STUDIES ON THE ANTHRACNOSE OF CHINA FIR*

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Introduction

The prevalence of anthracnose of China fir occuring in Pu-Li was found by one of the authors in 1963 (Chen 1966). This disease spreaded very quickly with large area. The presence of the disease is certainly destructive to their host plant which hold to be of sufficient economic importance to the forest products.

Isolation of pathogens from China fir showed that *Gloeosporium* and *Pestalotia* spp. were frequently isolated (Chen and Hsu, 1966). After inoculation to healthy China fir showed that *Pestalotia* sp. was little pathogenic. The genus name Gloeosporium were changed to *Colletotrichum* since the setae were found. Up to the present time there is no record regarding anthracnose of China fir caused by *Colletotrichum* sp. in Taiwan (Sawada).

By the method of single spore isolation, the authors has isolated eight groups of *Colletotrichum* which have distinguished culture types. These isolates were isolated during the spring of 1965 to the autumn of 1966. Preliminary studies on the fungus are reported in the present paper.

Material and Methods

Eight isolates of *Colletotrichum* sp. were used at this investigation. They were first isolated from China fir leaves by means of tissue plating method (Rawlins 1933). Monospore isolates were obtained by employing the dilution plate method with spore ooze. All the cultures were maintained on PDA at room temperature. These eight isolates can be differentiated by growth rates and other characters. Thus they were designed as; A1, A21, B11, C11, D1, E1, F11, G1.

Potato dextrose agar (PDA) was used as a routine medium for general purposes. Carrot agar, onion agar, bean agar, Czapek's agar, oat-meal agar,

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Lilly-Barnett's agar, malt extract-yeast extract agar, China fir juice agar, yeast extract solution, China fir juice were also applied for particular study (Riker and Riker 1936; Lilly and Barnett 1951).

Czapek's sucrose nitrate agar was used as a basal medium in the nutritional studies and the malt extract-yeast extract medium as a control medium. The media used in studying the effects of different nitrogenous compounds were prepared in similar manner. In the study of the effect of hydrogen ion concentration on the mycelial growth the basal medium was adjusted to different pH values by adding 6N sulphuric acid or 6N sodium hydroxide (Lilly and Barnett 1951). The inocula were mycelial blocks of 2 mm. diameter cut off from the margin of fresh colony of PDA.

Four media were used for studying the germination of conidia. They are potato dextrose solution, distilled water, China fir leaf extract and yeast extract solution. The China fir leaf extract were leaves of China fir grounded with distilled water in mortar and the filtrate was used.

Results and Disccussion

Cultural characters and morphological features

By growing the monospore isolate and on PDA, Czapek's agar and China fir decoction agar under room temperature, the authors got eight distinguished culture types of *Colletotrichum* sp., The characters of the eight isolates of *Colletotrichum* sp. on PDA were as follow;

Isolate A1: colony light gray, aerial mycelium densely felty, raised, deep green at central part of colony; acervulus sparse, spore ooze sparse, average width of hyphae is 3.74 μ; submerged hyphae deep green, oil drop present, average width of hyphae 9.35 μ.

Isolate A21: colony gray, aerial mycelium densely felty, raised; average width of hyphae 3.8 μ; spore ooze minute, scattered; submerged hyphae light gray, average width 5.78 μ.

Isolate B11: colony dark green, flat, margin gray at young and yellow at old age; colony scattered with acervulus, spore ooze scattered; aerial mycelium dark gray, growth sparse, oil drop present; submerged hyphae dark green at young, black at old, oil drop present, average width 6.42 μ; setae dense.

Isolate C11: colony light brown; acervulus dense, scattered; spore ooze minute, scattered; aerial mycelium dark gray, dansely felty, raised, average width 2.47 μ; submerged hyphae, sac shape, average width of hyphae 8.5 μ, sac 17.8 to 36.8 μ.

