On Two *Alternaria* Species Injurious to Cotton Fibers in Bolls.

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I. Introduction.

The Tyûgoku district, particularly the Okayama prefecture, had been known to produce cotton as a principal crop prior to the middle of Meiji Era, but with the import of foreign raw cotton, this industry had gradually declined to near extinction. The recent revival of cotton cultivation in this and other districts has brought the diseases of cotton into attention. As the industry is new, there are practically no record of the occurrence of cotton diseases up to the present time, and their exact description seems of great value. The present report is on the studies of *Alternaria* species affecting cotton bolls.

For some time diseases causing the discoloration of bolls, particularly of the fibers have been observed in the experimental field of the Institute. In 1939 which was comparatively a dry year, excellent vegetative growth occurred, but with the prolonged rain during the harvesting time heavy loss of clean lint

resulted. On September of 1939, specimens of nearly ripe bolls discolored grayish to almost black in the fibers were received from Mr. HUSATARO TANAKA of Simane prefecture. In his accompanying notes, he mentioned of the disease as being very destructive in his locality. A laboratory examination disclosed that they were caused by at least two species of *Alternaria* and three species of *Fusarium*. Subsequent inoculation experiments showed that the species of *Alternaria* were able to discolor the cotton fibers.

In connection with the present study, the writers wish to acknowledge their indebtedness to Mr. HUSATARO TANAKA and Professor HAJIME YOSHII for supplying a portion of materials used in the experiments, and to Mr. TAKAO NAKAYAMA for rendering into English the original paper which appeared in Ann. Phytopath. Soc. Japan 10:214-230, 1940 and in Nogaku-Kenkyu 32:469-494, 1941.

II. Symptoms of the Diseases Caused by the *Alternaria*.

The initiation of the diseases of cotton bolls in the Tyûgoku district apparently occurs during the month of September when rain is followed by prolonged warm moist period. Although the damage caused by *Alternaria* is by far greater on the boll, the occurrence of the causal organisms on the leaves is not uncommon. The minute brownish flecks on the leaves increase in size with each rain. They attain to a diameter of 4 to 7 mm. with a more or less distinct margin, and often regular concentric rings are formed which are caused by periodic mycelial and conidial stages of the fungus. The spores are found in more abundance toward the center of the lesion. Severely diseased leaves usually do not drop to the ground but remain hanging after death.

The symptom of the disease on the boll is the turning of the affected portion of the pericarp to purplish brown which usually has an irregular or indistinct margin. The unopened bolls are less frequently attacked by the disease, but in those that are beginning to open, it may become so severe that the discoloration involves the whole surface of the pericarp.

When the fibers are diseased, they are turned to corky, hard mass, discolored to ash or dark gray color. Bolls rotted to the fibers in this manner fail to open even after maturity.

III. Morphology of the Fungi.

The species of *Alternaria* found on diseased cotton plants can be distinguished into two groups as described below.

Group 1.

The *Alternaria* of this group occur on the boll as well as on the foliar portion. The conidiophores are brown although the intensity vary somewhat with maturity, 3 to 7 septate, 75-150 by 2.5 μ, and may have 2 or 3 branches; the apex

is provided with a distinct scar showing the place of attachment of conidium. The conidia are long-beaked, obclavate in shape, 35 – 130 by 7.5 – 23 μ, and have 3 – 7 constricted septations with 1 – 3 longitudinal cross walls, beak 15 – 20 by 3 – 5 μ with 2 – 3 cross walls; and the apex has a distinct scar.

Group 2.

The *Alternaria* of this group principally occurs on the cotton fibers. The conidiophore may be either simple or branched, bearing conidia in chain with the youngest conidium in contact with the conidiophore. The point of attachment of conidium is dark. The conidia are rarely solitary, usually in chain of 2 or 3 or often as many as of 5; clavate to oval, narrower at apex, and with or without short beak; brown to dark but lighter toward apex; and 13 – 55 by 5 – 15 μ in size.

Table 1.

