
<th>Commercial Name</th>
<th>Group</th>
<th>Systematic Name</th>
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<tbody>
<tr>
<td>Cauto</td>
<td>Brazilian</td>
<td>Gossypium braziliense</td>
</tr>
<tr>
<td>Elma</td>
<td></td>
<td>var. anispersum</td>
</tr>
<tr>
<td>St. Vincent Sea Island</td>
<td></td>
<td>G. peruvianum var.</td>
</tr>
<tr>
<td>Durango</td>
<td>Sea Island</td>
<td>G. barbadense var.</td>
</tr>
<tr>
<td>Acala</td>
<td>Upland</td>
<td>G. hirsutum vars:</td>
</tr>
<tr>
<td>Lone Star</td>
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Bolls of Canto, Pima, Sea Island, Lone Star, Durango and Acala were experimented with, but it was found that using more than four bolls in one bell jar was inconvenient for manipulation and unfavourable for the growth and metabolism of the bolls. Different combinations of the several varieties of cotton were employed and all gave similar results for each variety.

When Sea Island, Durango, Acala and Lone Star bolls were used, in two days after withdrawal from the zoospore suspension a rot was observed in all three jars on the Sea Island, but all of the Upland varieties remained normal. In three days rot was observed in the Durango boll in the St. Vincent jar and aerial mycelium was visible on the Sea Island. On this variety conspicuous sporangia were produced in four days and cell separation took place in the boll wall. The Durango boll was completely rotted by this time, but cell separation did not appear to have taken place so rapidly as in Sea Island. The boll wall was firm and hand sections could be cut with ease whilst it was almost impossible to cut sections of the Sea Island boll wall.

Aerial mycelium was developed on the Sea Island bolls in the Montserrat and Trinidad jars in 4 days and a few sporangia in 5 days. Cell separation was complete by this time.

In all bolls that became infected the rot had penetrated to the seed, in which the presence of phytophthora mycelium could be detected, and the lint only became stained after the seed had been attacked i.e. after the fungus had attacked the "root" of the hair. St. Vincent and Montserrat sporangia were observed in the lint and they germinated conidially after being left in a damp chamber for 24 hours, but when placed in sterile water in a watch-glass near an open window they discharged zoospores within 15 and 30 minutes respectively. The Trinidad strain
strain developed sporangia in the lint only after it was allowed to lie in water for 24 hours. This reluctance to produce sporangia by Trinidad is worthy of note since it appears to be a fairly constant character and the only observed point in which it differs from Montserrat.

To test the susceptibility of Pima cotton, an Egyptian variety of the same group as Sea Island, bolls of Cauto, Pima, and Lone Star were used. Pima, as expected, showed a rot in two days in all three bell jars. At the end of four days complete cell separation in the boll wall had taken place, the seed was attacked and rotted and the lint stained. Also aerial mycelium was in evidence on the surface of the boll, sporangia being produced by St. Vincent and Montserrat and a few by Trinidad. Sporangia were only observed in the lint of bolls infected by St. Vincent.

Lone Star was completely resistant to the fungus attack and Cauto to Montserrat and Trinidad; St. Vincent, however, brought about a rot of Cauto but only after 14 days and then no cell separation was evident. This infection must have occurred during the time of immersion of the bolls in the zoospore suspension, as they had not been in contact with any other source of infection since being withdrawn. On examination it was found that the seed and lint had been attacked; phytophthora mycelium being present in sections of the seed, but the lint was unstained. Hence it is natural to conclude that the fungus could not have entered the seed more than four days previously otherwise the lint hairs would have been killed and stained (c.f. Pima var.). The probable explanation of the prolonged period before the rot became evident would therefore seem to be that the fungus is capable of establishing itself in the boll wall and, either forcing a way through the tissues...
slowly, or else lying dormant until the damp atmosphere inside the bell jar so decreased the physiological resistance of the tissues as to allow an easy passage for the hyphae or bring about autolytic cell separation. This point will be further dealt with in discussing the physiological relations between the host and the parasite.

It may be remarked that in the above experiment a rot appeared at the stalk end of the Lone Star boll in the Montserrat jar after five days. This rot was due to Diplodia sp. and no evidence of Phytophthora could be found; the writer therefore concluded that it was due to external contamination which might have been picked up in the course of setting up the experiment. It may be mentioned here that, although every precaution was taken, yet several experiments were rendered useless through contamination by Colletotrichum, Diplodia and Fusarium spp., the infection probably taking place whilst attaching the cotton threads to the boll stalks, since in all cases the rot started at the stalk end of the boll and so could be distinguished from Phytophthora tip infection.

