Errata

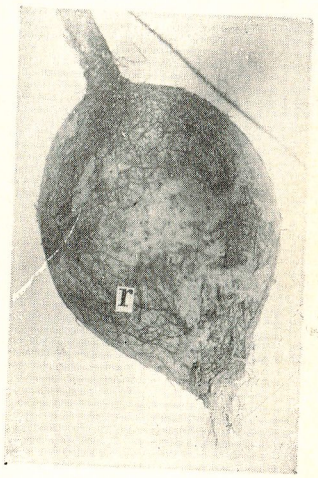
Page	line ↓	line ↑	erroneous	correct
8		2	follows.	follows:
11	8		text-book	text-book,
18		15	surfacer	surface
19	18		Vineceous	Vinaceous
21		11	experimet	experiment
26	5		clear the	clear that the
27		17	*Pruns*	*Prunus*
29	7		(Pl. VI, G).	(Pl. VI, H).
32		12	sporphore	sporophore
35		4	; Pl. VI, H).	; Pl. VI, G).
38	3		experimets	experiments
42	1		**Reletion**	**Relation**
54	6		an	a
	16		reserches	researches
60		8	colerless	colorless
61		15	the that	that
62	4		color or	color
65		4	35°, 40°, 50°,	35°, 40°, 45°, 50°,
		3	After 10	After 5–60
69	17		conentrations	concentrations
76	2		uable	unable
80	9		forme	form
	18		epiderm	epidermis

Errata (*Continued*)

Page	line ↓	line ↑	erroneous	correct
83	10		formed of	formed on
85	6		Text-figure 24.	Text-figure 27.
87	17		invstigators	inyestigators
88	5		reportad	reported
93	16		surved	served
		12	surve	serve
95	1		tha	the
	16		nerotic	necrotic
96		7	unchaged,	unchanged,
101	14		MEYAKE	MIYAKE
	21		(1920), by HIRMER	, by HIRMER
103		10	proceding	preceding
106	20		apple, tree,	apple tree,
107		1	evan	even
111		16	holzzerstör enden	holzzersörenden
119	13		*d:*	*p:*
		17	1937	1947
		5	E. Roots	F. Roots
		4	F. Ditto.	E. Ditto.
120	10		Scerotium-	Sclerotium-
		8	G. Hyphae	H. Hyphae
		6	H. Germinating	G. Germinating

A

B



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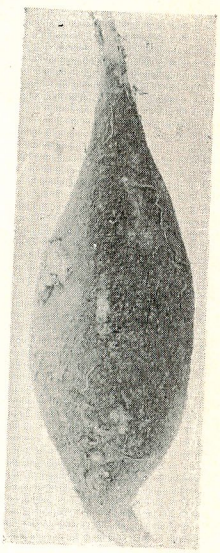

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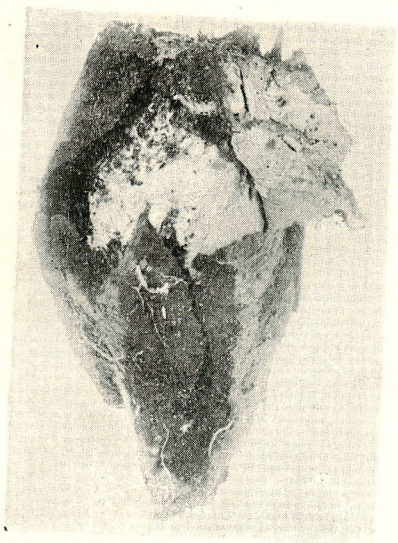

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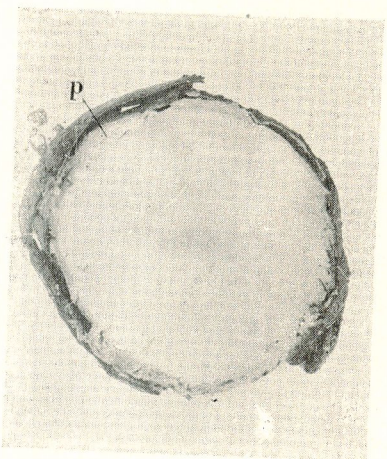

Plate II

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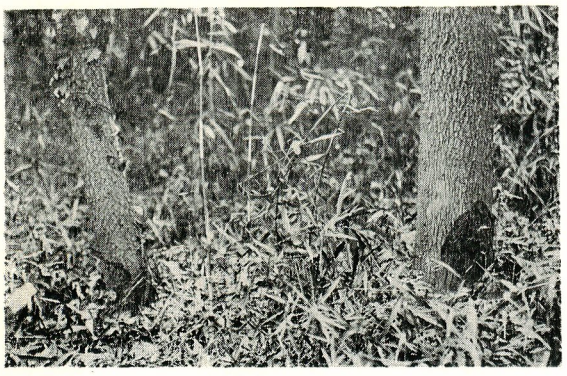

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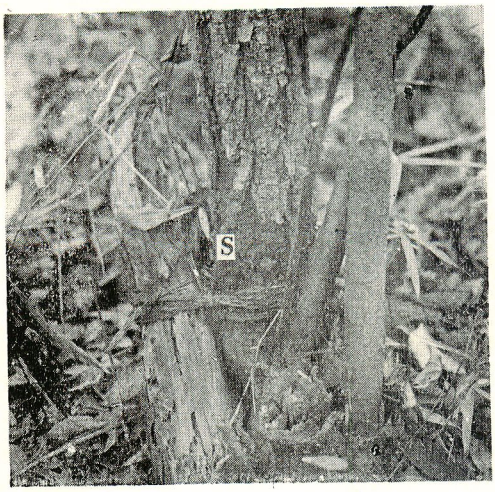

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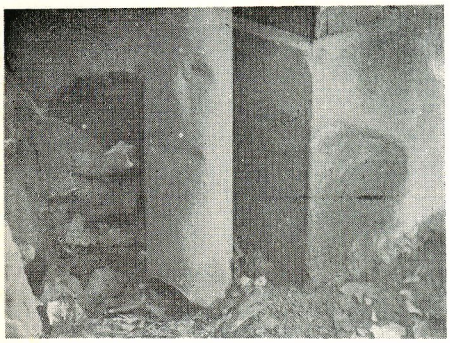

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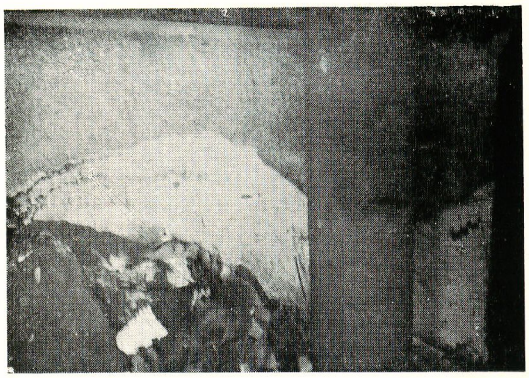

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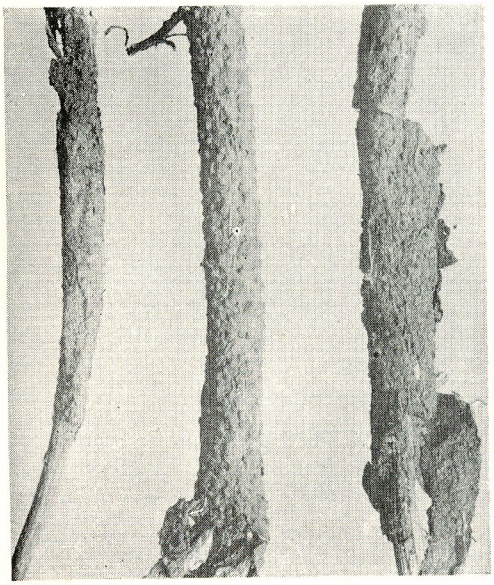

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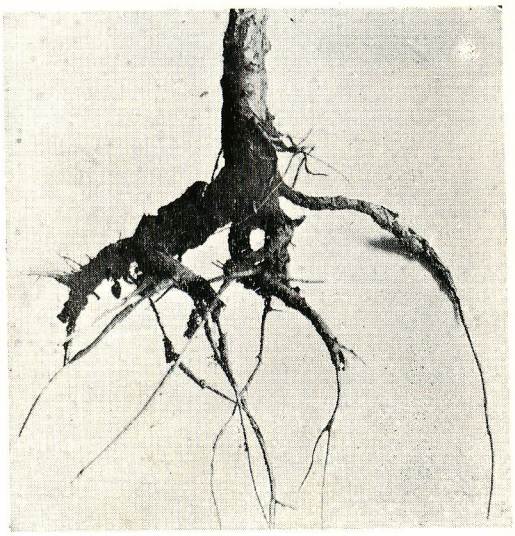

Plate IV

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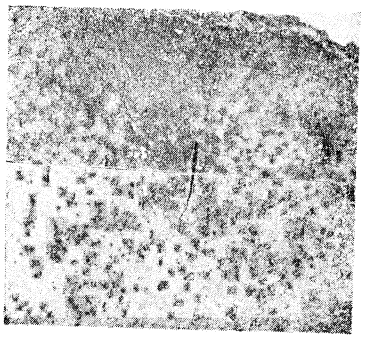

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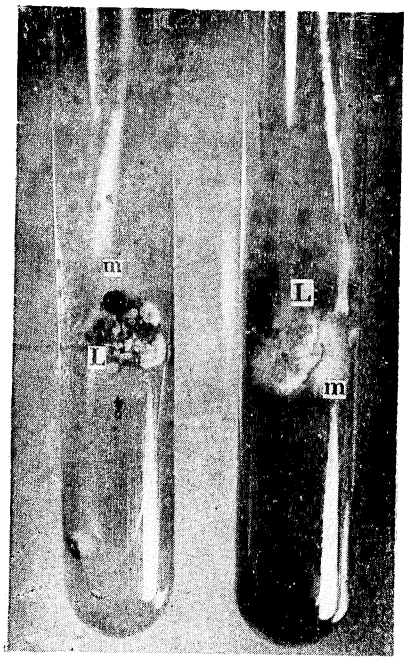

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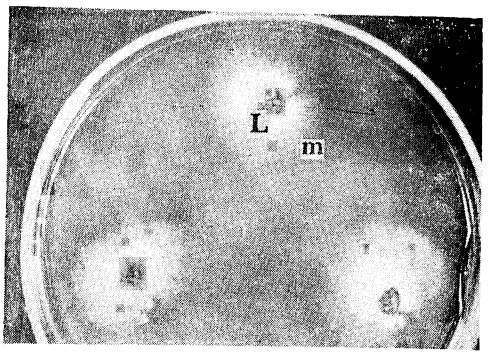

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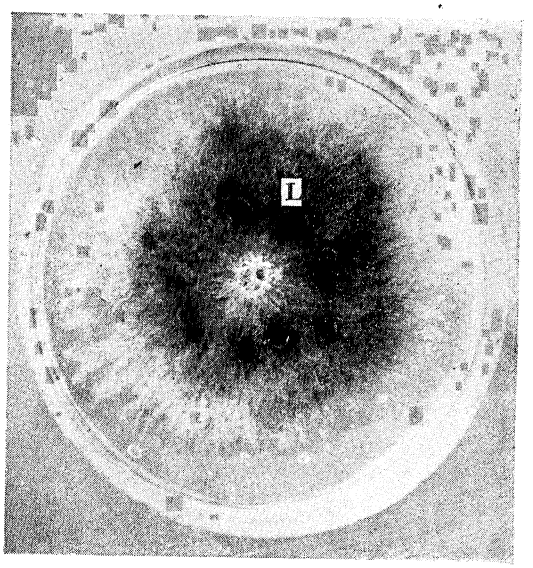

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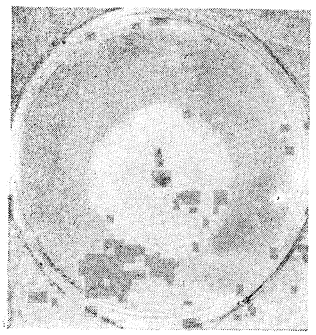

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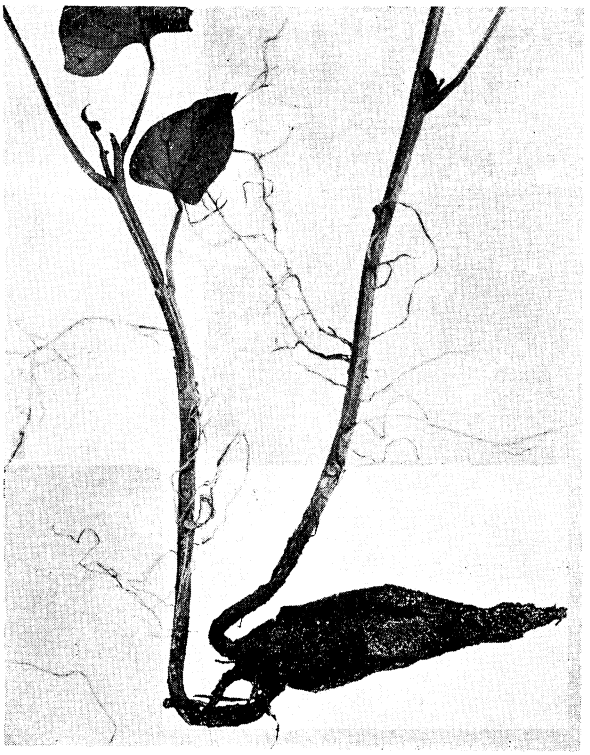

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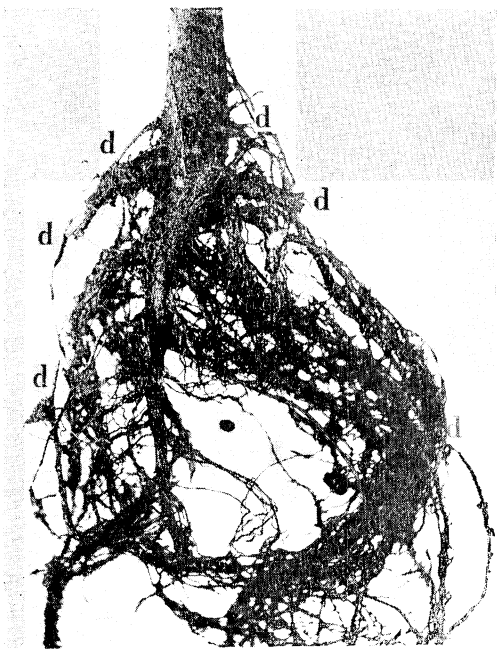

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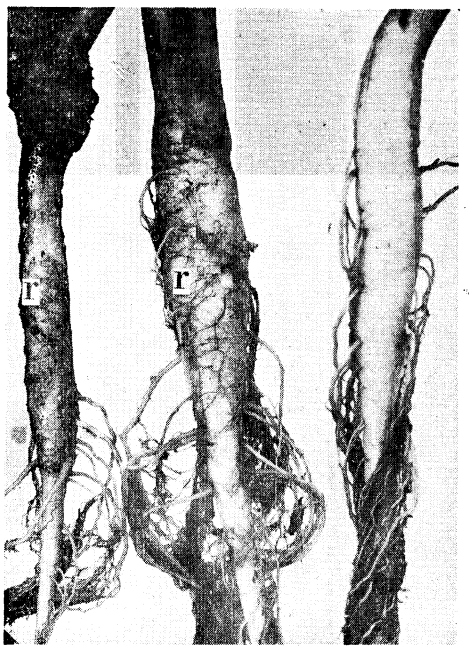

Plate VI

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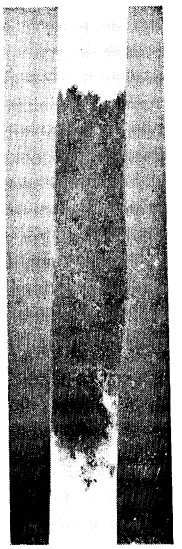

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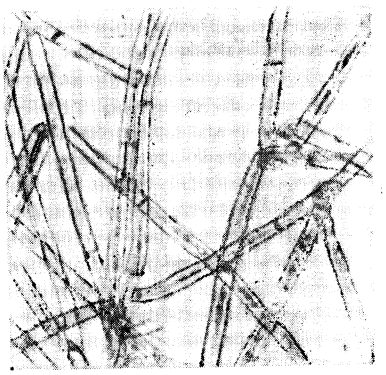

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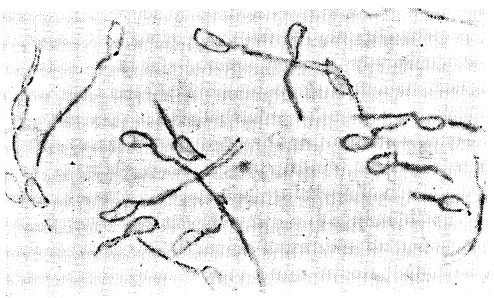

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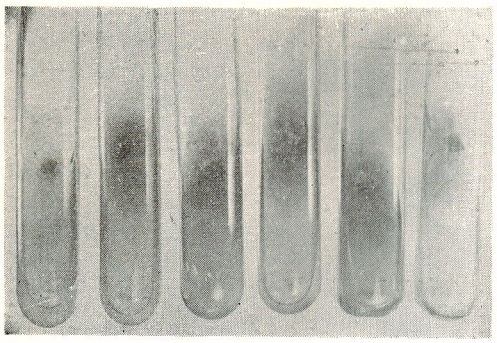

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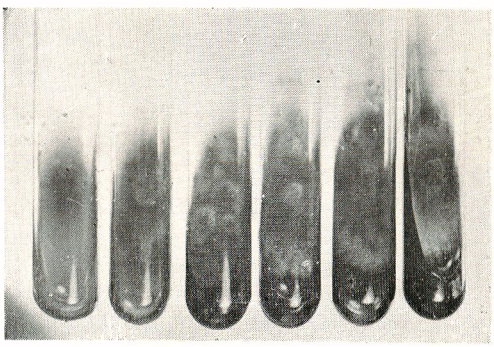

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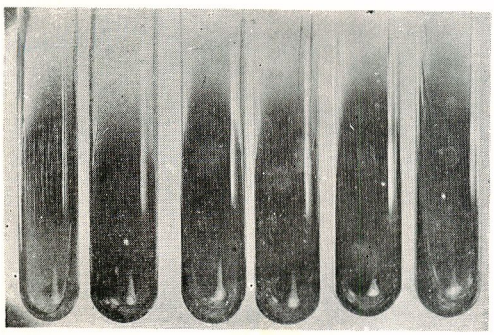

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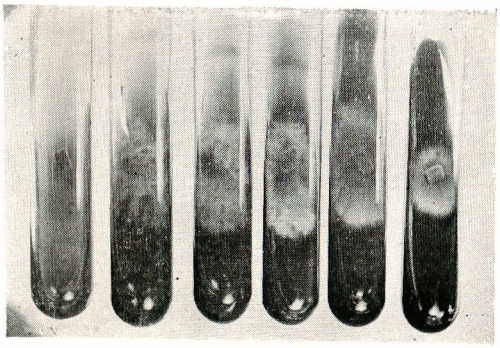

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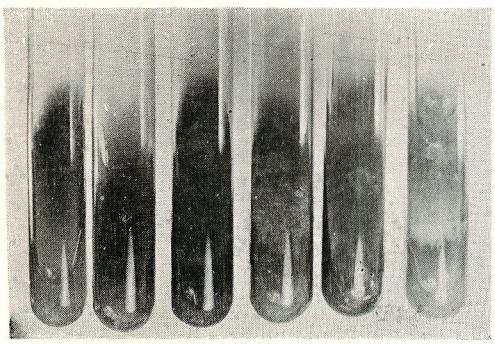

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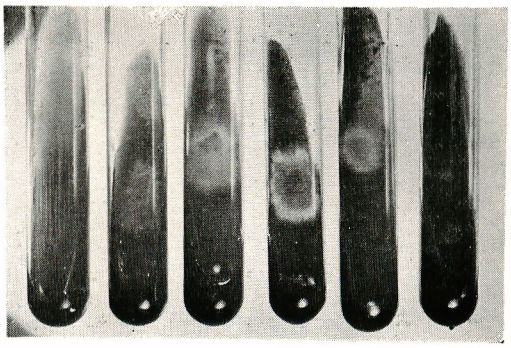

Plate VIII

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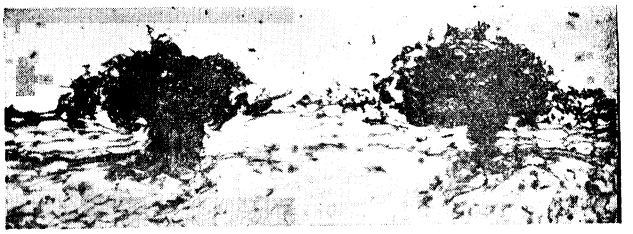

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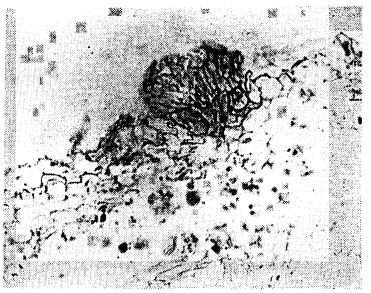

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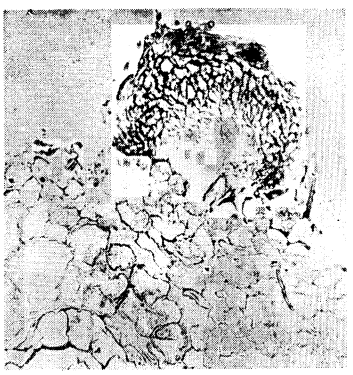

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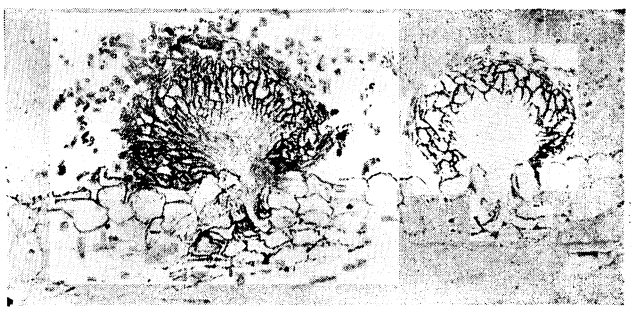

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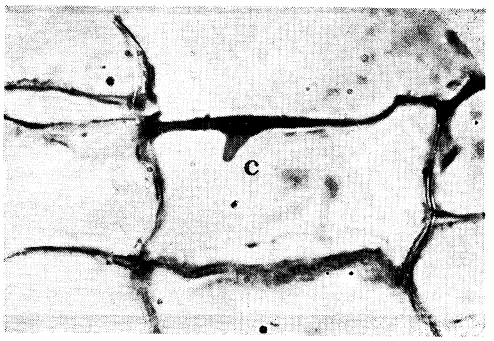

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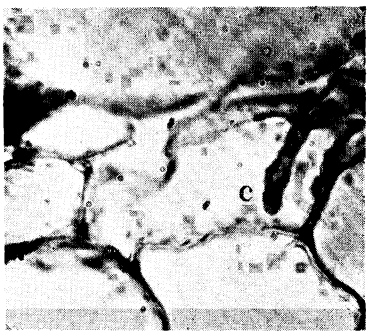

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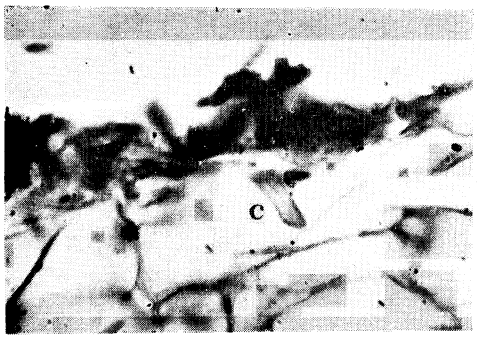

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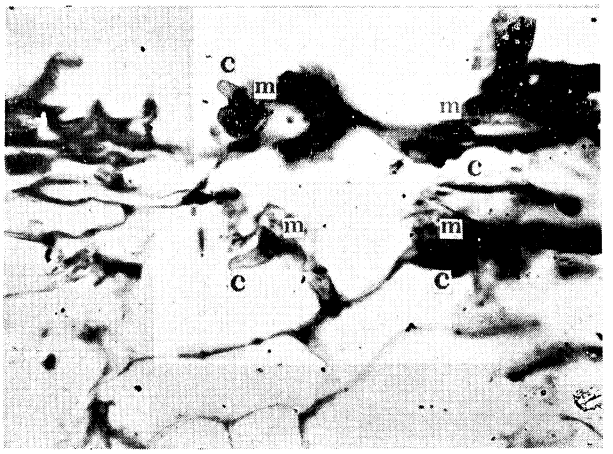

Plate X

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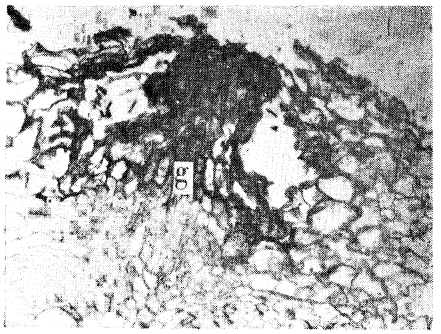

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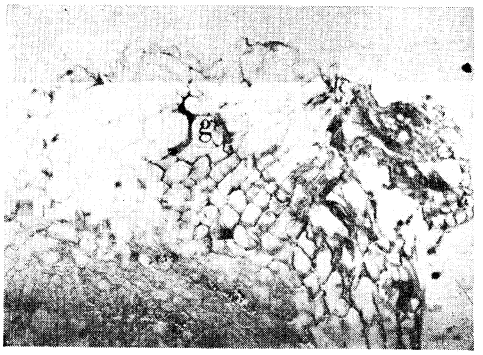

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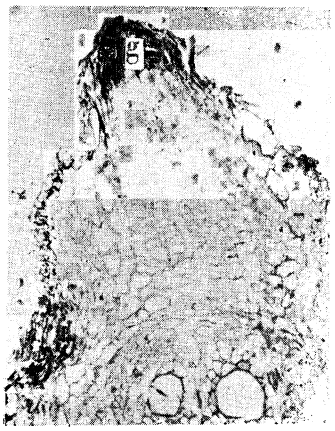

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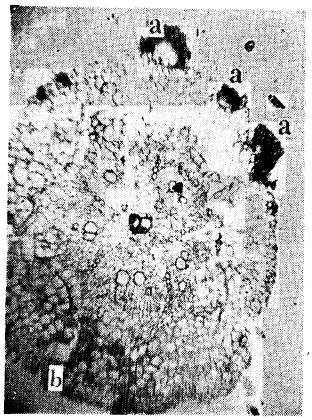

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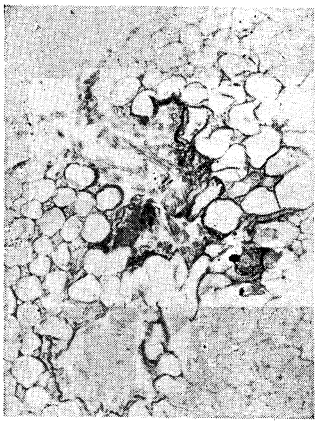

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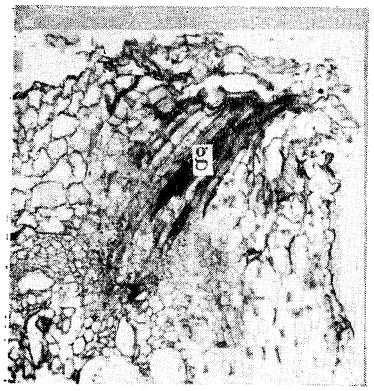

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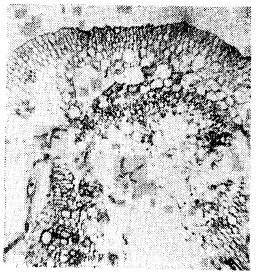

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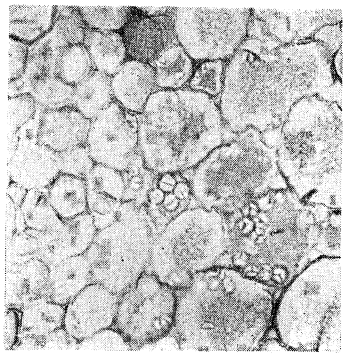

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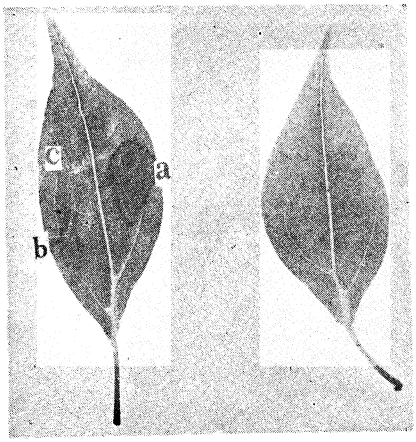

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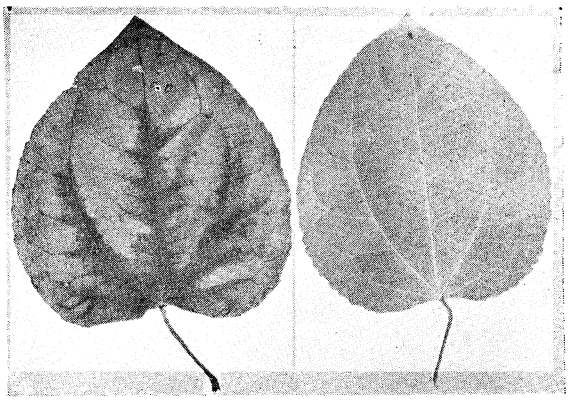

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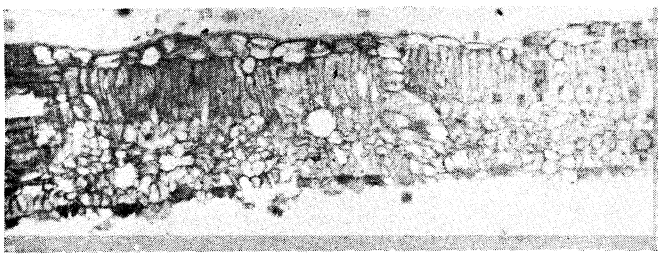

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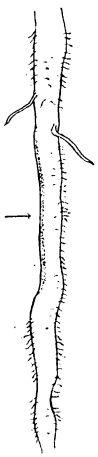

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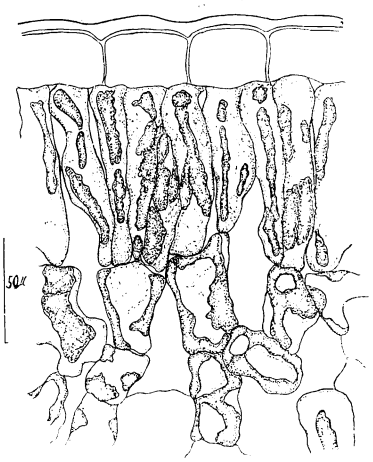

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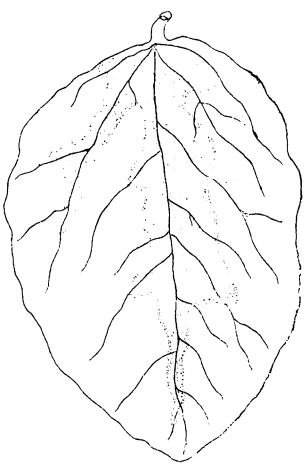

Plate XII

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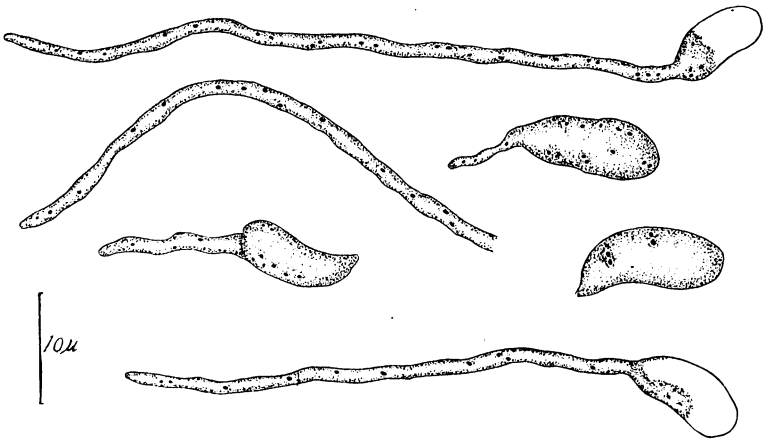

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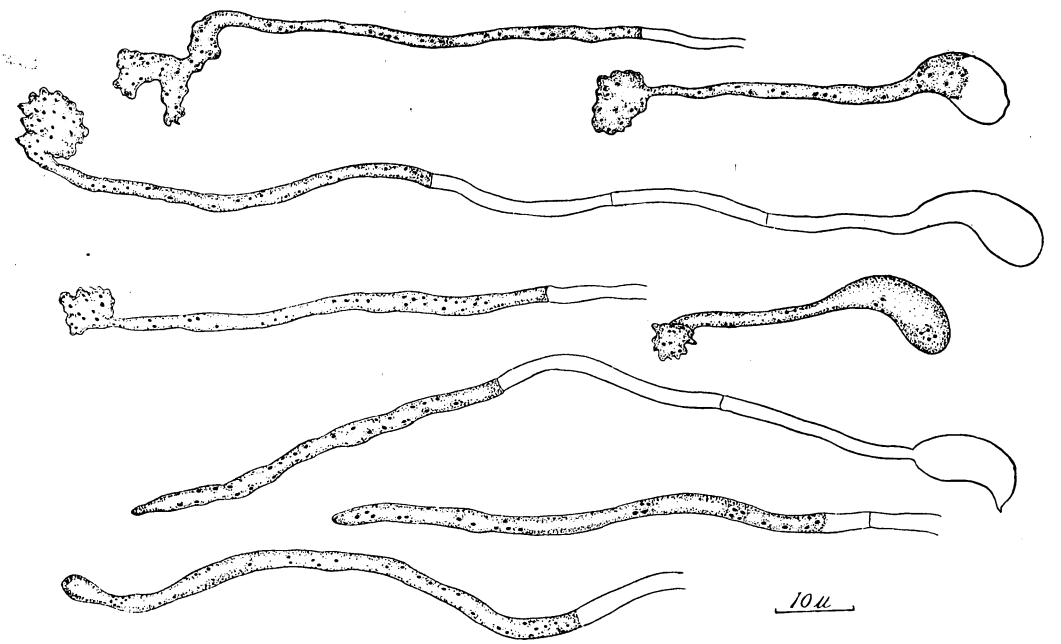

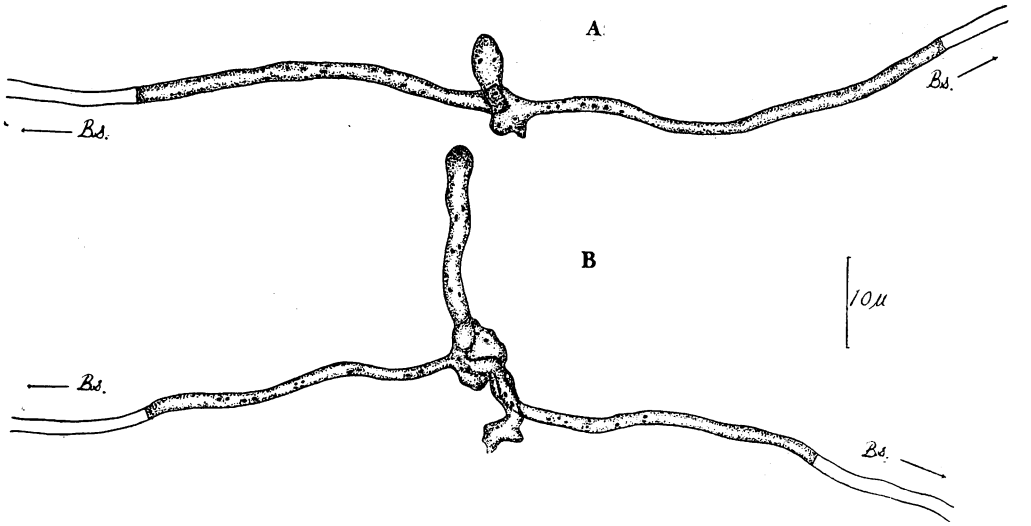
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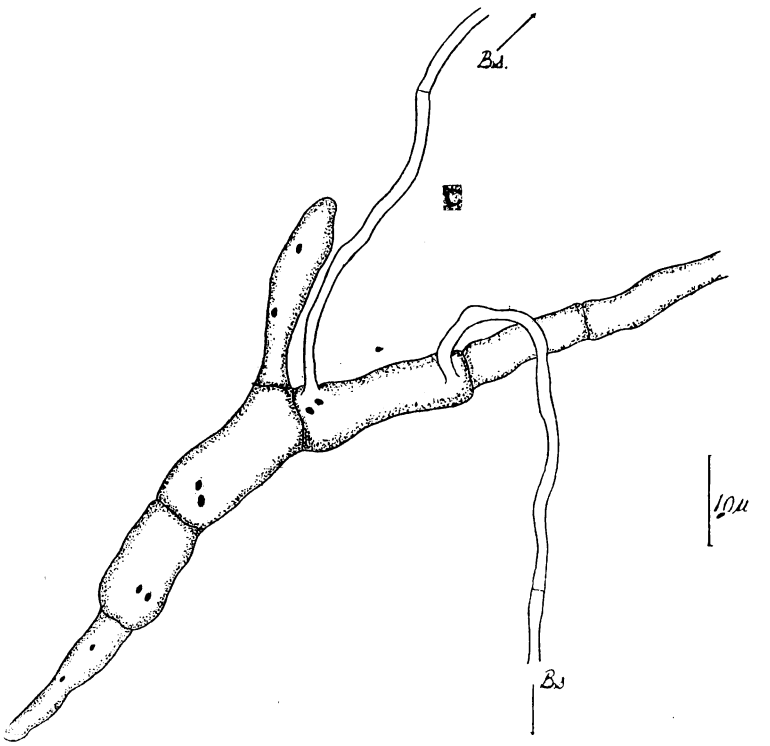
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Plate XIV