Isolate D1: colony white gray, acervulus scattered, spore ooze scattered; aerial mycelium white gray, densely felty, raised; oil drop presented, average

width 2.6 μ; submerged hyphae light yellow, oil drop present, sac shape at the interval of hyphae, average width of hyphae 6.9 μ, sac 30.6 μ to 23.8 μ; ascospore and ascus formed.

Isolate E1: colony light gray; acervulus scattered at central part, large spore ooze scattered at central part; aerial mycelium grey, dense felty at central part, raised, oil drop present, average width 3.37 μ; submerged hyphae few, dark, oil drop present, average width 5.9 μ.

Isolate F11: colony light brown, acervulus absent, spore ooze absent; aerial hyphae dark gray, sparse, flat, average width 2.5 μ; submerged hyphae, dark, some of them black boxing-glove shape and sac shape, sac 22.6 to 29.7 μ; setae absent.

Isolate G1; colony light yellow, acervulus absent. spore ooze yellow; aerial mycelium white, sparce, flat, average width 4.0 μ; submerged hyphae sparse, average width 6.6 μ.

Spore size measuring showed that there were variation among these isolates. A comparison of the variation of spore size of eight isolates is shown in Table 1.

Table 1. *Showing the spore size of eight isolates grown of PDA at room temperature for 20 days*

Isolate	Length and width of spore (μ)	Average length (μ)	Average width (μ)
A1	8.3-24.3×4.8-7.2	15.8	5.6
A21	13.0-21.4×3.5-5.9	17.3	4.8
B11	15.4-29.7×4.7-9.5	19.2	7.1
C11	10.7-16.6×4.7-7.1	15.1	6.2
D1	13.0-24.9×4.7-6.6	17.9	5.7
E1	15.4-28.5×4.2-5.9	19.3	5.6
F11	10.7-19.0×3.6-5.9	14.8	4.6
G1	11.4-26.2×4.8-10.7	18.1	6.6

The formation of acervulus, conidia, setae and asci on PDA at room temperature for 20 days is shown in Table 2. Except the A1 isolate, the others were found to be abundant in sporuration. Asci were found only in isolate D1. Setae were absent in F11, while long and abundant setae were found in B11.

The average length and width of setae of the isolate on PDA at room temperature for 20 days is shown in Table 3. B11 has longest setae, whereas A1 has the shortest setae. Setae are absent at F11.

Fffect of temperature on vegetative growth

Cultures of eight isolates in potato dextrose solution were incubated at

Table 2. *Showing the formation of acervulus, conidia, setae, and asci of siolates grown on PDA at room temperature for 20 days*

Isolate	Acervulus	Conidia	Ascus	Setae
A1	±	+	−	+
A21	+++	++	−	+
B11	+	++	−	+++
C11	+++	++	−	+
D1	++	++	+	+
E1	++	+++	−	+
F11	−	++	−	−
G1	−	+++	−	+

Table 3. *Showing the average length and width of the setae of isolates on PDA at room temperature for 20 days*

Isolate	Average length of setae (μ)	Average width of setae (μ)
A1	45.2	4.4
A21	49.5	3.8
B11	195.6	3.1
C11	81.3	4.2
D1	97.5	4.8
E1	129.4	3.6
F11	—	—
G1	69.0	3.0

Table 4. *Showing the dry weight of isolates grown in potato dextrose solution at different temperature for 10 days.* (*gm.*)

