ALTERNARIA GOSSYPINA (THÜM.) COMB. NOV. CAUSING A LEAF SPOT AND BOLL ROT OF COTTON

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(With Plate VI and 7 Text-figures)

The commercial cottons grown throughout Southern Rhodesia are of the American upland type and within recent years have been almost entirely reduced to one variety, known as U 4, which has been selected for its jassid resistant qualities and has replaced strains of "Improved Bancroft" which formerly enjoyed much popularity. A characteristic of both these cottons during the first few weeks of growth is a more or less severe spotting of the cotyledons and early leaves which is frequently mistaken by farmers for angular leaf spot (*Bacterium Malvacearum* E. F. Sm.). The lesions are minute at first, but are rendered conspicuous by a relatively broad margin of reddish purple which surrounds a pale brown, killed area (Pl. VI, fig. 1). When it first becomes noticeable, the whole affected area has a diameter rarely exceeding 0·5 mm. and the central brown tissue is barely perceptible without the aid of a lens. In 1927, material collected for microscopic examination showed the lesions to be invaded by hyphae of a fungus, an *Alternaria* being subsequently obtained in culture. Towards the end of the 1928 season, a leaf spot of mature cotton which had been growing under adverse conditions was observed and an *Alternaria* was isolated from diseased tissue, whilst again in 1929, *Alternaria* was isolated from a cotton boll affected by an internal rot. Recent study of these cultures and material has proved *Alternaria* to be the causal agent in each case, and has established the identity of all three isolations.

Symptoms

On the leaves a light brown area appears in the centre of the small purple spots and, as the lesion increases in size, the purple margin persists and may be half as wide as the killed area when this measures 1 mm. in diameter. The spots usually maintain a circular shape, are zonate and may be as large as 1 cm. in diameter, but rarely exceed this size; an average of twenty measurements equals 0·6 cm. As the fungus activity ceases, so the invaded tissues assume a vandyke brown colour, due in part to the production of conidiophores and conidia on the upper surface and the characteristic purple disappears

from the margin to be replaced by dark brown or black. On old leaves a small white spot may appear in the centre of the killed tissue, but this symptom is of rare occurrence and may be due to the presence of *Phyllosticta* sp. (*? gossypina*) which is sometimes found associated with *Alternaria*. Two or three spots may coalesce to form a dead area 2 × 1 cm. in size, the original lesions being clearly defined by the presence of zonations and the lighter colour of the intervening tissue. In severe attack, the earlier formed leaves may become somewhat chlorotic and under certain conditions (as was demonstrated when excessive quantities of potassic fertiliser were applied to the soil or during a period of prolonged heavy rain) may be shed (Pl. VI, fig. 3).

Examples of natural infection of bolls have been brought to my notice, but it would appear from field observations that the disease is of rare occurrence on fruits which eventually mature; the fungus, however, may be commonly found discolouring the exposed lint of ripe bolls after periods of rainy weather. Material which was examined in the laboratory showed small, almost black, depressed lesions about 1 cm. in diameter situated on the line of suture of the carpels, about 2 cm. from the tip of the boll, resembling closely lesions due to *Glomerella Gossypii* (Southw.) Edg. or *Bacterium Malvacearum* E. F. Sm. In the interior, a slight yellowish discoloration of the lint was observed and a dry rot had commenced immediately below the boll wall in two adjacent loculi bordering the seat of infection. The opened bolls were placed on one side and within forty-eight hours a copious sooty deposit of spores had appeared upon the exposed lint which, as will be seen, is characteristic of the boll rot phase of the disease.

According to Jones (1), who describes an almost identical affection due to *Alternaria macrospora* Zimmerm. on native cotton in Nigeria, fungus infection of bolls is principally confined to those a few days old and is concerned in their early shedding. Similar conditions appear to obtain in Rhodesia and a *Macrosporium** has been reported from completely rotted young bolls which, however, remained attached to the plant; this condition has also been produced experimentally as will be described later.

A leaf spot and boll rot, in which the lint is discoloured and "compacted" and covered with a black sooty deposit, have been recorded on three occasions by Eyles (2), who referred the causal agent to *Macrosporium nigricantium* Atk., but material is unfortunately not available for comparison of the two diseases.

* Unpublished.

