STUDIES ON THE GROWTH AND PERITHECIA FORMATION IN THE GENUS NECTRIA*

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Introduction

Fungi in the genus Nectria induce canker of trees. They have been known to be the causes of cankers of apple, pear, maple, birch, black walnut, oak, aspen, coffee etc. (Welch, 1934; Spaulding et al., 1936; Baxter, 1952). In Taiwan, several species of fungi in the genus Necteria were also described (Sawada, 1928, 1931, 1959; Chen. 1965, 1966), however, none of them have been studied extensively.

The presence of the disease is certainly destructive to their host plants which hold to be of sufficient economic importance to the forest products. The potential infectivity of cankered wood in the vicinity of other trees was clearly demostrated by the large numbers of ascospores caught on vaselined slides which were placed closely to the canker (Munson, 1939). A better understanding of the physiology of the growth and perithecia formation of the fungi are, therefore, necessary.

Materials and Methods

Seven specimens were collected from Taipei, Kan-kou, Chia-i, Lugrey, San-pin, and Heng-chun. Isolation of the fungi were made from a mass of perithecia by an ordinary means (Riker and Riker, 1936). Three days after incubation at 28°C, scattered colonies appeared on potato dextrose agar (PDA) and they were transferred and kept as stock cultures.

Among the seven isolates, #1 and #4; #2, #3, and #7 showed similar cultural characters. Thus, four isolates with distinct characters were chosen for the present investigation. They were the isolates #1 (*Necteria pterospermi* Saw.), #3 (*N. pterospermi* Saw. 75), #5 (*N. swieteinae-mahoganii* Chen) and #6 (*N. durantae* Saw.) They are summarized as follows:

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Isolates	Host	Locality	Collector	Date of collection
#1	Maple-leaved pterospermum	Chia-i	C. C. Chen	Aug. 6' 65
#2	Maple-leaved pterospermum	Sanpin	ditto	Aug. 21' 65
#3	Maple-leaved pterospermum	Lugrey	ditto	Aug. 21' 65
#4	Maple-leaved pterospermum	Taipei	P. H. Yu	Aug. 30' 65
#5	Mahogany	Heng-Chun	C. C. Chen	Aug. 20' 65
#6	Creeping sky flower	Kan-kou	C. C. Chne & P. H. Yu	Aug. 27' 65
#7	Apolo tree	Lugrey	C. C. Chen	Aug. 21' 65

Unless otherwise specified, stock cultures were maintained on PDA slants and mycelial mass and spores were used as an inoculum for solid and liquid cultures, respectively. All the experiments were repeated at least twice with triplicate. For the perithecia formation, all the cultures were incubated at 26°C, under the illumination of 40 foot-candles fluorescent lamp from a distance near 30 cm.

Results

Factors affecting the vegetative growth of Nectria

Medium—Eleven kinds of media were chosen to compare their cultural

Table 1. The diameter of the colonies of Nectria grown on different media at 26°C for 6 days*.

Medium	Diameter of colonies (mm.) and color of the agar			
	#1	#3	#5	#6
PDA	63.1 creamy yellow	44.5 light red	37.0 yellow	80.3 light orange
Corn meal	69.0** creamy yellow	43.0** light orange	44.5** yellow	70.0** creamy yellow
Bean	63.0 creamy yellow	33.0 light orange	42.5 yellow	67.5 creamy yellow
Lima-bean	65.5 light red	35.0 light orange	38.0 yellow	68.0 light yellow
Onion	61.3 light violet	30.5 light orange	35.3 yellow	70.0 creamy yellow
Carrot	63.0 creamy yellow	39.8 light orange	38.2 yellow	73.5 creamy yellow
Czapek's	18.7 red	17.0 light orange	16.7 yellow	17.5 orange
Uschinsky's	40.5 red	27.3 light orange	33.0 yellow	58.2 yellow
Steinberg	63.0 light yellow	40.0 light orange	38.2 yellow	70.0 yellow
Sabouraud's	53.0 red	41.0 orange red	33.0 yellowish green	60.0 red
Lilly & Barnett's	56.0 red	36.5 light orange	40.3 yellow	63.6 red

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

**Mycelia not densely developed.

characters (Riker & Riker, 1936; Lilly & Barnett, 1951; Rawlins, 1933). As shown in Table 1, their feature of colonies and discoloration of the agar media are quite different. Although isolates #1 and #3 were microscopically identified to be the same species, *N. pterospermi*, their cultural characters are not exactly the same. Presumably, geographic difference caused this variation. However, both isolates grew well on PDA, corn meal, lima-bean, carrot, and Steinberg's agar media. Contrarily, Czapek's agar medium was not utilized well by all the four isolates tested.