Temp. (°C)	A1	A21	B11	C11	D1	E1	F11	G1
13	84.3	347.7	196.0	57.2	178.5	165.0	242.7	239.1
16	258.0	356.0	212.2	78.0	211.0	238.0	333.4	306.2
19	301.7	412.2	275.2	97.2	319.0	299.1	339.5	340.5
22	302.5	425.4	397.3	195.6	423.9	373.2	333.9	346.2
25	398.2	434.2	404.1	195.0	397.0	382.3	327.3	326.2
28	288.9	437.1	413.5	187.0	350.3	340.1	333.2	316.7
31	118.4	226.8	262.5	146.0	174.7	221.2	313.2	180.4
34	—	—	—	42.9	—	—	29.7	—
37	—	—	—	36.0	—	—	22.5	—

the following temperatures: 13°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, and 37°C, for 10 days. They were measured by dry weight of mycelium grown in liquid medium. The optimum temperature for mycelial growth were varied, most of them were around at 25° to 28°C. The growth of the fungi were rather abruptly declined toward the temperature above the optimum or thereabout. The data show in Table 4 show the dry weight of the fungi in potato dextrose solution different temperatures.

Effect of different media on vegetative growth

Six media were chosen for this purpose (Riker and Riker 1936; Lilly and Barnett 1951). Czapek's agar and oat meal media gave good growth of several isolates. Yet they varied one another. The linear growth of the isolates in different media at room temperature for 5 days are shown in Table 5.

Table 5. *Showing the linear growth of eight isolates grown on different media at room temperature for 5 days. (mm.)*

Medium	A1	A21	B11	C11	D1	E1	F11	G1
Carrot	4	31	10	72	17	13	50	16
Onion	5	24	11	71	18	17	45	3
Bean	22	30	12	80	20	15	43	17
Czapek's	5	34	21	69	11	23	55	18
Oat-meal	13	30	11	85	25	26	56	21
PDA	28	22	18	75	20	22	60	16

Effect of the carbon source on vegetative growth

This experiment was designed to show the characters differ in their ability to utilize certain carbon source: Seven carbon compounds including mono- and disaccharides, were used in this experiment. Basal medium was Czpek's sucrose nitrate agar in which the carbon source—sucrose was substituted by other carbohydrates. These carbohydrates were sucrose, galactose, maltose, mannose, D-(−)-fructose, glucose, D-(−)-mannit. The control media was malt extract-yeast extract agar (Lilly and Barnett 1951). The pH of all media was adjusted to approximately 6.0 befor autoclaving. They were incubated at 25°C for 5 days and dried in an air oven at 80°C for one day. The results were based on the dry weight of mycelia. As shown in table 6, different isolates responded differently to various carbon sources for their mycelial growth. Yet it seems that maltose is the most available to most of them.

Effect of nitrogen sources on mycelial growth

Five nitrogenous compounds were used for comparing the ability of nitrogen source to support growth. The media were prepared in similiar manner to the carbon test. In this case the carbon source was kept constant

Table 6. *Showing dry weight of mycelial mats grown on different carbon sources at 25°C for 5 days. (mg.)*

Carbon sources	A1	A21	B11	C11	D1	E1	F11	G1
Sucrose	41.0	17.5	21.0	27.0	16.8	12.4	13.1	14.5
Galactose	20.2	17.5	17.6	9.1	14.0	8.5	9.5	26.2
Maltose	70.0	46.3	25.2	20.7	22.1	18.9	14.2	40.6
Mannose	37.9	23.3	38.8	15.5	6.2	9.7	10.1	5.7
D-(−)-Fructose	14.0	51.5	18.9	8.6	12.6	3.1	9.6	1.6
Dextroe	1.5	0.1	2.3	6.1	10.3	0.9	1.5	8.7
D-(−)-Mannit	8.3	4.7	6.2	10.3	0.8	5.7	5.8	18.8
Malt extract-yeast extract	52.2	66.0	30.5	29.1	51.7	42.7	120.4	74.0

and the nitrogen source varied with each medium. Malte extract-yeast extract medium was used again as a control. The pH of all media was ajusted to approximatly 6.0 before autoclaving. Then they were incubated at 25°C for 7 days. As shown in Table 7, L-asparagine was better then any other nitrogenous compounds for growth. But ammonium tartrate gave very good growth to isolate B11 too.