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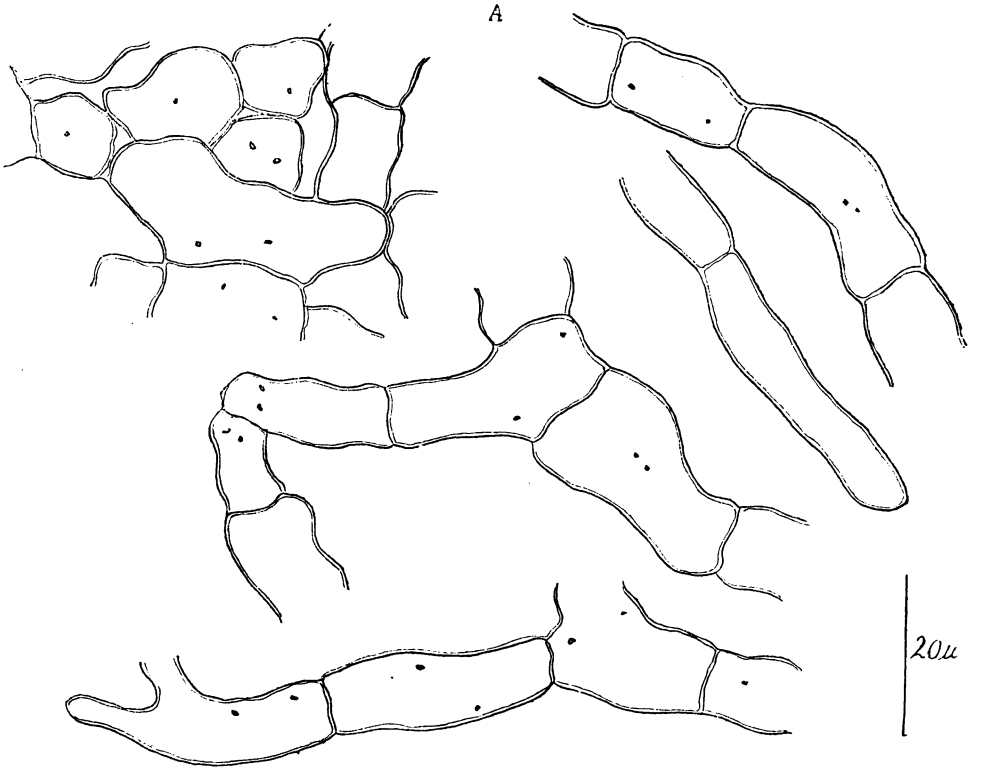

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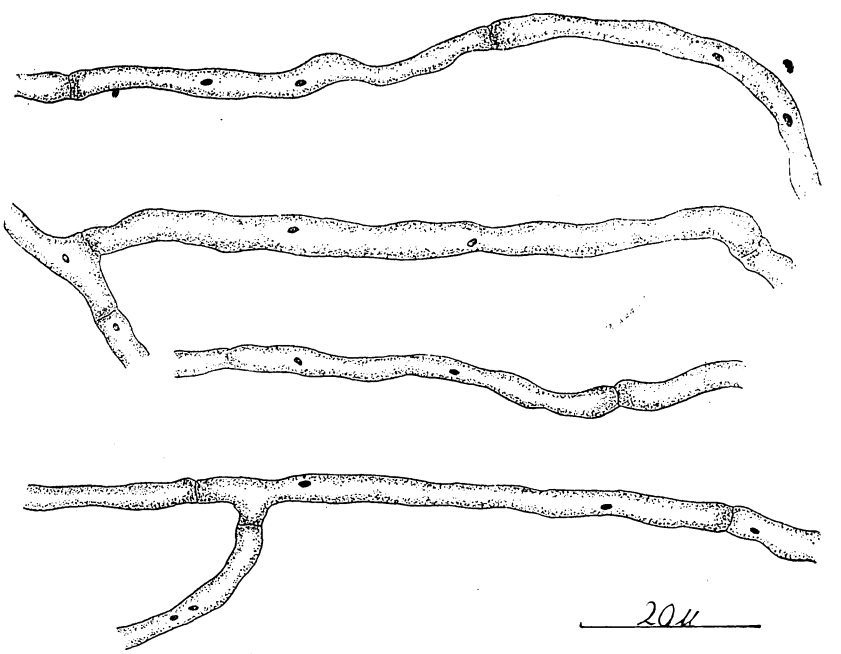
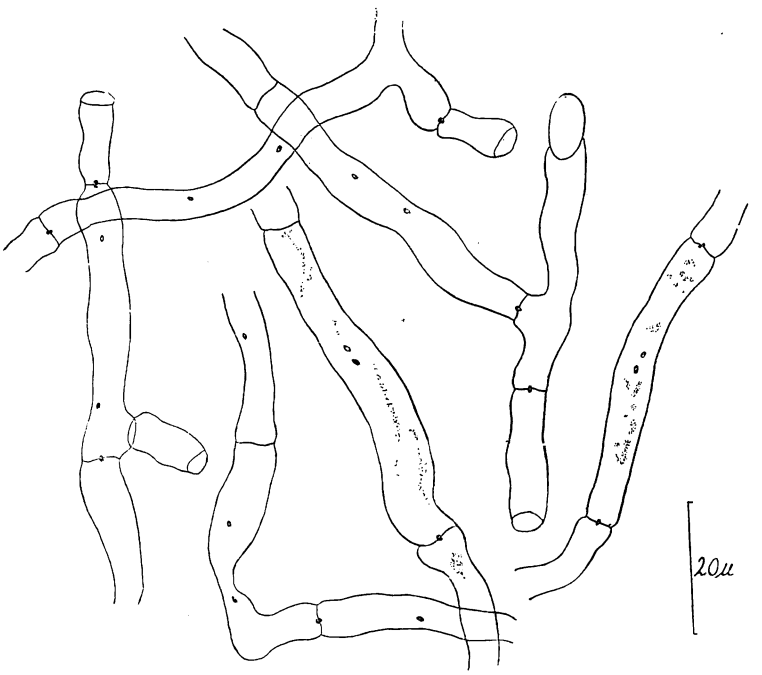


A



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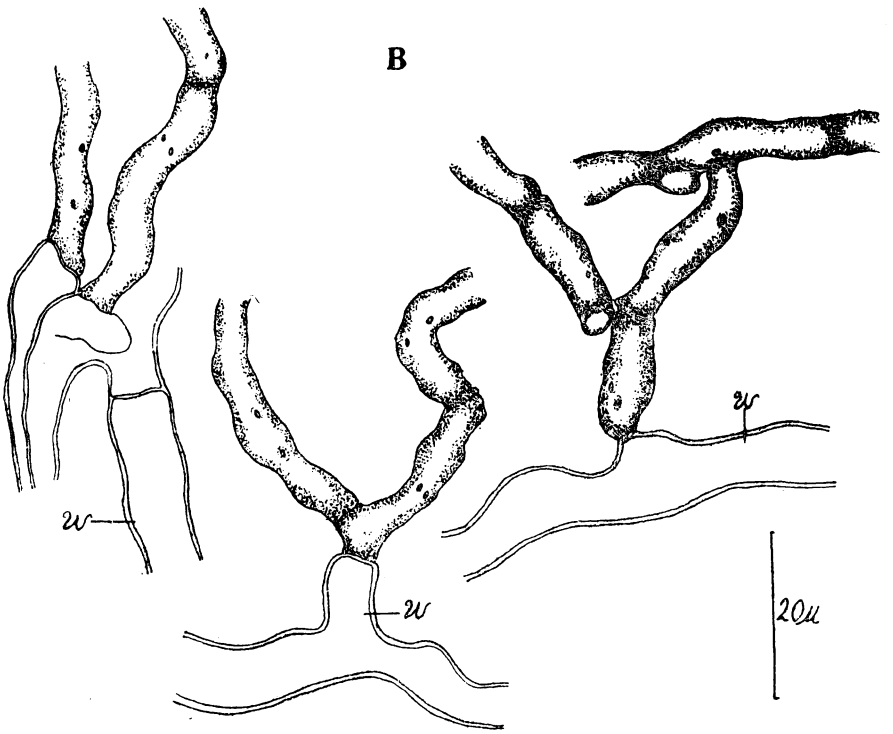
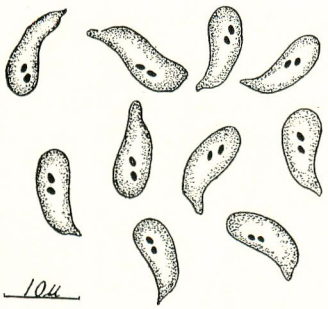
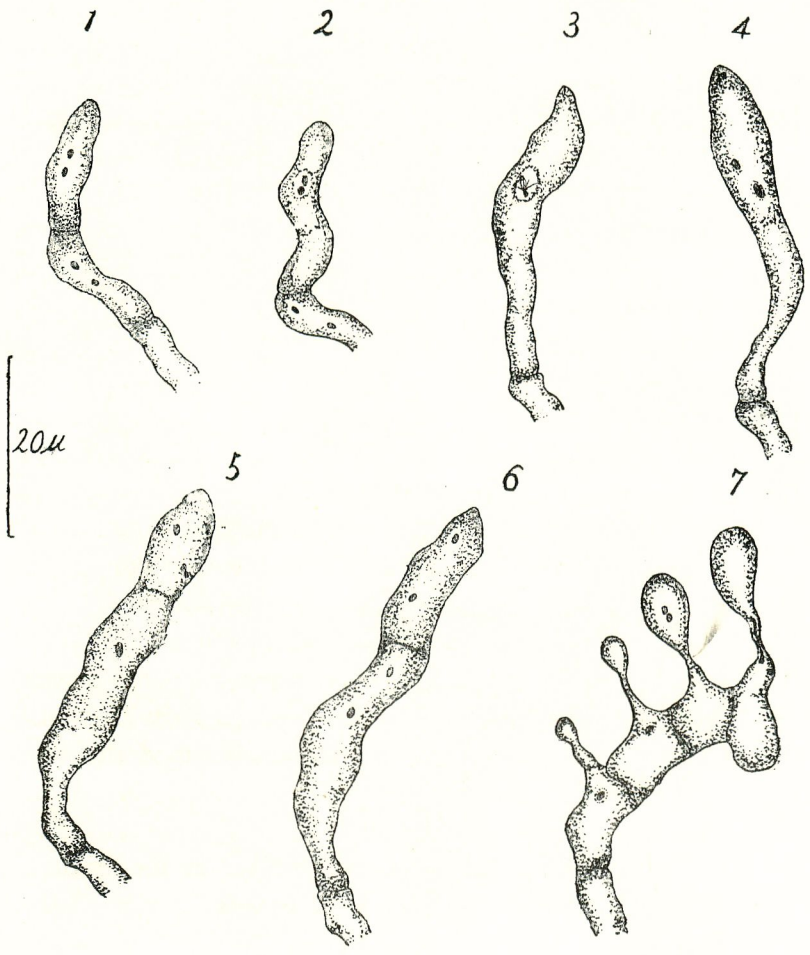


Plate XVI

A



B



INTRODUCTION

A root rot which has been well known under the name of "Murasaki-monpa byô"[1] among phytopathologists and growers is one of the most destructive diseases in Japan, because of its severe damage and of difficulties in its prevention.

In 1891, TANAKA, who studied this disease affecting mulberry trees which are cultivated on a large scale for rearing silkworms, described a new basidiomycetous fungus as the causal organism and named it *Helicobasidium Mompa.*

Since the disease was recognized to attack mulberry trees very frequently and induced a great deal of losses in economical standpoint, it has been noticed as "Japanese mulberry root rot" in European and American literature.

The fungus attacks not only mulberry trees, but also a large number of other kinds of plants, both wild and cultivated, belonging to many families. Consequently it is generally treated as one of the polyxenic pathogenes.

Some years after TANAKA's first report on the fungus, RACIBORSKI (1909) recorded erroneously its existence in Java and expressed his taxonomical opinion on this organism. SAWADA (1919) discovered also this fungus in Formosa and made some morphological and taxonomical notes.

The most important contribution to the fungus was made by MIYAKE (1920), especially in the morphological characters, but he could not obtain a pure culture from this pathogene.

In their handbooks, SHIRAI(1903), HORI(1910), MIURA(1917), NISHIDA(1921), BOKURA (1921), TSUJI(1926), IKATA(1927), ENDÔ(1927), HARA(1927, 1930, 1931, 1936), SUEMATSU(1930), KITAJIMA(1933), and HEMMI(1937) published some fragmentary notes of the fungus.

Despite of such a common disease, so far as can be ascertained by the writer, there has been no reliable account concerning the artificial culture of the fungus. It has been generally believed that the isolation and the pure culture of the fungus may be probably unable, notwithstanding several workers seem to have made some efforts. Accordingly it has been considered that there may be many problems on this disease that require further investigations, especially on the physiological and ecological characters of the fungus, and on the pathological and therapeutical studies of the disease.

Since 1942, the present writer has carried out the experimental studies on this disease and, during the course of his work, fortunately succeeded in the isolation and the artificial culture of the organism. Preliminary notes on these accounts were previously published by YOSHII and the present writer(1944) and by the present writer(1944).

In the present paper the writer deals with the results of the etiological and

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1) The Japanese word "murasaki" means purple; "monpa", a kind of nappy cloth; "byô", disease. Formerly this disease was called under the name of "Monpa-byô" in Japan, but at the present time this word includes at least two kinds of well known diseases, namely the one is "Shiro-monpa byô" ("Shiro" means white), and the other, "Murasaki-monpa byô". The former is caused by *Rosellinia necatrix* (HART.) BERL.

pathological studies on this disease which have been carried out mainly by the pure culture method.

The writer wishes to express his heartiest thanks to Prof. Dr. Hazime Yoshii, of Kyûshû University, under whose direction the present investigation was done, for the helpful suggestions and criticisms given throughout the study. He is also much indebted to Dr. Kôgo Togashi, Professor of Plant Pathology, of Yokohama National University, for many suggestions and careful reading of this manuscript, and to Dr. Kimizô Kitajima for his valuable advice and encouragement during the work. Finally, acknowledgements are also due to the facilities in connection with the supply of the experimental materials given by Dr. Seitô Takimoto, Mr. Hiroshi Hori, and Mr. Isao Tada.

Etiological Studies on "Murasaki-monpa" Disease

Geographic distribution and host plants of the fungus

The fungus has long been known to distribute widely throughout this country and to have a very large host range. Besides in Japan, Sawada (1919) recorded this fungus in Formosa and subsequently Nakata and Takimoto (1928) in Corea.

According to Raciborski (1909), the fungus was collected in Java by himself, but his fungus seems to be different from the Japanese species in the taxonomical point of view that will be discussed by the present writer in the later part of this paper. Sawada (1919) suggested that the fungus might be probably found in China. More recently, Nakata and Asuyama (1930) reported this fungus attacking the pear-tree in Manchuria.

Hereupon it may be safely inferred that the geographic distribution of this organism is limited to Japan, Formosa, Corea, and probably to certain districts of China.

As mentioned above, the fungus attacks a large number of plants, both mono- and dicotyledons and also gymnosperms, including many kinds of the valuable plants, such as mulberry tree, apple tree, sweet potato, soy beans, paper mulberry, paulownia tree, etc.

The host plants that have been recorded in the literature are assembled in the following table (Table 1), with several new additions and recognitions to the list (represented as "O") by the present writer himself.

Table 1. Host plants of *Helicobasidium Mompa*[1].

Host plant		Author	Remark
Scientific name[3]	Japanese name[3]		
Polypodiaceae			
1. *Pteridium aquilinum* Kuhn. *var. japonicum* Nakai	Warabi	Sawada ('19)[2]	

1) In the identification of the woody plants, the writer was much indebted to the late Mr. Yoshizô Yanagita, of our Experiment Station.
2) The numeral in parenthesis after the author, *e. g.* ('19) means 1919.
3) In using the scientific and Japanese names of the plants the writer chiefly followed Makino and Nemoto (1921).

Table 1. Host plants of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
Equisetaceae			
2. *Equisetum arvense* L.	Sugina	SAWADA('19)	
Ginkgoaceae			
3. *Ginkgo biloba* L.	Ichô	NANBU('17), SAWADA('19), HARA('27)	O
Pinaceae			
4. *Torreya nucifera* S. ET Z.	Kaya		O
5. *Chamaecyparis obtusa* S. ET Z.	Hinoki	MIURA('17), SAWADA('19), HORI('21), HARA('27)	O
6. *C. pisifera* S. ET Z.	Sawara	MIURA('17), SAWADA('19), HORI('21), HARA('27)	O
7. *C. pisifera* S. ET Z. *var. plumosa* MAST.	Shinobu-hiba		O
8. *Cryptomeria japonica* D. DON.	Sugi	SHIRAI('03), MIURA('17), SAWADA('19), HORI('21), HARA('27)	
9. *Pinus densiflora* S. ET Z.	Aka-matsu	SHIRAI('03), MIURA('17), SAWADA('19), HORI('21), HARA('27)	O
10. *P. parviflora* S. ET Z.	Himeko-matsu		O
11. *P. strobus* L.	Sutorôbumatsu		O
12. *Picea excelsa* LK.	Ôshû-tôhi		O
13. *Thuja occidentalis* L.	Nioi-hiba		O
Salicaceae			
14. *Populus Maximowiczii* A. HENRY	Doronoki	MIURA('17), SAWADA('19), HORI('21), HARA('27)	
15. *P. nigra* L.	Amerika-yamanarashi		O
16. *Salix Bakko* KIMURA	Bakko-yanagi		O
17. *S. vulpina* ANDERS.	Kitsune-yanagi	MIURA('17), SAWADA('19), HARA('27)	
Juglandaceae			
18. *Juglans Sieboldiana* MAXIM.	Oni-gurumi		O
19. *Platycarya strobilacea* S. ET Z.	Nobunoki		O
Fagaceae			
20. *Castanea crenata* S. ET Z.	Kuri		O
21. *Cyclobalanopsis myrsinaefolia* OERST.	Shira-kashi		O
22. *Quercus acuta* THUNB.	Aka-gashi	MIURA('17), SAWADA('19), HORI('21), HARA('27)	
23. *Q. acutissima* CARR.	Kunugi	MIURA('17), SAWADA('19), HORI('21), HARA('27)	

Table 1. Host plants of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
24. *Q. gilba* BLUME	Ichii-gashi		O
25. *Q. serrata* THUNB.	Konara	MIURA('17), SAWADA('19), HORI('21), HARA('27)	O
26. *Shiia Sieboldi* MAKINO	Suda-zii		O
Ulmaceae			
27. *Aphananthe aspera* PLANCH.	Mukunoki		O
28. *Cetis sinensis* PERS.	Enoki	SAWADA('19), HARA('27)	O
29. *Ulmus pumila* L.	No-nire		O
30. *Zelkowa serrata* MAKINO	Keyaki		O
Moraceae			
31. *Broussonetia Kazinoki* SIEB.	Kôzo		O
32. *B. papyrifera* VENT.	Kazinoki		O
33. *Ficus Carica* L.	Ichijiku	TAKIMOTO	
34. *F. elastica* ROXB.	Indo-gomunoki	SAWADA('19), HARA('27)	
35. *F. retusa* L.	Gajumaru	SAWADA('19), HARA('27)	
36. *Morus acidosa* GRIFF.	Shima-guwa	SAWADA('19)	
37. *M. alba* L.	Maguwa	TANAKA(1891), SHIRAI('03), MIYAKE('20), HORI('21), HARA('27), ENDÔ('27), NAKATA & TAKIMOTO('28)	O
Chenopodiaceae			
38. *Beta vulgaris* L. *var. Rapacea* C. KOCH.	Satô-daikon	NAKATA & TAKIMOTO('28)	
Eupteleaceae			
39. *Euptelea polyandra* S. ETZ.	Fusazakura		O
Magnoliaceae			
40. *Liriodendron tulipifera* L.	Hanten-boku		O
Anonaceae			
41. *Asimina triloba* DUN.	Pôpô		O
Lauraceae			
42. *Cinnamomum Camphora* N. ET E.	Kusunoki	NISHIDA('21), KITAJIMA('33)	O
Cruciferae			
43. *Brassica oleracea* L.	Habotan	HARA('27)	
44. *Raphanus sativus* L.	Daikon	SHIRAI('03), MIURA('17), SAWADA('19), NISHIDA('21), HARA('27)	O
Eucommiaceae			
45. *Eucommia ulmoides* OLIVER	Tochû		O

Table 1. Host plant of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
Platanaceae			
46. *Platanus orientalis* L.	Suzukake-noki		O
Rosaceae			
47. *Chaenomeles Maulei* LAVAL.	Kusaboke	MIURA('17), SAWADA('19), HORI('21)	
48. *Malus pumila* MILL. *var. domestica* SCHNEID.	Ringo	MIURA('17), SAWADA('19), HORI('21), NISHIDA('21), HARA('27), IKATA('27), NAKATA & TAKIMOTO('28), TOGASHI *et al.*('42)	O
49. *Pirus sinensis* LINDL.	Nashi	SAWADA('19), HARA('27), IKATA('27), NAKATA & ASUYAMA('39)	
50. *Photinia glabra* MAXIM.	Kanamemochi	MIURA('17), SAWADA('19), HORI('21), HARA('27)	
51. *Prunus Armenica* L. *var. Ansu* MAXIM.	Anzu	IKATA('27)	
52. *P. chikusienses* KOIDZ.	Ôshima-zakura		O
53. *P. Grayana* MAXIM.	Uwamizu-zakura		O
54. *P.* Mume S. ET Z.	Ume	TAKIMOTO	
55. *P. Persica* S. ET Z. *var. vulgaris* MAXIM.	Momo	SAWADA('19), IKATA('27)	
56. *P. serrata* LINDL.	Sakura	MIURA('17), SAWADA('19), HARA('27)	
57. *P. triflora* ROXB.	Sumomo	IKATA('27)	
58. *P. verecunda* KOEHNE. *var. typica* NAKAI	Kasumi-zakura		O
59. *Rhaphiolepis umbellata* MAKINO	Sharinbai	SAWADA('19), HARA('27)	
Leguminosae			
60. *Arachis hypogaea* L.	Nankin-mame	SAWADA('19), HARA('27)	
61. *Cercis canadensis* L.	Amerika-hanazuô		O
62. *Glycine Soja* BENTH.	Daizu	SAWADA('19), HARA('27), TOGASHI *et al.*('42)	O
63. *Robinia pseudoacacia* L.	Nise-akashia	MIURA('17), HARA('27)	O
64. *R. pseudoacacia* L. *var. umbraculifera* DC.	Togenashi-niseakashia		O
Rutaceae			
65. *Citrus spp.*		NISHIDA('21), IKATA('27) HARA('30)	
Euphorbiaceae			
66. *Mallotus japonicus* MUELL. ARG.	Akame-gashiwa		O
67. *Sapium japonicum* PAX. et HOFFM.	Shiraki		O

Table 1. Host plant of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
Anacardiaceae			
68. *Pistacia chinensis* Bunge.	Toneriba-hazenoki	Kitajima	
69. *Rhus javanica* L.	Nurude		O
70. *R. succedanea* L.	Hazenoki	Sawada('19), Hara('27)	
71. *R. verniciflua* Stok.	Urushi	Miura('17), Sawada('19), Hori('21), Nishida('21), Hara('27)	O
Celastraceae			
72. *Evonymus japonicus* Thunb. *var. aureovariegatus* Lowe.	Fuiri-masaki		O
73. *E. striata* Loes.	Mayumi		O
Aceraceae			
74. *Acer campestre* L.	Kobu-kaede		O
75. *A. pictum* Thunb. *var. dissectum* Wesmael.	Enkô-kaede		O
76. *A. pictum* Thunb. *subvar. eupictum* Pax.	Itaya-kaede		O
Sapindaceae			
77. *Sapindus Mukorossi* Gaert.	Mukuroji		O
Sabiaceae			
78. *Meliosma myriantha* S. et Z.	Awabuki		O
Vitaceae			
79. *Vitis vinifera* L.	Budô	Miura('17), Sawada('19), Hara('27)	
Sterculiaceae			
80. *Firmiana platanifolia* Schott. et Endl.	Aogiri		O
Theaceae			
81. *Eurya japonica* Thunb.	Hisakaki		O
82. *Thea sinenses* L.	Cha	Endô('27), Hara('30)	
Guttiferae			
83. *Calophyllum Inophyllum* L.	Terihaboku	Sawada('19), Hara('27)	
Thymelaeaceae			
84. *Edgeworthia papyrifera* S. et Z.	Mitsumata	Hori('10, '21), Miura('17), Sawada('19), Nishida('21), Hara('27), Togashi *et al.*('42)	
Araliaceae			
85. *Aralia elata* Seem.	Taranoki		O

Table 1. Host plant of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
Umbelliferae			
86. *Daucus Carota* L.	Ninjin	Shirai('03), Sawada('19), Hara('27)	
Cornaceae			
87. *Aucuba japonica* Thunb.	Aoki		O
88. *Cornus controversa* Hemsl.	Mizuki		O
89. *C. coreana* Wang.	Chôsen-mizuki		O
Ebenaceae			
90. *Diospyros Kaki* L. *f. domestica* Makino	Kaki	Shirai('03), Miura('17), Sawada('19), Hara('27) Ikata('27, '42)	
Styracaceae			
91. *Styrax Shiraiana* Makino	Ko-hakuunboku		O
Oleaceae			
92. *Ligustrum Ibota* Sieb. *var. angustifolium* Blume	Ibotanoki		O
93. *L. japonicum* Thunb.	Nezumimochi		O
Convolvulaceae			
94. *Ipomoea Batatas* Lam. *var. edulis* Makino	Satsuma-imo	Shirai('03), Miura('17), Sawada('19), Hori('21), Nishida('21), Hara('27), Hemmi('37)	O
Solanaceae			
95. *Solanum tuberrosum* L.	Jagatara-imo	Miura('17), Sawada('19), Hara('27)	
Scrophulariaceae			
96. *Paulownia tomentosa* Steud.	Kiri	Hara('27), Nakata & Takimoto('28)	O
Caprifoliaceae			
97. *Lonicera japonica* Thunb.	Suikazura		O
98. *Sambucus Sieboldiana* Blume *var. typica* Nakai	Niwatoko	Miura('17), Sawada('19), Hori('21), Hara('27)	O
99. *Viburnum pubinerve* Blume *f. intermedium* Nakai	Kanboku		O
Compositae			
100. *Arctium Lappa* L.	Gobô	Sawada('19), Hara('27)	
Araceae			
101. *Amorphophallus Konjac* C. Koch.	Konnyaku	Miura('17), Sawada('19), Hori('21), Nishida('21)	
Gramineae			
102. *Pleioblastus Simoni* Nakai	Medake	Suematsu('30)	

Table 1. Host plant of *Helicobasidium Mompa* (*Continued*).

Host plant		Author	Remark
Scientific name	Japanese name		
103. *Saccharum officinarum* L. Zingiberaceae	Satô-kibi	SAWADA('19)	
104. *Zingiber officinale* ROSE.	Shôga	SAWADA('19), HARA('27)	

As is shown in Table 1, 104 species in 76 different genera belonging to 45 families have been listed as the hosts of the fungus. Among them, 50 species were newly added to science by the present writer. Perhaps, more number of the species shall be found as the host plants by further studies.

The fungus is distributed throughout all the parts of Japan, and, in general, the greatest menace of the disease in the regions of high temperature may be inferred with probable certitude that the fungus is more frequently found in the southern districts than in the northern sections.

As described previously, the fungus is one of the most serious and destructive agents for the mulberry tree. It may be no more necessary to say that wherever is a mulberry tree, there occurs the fungus.

As well as the mulberry tree, other valuable plants such as apple trees, sweet potatoes, soy beans and "Mitsumata" (*Edgeworthia papyrifera* S. ET Z.) are affected most seriously by the fungus. HORI (1910) noted in his text-book that the "Mitsumata" (an excellent material for manufacturing Japanese paper) cultivated in Shizuoka Prefecture was seriously damaged by the fungus in the past and the growers were obliged to give up its cultivation.

In the autumn of 1942, the writer observed in Kagoshima Prefecture large heaps of heavily diseased fleshy roots of the sweet potato being piled up on fields. Such events may also be seen probably in other districts of our country.

In the case of soy beans, TOGASHI *et al.* (1942) reported the occurrence of serious damage caused dy this fungus in Iwate Prefecture.

Symptom and sign of the disease

A detailed description on the symptom of this disease of the mulberry tree was made by MIYAKE (1920), but as regards other host plants there has been scarcely and unsatisfactorily reported up to the present time, and accordingly the writer will state in detail the symptom and the sign of the disease affecting the sweet potato and some woody plants except the mulberry tree.

1. On sweet potatoes

Materials infected in nature or inoculated artificially show the the symptoms as follows.

The slender roots attacked by the fungus are rotted and later dried up; both small

and large fleshy roots are covered with networks of the rhizomorphs. The attack of the fungus has no relation to the size of fleshy roots affecting any one else. Among numerous fleshy roots produced from the same plant, there have been observed different degrees of infection.

In one case, the rhizomorphs on fleshy roots creep up, while in the other they creep down, showing no general rule in the direction of creeping.

Even though the fleshy root of the plant has been heavily attacked by the fungus, the plant may remain alive when its slender roots remain healthy. When all of the subterranean parts are destroyed completely, the plant may wilt and finally be dead. Small fleshy roots which were attacked by the fungus severely at the early stage of development are unable to continue their further growth.

In the case of extremely heavy damage, it is not uncommon that a great number of small sclerotia in pin-head size are produced on the basal portion of the stem below the ground level, and that purplish-brown mycelial mats with whitish margin are formed on the ground surrounding the diseased plants. The mycelial mat is velvety in appearrance, irregular in shape, variable in size (from 300 to 500 sq. cm.), and often embraces some extraneous substances, such as weeds, pebbles and soil particles near the plant. The mycelial mat practically seems to be a fruit-body of the causal fungus, but the formation of hymenial layers is not observed even in late autumn (Pl. I, A).

For the purpose of convenience, affected fleshy roots were divided into five classes by the severity of the damage macroscopically, viz.,

(1) Very slightly affected fleshy root (degree-α) : Only a small part of the fleshy root is covered with purplish rhizomorphs and the other parts seem to be healthy in appearance. Fleshy roots are not softened at all (Pl. I, B) .

(2) Slightly affected fleshy root (degree-β) : Rhizomorphs and purplish-brown mycelial mats cover the whole of fleshy roots, but the mycelial mats can be removed easily. Fleshy roots are not softened (Pl. I, C).

(3) Moderately affected fleshy root (degree-γ) : The whole surface of fleshy root is compactly covered with rhizomorphs and mycelial mats are dark purplish or deep purplish brown in color. The fleshy root is roughened and rugged in appearance, as its surface is covered compactly by the tangled rhizomorphs. The mycelial mat can not be easily separated from the skin. The skins are separated from the fleshy parts by slight force of pin-cette readily. There is not yet noticeable spaces between the skin and the fleshy substance. The fleshy tissue is more or less softened and becomes chalky.

(4) Heavily affected fleshy root (degree-δ): Rhizomorphs and mycelial mats cover the whole surface of fleshy root compactly, and they are blackish brown in color with the attaching of soil particles. As there is so remarkable space between the skin and the fleshy tissue that the skin is separated very readily. A large part of the fleshy tissue is rotted heavily, and softened conspicuously, giving out bad smell frequently. A great number of pin-head sized sclerotia are produced on the surface of the fleshy root(Pl. I,D,E;Pl.IV,A).

(5) Very heavily affected fleshy root (degree-ϵ): Soil particles adhere to the skin of the fleshy root so compactly that the mycelial mats are seen only after washing

with water again and again. The decayed fleshy tissue covered by the crumpled skin is sometimes contracted and dried up. On the under surface of the skin, numerous small sclerotia are observed.

According to RIDGWAY's (1912) color standard, the colors of the mycelial mat, of the rhizomorph, and of the fruit-body or sporophore will be described as follows :

1. Mycelial mat (in degree-β) ; Madder Brown,
2. Rhizomorph; Dark Vinaceous-Purple~Madder Brown,
3. Fruit-body or sporophore; the upper surface is Light Burnet Lake~Madder Brown, while the under surface Indian Purple~Blackish Red-Purple.

2. On woody plants

Results of the observations concerning some woody hosts such as *Robinia pseudo-acacia, Platycarya strobilacea, Acer campestre, Juglans Sieboldiana, Paulownia tomentosa, Liriodendron tulipifera* and *Ulmus pumila* will be noted briefly as follows:

Slender roots attacked by the fungus become yellowish brown or blackish brown in color; they are softened, rotted and finally disappear. In the case of severer damage, the cambial portion of large roots is heavily destroyed, only remaining the bark as well as woody tissue and leaving no trace of small roots. On heavily affected roots, a great number of sclerotia buried in the cork layer are seen very frequently.

Purple rhizomorphs creep up the surface of the roots and the trunk, increasing in diameter towards the ends. When the roots were destroyed severely, it is not rare that numerous small roots develop newly, and thus the plants may escape from death at least temporarily(Pl. III,E,F).

Soon after the aggregations of rhizomorphs reach the basal portion of the host, they develop here into a mycelial mat or a sporophore. The sporophore formed on the basal portion of the tree spreads its area upwards as well as sidewards covering the surface of the trunk (Pl. II, A,B,C; Pl. III, A).

It is of interest to note that the sporophores are produced not only on the host plants but also on no-living materials, such as stones, dead twigs, masses of soil and even on the concrete foundation of the house near the diseased tree (Pl. III, C).

During the period from May to July, the surface of the sporophore becomes powdery white, being caused by the formation of the hymenial layer (Pl. II,D; Pl. III,B,D).

The size of the sporophore may not always be influenced by the severity of damage, but rather by the environmental conditions, especially by the humidity. Consequently under an extremely humid condition, the sporophore reaches some times few decimeters in height from the ground level, sweating a great number of colorless watery drops upon the surface of it.

Many of the diseased trees show at first no remarkable changes in appearance above the ground, but, by and by, some of them are weakened noticeably and finally are led to death. In such trees, the leaves may become more or less smaller and fall earlier than in the healthy ones. In extreme cases the entire subterranean portion of the plant may be almost completely destroyed probably by the secondary invasion of wood-decaying fungi. Thus the plant becomes to offer no remarkable resistance

to external force.

Isolation of the causal fungus

As pointed out already, concerning the isolation and the artificial culture of the fungus in question, MIYAKE (1920) seemed to make a great deal of efforts, but failed to gain its pure culture. Thus he reached the conclusion that ["the germ tubes or the primary hyphae never develop to the large vegetative hyphae in artificial media, but probably only in favorable living host……"].

Prior to this report, MIURA (1917), in his text-book made a brief note on the isolation and the culture of the fungus without any description of his experimental method. However, his fungus is very doubtful in many respects as compared with the causal organism of this disease. Thus, so far as the present writer can ascertain, there has been hitherto no reliable account dealing with the subject.

The present writer has made an effort to solve this problem, and, after some failures, succeeded in artificial culture of the fungus from both diseased fleshy roots of sweet potatoes and basidiospores collected from woody hosts.

The experiments of the writer will be reported in detail as follows:

1. From the diseased fleshy roots of sweet potatoes

A. MATERIALS Materials obtained from the fields at Takarabe-machi, in Kagoshima Prefecture by the writer and those sent by Mr. HORI, of the Kumamoto Agricultural Experiment Station, were used in this experiment. As the sources of the isolation, the following materials were prepared;

(1) Mycelial mats found on the ground near the diseased sweet potato,

(2) Rhizomorphs or mycelial strands formed on fleshy roots of sweet potatoes,

(3) Mycelial mass in the soil near the diseased plants,

(4) Affected fleshy roots; according to the severity of damge, these were classified into five grades as noted in pages 9–10.

B. AGAR MEDIA Two kinds of agar media were prepared, namely

(1) Potato decoction with 2 per cent glucose, [1]

(2) Apricot decoction agar [2].