Growth Characters of Cotton *Alternaria* on Three Different Culture Media

Culture Medium	Fungous Strain	Growth of Mycelium in Mm. at		Texture of Mycelium	Amount of Aerial Mycelium	Chromogenesis		Conidia Formation
		2 Days	7 Days			Color*	Extent	
1% Mrlt Extract Agar	No. 2001	20.0	66.0	Thin and soft	±	Dark greenish olive	|||	+
	No. 2002	21.5	72.5	Do.	±	Dark ivy green	||	±
	No. 2003	21.0	71.0	Do.	±	Dark greenish olive	|||	±
	No. 2004	17.5	63.5	Do.	—	Do.	|||	||
Rice Straw Decoction Agar	No. 2001	20.0	78.0	Rather thick and soft	±	Dark greenish olive	||||	|||
	No. 2002	22.0	73.0	Do.	±	Do.	||	||
	No. 2003	20.5	78.0	Do.	±	Do.	||||	|||
	No. 2004	19.5	71.5	Do.	—	Do.	|||	|||
Potato Decoction Agar	No. 2001	22.5	76.5	Thick and hard	±	Dark greenish olive	||||	|||
	No. 2002	23.5	71.0	Do.	+	Olivaceous black (1)	|||	||
	No. 2003	25.0	74.0	Very thick and hard	±	Do.	||||	||
	No. 2004	21.0	78.0	Thick and hard	—	Dark greenish olive	||||	||||

*Colors are of R. Ridgway, Color Standards and Color Nomenclature. 1912.

IV. Cultural Characters.

A. Isolation and Source of Fungi Used in the Experiments.

The fungi were in every case isolated by the usual pour plate method. The strains studied were as follows:

Alternaria No. 2001. Isolated from a diseased cotton leaf collected in the experimental field of the Institute. The strain belongs in the previously described group 1.

Alternaria No. 2002. Isolated from the diseased fibers collected in the experimental field of the Institute. The strain also belongs in group 1.

Alternaria No. 2003. Isolated from the fibers of the interior of a diseased boll received from Mr. TANAKA. The strain corresponds to group 2.

Alternaria No. 2004. Isolated from the diseased surface portion of a boll received from Mr. TANAKA, and belongs to group 2.

B. Growth on Culture Media.

The four isolated strains of *Alternaria* were grown on three different culture media, namely, the malt extract agar, the rice straw decoction agar, and the potato decoction agar media. The diameter of growth and other cultural characters were recorded at the ends of 2 and 7 days. A summarized result is shown in Table 1.

V. Classification and Nomenclature of the *Alternaria.*

One of the earliest descriptions of parasitic *Alternaria* or *Macrosporium* on cotton plant is given in SACCARDO's Sylloge Fungorum, volume IV, 1886 under the name of *Macrosporium gossypium* THÜM. This name was altered by HOPKINS in 1931 to *Alternaria gossypina* (THÜM.) HOPKINS. Recently, BRIGHI (1937) reported of the fungus being pathogenic to bolls. A comparison of the writers' strains of the group 2 to this fungus shows that the two are not the same as made evident by the marked difference in the shape and the size of the conidia.

TAKIMOTO (1924) applied the name of ATKINSON's *Macrosporium* (*nigricans*) *nigricantium* ATK. to what he isolated from a diseased cotton plant in Heijo, Tyosen; and ZAPROMETOFF (1928) also used *M. nigricans* to the organism on the cotton fibers in Soviet Central Asia. Again, none of the writers' strains coincides with the above organism in description.

JONES (1928) mentions an *Alternaria* damaging the leaves and bolls of the indiginant *Gossygium peruvianum* and *G. vitifoium* species in British Africa. From his descriptions, the spores are large, 75 - 150 by 10 - 18 μ, dark colored with a small hyaline beak, and occur singly or in chain of two. From his comparative studies with other specimens, he classified it as *Alternaria macrospora* ZIMM.

NAKATA (1934) uses this name for the leaf blight of cotton. BRIGHI gives account of the pathogenicity of the fungus to not only the leaves but also to fibers as well. The authors' strains in the group 1 corresponds more closely with this *Alternaria macrospora* ZIMM. than to any others, and for the present this name shall be applied.

GASPARRINI in Italy reported on a disease of cotton caused by *Alternaria tenuis* NEES which also occurs on many different plants as a saprophyte. The authors' *Alternaria* of group 2 resemble fairly close except for the smaller size of the

conidia.

JACZEWSKI (1929) describes a new organism affecting the cotton fibers under *Macrosporium gossypii* n. sp. in Soviet Central Asia. Its mycelium is dark brown, creeping and nodose; the conidiophores are short, brown, septate, and dentate at the apex; the conidia are slightly clavate, frequently ovoid, light brown, with two or three transverse septa and sometimes one oblique or longitudinal septum, single or in short chains, and measure 22–27 by 9–11 μ. On agar a compact, black-purple mycelium develops and the substratum is blackened.

The authors' strains show close similarity to the above fungus except for the catenate formation of conidia. In applying the JACZEWSKI'S name, a new combination is therefore suggested—*Alternaria gossypii* (JACZ.) nov. comb. In the present study, the species are differentiated according to the relative size of the conidia—*A. macrospora* ZIMM. to the large spore group, and *A. gossypii* (JACZ.) nov. comb. for the short spore group.