Table 7. *Showing the dry weight of mycelial mats grown in the media containing different source of nitrogen. (mg.)*

Nitrogen source	A1	A21	B11	C11	D1	E1	F11	G1
Ammonium sulfate	35.2	50.4	51.8	42.0	39.3	46.6	58.8	48.8
L-Asparagine	71.8	135.1	132.8	76.4	53.2	59.1	126.0	38.6
Urea	33.6	37.7	127.3	32.7	33.1	28.0	180.2	27.2
No nitogen	17.0	3.2	13.1	13.0	12.4	12.0	10.5	12.4
Ammonium tartrate	31.7	62.3	333.7	42.2	39.3	39.9	73.8	35.5
Ammonium phcsphate, dibasic	38.4	63.7	113.3	40.9	38.4	60.9	100.9	36.2
Malte extract-yeast extract	71.9	182.6	112.5	34.0	127.4	64.4	268.4	57.4

Effect of hydrogen ion concentration on vegetative growth

The Czapek's dextrose nitrate solution was adjust to pH value within the range of 3.2, 4, 5, 6, 7, 8, 9, and distributed to 125 ml. Erlenmeyer flasks, 40 ml. for each. Then, they were inoculated and incubated at 25°C for 8 days. Dry weight of the mycelium was measured. As shown in Table 8, the optimum pH range for mycelial growth of isolates A21, E1, G1 was 6.0, 7.0 for isolates A1, B11, D1, F11. and 8.0 for C11.

Germination of conidia

Four media were used for studying the germination of conidia. The

Table 8. *Effect of hydrogen ion concentration on the vegetative growth.* (*mg.*)

Isolate	3.2	4	5	6	7	8	9
A1	50.5	60.2	79.6	130.9	192.6	99.4	61.2
A21	120.0	165.3	178.0	190.2	186.0	181.7	171.1
B11	109.4	110.3	116.2	124.3	153.8	95.1	86.3
C11	14.8	20.7	24.4	67.1	97.0	102.1	99.9
D1	120.3	133.5	125.2	144.9	155.5	149.0	138.8
E1	163.1	177.9	187.2	207.2	164.3	161.7	160.9
F11	107.5	147.8	154.0	166.9	167.4	145.6	137.6
G1	61.8	80.7	90.5	104.3	90.8	88.9	80.0

conidia of eight isolates were used in this experiment. Filter paper was placed on the bottoms of petri dishes, added water to moist the paper and autoclaved. Conidia collected from 10-day-old cultures on PDA were used to make up a spore suspension with potato dextrose solution, distilled water, China fir juice, and yeast extract solution. A few drops of this suspension were placed on the slide laid on the paper, which kept moist with sterile water. Then they were incubated at room temperature for 9 hours. The spore suspension on slide were treated with Iodinepotassium iodide. The percentage of germination were counted under a microscope. On China fir juice medium, the highest percentage of germination was obtained. Potato dextrose solution came to the next. Isolate A1 and F11 germinated well on yeast extract solution whereas isolates C11 and F11 germinated well on distilled water. The details are shown in Table 9.

Table 9. *Showing the percentage of conidia germination on four media under room temperature for 9 hrs*

Medium	A1	A21	B11	C11	D1	E1	F11	G1
PDA-A	93.0	86.3	29.0	80.8	74.7	59.1	83.2	76.6
Distilled water	2.7	7.4	7.4	76.6	23.4	2.3	92.1	4.8
China fir juice	99.7	100.0	99.0	100.0	95.4	99.3	100.0	94.5
Yeast extract solution	95.5	20.2	9.1	1.7	51.7	80.0	93.9	62.8

Besides the variation of percentage of germination, there were different germination types among eight isolates. Except the isolates C11, the other seven isolates of single-cell-conidia became two cell when germination occured. Appressorium was formed in isolate C11 on different media, but other isolates were not. The germination types in potato dextrose solution, China fir juice, yeast extract are shown in Fig. 2. Yet the germination types in distilled