An experiment was made to observe the comparative susceptibility of Durango and Sea Island bolls to the three strains of Phytophthora. St. Vincent caused a rot of both and Montserrat and Trinidad infected Sea Island within three days; after 5 days Durango in the Montserrat jar shewed a blackening of the boll wall which was due to Phytophthora. In the next experiment the effect of prolonged immersion of the bolls in the zoospore suspension was studied. Bolls of Sea Island, Durango, Acala and Lone Star were chosen as representing susceptible, slightly resistant and very resistant varieties. About 1/6th of the surface of the bolls was allowed to remain immersed for
four days and the results were somewhat striking.

St Vincent affected a complete rot of the Sea Island and Durango bolls in three days and Acala in seven days, whilst at the end of that time Lone Star had a peculiar brownish-red rot below the surface of the liquid, with a bright red circle on the waterline. When removed from the water the rot progressed for rather less than two days and then stopped. The boll was allowed to remain for another two days in the bell jar but no further developments were observed. A careful examination revealed the presence of no organism, yet the rot had penetrated to the seed.

Sections of the boll wall across the limit of rot shewed cells with their contents broken down and exhibiting a bright red colour, the cell walls swollen and intercellular spaces filled with fluid, in marked contrast with the healthy tissue in which the intercellular spaces were filled with air, and appeared as dark patches throughout the section (Fig. 1). The margin of the affected tissue could be distinctly seen owing to its brilliant red colour in contrast with the normal green tissue beyond. Farther back cell separation began, and near the tip of the bolls, was quite complete. The liquid, which originally contained the zoospores, assumed a pale red coloration the pigment being completely water-soluble and having presumably diffused from the broken-down cells of the boll wall.

Corresponding results were obtained in the Montserrat and Trinidad jars. Sea Island was alone infected by phytophthora and that within three days, aerial mycelium appearing on the fourth. The remainder of the bolls shewed a red rot which appeared to be identical with that in the St. Vincent jar except that the rot had progressed further and consequently the liquid
in the dishes had assumed a more brilliant eosin-red coloration, more intense in Montserrat than in Trinidad. In five days the Durango boll in the Montserrat jar showed signs of phytophthora infection; but Acala and Lone Star bolls effectively resisted the fungus. The bolls in the control bell jars, though immersed for the same length of time, showed no signs of rot but on the contrary were perfectly healthy, so that the red rot must have been due to action on the part of the fungus i.e. was enzymic.

In another experiment the resistance of Cauto was again demonstrated by employing bolls of Cauto, Pima, Acala and Lone Star, and again it was found that Pima exhibited highest susceptibility; Cauto showed a delayed rot after 16 days by St. Vincent but not by Montserrat or Trinidad, whilst Acala and Lone Star were totally resistant.

The table which follows shows the order of the resistance of the varieties tested to phytophthora rot.

<table>
<thead>
<tr>
<th>Variety of Cotton</th>
<th>St. Vincent</th>
<th>Montserrat</th>
<th>Trinidad</th>
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<tbody>
<tr>
<td>Sea Island</td>
<td>+ 2 days</td>
<td>+ 2 days</td>
<td>+ 2 days</td>
</tr>
<tr>
<td>Finja</td>
<td>+ do</td>
<td>+ do</td>
<td>+ do</td>
</tr>
<tr>
<td>Durango</td>
<td>+ 3-4 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Acala</td>
<td>+ 7 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cauto</td>
<td>+ 12-16 days</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lone Star</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: Young bolls were found to be more resistant than those more mature and no infection was brought about on any Upland bolls less than half grown. Sea Island and Pima bolls, however, appear to be susceptible in all stages of development.

The table which follows shows the order of the resistance of the varieties tested to phytophthora rot.
The positive and negative signs in the susceptibility column are used as standards of comparison. Thus + represents a variety of cotton in which infection took place in the bell jars in every case, no matter what period of time elapsed before the rot became evident. When ± is used, it represents occasional infection in a short time and after a prolonged period; whilst — shows no infection was obtained.

**Microscopic.**

In all the diseased bolls from the infection experiments, the course of the fungus was followed through the tissues and its action upon them studied.

The fungus enters the boll wall by the germ tube penetrating the epidermis, or sometimes entering through a stoma. The hyphae grow through the intercellular spaces of the tissue (Fig. 3), but later become intracellular (Figs. 4 & 5). It is only in the older diseased tissue that the latter condition is found, and it is evidently brought about by late formed lateral branches from the intercellular hyphae in tissues already dead or dying. The fungus passes through the boll wall and enters a loculus in which it appears to attack the seed first, because it is possible to distinguish the mycelium amongst the lint hairs, yet no staining of the lint occurs in newly diseased bolls. In bolls that have been infected for a longer period (about five days) stained lint is plentiful and hyphae can be observed in the lumina of the hairs (Figs. 7 & 9).