VI. Pathogenicity.

The pathogenicity of the two groups of the isolated fungi was tested on cotton bolls and fibers by inoculation experiments.

Experiment 1.

A pure culture of *A. gossypii* was inoculated on the fibers with bolls in various stages of maturity. A summarized results are given in Table 2 and 3. The results show that the fungus is weakly pathogenic to the fibers in young unopened bolls and in matured fully opened bolls, but it is highly pathogenic to the bolls that have just begun to open.

Table 2.

The Result of Inoculation of *Alternaria gossypii* on Cotton Boll. (1)

Condition of Boll	Days After Inoculation	Application of Water						Remarks
		Once Each Day			Continuous			
		No. of Bolls Used	No. of Bolls Diseased	Percent Diseased Boll	No. of Bolls Used	No. of Bolls Diseased	Percent Diseased Boll	
Opened and the fibers extended	7	7	1	14.3	3	0	0	Discoloration at the base of the pericarp, no conidia formed.
	10	7	2	28.7	3	3	100.0	
Beginning to open	7	8	1	12.5	4	2	50.0	Black discoloration of the pericarp and the fibers. Conidia formed.
	10	8	4	50.0	4	3	75.0	
Closed	7	4	0	0	12	0	0	Beginning to rot but not caused by the *Alternaria*.
	10	4	0	0	12	2	16.7	

Table 3.

The Result of Inoculation of *Alternaria gossypii* on Cotton Boll. (2)

Condition of Boll	Fungous Strains Used						Remarks
	No. 2003-1			No. 2003-2			
	No. of Bolls Used	No. of Bolls Diseased	Percent Diseased Boll	No. of Bolls Used	No. of Bolls Diseased	Percent Diseased Boll	
Opened and the fibers extended	7	5	71.4	5	3	60.0	Very suceptible to the disease
Beginning to open	5	5	100.0	6	6	100.0	Very suceptible to the disease
Closed	6	0	0	5	0	0	Almost insuceptible to the disease

Experiment 2.

Pure cultures of *A. gossypii* and *A. macrospora* were each inoculated on the following kinds of cotton fibers. 1, fibers removed from an interior of the unopened boll; 2, similarly from a slightly opened boll; 3, from a fully opened but inmature boll; 4, from a fully opened and mature boll; 5, filter paper; 6, commercial cotton fibers; and 7, commercial absorbent cotton fibers. As given in Table 4, the result showed that the two groups of fungi readily discolored the various fibers excepting the absorbent cotton. The discoloration was most severe on the fibers removed from the unopened and the slightly opened bolls.

Table 4.

Inoculation of *Alternaria macrospora* and *A. gossypii* to Cotton Fibers.

Kinds of Fibers	Condition of Fibers	*A. macrospora*		*A. gossypii*
		No. 2001	No. 2002	No. 2003
(1) From interior of an unopened boll	Unsterilized	##	±	##
	Sterilized		##	##
(2) From a slightly opened boll	Unsterilized		+	+
	Sterilized		##	##
(3) From a fully opened but inmature boll	Unsterilized	##	##	##
	Sterilized		##	##
(4) From a fully opened and mature boll	Unsterilized	±	+	±
	Sterilized		##	+
(5) Filter paper	Unsterilized	±	+	±
	Sterilized		##	+
(6) Commercial cotton fibers	Unsterilized	±	±	±
(7) Commercial absorbent cotton fibers	Sterilized	—	—	—

VII. Growth in Relation to Environments.

A. Germination of Conidia in Relation to Temperature.

A study on the influence of temperature on the germination of conidia of *Alternaria macrospora* and *A. gossypii* was made. The method of study consisted of germinating the conidia which was fixed on the surface of small glass plates by drying, and after covering by a drop of 1 per cent malt extract solution. Results of observations after 1, 3, 6, 24 and 48 hours are shown in Table 5, expressing the degree of germination by + and − signs. The table shows that the two fungi are almost identical in their response to temperature. At 5 °C. the germination was seen only after 48 hours, while at 10 °C. it was after 6 hours. The result would suggest that the minimum temperature of germination lies in the neighborhood of 5 °C. Between 24 and 35 °C. few conidia germinated at 3 hours, but the optimum apparently lies near 27 to 30°C. A positive germination at 35° but none at 38° shows that the maximum temperature for germination is near 36 °C.

Table 5.

The Influence of Temperature on the Germination of Conidia of *Alternaria macrospora* and *A. gossypii*.