C. EXPERIMENTAL METHODS (1) Small bits of the mycelium from the inner part of the mycelial mat and those from rhizomorphs were placed on agar media after washing with sterilized distilled water or without any treatment.

(2) The surface of apple fruit was treated with 80 per cent alcohol, 0.1 per cent corrosive sublimate solution and sterilized distilled water. After the apple fruit was cut into desirable size using aseptic technique, some pieces of them were placed in sterilized Petri dishes, and then the rind was opened by a sterilized knife. Within the openings, the following materials were inserted respectively with or without treatments mentioned above: Small bits of the mycelial mat, fragments of rhizomorphs and the mycelial mass from the soil.

(3) After the skin of the diseased fleshy root was removed with a sterilized

1), 2) TAKIMOTO (1932) p.59, p.62.

needle, small pieces of fleshy tissue beneath the skin were picked up and then transferred to agar medium. In this experiment the diseased fleshy roots belonging to each of four degrees of severity (degree-α, β, γ and δ) were used.

(4) The glass-ring method employed generally for isolating fungi from contaminated bacteria was used.

(5) Method using the boiled skin of fleshy root of sweet potatoes as a filter. After boiling, the skin of fleshy root was peeled off carefully, and small pieces of it were placed on sterilized agar medium in Petri dishes. Setting sources of isolation on the skin, the writer expected the growth of the mycelium on agar through the filter.

(6) A small amount of sterilized agar medium was poured on pieces of apple fruit or on those of boiled fleshy root in Petri dishes, making a thin film covering the surface of them. On the agar film sources of isolation were placed. Thus, it was expected that the mycelium of the fungus would develop on the pieces of apple fruit or those of fleshy root passing through the thin agar film.

Each of the above-mentioned experiments was at least triplicated, and in one experiment, 30 Petri dishes were employed.

D. Results of the experiments Data of the experiments will be briefly shown in Table 2.

Table 2. Results of the isolation experiments from diseased sweet potatoes.

Source of isolation / Experimental method	Sporophore	Rhizomorph	Mycelial mass in the soil	Affected fleshy root			
				Degree-α	Degree-β	Degree-γ	Degree-δ
Potato agar with 2% glucose	—	—	—	—	—	+	—
Apricot decoction agaar	—	—	—	—	—	1)	—
Using apple fruit	—	—	—	—	—		—
Using agar-film on apple fruit or fleshy root	—	—	—	—	—		—
Glass ring method	—	—	—	—	—		—
Using boiled skin of fleshy root	—	—	—	—	—		—

1) Blank space, not tested; +, suceeded; —, failed.

As shown in Table 2, the writer gained pure culture of the fungus in question only in the case that small bits of fleshy root affected moderately (degree-γ) were placed on agar medium without any pretreatment, while failed in all other cases by

the contamination with various microorganisms.

This strain of pure culture of the fungus was designated M-1. Concerning the causes of success of the pure culture from the moderately diseased fleshy root(degree-γ) the writer will make some discussions in a the later part of this paper.

2. From the basidiospores

A. Experiment-1.

a. Materials Basidiospores used in this experiment were obtained from the hymenial layer on some woody plants, such as *Liriodendron tulipifera*, *Prunus Grayana* and *Thuja occidentalis* in the yard of Forest Experimet Station at Meguro, Tôkyô.

b. Experimental method The procedure was as follows: Ten cc. of 2 per cent glucose agar which had been sterilized after filtering through absorbent cotton were poured in the sterilized Petri dish and left to harden. On the inner surface of the upper lid small pieces (ca. 5 mm. sq.) of the fresh sporophore were attached quickly by means of hard vaseline in such a way that the hymenial surface was hanged thereupon the agar, when the lid was put in position. The Petri dish in this condition was kept in the incubator of 15°C.

When the basidiospores had adequately fallen from the hymenium upon the surface of the agar in the dish, the pieces of the hymenium were removed out.

At the end of about 18 hours, waiting for some growth of germ tubes, the portions of the growing germ tubes which had not been contaminated with other microorganisms were marked on the bottom of the dish under the low power microscope. A very small amount of this portion was picked up with a sterilized platinum needle and transplanted on a new 2 per cent glucose agar in Petri dishes and then placed in the incubator of 27° C. After incubated for 2 days at 27°C., the minute bits of the uncontaminated primary mycelium were transferred again to a new 2 per cent glucose agar and placed at 27°C.

Furthermore, after 2 days, with the same manners a small amount of the primary mycelium was transplanted on each of the following agar media, and then placed in the incubator of 27°C.:

(1) Potato decoction agar with 2 per cent glucose,

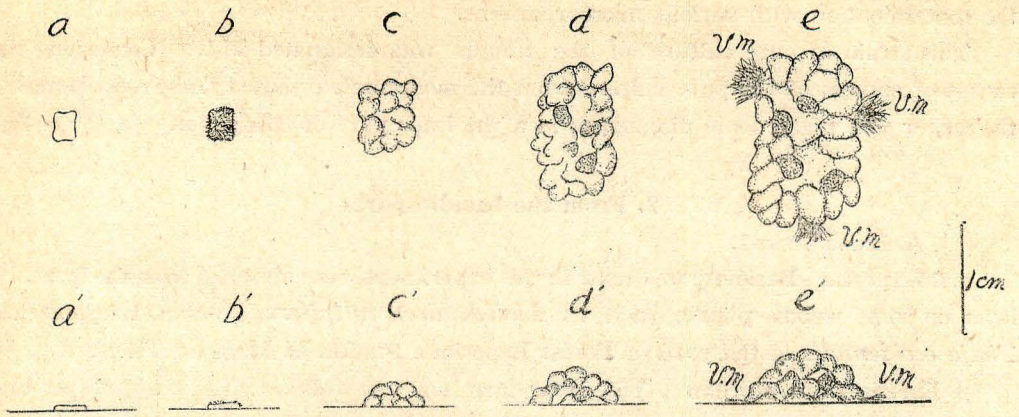
(2) mulberry bark decoction agar with 2 per cent glucose[1]),

(3) 2 per cent glucose agar, and

(4) 2 per cent peptone (Teruuchi) agar.

c. Results of the experiment By the above-mentioned procedure, the writer expected that a new growth of vegetative hyphae would take place directly from the small mass of the inoculated hyphae in a few days, as seen in the normal fungous culture however such a fact has not been observed at all beyond his anticipation, but a peculiar phenomenon occurred. Namely, on both potato glucose agar and mulberry decoction agar, the inoculated small mass of primary hyphae increased in its size gradually and became a leathery sclerotium-like body with deep purplish brown papulae. This

1) Dried bark of mulberry tree 100g., distilled water 1,000 cc., glucose 20g., agar-agar 20g.



Text-fig. 1. Stages in the development of the sclerotium-like body and the vegetative mycelium from small mass of primary mycelium on potato-glucose agar.
a-e : plane figures, á-é : side view of a-e, v.m. : vegetative mycelium.

body was very compact in tissue, protuberant, conspicuously rugged and light purplish brown in color. When the sclerotium-like body reached the size of a soy bean or that of a French bean after 7 or 10 days at 27°C., then from the bodies white cottony vegetative hyphae began to develop on the agar in contact with the sclerotium-like body (Text-fig. 1; Pl. IV, C, D). After this stage an isolation of this fungus was readily secured by transplanting a small bit of the white mycelium on potato glucose agar or mulberry decoction agar. While, on either 2 per cent glucose agar or peptone agar, such a phenomenon could not been observed in the period of this experiment. Thus three pure cultures of the fungus were gained from basidiospores on the following woody plants:

Prunus Grayana·····Strain M-2,
Liriodendron tulipifera····· Strain M-3,
Thuja occidentalis····· Strain M-4.

B. Experiment-2.

a. Materials Basidiospores used in this experiment were collected from the following diseased woody hosts: *Robinia pseudoacacia var. umbraculifera, Populus nigra,* and *Salix Bakko.* All these hosts were found in the yard of Kôma Local Forest Experiment Station in Iwate Prefecture.

b. Experimental method The method employed in this experiment is the same as in the preceding one, and the last agar media used are as follows:

(1) Potato decoction agar with 2 per cent glucose,
(2) glucose-peptone agar[1],
(3) 2 per cent peptone agar, and
(4) 2 per cent glucose agar.

These agar media planted with the bits (2-3 mm. sq.) of the primary mycelium were kept at room temperature (15°-25°C.).

c. Results of the exreriment Results of the experiments are shown in Table 3 and

1) Distilled water 1,000 cc., glucose 10g., peptone (Teruuchi) 10g., agar-agar 20g.

Table 4.

Table 3. Development of the planted bits of the primary mycelium on various agar-media (1). After 17 days at room temperature (June 16-July 3, 1946).

Agar medium / The fungus	Potato agar	Glucose-peptone agar	2 % peptone agar	2 % glucose agar
Size of sclerotium-like body (mm) 1)	12.6×10.0	8.3×6.6	3.3×2.7	2.3×2.0
Shape of sclerotium-like body	Protuberant remarkably	Protuberant remarkably	Protuberant slightly	Protuberant slightly
Color of sclerotium-like body	Light purplish brown	Light purplish brown	Light purplish brown	Light brown
Growth of hyphae	Typical vegetative hyphae present	Typical vegetative hyphae absent, but feeble creeping ones pesent	Typical vegetative hyphae absent, but feeble creeping ones present	Typical vegetative hyphae absent, but feeble creeping ones present

Table 4. Development of the planted bits of the primary mycelium on various agar-media (2). After 24 days at room temperature (June 16-July 10, 1946).

Agar medium / The fungus	Potato agar	Glucose-peptone agar	2 % peptone agar	2 % glucose agar
Size of sclerotium-like body (mm) 1)	15.3×12.8	11.3×9.3	5.0×4.0	3.5×3.0
Shape of sclerotium-like body	Protuberant and rough extremely	Protuberant and rough extremely	Protuberant slightly	Protuberant slightly
Color of sclerotium-like body	Light purplish brown	Grayish brown	Light grayish brown	Light brown
Growth of hyphae	Typical vegetative hyphae present	Typical vegetative hyphae present	Ashy feeble hyphae creeping on the agar present, but typical vegetative ones absent	Vegetative hyphae present, but very feeble

1) Showing the average diameter of 10 sclerotium-like bodies.

From Tables 3-4, it is obvious that the development of the vegetative hyphae from the inoculated primary mycelium was observed on the three agar media, viz., potato-glucose agar, glucose-peptone agar and 2 per cent glucose agar, while the growth of the vegetative hyphae did not occur on 2 per cent peptone agar.

It is also known that potato glucose agar is very suitable for the isolation of the

fungus and the other agar except glucose-peptone agar are undesirable because of requiring long period for the fungous growth.

By transplanting of the small piece of the vegetative mycelium developed from the sclerotium-like body on new potato glucose agar, several strains of pure cultures of the fungus were obtained from each of the three hosts as follows :

(1) Strain M-6 ·····*Salix Bakko*,

(2) Strain M-7 ·····*Robinia pseudoacacia var. umbraculifera*,

(3) Strain M-8 ·····*Populus nigra*.

In order to make clear the cause of the feeble mycelium doveloped from the sclerotium-like body on both 2 per cent glucose agar and 2 per cent peptone agar, the writer made further simple tests. A small piece of the feeble mycelium on 2 per cent peptone agar was transplanted to a new potato glucose agar and kept at room temperature. It became larger in size gradually and formed such a sclerotium-like body as noted previously. From this sclerotium-like body the typical vegetative hyphae grew abundantly. While, on the contrary, the feeble mycelium from 2 per cent glucose agar developed directly to the typical vegetative mycelium on potato glucose agar without formation of the sclerotium-like body.

The feeble hyphae produced on 2 per cent peptone agar and 2 per cent glucose agar were observed under the microscope. The formers were irregular in shape, variable in diameter, shorter in length, rugged, rich in vacuole and, in a word, they had abnormal forms, while the latters normal in shape.

The phenomena found in the isolation experiments from basidiospores made hitherto will be summarized in Table 5.

Table 5. Various stages in the isolation of the fungus from basidiospores.

I	II	III	IV		V	
Germinating basidiospores	Primary mycelium	Primary mycelium	Sclerotium-like body	Vegetative mycelium	Sclerotium-like body	Vegetative mycelium
2% glucose agar	2% glucose agar	2% glucose agar	potato glucose agar	potato glucose agar		
			→(+)	→(+) →(—)		→(+)
			mulberry decoction agar	potato glucose agar		
			→(+)	→(+) →(—)		→(+)
			2% glucose agar	potato glucose agar		
(+)	→(+)	→(+)	→(+)	→(±) →(—)		→(+)
			glucose-peptone agar	potato glucose agar		
			→(+)	→(+) →(—)		→(+)
			2% peptone agar	potato glucose agar		
			→(+)	→(±)	→(+)	→(+)

Note : (+)·····present, (—)·····absent, (±)·····indistinct.

It seems evident from Table 5 that the carbohydrate, such as glucose, plays an important rôle on the development of the vegetative mycelium from basidiospores of *Helicobasidium Mompa.*

C. Consideration

In spite of detailed experiments on the germination of basidiospores, Miyake (1920) failed to secure the pure culture of this fungus, perhaps, because of his failure to pursuit the development of germ tubes or primary hyphae extensively owing to the contamination of other microorganisms. In order to keep the fungus free from biological contamination for a long period, the writer examined the above-mentioned procedure suggested by the fact that the basidiospores of the fungus germinated very readily at relatively lower temperatures and they required no special substance for the germination bed.

Up to the present time, the fact that the vegetative hyphae do not develop directly from the primary hyphae, but grow after the formation of the sclerotium-like body has not been recorded, and, therefore, it might be well considered that Miyake and other investigators would probably fail to gain pure culture.

Inoculation experiments

In order to make clear the pathogenicity of the fungus isolated by the present writer, inoculation experiments were carried out on three kinds of plants, *i. e.* sweet potato, soy beans and radish.

1. Sweet potato

Twenty pots were filled with the field-soil and steamed for one hour at 15 pounds pressure. After cooling down, in the eary spring of 1943, they were planted with fleshy roots of the sweet potato (var. Okinawa Hyakugô) which had been treated with 80 per cent alcohol, 0.1 per cent corrosive sublimate solution and then thoroughly washed with sterilized water. These pots were placed in the green house at least during the cold season.

On July 14, when the formation of newly developed fleshy roots was observed, 15 pots of them were inoculated with several small bits of the mycelial colony of the fungus isolated from the diseased fleshy root (Strain M-1) growing on potato-glucose agar plate, while others were left uninoculataed to serve as controls.

After about 2 months (on September 16) the plants were examined in detail by the naked eyes. Most of the plants in the inoculated pots, except the ones in the condition of insufficient supply of water, were infected in different degrees of severity, while no sign of the disease was observed in the controls.

On the surface of the infected roots and stems, numerous purplish brown rhizomorphs were formed in network. In the case of heavy affection, a great many of sclerotia in size of pin-head were observed not only on the fleshy roots but also on the stems. Finally the infected fleshy roots were decayed severely.

Most of the infected plants showed no remarkable change in the appearance above the ground, but a few of them those subterranean portions had been perfectly destroyed were led to death afterwards (Pl. V,A) .

2. Soy beans

On April 30, 1943, seeds of soy beas which had been sterilized with 80 per cent alcohol, 0.1 per cent corrosive sublimate solution, and then washed several times with sterilized water were sown in each of the 15 autoclaved pots. On June 14, in 10 pots of them inoculation was made by placing small bits of mycelium obtained from a pure culture of the fungus (Strain M-1) and other pots were remained as controls.

On September 16, the effect of the inoculation upon the soy bean plants was examined. Both large and slender roots of the soy beans were covered with purplish brown rhizomorphs of the fungus. Especially on the large roots, the rhizomorphs were observed obviously. In some parts of the heavily attacked roots, the parenchymatous tissues were destroyed so completely that only the periderm and the stele portion remained their original shape. On the surface of the roots pin-head-shaped sclerotia were formed very abundantly. On the contrary the controls gave no signs of infection(Pl. V, B).

3. Radish

Seeds of radish (var. Tokinashi) were prepared for inoculation test. With the same manners mentioned above, the seeds were sterilized and sown in the pots on April 30, 1943. On June 14, inoculation was made on the soil of the pots near the growing radish plants. At the end of one month after inoculation, on July 13, the roots of radish were covered with some of the typical rhizomorphs of the fungus, but were not yet softened or decayed at all. In controls no remarkable changes were observed (Pl. V,C).

Regarding the other strains of the fungus isolated from basidiospores obtained from woody plants (Strain M-2, M-3, and M-4), the writer made also some inoculation experiments on soy bean seedlings and could prove their pathogenicity.

Cultural characteristics of the fungus

In order to know the general cultural characters; rate of growth, type of growth, consistency of colony, surfacer character, and pigmentation, the writer made some cultural experiments on various kinds of substrata, both liquid and solid. In the experiments the strain M-1 of the fungus was used.

1. On agar media

Ten cc. of the medium were poured into each of 5 test tubes (17 mm. in diameter), and they were sterilized under 15 pounds of steam pressure for 15 minutes and slanted. For the inoculum the margin of the mycelial colony on potato sucrose agar plate was cut with a sterilized needle into small pieces about 4 mm. sq., and these were planted and kept at 27°C. for 2 months.

The kinds of culture media used for the experiment and each of their formulae[1] are as follows:

Czapek's agar; Distilled water 1,000 cc., $MgSO_4 . 7H_2O$ 0.5g., K_2HPO_4 1g., KCl 0.5g., $NaNO_3$ 2g., sucrose 30g., $FeSO_4$ 0.01g., agar-agar 20g.

Bouillon agar; Distilled water 1,000 cc., peptone 10g., Liebig's meat extract 10g.,

1) Takimoto (1932).

NaCl 5 g., aga-ager 20g.

WAKSMAN's agar; Distilled water 1,000 cc., glucose 10g., peptone 5 g., KH_2PO_4 1 g., $MgSO_4 . 7H_2O$ 0.5g., agar-agar 20g. (pH 5.6).

Apricot agar; Water 1,000 cc., dry apricot 25g., agar-agar 20g.

Potato-sucrose agar; Potato decoction 1,000 cc., sucrose 20 g., agar-agar 20g.

Carrot-sucrose agar; Carrot decoction 1,000 cc., sucrose 20g., agar-agar 20g.

Results of the observation will be briefly described as follows:

(a) On CZAPEK's agar: After 7 days, the white mycelium grows scarcely, and the agar becomes light Dark Grayish Brown in color. After 1 month, the growth of mycelium well develops, and covers almost all surface of the medium. Aerial hyphae are abundant. Color of the colony is Sorghum Brown, with Mars Orange amorphous bodies among the hyphae. Agar medium becomes Olivaceous Black to Fuscosus Black in color, but the lower layer of it colorless. After 2 months, the colony is colored Verona Brown in cottony appearance. Mycelia near the inoculum are rather compact and deep in color. Mars Orange amorphous bodies are abundant. Later, the media change the color to Blackish Brown, and the lower portion of the test tube is very light in color.

(b) On bouillon agar: After 7 days, the mycelium is very feeble and Light Purple-Drab in color. The color of agar is Dark Vineceous Drab in small area. After 1 month, the growth of colony is not well, near the inoculum aerial hyphae present and light Indian Red in color, while the white mycelium from the marginal portion of the colony is creeping on the agar. After 2 months, the colony of the fungus is not large, Hessian Brown in color. Mars Orange amorphous bodies are absent. Color of the agar is Warm Blackish Brown, but it is limited to the portion just under the inoculum.

(c) On WAKSMAN's agar: After 7 days, the mycelial growth is well in Pale Grayish Vinaceous color, the agar changing from Cinnamon-Buff to Pinkish Cinnamon. After 1 month, the growth of mycelium well develops, aerial hyphae are dense, hyphae near the inoculum being bushy and Prussian Red in color. The marginal portion of the colony accompanied with numerous little watery drops is white. Mars Orange amorphous bodies among hyphae are present abundantly. The color of the agar is Dark Indian Red, while lower ends of it colorless. After 2 months, the colony is rather compact, Hessian Brown, especially near the inoculum, small watery drops are formed here and there on the mycelium. Numerous Mars Orange amorphous bodies are found, and color of the agar is Diamine Brown.

(d) On apricot agar: After 7 days, the growth of mycelium is very scanty, aerial hyphae relatively much, while creeping hyphae scarce, the mycelium is Pale Grayish Vinaceous in color, and agar almost colorless. Even after 1 month, the colony of the fungus is small in diameter, light Indian Red aerial hyphae are found near the inoculum. Near the inoculum the creeping hyphae are present, and a small number of Mars Orange amorphous bodies are seen, agar being slightly brownish. After 2 months, the mycelial growth is rather poor as it was after 1 month, Dark Vinaceous Brown in color, and agar under the inoculum is colored.

(e) On potato-sucrose agar: After 7 days, as in the case of WAKSMAN's agar the

mycelial growth is well, while discoloration of the agar is lighter than the former case. After 1 month, growth of the mycelium is very vigorous, aerial hyphae well develop, having a tendency to grow erect, the mycelial felt in velvety appearance is Ocher Red in color, and on the mycelial colony sclerotia being Madder Brown in color are formed. Numerous Mars Orange amorphous bodies are present among hyphae, even on the portion of the agar and the glass wall where there is no growth of the mycelium. The agar is brilliantly Dark Corinthian Purple in color, except the ends of the tube which are Deep Hellebore Red. After 2 months, remarkable differences in the colony are not recognized, but sclerotia develop. The whole agr medium is colored in Hessian Brown to Dark Corinthian Purple (Pl. IV,E,F) .

(f) On sweet potato-sucrose agar: After 7 days, the growth of the colony well develops, agar being almost no colored. After 1 month, the mycelial growth is very vigorous, aerial hyphae well develop, and Prussian Red to Brick Red in color. On the colony there are some brick reddish patches of mycelial mass, but less compact than those on potato-sucrose agar. Mars Orange amorphous bodies are abundant, color of the agar being Neutral Red to Indian Purple. After 2 months, all the aerial hyphae tumble down on the medium, color of the colony is Chestnut Brown, and agar Maroon to Hessian Brown in color.

(g) On carrot-sucrose agar: After 7 days, the growth of the mycelium well develops, with resembrances to that on potato-sucrose agar in color of the mycelial colony and the agar. After 1 month, the mycelial growth is vigorous, but less than in the case of potato-sucrose agar. The colony is cottony, Ocher Red in color, with numerous Mars Orange amorphous bodies, and whole agar medium becomes Corinthian Purple in color. After 2 months, the cottony aerial hyphae develop abundantly with Chocolate color. Mars Orange amorphous bodies are enormously present, and discoloration in the agar is Hay's Maroon.

2. On liquid media

One hundred cc. of each of the three kinds of the solution, viz., sweet potato-sucrose solution, CZAPEK's solution and modified RICHARDS' solution were poured into 150 cc. Erlenmeyer flasks, and each solution was set up at least in quadruplicate. After sterilization they were inoculated with the fungus (Strain M-1), and were kept at about 27°C. At the end of a month's culture notes were taken and the results were as follows:

(a) On sweet potato-decoction with 2 per cent sucrose: Growth of the mycelium well develops, the colony appears to be light brownish purple and rather compact. The mycelium extending in solution is very scarce, and the culture solution becomes Dragon's blood Red in color.

(b) On CZAPEK's solution: The mycelial growth is not less vigorous than the preceding solution. The loose colony covers only a small part of the medium surface, and it is light Diamine Brown in color. In the solution some hyphal masses are present. The solution is colored in light Sorghum Brown.

(c) On modified RICHARDS' solution: The composition of this solution is as follows:

Distilled water 1,000 cc., KNO_3 5g., KH_2PO_4 2g., $MgSO_4.7H_2O$ 1g., glucose 10g. The result of the experiment was observed after both 20 and 40 days.

At first the mycelial growth well develops, but after two weeks it stops further growth. Forty days after inoculation the colony is brownish, and turns the color of the medium to light brown. Mars Orange amorphous bodies are formed abundantly. By MOLISCH's reaction, the existence of sugar in the staled culture solution is affirmed. By adding small mounts (0.5-1.0%) of calcium carbonate in the culture solution, the retardation of the mycelial growth with the lapse of time is not remarkable, and consequently the harmful action is probably due to substances produced by the fungus in the course of metabolism.

3. On various solid media

(a) Steamed potato tuber: After washing thoroughly with water, potato tuber was cut into pieces (15×10×10 mm.). Two or three pieces with or without skins were placed in each of Erlenmeyer flasks of 150 cc. capacity, and small amount of distilled water was poured into the flasks. They were sterilized by the ordinary steam autoclave, inoculated, and kept at 25°C. Uninoculated flasks were prepared as controls.

The mycelial growth develops well, the colony is at first almost colorless, and with the lapse of time turns to deep brown. At the end of 55 days, the inner part of potato pieces is colored to deep brownish purple, and softened remarkably. The corner of the block is disappeared by the dissolving action of the fungus, while not infected ones unchanged. On the blocks with skin the mycelial masses of rather compact structure are found, but none of them are found on the blocks without skin.

(b) Steamed fleshy root of sweet potato

As compared with the case of potato tuber, there is no remarkable difference between the two experiments.

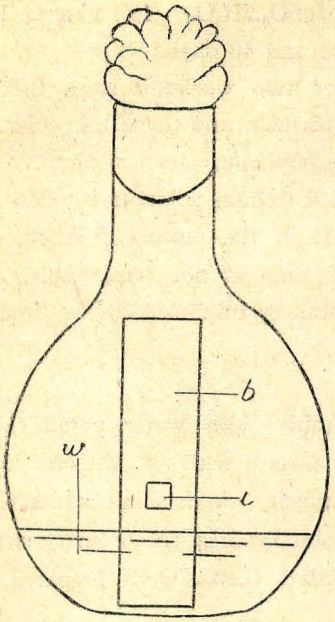
(c) Sterilized soil

As substratum the following materials were placed in test tubes: Ten volumes of sand, 3 volumes of humus, 1 volume of sectioned fleshy root of sweet potato and sweet potato decoction. After sterilization with the ordinary manners, the fungus was inoculated and kept at 27° C. in the dark room for 30 days.

At the end of the experimet, the mycelium develops well and the mycelial masses are formed in the spaces of the substratum. Between the substratum and the wall of glass, numerous mycelial strands or rhizomorphs, Indian Purple to Mars Violet in color, are found. These mycelial strands resemble considerably those formed on the infected fleshy roots of sweet potato in nature.

(d) Steamed bark of mulberry tree

The bark of mulberry tree was cut into adequate size (ca. 15×60 mm.). One piece was placed in each of KITAJIMA's flasks (1930) containing a small amount of distilled water. After sterilization by the ordinary steaming method for 20 minutes, a bit of the mycelium of the fungus was inoculated on the inner part of the bark and then kept at 25°C.

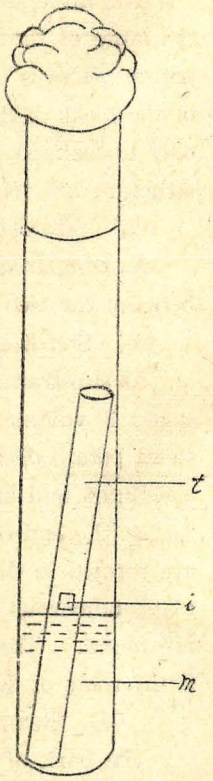


Text-fig. 2. Culture of the fungus on mulberry bark.
b: bark of mulberry tree, w: distilled water, i: inoculum of the fungus.

In Text-figure 2, *b* is a piece of mulberry bark, *w*, distilled water and *i*, an inoculum of the fungus.

The mycelial growth on the mulberry bark is very vigorous, having abundant aerial hyphae. At the end of 8 weeks the colony becomes brownish purple in color, accompanying with some deep brownish sclerotia. From the fact mentioned above, the bark of mulberry tree seems to be a very favorable medium for the growth of the fungus.

(e) Steamed twig of tree As shown in Text-figure 3, twenty-five cc. of potato decoction with 1 per cent sucrose (*m*) were poured into each of large test tubes (200 mm. long, 20 mm. diam.), a twig of tree (*t*), 15 cm. long and 10 to 15 mm. in diameter, was inserted in the medium. After steaming with the ordinary manners, a small bit of the fungus (*i*) was inoculated on the surface of the culture solution, and then incubated at 25°C.



Text-fig. 3. Culture of the fungus on twig of tree.
t: twig of tree,
m: culture solution,
i: inoculum of the fungus.

In this experiment seven kinds of plants were used; viz., *Cryptomeria japonica*, *Liriodendron tulipifera*, *Robinia pseudo-acacia*, *Cinnamomum Camphora*, *Prunus yedoensis*, *Quercus myrsinaefolia*, and *Pseudosasa japonica*. In each kind of plants, the experiments were done at least triplicated. The results observed at the end of 3 months are as follows:

(i) On the twig of *Cryptomeria japonica*: Under portion of the twig is covered with the mycelial mat. It is brownish and rather compact near the surface of the solution, while the remaining portion with abundant aerial hyphae is pinkish. A few sclerotia are formed (Pl. VI, B).

(ii) On the twig of *Liriodendron tulipifera*: In the apperance, the growth of the mycelium, coloration of the colony, and formation of the sclerotium are similar to the case of *Cryptomeria japonica*. Furthermore, in this case, deep brownish watery-drops are formed on the mycelial mat.

(iii) On the twig of *Robinia pseudoacacia*: The mycelial mat developed on the twig is purplish, especially the upper portion being deep in color, while the margin of the mycelial felt becomes brownish purple. No sclerotium occurs.

(iv) On the twig of *Cinnamomum Camphora*: The growth of the mycelium is rather scanty, the mycelial felt with the light pinkish margin is as a whole brown in color. Sclerotia are not formed.

(v) On the twig of *Prunus yedoensis*: The development of the mycelial mat is not good, and the portion near the solution is brownish purple, while the margin of the mat still white, aerial hyphae are scarce, no sclerotium being formed,

(vi) On the twig of *Quercus myrsinaefolia*: The mycelial growth is good, and the upper portion of the colony is light pinkish, while the remaining part deep brownish purple, aerial hyphae feeble, and some sclerotia are found.

(vii) On the stem of *Pseudosasa japonica*: The mycelium develops vigorously, the stem is covered uniformly with the brownish mycelial mat. Mycelial strands or rhizomorphs are formed on the surface of the mat, and several sclerotia are seen distinctly (Pl. VI, A).

From above-mentioned data and the appearances showing in the photographs (Pl. VI, A-B) it is interesting to note that the mycelial mats produced on the twig in this experiment are very similar in many respects to the fruit-bodies found on the host plants in nature, though the formers are rather soft and loose in structure as compared with the latters. But, the formation of the hymenial layers on the twigs in test tubes has not ever been discovered up to the present. Why the mycelial mats on the twigs in test tubes are looser in structure than those found in nature ? Why the hymenium has not been formed in this experiment ? Although the present writer has been unable to explain these questions exactly, they may be probably due to the environmental influences in the tubes, especially to humidity and temperature conditions.

(f) Ungrazed ceramic rod soaked in the solution

By applying the same method described in the preceding pages, but by using ungrazed ceramic rods (10 cm. in length, 10 mm. in diameter) instead of twigs, the similar cultural experiment was undertaken. In this case, as culture medium, sweet potato decoction with 1 per cent sucrose was poured in the test tubes. After 2 months at 27°C. the experiment was finished.

The results of the experiment are briefly noted as follows: The mycelium covered over the rod is rather compact and deep brownish purple in color. Mycelial strands or rhizomorphs are formed on the superficial portion of the mat. These appearances on the rod bear remarkable resemblances to the fleshy root of sweet potatoes attacked by the fungus in nature (Pl. VI, C).

Morphology of the fungus

As regards the morphology of the fungus in question, since TANAKA's first record, some notes have been made by SAWADA (1919), MIYAKE (1920) and some authors in their hand-books. Especially MIYAKE's one is very sufficient and accurate in description, and, therefore, there seems to be hardly necessary to take further explanation on it. The writer will make only a brief description of the morphological characters of the fungus, chiefly of those untouched by MIYAKE (1920).

1. Sporophores or fruit-bodies

The sporophore of the fungus develops surrounding the basal portion of the trunk of the diseased tree up to a height of 10 cm. or more, sometimes leaving here and there small narrow parts uncovered, and often embracing some extraneous matters, such as decayed leaves, twigs, weeds, gravels and particles of soil.

The size of the sporophore covered the trunk of the diseased tree depends mainly on the degree of nutrition and environmental conditions, especially on the atmospheric moisture. In the thick forest it is not rare that the fruit-body creeps up the trunk as far as several decimeters above the ground (Pl. II, A,B,C; Pl.III,A, etc.).

The fruit-body is sessile, resupinate, often irregularly lobed, velvety, and membranaceous. The surface of the fruit-body which was deep purplish brown in early spring becomes whitish or light pink in color during the later part of the same season without developing its size, appearing as if white powder being scattered. This is due to the formation of the hymenial structures (Pl. II, D; Pl. III, B,D).

In Tôkyô, the formation of basidiospores is observed in late May to early July, and after spores are discharged, the hymenial surface becomes gradually purplish brown again. Subsequently the surface is covered by whitish mycelium and the fungus continues its further growth. Early in September, the whitish mycelium develops vigorously and in the later part of the same month it encloses sometimes adjacent decayed leaves and dust, and finally it becomes brilliant purplish brown in color. The purplish brown mycelium overwinters and, in the next spring, the basidial stage will be formed again on the surface of this mycelial mat (Pl. II, D,E,F; Pl. III, A,B,C,D).

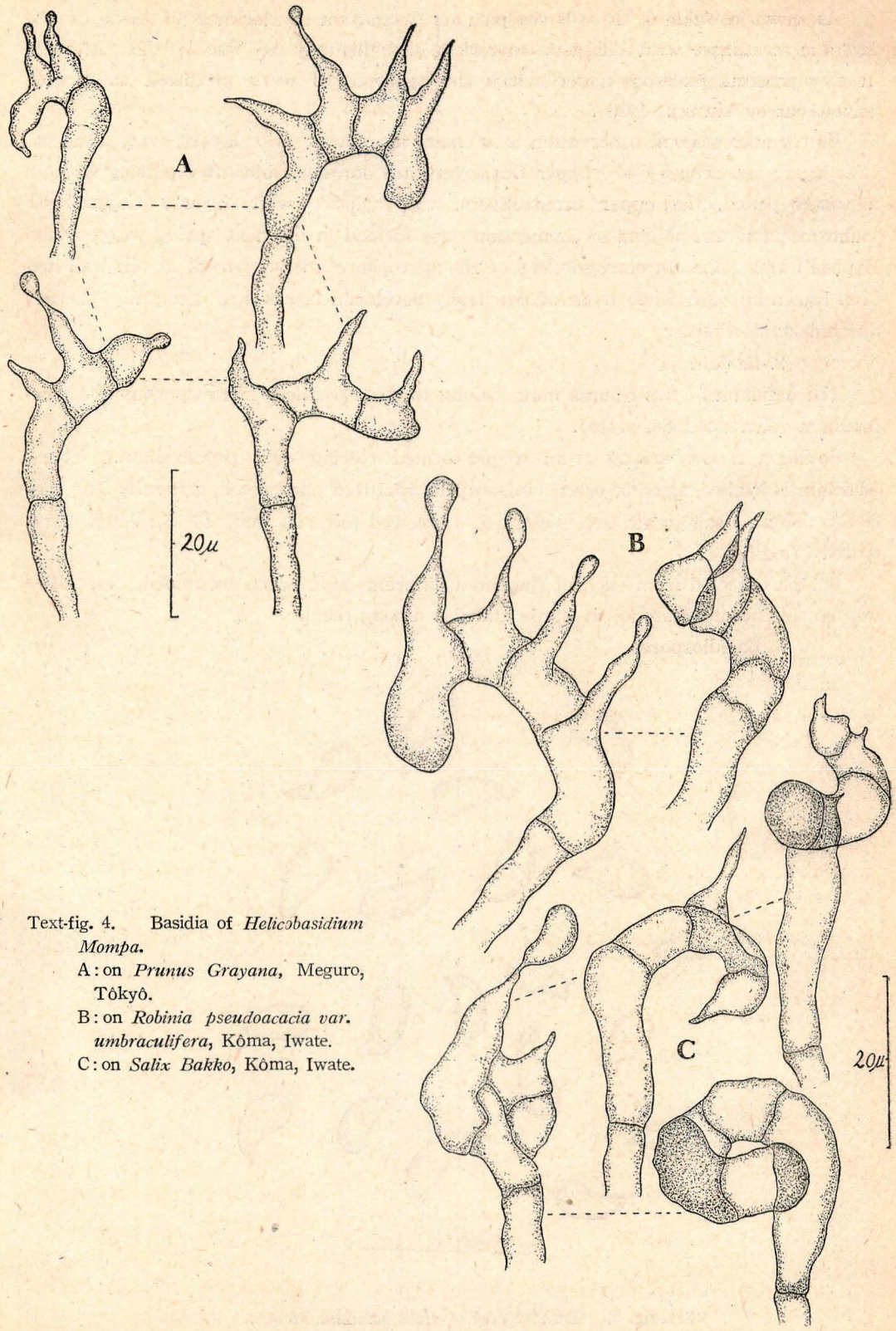
By sectioning microtomically, the spore-bearing sporophores were examined carefully under the microscope, and as already pointed out by MIYAKE (1920), four or five layers were distinguished. MIYAKE named each of them from inside to outside as follows: Henpei-sô (flat layer), henpei-jô-sô (upper flat layer)[1], ishi-sô (weft layer), shijitsu-ka-sô (subhymenium), and shijitsu-sô (hymeninm); the first two layers may be probably unified under the name of subiculum.

