Studies on the Effect of Kinds of Tree in Culture Medium upon the Growth of *Cortinellus Berkeleyanus*.

I. The Mycelial Growth in Pure Culture on the Sawdust Medium prepared of Various Kinds of Tree.

By

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I. Introduction.

The mushroom production of the Siitake fungus, *Cortinellus Berkeleyanus* has become very popular in recent years in Japan. This has been brought about by the development of a simple and yet efficient technique for making the Siitake fungus, (*Cortinellus Berkeleyanus* Ito et Imai) establish in the culturing log. As a source for inoculum, the fungus is grown in pure state in sawdust medium, (Nisikado, 1935). Logs are prepared principally from trees in the families of Fagaceae, Betulaceae, or Juglandaceae, commonly represented by *Quercus serrata* Thunb., *Quercus acuta* Thunb., *Carpinus carpinoides* Makino or *Petrophiloides strobilacea* Reid et Chandler. Sawdusts prepared from them no doubt best serve their purpose if used as culturing medium ; but if they are not always available, it is of importance to make an analysis on the kinds of wood that can be successfully substituted in their place. The results obtained from such an analysis can further be employed in testing the suitability of various kinds of log for Siitake mushroom production. With these purposes in mind, the present authors conducted this experiment.

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II. Materials.

Various kinds of sawdust used in this experiment were prepared from the logs listed in Table I and II. The first batch of which was sent to us through the kindness of Directors H. Nakayama and I. Tanizawa of Hirosima and Nitibara Forestry Stations, respectively March, 1935, and the second was received from the Tuyama Forestry Station through the kindness of Director M. Kudo in May, 1936. The authors also wish to thank the above three Directors for supplying the materials and making this investigation possible.

Table I.

Trees used in the Experiments on the Mycelial Growth of *Cortinellus Berkeleyanus* in Pure Culture on the Sawdust Medium.

The materials were sent to the writers from the Hirosima and Nitihara Forestry Stations in May, 1935.

No.	Scientific name	Japanese name	Family	Locality, age and diameter of the log used
1	*Cryptomeria japonica* D. DON.	Sugi	Pinaceae	Hirosima
2	„ „	„	„	Nitihara 40 years (24 cm.)
3	*Pinus densiflora* SIEB. et ZUCC.	Aka-matu	„	Hirosima
4	*Abies firma* SIEB. et ZUCC.	Momi	„	„
5	*Tsuga Sieboldii* CARR.	Tuga	„	„
6	*Petrophiloides strobilacea* REID et CHANDLER	No-gurumi	Juglandaçeae	„
7	*Betula carpinifolia* SIEB. et ZUCC.	Mizume	Betulaceae	„
8	„ „	„	„	Nitihara 100 (44)
9	*Carpinus carpinoides* MAKINO	Kuma-side	„	„ 80 (30)
10	*Fagus crenata* BLUME	Buna-no-ki	Fagaceae	Hirosima
11	„ „	„	„	Nitihara 150 (52)
12	*Castanea crenata* SIEB. et ZUCC.	Kuri	„	Hirosima (Chiefly sapwood)
13	„ „	„	„	Nitihara 130 (52) (Chiefly heartwood)
14	*Quercus serrata* THUNB.	Konara	„	Hirosima
15	„ „	„	„	Nitihara 80 (36)
16	*Zelkowa serrata* MAKINO	Keyaki	Ulmaceae	Hirosima
17	„ „	„	„	Nitihara 90 (32)
18	*Cercidiphyllum japonicum* SIEB. et ZUCC.	Katura	„	„ 60 (26)
19	*Prunus serrulata* LINDL. var. *spontanea* MAKINO	Yama-sakura	Rosaceae	„ 44 (24)
20	*Phellodendron amurense* RUPR.	Kihada	Rutaceae	„ 120 (48)
21	*Aesculus turbinata* BLUME.	Toti-no-ki	Hippocastanaceae	Hirosima
22	„ „	„	„	Nitihara 40 (26)
23	*Acanthopanax sciadophylloides* FR. et SAV.	Kosiabura	Araliaceae	„ 60 (28)
24	*Kalopanax ricinifolium* MIG. var. *typicum* NAKAI	Harigiri	„	Hirosima
25	„	„	„	Nitihara 100 (56)
26	*Cornus controversa* HEMSL.	Mizuki	Cornaceae	„ 40 (24)
27	*Fraxinus japonica* BLUME	Siozi	Oleaceae	Hirosima
28	„ „	„	„	Nitihara 100 (44)

Table II.