Temperature (°C.)	Time of Treatment in Hour									
	Alternaria macrospora (No. 2001)					*Alternaria gossypii* (No. 2003)				
	1	3	6	24	48	1	3	6	24	48
5	−	−	−	−	+	−	−	−	−	+
10	−	−	±	++	+++	−	−	±	+++	+++
15	−	−	±	+++	++++	−	−	+	+++	++++
20	−	−	+	++++	++++	−	−	++	++++	++++
24	−	±	++	++++	++++	−	±	++	++++	++++
27	−	±	++	++++	++++	−	±	+++	++++	++++
30	−	±	++	++++	++++	−	±	+++	++++	++++
33	−	±	±	+++	++++	−	±	++	+++	++++
35	−	±	±	++	++++	−	±	+++	++	++++
38	−	−	−	−	−	−	−	−	−	−

B. Growth of Mycelium in Relation to Temperature.

The mycelial growth of *A. macrospora* and *A. gossypii* was studied. The results of growth on different culture media at various temperatures are given in Tables 6 and 7 and Figures 1 and 2. Both *A. macrospora* and *A. gossypii* begin growth at 5 °C. and reach the optimum of 27 to 30°, after which the growth declines rapidly till 40° is reached where it completely ceases. Therefore, the maximum temperature for growth seems to lie between 37 and 40°C. Other cultural characters are recorded in Table 8.

Table 6.

The Mycelial Growth of *Alternaria macrospora* and *A. gossypii* on Three Different Culture Media at Various Temperatures.

Temperature (°C.)	Growth in Mm. at 4 Days						Growth in Mm. at 7 Days					
	Malt Extract Agar		Potato Decoction Agar		Rice Straw Decoction Agar		Malt Extact Agar		Potato Decoction Agar		Rice Straw Decoction Agar	
	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003
5	—	—	—	—	—	—	±	+	±	+	±	—
10	23.0	25.0	24.5	24.5	21.5	18.5	51.5	45.0	49.5	50.0	44.5	43.5
15	24.0	22.5	24.0	24.0	17.0	19.0	45.5	44.5	46.0	47.5	39.0	39.0
20	24.5	28.0	26.0	27.0	22.0	22.5	43.5	49.0	54.0	50.0	44.0	43.5
24	35.0	35.5	43.5	36.0	35.0	33.0	65.0	72.0	74.0	65.0	64.5	63.5
27	36.0	35.0	38.0	37.5	37.5	33.5	64.0	59.0	68.0	66.5	65.0	64.5
30	34.0	36.0	34.5	38.5	35.0	36.5	54.5	58.0	62.0	68.0	62.5	66.5
33	26.5	32.5	35.0	34.0	31.0	36.5	53.0	54.0	65.0	52.0	59.0	64.5
38	13.0	11.0	14.0	10.5	9.5	13.0	25.0	17.5	33.5	24.0	24.5	33.0

No. 2001 is *A. macrospora* and No. 2003 is *A. gossypii*.

Table 7.

The Mycelial Growth of *Alternaria macrospora* aud *A. gossypii* on Two Different Culture Media at Various Temperatures. (2)

Temperoture (°C.)	Growth in Mm. at 3 Days				Growth in Mm. at 5 Days			
	Malt Extract Agar		Potato Decoction Agar		Malt Extract Agar		Potato Decoction Agar	
	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003	No. 2001	No. 2003
5	—	±	—	±	±	10.0	±	11.0
10	9.3	17.3	12.3	19.3	18.3	31.5	23.5	32.5
15	15.0	26.5	20.5	30.0	30.5	46.5	38.8	47.0
20	17.0	27.8	22.8	31.8	35.0	50.5	43.3	51.0
24	19.0	30.3	26.0	34.5	38.8	53.8	48.5	56.8
27	23.0	33.5	29.8	37.8	42.0	58.3	54.8	62.8
30	23.3	40.0	30.0	36.5	40.5	57.0	55.8	60.8
33	22.0	29.5	28.5	33.5	35.8	50.3	51.8	54.3
35	18.0	24.3	23.3	27.8	28.0	38.8	40.5	43.0
36	9.5	14.0	12.5	16.3	15.5	21.0	21.8	24.3

No. 2001 is *Alternaria macrospora* and No. 2003 is *A. gossypii*.

Table 8.

The Cultural Characters of *Alternaria macrospora* and *A. gossypii* on Three Different Culture Media at Various Temperatures.