Concerning four sporophores collected from the same numbers of hosts, the measurements of thickness of these layers were undertaken by the present writer, and the results will be given briefly in Table 6.

Table 6. Measurement of thickness of layers organizing the sporophore.

Kind of layer	Host							
	Liriodendron tulipifera		*Prunus Grayana*		*Robinia pseudoacacia*		Concrete-foundation	
	Range	Average	Range	Average	Range	Average	Range	Average
2) Hymenium / 3) Subhymenium (μ)	46–92	72.5	25–50	39	37–62	50	87–125	92
4) Weft layer (μ)	46–125	80.0	37–75	54	37–75	54	92–125	117
5) Upper flat layer (μ)	92–152	141.0	–	–	92–187	138	187–225	210
6) Flat layer (μ)	400–600	–	500–800	–	800–1200	–	800–1200	–

1) This layer is sometimes indistinct. 2), 3), 4) The upper three layers are formed in May to July. 5), 6) The lower two layers have passed the winter.



Text-fig. 4. Basidia of *Helicobasidium Mompa.*
A: on *Prunus Grayana,* Meguro, Tôkyô.
B: on *Robinia pseudoacacia var. umbraculifera,* Kôma, Iwate.
C: on *Salix Bakko,* Kôma, Iwate.

As shown in Table 6, there is conspicuous fluctuation in thickness of each of the layers in accordance with different materials, and this may be due to the difference in environmental conditions under which the sporophores were produced as already pointed out by MIYAKE (1920).

By the microscopical observation it is clear the lower two layers, viz., henpei-sô (flat layer) and henpei-jô-sô (upper flat layer) are dormant, and after passing through a winter period, the upper three layers, viz., ishi-sô (weft layer), shijitsu-ka-sô (subhymenium) and shijitsu-sô (hymenium) are formed in the next spring successively. Hyphae forming the dormant portions of the sporophore are very thick in cell wall and deep brown in color, while those of the newly developed layers are generally slender, and almost colorless.

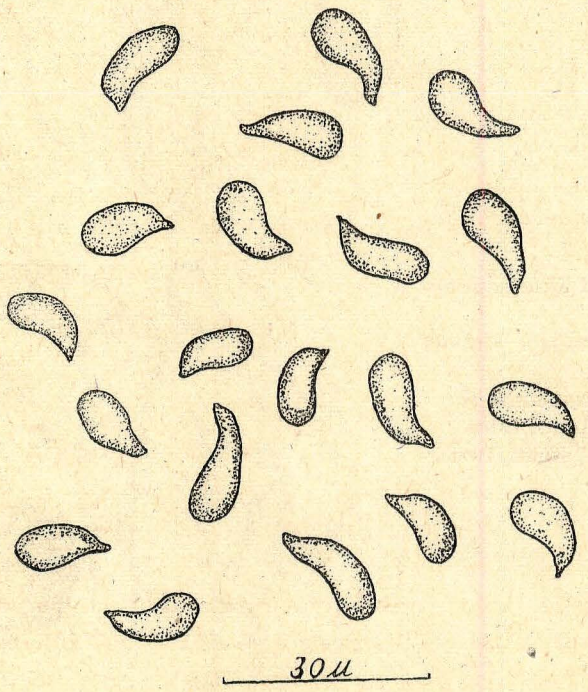
2. Basidia

The basidium of this fungus may belong to the so-called pleurosporous phragmobasidium (GÄUMANN 1928, p.414).

Basidium is not arising from a pre-formed resting cell, probasidium. Young basidium is hyaline, smooth, erect, club-shaped; matured one curved, generally 3-septate, 30–50×5–10μ: 4 sterigmata are elongated, narrowed towards end, 10–15μ long, tetraspored (Text-fig. 4).

Basidia collected from each of the three different hosts were examined, but there was no significant difference in shape and size among them.

3. Basidiospores



Text-fig. 5. Basidiospores of *Helicobasidium Mompa.*

Basidiospores are hyaline, ovoid, slightly curved and filled with homogeneous protoplasm; granules and vacuoles are not seen generally. Cell wall is smooth, thin, but the attaching portion of it is more or less thick (Text-fig. 5). Sometimes the spores of abnormal shape bearing large vacuoles are observed, but these are unable to germinate.

The measurements of spore size on materials obtained from various hosts and localities were carried out. The results will be briefly shown in Table 7.

Table 7. Biometric data for length and width of basidiospores.

(a) Length in microns

Host plant	Locality	Number measured	Range	Mean	Standard deviation	Coefficient of variability
Thuja occidentalis	Meguro, Tôkyô	100	14.8—24.4	18.23±0.12	1.83±0.08	10.00±0.47
Prunus Grayana	do.	100	14.0—22.0	17.77±0.11	1.62±0.07	9.13±0.48
Liriodendron tulipifera	do.	100	14.0—21.6	17.54±0.10	1.52±0.07	8.71±0.41
Salix Bakko	Kôma, Iwate	100	12.6—23.8	16.36±0.15	2.21±0.11	13.50±0.65
Populus nigra	do.	100	12.2—22.2	17.12±0.12	1.71±0.08	9.98±0.48
Robinia pseudoacacia var. umbraculifera	do.	100	12.0—20.4	16.14±0.11	1.68±0.08	10.40±0.50

(b) Width in microns

Host plant	Locality	Number measured	Range	Mean	Standard deviation	Coefficient of variability
Thuja occidentalis	Meguro, Tôkyô	100	5.2—9.2	6.39±0.06	0.85±0.04	9.33±0.45
Pruns Grayana	do.	100	5.6—8.0	6.56±0.05	0.82±0.04	12.42±0.63
Liriodendron tulipifera	do.	100	6.0—8.4	6.64±0.04	0.57±0.03	8.55±0.41
Salix Bakko	Kôma, Iwate	100	5.2—8.2	6.36±0.05	0.79±0.04	12.34±0.62
Populus nigra	do.	100	4.8—7.2	6.80±0.05	0.67±0.03	9.85±0.47
Robinia pseudoacacia var. umbraculifera	do.	100	4.2—6.6	5.48±0.05	0.65±0.03	12.59±0.63

As is obviously seen from Table 7, there are considerable fluctuations in the dimension of the basidiospores, but statistically no remarkable differences are recognized among the materials from various hosts and localities.

Size of the basidiospores of *Helicobasidium Mompa* measured by several workers is shown in Table 8.

Table 8. Dimension of basidiospores of *Helicobasidium Mompa* given by other investigators.

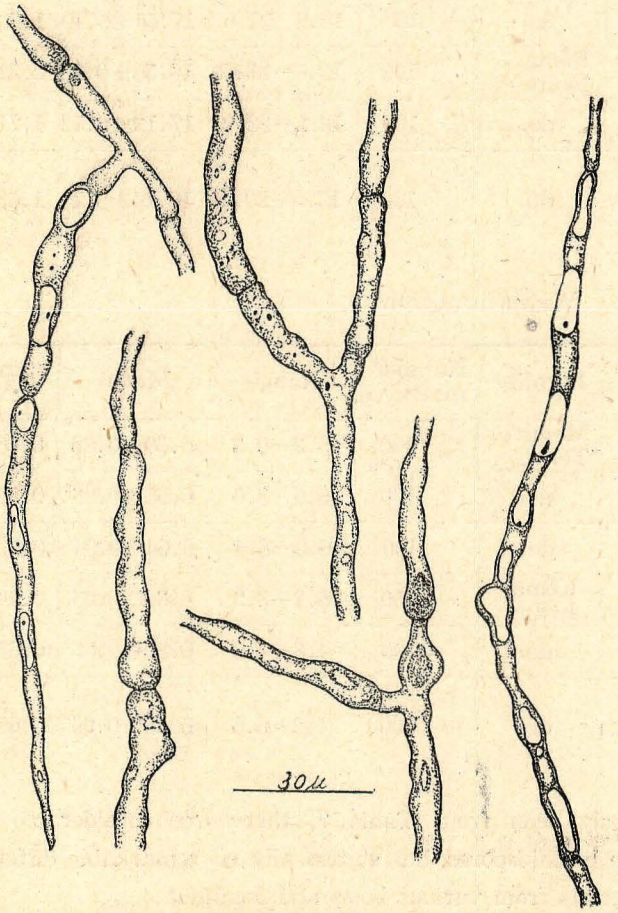
Investigator	TANAKA(1891)	SAWADA(1919)	MIYAKE(1920)
Size of basidiospores (μ)	10—12×5—7	13—25×4.5—7	15—29×5.5—7

The results of the measurement made by the present writer are nearly agree with SAWADA's one. As discussed already by MIYAKE (1920), the dimension noted by TANAKA (1891) seems to be too small.

4. Hyphae

Germ tubes or primary hyphae are very slender at first, but after some lapse of time suddenly increase in their width, if the condition is favorable for them. Hyphae which form sclerotia are very irregular in shape.

Shape and color of the vegetative hyphae on culture media are considerably



Text-fig. 6. Hyphae of *Helicobasidium Mompa* growing in extremely small amounts of free oxgen.

variable according to the cultural conditions and, for example, hyphae growing in extremely small amouts of free oxygen are as shown in Text-figure 6.

Hyphae composing the inner part of sclerotia produced on culture media are very large in width. Clamp connections are not seen in all kinds of hyphae, both primary and vegetative.

Morphology and dimension of the hyphae of the fungus (Strain M-1) are briefly shown in Table 9 (Pl. VI, G).

Table 9. Morphological notes and dimension of hyphae on culture media.

Hyphae	Diameter in microns			Length in microns			Remarks
	Max.	Min.	Mode	Max.	Min.	Mode	
Young hyphae on sweet potato-glucose agar	7.2	1.4	4.4	288	48	60	Almost colorless, range of dimension is wide, granular in content; branchings at a right angle and H-shaped hyphae are seen.
Old hyphae on sweet potato-glucose agar	7.2	1.8	5.2	132	50	101	Brownish, smooth branchings at right angle and anastomosings in H-shape present.
Hyphae forming mycelial strand on soil medium	9.6	2.4	5.2	150	28	82	Purplish brown hyphae abundant, branchings at a right angle with the main axis present.
Hyphae of the under layer of the mycelial mat on ungrazed ceramic rod	11.2	2.4	5.4	—	—	—	Purplish brown, granular in contents, very irregular in shape, having frequently globose cells; right-angle branchings present.
Hyphae of the upper layer of the mycelial mat on ungrazed ceramic rod	7.2	1.8	5.4	130	76	92	Brownish, range of dimension small; smooth in appearance, right-angle branchings and anastomosings in H-shape present.

5. Sclerotia

a. Sclerotia formed on diseased plants

Concerning sclerotia formed on fleshy roots of sweet potatoes and roots of soy beans which had been naturally or artificially inoculated, a comparative study was made by the writer, showing no significant difference among them.

Sclerotia are typically mushroom-shaped, spherical or semi-globular in the upper

portion, but frequently are irregular and unite with others (Pl. VI, D).

The dimension of sclerotia obtained from the diseased sweet potato will be shown in Table 10.

Table 10. Dimension of sclerotia on sweet potato.

Sclerotium	On fleshy roots			On stems		
	Max.	Min.	Average	Max.	Min.	Average
Diameter of the upper portion, "pileus" (μ)	450	150	300	290	200	240
Diameter of the foot-portion, "stipe" (μ)	180	45	90	100	40	60
Height of the whole sclerotium (μ)	300	150	220	310	150	240

When examined the microtomic sections of the sclerotium, four parts are differentiated as follows:

(1) Vegetative mycelium layer; the external portion composed of loose purple hyphae.

(2) Rind; very firm tissue of deep brownish hyphae, which are thick-walled and large in diameter.

(3) Pseudoparenchymatous part; that is composed of light brownish pseudoparenchymatous hyphae, cell walls of them are thinner than those of the rind.

(4) Prosenchymatous or prosoplectenchymatous core; the greater part of the sclerotium occupied and formed of laminated hyphae. Hyphae of this portion are colorless and slender, and connected with invading hyphae.

b. Sclerotia on culture media

Sclerotia produced on potato agar are at first small, white, and form loose mycelial masses, then become white knots without stalklets by which they attach to aerial hyphae or substrata. During the course of their ripening some watery drops of deep brown color are exuded from the surface of sclerotia. They increase gradually in size and become more compact, hard in tissue and deep brown in color. Watery drops on the surface of sclerotia finally disappear.

Matured sclerotia are convex, covered by velvety brown hyphae, having some small pores through which watery drops were exuded. They are $3.0-4.5 \times 3.0-8.5$ mm. in dimension, and the inner part of them is charcoal black (Pl. IV, E).

Comparing with sclerotia found on diseased plants, those produced on agar media show some differences in several respects, viz., in size, color, and solidity. The writer examined microtomic sections of them under the microscope.

In the case of sclerotia on diseased plants, there are some differentiations among their tissues, while, on the contrary, no remarkable differentiations are seen in this case, *i.e.*, the latters are covered by small amounts of loose purplish brown hyphae on the

surface and, in the inner part of them, are filled equally with large deep brown hyphae, which are frequently more than 30μ in diameter (Pl. VI, E).

Consequently, it may be probably concluded that there is conspicuous difference between the sclerotium on the diseased plant and the one on culture media.

6. Mycelial strands or rhizomorphs

Mycelial strands creeping on the diseased plants and those on the artificial media are purplish brown and have a wide range of diameter from 0.1 mm. to 1.2 mm. (Pl. I,B ; Pl.IV, B ; Pl. V, C).

Comparing with that of *Armillaria mellea*, the rhizomorph of the fungus in question is very simple in structure and there is no noticeable differentiation in tissues, and consequently it must be said that it is only an aggregation of numerous entangled hyphae (Pl. VI, F).

On the taxonomy of the fungus

For the fungus in question following scientific names have been used by various authors :

1. *Helicobasidium Mompa* Tanaka

Tanaka (1891), Shirai (1903), Massee (1910), Miura (1917), Sawada (1919), Endô (1927), Nakata and Takimoto (1928), Hara (1927, 1930, 1931, 1936), Sorauer (1932), Bokura (1934), Hiura (1939), Nakata (1941), Hino (1942), Togashi *et al.* (1942).

2. *Stypinella Mompa* (Tanaka) Lindau

Lindau (1908), Nanbu (1917), Miyabe and Ideta (1923), Kitajima (1933), Tochinai (1938).

3. *Stypinella purpurea* (Tul.) Schroet.

Hori (1921), Nishida (1921).

4. *Septobasidium Mompa* (Tanaka) Racib.

Raciborski (1909), Saccardo (1912), Miyake (1920), Bokura (1921), Tsuji (1926), Engler und Prantle (1928), Kitajima (1938).

As mentioned above, this fungus was described by Tanaka in 1891 as a new species to science under the name of *Helicobasidium Mompa*. Later, by Lindau (1908), it was transferred to the genus *Stypinella* which was erected by Schroeter in 1887. However, now it is the general opinion that the genus *Stypinella* is to be treated as a synonym of the genus *Helicobasidium* which was established by Patouillard in 1885 (Saccardo 1888, Patouillard 1900, Engler u. Prantle 1928, Gäumann 1928, etc.).

Raciborski (1909), discovering a fungus which is said to be the same to the present fungus in Java, transferred it to the genus *Septobasidium* and emended the name as *Septobasidium Mompa* (Tanaka) Racib. He noted also that *Septobasidium bogoriense* Pat. might be the same as this fungus. Concerning the scientific name of this fungus Saccardo (1912) adopted Raciborski's opinion.

Miyake (1920), making a detailed study on the fungus, reached the conclusion that his result was well coincident with Raciborski's opinion, but not with Tanaka's one. Miyake's conclusion is chiefly based upon the character of the sporophore of the

fungus, which is not gelatinous, but subcoriaceous.

According to SACCARDO (1888, 1895) and PATOUILLARD (1900) the descriptions on the genera *Helicobasidium* and *Septobasidium* are as follows:

The genus *Helicobasidium*.

SACCARDO (1888)

Fungi resupinati, incrustantes, ceraceo-molles. Basidia initio recta, dein sursum eximie incurvata et ex arcu sursum sterigmata 2-4 gerentia, septulata. Sporae ovoideae, curvulae, hyalinae. .....

PATOUILLARD (1900)

Réceptacle floconneux, indeterminé, non gélatineux, basides cylindriques plus ou moins incurvées, à stérigmates subules, placés sur la partie convexe; spores incolores, lisses, ovoïdes, germant par renovation ou par production directe du filament mycélien. .....

The genus *Septobasidium*.

SACCARDO (1895)

Pileus effusus, resupinatus, coriaceus, non gelatinosus. Hymenium disjunctum; basidiis primitus globuiosis et simplicibus dein cylindraceis et transverse septatis, rectis v. curvulis, sterigmata inconvexitate gerentibus. Sporae continuae, oblongae, hyalinae .....

PATOUILLARD (1900)

Réceptacle étalé, sec, rayonnant, crustacé, à subiculum de fibres rigides dressées, supportant une croûte membraneuse mince, fragmentée. Probaside à bourgeonnement tardif, persistante ou transitoire et s'allongeant en baside cylindrique droite ou courbée, septée transversalemant. Spores acro-pleurogènes, incolores, ovoïdes ou virgultiformes, germant en se renouvelant en même temps qu'elles prennent 1 — 3 cloisons transversales. .....

As underlined by the present writer, there are seen somewhat differences in the character of the sporphore between SACCARDO's description and PATOUILLARD's one. It seems to be correct for us to follow the description of PATOUILLARD, by whom these two genera were established. Hereupon, MIYAKE's opinion must be criticized thoroughly.

As pointed out by COUCH (1929), it is quite difficult to separate the genus *Septobasidium* from *Helicobasidium* satisfactorily with decision but it seems best, for the present at least, to follow PATOUILLARD's (1900) opinion that the presence of the probasidium in *Septobasidium* and its absence in *Helicobasidium* are the chief distinguishing characters between the two genera. GÄUMANN (1928), etc. also recognized the formation of the probasidium in fungi to be one of the most important characters from the phylogenetic standpoint of view.

In the case of the fungus in question, no formation of the probasidium has been

observed by earlier workers up to the present time. In spite of the careful investigations by examining of microtomic sections of the sporophores of the fungus collected from various kinds of hosts, the present writer has not yet found the formation of the probasidium.

Furthermore, it may be doubtful in many respects whether RACIBORSKI's fungus in Java is the same as the causal organism of the "Murasaki-monpa" disease or not, because, for example, *Septobasidium bogoriense* PAT. which had been identified with the present fungus by him has been cleared to be quite different from the fungus by the studies of HENNINGS(1899)[1], GÄUMANN (1922), and HARA(1927, 1930).

More recently, by examining a collection of RACIBORSKI labelled *Septobasidium mompa* (TANAKA) RACI., Herb. Cracov and U. N. S. Herb., COUCH (1938) identified it as *Septobasidium bogoriense* PAT.

From the facts mentioned above, *Helicobasidium Mompa* TANAKA, the causal fungus of the "Murasaki-monpa" disease, is clearly a different species from *Septobasidium mompa* (TANAKA) RACIB. identified by RACIBORSKI (1909).

There may be some notes to be added by the present writer: —— Concerning the "Kôyaku-byô[2]" which is frequently found on the upper parts of the stems and branches of old mulberry trees, TANAKA (1891) stated in his conclusion (p. 210) that "whether the orbicular patches [Kôyaku byô] just described simply represent a form of the present species [*Helicobasidium Mompa*] or not can only be determined after further investigation. But I venture to say that it is probably a poorly nourished form of the latter".

However, by the investigations of SAWADA (1912-a, 1912-b) and HARA (1931) it has been demonstrated that there are two kinds of "Kôyaku byô" which are found commonly on the mulberry tree, *i. e.*, the one is the "Kasshoku[3]-kôyaku byô" and and the other the "Haiiro[4]-kôyaku byô". Concerning the causal organisms of these diseases the following two fungi were identified by the investigators as *Helicobasidium Tanakae* MIYABE (Kasshoku-kôyaku byô) and *Septobasidium pedicellatum* (SCHW.) PAT. (Haiiro-kôyaku byô).

These fungi which have been known as epiphytes are frequently found on the twigs, the branches, and on the upper parts of the stems, but never on the basal portions of the tree and the roots. On the contrary, *Helicobasidium Mompa* attacks the underground parts of the tree heavily and produces its sporophore on the lower portion of the trunk near the ground level.

Judging from TANAKA's description, his fungus of the "Kôyaku byô" seems probably to agree with *Helicobasidium Tanakae* MIYABE (SAWADA 1912-b).

From the facts mentioned already, it is generally considered at present that there is no relationship between the "Kôyaku byô" and the "Murasaki-monpa byô", and accordingly TANAKA's opinion on the fungus of the "Kôyaku byô" quoted above may

1) Cited from SACCARDO (1902).

2) Japanese word "Kôyaku" means a medical plaster; "byô", disease.

3), 4) "Kasshoku" means brown in English; "Haiiro", gray.

be denied.

GÄUMANN (1928) working with *Septobasidium bogoriense* PAT. felt a great deal of difficulties in comparison with *Helicobasidium Mompa*, because, perhaps, the above-mentioned facts had not been fully recognized by him, but, at least, his following conclusion (p. 172) may be correct: "Zusammenfassend können wir also sagen, dass der polyphage und beträchtlich polymorphe Pilz *Septobasidium bogoriense* PAT. diesen Namen zu Recht trägt und dass die Kombination *Septobasidium Mompa* (TAN.) RAC. in die Synonymik zu verweisen ist. Das *Helicobasidium Mompa* TANAKA ist mit dem *Septobasidium bogoriense* PAT. nicht identisch und muss daher vorläufig bei der Gattung *Helicobasidium* verbleiben".

The present writer must make clear whether or not *Helicobasidium Mompa* TANAKA is a different species from an allied fungus, *Helicobasidium purpureum* (TUL.) PAT. which is widely distributed through Europe and America.

By comparing the causal fungus of the "Murasaki-monpa" disease with the description of *Helicobasidium purpureum* in SACCARDO's Fungorum(1888), TANAKA(1891) distinguished the former from the latter chiefly by the color of the pileus and the number of basidiospores on a basidium, and thus he described it as a new species to science.

According to SACCARDO (1888) the description of *Helicobasidium purpureum* (TUL.) PAT. is as follows: "Effusum, tenue, primo roseo-violaceum, dein violaceo-purpureum, albo-pruinosum; basidiis generis circinatis, ex arcu bisporis; sporis ellipsoideo-reniformibus, nubilosis, hyalinis, 10–12~6–8 μ". While TANAKA's fungus was purplish brown in the color of the pileus and tetraspored.

BUDDIN and WAKEFIELD (1927, 1929) expressed the opinion that *Helicobasidium purpureum* (TUL.) PAT. is the perfect stage of *Rhizoctonia crocorum* (PERS.) DC.,[1] the causal organism of the violet root rot of clover and alfalfa, etc. They noted also that on germination the basidiospores of *Helicobasidium purpureum* produced a branched purplish hypha, and conidia belonging to the Imperfect form-genus *Tuberculina* were frequently observed in culture.

As already mentioned by the present writer, in the case of the causal fungus of the "Murasaki-monpa" disease, germ tubes or primary hyphae are colorless, and secondary spores, such as conidia of *Tuberculina* type, are not found in the culture.

Furthermore, in symptom, pathogenicity and parasitism, there seems to be some differences between the two fungi (DUGGAR 1916, FARIS 1921, HEALD 1911, FROMME 1916, DIEHL 1916, BUDDIN and WAKEFIELD 1927, 1929, WIEMER 1940).

From these reasons, though a direct comparison has not yet been made unfortunately, so far as it is judged from the literature, the present writer comes to the conclusion that the causal fungus of the "Murasaki-monpa" disease is clearly distinct from

1) The existence of *R. violacea* TULASNE (=*R. crocorum* (PERS.) DC.) in Japan was recorded by HARA (1931, p. 167–171), and it was considered as the causal fungus of the "Kuriiro-monpa" disease ("Kuriiro" means chestunt color) affecting tea- and citrus-trees. Though he could not discovered the perfect stage, he distinguished this fungus from *Helicobasidium Mompa* TANAKA by the color of the mycelium and the symptom.

Helicobasidium purpureum, and *Helicobasidium Mompa* TANAKA is to be adopted as the binominal for this fungus.

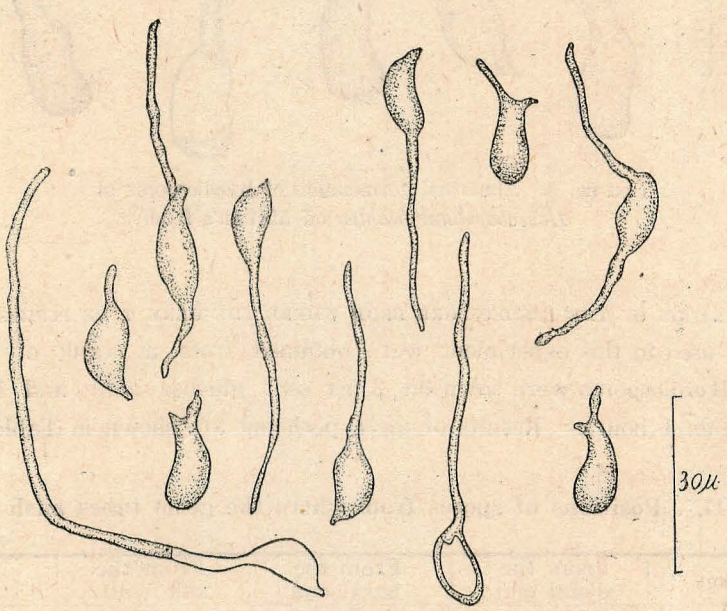
Physiology of the fungus

As regards the physiology of the fungus, there has been no report except MIYAKE's (1920) one which was, however, not sufficiently described, because he could not gain its artificial culture. Consequently, it may be apparently considered that there are many problems still requiring further extensive investigations.

1. Germination of the basidiospores

Basidiospores found on the woody plants in the yard of Forest Experiment Station at Meguro in Tôkyô were used as materials for this experiment.

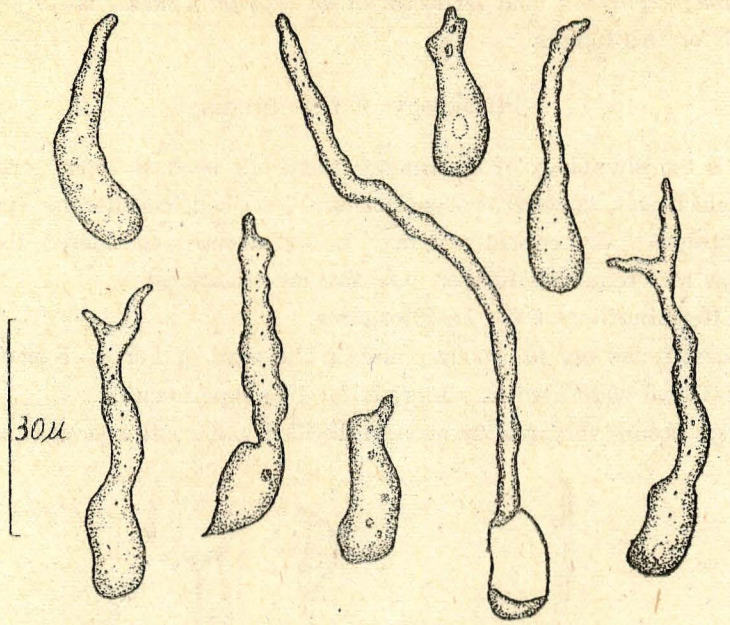
Germination occurs very readily even in distilled water after several hours.



Text-fig. 7. Germinating basidiospores of *Helicobasidium Mompa* on 2 per cent glucose agar.

(a) MODE OF GERMINATION

On 2 per cent glucose agar, it is most common for the germination to start from the distal end as well as from the basal end (apiculus) and rarely from the side wall. Usually one germ tube, uncommonly two tubes, pushes out from a basidiospore. With advancement of germination the protoplasm of the spore removes to the germ tube, and gradually a vacuole is formed; at last the spore becomes an empty sack. The germ tubes, having 1.5-3.0μ in width, are granular, soon septate, and branch out very lately (Text-fig. 7; Pl. VI, H). While, on MAYER's fixative (white of egg 50 cc., glycerine 50 cc., salicylate of soda 1 g.) the germ tubes are irregular in shape, frequently branch off soon after germination and are larger in width than those on 2 per cent glucose agar (Text-fig. 8).



Text-fig. 8. Abnormal germination of basidiospores of *Helicobasidium Mompa* on MAYER's fixative.

(b) POSITION OF THE BASIDIOSPORE BROM WHERE THE GERM TUBE PUSHES OUT

Materials used in this experiment were obtained from a trunk of *Liriodendron tulipifera.* Basidiospores were sown on 2 per cent glucose agar and kept at 15°C. in dark room for 6 hours. Results of the experiment are shown in Table 11.

Table 11. Positions of spores from where the germ tubes push out[1].

Germination	From the distal end	From the basal end	From the side wall	Total
Numbers	737	280	49	1066
Percentage(%)	68.7	26.6	4.7	100

1) Spores pushing out two germ tubes were omitted.

As is obviously seen in Table 11, the germination of the basidiospores starts most frequently from the distal end, less commonly from the basal end, and rarely from the side wall.

(c) EFFECT OF TEMPERATURE UPON THE GERMINATION OF THE BASIDIOSPORES

Materials used for this experiment were collected from a fruit-body formed on *Thuja occidentalis.* The basidiospores were sown on 2 per cent glucose agar in Petri dishes and kept in incubators at desired temperatures for 20 hours. The details of the experiment will be given in Table 12.

Table 12. Germination of the basidiospores at various temperatures.

Temperature (°C)	17	22	25	27	31	35	45
Germination percentage(%)	ca. 90	ca. 90	ca. 90	ca. 90	ca. 90	ca. 90	0
Maximum length of germ tubes (μ)	250	350	500	630	385	70	-

It seems evident from Table 12 that there are no remarkable differences in the percentage of germination at temperatures ranging from 17°C. to 35°C., but considering from the maximum length of germ tubes the optimum may lie about 27°C. No germination occurs at 45° C.

(d) Effect of H-ion concentration upon the germination of the basidiospores

Materials were obtained from *Robinia pseudoacacia* and *Liriodendron tulipifera.* Two per cent glucose agar (agar-agar 2 per cent) was prepared. A range of pH value was obtained by additions of regulated amounts of HCl or NaOH. The pH value was determined according to the method described by Kolthoff (1925)[1]. The pH values of agar medium in this experiment were denoted after steaming sterilization. The basidiospores were sown on agar and placed in the laboratory or in the incubators respectively.

The results of the experiments will be shown in Tables 13-14.

Table 13. Germination of basidiospores on media of various pH values (-a).
Material: from *Robinaia pseudoacacia,*
Temperature: 20°-22° C. (in the laboratory),
Experimental period: 20 hours.

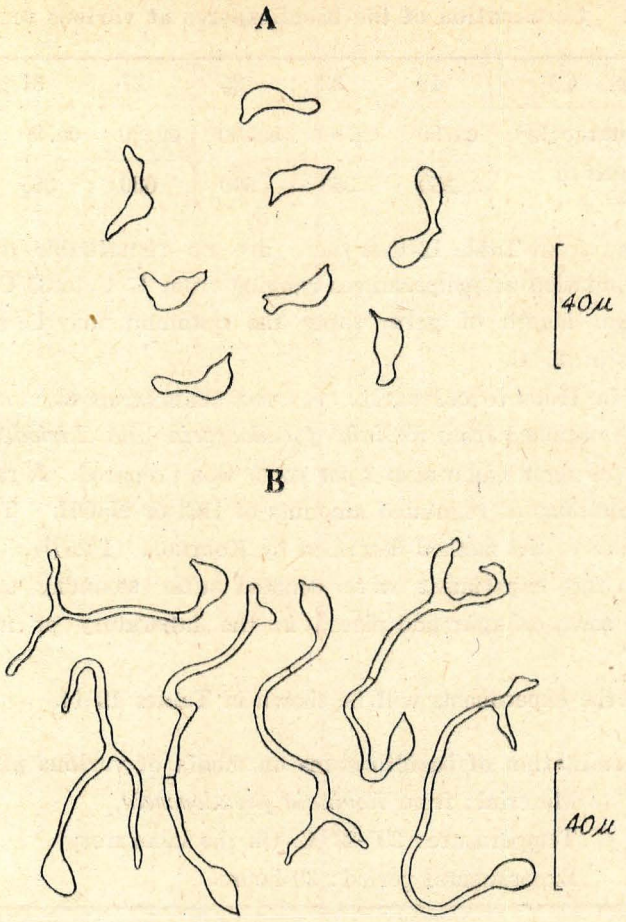
pH value	2.6	3.2	4.8	5.6	6.2	7.2	7.8	9.0
Percentage of germination(%)	+	+	40	80	90	90	90	90
Maximum length of germ tube (μ)	70	70	140	210	350	250	210	210

Table 14. Germination of basidiospores on media of various pH values(-b).
Material: from *Liriodendron tulipifera,*
Temperature: 28° C. (in the incubator),
Experimental period: 15 hours.

pH value	2.0	3.0	4.0	5.0	6.2	6.8	7.8	9.0	9.6
Percentage of germination(%)	80	80	80	80	80	80	80	80	80
Maximum length of germ tube(μ)	24	150	210	240	420	330	270	210	180

From the data shown in Tables 13-14, it may be said that the optimum H-ion concentration for the germination of the basidiospores of the fungus is about pH 6.2; while the percentage of germination is not so strikingly effected by the change of H-ion

1) Cited from Takimoto (1932).



Text-fig. 9. Abnormal germ-tubes of *Helicobasidium Mompa* on the exteme acid sides of medium. A : pH 2, B : pH 3.

concentration within the limits studied. In the course of experimets, the writer observed some interesting phenomena that on the extreme acid side (viz., pH 2.0 and 3.0), as well as on the extreme alkaline side (viz., pH 9.0 and 9.6) the shape of the germ tube became irregular and branched very frequently (Text-fig. 9).

2. Effect of temperature on the mycelial growth

In order to know the temperature relations of the fungus in culture, estimating the cardinals for the mycelial growth some experiments were undertaken. For the purpose of this study the plate culture method with Petri dishes was used. For the inoculum, the margin of the mycelial colony (Strain M-1) on potato agar plate was cut with a sterilized platinum needle into small pieces (ca. 4 mm. sq.), and these were placed on the agar media in Petri dishes. The inoculated dishes were kept in incubators regulated at desired temperatures for 10 days.

Three culture media were used for the experiments, viz., sweet potato decoction agar with 2 per cent glucose, WAKSMAN's agar, and potato decoction agar with 2 per cent glucose.

The results of the experiments at each temperatures were determined by taking the averaged diameters of five or six colonies in Petri dishes.

The influence of temperatures upon the mycelial growth will be summarized in Table 15.

Table 15. Effect of temperatures upon the mycelial growth.

Experiment-1: On sweet potato decoction agar with 2% glucose

Temperature(°C)	13-15	20	23	25	27	29	35	40
Diam. of colony (mm.)	9.3	22.6	34.4	38.3	43.6	41.0	±	-

Experiment-2: On sweet potato decoction agar with 2% glucose

Temperature(°C)	13-15	20	23	25	27	29	35	40
Diam. of colony (mm.)	10.0	25.3	35.5	41.8	47.0	40.0	±	-

Experiment-3: On WAKSMAN's agar

Temperature(°C)	15-18	20	23	25	27	29	35	40
Diam. of colony (mm.)	8.0	16.4	20.0	22.6	29.0	19.3	±	-

Experiment-4: On potato decoction agar with 2% glucose

Temperature(°C)	15-18	20	23	25	27	29	32	35	40
Diam. of colony (mm.)	6.0	25.4	42.1	44.2	47.5	33.6	+	±	-

Experiment-5: On potato decoction agar with 2% glucose

Temperature(°C)	5	8	13
Diam. of colony (mm.)	-	+	8.3

Experiment-6: On potato decoction agar with 2% glucose

Temperature(°C)	5	8	13
Diam. of colony (mm.)	-	+	9.0

It will be seen from Table 15 that the fungus grows favorably at the temperatures ranging from 20° to 29°C. with an optimum at 27° C., and the maximum and minimum temperatures for the growth are 8° and 35°C., respectively. At 5° and 40° C. no growth is observed even after 2 weeks' incubation.

3. Effect of H-ion concentration on the mycelial growth

For the culture solution, potato decoction (potato 100g, distilled water 1,000 cc.) was prepared by adding 1 per cent sucrose; the range of the pH value was obtained by addition of regulated amounts of HCl or NaOH according to the method described previously. By the preliminary tests the influence of sterilization on the change of pH value of the solution was determined.