Infection Experiments

Plants of U 4 cotton were grown in pots in the glasshouse and subsequently placed outside under cages of book muslin, which served the dual purpose of affording protection against insect attack and acting as incubation chambers when the fabric was kept constantly wet. Three methods of inoculation with suspensions of *Alternaria* spores in sterile water were attempted upon bolls four days old and about sixteen days old, the surface of the bolls having been previously washed with 0·01 per cent. corrosive sublimate solution, followed by sterile water.

(1) Upon uninjured bolls.

(2) Upon bolls the surface of which had been scratched by a needle.

(3) By introducing the inoculum into the interior of the boll with a fine needle.

Control plants were used throughout.

Young bolls were picked after four days and no disease was observed in those which had received no injury. Two out of three bolls in class (2) showed a faint yellow discoloration of lint in the loculus immediately beneath the point of inoculation, and fungus mycelium was detected in both the lint and the tissues of the carpel. No spores were observed. The bolls which had been inoculated internally exhibited a reddish brown, soft rot of the seed and a soft, pulpy, yellow rot of the lint. Mycelium was present in abundance, but no spores were seen. After fourteen days, no disease had developed upon the remaining uninjured bolls, but those which had been scratched superficially had dry, shrivelled carpels, whilst the interior consisted of a black powdery mass of disintegrated tissue and fungus spores. In one boll the rot was confined to one infected loculus, the remainder having developed normally, thus giving the boll a distorted appearance. All infected fruits, even though completely disorganised internally, remained firmly attached to the plant and were not shed by the time that healthy bolls had ripened and split.

Sixteen days' old bolls were unaffected in classes (1) and (2) after twenty days from the date of inoculation, but where the inoculum had been introduced into the interior, a yellowish brown rot of the lint had begun after five days. In ten days the rot had invaded about two-thirds of the contents of the inoculated loculus, a small portion of the lint still retaining its white colour; the remainder was affected by a light brown, dry rot which had also reached the seed and produced a brownish discoloration of the immature cotyledons. Fungus mycelium was observed in both "stained" and unstained lint and also in the interior of the seed but did not appear to be present in the carpel except in the tissues adjacent to the inoculation wound. Within

forty-eight hours of the opening of the bolls, the characteristic sooty deposit of spores appeared in patches on both diseased and unstained lint. After twenty days, the carpels of all infected loculi had become dry and shrivelled, but the majority failed to open. In a few, however, the lips of the carpels curled away from the sutures revealing the typical dry rot of lint and sooty deposits of spores which appear to be symptomatic of the disease (Pl. VI, fig. 2). The interior of the seed was usually completely disintegrated but the seed coat mostly remained firm and hard. It was black and could be easily crushed between thumb and forefinger exposing a sooty mass of spores superficially resembling the smuts of cereals.

Similar infection was obtained in preliminary experiments in glass dishes with very young and half-grown bolls, and it was again found that uninjured fruits resisted the fungus but that surface wounding induced infection in the larger bolls after a prolonged period of time. It therefore appears that there is distinct physiological resistance to *Alternaria* in the boll walls, for even under extremely favourable conditions for fungus attack, provided that the epidermis is not ruptured, resistance is still maintained, whilst, even when the latter condition is not fulfilled, sufficient time must elapse for autolysis to set in before invasion of the carpel tissues is possible. This is in agreement with the behaviour of *Phytophthora* (3) on certain varieties of cotton having marked field resistance, which become susceptible to attack after being kept for some twelve days in glass vessels, and indicates the limited use of this method of testing pathenogenicity with any but quickly developing diseases.

Typical lesions were produced upon young, growing leaves by spraying on a suspension of spores by means of an atomiser or by placing fragments of hyphae and spores on the upper surface. A few *Alternaria* spots developed upon the leaves of the control plants and were attributed to natural infection, the disease being difficult to control on any plants grown in the open. The fungi were isolated from naturally and artificially produced lesions and found to be identical in culture.

Microscopic

A considerable polymorphism was exhibited by the spores taken from different parts of the cotton plant (*i.e.* lint and interior of the seed), and those produced in culture, but, on the whole, their dimensions and the production of long chains suggests an affinity with *A. tenuis* Nees. The presence of a long-beaked form would appear to place the fungus outside the *A. tenuis* group and a comparison with *A. macrospora* Zimmerm. shows it to be distinct from that species, so that it is proposed to refer the Rhodesian organism to *Alternaria gossypina* (Thüm.) comb. nov., since Mason (4) has pointed out that

the fungus on authentic material of von Thümen's (Mycotheca Univ. No. 1469 in Herb. Kew) is an *Alternaria*.