Temperature—Potato dextrose agar was used in this experiment. The agar plates inoculated with mycelial mass were incubated at different temperatures (Table 2). The range of optimum temperature for mycelial growth was within 28°–31°C.

Table 2. Effect of temperature on the mycelial growth of Nectria*.

Temperature (°C)	Diameter of colonies (mm.)			
	#1	#3	#5	#6
19	22.5	17.0	16.5	26.5
22	24.0	19.0	18.5	34.5
25	31.5	24.5	22.0	40.5
28	34.5	26.5	23.5	44.0
31	37.5	31.5	23.0	45.0
34	30.0	20.5	18.0	26.5
37	20.5	19.5	17.5	25.0

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

Hydrogen ion concentration—Lilly and Barnett's solution was used. When 10 days old cultures were compared, isolates #1 and #6 grew well near neutral,

Table 3. Effect of hydrogen ion concentration on the growth*

Hydrogen ion concentration (pH)	Dry weight of the fungi (mg.)			
	#1	#3	#5	#6
2	8(3.2)**	527(7.6)	228(5.4)	61(3.4)
3	8(3.2)	554(7.6)	340(6.8)	117(3.6)
4	11(3.6)	571(7.6)	591(6.8)	220(4.2)
5	92(4.0)	637(7.0)	518(6.2)	252(4.8)
6	113(4.2)	234(4.2)	263(6.0)	236(5.0)
7	141(4.4)	195(4.0)	222(6.0)	263(5.6)
8	136(6.0)	194(4.2)	140(5.4)	260(5.8)

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

**Final pH values are in parentheses.

however, both #3 and #5 preferred acidic condition for their initial pH values. It is worthy to note that both #3 and #5 tended to shift pH value from acidic to basic side when both initial and final pH of the culture medium were compared (Table 3).

Carbon source—Lilly and Barnett's agar and Czapek's nitrate agar without dextrose were used as the basal medium in this experiment. As shown in Table 4, maltose and lactose were most readily utilized by the four isolates tested when the Lilly and Barnett's agar without dextrose was used as a basal medium. However, it was no longer valid when Czapek's agar was used instead of Lilly and Barnett's agar as a basal medium. Four isolates differed in their rosponse to the various carbon sources are shown in Table 5. Mannit, mannose,

Table 4. The linear growth of Nectria on Lilly and Barnett's agar with different carbon sources at 26°C for 5 days.*

Carbon source	Diameter of colonies(mm.)			
	#1	#3	#5	#6
Glycerol	28.6	26.0	23·3	43.0
Arabinose	33.0	24.0	26.6	50.0
Xylose	29.5	25.6	27.5	45.0
Dextrose	32.1	26.6	23.0	47.3
Mannose	31.3	26.3	22.0	48.3
Galactose	34.5	21.0	24.0	49.0
Fructose	29.3	23.3	21.6	44.0
Mannitol	34.3	25.6	25.3	45.0
Saccharose	30.5	24.0	22.0	47.0
Maltose	42.5	31.3	32.0	52.6
Lactose	40.3	30.3	27.3	51.6
Soluble starch	29.0	28.0	24.6	47.0

* Isolates #1 and #3 are *N. pterospermi*: #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae*.

and glycorol seemed to be the best carbon sources for the mycelial growth of isolate #1 (*N. pterospermi*) while dextrose, saccharose, and fructose were suitable for other isolate of *N. pterospermi* (#3). On the other hand, isolate #5 (*N. swieteinae-mahoganii*) preferred maltose, fructose, and xylose for better growth whereas #6 (*N. durantae*) grew better on soluble starch, glycerol, and dextrose for the carbon sources.