One hundred cc. of each of the above-mentioned solution were poured into 200 cc. Erlenmeyer flasks. After sterilization, quadruplicates of all these solutions were

inoculated with the fungus and then placed in the laboratory for 36 days (May 20 to June 25).

At the end of the experiment, the mycelial colonies were thoroughly washed with distilled water and dried up in the oven. The dry weight of the mycelium was measured in miligrammes and averaged for four flasks. Thus, the effect of the H-ion concentrations upon the mycelial growth of the fungus was determined.

The results of the experiments will be summarized in Table 16.

Table 16. Growth of mycelium on the media having various pH values.

pH value			Dry weight of mycelium (mg.)
Initial (after sterilization)	After experiment	Control (not cultured)	
3.0	2.6	3.0	70.8
3.4	2.8	3.4	74.2
4.2	2.8	4.2	123.5
5.2	2.6	5.2	204.3
6.0	2.6	6.0	199.5
6.4	2.6	6.4	206.6
7.0	2.6	7.0	161.2
7.4	2.8	7.4	146.2
7.8	2.8	7.8	104.5
8.2	2.8	8.2	85.0

Table 16 shows that influence of H-ion concentration is not remarkable in the solution with exponents ranging from pH 4.2 to pH 7.8, but, in all probabilities, the maximum growth of the fungus may be obtained on the media having pH 5.2-6.4. However, in every medium studied, the pH value is apt to become strikingly low during the growth of the mycelium, and therefore the writer can not lead the definite conclusion by such a simple experimental method.

4. Effect of diffused sunlight on the mycelial growth

Petri dishes containing sterilized potato-glucose agar were prepared, and, after inoculation, half of the cultures were covered thoroughly with red paraffin paper and black paper, and another half untreated. They were placed on a disk in the laboratory for 3 weeks, and the experiments were at least duplicated.

From the results of the experiment it is shown that the diffused sunlight may induce almost no visible effect upon the growth of the fungus.

5. Effect of free oxygen on the mycelial growth

In order to make clear whether the fungus grows under the condition without free oxygen or not, an experiment was carried out using BUCHNER's apparatus as follows: In each glass tubes of 100 cc. in the capacity 1 g. of pyrogallol and 10 cc. of 10 per cent water solution of KOH were added, then a culture tube inoculated with the

fungus was immediately inserted into it; and the large tube was stopped with gum and sealed with melted paraffin. In the small tube, potato glucose agr had been poured. Thus, the prepared apparatus was placed in the incubator of -5° C. for 24 hours and then kept at 25° C. Of course, some checks were prepared adding with the same amount of distilled water instead of pyrogallol and KOH-solution.

Even after 7 weeks no mycelial growth occurred in BUCHNER's apparatus; but as soon as the small tubes is taken out of the large one in the air, the mycelial growth from the inoculum started vigorously.

From the facts mentioned above, it may be concluded that this fungus can not grow under the condition without free oxygen at all. Accordingly this is an obliged aerobic microorganism as most other fungi, while the fungus is not killed even after 7 weeks without free oxygen.

6. Growth of the fungus in relation to various kinds of sugars

As carbohydrates, sucrose, glucose, lactose and galactose were used. Agar media (agar-agar 2 per cent) containing 2 per cent of each of these carbohydrates were inoculated with the fungus and kept at 27° C. for 10 days.

The result of the experiment will be briefly shown in Table 17.

Table 17. Relation between the mycelial growth and each of various kinds of sugars.

Carbohydrate	Sucrose	Glucose	Lactose	Galactose
Degree of mycelial growth	+++++ +++++	+++++	++++	+

As shown by Table 17, the mycelial growth of the fungus is most vigorous on agar containing sucrose and very feeble on galactose.

Another experiment was done as follows: CZAPEK's solution without sugar was prepared. As a sourse of carbohydrate each of 2 per cent glucose, lactose, and sucrose was added in each of flasks. Also, in this case, the same result as shown in Table 17 was gained.

7. Growth of the fungus in relation to various kinds of alcohols

In this experiment, methyl alcohol, ethyl alcohol, n-butyl alcohol, iso-propyl alcohol and glycerine were prepared. Eight cc. of hot distilled water containing 2 per cent agar-agar were poured in test tubes and autoclaved by the ordinary manners. While the agar was still hot, one cc. of each of alcohols, except glycerine, was poured into test tube by using aseptic technique. In the case of glycerine, medium containing 2 per cent of it was used. These test tubes are inoculated with the fungus and kept at 25°C.

The results of the experiment obtained at the end of 7 weeks will be shown in Table 18.

Table 18. Reletion between the mycelial growth and each of various kinds of alcohols.

Kind of alcohol	Methyl alcohol	Ethyl alcohol	N-butyl alcohol	Iso-propyl alcohol	2% glycerine	2% glucose
Mycelial growth	+	+++++ ++	−	+++++	+++++ +++++ +++++	+++++ +++++
Aerial hyphae	±	+	−	−	+++	+

The mycelial growth of the fungus occurred on all alcohols used in the experiment, except n-butyl alcohol. The fungus grew very scanty on methyl alcohol and no mycelial growth was observed on n-butyl alcohol.

On 2 per cent glycerine, the fungus at first grew feebly, but with the lapse of time, developed more vigorously than on 2 per cent glucose.

Strictly speaking, the experimental method mentioned above is rather imperfect in some respects, viz., concentration of alcohol and neglecting of evaporation of alcohol, but it may be probably said that some alcohols are able to be utilized by the fungus, especially glycerine is one of the most favorable carbohydrates for this fungus.

8. Growth of the fungus in various concentrations of glucose, peptone and the combination of them

This experiment was carried out to know the relation between the amount of the mycelial growth and each of the following three respects: (1) Concentration of glucose in agar, (2) that of peptone, (3) that of both glucose and peptone.

The data of the experiment after 20 days at 25° C. will be given in Table 19.

Table 19. Effects of various concentrations of glucose and peptone on the mycelial growth.

Glucose(%) / Peptone(%)	0.0	0.5	1.0	2.0	5.0	10.0
0.0	20.0 [1] 1) 2)	34.0 [4]	36.0 [5]	37.0 [4]	37.0 [3]	38.0 [3]
0.5	36.0 [5]	50.0 [40]	72.0 [45]	68.0 [40]	60.0 [38]	54.0 [25]
1.0	20.0 [4]	52.0 [32]	54.0 [35]	52.0 [30]	52.0 [28]	48.0 [25]
2.0	17.0 [3]	44.0 [24]	44.0 [28]	42.0 [25]	44.0 [22]	44.0 [20]
5.0	15.0 [2]	36.0 [20]	40.0 [25]	34.0 [24]	32.0 [20]	18.0 [10]
10.0	15.0 [1]	26.0 [10]	28.0 [18]	18.0 [14]	12.0 [10]	0 [0]

1) The number shows the averaged diameter of 5 mycelial colonies in milimeters.

2) The number in brackets represents the relative amount of total mycelium per unit area approximately measured, in each ease [1] representing a minimum positive quantity.

From Table 19 the conclusions will be given as follows:

(1) On various concentrations of glucose ranging from 0.5 per cent to 10 per cent, there were no remarkable differences in the mycelial growth, probably with the maximum of 1.0 per cent.

(2) In the case of peptone the maximum growth was shown in 0.5 per cent, and the amount of mycelium in a given time decreased considerably with the increase of the concentration of peptone.

(3) On glucose agar by adding small amount of peptone, or *vice versa*, the growth of the fungus was promoted conspicuously.

Furthermore, the amount of aerial hyphae produced on various concentrations of glucose and peptone will be shown in Table 20.

Table 20. Relative amount of aerial hyphae on various concentrations of glucose and peptone.

Glucose(%) / Peptone(%)	0.0	0.5	1.0	2.0	5.0	10.0
0.0	[0]	[1] [1)]	[3]	[2]	[2]	[1]
0.5	[12]	[9]	[8]	[7]	[6]	[4]
1.0	[8]	[10]	[8]	[7]	[5]	[4]
2.0	[8]	[10]	[8]	[8]	[5]	[4]
5.0	[2]	[7]	[7]	[6]	[5]	[4]
10.0	[1]	[5]	[5]	[5]	[4]	—

1) The numbers in brackets represent relative amount per unit area, in cach case [1] representing a minimum quantity.

As shown in Table 20, aerial hyphae were scanty on glucose agar, however, very abundant on peptone agar, especially in its lower concentrations.

9. Acid production of the fungus

The remarkable shifting of acidity of the solution during the growth of the fungus was already described by the writer in this paper (p. 40). In order to know this fact more accurately some experiments were carried out.

(a) In potato-sucrose agar

Potato-sucrose agar(potato 100g., distilled water 1,000 cc., sucrose 10 g., agar-agar 20g.) was prepared. PH value of agar after first sterilization was adjusted to pH 7.0 by adding regulated amount of NaOH. Each dye solution was added to the neutral melted agar in a certain proportion, and after thoroughly mixed, 8 cc. of agar were distributed into sterilized test tubes (9 mm. in diameter), and then these were autoclaved at 15 pounds' pressure for 15 minutes. The fungus (Strain M-1) was inoculated on agar and incubated at 27° C. Details will be given in Table 21.

Table 21. Acid production on potato-sucrose agar with the age of culture showing color reactions.

After inoculation (day)	Dye added									
	Brom cresol purple		Methyl red		Brom phenol blue		Thymol blue		Lacmus	
	1) Cont.	2) Inoc.	Cont.	Inoc.	Cont.	Inoc.	Cont.	Inoc.	Cont.	Inoc.
1	violet	slight-yellow	yellow	yellow	blue	blue	yellow	yellow	blue	blue
3	do.	yellow	do.	slight red	do.	do.	do.	do.	do.	red
5	do.	do.	do.	red	do.	slight yellow	do.	do.	do.	—
12	do.	yellow (whole agar)	do.	red (whole agar)	do.	do.	do.	do.	do.	red (whole agar)
17	do.	do.	do.	do.	do.	yellow	do.	slight red	do.	—

1) Cont.……Control, 2) Inoc.……Inoculated.

From Table 21, it may be clear that pH value of agar at least near the inocula becomes about 2.8 after 17 days at 27° C.

(b) In Waksman's agar

Waksman's agar and that without glucose were prepared. The reactions of these media adjusted to pH 7.0 with NaOH, using brom-thymol blue and brom-cresol purple as indicators.

Five days after inoculation at 27° C., the remarkable shifting of acidity was occurred in Waksman's agar with perfect composition, but, on the contrary, no acid production was observed in that without glucose even after 30 days.

From the fact mentioned above, it may be said that the rôle of glucose in acid-production of the fungus is very important.

(c) Relation between acid-production and various carbohydrates

Waksman's agar media with various sugars were prepared, containing each of the following compounds as a source of carbohydrate: 2 per cent glucose, 2 per cent sucrose, 2 per cent lactose, 2 per cent galactose and 2 per cent soluble starch. The experiment was carried out by the same method described in the previous page.

Six days after inoculation at 27° C., the shifting of acidity was observed considerably in the agar containing each of glucose and sucrose, but was slightly in galactose and starch.

(d) In various alcohols

Acid production occurred also in media containing each of the following alcohols: Methyl alcohol, ethyl alcohol and glycerine.

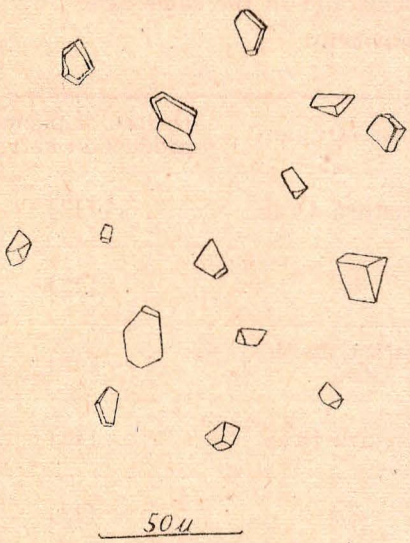
10. Oxalate and phenol-like substance produced by the fungus

As already described (p. 19), the writer observed at least two kinds of crystal-like

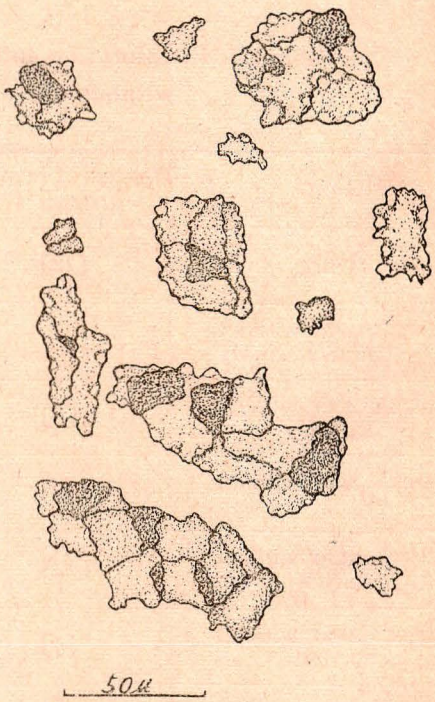
bodies in media cultured with the fungus, the one was small and colorless, and the other large and yellow under the microscope (Text-fig. 10, 11). Some tests were carried out by the writer to make clear the nature of these substances.

(a) OXALATE PRODUCED BY THE FUNGUS

An adequate review of the literature with regard to oxalate production of micro-organisms was given by HAMADA (1940), etc., and, therefore, it may be unnecessary to



Text-fig. 10. Oxalate produced by *Helicobasidium Mompa* on sweet potato decoction plus 2 per cent sucrose agar.



Text-fig. 11. Mars orange amorphous bodies found on the colony of *Helicobasidium Mompa*.

include such review in this paper.

From the results of the microchemical tests following MOLISCH's (1913) procedure, the small colorless crystals mentioned above (Text-fig. 10) seems to be oxalate.

Furthermore, another experiment was carried out: Modified RICHARDS' solution cultured with the fungus for one month at 25° C. was tested by acid potassium permanganate solution, and in a few moments, the color of the solution faded and numerous bubbles were formed, while, on the contrary, no changes occurred in the controls. From these facts, it may be probably concluded that the fungus produces oxalatte in cultured media.

(b) PHENOL-LIKE SUBSTANCE PRODUCED BY THE FUNGUS

As mentioned already (p. 19), the discoloration in CZAPEK's agar was different from

that of other agar, namely the former was Olivaceous Black in color, but the latters brownish purple. In order to make clear the cause of this phenomenon, some experiments were carried out.

Experiment-1. CZAPEK's solution and that lacking one of its components were prepared. They were added with 2 per cent agar-agar and then inoculated with the fungus. The results of the experiment obtained at the end of 23 days' culture at 25°C. in the dark room, will be shown in Table 22.

Table 22. Discoloration in CZAPEK's agar and in the same agar without one of each component.

Agar	Degree of mycelial growth	Color of agar	Degree of pigment production in agar
CZAPEK's agar	[12]	Chaetura Drab	[12]
CZAPEK's agar without $MgSO_4.7H_2O$	[12]	do.	[12]
CZAPEK's agar without K_2HPO_4	[8]	Dark Grayish Olive	[10]
CZAPEK's agar without KCl	[12]	Chaetura Drab	[12]
CZAPEK's agar without $NaNO_3$	[12]	do.	[12]
CZAPEK's agar without sucrose	[1]	Pale Ecru-Drab	[1]
CZAPEK's agar without $FeSO_4$	[10]	Light Cinnamon Drab	[8]

As shown in Table 22, the agar containing iron sulphate ($FeSO_4$) were dark gray in color, while those without it brownish purple; the degree of discoration was increased by the addition of iron sulphate.

Experiment-2. One hundred cc. of 2 per cent glucose agar containing 2 cc. of each of the following materials were prepared: Namely 0.5 per cent iron acetate (basic), 0.5 per cent iron sulphate, 0.5 per cent iron phosphate ($Fe_3(PO_4)_2.8H_2O$) and 0.5 per cent iron trichloride ($FeCl_3$). They were distributed in test tubes respectively, autoclaved, inoculated with the fungus and then incubated at 26° C. for 27 days. As controls, the same agar without ferrous or ferric compounds was used. Results of the tests will be briefly shown in Table 23.

Table 23. Cultural experiment on glucose agar containing ferrous or ferric compounds.

Agar	Degree of mycelial growth	Color of agar	Degree of pigment production
2% glucose agar	+++	Light Cinnamon Drab	++
2% glucose agar containing iron acetate	++	Chaetura Drab	++++
2% glucose agar containing $FeSO_4$	+++	do.	++++
2% glucose agar containing $Fe_3(PO_4)_2.8H_2O$	++	Drab	+++
2% glucose agar containing $FeCl_3$	+++	Chaetura Drab	++++

As shown in Table 23, the agar containing a trace of ferrous or ferric compounds became browish green in color.

Experiment-3. Potato decoction with 2 per cent sucrose, as well as the modified RICHARDS' solution, in which the fungus had been cultured for 25 days were tested by adding a few drops of iron trichloride solution. The color of these solutions became brownish green, while the controls remained light yellow. Furthermore, the large brown amorphous bodies formed on the mycelial colony(p. 45) were tested by the same manners mentioned above and the tests indicated evidently phenol reaction.

These bodies were produced not only on the culture media, but also on the mycelium, and they were observed even on the glass wall being a little distant from the creeping hyphae of the fungus. Accordingly the formation of these bodies are not considered to be due to the chemical changes of the culture media caused by the fungus.

Judging from the above-mentioned facts, it may be probably concluded that the fungus produces phenol-like substance in the course of the metabolism, though, so far as the writer can ascertain, there has been hardly any report concerning the production of such substance by pathogenic fungi.

After sufficient accumulation of more data a complete consideration in connection with this problem will be reported in future.

11. Preliminary tests on enzymes of the fungus

Very large numbers of work have been reported by various investigators on enzymes secreted by various fungi, and, in Japan, an adequate review of the literature related to this special subject has been made by MATSUMOTO(1923, 1924) and TOGASHI (1931), etc. Therefore, the writer will permit himself only a brief review of the literature most closely connected with his present investigation.

As regards the enzymic actions of the fungus in question no report has been made up to the present time, except MIYAKE's (1920) brief note, which was carried out by

using the mycelium from the natural fruit-body as materials for the reason of failing in artificial culture. Hereupon, the present writer started work in 1944, and some preliminary experiments have been finished.

The objects of the present studies were primarily to ascertain what kind of enzymes *Helicobasidium Mompa* secretes, but the scope of the present study is limited only to detect the enzymes concerned in pathogenicity.

The occurrences of some of the following kinds of enzymes, namely esterase, amidase, catalase, carbohydrase and oxidase were examined in the culture media and in the extractions of the mycelium of the fungus.

Two kinds of experimental procedures employed by the writer are very simple and convenient, but are rather inaccurate. Classification and arrangement of enzymes are followed ÔTANI's (1939) handbook.

(1) TEST OF ENZYMES BY "CULTURAL METHOD"

The term "cultural method" here means the following procedure: The fungus was cultured in media containing low percentage of certain substratum at 25° C. for several days, and as compared with checks, the change in media was tested by suitable reagents. The presence of enzymes was indirectly ascertained.

A. Esterase

a. Tannase The fungus was inoculated on 0.5 per cent tannic acid (Merck) solution, and after 12 days some growth of mycelium occurred.

B. Amidase

a. Asparaginase Soluble starch (Takeda) solution (0.1 per cent) in which the fungus had been cultured for 8 days was added with small amount of 1 per cent asparagin solution, and after a few minutes, the solution was tested by NESSLER's reagent. The reaction of ammonium was shown distinctly.

b. Urease In the soluble starch solution containing small amount of 1 per cent urea (Merck) solution, the fungus was cultured for 8 days. The presence of ammonium in the culture solution was ascertained by a few drops of NESSLER's reagent.

C. Catalase

The fungus was cultured in 2 per cent glucose (Merck) solution for 9 days, and then a few drops of H_2O_2-solution were added to the solution. As soon after that, numerous bubbles were seen.

D. Carbohydrase

a. Sucrase Two per cent saccharose (Merck) solution in which the fungus had been cultured for 9 days was tested by BARFOED' and FEHLING's solutions. Cuprous oxide was produced.

b. Maltase The fungus was cultured in 2 per cent maltose (Merck) solution. After 9 days' incubation at 25° C., it was tested by BARFOED's solution. Cuprous oxide was observed.

c. β-phenol-glucosidase (Emulsin) Two per cent amygdalin (Merck) solution in which the fungus had been cultured for 9 days was tested by FEHLING's solution. The presence of cuprous oxide was observed. Furthermore, by SMITH's method, the

production of HCN in cultured solution was ascertained.

d. Lactase (β-d-galactosidase) Two per cent lactose (Merck) solution was infected with the fungus. After 9 days' incubation at 25°C., it was tested by BARFOED's solution, but the reaction was not clear.

e. Amylase The fungus was cultured on 2 per cent soluble starch agar (2 per cent agar-agar) for several days, and, then iodine alcohol was poured on the surface of the agar. The result shows as follows: Agar under and near the mycelial colony was colorless, while the other portion purple in color.

f. Cellulase No mycelial growth occurred on colloidal cellulose prepared by SCHWEITZER's reagent.

g. Pectinase Two per cent pectin[1]) was inoculated with the fungus. After several days the cultured solution was tested by FEHLING's solution and the production of sugar was ascertained.

h. Gelase(?) Agar media on which the fungus has been cultured for a long time are softened, watersoaked and somewhat dissolved. To make clear whether this phenomenon is due to the agar-dissolving enzyme or not, the following experiment was made.

The fungus was cultured on 0.5 per cent agar for 3 days. Mycelial growth occurred evidently. The agar was tested by FEHLING's solution, and the presence of sugar was determined.

E. Dehydrogenase

a. Succinic acid-dehydrogenase Experiment-1. Eight cc. of 1 per cent succinic acid (Merck) containing one drop of 0.5 per cent methylene blue solution were inoculated with the fungus. After 2 days' incubation at 25°C., the color of the solution was disappeared evidently, while in controls no change occurred.

Experiment-2. Eight cc. of soluble starch solution containing one cc. of 2 per cent succinic acid solution were added with one drop of 1 per cent methylene blue solution. The fungus was cultured in this solution for 20 days. The color of methylene blue was conspicuously faded.

b. Glucose-dehydrogenase The fungus was cultured in 2 per cent glucose (Merck) solution containing a few amounts of brom cresol purple solution. After 7 days, the color of agar became yellow and consequently the production of acid in the solution was ascertained.

c. Amino acid-dehydrogenase Two per cent glucose- and the same percentage of sucrose-solution were prepared and inoculated with the fungus. After 12 days, the solutions were added with small amounts of asparagin acid solution, then tested by NESSLER's reagent, and in a few moments the production of ammonium was shown.

F. Oxidase

Concerning the fungus in question, the so-called BAVENDAMM's (1928) reaction, which had been used for diagnosis of wood-destroying fungi by him and other investigators,

1) Pectin obtained from the rind of summer orange was kindly supplied by Mr. K. TAKUBO, of our Experiment Station.

was tested as follows: Potato sucrose agar containing low percentage of tannic acid (Merck) or that of gallic acid (Merck) was poured in Petri dishes, inoculated with the fungus, and incubated at 25°C. for 8 days. The results of the experiment will be shown in Table 24.

Table 24. The fungus on tannic or gallic acid media.

Agar medium	Diameter of colony (mm.)	Brown zone on agar
Potato-sucrose agar (control)	41.7[1)]	absent
Potato-sucrose agar containing 0.1 per cent tannic acid	33.0	do.
Potato-sucrose agar containing 0.1 per cent gallic acid	34.3	do.

1) Figures in the table show the averaged diameters of five Petri dishes.

As shown in Table 24, the discoloration on agar containing tannic or gallic acid was not observed; namely BAVENDAMM's "Oxydationszone" was not formed.

a. Monophenol oxidase (Tyrosinase)

The fungus was cultured on potato-sucrose agar containing a trace of each of thymol and carbolic acid. Table 25 gives the results of the experiment after 5 days.

Table. 25. The fungus on potato-sucrose agar containing a trace of thymol or carbolic acid.

Agar medium	Diameter of colony (mm.)	Brown hallow on agar
Potato-sucrose agar containing thymol	0	absent
Potato-sucrose agar containing carbolic acid	33.2	present

b. Polyphenol oxidase (Laccase) Experiment-1. The fungus was cultured in Petri dishes, each of which contained 9 cc. of potato-sucrose agar with each of the following phenol-compounds, *i. e.* 0.1 per cent resorcine, 0.1 per cent hydroquinon(Merck), and 0.1 per cent phloroglucin (Merck). The results obtained are shown in Table 26.

Table 26. The fungus on potato-sucrose agar containing small mounts of various phenol-compounds.

Phenol compound	Diameter of colony (mm.)	Brown hallow around the colony
Resorcine	0	absent
Hydroquinon	6	present
Phloroglucin	1.4	present

Experiment-2. Two per cent saccharose (Merck) solution in which the fungus had been cultured for 12 days was added with a few drops of 2 per cent guaiacol alcohol. The solution became slightly brown, while no change occurred in control.

(2) TEST OF INTRACELLULAR ENZYMES

One hundred cc. of potato-sucrose solution (potato 100g., sucrose 10 g., distilled water 100 cc.) were placed in each of Erlenmeyer flasks of 150 cc. capacity, autoclaved by the ordinary method, and inoculated with the fungus in question. After 45 days' incubation at 25° C. the mycelial mats grown in culture were gathered on the filter paper of known weight, having been washed thoroughly with distilled water, first dried in an electric oven at 30° C. for 3 days, then in a calcium chloride desiccator for 2 days, and weighed. The dried mycelia were powdered in a ceramic mortar, ground again with a small amount of distilled water, diluted to a concentration of 0.2 per cent, and filtered through absorbent cotton. The mycelial extraction containing intracellular enzymes is light brownish purple in color, rather opaque, and has mushroom-like smell and fluorescence-like character. As the antiseptic, toluol in the proportion of 1 per cent was added in it.

A. Esterase

a. Tannase Eight cc. of 1 per cent tannic acid solution were added with 1 cc. of the enzyme solution. After 2 days at 26°C., they were tested by RIND-SMITH's method, *i. e.*, indigocarmine-$KMnO_4$ method, and it was cleared that the quantity of tannic acid in the solution added with enzyme solution decreased remarkably as compared with controls.

B. Amidase

a. Asparaginase To 1 per cent asparagin solution, a small amount of the enzyme extraction was added. After 2 days, it was tested by NESSLER's reagent. Thus the presence of ammonium was determined.

b. Urease One cc. of the enzyme extraction was poured in 8 cc. of 1 per cent urea solution, and after 2 days tested by NESSLER's reagent. The reaction of ammonium was noticed.

C. Catalase

The enzyme extraction was added with some drops of H_2O_2-solution and in a few moments numerous bubbles were formed evidently.

D. Carbohydrase

a. Sucrase Two per cent saccharose solution was added with small amounts of enzyme extraction. After 2 days, it was tested by FEHLING's solution and by BARFOED's one. The production of cuprous oxide was determined.

b. Maltase Nine cc. of 2 per cent maltose solution containing 1 cc. of the enzyme extraction were placed in the incubator of 25° C. for 2 days, and then tested by BARFOED's solution. Cuprous oxide was produced.

c. β-phenol-glucosidase (Emulsin) Two per cent amygdalin solution was added with small amounts of the enzyme solution. After 2 days, it was tested by FEHLING's solution. The existence of cuprous oxide was ascertained. Furthermore, by SMITH's

method, the reaction of cyanic acid was detected.

d. Lactase (β-d-galactosidase) Using 2 per cent lactose, the same experiment was made. When used by BARFOED's solution, the cuprous oxide was ascertained evidently.

e. Amylase Eight cc. of 2 per cent soluble starch solution were added with 1 cc. of the enzyme extraction. After 2 days, the starch solution was tested by FEHLING's solution for the sugar determination, and thus the hydrolysing power of the enzyme was ascertained. Furthermore, by adding some drops of iodine alcohol, it was clear that the reaction of starch in the solution containing the enzyme solution was considerably weakened as compared with the control.

f. Cellulase The distilled water containing colloidal cellulose prepared with SCHWEITZER's reagent was added with small amounts of the enzyme extraction. After 2 days, the solution was tested by FEHLING's solution. The hydrolysing activity of the enzyme was seen apparently.

g. Mannase The powder of "Konnyaku" (*Amorphophallus Konjac*) was washed with distilled water several times, and soluble starch and sugar were removed as much as possible. Distilled water containing small amounts of "Konnyaku" powder was added with the enzyme solution. After 2 days, the solution was tested by FEHLING's solution. The hydrolysing power of the enzyme was noticed.

h. Pectinase Two per cent pectin solution was added with some amounts of the enzyme solution. After 2 days the mixed solution was tested by FEHLING's solution, and the hydrolysing activity was detected obviously.

E. Dehydrogenase

a. Succinic acid-dehydrogenase Eight cc. of 1 per cent succinic acid solution containing a drop of 0.5 per cent methylene blue solution were added with 1 cc. of the enzyme solution. After 2 days, the color of the solution was slightly faded, but not distinctly.

b. Glucose-dehydrogenase Concerning 2 per cent glucose solution, the same test described above was carried out, but no remarkable reaction was determined.

C. Amino acid-dehydrogenase Experiment-1. Two per cent asparagin acid solution being added with small amounts of the enzyme extraction was tested by NESSLER's reagent, and thus the reaction of ammoninm was seen.

Experiment-2. Concerning glycin, the same test was made, and consequently the presence of ammonium was noticed very evidently.

F. Oxidase

a. Monophenol-oxidase (Tyrosinase) The enzyme extraction was poured in each of the test tubes containing 0.1 per cent carbolic acid or the same amount of thymol, but even after 2 days, no remarkable reaction was observed.

b. Polyphenol-oxidase (Laccase) Some amounts of the enzyme solution were added with a few drops of guaiacol alcohol, after 15 hours, the solution was increased in color.

(3) Conclusion

The results obtained in the present series of experiments may be summarized in Table 27. Experiments concerning this subject are still in progress. Therefore, a sufficient discussion and a definite conclusion will be left for the further investigation.

Table 27. Kinds of enzymes of the fungus tested by the writer.

Kind of enzyme		Result of the experiment	
		by "cultural method"	by mycelial extraction
Esterase	Tannase	±	+ +
Amidase	Asparaginase	+	+ +
	Urease	+	+++
Catalase		+++	+++
Carbohydrase	Sucrase	+++	+++
	Maltase	+	++
	β-phenol glucosidase (Emulsin)	++	++
	Lactase	?	++
	Amylase	+++	+++
	Cellulase	?	++
	Mannase	.....	+++
	Pectinase	++	++
	Gelase(?)	+	.....
Dehydrogenase	Succinic acid dehydrogenase	++	±
	Glucose-dehydrogenase	++	?
	Amino-acid dehydrogenase	+	+++
Oxidase	Monophenol-oxidase (Tyrosinase)	±	?
	Polyphenol-oxidase (Laccase)	+	±

Pigment Production of *Helicobasidium Mompa* Tanaka

Since the end of the nineteenth century some mycologists and pathologists have called attention to coloraton and pigment production of fungi. As regards chemical natures of pigments produced by various kinds of fungi a great many works have been published by various investigators. While, however, from the physiolgical and ecological points of view, a few reports have been made, especially on pathogenic fungi.

An extensive review of the literature relating to this special subject was given by NAKAMURA (1927), and therefore it may be unneccesary to cite the earlier literature, except the ones more closely connected with the present investigation.

One of the most important studies on pigment-production of fungi with special relation to the environmental factors made by BESSEY (1904) on *Fusarium sp.* and *Neocosmospora sp.* isolated from sesame plants. DANILOV (1925) gave an noticeable contribution on this subject in his excellent work of *Isaria virescens.*

In Japan, KAWAMURA (1924) reported some interesting facts relating to the pigmentation of the fungus in the course of his experiments on *Cercospora* (*Cercosporina*) *Kikuchii*, the causal organism of purple speck of soy beans. Some notes on this subject were published by NISHIKADO (1926,1928) on *Piricularia oryzae* and *Helminthosporium gramineum.* More recently HAMADA (1940) expressed an opinion that, in the case of *Armillaria mellea*, brown coloration of the mycelial colony and brown pigmentation of culture media were depend on the concentration of glucose and peptone, and finally he reached the conclusion that this phenomenon might be related to N/C-ratio.

Concerning *Helicobasidium Mompa* TANAKA, no noteworthy reserches have been made, except a brief chemical test by MIYAKE (1920).

The present writer has carried out some experimental studies in order to make clear the environmental conditions influencing the pigment production and its biological signification.

Experiments on pigment production of the fungus in various cultural conditions

1. Effect of temperature on pigment production

a. COLOR OF THE MYCELIAL COLONY

Three kinds of agar media were used as follows: Sweet potato decoction agar with 2 per cent glucose, potato decoction agar with 2 per cent glucose, and WAKSMAN's agar. Each of these media was distributed in Petri dishes, a small bit of the mycelium (Strain M-1) was inoculated on it, and then placed in incubators having been regulated at each of the following temperatures; about 15°, 20°, 23°, 25°, 27°, 29°, 32°, 35°, and 40°C.

Ten days after inoculation the observations were made. The coloration of the mycelial colony is very slight at the lower temperatures, while, with the rise of temperatures, it becomes deeper gradually. Outstanding coloration is noticed at 29°C., which is higher than the optimum for the mycelial growth of the fungus. At higher temperatures, such as 32°C. and 35°C., the mycelium on and near the inoculum is colored remarkably in spite of its feeble growth.

The above-mentioned facts are clearly seen especially on sweet potato decoction agar with 2 per cent glucose.

b. PIGMENTATION OF CULTURE MEDIA

Results of the observations will be given in Table 28.

Table 28. Effect of temperatures on pigment production in culture media [1].

Experiment-1: On sweet potato decoction plus 2% glucose agar.

Temperature(°C)	13—15	20	23	25	27	29	35	40
Color of agar	colorless	colorless	almost colorless	slightly colored	slightly colored	slight Etuscan Red	Ocher Red	colorless

Experiment-2: On potato decoction plus 2% glucose agar.

Temperature(°C)		15—18	20	23	25	27	29	32	35	40
Color of agar	The portion under the inoculum	almost colorless	Vinaceous Cinnamon	Onion skin Pink	Vinaceous Russet	Vinaceous Russet	Prussiian Red	Dark Mineral Red	Blackish Red~ Purple	Colorless
	The remaining portion	almost colorless	Light Pinkish Cinnamon	Light Vinaceous Cinnamon	Pinkish Cinnamon	Pinkish Cinnamon	Ocher Red	Livid Brown	Argyle Purple	Colorless

Experiment-3: On WAKSMAN's agar.

Temperature(°C)		15—18	20	23	25	27	29	35	40
Color of agar	The portion under the inoculum	Russet	Russet	Russet Brown	Russet Brown	Russet Brown	Mars Brown	Mars Brown	Colorless
	The remaining portion	Cream Color	Maiz Yellow	Warm Buff	Antimony Buff	Antimony Buff	Ochraceous Tawny	Mars Brown	Colorless

In the experiment with WAKSMAN's agar, an attempt was made to state in figuring the relation between the mycelial growth and the pigment production at various temperatures. The result will be given in Table 29. Figures of diameters show average values from 5 Petri dishes in milimeters (the inoculum is 4 mm. sq.).

Table 29. Relation between the mycelial growth and the pigment production at various temperatures.

Temperature(°C)	15—18	20	23	25	27	29	35	40
Diameter of the mycelial colony (α) (mm.)	8.0	16.4	20.0	22.6	29.0	19.3	[2] 4.0	[3] 4.0
Diameter of the deep colored portion of agar (β) (mm.)	4.0	7.6	10.5	15.8	21.0	18.0	7.0	0
β/α x100 (%)	50.0	46.4	52.5	74.6	72.4	93.2	175.0	0

1) Descriptions of color were designated according to RIDGWAY's (1912) color standard.
2) Mycelial growth is very scanty; 3) mycelial growth absent.

From Tables 28—29, it may be said in general that the pigment production of the fungus is scarce at lower temperatures, but is abundant at higher temperatures than the optimum for the mycelial growth, namely 29°—35°C.

2. Relation between pigment production and H-ion concentration

a. COLOR OF THE MYCELIAL COLONY

The fungus was cultured in potato decoction with 1 per cent sucrose adjusted to desirable H-ion concentrations using NaOH or HCI at room temperature.

Table 30. Effect of H-ion concentrations on the color of the colony.

PH	Color of the colony
3.0	Russet-Vinaceous
3.4	do.
4.2	do.
5.2	Mikado Brown
6.0	do.
6.4	do.
7.0	do.
7.4	do.
7.8	Russet-Vinaceous
8.2	do.

At the end of 36 days (from May 20 to June 25) after inoculation, the observation was made. The result will be given in Table 30.

From Table 30, it seems clear that the color of the mycelial colony is deeper at H-ion concentration ranging pH 5.2 to pH 7.4.

b. PIGMENTATION OF CULTURE MEDIA

(1) Potato decoction agar with 1 per cent sucrose was used in this experiment. After 10 days' incubation at 27° C., the observation was made, and the result will be summarized in Table 31.