Culture Medium	Temperature (°C.)	Amount of Aerial Mycelium		Chromogenesis				Conidia Formation		Thicknes of Mycelium	
				No. 2001*		No. 2003					
		No. 2001	No. 2003	Color	Degree	Color	Degree	No. 2001	No. 2003	No. 2001	No. 2003
Potato Decoction Agar	5	—	—	—		—	—	—	—		
	10	—	##	Dark ivy green	###	Chaetura black	###	###	##	Medium	Thick
	15	—	##	Do.	###	Do.	###	###	##	Do.	Do.
	20	—	##	Do.	###	Do.	###	###	##	Do.	Do.
	24	+	##	Olivaceous black	###	Do.	###	###	##	Do.	Do.
	27	+	###	Do.	###	Do.	###	###	##	Do.	Do.
	30	+	###	Do.	###	Do.	###	###	##	Do.	Do.
	33	±	##	Do.	###	Do.	###	###	##	Do.	Do.
	36	##	###	Do.	###	Do.	###	##	##	Thick	Very thick
Malt Extract Agar	5	—	—	—	—	—	—	—	—		
	10	—	—	Dark ivy green	###	Chaetura black	##	###	#	Thin	Thin
	15	—	—	Do.	###	Do.	##	###	#	Do.	Do.
	20	—	—	Do.	###	Do.	##	###	#	Do.	Do.
	24	—	—	Do.	###	Do.	###	###	#	Do.	Do.
	27	—	—	Do.	###	Do.	###	###	#	Do.	Do.
	30	±	—	Do.	###	Do.	###	###	#	Do.	Do.
	33	±	±	Do.	###	Do.	###	###	#	Do.	Do.
	36	+	##	Light grayish olive	###	Hair brown	±	±	#	Do.	Do.
Rice Straw Decoction Agar	5	—	—	—		—	—	—	—		
	10	—	±	Dark ivy green	###	Dark ivy green	##	###	#	Rather thin	Rather thin
	15	—	±	Do.	###	Do.	##	###	#	Do.	Do.
	20	—	±	Do.	###	Do.	##	###	#	Do.	Do.
	24	—	±	Olivaceous black	###	Olivaceous black	###	###	##	Do.	Do.
	27	—	±	Do.	###	Do.	###	###	##	Do.	Do.
	30	—	±	Do.	###	Do.	###	###	##	Do.	Do.
	33	—	±	Do.	###	Do.	###	###	##	Do.	Do.
	36	##	##	Dark ivy green	###	Dark ivy green	##	##	+	Do.	Do.

* No. 2001 is *Alternaria macrospora* and No. 2003 is *A. gosypii*.

C. Effect of Reaction of Media to Mycelial Growth.

The malt agar medium with various hydrogen-ion concentrations was prepared

by adding varying quantities of 1/5 normal hydrochloric acid. The adjusted medium was poured in Petri dishes and *Alternaria macrospora* and *A. gossypii* were inoculated at the center and incubated at 24°C.

The results obtained are given in Table 9 and Figure 3. Both species began growth at pH 2 and reached the optimum at about pH 5; further rise in pH reduced the growth but even at pH 10 some growth was observed.

Table 9.

The Effect of the Reaction of the Culture Medium to the Growth of *Alternaria macrospora* and *A. gossypii*.

pH of Medium	Growth of *A. Macrospora* at		Growth of *A. gossypii* at	
	3 Days (Mm.)	7 Days (Mm.)	3 Days (Mm.)	7 Days (Mm.)
1.45	—	—	—	—
1.70	—	—	—	—
1.95	—	8.0	±	7.8
2.30	5.3	13.0	5.2	12.0
2.60	9.1	21.5	9.3	19.7
3.40	14.0	35.4	14.7	35.4
4.20	20.0	53.9	20.9	58.1
5.00	23.0	61.7	24.5	68.3
5.90	22.9	60.1	25.7	64.8
6.30	21.4	55.8	25.2	57.1
7.00	19.6	49.9	21.0	51.2
8.20	15.7	40.6	16.7	42.8
10.00	8.3	23.1	8.7	23.4

VIII. Fungicidal and Growth Inhibition Studies.

Table 10.

The Lethal Effect of High Temperatures on the Conidia of *Alternaria macrospora* and *A. gossypii*.

Fungus	Period of Treatment in Minutes	Temperatures in °C.							
		Control	44	46	48	50	52	54	56
Alternaria macrospora	5	+	+	+	+	+	+	—	—
	10	+	+	+	+	—	—	—	—
	15	+	+	+	—	—	—	—	—
	20	+	+	+	—	—	—	—	—
Alternaria gossypii	5	+	+	+	+	+	—	—	—
	10	+	+	+	+	—	—	—	—
	15	+	+	+	+	—	—	—	—
	20	+	+	+	—	—	—	—	—

A. Reaction of Conidia to Physical and Chemical Treatments.

The water suspensions of *Alternaria macrospora* and *A. gossypii* were exposed to the temperatures of 44, 46, 48, 50, 52, 54 and 56°C. for 5, 10, 15 and 20 minutes by dipping the conidia suspension into the adjusted water bath. Loop-full of treated spores were then smeared over the freshly poured rice straw decoction agar medium in Petri dishes and incubated for 7 days at 27°C. The results on the viability of conidia of two fungi are given in Table 10.