It occurred to the writer that it would be interesting to observe the behaviour of zoospores on the epidermis of the boll and so a modified hanging-drop culture was prepared as follows:

A small cell about 2 m.m. in diameter was made on a coverslip by painting a circle of vaseline with a fine camel hair
brush. Into this was placed a drop of sterile water containing motile zoospores and the whole covered with a piece of epidermis stripped from the lower side of a cotton leaf. The coverslip was mounted in the usual way for hanging-drop cultures and the behaviour of the zoospores could then be easily followed since the thin epidermis offered no obstacle to the passage of light. The zoospores swam about apparently in an aimless fashion for about five minutes, then settled down suddenly, without any sluggish movements, and rounded off. Within five minutes the margin of the spore became more refractive due apparently to the formation of a wall, and about twenty minutes later germination began by a small papilla being pushed out from the spore (Fig. 15). The wall of the papilla was thinner than that of the spore and appeared to be the spore wall stretched. The germ tube remained thus for ten minutes, when movement was observed in the protoplasm of the spore and the tube began to grow out. Again the growing tip had a wall much thinner than the remainder of the tube. Growth continued for about 10-15 minutes and again ceased, when the germ tube measured 6.3 μ in length. Another period of rest followed during which the tube tip thickened and a streaming movement was noticed in the protoplasm; later growth continued in the same fashion. Finally after a period of rest the tip of the germ tube appeared to be blown out as a bladder (Fig. 15 b) not in the original direction of growth, but alternately first to one side and then to the other (Figs. 15 c & d). No penetration of the epidermis was noticed and only one case of the germ tube passing through a stoma (Fig. 13). The majority of hyphae grew over the surface of the epidermis (Fig. 14) and were observed as a tangled web of mycelium after 24 hours. The stimulus responsible for inducing the fungus to enter the boll was apparently absent in the above experiment, but by substituting tangential
tangential sections (about 3 cells in thickness) of the boll wall for the leaf epidermis were observed (Figs. 17 & 16). In one case the hypha entered after passing over a stoma, and forced its way through in the centre of a cell, whilst in the second case, entrance was effected by forcing a passage between two epidermal cells. It is perhaps allowable to conclude from this that penetration of the boll epidermis was in response to some stimulus which was present in the section of the boll and not in the stripped lower epidermis of the leaf.

Having entered the host, the hypha passes through the intercellular spaces of the boll wall, causing a break down of the cells as it progresses. After a while the cell walls become swollen, at first irregularly with numerous constrictions, and eventually become quite homogeneous in structure, staining a pale violet colour with chlor-zinc iodine (Fig. 11). The walls after being weakened in this way are unable to resist the hyphae which force their way into the cells causing a complete disintegration of the contents to reddish or reddish-brown products.

A distinction must here be pointed out between resistant and susceptible varieties of cotton. As mentioned in an earlier part of the paper, susceptible varieties showed a complete cell separation in the boll wall two days after infection, whilst a corresponding break down of tissue is not observed in resistant bolls. Microscopic examination revealed the fact that the swelling of the cell wall was common to both types and appeared to take place in the same way, but complete cell separation with solution of the middle lamella was considerably delayed in Upland and Santo bolls. It follows therefore that the passage of the hyphae is obstructed to a great extent by the non-disintegration of the tissue and this must to some extent be responsible for the resistance exhibited by these cottons. Indeed, it is quite reasonable
to suppose that this may be a limiting factor in varietal susceptibility, especially when it is remembered that infection (i.e. penetration) can take place within two days, but that boll rot (i.e. disintegration and cell separation) may be delayed for as long a period as 16 days.

Infected tissues were examined for haustoria. Dastur figures haustoria-like processes in cells of Castor infected by P. parasitica, and the writer has found similar structures in cells of the boll wall (Fig. 5), but agrees with Dastur that these have more the appearance of hyphal branches than of true haustoria, as intracellular mycelium was common in older diseased tissue. The case is, however, different in considering infection of the cotton lint. The mycelium grows amongst the lint hairs for some time without penetrating them, but attacks the seed. Examination of diseased bolls in a more advanced stage shewed numerous cases of hyphae in the lumina of the lint hairs and Fig. 7 shews what the writer regards as a true haustorium which has just penetrated the cellulose wall. What appears to be an appressorium-like structure at the hypha tip is in reality the optical effect produced by the hypha turning and growing at right angles to the plane in which the drawing is figured.