Table 10. *Showing the percentage of germination at different temperatures on China fir juice media for 9 hrs.*

Isolate	13°C	16°C	19°C	22C°	25°C	28°C	31°C	34°C
A1	0.3	1.3	77.0	78.8	94.3	99.5	96.5	72.3
A21	0.6	1.3	50.5	97.5	99.6	97.5	95.6	78.9
B11	0	3.8	15.2	80.3	80.6	98.6	33.3	0
C11	0	2.2	29.9	77.4	96.3	99.3	96.9	96.7
D1	0	8.5	81.0	98.2	98.5	92.4	90.0	15.3
E1	0	0	74.4	99.3	98.9	99.4	91.8	87.0
F11	0.3	0.6	25.3	75.2	98.8	52.1	44.6	40.0
G1	0	7.1	80.7	85.8	90.0	96.1	79.0	25.8

Table 11. *Showing the percentage of infection by wound inoculation at room temperature in autumn 1966*

Isolate	2 days	4 days	6 days	8 days
A1	0	13.6	27.2	39.6
A21	0.6	15.5	33.7	38 5
B11	2.1	8.0	28.8	43.1
C11	0	3.6	15.9	25.3
D1	7.4	44.1	92.2	92.2
E1	10.6	48.5	55.9	58.8
F11	1.5	7,7	9.9	21.4
G1	18.0	42.6	54.6	88.0
CK	0	0	0	0

Table 12. *Showing the percentage of infection by wound inoculation at room temperature in spring 1967*

Isolate	2 days	4 days	6 days	8 days
A1	6.6	34.8	48.1	51.8
A21	15.1	55.3	67.4	75.4
B11	7.6	39.3	57.4	59.8
C11	1.9	21.5	22.4	22.4
D1	12.3	59.9	70.4	95.5
E1	38.4	77.6	90.9	100.0
F11	0	14.5	41.6	54.7
G1	40.2	77.3	87.4	96,8
CK	0	0	0	0

Table 13. *Showing the percentage of infection by wound inoculation at various temperatures*

Temperature (°C)	Isolate	2 days	4 days	6 days	8 days
	A1	0	0.7	8.6	30.4
	A21	0	5.4	41.2	53.2
	B11	0	0.5	10,5	17.4
	C11	0	2.0	5.6	9.3
15	D1	0	5.2	52.9	71.6
	E1	0	1.4	58.4	77.4
	F11	0	1.0	1.5	3.2
	G1	0	1.6	34.8	57.8
	CK	0	0	0	0
	A1	0	0.8	3.2	4.8
	A21	0.9	15.5	20.3	26.2
	B11	1.0	6.3	11.5	17.8
	C11	3.3	4.2	5.1	17.6
20	D1	7.1	58.9	60.7	60.7
	E1	18.4	59.2	64.0	69.9
	F11	0	8.6	10.3	27.5
	G1	9.1	25.8	33.3	33.3
	CK	0	0	0	0
	A1	2.5	9.4	14.5	17.5
	A21	45.4	75.4	100.0	100.0
	B11	3.0	30.6	41.8	41.8
	C11	11.1	18.5	33.3	38.2
25	D1	30.3	45.4	50.5	63.6
	E1	27.7	53.3	60.0	92.2
	F11	5.5	16.5	25.5	37.7
	G1	43.9	65.1	69.6	69.6
	CK	0	0	0	0
	A1	2.1	7.6	10.8	13.0
	A21	29.3	47.8	56.5	65.2
	B11	4.3	14.2	21.9	25.2
	C11	3.1	5.2	20.8	20.8
30	D1	38.5	51.5	59.1	60.0
	E1	37.8	52.7	64.8	64.8
	F11	14.5	46.6	47.5	54.3
	G1	59.0	68.0	68.0	69.6
	CK	0	0	0	0

water were not the same as that in above media. All the isolates on distilled water revealed the same germination type as that of C11 forming appressorium.