On the plant

The hyphae on the leaf are dark brown, slightly constricted at the septa, with cells averaging 20 × 3 μ, mainly in the leaf tissues, but sometimes growing on the surface. The conidiophores are at first

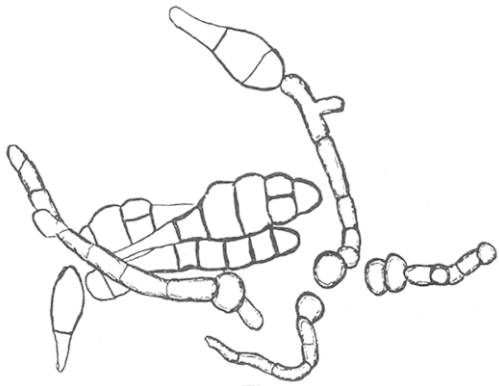


Fig. 1.

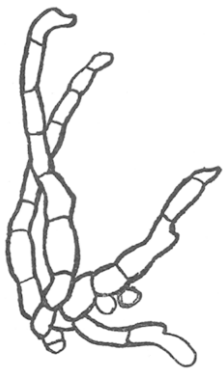


Fig. 2.

Text-fig. 1. Group of conidiophores and spores on upper surface of the leaf.
Text-fig. 2. Cluster of conidiophores showing geniculations.

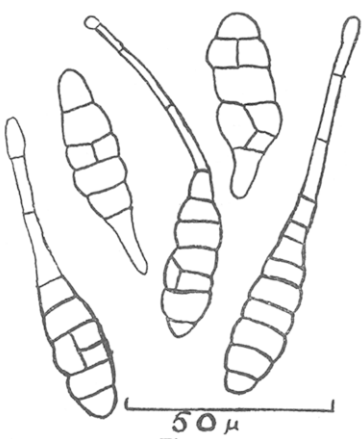


Fig. 3.

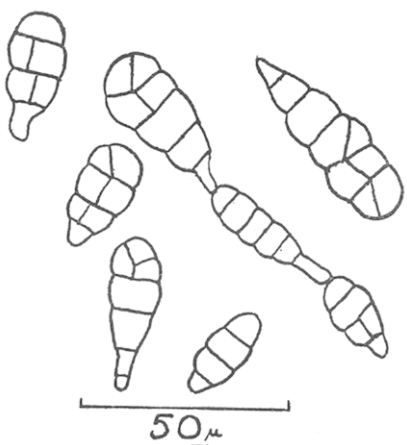
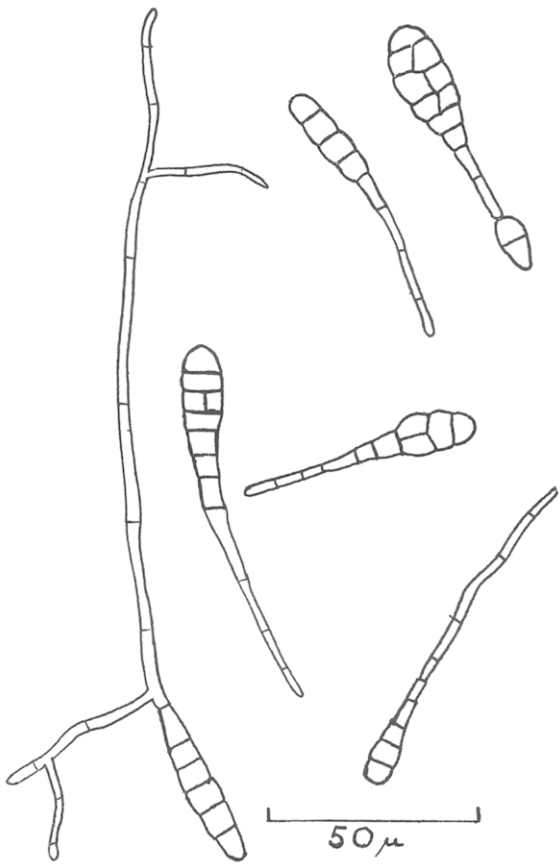


Fig. 4.