Table 31. Effect of H-ion concentrations on the pigmentation of the culture agar.

PH	4.0	4.2	5.0	5.6	6.0	6.8	7.2	7.6
Color of agar	Bone Brown	Buffy Brown	Buffy Brown	Natal Brown	Army Brown	Natal Brown	light Prussian Red	light Ocher Red

As shown in Table 31, the coloration of agar is most remarkable at pH 6.8.

(2) In potato decoction with 1 per cent sucrose which was adjusted to the desirable pH-values, the fungus was cultured. The results of the experiment obtained after 36 days is shown in Table 32.

Table 32. Effect of H-ion concentrations on the pigmentation of the culture solution.

PH	Color of the solution
3.0	Orange Cinnamon
3.4	do.
4.2	do.
5.2	Verona Brown
6.0	do.
6.4	do.
7.0	do.
7.4	light Mikado Brown
7.8	do.
8.2	do.

The optimum pH-value for the pigment production seems to lie at pH 6.0-7.0.

3. Effect of the diffused sunlight on the pigment production

The diffused sunlight is apt to promote more or less the pigment production of the fungus, but the effect of it is not remarkable.

4. Effect of free oxygen on the pigment production

a. EXPERIMENT-1. One hundred cc. of potato decoction with 1 per cent sucrose were distributed into each of 150 cc. Erlenmeyer flasks, after sterilization by the ordinary manners, they were inoculated with the fungus and incubated at 25° C. (in the dark room). At the time of inoculation, the following two groups were prepared: (a) Inocula were floated on the surface of the culture solution, and (b) inocula were sunk in the bottom of the flasks. As controls uninoculated flasks were prepared.

The results obtained after 25 days' incubation are as follows: (i) In the case of (a), the growth of the mycelium is abundant and the colony on the surface of the solution as well as the culture solution becomes purplish brown, while the dipped portion of the mycelium is almost colorless; (ii) in the case of (b), on the contrary, a feeble growth of the white mycelium is observed and the solution almost colorless. In controls, no change occurs.

b. EXPERIMENT-2. With the same manners mentioned above, each of flasks containing potato decoction with 1 per cent sucrose was inoculated with a bit of the fungus, and then the inoculum was sunk in the bottom of the flask.

After 25 days' incubation at 27°C., waiting some growth of the cottony mycelium, the flasks were grouped as follows: (i) The ones, those mycelial colonies in the bottom of them were floated on the surface of the solution by shaking slightly with a hand, and (ii) the others were left as they were.

In the former case, the growth of the mycelium became vigorous, and, after several weeks, the color of the solution as well as the colony changed in purplish brown. In the latter case, pigmentation of the colony or the solution was not observed even after 10 weeks.

c. EXPERIMENT-3. In this experiment, BUCHNER'S apparatus was used. For the purpose of supplying a very small amount of free oxygen in the apparatus, a slender needle was inserted into the large tube through the gum stopper; and, by moving the needle very slightly, a small amount of free oxygen was given in it. In the small

tube, potato decoction agar with 2 per cent glucose was distributed. The results of the observation at the end of 50 days after inoculation at 25°C. were as follows: In the case of no free oxygen, the mycelial growth did not occur at all, while, in that supplied very small amount of free oxygen, the mycelium grew slowly and both the colony and the agar were almost colorless. The colony which developed under the conditon of sufficient oxygen was deeper in color.

Seeing from the results of Experiments 1—3, it may be probably said that the pigment production of the fungus occurs only under the condition of sufficient supply of free oxygen.

5. Relation between the pigmentation and each of the components of WAKSMAN's agar

WAKSMAN's solution and those lacking one of its components were prepared. They were added with 2 per cent agar-agar and adjusted to pH 5.6 with HCl or NaOH. The results obtained at the end of 10 days' incubation at 27°C. will be given in Table 33.

Table 33. Pigmentation of WAKSMAN's agar lacking each one of the components (−1). After 10 days.

Agar medium	Perfect component	Without glucose	Without peptone	Without KH_2PO_4	Without $MgSO_4.7H_2O$
Degree of mycelial growth	++++	+	++++	++++	++++
Degree of coloration of agar	+++	++	++++	+++	+++
Color of agar	light purplish brown	light purple	brilliant purplish brown	light purplish brown	light purplish brown

Table 33 gives that the color of agar without glucose is lighter and is more purplish than the others. After 18 days, the colors of the agar media become very different from the above-mentioned data as shown in Table 34.

Table 34. Pigmentation of WAKSMAN's agar lacking each one of the components (−2). After 18 days.

Agar medium	Perfect component	Without glucose	Without peptone	Without KH_2PO_4	Without $MgSO_4.7H_2O$
Degree of mycelial growth	++++ ++++ ++++	+++	++++ ++++ ++	++++ ++++ ++	++++ ++++ +++
Degree of coloration of agar	+++ +++ +++	+++ +++ +++	+++ +++ +++	+++ +++ +++	+++ +++ +++
Color of agar	Verona Brown	Perilla Purple	Verona Brown	Verona Brown	Verona Brown

As shown in Table 34, the mycelial growth on the agar without glucose is very feeble, but the coloration is not inferior to the other agar media. The color of the agar

lacking glucose is purplish, while that of the others brownish.

6. Relation between the pigment production and various carbohydrates

a. VARIOUS KINDS OF SUGARS

Sucrose, glucose, lactose and galactose were used. Agar media (agar-agar 2 per cent) containing 2 per cent of each of these sugars were respectively inoculated with the fungus and incubated at 27°C. The results obtained at the end of 10 days are given in Table 35.

Table 35. Relation between the coloration and various sugars.

Kind of sugar	Sucrose	Glucose	Lactose	Galactose
Degree of the mycelial growth	+++++ +++++	++ +++++	++++	+
Degree of the pigment production	+++++	++++	+++	+
Color of agar	dark purplish brown	yellowish brown	light purplish brown	light purplish brown

Table 35 gives that the coloration is strongest on sucrose agar, and weakest on galactose one.

b. VARIOUS KINDS OF ALCOHOLS

With respect to five kinds of alcohols, viz., methyl alcohol, ethyl alcohol, n-butyl alcohol, iso-propyl alcohol and glycerine, the experiment was carried out by the same procedure described in the proceding page (p.41). After 50 days' incubation at 25°C., the results were observed and are summarized in Table 36.

Table 36. Relation between the pigment production and various kinds of alcohols.

Kind of alcohol	Methyl alcohol	Ethyl alcohol	N-butyl alcohol	Iso-propyl alcohol	Glycerine	Glucose (as control)
Color of the mycelial colony	almost color-less	Fawn Color	1)	Vinaceous Buff	Sorghum Brown	Wood Brown
Degree of coloration of agar	+	+++++	-	++	+++++ +++++	+++++ ++
Color of agar	Pale Grayish Vinaceous	Vinaceous-Fawn	-	Light Vinaceous Fawn	Dark Vinaceous Brown	Wood Brown

1) No growth.

As shown in Table 36, the pigment production of the fungus is better on the agar containing glycerine than that containing glucose, and is obvious on the medium containing ethyl alcohol.

7. Relation between the pigment production and each of various concentrations of glucose, peptone, and the combination of them

The observations on this subject were made by the writer simultaneonsly with the experiments bescribed in the preceding page (p.42).

a. Color of the mycelial colony

Results gained at the end of 20 days are given in Table 37.

Table 37. Effect of various concentrations of glucose and peptone on the coloration of the mycelial colony.

Glucose(%) / Poptone (%)	0.0	0.5	1.0	2.0	5.0	10.0
0.0	very slight purple	Pinkish Buff	Pinkish Buff	Pinkish Buff	Pinkish Buff	Pale Pinkish Buff
0.5	Dark Livid Brown	Fawn Color	Army Brown	Nutal Brown	Army Brown	Army Brown
1.0	Deep Livid Brown	Fawn Color	Fawn Color	Army Brown	Army Brown	Army Brown
2.0	Vina-ceous Brown	Vina-ceous Fawn	Vina-ceous Fawn	Light Russet Vinaceous	Army Brown	Fawn Color
5.0	almost color-less	Vina-ceous Buff	Vina-ceous Buff	Vina-ceous Buff	Vina-ceous Buff	Vina-ceous Buff
10.0	almost color-less	Tilleul Buff	Tilleul Buff	Tilleul Buff	Tilleul Buff	...

From Table 37, the following conculsions may be given:

(1) On various concentrations of glucose ranging from 0.5 per cent to 10 per cent, there are no visible differences in the coloration of the mycelial colony, but at the highest percentage, the color is more or less lighter than the others.

(2) In the case of peptone, the coloration is remarkable at the lower concentrations (from 0.5 per cent to 2 per cent), especially, at 0.5 per cent. While at the higher concentrations, the mycelial colonies are almost colerless.

(3) On glucose agar by adding with small percentage (0.5-1.0 per cent) of peptone, the color of the mycelial colony becomes brownish.

(4) On 2 per cent peptone agar containing the lower concentrations (0.5-2.0 per cent) of glucose, the color is brilliant brownish purple.

(5) Generally speaking, at the lower concentrations of peptone only, the color is deep and purplish, while by adding with glucose, the degrees of the coloration are apt to decrease more or less.

b. PIGMENTATION OF CULTURE MEDIA

The results are summarized in Table 38.

Table 38. Effect of various concentraions of glucose and peptone on the coloration of agar medium.

Glucose(%) / Peptone(%)	0.0	0.5	1.0	2.0	5.0	10.0
0.0	Colorless [0]	Pinkish Buff [1]	Pinkish Buff [3]	Pinkish Buff [2]	Pinkish Buff [2]	Pinkish Buff [1]
0.5	Deep Livid Purple [10]	Army Brown [8]	Army Brown [12]	Army Brown [12]	Army Brown [12]	Army Brown [10]
1.0	Dark Livid Purple [15]	Army Brown [8]	Army Brown [12]	Army Brown [12]	Army Brown [12]	Army Brown [8]
2.0	Dark Vinaceous Drab [13]	Nutal Brown [8]	Nutal Brown [10]	Warm Sepia [12]	Warm Sepia [12]	Warm Sepia [10]
5.0	Colorless [0]	Warm Sepia [7]	Warm Sepia [8]	Warm Sepia [10]	Warm Sepia [8]	Warm Sepia [5]
10.0	Colorless [0]	Colorless [0]	Colorless [0]	Colorless [0]	Colorless [0]	..... [0]

Notes: The numbers in brackets denote the degrees of the pigment production; namely in the case of [1], a minimum positive quantity.

The portion enclosed with the broken line is better purplish in color, while the that enclosed with the solid line rather brownish.

From Table 38, the following conclusions may be given:

(1) No differences in color are observed in all percentages of glucose, but the degree of the coloration is best at 1 per cent.

(2) In the case of peptone only, pigment production is remarkable at the lower concentrations (from 0.5 per cent to 2 per cent), but at higher ones it is almost absent.

(3) Within lower concentrations of peptone (0-0.5%) the color is purplish, while, increasing the concentration (1-2%), it becomes dark. Degree of the pigmentation is best at 1 per cent.

(4) On higher percentage of glucose (2 per cent to 10 per cent) agar with small percentage of peptone (0.5 to 1.0 per cent), there are no remarkable differences in color.

It may be said in general that the lower concentrations of peptone are apt to promote the pigment-production and to make the color purplish (Pl. VII).

8. **Substances promoting the pigment production**

a. METHYL RED When the fungus was cultured on potato decoction agar with 2 per cent sucrose, containing a few drops of 0.04 per cent methyl red solution, the color or of the mycelial colony was conspicuously deeper, namely it became deep grayish brown.

b. CALCIUM CARBONATE Potato decoction with 1 per cent sucrose and the modified RICHARDS' solution[1]) were prepared, and each of these was added a small amounts of calcium carbonate(0.5–1.0 per cent). After sterilization, they were inoculated with the fungus. At the end of several weeks' incubation at 25°C., it is noticed that the pigment production is strikingly better in the media containing calcium carbonate than in the one without it.

c. MILK In cow's milk, the pigment production of the fungus is very pronounced, and the color is brilliant Dahlia Purple.

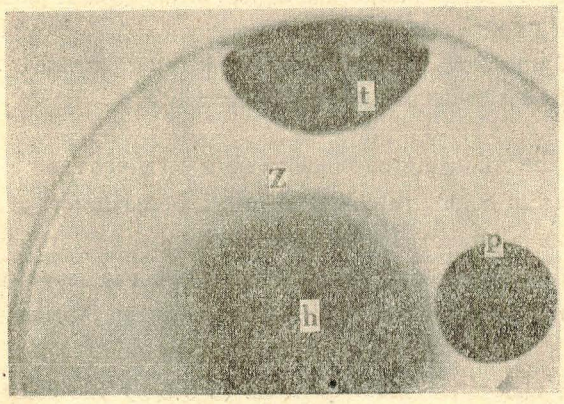
Augmentation in pigment production of the fungus by mixing with other microorganism

In the course of culture experiments the writer observed frequently the facts that the coloration of the mycelial colony and the culture medium were promoted noticeably by mixing culture with other fungi or bacteria.

On these facts some notes will be given as follows:

1. **By mixing culture with fungi**

a. OBSERVATION-1. On potato decoction agar with 2 per cent sucrose, the coloration of the colony and the agar medium became deep purplish by culturing



Text-fig. 12. Formation of the deep purplish zone on the culture of *Helicobasidium Mompa* by mixing with other fungi. x 6/5.
h: colony of *Helicob. Mompa*, t and p: contaminated fungi, z: deep purplish zone.

1) Distilled water 1,000 cc., KNO_3 5 g., KH_2PO_4 2 g., $MgSO_4$ 1 g., glucose 10g.

together with some species of *Penicillium* and *Trichoderma*. The coloration was especially remarkable on the portion near the growth of foreign fungus. Furthermore, there has been seen often a deep purplish zone between the colonies of the fungus and the foreign fungus (Text-fig. 12).

The pigment production was not always promoted by all fungi used, but by a few of them, and therefore it may be concequently said that there were many fungi which could not influence the coloration at all.

b. OBSERVATION-2. On boiled potato tuber, the part of the mycelial colony of the fungus adjacent to the colony of *Penicillium sp.* became Deep Livid Brown or Sorghum Brown.

2. By culturing with bacteria

a. EXPERIMENT-1. One hundred cc. of potato decoction with 1 per cent sucrose were poured into each of 150 cc. Erlenmeyer flasks. After sterilization by the ordinary method, they were inoculated with the fungus and incubated at 25° C. After several days, waiting for some mycelial growth, a loop of *Bacillus mesentericus* was added in each of the flasks and observations were continued. By adding *B. mesentericus*, the color of the culture solution became remarkably deeper and dark brown in color.

b. EXPERIMENT-2. *Bacillus mesentericus* was cultured in 150 cc. Erlemeyer flasks containing 100 cc. potato decoction with 1 per cent sucrose. After 3 days' incubation at 25°C. the culture was filtered by BERKEFIELD's apparatus. Five kinds of agar media containing various amounts of the filtrate of *B. mesentericus* were prepared as shown in Table 39.

Table 39. Compositions of agar media containing the filtrate of *B. mesentericus*.

Kind of media	No.	No. 2	No. 3	No. 4	No. 5
Filtrate of *B. mesentericus* (cc.)	50	25	10	5	0
Potato decoction plus 1 % sucrose solution (cc.)	0	25	40	45	50
Soluble starch (g.)	0.5	0.5	0.5	0.5	0.5
Agar-agar (g.)	1	1	1	1	1

Ten cc. of the above mentioned media were poured into each of KITAJIMA's (1930) small flasks, which were inoculated with the fungus and then incubated at 25°C. Results of the experiment at the end of 12 days are given in Table 40.

Table 40. Pigmetation of agar media containing various amounts of the filtrate of *B. mesentericus* (for each media, 5 flasks were prepared).

Kind of media	No. 1	No. 2	No. 3	No. 4	No. 5
Diameter of the colony (mm.)	12.5	15.0	22.5	26.0	36.0
Degree of coloration in agar medium	+++++	++++	++++	+++	++
Color of agar medium	Olive Brown	Olive Brown	Olive Brown	Nutal Brown	Fawn Color

It may be apparent from the results given in Table 40, that, by adding the filtrate of *Bacillus mesentericus* the pigment production of agar media is promoted; and it may be said that there is certain substances stimulating the coloration in the filtrate.

Differences between white young hyphae and colored old ones in the behavior towards external conditions

As already mentioned, the mycelium of the fungus is at first white, and with the lapse of time it becomes brownish purple. In order to know how much differenes are seen between these two kinds of mycelia in their physiological characters, the writer has made some experiments as follows:

1. Time required for the recovering of growth

Both white young colonies and brown old ones of the fungus cultured on potato agar were prepared; the formers' age of culture was 7 days, and the latters' 50 days. These colonies were cut into small pieces (4 mm. sq.) and washed with sterilized distilled water. One piece of each of the colonies was placed on new potato agar in Petri dish and incubated at 25° C. Four dishes were used in each case.

After 24 hours, visible growth of mycelium from the young inoculum was observed, while it took 4 to 5 days in the case of the old inoculum. For example, the size of the colonies which were started from both of the young and old inocula measured at the end of 6 days' incubation is given in Table 41.

Table 41. Width of colonies after 6 days started from the young and the old inocula.

Kind of inocula	Width of newly developed colony (mm.)				
	No. 1	No. 2	No. 3	No. 4	Averaged
White young inoculum	11.0	10.5	10.5	11.5	10.9
Colored old inoculum	1.5	1.5	1.5	0.5	1.3

2. Resistance to low temperatures

Deep purplish-brown old colonies (the age of culture, 90 days) and white young ones (the age of culture, 10 days) were prepared, and then they were cut into small pieces in size of 3 mm. sq. Each of these pieces was placed in each of test tubes containing potato decoction agar with 2 per cent sucrose, and they were kept in incubators set at each of the following temperatures: −3°, −15°, and 25° C. (check).

After each of desirable periods of exposure to the low temperatures, the test tubes were transferred to incubator and kept at 25° C. If no new growth of the mycelium occurred even after 30 days' incubation at 25° C., it was presumably determined that the inoculum had been killed by the effect of the low temperatures.

The results of the experiment are summarized in Table 42.

Table 42. Differences in durability to low temperatures between white young mycelium and colored old one.

Temp. (C°)	Kind of mycelium	Period of exposure to temperatures								
		18h.	1d.	2d.	3d.	7d.	14d.	21d.	28d.	35d.
-3	Y.M	+	+	+	+	+	+	+	+	+
	O.M	+	+	+	+	+	+	+	+	+
-15	Y.M	−	−	−	−	−	−	−	−	−
	O.M	+	+	+	+	+	+	+	+	+
25	Y.M	+	+	+	+	+	+	+	+	+
	O.M	+	+	+	+	+	+	+	+	+

Notes: Y.M.....White young mycelium, O.M.....Brown old mycelium, 18h.....18 hours, 1d.....1 day, +.....living, −.....died.

From Table 42, it may be clear that both old and young mycelia start to grow again even after 35 days' exposure at -3° C., while at -15° C., the white young mycelium is killed by 18 hours' exposure, whereas the colored old one seems to be resistant to low temperature and is not put to death even after 35 days at -15° C.

3. Resistance to high temperatures

By the same manners noted above, small pieces (5 mm. sq.) of both young and old mycelial colonies were prepared. Each of these inocula was sunk quickly in sterilized distilled water in test tubes. These tubes were inserted into hot water which had been regulated at the following desirable temperatures respectively: 35°, 40°, 50°, and 60° C. After 10 minutes, these inocula were transferred into cold sterilized water and then placed on potato agar, incubating at 25° C. Some checks were prepared. The results are given in Table 43.

Table 43. Durability of the mycelia to high temperatures.

Experiment-1.

Kind of mycelium		White young mycelium					Colored old mycelium				
Temperature(°C)		35	40	45	50	60	35	40	45	50	60
Period of exposure (minutes)	5	+	+	+	—	—	+	+	+	—	—
	10	+	+	+	—	—	+	+	+	—	—
	15	+	+	+	—	—	+	+	+	—	—
	20	+	+	+	—	—	+	+	—	—	—
	30	+	+	—	—	—	+	+	—	—	—
	60	+	+	—	—	—	+	+	—	—	—

Experiment-2.

Kind of mycelium		White young mycelium					Colored old mycelium				
Temperature(°C)		35	40	45	50	60	35	40	45	50	60
Period of exposure (minutes)	5	+	+	+	—	—	+	+	+	—	—
	10	+	+	+	—	—	+	+	+	—	—
	15	+	+	+	—	—	+	+	+	—	—
	20	+	+	+	—	—	+	+	+	—	—
	30	+	+	+	—	—	+	+	+	—	—
	60	+	+	—	—	—	+	+	—	—	—

Notes: +.....living, —.....died.

From the results in Table 43, it may be probably said that the mycelia of the fungus are killed by heating at 45°C. for 15–30 minutes, and at 50°C. for 5 minutes. It is noteworthy that there has been hardly any difference in durability to high temperatures between the white young mycelium and the brown old one.

Consideration and conclusion

1. Effect of temperature

BESSEY(1904) reported that *Fusarium sp.* and *Neocosmospora sp.* isolated from sesame plants produced no pigment at extremely high temperatures. Concerning *Cercospora Kikuchii*, KAWAMURA (1924) observed no special relations between pigment production and temperatures. DANILOV (1925) working with *Isaria virescens* indicated the fact that the production of dark-brownish color was promoted remarkably at higher temperatures.

In the case of the fungus in question, the pigmentation of meida becomes deeper at higher temperatures and is found in abundance at the maximum temperature for the mycelial growth, and also the mycelial colny is colored most conspicuously at 29°C.

This is considered to be apparently due to the effect of temperature, because the pigment production at 29°C. is better than that at 25°C., while the mycelial growth is almost equal at these temperatures.

Judging from the above mentioned facts, in the color production the fungus resembles *Isaria virescens* studied by DANILOV.

2. Effect of reaction in culture media

BESSEY(1904), as regards *Fusairum sp.* and *Neocosmospora sp.*, noted the following data: (1) The two kinds of pigments, namely reddish and purplish, were produced only when the fungi had been cultured on acid medium and then transferred to slightly alkaline one, but no pigmentation occurred, when they had been cultured on acid or basic media from the first. (2) Another orange pigment was not effected by the reactions of culture media. Furthermore, he noticed that *Fusarium culmorum* infected wheat produced red-purplish pigment on basic media, and yellowish one on acid media, but no coloration was observed on weaker basic media as well as on weaker acid ones.

SMITH AND SWINGL (1904) [1] stated that the production of light reddish color by *Fusarium oxysporum* was retarded by alkalis, but promoted by acids.

According to KAWAMURA(1924), *Cercospora Kikuchii* produced the reddish purple pigment in media of all reactions, but the color is strongest especially in acid side.

Concerning *Helminthosporium gramineum*, NISHIKADO(1928) made some studies on the coloration relating to H-ion concentrations and he reached the conclusion that pigmentation did not take place at pH 4.8, and, with increasing of pH value, it was produced and became brilliant red at near pH 7.

In the case of *Helicobasidium Mompa*, the pigment production was more or less better at pH 5-7, but it may be said that the effect of H-ion concentration on the coloration of this fungus is not so remarkable as of that.

3. Effect of light

KAWAMURA (1924) observed that *Cercospora Kikuchii* produced reddish purple

1) Cited from NAKAMURA (1927).

pigment equally in both dark and light rooms. Studying some kinds of pigments of *Isaria virescens*, DANILOV (1925) divided them into two groups, the one produced only under existence of light and the other required no light.

Later, NISHIKADO (1926) reported that the coloration of the colony of *Piricularia oryzae* was better in light place than in dark one. According to NAKAMURA (1927), the pigment production of *Septoria callistephi* was not influenced by light at all.

Helicobasidium Mompa, seeing from the results of the experiments made by the present writer, produces the pigment both in the dark and in the light conditions very abundantly, but there may be a tendency to be promoted by the diffused light more or less. Accordingly, the case of the fungus may be similar to that of *Piricularia oryzae* reported by NISHIKADO (1926).

4. Effect of free oxygen

BESSEY (1904) described that *Fusarium sp.* and *Neocosmospora sp.* repuired free oxygen absolutely for their pigmentation, but on the contrary, in the case of *Fusarium culmorum* free oxygen was not necessary.

UEDA (1920) 1) reported that *Monoascus sp.* never produced the reddish pigment unless free oxygen was presnt.

With respect to *Cercospora Kikuchii*, KAWAMURA (1924) noted that its pigmentation was not effected by the presence of free oxygen.

Judging from the above-mentioned literature, there may be two groups of fungi, the one, for pigmentation free oxygen is absolutely necessary and the other, unnecessary.

Helicobasidium Mompa, according to the present writer's investigation, may belong to the former group.

5. Effect of nutrients of culture media

BRENNER (1918) 2) reported that *Penicillium purpurogenum* required magneium for its coloration.

As regards *Cercospora Kikuchii*, KAWAMURA (1924) reported that the pigment was produced very abundantly on media containing each of glucose, sucrose and glycerine, but effects of $CaCl_2$ and $BaCl_2$ were extremely slight.

Concerning *Isaria virescens*, DANILOV (1925) indicated the following facts: (1) There was close connection between the pigmentation and the amounts of nitrogenous compounds, (2) the pigment production was promoted by each of magnecia, phosphoric acids, sugars, proteins and peptone, but retarded by licium.

In the case of *Helicobasidium Mompa*, the coloration was very remarkable on media containing each of $CaCO_3$, peptone, protein, sugar, and alcohols (especially glycerine), but it was scarcely influenced by each of $MgSO_4$, KH_2PO_4, KCl, and $NaNO_3$.

6. Effect of concentrations of culture media

As already mentioned, DANILOV (1925) reported that the pigmentation of *Isaria virescens* was hindered gradually with increase in quantity of nitrogenous compounds, and at the limited concentration it was not observed. For example, on a medium containing only 0.016 per cent of $(NH_4)_2SO_4$ no coloration occurred, and also in the

1), 2) Cited from NAKAMURA (1927).

case of nitrates, about 0.04 per cent.

BESSEY (1904), studying *Fusarium sp.* and *Neocosmospora sp.* stated that reddish and purplish pigments were not produced at a certain limit of osmotic pressure of media, but orange pigment was found at higher pressure.

According to NISHIKADO(1926), color of the colonies of *Piricularia oryzae* was variable with amounts of carbohydrates in media, namely it was blackish orange on agar containing 0.5–3.0 per cent glucose; when the agar contained 15-30 per cent glucose, dark greenish orange, and when 40–50 per cent, colorless.

More recently HAMADA (1940), in his studies on *Armillaria mellea*, made an extensive study on the relation between the coloration and concentrations of both glucose and peptone. Results of his observaitons are summarized as follows: (1) The brown coloration of the colony was not remarkably influenced by the differences in the concentrations of glucose alone, whereas, it was strikingly effected by those of peptone, namely the optimum concentrations were 0.5–1.0 per cent, while at 4–8 per cent no coloration was observed. Generally speaking, the coloration of medium containing peptone was very slight comparing with that of glucose medium. (2) The brown pigmentation of agar media was variously influenced by the conentrations of glucose and peptone, viz., the pigment production was best at the concentrations of 1-2 per cent of glucose and at the higher concentrations it became scarce gradually. (3) When *Armillaria mellea* was cultured on agar media containing both glucose and peptone, the following facts were obtained; (a) the color of the colony was very deep at the combinations of lower per cent peptone and higher per cent glucose, while the pigmentation of agar media was better at the combinations of higher per cent peptone and lower glucose. In the latter case, some exceptions were observed.

Conseqently HAMADA came to the conclusion that the above-mentioned relationships might be denoted as N (peptone): C (glucose)-ratio, namely "Braunhautbildungen" was best at N/C=1/4, but "Bräunung der Nährböden" was not closely connected with N:C-ratio.

Concerning *Helicobasidium Mompa*, as pointed out already, the coloraton of the colony was not influenced remarkably on agar media having various concentrations ranging from 0.5 to 10 per cent of glucose and the color was not brilliant purplish brown, but light brown. In the case of peptone alone, the coloraton was deeper at lower concentrations (0.5-2.0 per cent) and especially it was deepest at the concentration of 0.5 per cent, but no coloration was observed at higher concentrations (5 per cent and 10 per cent). At 0.5 per cent, the color was deep purple, but became rich in brown at the higher concentration (1 per cent and 2 per cent).

The above-mentioned facts on the fungus are different from those reported by NISHIKADO(1926) as regards *Piricularia oryzae*, but are rather similar to HAMADA's (1940) description on *Armillaria mellea*.

In the case of the combination of various concentrations of both glucose and peptone, the coloration of the colony is influenced by peptone more conspicuously than by glucose. The color is apt to become purplish by adding peptone in low con-

centrations. On the other hand, there is a tendency to become lighter in color by mixing with glucose.

The pigmentation of medium is betten when it contains low concentrations of both glucose and peptone, furthermore different colors were observed according to various combinations of them.

Though the pigment production of the fungus may be indeed promoted by a certain combination of both glucose and peptone, as it is so variable that the writer can not come to such a definite conclusion as N/C-ratio offered by HAMADA (1940).

Considering from the reactions of *Helicobasidium Mompa* on media, the writer cannot agree with BESSEY's opinion that the coloration is depend upon the osmotic pressure of media alone. It is considered by the present writer that the coloration is a special character of *Helicobasidium Mompa* in certain concentrations of carbohydrates and organic nitrogenous compounds.

7. Signification of pigment production

As regards the signification of the pigment production in fungous world, very few reports have been published up to the present time.

LINOSSIER (1891) 1), in his study on *Aspergillus niger*, stated that the dark color of conidia was relating to respiration. Later, DANILOV (1925), in his investigation of *Isaria virescens*, called attention to the fact that the pigment production was a protective action of the fungus against the light because the pigment was produced only on the surficial portion of colonies, but not on the inner portion.

Judging from the result of the present writer's experiment that *Helicobasidium Mompa* requires sufficient free oxygen for its pigmentation, and that in a very small amounts of free oxygen the feebly developed mycelium of the fungus is colorloss, it may be considered that the pigment is not, perhaps, produced for the purpose of respiration, but produced as the result of sufficient respiration; and accordingly, at least in the case of this fungus, the writer can not agree with LINOSSIER's opinion.

Furthermore, the present writer has no reason to support DANILOV's theory, because the pigment production of the fungus is made almost equally both dark and light conditions.

When the fungus is mix-cultured with other microorganisms, the pigmentations of the colony and of the medium become better strikingly, and, in most cases, the growth of the mix-cultured microbes is hindered conspicuously. These phenomena do not always occur with every kind of mix-cultured microorganisms; namely some of them are not relating to the coloration.

In cases of mixed culture with certain microorganisms a deep purplish zone is formed halfway between two colonies. Furthermore, the coloration of the fungus is promoted by adding staled solution of the microorganisms. From these facts the writer can not confirm that the pigment of *Helicobasidium Mompa* is produced for the purpose of defence against other microorganisms. Turning to the factors influencing the pigment production of the fungus, favorable nutritions, free oxygen, suitable tem-

1) Cited from NAKAMURA (1927).

peratures and some periods are indispensable conditions for the sufficient production of the pigment of the fungus.

The old purplish-brown mycelia developed under favorable conditions may be "Dauermyzelien" (FISCHER u. GÄUMANN 1929), because of following facts:

(1) They take several days for starting to grow again,

(2) they are highly resistant to low temperatures below zero,

(3) after overwintered, hymenial structures are formed on them (p.24).

Taking into consideration the above-mentioned facts, the writer comes to the conclusion that the pigment production is a unique character of the fungus following its maturity as a result of metabolism. The pigment may have some physiological and ecological significance in respect that when it becomes darker the mycelium becomes also more resistant to low temperatures and can pass the winter safely.

PATHOLOGICAL ANATOMY OF THE AFFECTED PLANTS

No experimental study on the infection of the host by *Helicobasidium Mompa* TANAKA has been published up to the present time. Concerning the pathological changes in the plant tissues, MIYAKE (1920) made a brief report on the roots of the mulberry tree attacked by the fungus.

The present writer has made some experiments to know the mode of penetration of the fungus and the anatomical observations on the tissues affected by *Helicobasidium Mompa.* A report was preliminarily published by YOSHII and the present writer (1944).

Plants used were sweet potatoes, soy beans, mulberry trees and potatoes. More extensive studies were carried out on the former two.

Mode of penetration

Before making a detailed study of the relation of the fungus to the various host tissues it was considered of fundamental importance to know how it enters the host. There seems to be no definite information as to the exact mode of entrance of the fungus into its hosts. It was hoped that by using pure culture methods penetrations of various plants by this fungus might be obtained so that they could be detected by the aid of microscope.

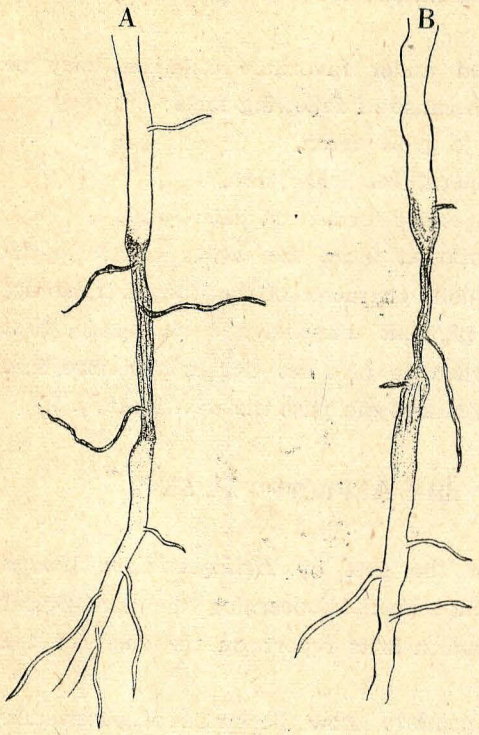
For this purpose the following four of plants, viz., mulberry seedlings, potatoes, soy beans and sweet potatoes were used as materials for inoculation, but the first two were omitted in this report, because these were similar to the latter two.

1. Young root of sweet potato

a. MATERIALS AND METHODS

Two varieties of the sweet potato, Okinawa Hyakugô and Genji, were used as fundamental materials of this experiment.

Seedlings which had been cultured in sand-bed were rooted up carefully and the



Text-fig. 13. A. Young root of sweet potato infected by *Helicobasidium Mompa*. After 3 days at room temperature. x 8/3. B. Young root of mulberry seedling infected by *Helicobasidium Mompa*. After 2 days at room temperature. x 8/3.

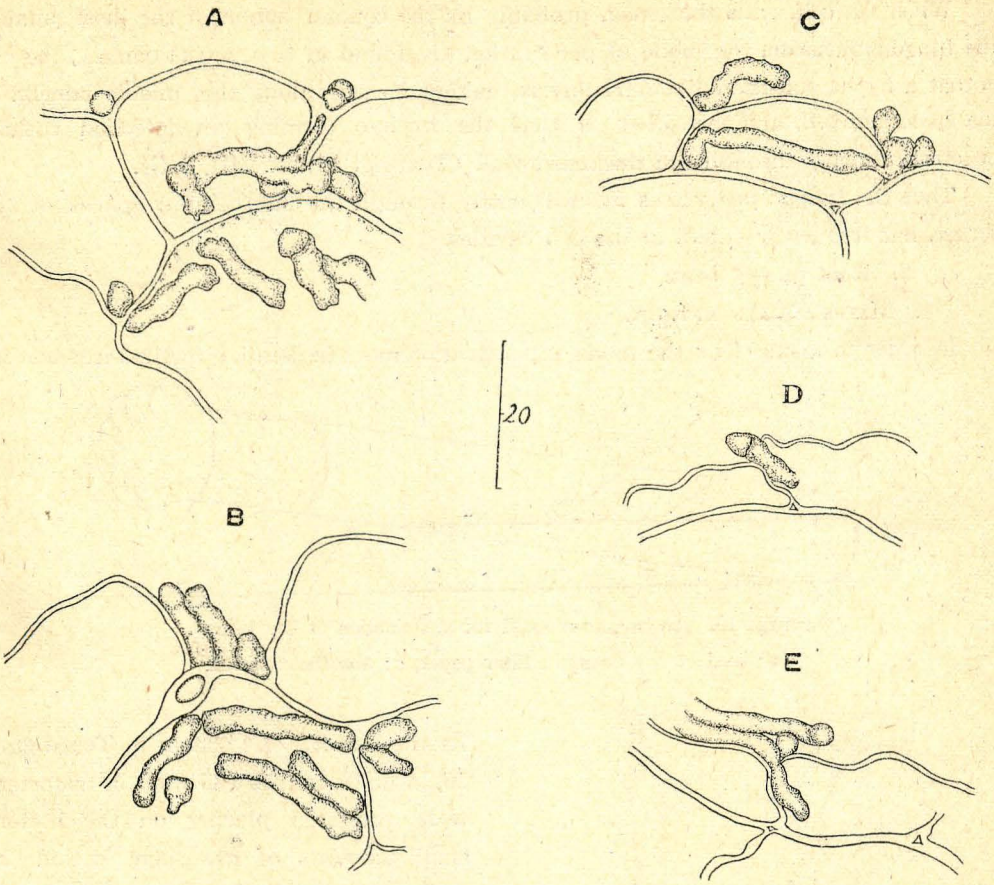
roots of them were washed several times with sterile water, and then placed in the moist chambers, where filter papers wetted with water were prepared. As inocula small squares cut from the colonies of the fungus (Strain M-1) growing on potato-glucose agr plate were placed on the young roots of the seedlings.