The effects of temperature on conidia germination were determined by using China fir juice media under the range of temperature: 13°, 16°, 19°, 22°, 25°, 28°, 31°, 34°, C. for 9 hrs. The result showed that there were different range of temperature for abundant germination i. e. 22° to 28°C for eight isolate, excepting that 28°C was unsuitable for F11. The details shows in Table 10.

Plant inoculation

Spore suspension was made by spore ooze obtained from 10-day-old PDA culture. China fir was wounded by needle. Then the host plant were inoculated by spore suspension by an automizar and covered by plastic bag for three days. The results showed that the symptom was the same as that in natural condition. All eight isolates were pathogenic, especially D1, E1 and G1 attained to 95 percent infection. Yet the percentage of infection varied from season to season. As we can see from Table 11 and Table 12. The percentage of infection in spring 1967 was higher than that in autumn 1966. It may be due to the susceptibility of young growing leaves. The results obtained from plant inoculation showed that eight isolates hardly infect on China fir when the plants were unwound, i. e. below 5%.

The relation between infection and temperature, was also investigated. The results showed that the highest percentage of infection occurred at 25°C. The establishment of infection at 15°C was lower than that at above 15°C. The details are shown in Table 13.

Sumarry

The authors regarded the anthracnose of China fir was caused by *Colletotrichum* sp.. Eight isolates of *Colletotrichum* sp. were obtained from the anthracnose of China fir. The general physiological characters of eight isolates were studied. They were different in growth rate and other characters. The spore size, length of setae, formation of ascus, density of spore ooze, density of acervulus varied among eight isolates. The optimum temperature for mycelial growth varied, most of them were around at 25°C to 28°C. Nutrition studies showed that they responded differently to various media for their mycelial growth. Czapek's agar and oat meal media gave good growth of those isolates except A1 isolate. Maltose and L-asparagine were most available to most of them. The optimum pH range for mycelial growth of isolates A21, E1, G1, was 6.0, 7.0 for isolates A1, B11, D1, F11 and 8.0 for C11. China fir juice media revealed the highest percentage of conidia germination. There were different conidia germination type among eight isolates. Except the

isolate C11, the other seven isolates of single-cellconidia became two cell when germination occurred. Temperatures of 22° to 28°C was good range for germination. All eight isolates were found to be pathogenic, especially D1, E1 and G1 attained to 95% of infection. Yet the percentages of infection varied from season to season. Plant inoculation showed that eight isolates hardly infected China Fir without wounding of the host plants.

福 州 杉 炭 疽 病 之 研 究

陳 其 昌　徐 漢 勇

南投埔里林區之福州杉病害，著者認爲其爲由 *Colletotrichum* sp. 所引起之炭疽病(anthracnose)。據初步之研究，由該病害植物上能分離出8種不同特性之 *Colletotrichum* sp. 羣 (isolates)。本報告爲此8種不同之 isolates 之普通生理實驗。其生長速度，分生胞子大小，剛毛 (setae) 長度，子囊胞子囊 (ascus) 之形成，胞子角 (spore ooze) 之密度，胞子褥 (acervulus) 之密度等皆不同。其最適生長速度在25°C至28°C 之間，按各 isolates 而異。營養方面之實驗，知各 isolates 有其較適宜之培養劑，除了 isolates A1 外，其他7個 isolates 皆能在 czapek's agar 及 eat meal media 上生長良好。碳素及氮素營養方面，maltose 及 L-Asparagine 皆適合於大部份 isolates 之生長。pH 之實驗，A21，E1，G1，最適於 pH 6.0；A1，B11，D1，F11 則最適於 pH 7.0；而 G_{11} 則最適於 pH 8.0。福州杉原汁 (china fir juice) 對於分生胞子之發芽最適宜，可達很高之發芽百分率。除了發芽率之不同外，8個 isolates 之發芽情形 (germination type) 亦有不同。除了 C_{11} isolate 外，其他7個 isolate 在胞子發芽時能立刻由單胞胞子變爲雙胞胞子。22°C至28°C 最適宜分生胞子之發芽。所有8個 isolates 皆有病原性，尤其D1，E1，G_{11} 之發病率可達95%之多。但發病率按季節而異。寄主未刺傷 (unwound) 之接種實驗很難有感染之現象。