Text-fig. 3. Spores on leaf surface.
Text-fig. 4. Spores from interior of seed.

submerged and then erumpent, apparently bursting the epidermis, in clusters or single, 38–95 × 6 μ, brown, septate, slightly constricted at the septa, geniculate and the basal cell swollen (Text-figs. 1 and 2).

The spores are dark brown, obclavate, with up to nine transverse and usually one or two longitudinal septa, constricted at the septa, 32–46 × 12–15 μ with a hyaline, sparingly septate beak 9–52 μ in length, giving a total measurement of 41–98 μ (Text-fig. 3). Catenulate spores were observed in the seed and in cultures and are shown in Text-figs. 4, 5 and 6. Jones's (1) measurements for spores on the leaf



Text-fig. 5. Elongation of beak due to germination of terminal cell. Spores from aerial mycelium of vigorous young culture.

surface are 34–50 × 10–18 μ with a beak 40–100 μ. It will be seen that the two spore measurements (with the beak), are in close agreement, but that the beak of the Rhodesian fungus does not attain the great length of the Nigerian one. On the lint, however, the measurements approximate to those of Jones's Trinidad fungus, the spore being 34–61 × 12–15 μ with a beak 12–73 μ, giving a total length of 46–134 μ, but the apparent long beak is made up of a certain

amount of hypha due to the germination of the terminal cell of the neck (Text-fig. 5). Spores obtained from the interior of seeds and cultures of different ages again possessed different dimensions and, in the table which follows, are compared with the Trinidad and Nigeria organisms.

Table I. *Comparison of spores from different parts of the cotton plant with those of Nigeria and Trinidad fungi*

Origin	Spore μ	Beak μ	Total length μ	Width μ
Leaf	32–46	9–52	41–98	12–15
Lint	34–61	12–73	46–134	12–15
Interior of seed	28–50	8–12	36–62	16
Young culture	35–54	29–46	64–100	10–15
Six weeks' old culture	25–52	23–65	45–117	12–16
Six weeks' old culture (perimeter)	23–41	9–16	32–57	14
Nigeria	34–50	40–100	74–150	10–18
Trinidad	37–60	80	140	13–18

In culture

After five days on potato dextrose agar at room temperature (20°–27° C.) the growth measured 2·5 cm. in diameter, and was almost

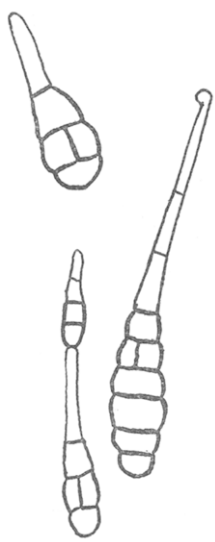


Fig. 6.

Fig. 7.

Text-fig. 6. Spores from centre of six weeks' old culture.
Text-fig. 7. Spores from edge of same culture.

completely covered by a thick, olive-green mass of spores with an area of white aerial hyphae, 1 cm. in diameter, in the centre. The hyphae were hyaline, vacuolate and granular at the edge of the colony,

becoming olive-green and then light brown. Marked constrictions of secondary hyphae were present at the points of branching. After six weeks, the culture was covered by an almost black sooty deposit of spores with an increased amount of white aerial mycelium in the centre of the plate. The hyphae were dark brown, constricted at the septa, the cells averaging 20 × 3 μ, and considerable variation in spore measurements existed (Text-figs. 6 and 7) as indicated in Table I. After being transferred successively to corn meal agar, lima bean agar, oat juice agar and potato dextrose agar over a period of ten months, no pigment was produced in the medium, no diminution of spore production was noticed and no recognisable saltant appeared. The behaviour of the fungus in culture, therefore, differs from that of both the Nigeria (which ceases to form spores in culture) and the Trinidad (which is strongly chromogenic) organisms.

Discussion

The characters of the leaf spots produced by *Alternaria macrospora*, a probably identical *Alternaria* in Trinidad, and Reichert's *Sporodesmium longipedicellatum* (now referred to *A. macrospora*) in Tanganyika are almost the same as those of the Rhodesian disease, except for a variation in the persistence of the purple margin which may well be determined by environment or differences in the varieties of the host plants, whilst the rot of young bolls is a symptom common to the Nigerian and Rhodesian diseases. The fact that shedding of "squares" is not caused by the latter can be attributed to the hereditary character of the U 4 strain of cotton, which has been selected for its low shedding qualities; the young bolls still remain attached to the plant even after boll-worm attack. As far as can be ascertained from available descriptions, the chief differences between the affections lie in the fungi concerned, two distinct species of *Alternaria* being involved.