Only after staying 24 hours at 19° to 27°C. (On June 1-2), the inoculated portions of the root changed already to light yellowish brown and further, after two days, they were more or less withered, becoming purplish brown. The severely infected rootlets became finally blackish brown in color (Text-fig. 13). The inoculated portions were fixed in chromo-acetic acid solution (chromic acid lg., glacial acetic acid lcc., distilled water 100 cc.).

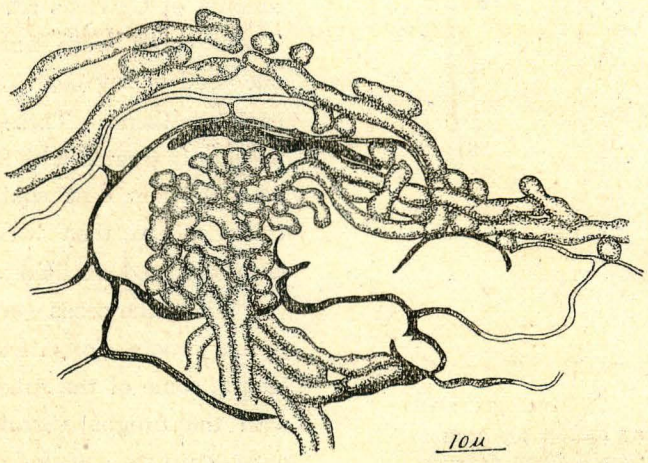
By ZIRKLE's (1930) n-butyl alcohol method sections were made from 8 to 10 μ in thickness, and stained with FLEMMING's triple stain. As controls, uninjured roots were fixed and stained in similar manners.

b. OBSERVATIONS

The mycelium covers the whole surface of root near the inoculum and frequently grows along the suture portions of the cells of epidermis. Usually the hypha of the fungus penetrates through the suture of epidermal cells. It is rare to observe the hypha actually piercing the epidermal wall. In these cases, little or no constriction of the hypha is noticed at the point where penetration occurs (Text-fig. 14, A-C).



Text-fig. 14. Mode of penetration of *Helicobasidium Mompa*.
A-C : Young root of sweet potato,
D-E : Young root of mulberry seedling.



Text-fig. 15. Penetration of the thickened cell wall of young root of sweet potato by forming an infection cushion.

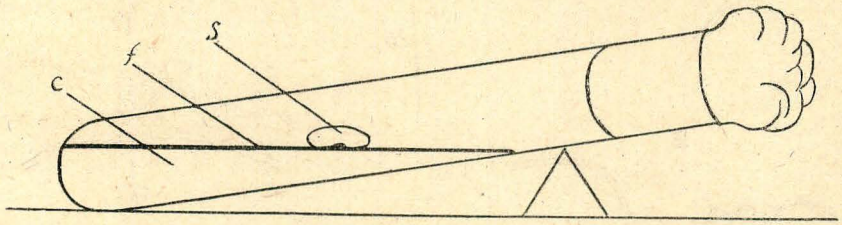
When the cell walls thickened, probably by the counter action of the host against the fungous invasion, the mode of penetration are found in two ways; namely, the one is that a hypha constricted remarkably is making its way along the middle lamella of the epidermal cell, and the other is that the hyphae forming an infection cushion penetrate forcibly through the thickened wall (Text-fig. 15; Pl. VIII, B, C).

Thus the fungus progresses inward mostly through the intercellular spaces of the cortex, but frequently enters in the cell cavities.

2. Root of soy bean

a. MATERIALS AND METHODS

In order to make clear the mode of penetration more in detail, a method was deviced



Text-fig. 16. An aseptic method for germination of soy bean.
s: seed of soy bean, f: filter paper, c: absorbent cotton.

Text-fig. 17. Young root of soy bean inoculated with *Helicobasidium Mompa*.
A. After 4 days at room temperature. x 1
B. After 8 days at room temp. x 3

by the writer. As shown in Text-figure 16, large test tubes (35 mm. in diameter) were prepared placing on the bottom small amounts of absorbent cotton (*c*) and a sheet of filter paper (*f*) in layers which were moistened and rendered as a medium for the growth of both fungus and seedling by pouring an adequate amount of CZAPEK's solution over them. These tubes were autoclaved and then one seed of soy bean (*s*) was placed in each of them. These seeds had been previously treated with 80 per cent alcohol, with 0.1 per cent solution of mercuric chloride, and then washed several times with sterilized distilled water.

When the seeds germinated and the primary roots elongated to an adequate length, some of the tubes were inoculated with the fungus (Strain M-1) from a pure culture, while others were left uninoculated to serve as controls.

The inoculated portion colored in light

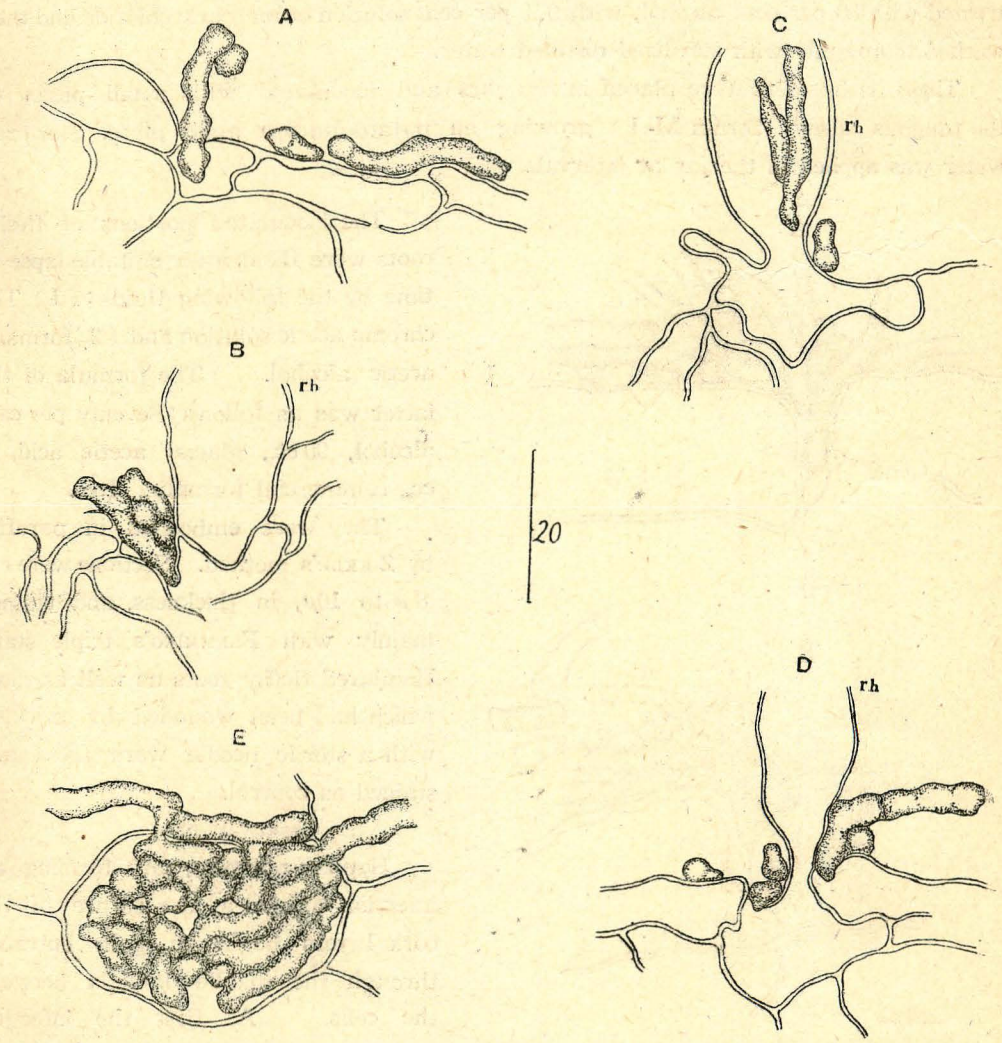
brownish gradually and at the end of four days (on May 14-18) the heavily infected part became conspicuously slender as shown in Text-figure 17.

By the same manners mentioned above, the affected roots as well as uninoculated ones were sectioned and stained.

With respect to the old root of matured plants, the materials obtained from the inoculation experiment in the pot were used.

b. Observation

Hyphae creep along the suture of the epidermal cells and gain entrances from this portion very frequently (Text-fig. 18). Slight constriction of the hypha is observed at the point where the penetration occurs.



Text-fig. 18. A–D: Hyphal penetration of the epidermis of soy bean root.
rh: root hair.
E: Hyphae in the cortical cell of the young root of soy bean.

The hyphae which penetrated the epidermal cells develop intercellularly as well as from cell to cell in the cortex (Text-fig. 18,E) , but they are uable to attack the well-lignified xylem portion in vascular system.

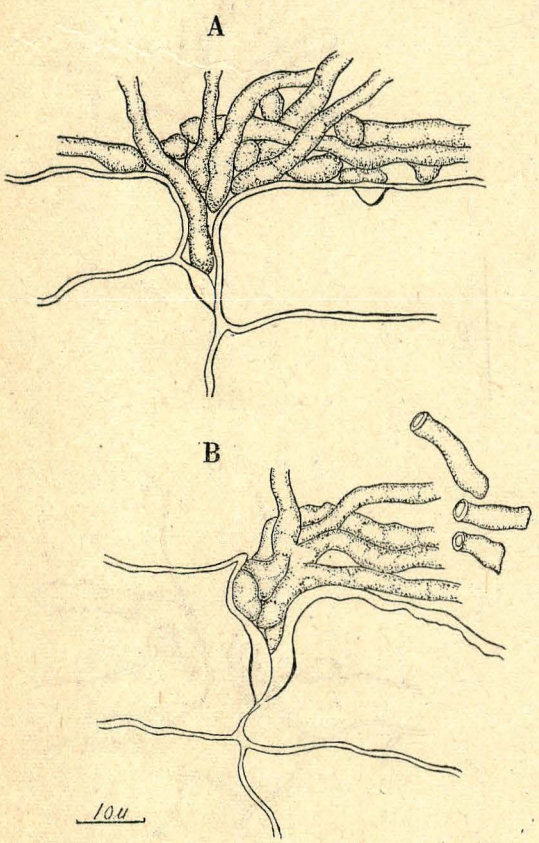
In the case of penetration of the old suberized root, after forming a mycelial cushion the hyphae invade the periderm through the suture portion (Pl. VIII, G).

3. Fleshy root of sweet potato

a. Materials and methods

In this experiment, three varieties of sweet potato were selected as follows : Okinawa Hyakugô, Nôrin Nigô and Genji. The glass jars with covers which had been placed on the bottom a sheet of filter paper and small squares of absorbent cotton moistened with water were autoclaved. The surface of the fresh fleshy roots to be used was treated with 80 per cent alcohol, with 0.1 per cent solution of mercuric chloride and then washed thoroughly with sterilized distilled water.

These fleshy roots were placed in the jars and inoculated with small pieces of the fungous colony (Strain M-1) growing on potato-glucose agar plate. Sterilized water was applied in the jar at intervals.



Text-fig. 19. Early stage of infection at the phellem of fleshy root of sweet potato (A–B).

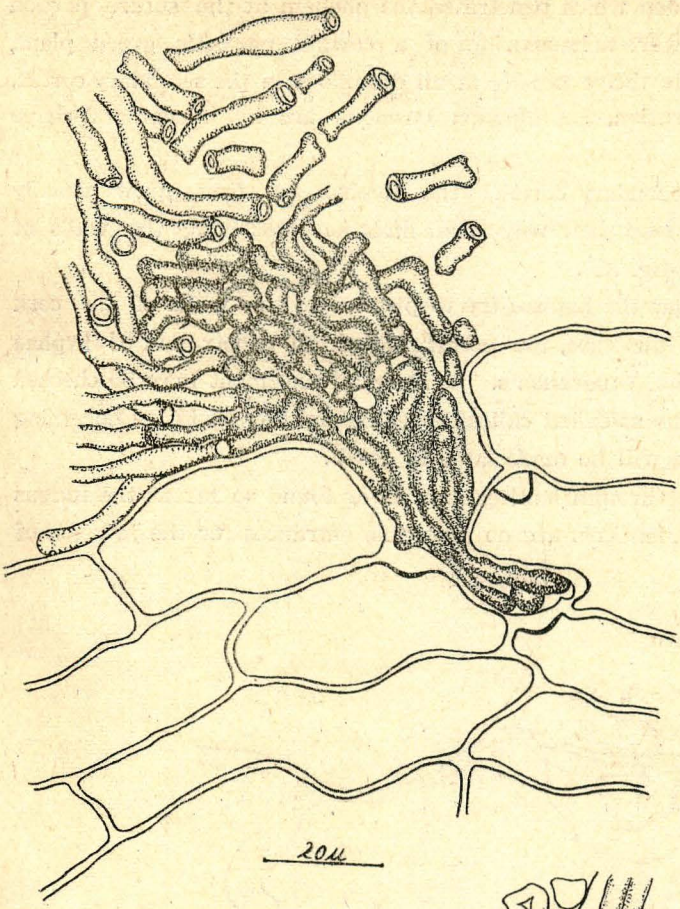
The inoculated portions of fleshy roots were fixed after suitable lapse of time by the following fluids : (1) The chromo-acetic solution and (2) formalin acetic alcohol. The formula of the latter was as follows : Seventy per cent alcohol, 90 cc., glacial acetic acid, 5 cc., commercial formalin, 5 cc.

They were embedded in paraffin by Zirkle's method. Sections were cut 8 μ to 10 μ, in thickness, and stained mainly with Flemming's triple stain. Uninjured fleshy roots as well as those which had been wounded by pricking with a sterile needle were fixed and stained as control.

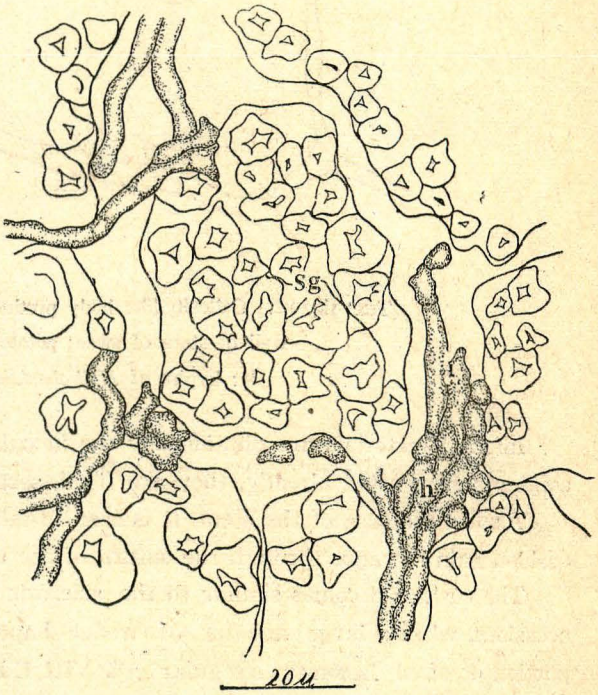
b. Observation

Hyphae amassing and forming an infection cushion at the suture of the cork layers begin to gain entrance through the middle lamella between the cells. At first the infection cushion is only a bundle of hyphae, but gradually becomes sclerotium-like in shape as the invasion progresses.

As soon as the hyphal bundle of



Text-fig. 20. An infection cushion of *Helicobasidium Mompa* penetrating the phellem of fleshy root of sweet potato.



Text-fig. 21. Hyphae of *Helicobasidium Mompa* in the secondary cortex (fleshy tissue) of fleshy root of sweet potato.
sg: starch grain, h: hyphae of the fungus.

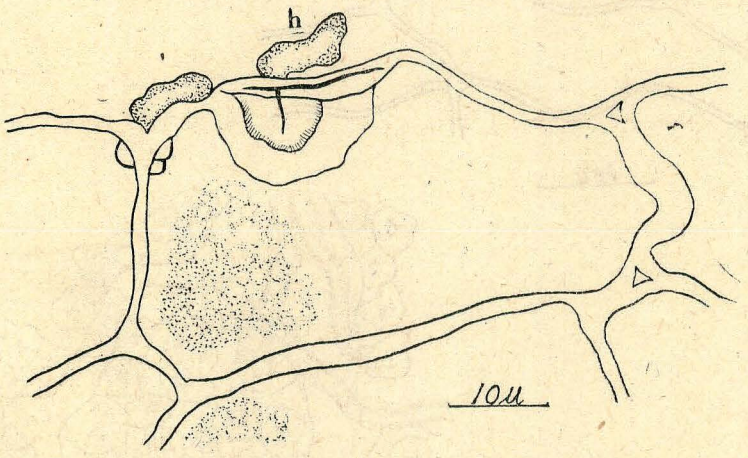
the under side of infection cushion which penetrated the phellem at the suture portion reaches the phellogen, as if it were a haustorium of a certain parasitic green plant, the apical hyphae of the bundle thrive rapidly in all directions in the secondary cortex. In the final stage of the penetration, the infection cushions are in "mushroom"-shape (Text-fig. 19, 20; Pl. VIII, A,D).

In the fleshy tissue (the secondary cortex) the hyphae at first grow usually intercellularly, but later they make their way in all directions dissolving the walls of the parenchymatous cells (Text-fig. 21).

It is commonly observed that the hyphae try to pierce into the walls of the cork cells (phellem) directly, but, in this case, the occurrence of the penetration of hyphae is never found throughout the experiments. Their advancement may be checked perfectly by the formation of the so-called callosities (Text-fig. 26; Pl. IX). Concerning the callosities further discussion will be made later in detail.

The invasion of the hyphae through lenticels has never found so far as the fungus is concerned. It seems that lenticels are no favorable entrances for the invasion of the fungus in question.

4. Stem of sweet potato



Text-fig. 22. Callosity-like body produced on the epidermal cell wall of stem of sweet potato by artificial inoculation.
h: hypha of *Helicobasidium Mompa*.

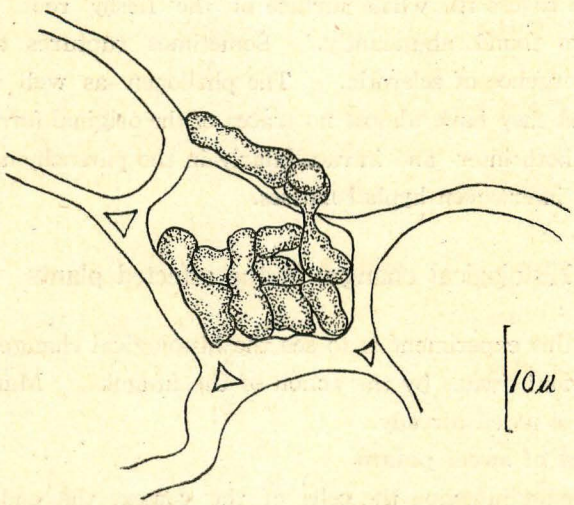
Infected materials are obtained by the inoculation experiment in the pots. By the same manners noted already, they are fixed, sectioned and stained.

Also in the case of the stem, it is seen usually that hyphae forming an infection cushion gain entrance through the suture of the cutinized cell wall of the epidermis.

The cushion becomes similar to the sclerotium in shape generally, but, in some occasions, when a large amount of wedge-shaped mycelial mass invade the suture portion it often becomes irregular (Pl. VIII, E-F; Pl. X,E, F). Sometimes an actual piercing of the epidermal wall by the fungus is noticed, but the penetration is commonly

checked by the callosity (Text-fig. 22) .

Rarely a conspicuously constricting hypha enters the cell cavity of the epidermis and some branchings occur in the cell, but soon the hyphae are enclosed by the remarkable thickening of the cell wall and no instances are observed further invasion of the hyphae into the cortex (Text-fig. 23).



Text-fig. 23. Penetration of hypha in the epidermis of stem of sweet potato.

Anatomical observations on the diseased fleshy roots of sweet potato in various degrees of infection

The affected fleshy roots in various degrees of infection which had been classified in the proceding page (p. 9) were fixed, sectioned, and stained. The results of observations on each of these degrees are noted briefly as follows:

(1) Very slightly affected fleshy roots (degree-α): Small wedge-shaped mycelial cushions are formed on the suture portions of the periderm attached with rhizomorphs. The mycelium enters only one or two layers of the phellem. In general, a very few infection cushions are observed and no change occurs in the periderm except the portion of the cushions.

(2) Slightly affected fleshy roots (degree-β) : Almost all surface of the fleshy root is covered with the purplish mycelial mat and many infection cushions are observed. Some of the infection cushions invade several cork layers, but there are not still seen cushions which penetrate the periderm perfectly. In the outer layers of the periderm, the thickening of the cells and the formation of callosities are noticeably observed.

(3) Moderately affected fleshy roots (degree-γ) : The surface of the fleshy root is covered with the thick mycelial mat of purplish brown in color. A great number of infection cushions penetrate the cork layers and the hyphae invade the phellogen and the phelloderm. Here, they thrive conspicuously and further develop in the secondary cortex (fleshy tissue) mostly intercellularly, but frequently enter also in the cell cavities.

The cell walls of both the phellogen and the phelloderm are fairly dissolved, but some of them retain still their original forms. The cell walls of the outer layers of the periderm are remarkably thickened and a great many of callosities are formed on the cork cells. The infection cushions become typically mushroom-shaped.

(4) Heavily affected fleshy roots (degree-δ) : The thick mycelial mat colored in deep purplish brown covers the whole surface of the fleshy root. The mushroom-shaped sclerotia are found abundantly. Sometimes ruptures are produced in the periderm by the emergence of sclerotia. The phellogen as well as phelloderm are entirely dissolved and they have almost no traces of the original forme. The mycelium develops vigorously both inter- and intracellularly in the parenchymatous tissues of the fleshy part, forming interwoven hyphal masses.

Histological changes in the affected plants

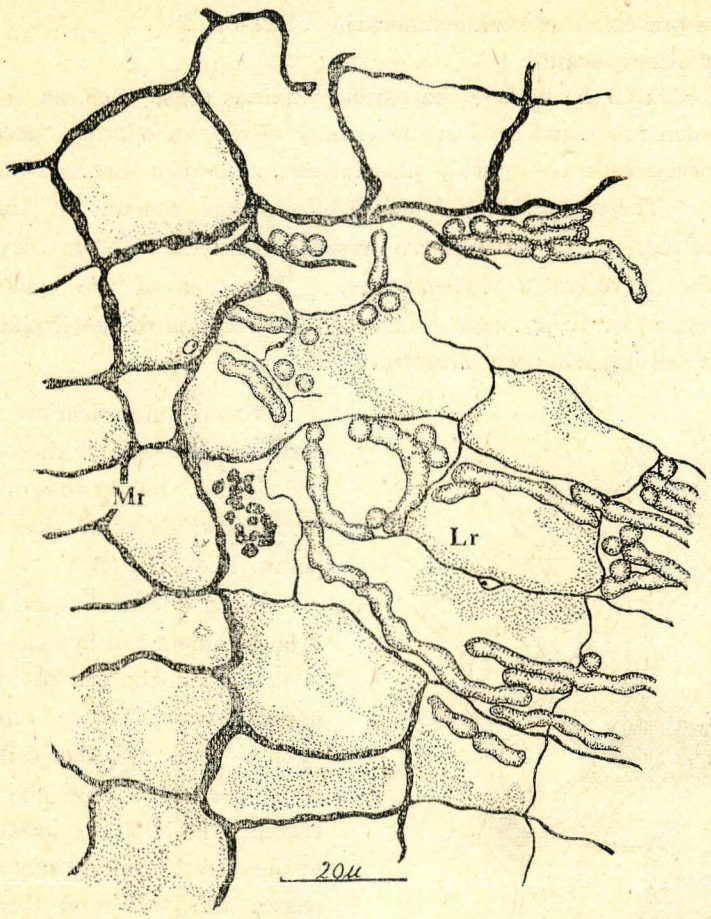
The purpose of this experiment is to see the histological changes which take place in the infected cells and tissues by the action of the fungus. Materials and methods are the same as those noted already.

1. Young root of sweet potato

In the case of heavy infection the cells of the cortex, the endodermis and other parenchymatous tissues are dissolved seriously and only those of the epiderm and the xylem remain their originl shapes. The most conspicuous change in the host tissues is markedly increased tendency of the stainability of the cells walls; when the invasion is comparatively slow, the walls of the cortical cells as well as the endodermis become thickened remarkably and become very stainable with safranin. The thickening or the swelling of these walls may be found to be associated with invasion or before the hyphal penetration. Frequently, the fungus forming a bundle of hyphae penetrates forcibly the heavily thickened cell wall of the cortex, resulting the raising of cell walls of the cortex and the epidermis by the mass action of the mycelium (Pl. VIII. B, C).

The region of the thick cell-walls stained strongly is seen to extend over a considerable distance beyond the limit of fungal penetration. The invasion of the fungus is sometimes observed to be checked, perhaps, temporarily, by the thickened walls of the endodermal cells.

Accompanying the changes of the staining reactions of the cell walls the changes in the protoplasm itself are observed; the protoplasm of the host cells not only immediately surrounding the portion of invasion, but also those at some distance from the fungus becomes plasmolyzed and granularized in appearance. Furthermore there are often some small masses stained with safranin in the affected cells. The nucleus of the cell near the fungous invasion becomes less stainable, but the peculiarity of the nuclear orientation is not seen.



Text-fig. 24. The original portion of the lateral root of sweet potato which had been infected heavily by *Helicobasidium Mompa*.
Mr: main root, Lr: lateral root.

The lateral roots of the sweet potato are affected readily and the parenchymatous cells of them are dissolved rapidly. In the incipient stage of infection the walls are thickened and wound-gummified conspicuously. The exudation of the wound-gummy substance is also occurred in the intercellular spaces.

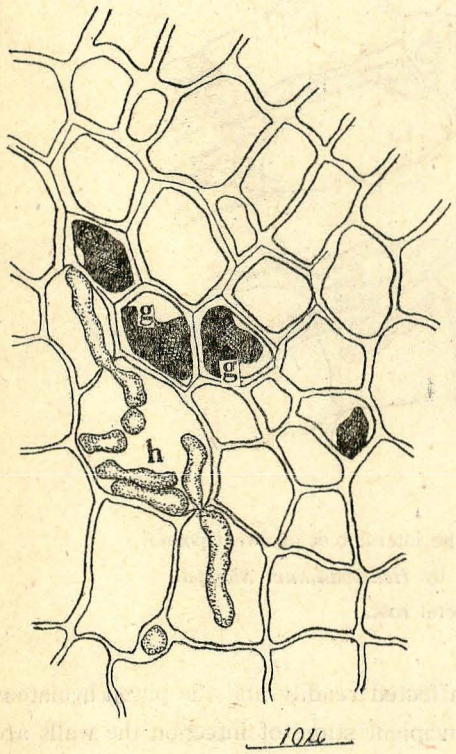
In the final stage, these roots are discolored in dark blackish brown and fall off, and the wound-gummification takes place in large quantity in the tissues adjacent to the rotted portions as far as several cell layers in the cell cavities as well as in the intercellular spaces. Thus, with the aid of thickening of the cell walls, it seems likely that the advance of the parasite is checked for no further development of the hyphae beyond these barriers (Pl.X. B,C,D). Plasmolysis and degeneration in the cells occur beyond the hyphae and at a great distance from the fungus.

When the hyphae make advance deeply in the basal portion of the lateral root, the cells in the vicinity of the pericycle of the main root, the origin of the lateral

root, are remarkably wound-gummified and here, not rarely, further invasion of the fungus may be prevented at least temporarily (Text-fig. 24).

2. Root of soy bean

By the invasion of the hyphae the parenchymatous cells, such as the hypodermal layers of the young root and cells in the vicinity of the endodermis, including cortical as well as pericycle cells are quickly plasmolyzed and then brought to the granular degeneration. The cortical cells attacked by a large amount of the hyphae are sometimes dissolved, but, in general, are pressed to the stele with the cell walls becoming slender, thus they leave traces of themselves. The cells of the epidermis and the endodermis keep rather their original shape. Penetration of the hypha through the lignified xylem cell has not been observed.



Text-fig. 25. Cross section of the lateral root of soy bean infected by *Helicobasidium Mompa*.
g: wound gum, h: hyphae of the fungus.

Wound-gummification is often observed in the cells of the cortex and the endodermis, but in these cases, it is not so remarkable as in the root of sweet potato (Text-fig. 25).

In the infected lateral roots there are a large amount of hyphae in the cortical layer both in the cell cavities and in the intercellular spaces. Furthermore the hyphae penetrate the cell walls of the stelar portion except the xylem cells. Usually, the hypha is greatly constricted at the point of penetration; a fine filament passes the wall and then immediately swells up again to its original size after gains its position in the new cell cavity.

So far as the present experiment is concerned, it appears that the hyphae are unable to attack the lignified xylem cells, which are frequently filled with plugs of wound-gummy substance (Text-fig. 25).

In the case of heavily infected old roots, the parenchymatous cells of the hypodermal tissues are dissolved severely and almost have no trace of the original form, and consequently there exists a noticeable space between the periderm and the stele.

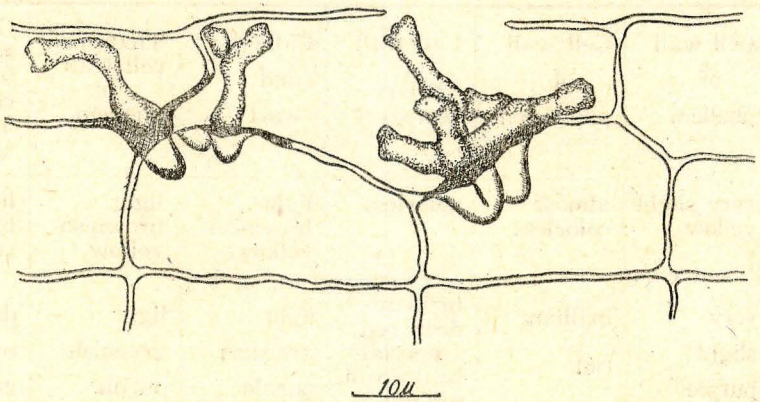
3. Fleshy root of sweet potato

a. Microscopic observation

For this purpose both artificially and naturally infected fleshy roots were used. They were fixed in formalin-acetic alcohol or in chromo-acetic acid solution, then were

cut from 8 μ to 10 μ in thickness by the manners of ZIRKLE (1930) and stained mostly with FLEMMING's triple stain.

The hyphae which entered through the suture portion of the cork layer, forming an infection cushion, develop vigorously in the phellogen and the phelloderm, and here dissolve the cell walls conspicuously. Furthermore, making advance inward, they invade the cortical tissues of the fleshy portion. At first they grow here only intercellularly, but soon destroy the cell walls. Thus the upper part of the fleshy root are more or less softened. There are formed so noticeable spaces between the phellem and the fleshy part that the cork layers can be separated readily.



Text-fig. 26. Callosities formed of the cork cells of fleshy root of sweet potato by artificial inoculation of *Helicobasidium Mompa*.

Starch grains in the cortex near the heavily invaded portion are less stainable, and their outline becomes indistinct.

It is noteworthy to state that the rapid soft-rot of the fleshy root may be chiefly due to the secondary invasion of decaying microorganisms inhabiting in the soil, because they gain entrance through the ruptures of the phellem near the mushroom-shaped sclerotia and thrive mostly in the cortical cells intercellularly, and finally dissolve the tissue.

In the phellem, thickened or swollen cell walls are commonly observed in association with invasion of the infection cushion of the hyphae, and a great many of callosities are also produced on the opposite side of the walls pierced by the hyphae. Invasion of the hyphae is checked by the formation of the callosities without exception, and the hyphae never grow out of the callosities (Text-fig. 26. Pl; IX.).

As well as callosities, deposits stained with safranin are seen frequently on the walls of cork cells. Seeing from their appearances, they may be probably the same bodies reported by ALLEN (1927) as "warts".

In order to make clear the optical character of the swollen bodies, such as callosties, warts and thickened cell walls, the writer examined them by Nicol's prism under the microscope. The result of the examinations showed that these bodies were inactive optically.

As regards the callosity, further detailed descriptions will be made in the later part of this paper.

b. Microchemical test

Several thousand sections of artificially or naturally infected fleshy roots were made 8 μ to 12 μ in thickness by the microtome, and their pathological changes were examined by microchemical tests and compared with healthy ones.

The results of the experiments will be summarized in Table 44.

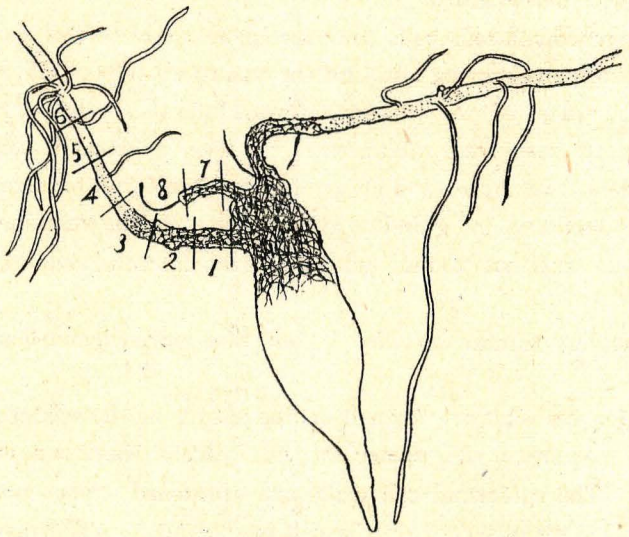
Table. 44. Microchemical reactions of the diseased fleshy root of sweet potato.

Kind of tissues / Stains	Cell wall of phellem	Cell wall of phellogen	Cell wall of 2ndary cortex	Callus and wart	Thickened cell wall of phellem	Gum like substance in cell cavities and intercellular spaces
No stain	very slight yellow	almost colorless	colorless	light brownish yellow	light brownish yellow	light brownish yellow
Ruthenium red and methyl green	very slight purple	brilliant red	brilliant deep red (especially deep in middle lamellae)	light greenish purple	light greenish purple	deep brownish green
Sudan III and methyl green	light red	middle lamellae are slight red	middle lamellae are slight red	purplish green (some of them are reddish)	purplish green	deep green
Chloro-iodide of zinc	slight yellow	almost colorless	almost colorless	yellow	yellow	brownish yellow
Methylene blue	greenish blue	brilliant blue	light blue	brownish green	brownish green	deep blue
Congo red (ammonium solution)	slight red	light red	outer lamellae are red, but middle lamellae almost colorless	almost colorless	almost colorless	very slight red
Safranin	deep red	orange yellow	orange yellow (especially middle lamellae)	deep red ~ orange red	deep red ~ orange red	deep orange red
Determination	Suberin, Pectin (middle lamellae)	Pectin (especially middle lamellae)	Pectin (especially middle lamellae)	Wound-gum, Pectin, Lignin ?	Wound-gum, Pectin, Lignin ?	Wound-gum, Pectin, Lignin ?

4. Serial cross section of the affected stem of sweet potato

a. MATERIALS AND METHODS

The infected stems of the seedling which had been inoculated with the fungus in



Text-fig. 27. Heavily infected sweet potato inoculated by *Helicobasidium Mompa* artificially. x 2/3.

the pot were cut into pieces of suitable length as shown in Text-figure 24. These pieces were numbered serially and placed in separate fixing vials containing formalin chromic acid solution (formalin 3 cc., chromic acid 1g., distilled water 100cc.). With the same manners mentioned already, the materials were sectioned transversally from 8 μ to 10 μ in thickness and stained with FLEMMING's triple stain. Thus the histological changes as well as the mycelial development in the tissues of these sections were examined systematically under the microscope comparing with their macroscopic appearances.

b. MACROSCOPIC APPEARANCES

No. 1 [1]……The epidermis with abundant rhizomorphs and sclerotia is separated from the stele and the parenchymatous tissues are destroyed heavily.

No. 2……This is very similar to No. 1.

No. 3……Rhizomorphs and sclerotia are not seen at all. The portion beneath the epidermis is colored in blackish brown.

No. 4……No remarkable changes are noticed.

No. 5…… do.

No. 6…… do.

1) This number designates that of the piece in Text-figure 27.

No. 7 ……Numerous rhizomorphs and sclerotia are present, but the epidermis is not yet separated.

No. 8 ……The epidermis falls off completely and the parenchymatous tissues disappear. Only the central portion remains.

c. Microscopic observations

No. 1 ……The parenchymatous cells near the phloem and of the cortex are affected heavily by the mycelium entering through the suture portion of the epidermis and they leave almost no trace of the original form, but the xylem remains unaffected. Matured sclerotia are seen very abundantly. The epidermal cell walls near the sclerotia are thickened remarkably and are stained deeply with safranin. The xylem cells are often filled with wound gum-like substances. In the remained parenchymatous cells, starch grains are not found, but bacterial masses invading secondarily are noticed.