Further experiment on the effect of carbon source showed that higher concentrations of dextrose promoted mycelial growth of the fungi (Table 6), yet, there was an inhibitory effect on the growth of isolate #3 when concentration of dextrose went up 2%. Simultaneously, a lot of dark pigment was

Table 5. The linear growth of Nectria on Czapek's agar with different carbon sources at 26°C for 5 days*

Carbon source	Diameter of colonies (mm.)			
	#1	#3	#5	#6
Glycerol	19.5	12.9	16.5	13.1
Arabinose	16.2	12.7	16.5	12.5
Xylose	15.8	12.7	17.3	12.8
Dextrose	16.6	14.2	16.3	13.3
Mannose	19.5	12.1	15.7	12.5
Galactose	16.4	12.3	15.5	12.7
Fructose	15.8	13.0	17.5	12.7
Mannitol	20.3	12.8	16.7	12.7
Saccharose	14.5	13.8	16.0	12.9
Maltose	17.5	12.7	18.5	12.2
Lactose	14.1	11.7	14.8	11.6
Soluble starch	15.5	12.8	15.5	13.0

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

Table 6. The diameter of the colonies grown on PDA containing various concentrations of dextrose at 26°C for 4 days.*

Concentration of dextrose	Diameter of colonies (mm.)			
	#1	#3	#5	#6
0.5%	46.0	32.0	27.5	46.0
1.0%	47.0	35.0	30.0	50.0
2.0%	45.5	29.0	30.6	53.5
5.0%	50.0	29.0	31.0	53.0
7.5%	51.0	28.0	33.0	54.0
10.0%	51.0	28.0	34.0	54.0

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

accumulated under the disturbance of the mycelial growth.

Nitrogen source—The demand of nitrogen sources for the growth of the four isolates varied one another (Table 7). In the present experiment, Lilly and Barnett's medium without asparagine was also used as a basal medium and the pH value was adjust to 6.0.

Light—As shown in Table 8, continnous illumination caused disturbance of mycelial growth of isolates #1 and #5 while no such a phenomenon was observed in the case of isolate #6. However, 2 minutes illumination of ultraviolet light was certainly detrimental to the mycelial growth of the all isolates tested.

Table 7. The diameter of the colonies grown on the media with different nitrogen sources at 26°C for 4 days*.

Nitrogen source	Diameter of colonies (mm.)			
	#1	#3	#5	#6
KNO_3	37.0	22.0	13.0	37.0
NH_4NO_3	32.0	29.5	13.5	38.0
Urea	40.5	20.0	14.5	35.0
Leucine	32.5	18.5	14.0	34.5
Tyrosine	27.0	16.0	13.0	28.0
Arginine	32.0	20.0	13.0	32.5
Tryptophan	31.5	13.5	12.5	22.0
Asparagine	36.0	23.5	16.0	31.0
Minus-nitrogen	—	—	—	—

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

Table 8. Effect of light on the linear growth of the fungi at 26°C for 5 days*

Light	Diameter of colonies (mm.)		
	#1	#5	#6
Total darkness	55.5	37.6	59.0
Total light	48.2	30.0	60.0
12 hrs. alternation of light & darkness	53.0	34.5	59.0
UV treatment**	40.3	28.0	47.3

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

**Illumination of 2 and 4 days old cultures with a Si-kwang type No. GR-1501 ultra-violet lamp for 2 minutes.

Factors affecting the perithecia formation of Nectria

The perithecia and the ascospores of Nectria were known to be the main source of inoculum, a better understanding of the condition for the sexual reproduction might be paramount importance with regard to epidemiology of the diseases. The present experiment was carried out in order to search for a medium of known composition and condition suitable for the formation of the perithecia of the fungi.

Medium—Wood-chip alone, or wood-chip with rice-bran plus either dextrose, thiamin, or biotin were used as culture media in the present investigation. All the cultures were kept on laboratory desk at room temperature. From the resultant data showed that wood-chip with rice-bran, wood-chip with rice-bran and dextrose, and wood-chip with rice-bran, dextrose, thiamin and biotin promoted perithecia formation (Fig. 1, Table 9).

Table 9. The formation of perithecia on wood-chip with rice-bran at room temperature in day light*

Medium	Production of perithecia			
	#1	#3	#5	#6
Wood-chip alone	—	—	—	—
Wood-chip+rice-bran (3:1)	+	++	+	+
Wood-chip+rice-bran (3:1)+ 1% dextaose	++	++	+	++
Wood-chip+1% dextrose	—	—	—	—
Wood-chip+1% dextrose+ thiamin & biotin	—	—	—	—
Wood-chip+rice-bran (3:1)+ 1% dextrose+thiamin & biotin	++	++	+	++

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swietetnae-mahoganii* and *N. durantae.*

Hydrogen ion concentration—The hydrogen ion concentrations of the medium within the usual limits, pH 4–8, failed to influence perithecial formation.

Carbon source—As shown in the foregoing experiments, different isolates responsed differently to various carbon sources for their mycelial growth, however, this was not entirely true in the case of perithecial production. In general, dextrose, fructose, galactose, and maltose were sufficient to support their production (Table 10).