The results of Table 10 show that *Alternaria macrospora* and *A. gossypii* have similar reaction to hot water. That is, while the former died at 54° the latter died at 52°C. when the duration of exposure was 5 minutes. At 10 and 20 minutes exposures the death occurred respectively at 50 and 48°C. Concluding, the cotton *Alternaria* lose their viability easily when treated by hot water.

B. Effect of Fungicides on the Viability of Conidia.

Table 11.

The Effect of Fungicides on the Viability of Conidia of *Alternaria macrospora* and *A. gossypii*.

Fungicide	Concentration	Treatment in Hour							
		A. macrospora				*A. gossypii*			
		1	3	6	24	1	3	6	24
Mercuric Chloride	1/ 5,000	−	−	−	−	−	−	−	−
	1/ 10,000	−	−	−	−	−	−	−	−
	1/ 30,000	+	−	−	−	+	−	−	−
	1/120,000	+	+	+	−	+	+	−	−
	1/240,000	+	+	+	+	+	+	+	+
	Control (Water)	+	+	+	+	+	+	+	+
Formalin	1/ 80	−	−	−	−	−	−	−	−
	1/ 160	−	−	−	−	−	−	−	−
	1/ 320	+	+	−	−	+	+	−	−
	1/ 640	+	+	+	−	+	+	+	−
	1/1,200	+	+	+	+	+	+	+	+
	Control (Water)	+	+	+	+	+	+	+	+
Uspulun	1/ 2,000	−	−	−	−	−	−	−	−
	1/ 5,000	−	−	−	−	−	−	−	−
	1/10,000	−	−	−	−	−	−	−	−
	1/20,000	+	−	−	−	+	+	−	−
	1/40,000	+	+	±	−	+	+	+	−
	Control (Water)	+	+	+	+	+	+	+	+
Copper Sulphate	1/10	+	+	+	−	+	+	+	−
	1/20	+	+	+	−	+	+	+	−
	Control (Water)	+	+	+	+	+	+	+	+

To observe the fungicidal effects various fungicides, conidia of the two fungi were exposed to various concentrations of fungicides for different length of time. The method of experiment consisted in preparing small glass plates with fixed conidia on one surface by smearing and drying. These plates were immersed into the various concentrations of fungicides for required period of time; and after treatment, the glass plates were carefully washed or rinsed in sterile distilled water to remove the adhering solution of fungicide. The treated conidia were placed over the surface of the freshly poured malt extract agar medium by inverting the glass plates over the medium. The results of Table 11 were noted at the end of 7 days of inculation at 30°C. for the appearance of the mycelium.

According to the table, the conidia of the two fungi were not viable after the following treatments:

1. 3 hours in 1:30,000 or 24 hours at 1:120,000 solutions of mercuric chloride.
2. 1 hour at 1:160 or 6 hours at 1:320 solutions of formalin.
3. 1 hour at 1:10,000 or 3 hours at 1:20,000 solutions of Uspulun.
4. 1 hour at 1:10 solution of copper suphate was still not effective enough to inhibit the germination of the conidia.

C. Inhibition of Mycelial Growth by Fungicides.

In the present experiment varying quantities of fungicides were added to the culture medium and the growth of the fungous mycelium was noted. The culture was made on the 1% malt extract agar medium by incubating at 24°C.

Table 12.

The Inhibitory Effect of Fungicides on the Growth of *Alternaria macrospora* and *A. gossypii*.

Fungicides	Fungus	Concentration								
		Control	10^{-4}	5×10^{-4}	10^{-5}	5×10^{-5}	10^{-6}	5×10^{-6}	10^{-7}	5×10^{-7}
Mercuric Chloride	No. 2001	+	−	−	−	+	+	+	+	+
	No. 2003	+	−	−	±	+	+	+	+	+
Uspulun	No. 2001	+	−	−	−	−	±	+	+	+
	No. 2003	+	−	−	−	−	−	+	+	+

Fungicides	Fungus	Concentration								
		Control	10^{-3}	5×10^{-3}	10^{-4}	5×10^{-4}	10^{-5}	5×10^{-5}	10^{-6}	5×10^{-6}
Copper Sulphate	No. 2001	+	−	−	−	±	+	+	+	+
	No. 2003	+	−	−	−	±	+	+	+	+
Iron Sulphate	No. 2001	+	−	−	−	±	+	+	+	+
	No. 2003	+	−	−	±	+	+	+	+	+

No. 2001 is *Alternaria macrospora* and No. 2003 is *A. gossypii*.

for 7 days. The results obtained by mercuric chloride, Uspulun, copper sulphate, and iron sulphate are tabulated in Table 12. The results of mercuric chloride and Uspulun show that the lethal and growth inhibiting concentrations are almost the same ; while in copper sulphate and iron sulphate a very low concentration was sufficient in inhibiting the mycelial development.