In the seed, the action of the fungus is similar to that in the boll wall. Hyphae penetrate to the endosperm and cotyledons causing a breakdown of the cells to a brown pulpy fluid, the testa alone retaining its firmness. Later still the lint hairs die and become stained a deep brown also. The method by which the hyphae grow out from the host tissues corresponds to that generally described for phytophthoras. Fig. 6 shews a mycelial aggregate beneath the epidermis of the seed coat. This apparently increases in size and eventually ruptures the epidermis, although
although this stage was not observed by the writer. Tangential sections shewed numerous branched hyphae growing out from stomata on the bolls infected with St. Vincent (Fig. 12) but in no cases were hyphae observed to grow from stomata of bolls infected with Trinidad or Montserrat, escape being accomplished by rupture of the epidermis or by forcing a passage between cells.

It is now convenient to draw a comparison between the rot brought about by the fungus in the tissue of the boll and the red rot, already described, in which no organism could be detected and which was presumably due to enzymes.

The macroscopic appearance of bolls affected with these two rots presents a strong contrast. The fungus growth causes a typical blackening of the boll tip which progresses throughout the tissue and is complete in from two to three days; the second appears as a light brownish-red rot with a bright red margin, which does not spread through the boll but is arrested soon after the boll is removed from the inoculated liquid. Microscopic examination however, shows solution of the cell walls, first as a swelling with numerous constrictions and eventually a complete separation as described already for the phytophthora rot and as shewn in Figs. 3, 10 & 11. The effect upon the seed and lint is also similar in that complete disintegration is brought about, resulting in stained and ultimately rotted lint and a completely disorganised seed. It is evident therefore that the fungus excretes an enzyme capable of causing disorganisation of the boll tissues and cell wall solution. The suggestion that it is a cellulose-dissolving enzyme is further substantiated by the fact that germ tubes of sporangia germinating conidially or of undischarged zoospores germinating in situ grow through the sporangial wall, which is of pure cellulose. The papilla, the
plug of which, according to Dastur (6) consists of callose, is never punctured, although the germ tubes may form a rosette round it. The germ tubes of undischarged zoospores may, however, grow out through the open papilla.

Control.

Possibilities of control by spraying with Bordeaux Mixture have been demonstrated by Harland in St. Vincent during an attack by the disease upon his boll-sheding experimental plots, but this method has not been tried extensively in the fields although theoretically it should prove effective. (7)
Summary.

I. At least two and possibly three different strains of phytophthora are responsible for the Soft-Rot disease of cotton bolls in the West Indies.

II. Varying powers of virulence have been established for these fungi and details of the relative susceptibility or resistance of six varieties of cotton described.

III. It has been shewn that the St. Vincent, Montserrat and Trinidad strains of phytophthora excrete a cellulose dissolving enzyme which can cause a rot and cell separation in the absence of the fungus mycelium from the host tissue.

IV. The behaviour of zoospores, germination of the resting spore and growth of the germ tube is described and details of the technique employed to illustrate their behaviour on the surface of an epidermis are furnished.

V. Penetration of the boll and the passage of the fungus through the host tissues is followed and the significance of certain structures discussed.

VI. The rot resulting from growth of the fungus in the tissue and that due to enzymes are compared and the similarity between the two pointed out.

VII. The possibility of control by spraying with Bordeaux Mixture is indicated.
References.


(3) Diseases of Crop Plants in the Lesser Antilles. West India Committee p. 271.


Fig 1. Tangential Sect. of Cell.
Healthy tissue.

Fig 2. Tangential Sect. across rotted area.
Diseased tissue

S = Intercellular spaces filled with liquid

Cells with broken down contents giving characteristic appearance of red rot.
Fig 3.
Hypha in intercellular spaces.
X 360.

Fig 4.
Intracellular hyphae
C = constriction
X 400

Fig 5.
Haustorium-like process in cell of boll wall
X 360

Fig 6.
Mycelial aggregate beneath epidermis of seed coat.
X 360
h = hyphae
Fig 7. Penetration of lint hair x 520

Fig 8. Solution of cell wall. W = partly dissolved wall x 520

Fig 9. Hyphae in lint hair x 520

Fig 10. Red rot: enzymic action on cell walls & cell contents x 360

Fig 11. Action of hyphae in the tissues of the goll wall (Sea Island & Pima varieties)

Fig 12. Hyphae growing out from a stoma x 360
Fig. 13. Germ-tube entering a stoma. 
X360

Fig. 14. Germ-tube on surface of epidermis - no penetration. 
X360

Fig. 15. Germination of a spore.

Fig. 16. Penetration between two cells of the epidermis. 
X430
Wax covering epidermis.

Fig. 17. Penetration through a cell of the epidermis. 
X360.