Table 10. Effect of carbon sources on the production of perithecia at 26°C.*

Carbon source	Production of perithecia			
	#1	#3	#5	#6
Glycerol	+	+	—	—
Arabinose	++	—	—	—
Xylose	+	+	—	—
Dextrose	+++	++	++	++
Mannose	++	—	—	—
Galactose	+	+++	+++	+
Fructose	++	++	++	++
Mannitol	±	+	—	—
Saccharose	+	+	—	—
Maltose	+	++	+++	+
Lactose	+	+	++	—
Soluble starch	+	—	+	++

*Isolates #1 and #3 are *N. pterospermi:* #5 and #6 are respectively *N. swietetnae-mahoganii* and *N. durantae.*

Nitrogen source—As shown in Table 11 and Fig. 2, asparagine, leucine, arginine and NH_4NO_3 were suitable nitrogen sources for their production of perithecia.

Table 11. Effect of nitrogen sources on the production of perithecia at 26°C in day light*

Nitrogen source	Production of perithecia			
	#1	#3	#5	#6
Asparagine	+	+	++	++
Urea	±	—	++	++
Tyrosine	++	++	—	+
Leucine	±	++++	+++	—
Tryptophan	—	++	—	—
Arginine	+	++	+	++
KNO_3	—	++	—	+
NH_4NO_3	++	+	++	+
Minus-nitrogen	—	—	—	—

*Isolates #1 and #3 are *N. pterospermi;* #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae.*

Since leucine was most effective for the perithecia formation, different concentrations of leucine were tested for this purpose. As shown in Table 12. and Fig. 3, 0.25% leucine in Lilly and Barnett's medium was the best among the concentrations examined.

Table 12. The production of perithecia on Lilly and Barnett's medium with different concentrations of leucine*

Concentration of leucine	Production of perithecia	
	#3	#5
0.10%	+	++
0.17%	++	++
0.25%	+++	+++
0.50%	—	+++
0.75%	—	++
1.00%	—	—

*Isolates #3 and #5 are *N. pterospermi* 75 and *N. swieteinae-mahoganii* respectively.

The concentrations of leucine above 0.5% provided a better mycelial growth for isolate #3 (*N. pterospermi* 75) without perithecia formed, while no perithecia were found in the case of isolate #5 (*N. swieteinae-mahoganii*) at higher concentrtion of leucine, namely over 1%.

Combinations of carbon and nitrogen sources—The isolates #3 (*N. pterospermi*) and #5 (*N. swieteinae-mahoganii*) were grown on the media of Lilly and Barnett's containing 0.25% leucine as nitrogen source and gradient concentrations of dextrose. The results showed that higher concentrations of dextrose inhibited the formation of perithecia (Table 13). The fungi excreted yellowish brown pigment in agar and colonies appeared to be water-soaked when the concetration of dextrose was above 2%.

Table 13. The production of perithecia on Lilly and Barnett's medium with 0.25% leucine and different concentrations of dextrose*

Concentration of dextrose	Production of perithecia	
	#3	#5
0.2%	±	+
0.5%	+	++
1.0%	++	++
2.0%	—	+**
3.0%	—	+**
5.0%	—	—
PDA Ck	+	+

*Isolates #1 and #3 are *N. pterospermi* 75; #5 and #6 are respectively *N. swieteinae-mahoganii* and *N. durantae*.
**Perithecia formed on the margin of the slant.

Light—Light plays an important role in the formation of perithecia. Perithecia were not found when the cultures of Nectria were incubated in darkness. However, exposing the abundantly grown cultures from the dark to the light, perithecia were produced within 10 days. On the other hand, one day cultures were irradiated with a Si-kwang type No. 1501 ultraviolet lamp for 2 minutes every two days, the formation of a violet ring around the colonies was observed. Yet, no perithecia formation was promoted by this means.

Discussion

The production of fertile perithecia by Nectria in synthetic agar media is a rare occurence. Cayley (1921) found that the only media on which *Nectria galligena* completed perfect stage were those containing starch or some derivatives of starch with 1% glycerine and potato slopes in glycerine. Killian (1925) found maize flour agar or salep agar were best for *N. pyrosphaera* to ripen. Potassium nitrate was the only substance that appeared to favour their production in synthetic media, provided that the C/N ratio was maintained at a suitable level. Lortie (1964) used oat and wheat grains and powdered birch bark media with 1% agar to promote the formation of perithecia.