IX. Control Measures.

From the results of this study, following control practices seem to be effective in controlling the cotton diseases caused by *Alternaria*.

1. To cultivate cotton in regions that are free from excessive rain during the period following the opening of the boll.

2. In areas that cannot escape rain during this period, Asiatic cotton *Gossypium herbaceum* L. should be grown as its bolls develop facing down.

3. On account of copper sulphate being remarkable in inhibiting the mycelial growth of the fungi at a very low concentration, spraying fungicides containing copper sulphate such as the Bordeaux mixture are highly promising.

X. Summary.

1, The present paper deals with two species of *Alternaria* injurious to cotton fibers in Western Japan during the latter season when the bolls are near ripe.

2. According to the various characters of the two fungi, they were classified as *Alternaria macrospora* Zimm. and *A. gossypii* (Jacz.) n. comb. The former attacks the cotton leaves, bolls, and fibers, while the latter attacks mainly the cotton fibers.

3. Inoculation of the two fungi on : 1, cotton fibers taken from bolls at various degrees of maturity ; 2, commercial cotton fibers ; and 3, absorbent cotton proved the positive discoloration or blackening of the fibers in all cases excepting the absorbent cotton.

4. The relations of temperature and hydrogen-ion concentration to the growth were compared in both species. The minimum, optimum and maximum temperatures for the mycelial growth were same for both species ; they were respectively, about 5°, 27 – 30°, and 36°C. ; and the pH limits for the both groups were 2 and above 10 with the optimum of about 5. Conidia of *A. macrospora* and *A. gossypii* were killed at the respective treatments of 5 minutes at 54° and 52°, and of 10 minutes at 50°C. for both.

5. By the treatments of 3 hours, following solutions proved lethal to the conidia of both species. Mercuric chloride, 1 : 30,000 ; formalin, 1 : 160 ; Uspulun, 1 : 20,000. Copper sulphate did not kill the spores at even high concentration but inhibited the mycelial growth at 1 : 100,000.

6. Asiatic cotton should be grown in localities that cannot escape the rain during the development of bolls, and Bordeaux mixture should be applied when the disease begins to develop.

PLATE XVII.

Fig. 1.

Fig. 3.

Fig. 2

PLATE XVIII.

Fig. 4.

Fig. 5.

Fig. 6.

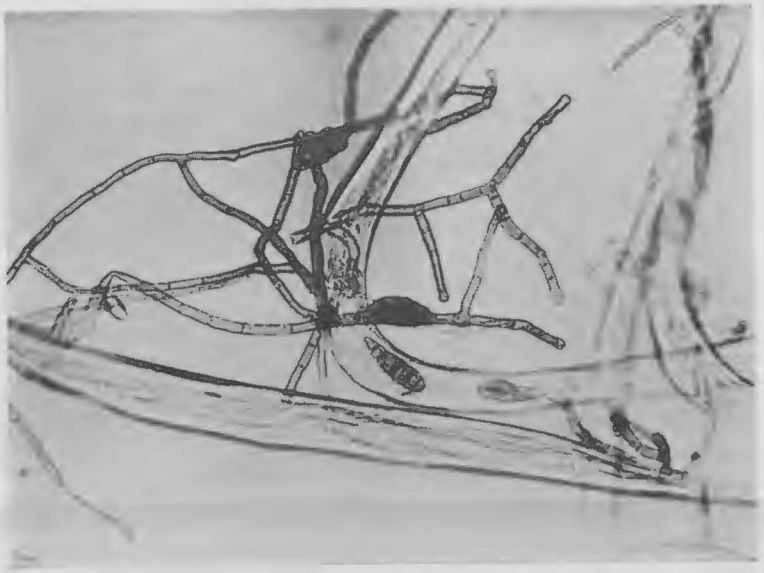
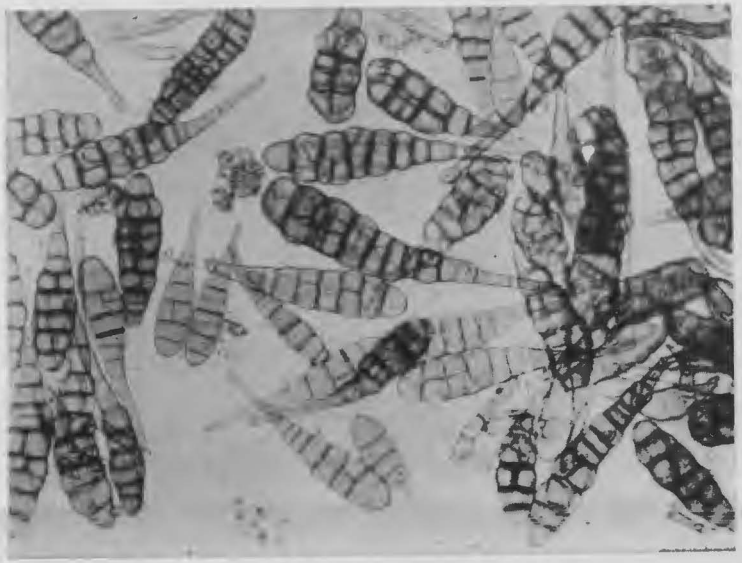