Saccardo's description of *Macrosporium gossypinum* Thüm., from cotton stems, with broadly clavate, six to eight septate conidia, 36–40 × 14–16 μ borne on short, septate stalks, agrees well with the Rhodesian fungus in culture and on the leaf if it is conceded that the terminal beaks of the long-beaked form of spore were mistaken for proximal stalks and were probably omitted from the length measurement in the original description. That such an assumption is allowable is substantiated by the fact that the model form of the spores of the Rhodesian fungus is typically that of the *A. tenuis* group, particularly in the interior of the seed and in culture where long chains are formed in abundance. The presence of the long-beaked spore, which was consistently obtained in single spore cultures, would, however, place it outside this species and it appears desirable to apply the binomial *Alternaria gossypina* (Thüm.) comb. nov. at any rate until a closer relationship is established.

It was at one time thought that this fungus was a strain of *A. macrospora* owing to the similarity in the symptoms of the diseases produced and also to the presence of spores in the cotton lint whose length apparently attained as much as 134 μ, thus comparing favourably with the Trinidad fungus. It has since been ascertained, however, that the very long extensions observed, both on the lint and in the aerial mycelium of some cultures, were the result of the germination of the terminal cell of the beak and that in reality the measurements included a certain length of germ tube. Such spores are shown in Text-fig. 5. In most instances the germ tubes are not as conspicuous as those in the illustration, but can usually be distinguished from the true beaks by the presence of a terminal swelling in the latter. Since Mason (4) has proposed to reserve the species *macrospora* for those *Alternaria* spp. on cotton whose spores (with beak) can attain a length of 200 μ, it is certain that the Rhodesian fungus is distinct from the Nigerian and it is therefore of some interest to note the close similarity in the symptoms of the diseases caused by these two organisms.

My thanks are due to the Director and Staff of the Imperial Bureau of Mycology for considerable assistance.

Summary

1. A common leaf spot of cotton in Rhodesia is attributed to *Alternaria gossypina* (Thüm.) comb. nov., which is also found associated with a rot of bolls.

2. Symptoms of the disease on leaves and bolls are described.

3. Details are given of inoculation experiments on both bolls and leaves by which the disease was produced on growing plants.

4. Microscopic characters of the fungus are compared with those of *Alternaria macrospora* causing a similar disease in Nigeria and Trinidad and certain cultural phenomena are noted.

5. Reasons are given for referring the Rhodesian fungus to *Alternaria gossypina* (Thüm.) comb. nov.

EXPLANATION OF PLATE VI

Fig. 1. Young *Alternaria* lesions on cotton leaf. × 1½.
Fig. 2. Typical sooty deposit on lint in inoculated boll. × 1½.
Fig. 3. Advanced stage of leaf spotting. The leaf is chlorotic and blotched with purple. × 1½.

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Fig. 1.

Fig. 2.

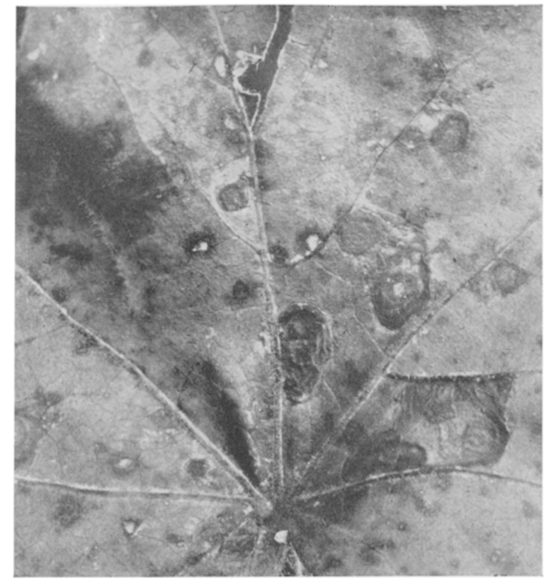

Fig. 3.

Alternaria Gossypina (Thüm.) Hopkins.