In our experiments the perfect stage of the 4 isolates were readily obtained on PDA slant. On the PDA slant culture at 26°C, under 40 foot-candles continuous illumination, the minimum duration for the perithecia formation were 17 days for #3 (*N. pterospermi* 75); 25 days for #5 (*N. swieteinae-mahoganii*); 35 days for #6 (*N. duarantae*); 38 days for #1 (*N. pterospermi*). The perithecia of *N. swieteinae-mahoganii* tended to aggregate on the media and the other three were scattered. The perithecia could be formed abundantly on synthetic media if suitable nitrogen and carbon sources were supplied under favorable condition. Yet, wood-chip mixed with rice-bran and dextrose gave the best production of perithecia for the four isolates.

On the synthetic media, the perithecia formation was found to depend a great extent on the nitrogen and carbon contents of the media, the former being more important than the later (Krause, 1930). Toussoun (1962) discovered that isoleucine isomers favored the formation of perithecia of *Fusarium solani* f. *cucurbitae*. In our experiments, leucine also served as a favorable nitrogen source for the production by perithecia of isolates #3 (*N. pterospermi* 75) and #5 (*N. swieteinae-mahoganii*), but not by #1 (*N. pterospermi*) and #6 (*N. durantae*). The production of perithecia may be due to inherent characters.

In the higher concentrations of leucine, mycelial growth was abundant without formation of perithecia. The similar results were obtained by Hanlin (1961) that the concentrations of dextrose above 2% impaired the perithecia formation. In the case of isolate #3 (*N. pterospermi* 75), dark pigment was accumulated in agar simultaneously with the increase of dextrose concentration above 2%.

The effects of carbon sources on the uegetative growth is not a sole event, it must combine with other factors. In fact, carbon nutrition of the fungi grown on Lilly and Barnett's and Czapek's basal media revealed the different response. The best sources of carbon or nitrogen for the peritheica formation was not always the same which yielded maximum vegetative growth. Isolates #1 and #3 were isolated from same species of host plants grown in different localities. This might cause the changes in physiological and cultural characteristics of the fungi. Thus, the responses of the fungi in the genus of Nectria were so divergent that no general conclusion can be drown from the present investigation.

Summary

The optimum temperatures for the mycelial growth of the four isolates, i. e. #1 (*Nectria. pterospermi*), #3 (*N. pterospermi* 75), #5 (*N. swieteinae-mahoganii*), and #6 (*N. durantae*) were respectively 31°C, 31°C, 28°C, and 31°C.; and the optimum pH values were 7, 5, 4, and 7 respectively. The demands of carbon

and nitrogen sources for mycelial growth and perithecia formation were different one another. Changes in hydrogen ion concentration failed to influence the formation of perithecia whereas mycelial growth was largely affected. Light played a subtle role in the formation of perithecia. Mycelial growth was favoured by incubating the cultures in the dark. Ultraviolet light did not stimulate the production of perithecia.

Large amount of perithecia were obtained on the wood-chip with rice-bran. However, perithecia formation was also possible on the synthetic media provided that suitable nitrogen and carbon sources were presented in appropriate ratio. Leucine served as a good nitrogen source for the production of perithecia. However, higher concentrations of dextrose and leucine impaired the production of perithecia.

樹木癌腫病菌生長與子囊殼生成之研究

于 浩 陳其昌 吳龍溪

樹木癌腫病菌 、 #1 (*Nectria pterospermi*) 、 #3 (*N. pterospermi* 75) ， #5 (*N. swieteinae-mahoganii*) ，及 #6 (*N. durantae*) ，其菌絲營養生長的最適溫度分別爲 31°C 31°C ， 28°C 及 31°C ，最適 PH 值爲 7 ，5 ，4 ，及 7 。生長及子囊殼的形成對碳素源及氮素源的需求互相差異很大。改變氫離子濃度影響子囊殼的形成效果不大，但對菌絲的生長之影響甚鉅。在暗處，菌絲生長較佳。紫外光線未能促進子囊殼的形成。

在木屑與米糠的培養基上，子囊殼可大量形成，然而在合成培養基上，如果供給適當的氮素源及碳素源，亦可形成子囊殼 。對於子囊殼的形成， Leucine 爲一種很好的氮素源，然而濃度過高反會阻止子囊殼的形成。過多的葡萄糖亦同樣地會阻止子囊殼形成。