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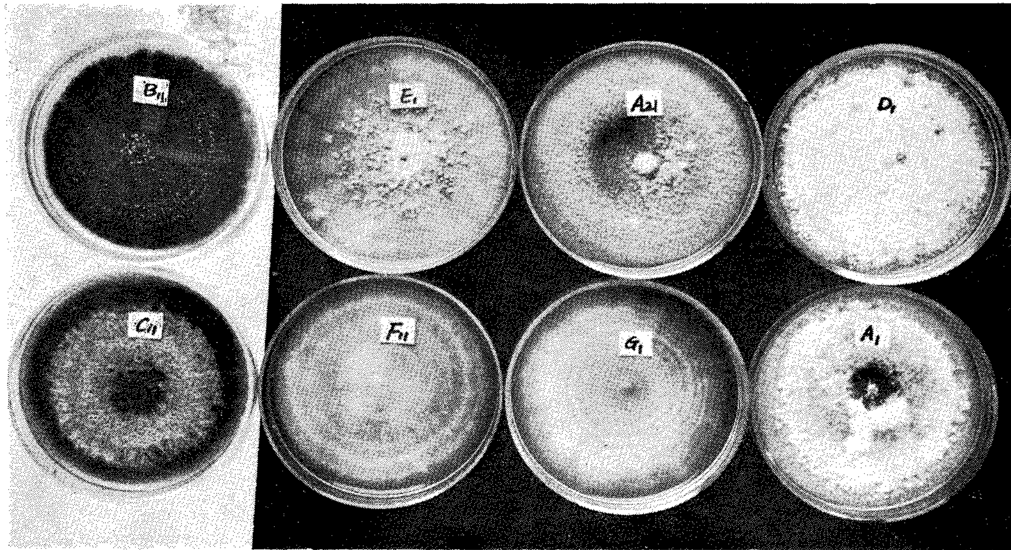

Fig. 1. Showing the growth features of eight isolates of *Colletotrichum* sp. grown on PDA under room temperature for 10 days.

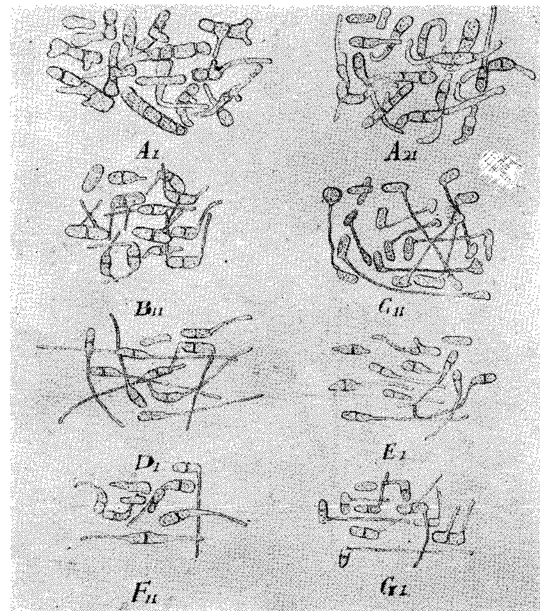

Fig. 2. Showing the germination types of eight isolates on media potato dextrose solution, China fir juice, and yeast extract.

Fig. 3. Showing the symptoms of China fir inoculated by wounding and incubated at room temperature for 2 weeks.

Fig. 4. Showing the symptoms of China fir inoculated by wounding and incubated at 15°C for 2 weeks.