Fig 7



XI. Literature Cited.

Brighi, A. (1937): Una mummificazione del cotone causata da *Alternaria*. Boll. Stz. Pat. Veg. Roma N. S., 17 (4): 475 – 496. (R.A.M. 17: 674).

Hara, K. (1927): Toa-Kinrui-Si. Sizuoka-Ken Nôkai Hô. No. 364.

_____ (1930): Zikken-Sakumotu-Byôrigaku. pp. 445, 454.

Hopkins, J. C. F. (1931): *Alternaria gossypina* (Thüm.) comb. nov., causing a leaf spot and boll rot of cotton. Trans. Brit. Mycol. Soc. 16: 136 – 144. (R.A.M. 11: 371).

Jaczewski, A. A, (1929): Some diseases of cotton fibres. Microbiol. Journ. 9: 159 – 167. (R.A.M. 9: 307).

Jones, G. H. (1928): An Alternaria disease of the cotton plant. Ann. Bot. 42: 168: 935 – 947. (R.A.M. 8: 171).

Nakata, K. (1934): Sakumotu-Byôgai-Zuhen. pp. 249 – 250.

_____ and S. Takimoto (1928): Tyôsen Sakumotu-Byogai-Mokuroku.

Nisikado, Y., K. Kimura and Y. Miyawaki (1940): Menzwo wo singaisuru 2 Syu no *Alternaria* Kin ni tuite. Ann. Phytopath. Soc. Japan. 10: 214 – 230.

Nisikado, Y., K. Kimura and Y. Miyawaki (1941): Menzwo wo singaisuru *Alternaria* Zoku Kin ni tuite. Nôgaku-kenkyû. 32: 469 – 494.

Takimoto, S. (1924): Wata no Byôgai. Byôtyu-Gai Zassi. 11: 559 – 564, 616 – 619.

Zaprometoff, N. G. (1928): Materials for the mycoflora of Central Asia, II. Usbekistan Exp. Plant Prot. Sta. Tashkent. Publ. 11: pp. 70 (R.A.M. 8: 338).

Zimmermann, A. (1904): Untersuchungen über tropische Pflanzenkrankheiten. Ber. Land. u. Forstwirt. Deut. Ostafrika. 2: 11 – 36.

Explanation of Plates.

Plate XVII.

Fig. 1. Leaf spots of cotton caused by *Alternaria macrospora* Zimm.

Fig. 2. Cotton bolls discolored by *Alternaria gossypii* (Jacz.). The boll at the right is unaffected.

Fig. 3. The fibers removed from the bolls showing the discoloration caused by *Alternaria gossypii* (Jacz.). The fibers at the extreme right are unaffected.

Plate XVIII.

Fig. 4. Results of inoculation of *Alternaria gossypii* (Jacz.) to various kinds of cotton fibers. (1). On absorbent cotton showing no discoloration; (2), on commercial cotton fibers with discoloration; (3), on fibers from slightly ripened boll showing greater discoloration than of (2); and (4), on inmature fibers removed from a boll showing the most severe discoloration.

Fig. 5. Conidia and conidiophores of *Alternaria gossypii* (Jacz.) formed on cotton fibers. (x 400).

Fig. 6. Conisia and conidiophores of *Alternaria macrospora* Zimm. formed on cotton fibers. (x 300).

Fig. 7. Conidia of *Alternaria macrospora* Zimm. formed on a diseased cotton leaf. (x 600).