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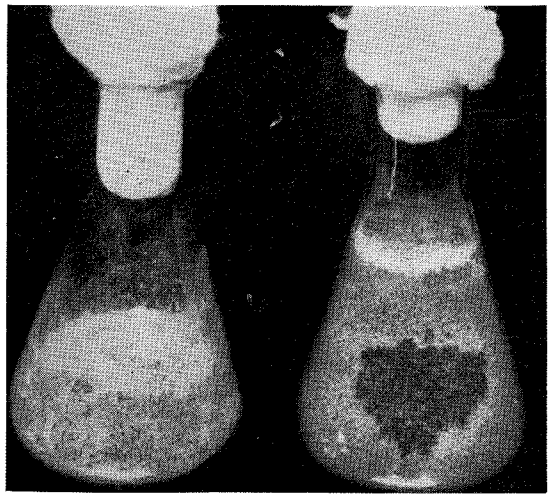

Fig. 1. Showing the production of perithecia of isolate #1 (*N. pterospermi*) (right) and #3 (*N. pterosperrni* 75) (left) on wood-chip with rice bran for two months.

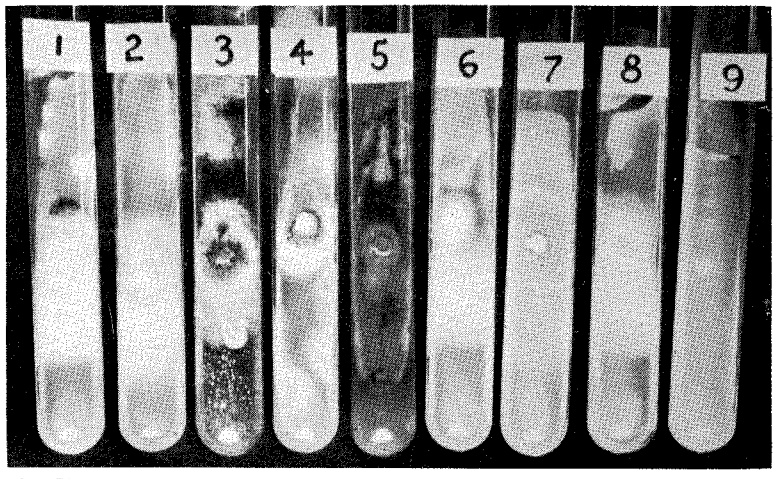

Fig. 2. Showing the effect of nitrogen sources on the perithecia formation of isolate #3 (*N. pterosperrni* 75) grown on Lilly and Barnett's agar containing, asparagine (1), urea (2), tyrosine (3), leucine (4), tryptophan (5), arginine (6), KNO_3 (7), NH_4NO_3 (8), and Minus-nitrogen (9).

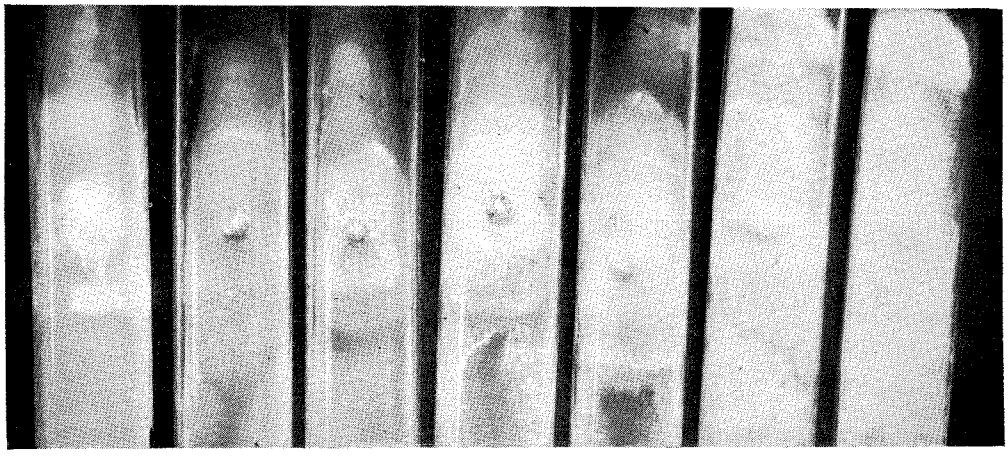

Fig. 3. Showing the effect of the cocentrations of leucine on perithecia formation of #3 (*N. pterospermi* 75). From left to right the concentrations of nitrogen are: 0.5 % KNO_3 (check); 0.1 %, 0.17 %, 0.25 %, 0.5%, 0.75 %, and 1.0 %, of leucine.