No. 2 ……This is very similar to No. 1, but the parenchymatous tissues are less dissolved.

No. 3 ……Infection cushions are formed on the suture of the epidermis and many of them do not still penetrate the epidermis, but rarely there are some entered the cortical tissues. The epidermal cell walls are thickened conspicuously and a large number of callosities are observed on the cell membrane. The mycelial development in the cortex is limited to small range. The protoplasm in esch cell of several layers of the cortex is plasmolized and becomes granular in appearance. In the small regions near the fungal invasion, the cell walls are more or less dissolved and the wound gummification occurs. In the parenchymatous tissues, there are numerous bacterial masses both in the cell cavities and in the intercellular spaces (Pl. X,H).

No. 4 ……Neither the fungal penetration of the epidermis, nor the mycelial thriving are observed. Bacterial precipitations are seen in some cells of the phloem.

No. 5 ……This resembles to the case of No. 4, but bacterial precipitations are rare (Pl. X, G).

No. 6 ……This is healthy and no histological change is observed.

No. 7 ……The mycelia thrive in the cortex both inter-and intracellularly. The cell walls attacked by the fungus heavily are dissolved and disappear. The hyphae are generally checked to invade the steler tissue at the endodermis, but sometimes a few enter the cells of the pericycle. Wound-gummy deposites are formed remarkably in the cell cavities of the cortex as well as in the intercellular spaces. Almost no histological change is observed in the stele (Pl. X, E,F).

No. 8 ……The heavily decayed cortical tissues disappear. The cell walls of the some other parenchymatous cells remain still no injured, though they are surrounded by the hyphae of the fungus. The xylem portions are not attacked at all, keeping their original forms. The phloem portions are filled with brownish wound-gummy substance both in the cell cavities and in the spaces, while in the xylem very scarce. Some bacterial massses are present in the stele.

d. Summary of the observations

(1) The hyphae penetrated the suture of the epidermis make growth in the cortical layers at first intercellularly and then in the cell cavities. In the case of heavy infection the cell walls are finally dissolved. The fungus enters the phloem, but never the xylem.

(2) Degeneration of the protoplasm and the production of the wound-gummy substance are seen even in the cells at considerable distance apart from the hyphae. In severely attacked tissues, a great amount of bacterial masses which, perhaps, invaded the host secondarily are observed.

(3) The range of the hyphal enlargement in the longitudinal direction is so small beyond expectation that there are seen no mycelia in the sections at some distance (1–3 cm.) from the heavily rotted portion. While, on the contrary, the secondarily invaded bacteria are seen to extend for a considerable distance beyond the limits of the infected portion by the fungus.

Discussion and conclusion

(1) On the mode of penetration

As regards the mechanism of the penetration of the tissues by parasitic fungi, a large number of works have been reported by various invstigators since the time of DeBary. The greater part of the discussion has concerned the question of the penetration of cuticularized plant surface. Some studies, however, have been made of the penetration of cell membranes within the host.

In order to explain the question as to the exact means by which fungous hyphae penetrate cell membranes, as being well known, two theories have been advanced; the one is the so-called chemical theory supported by DeBary (1886), Ward (1888-89), Büsgen (1893), Behrens (1898), Smith (1902), Voges (1910), Brown (1915), Harter and Weimer (1921), Higgins (1927), Paintin (1928), etc., and the other is the mechanical theory offered by Blackman and Welsford (1916), Hawkins and Harvey (1919), Dey (1919, 1933), Boyle (1921), Waterhouse (1921), Brown and Harvey(1927), etc.

Some investigators, such as Leach (1923), Matsumoto (1923), Pearson (1933), Ikata (1933), Hori (1934), Butler (1935), etc., have expressed the opinion that fungal hyphae may penetrate through the cell membrane by neither the chemical means nor by the mechanical process alone, and by the combined action of these two processes the penetration may be completed.

Concerning the mechanism of the penetration of *Helicobasidium Mompa* some discussions will be made by the present writer:

(a) In the case of the penetration of the non-cutinized or the non-suberized cell walls, as mentioned already, the following data are obtained, namely (i) no conspicuous constriction of the hypha at the point of penetration is generally seen, (ii) the fungus secretes a large quantity of pectinase and cellulase (p.52).

Taking into consideration these data shown by the fungus and the microchemical reactions of the cell membranes of the hosts, it is considered that the penetration may take place merely in a chemical way. But, it may be, perhaps, unable to explain

by the chemical process alone, because of the fact that hyphae constrict at the thicker cell walls.

(b) As noted already, the hyphae of the fungus can penetrate the cutinized wall by forming an infection cushion. Similar examples of penetration have been reportad by ULLSTRUP (1936) in the case of infection of China aster leaves by *Rhizoctonia solani* and NAKAYAMA (1940) in the infection of cotton seedlings by the same fungus. ULLSTRUP (1936) concluded, from his observation, that passage of *Rhizoctonia solani* through the cuticle is entirely a mechanical process.

According to BUDDIN and WAKEFIELD (1927), the hyphae of *Helicobasidium purpureum*, the perfect stage of *Rhizoctonia crocorum*, may penetrate the roots of red clover and other plants by making an infection cushion.

So far as the writer can ascertain, there are a few accounts concerning the fungal penetration of healthy cork layers. WATKINS (1938), in a study of *Phymatotrichum* root rot of the cotton, reported that *Phymatotrichum omnivorum* making a hyphal mass dissolved the phellem cells and developed into the phellogen, the phelloderm and finally into the cortex rapidly. He was not sure of the detailed process of the penetration, but suggested that the fungus might probably secrete a substance capable of dissolving suberin.

SCHAAL (1939) made some observations of the actual growth of the hyphae of *Rhizoctonia solani* entering the uninjured skin of potato tuber and reported that some wedge-shaped infection cushions of the fungus were invading a few layers of the phellem, but no perfect penetration of the skin was found. Thus he concluded, without any experimental proofs, that the fungus may probably gain entrance only through the lenticels of the healthy potato tuber. Somewhat similar phenomenon was noted by FARIES (1921) in the case of potato tuber attacked by *Rhizoctonia crocorum*.

Judging from the general opinions, *Corticium salmonicolor* seems to penetrate the uninjured periderm of the para rubber tree, but no experimental studies have been made up to the present time.

It is noteworthy to state the fact that *Helicobasidium Mompa*, as shown previously, can penetrate the cork layers of the hosts only through the suture portions, forming infection cushions. A study of the numerous examples of penetration through the phellem does not furnish evidence sufficient to justify the conclusion that penetration is either by a chemical or by a mechanical process alone. Hyphae making a mycelial bundle are observed closely amassed along the suture and seem to force open the way of penetration, as though they were exerting a pressure, and accordingly this fact, as well as the appearances of development of the infection cushion, may support the theory of mechanical action alone. However, there seems to be some slight indication that the chemical action occurs also in the course of the penetration, for the reasons of striking swelling of the middle lamellae at the point of penetration.

A part of the middle lamellae of the cork cells of sweet potatoes seems to be pectin-like materials, which may be acted chemically by the fungus and useful as food for the fungus. Enzymes, including pectinase and cellulase, secreted by the fungus

may soften the middle lamellae so severely that the fungus may be able to penetrate them by the hyphae with comparatively slight effort.

The greatly thickened or swollen walls met with so frequently do not offer so much resistance as might be supposd at first glance, for the chemical action on the walls in these instances may partly result in the production of substances that swell considerably. There appears some evidence that penetraton is at least in part a chemical process.

(c) The fungus can not penetrate the lignified cells and consequently the xylem remains its original form for a long time.

Sometimes, a few fragments of the hyphae are seen in the root hairs of the soy bean, but the instances which hyphae invade the cortical cells are not seen, and thus at least in the case of this fungus the writer can not assume TISDALE's (1917) opinion that the certain fungi, for example *Fusarium lini*, penetrate the host through root hairs.

From what has been discussed in the preceding pages the following conclusion may be induced: Both mechanical and chemical processes may be concerned in bringing about the passage of the fungus through the cell walls of the host. The chemical compositions of the cell walls of the host may play an important rôle in determining which process will predominate. The non-cutinized or the non-suberized cell walls which are partly made of pectin-like substance and hemicellulose hydrolysed by the fungus undoubtedly are acted chemically in wall penetration chiefly, whereas the cutinized walls as well as the suberized walls are less subject to chemical action of this parasite and their penetration may result mainly by the mechanical process.

The more or less similar conclusions were already drawn by PEARSON (1931) studying the seedling blight of Indian corn by *Gibberella saubinetii*.

(2) On the rot of affected plants

Roots and stems of the hosts attacked by the fungus are decayed heavily. Especially in the case of sweet potato, fleshy roots as well as stems are brought to conspicuous soft-rot. This is due not only to the action of the fungus, but also to the secondary invasion by saprophitic microorganisms, which are occupied mainly by bacteria and *Rhizopus spp.* Fleshy roots attacked by the fungus alone do not become soft conspicuosly, but rather chalky.

The greater part of difficulties in isolation of the fungus from the affected fleshy roots may lie in the above-mentioned facts. The writer could gain the pure culture of this fungus by the careful selection of the suitable materials (p.12).

It is also observed commonly that the cambial portion of the roots of woody hosts is first decayed by the fungus and then secondarily the lignified portion is destroyd severely by the invasion of wood-rotting fungi.

(3) On the formation of "callosity"

It has been well known among phytopathologists that the so-called callosities or calli are sometimes formed in plant bodies attacked by certain fungi.

According to YOUNG (1926), MANGIN, in 1899, was the first to record the callosity in wheat infected by *Septoria gramineum*, and afterwards FRON, in 1912, reported the

production of the same bodies in the infection of wheat by *Leptosphaeria sp.* Young (1. c.), furthermore, reported the extensive formation of callosities when wheat, oat, rye, and other seedlings were inoculated by each of the following parasites: *Alternaria spp., Cephalosporium acremonium, Diplodia zeae, Colletotrichum nigra,* and *Acrothecium sp.*

Similar structures were noted by Fellows (1928) in the infection of wheat by *Ophiobolus graminis* and by Simmons (1928) in the infection of oat by *Fusarium culmorum.* The callosities formed varied considerably in size from small round ones to elongated structures which followed the penetrating hyphae.

Pearson (1931) made some notes on the formation of callosities in corn seedlings infected by *Gibberella saubinetii.* Kusano (1936) who studied the parasitism of *Olpidium* on pea, broad bean, etc., made a great contribution to this subject, especially on the mechanism of the formation of callosities.

As regards the prothallium of fern attacked by a fungus, the callosity-like bodies were observed by Fritz (1937).

Similar observations were reported by Ikata (1939) in the cases of the infection of rush plant by *Stagonospora sp., Gloeosporium Kaki* and *Sclerotinia minor,* and by Iwata (1940) in the infection of leaves of *Sonchus oleraceus* and *Nasturtium sublyratum* by *Peronospora Aparines.*

Allen (1927) stated the formation of similar globules or "warts" in the case of the infection of Malakoff wheat by *Puccinia triticina* physiologic form 11. She found that these structures took up stains that were generally supposed to stain pectic materials, and concluded that they might be the result of a hydrolysis of the middle lamellae of the host cells by the action of the enzymes produced by the fungus.

Johann *et al.* (1931) described a similar accumulation of materials in the walls and intercellular spaces of cells surrounding lesions produced by *Penicillium oxalicum* in corn mesocotyls, and they reached the conclusion that the formation of these bodies were due to oxalic acid secreted by the causal fungus.

Young (1926) and Simmonds (1928) did not definitely state what they considered to be the method of formation or the chemical composition of the callosities.

By the microchemical tests, Fellows (1928) could not ascertain the existence of callose, but observed the clear reaction of lignin and thus he used the term "lignituber" instead of "callosity".

Concerning the mechanism of the formation of callosities, a great many of investigators, including Young (1926), Fellows (1928), Pearson (1931) and Fritz (1937), have considered that they are produced mainly from the cell walls secondarily, but Kusano (1936) has taken a different opinion that they are formed directly by the accumulation and the aggregation of the protoplasm of the host cells.

With respect to the significance of the formation of callosities no definite conclusion except Kusano's one has been given to the present time, but it has been considered in general that they may serve at least as a partial barrier against fungous penetration.

However, Kusano (1939), studying *Olpidium* and *Synchytrium,* reached the

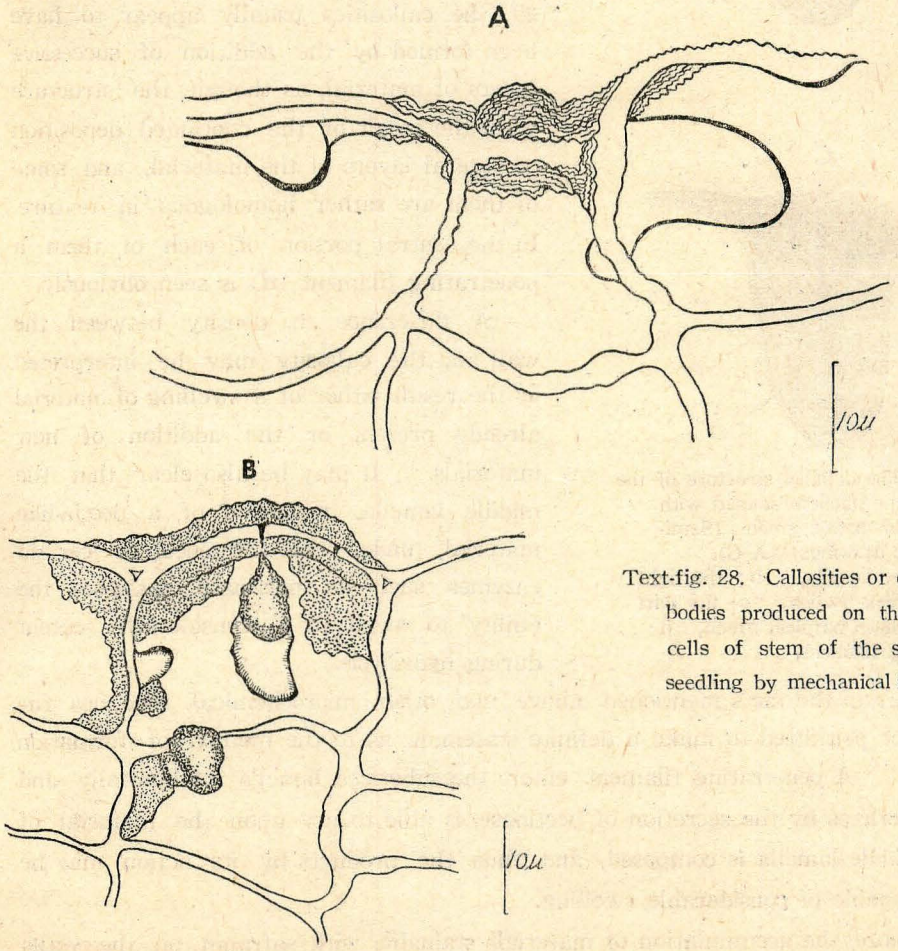
conclusion that the callosities were not formed in order to check the fungous invasion, but to heal the wounds injured by the causal organism, and accordingly there were no close connections between the formation of the callosity and the resistance of the plant.

More recently IWATA (1940), in his study on *Peronospora Aparines*, stated also that the callosities were not considered to play an important rôle in the infection of the fungus.

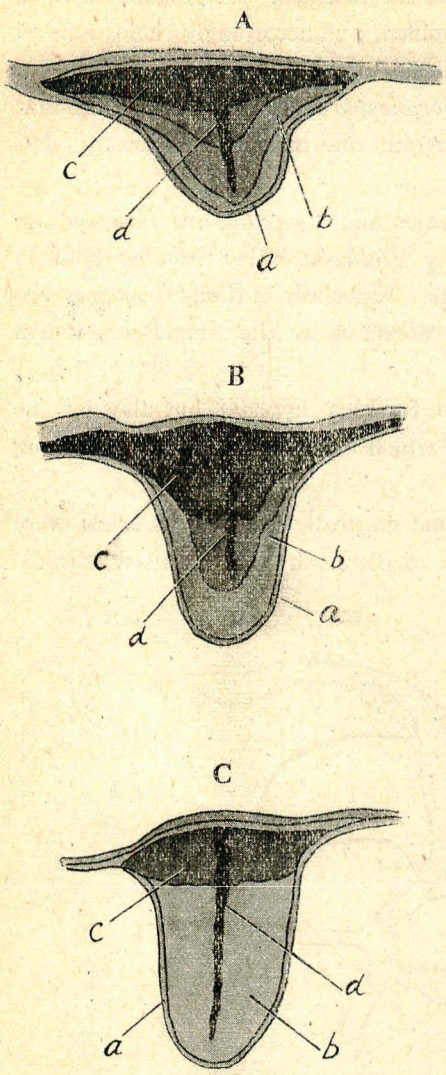
As previously stated, a great number of callosities and "warts" are observed on stems and fleshy roots of sweet potato attacked by *Helicobasidium Mompa* both in the artificial inoculation and in the natural infection. Especially in fleshy roots they are formed very abundantly. The formation of the callosities by the fungus in question is limited to the cutinized or suberized cell walls.

The callosities are formed not only by the fungous hyphae, but also by the mechanical injuries alone, some examples of those which were produced by pricking with a sterile needle are shown in Text-figure 28.

Several thousand sections of both artificially and naturally infected materials were examined very carefully, but no hypha is observed to pierce through a callosity formed in the wall of phellem cell.



Text-fig. 28. Callosities or callosity-like bodies produced on the epidermal cells of stem of the sweet potato seedling by mechanical injury (A-B).



Text-fig. 29. The detailed structure of the callosity on the phellem stained with Sudan III and methyl green (Semi-diagrammatic drawings) (A–C).
a: the outer lamella, b: the light purplish green portion, c: the part colored in deep purplish green, d: the pirecing filament.

For the study of the details of the structure of the callosities on the phellem, sections were stained with Sudan III and methyl green (Yoshii, 1933). This staining method seemed to be very well for the differentiation of the minute structures.

As shown in Text-figure 29, by this stain three portions of the callosity are differentiated clearly, namely the outer membrane (*a*) is light reddish, the basal part (*c*) deep purplish green, and the other portion (*b*) is light purplish green. In many cases, careful examinations indicate that the greater part of the swelling is due to the increase in the size of the middle layer. In the example shown in Text-figure 29, the callosities usually appear to have been formed by the addition of successive layers of material, as though the structure were the result of the continued deposition of several layers of the material, and some of them are rather homologous in texture. In the central portion of each of them a penetrating filament (*d*) is seen obviously.

A difference in density between the wall and the callosity may be interpreted as the result either of a swelling of material already present or the addition of new materials. It may be also clear that the middle lamella, which is of a pectin-like material, under the influence of certain enzymes such as pectinase possesses the ability to swell to a considerable extent during hydrolysis.

Judging from the facts mentioned above and other microchemical reactions the writer may be permitted to make a definite statemant as to the method of formation of callosities. A penetrating filament enters the suberized lamella mechanically and the hypha, perhaps by the secretion of pectinase, is able to act upon the material of which the middle lamella is composed, and thus the products by its action may be substances capable of considerable swelling.

Furthermore, the accumulation of materials stainable with safranin on the walls

could then be interpreted as a hydrolysed material of pectin-like substance by the chemical action of the hypha.

At least the callosities found on the cork cells are produced directly from the walls and accordingly the writer, in the present case, cannont agree with KUSANO's (1936) opinion that the callosities are formed immediately from the protoplasm.

In the case of the cutinized cells of stems, it is possible that the increase in swelling may be due in part to an accumulation of products from the protoplasm in the cells and in part to the hydrolysis of the middle lamellae.

Concerning the significance of the formation of the callosity, KUSANO (1939) has published a theory as follows: "[The host plants produce the callosities to heal the injuries caused by the causal organisms, but never to check the penetration of them, and whether the fungi grow out of the callosity and continue further development or not is wholly due to a certain substance in cytoplasm. According to the kind of the plant, this unknown substance is different in both quantity and quality and consequently in the plants which give a great deal of unfavorable action to the entered organisms the invasion appears to be stopped earlier, as if the callosities surved to check the penetration of the fungi]". In short, KUSANO's theory is that the fungous penetration of the cell wall is not checked by the callosity, but by the cytoplasm.

In the case of the present writer, seeing from the fact that the callosities are also produced on the cell walls wounded by the mechanical injury alone, KUSANO's theory may be true at least in part. However, the formation of callosities in the plant seems to be not merely a healing process, but also a response to the fungous action.

In the case of the callosities formed in the cork cells, it may be impossible to say that the cause of impediment to the fungous penetration at the cell wall is attributable to the cytoplasm; this must be considered to be entirely due to the function of the callosity originated from the cell wall.

Taking into consideration what has been discussed the writer comes to the conclusion that the callosities produced in sweet potato may surve a defensive function against the hyphal penetration of *Helicobasidium Mompa* at least in those places, but, on the whole, they are of little use for this purpose, because the fungus makes entrance forcibly through the suture portion of the cells forming the infection cushion.

TOXIC EFFECT OF THE STALED CULTURE MEDIUM OF *Helicobasidium Mompa* TANAKA UPON HIGHER PLANTS

In order to make clear the mode of infection of *Helicobasidium Mompa* TANAKA the writer placed an inoculum from the fungous colony growing on agar-medium on a young leaf of the camphor tree (*Cinnamomum camphora*). It was observed that the portion of the leaf to which the agar medium was attached was discolored soon after and finally became necrotic. Thus the writer knew incidentally the injurious effect of the staled culture medium of the fungus to the higher plant. Hereupon the writer

has carried out some experiments on this subject.

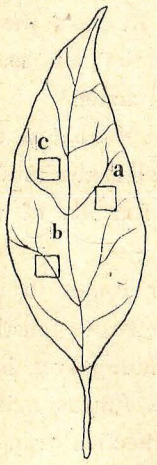
Concerning the poisonous substance produced by causal fungi of wilt-diseases in the staled culture solution, since the first report of HUTCHINSON (1913) many works have been published by various investigators: BRANDES (1919), AJREKAR and BAL (1921), DUFRÉNOY (1921), BEWLEY (1922), FAHMY (1923), NEAL (1927), WHITE (1927), HAYMAKER (1928), KULKARNI and MUNDKUR (1928), LINFORD (1931), YOSHII and MASANO (1935) and YOSHII (1935), etc.

BARNUM (1924) reported that the production of a toxic principle in culture solutions is not limited to plant pathogenes such as *Fusaria*, but that a saprophytic fungus like *Penicillium expansum* LINK acts a similar manner. As for the detailed historical review on the subject, the reader may see the account in YOSHII's (1935) paper.

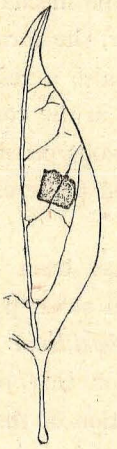
Toxic effect by attaching with the staled culture agar

a. LEAF

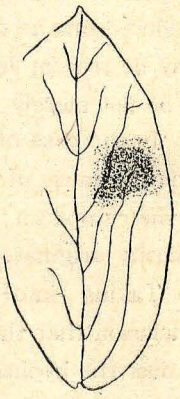
Experiment-1. A shoot of the camphor tree having several leaves was inserted in an Erlenmeyer flask containing water. On the under surface of the leaf, were placed small pieces (about 4 mm. sq.) of the staled potato agar with (Text-fig. 30, *a*) or without the mycelium (Text-fig.30, *b*) on which *Helicobasidium Mompa* TANAKA



Text-fig. 30.
a: Staled potato agar with the mycelium of *Helicobasidium Mompa*.
b: Staled potato agar without the mycelium of the fungus.
c: Uninoculated potato agar. x 2/3



Text-fig. 31.
Toxic effect of the staled culture agar on the leaf of camphor tree. x 2/3



Text-fig. 32.
Toxic effect of the staled culture agar on the leaf of broad bean. x 1

(Strain M-1) had been cultured for 26 days at 27°C. As control, a piece of uninoculated potato agar was placed on the opposite half of the leaf (Text-fig. 30, *c*). As soon as the pieces of the staled agar were placed on the leaf, the flask in which the shoot had been kept was covered with a large bell-jar to keep from drying.

After 24 hours at room temperature (19°-25°C.) the portions of the leaves on which the staled culture agar were placed changed distinctly from green to brown and in 4 days

they became brown and dry. Outside of tha necrotic portion was surrounded by a deep brownish zone (Text-fig. 31). Macroscopically, the appearances of the necrotic part were closely similar to the leaf fixed in chromic acid solution. No change was observed in the control (Pl. XI,A).

Similar test was made on a leaf of the broad bean (*Vicia Faba*). After 7 days the portion under the staled agar became brown, but in this case the change occurred more slowly than in the former (Text-fig. 32).

Experiment-2. For the purpose of testing the relation between age of culture and toxicity, a similar experiment as the preceding one was carried out with the cultured agar media having each of the following culture-age at 27° C.: 10, 20, 30, and 50 days. This test indicated that the toxic action is increased with the age remarkably.

Experiment-3. The staled agar which had been boiled for one hour and the untreated one were placed on a leaf of the camphor tree simultaneously. After 24 hours necrosis was formed by each of the above two cases and no considerable difference in the toxicity was seen between the boiled medium and the untreated one.

Experiment-4. The nerotic portions of leaves of the camphor tree caused by the staled medium were cut into small pieces and fixed in formalin-acetic acid-alcohol solution (70 per cent alcohol 90 cc., glacial acetic acid 5 cc., formalin 5 cc.). By the n-butyl alcohol method reported by ZIRKLE (1930), sections were made 8μ to 10μ in thickness and stained with FLEMMING's triple stain. As control, the healthy part of the leaf was also fixed and stained in the same manners.

As shown in Plate XI, E, at the incipient stage of necrosis, the plasmolysis occurred in the cells of palisade and spongy tissues, and then protoplasm swelled and stained with safranin brilliantly. The tissues found in the final stage of necrosis were contracted and collapsed (Pl. XI, D).

b. YOUNG ROOT

On the young root of soy bean (*Glycine Soja*) which had been cultured by the large tube method noted by the present writer previously (p. 74), a small piece (4 mm. sq.) of the staled CZAPEK's agar (3 weeks old, at 27° C.) was placed. After 8 days the part attached with the staled agar was discolored slightly, but no severe necrosis was observed (Pl. XI, G).

Toxic effect by absorption of the staled culture solution

EXPERIMENT-1. One hundred cc. of potato decoction with sucrose (potato 100 g., distilled water 1,000 cc., cane sugar 10g.,) were poured into 200 cc. Erlenmeyer flask and sterilized with the ordinary manners. A small piece of the fungous colony was planted in the flask and the culture incubated at room temperature.

After 36 days the solution was decanted from the flask and filtered by suction through BUCHNER's funnel, using double sheets of filter paper and a heavy mass of absorbent cotton. Microscopic observation of the filtrate showed the absence of the fragments of the mycelium. This original solution was diluted with distilled water and identified by the number as shown in Table 45.

Table 45. Solutions containing various amounts of the staled culture solution of *Helicobasidium Mompa*.

No.	Culture solution (cc.)	Distilled water (cc.)	Total (cc.)	pH
0	0	100	100	—
1	2	98	100	6.6
2	5	95	100	6.4
3	15	85	100	5.8
4	30	70	100	5.4
5	50	50	100	5.0
6	70	30	100	4.8

Small branches of the camphor tree, the mulberry tree and the soy bean were inserted in each of the above-mentioned solutions. Observations made for 7 days and the changes in the appearance of the leaves are given in Table 46.

Table 46. Toxic action of the staled culture solution of *Helicobasidium Mompa* upon plants.

a. Soy bean

No. of solution	Days						
	1	2	3	4	5	6	7
0	—	—	—	—	—	—	—
1	—	—	—	(1)	(1)	(1)	(1)
2	—	—	(1)	(2)	(2)	(3)	(3)
3	—	±	(2)	(3)	(3)	(3)	(4)
4	—	±	(2)	(4)			
5	—	±	(2)	(4)			
6	—	±	(2)	(4)			

Notes:
—.....unchaged,
±.....wilted slightly,
(1) .....wilted distinctly,
(2) .....wilted severely,
(3) .....distinct soaked area present and somewhat dry,
(4) .....dry and curled severely.

b. Camphor tree

No. of solution	Days						
	1	2	3	4	5	6	7
0	—	—	—	—	—	—	—
1	—	—	—	(1)	(2)	(3)	(3)
2	—	—	—	(2)	(3)	(3)	(4)
3	±	(2)	(2)	(3)	(3)	(4)	
4	(1)	(2)	(2)	(4)			
5	(1)	(2)	(2)	(4)			
6	(2)	(2)	(3)	(4)			

Notes:
—.....unchanged,
±.....wilted slightly,
(1) .....wilted distinctly,
(2) .....wilted severely,
(3) .....changed into purple and curled,
(4) .....all leaves fell.

c. Mulberry tree

No. of solution	Days						
	1	2	3	4	5	6	7
0	—	—	—	—	—	—	—
1	—	—	(2)	(2)	(4)		
2	—	—	(2)	(3)	(4)		
3	—	—	(2)	(3)			
4	±	(2)	(3)	(4)			
5	(1)	(2)	(3)	(4)			
6	(1)	(2)	(3)	(4)			

Notes:
—.....unchanged,
±.....wilted slightly,
(1) .....wilted distinctly,
(2) .....wilted severely,
(3) .....dried and curled,
(4) .....all leaves fell.

It seems evident from Table 46 that the higher the concentration of the staled culture solution is, the severer the toxic action to the higher plants. By the toxic effect of the staled culture solution all of the plants used were wilted at first, but afterwards the appearances in the leaves are different according to the kind of plant.

In the case of soy bean plant the yellowish soaked parts are formed surrounding the veins (Pl. XI, F) and the leaves of camphor tree change their color from green to purple, though no discoloration is observed in the case of mulberry tree. Generally speaking, the toxic action is seen more rapidly in the camphor- and the mulberry-tree than in the soy bean.

Experiment-2. The fungus cultured in potato decoction with 1 per cent sucrose for about 7 months at 25° C. The culture solution was filtered with the same manners noted previously and diluted two times with distilled water. After the filtrate was poured into two large Erlenmeyer flasks, the one was boiled for an hour and the other untreated. Distilled water and 0.2 per cent oxalic acid solution were prepared as controls.

In each of these flasks cut stems of the following kinds of plants were inserted:

Pleioblastus variegata var. viridis f. glabra	Nezasa,
Chamaecyparis pisifera	Sawara,
Larix kaempheri	Kara-matsu,
Cercidiphyllum japonicum	Katsura,
Morus alba	Kuwa,
Robinia pseudoacacia	Nise-akashiya,
Fagus japonica	Inu-buna,
Liriodendron tulipifera	Hanten-boku,
Sambucus Buergerina var. typica	Niwatoko,
Platanus orientalis	Suzukake-no-ki,
Ipomoea batatas	Satsuma-imo.

Changes in leaves after 2 days at room temperature will be shown as follows:

(1) *Pleioblastus variegata var. viridis f. glabra*	(2) *Chamaecyparis pisifera*
S-1 [1]···Leaves are dried slightly and somewhat curled.	S-1·····No change occurred.
S-2 [2]···do.	S-2·····do.
S-3 [3]···No change was observed.	S-3·····do.
S-4 [4]···do.	S-4·····do.
(3) *Larix kaempferi*	(4) *Cercidiphyllum japonicum*
S-1·····No change was observed.	S-1·····Dark brownish soaked part was formed surroundng veins of leaf. Leaves were dried and curled slightly.
S-2·····do.	S-2·····do., but the grade of toxicity was weaker than that of S-1.
S-3·····do.	S-3·····Leaves were dried slightly.
S-4·····do.	S-4·····No change occurred.
(5) *Morus alba*	(6) *Robinia pseudoacacia*
S-1·····Leaves were wilted distinctly.	S-1·····Light brownish soaked parts were formed around the veins. Here and there dark brownish spots in size of pin-head were seen.
S-2·····Leaves were wilted slightly.	S-2·····do., but the toxic action was weaker than that of S-1.
S-3·····Leaves were wilted severely. Light brownish part near veins are seen.	S-3·····Unchanged.
S-4·····Unchanged.	S-4·····do.
(7) *Fagus japonica*	(8) *Liriodendron tulipifera*
S-1·····Almost all parts of leaf were changed in color from green to light brown. Leaves were dried and curled.	S-1·····Large soaked parts were formed. Leaves were dried.
S-2·····do.	S-2·····do., but the grade of the toxicity was lower than that of S-1.
S-3·····A small area of soaked part was formed.	S-3·····Unchanged.
S-4·····Unchanged.	S-4·····do.

1) Two times diluted staled solution.
2) Boiled two times diluted staled solution.
3) 0.2% oxalic acid solution.
4) Distilled water.

(9) *Sambucus Buergerina var. typica*	(10) *Platanus orientalis*
S–1……The marginal and apical portions of leaf were changed the color from green to purple by soaking in the staled solution. Leaves were more or less dried. S–2……do., but the toxic action was weaker than of S–1. S–3……Leaves were wilted and the soaked portions were observed scarcely. S–4……Unchanged.	S–1……Almost all parts of the leaf became light brown in color and were dried. S–2……do. S–3……Wilted slightly. S–4……Unchanged.
	(11) *Ipomoea batatas*
	S–1……No change occurred. S–2……do. S–3……do. S–4……do.

EXPERIMENT–3. This experiment was carried out by the same method as the previous one, but, instead of potato-sucrose solution, the modified RICHARDS' solution (KNO_3 5g., KH_2PO_4 2g., $MgSO_4$ 1g., glucose 10g., distilled water 1,000 cc.) was used.

The kinds of plants used in this experiment are listed as follows:

Pleioblastus variegata var. viridis f. glabra	Nezasa,
Larix kaempheri	Kara-matsu,
Cercidiphyllum japonicum	Katsura,
Robinia pseudoacacia	Nise-akashiya,
Fagus japonica	Inu-buna,
Platanus orientalis	Suzukake-no-ki,
Morus alba	Kuwa,
Ipomoea batatas	Satsuma-imo.

The effect of the staled culture solution upon the plants were tested after 2 days at room temperature. The results will be shown as follows:

(1) *Pleioblastus variegata var. viridis f. glabra*	(2) *Chamaecyparis pisifera*
S'–1 1)…Leaves were dried and curled severely. S'–2 2)…do. S'–3 3)…Leaves were dried and curled slightly.	S'–1……No change occurred. S'–2……do. S'–3……do.
(3) *Larix kaempheri*	(4) *Cercidiphyllum japonicum*
S'–1……No toxic effect was observed. S'–2……do. S'–3……do.	S'–1……Veins of the leaf became deep brown and leaves were dried. S'–2……do., but the toxic effect was more or less weak. S'–3……No toxic effect was observed.

1) Two times diluted staled solution. 2) Boiled two times diluted staled solution.
3) Uncultured two times diluted solution.

(5) *Morus alba*	(6) *Robinia pseudoacacia*
S'–1……Leaves were dried and curled. Veins became necrotic and brown.	S'–1……No toxic effect was observed.
S'–2……do., but the toxic action was weaker than that of S'–1.	S'–2……do.
S'–3……Marginal portion of leaves became light brown.	S'–3……do.
(7) *Fagus japonica*	**(8) *Platanus orientalis***
S'–1……A great amount of brown necrotic portion was formed in the veins and the marginal portions of the leaf. Leaves were curled.	S'–1……A great amount of necrosis was formed. Leaves were somewhat dried and curled.
S'–2……do., but the grade of the toxicity was weaker.	S'–2……do., but the toxic action was weaker than the case of S'–1.
S'–3……Slight discoloration was observed.	S'–3……Almost no change occurred.
(9) *Ipomoea batatas*	
S'–1……Unchanged.	
S'–2……do.	
S'–3……do.	

From the results of the experiments 2–3, the following facts may be known:

(i) The toxic action of the staled potato solution upon the higher plants is stronger than that of the staled RICHARDS' solution (somewhat modified).

(ii) By boiling for one hour, the toxicity is apt to be decreased more or less.

(iii) The toxicity of 0.2 per cent oxalic acid solution is not recognized so clearly as that of the staled solutions.

(iv) The severity of the toxic effect is different according to the species of the plants as follows;

(a) No visible toxic effect is seen in the following species, *Chamaecyparis pisifera*, *Larix kaempheri*, and *Ipomoea batatas*,

(b) Moderate toxic effect is seen in the following species, *Sambucus Buergerina var. typica*, *Liriodendron tulipifera*, *Robinia pseudoacacia* and *Morus alba*.

(c) Severe toxicity was induced in the following species, *Pleioblastus variegata var. viridis f. glabra*, *Platanus orientalis*, *Fagus japonica* and *Cercidiphyllum japonicum*.

The toxic action of culture solution on which *Helicobasidium Mompa* TANAKA had been grown upon the higher plants was observed distinctly, and the grade of toxicity induced was not equal according to the species of the plants. Judging from the results of the experiments there may be probably no noteworthy correlation between the toxic injury by the staled solution and the susceptibility to the "Murasaki-monpa" disease.