Trees used in the Experiments on the Mycelial Growth in Pure Culture on the Sawdust Medium.

The materials were sent to the writers from the Tuyama Forestry Office in May, 1936.

No.	Scientific name	Japanese name	Family
1	*Petrophiloides strobilacea* REID et CHANDLER	No-gurumi	Juglandaceae
2	*Betula ulmifolia* SIEB. et ZUCC.	Yoguso-minebari	Betulaceae
3	*Carpinus carpinoides* MAKINO	Kuma-side	,,
4	*Castanea crenata* SIEB. et ZUCC.	Kuri	Fagaceae
5	*Quercus acuta* THUNB.	Kasi	,,
6	*Quercus serrata* THUNB.	Konara	,,
7	*Zelkowa serrata* MAKINO	Keyaki	Ulmaceae
8	*Euptela polyandra* SIEB. et ZUCC.	Husa-zakura	Eupteleaceae
9	*Magnolia obovata* THUNB.	Hoho-no-ki	Magnoliaceae
10	*Actinodaphne loncifolia* MEISSN	Kago-no-ki	Lauraceae
11	*Cinnamomum japonicum* SIEB.	Yabu-Nikkei	,,
12	*Machilus Thunbergii* SIEB. et ZUCC.	Tabu-no-ki	,,
13	*Prunus serrulata* LINDL. var. *spontanea* MAKINO	Yama-zakura	Rosaceae
14	*Albizzia Julibrissin* DURAZZ. var. *speciosa* KOIZ.	Nemu-no-ki	Leguminaceae
15	*Cladrastis platycarpa* MAKINO	Huziki	,,
16	*Picrasma quassioides* BENN.	Nigaki	Simarubaceae
17	*Acer rufinerve* SIEB. et ZUCC.	Urihada-kaede	Aceraceae
18	*Acer palmatum* THUNB.	Momizi	,,
19	*Acer pictum* THUNB. var. *Paxii* SCHW. subvar. *eupictum* PAX.	Itaya-kaede	,,
20	*Meliosma myriantha* SIEB. et ZUCC.	Awabuki	Sabiaceae
21	*Camellia japonica* L. var. *hortensis* MAKINO	Tubaki	Theaceae
22	*Idesia polycarpa* MAXIM.	Iigiri	Flacourtiaceae
23	*Aralia elata* SEEM.	Tara-no-ki	Araliaceae
24	*Aucuba japonica* THUNB.	Aoki	Cornaceae
25	*Cornus controversa* HEMSL.	Mizuki	,,
26	*Clethra barbinervis* SIEB. et ZUCC.	Ryôbu	Clethraceae
27	*Pieris japonica* D. DON.	Asebi	Ericaceae
28	*Diospyros kaki* THUNB. var. *silvestris* MAKINO	Yama-gaki	Ebenaceae
29	*Pterostyrax corymbosum* SIEB. et ZUCC.	Asagara	Styracaceae
30	*Styrax japonica* SIEB. et ZUCC.	Ego-no-ki	,,

III. Experiments on Sawdust Culture Medium.

(1) Culture in Large Mouth Glass Bottles.

A. Methods.

The first part of this experiment on the effect of kind of sawdust used as culture medium on the growth of Siitake mycelium was conducted by using the large mouth glass bottles having a capacity of one pound. The bottles have been employed in culturing the fungous mycelium in this laboratory for the past several years.

Each sawdust medium was prepared by adding sufficient 2% sugar solution to previously sun-dried sawdust in such a mount that free water will not drip after squeezing it with a hand. This mixture was packed into the glass bottles, plugged with cotton and autoclaved. No hole was made in the center of the medium as was ordinarily done. The bottles were inoculated with the pure culture of Shiitake mycelium grown on ordinary sawdust mycelium and were incubated at a temperature of 24 °C. for 20 to 30 days, after which time, records were taken by noting the growth on the inner side of the bottle. Since the growth was not uniform all around, measurements were taken representing both the greatest and the least growths and these were averaged to represent each bottle.

B. Results of the Experiments.

The observation of the results was made after incubating at 24 °C. for 10 and 20 days in the first experiment. The results are given in Table III. In the

Table III.

Growth of Siitake Mycelium on Various Kinds of Sawdust. Experiment I.
The media contained 2% sugar. Siitake fungus, strain No. 434, isolated from a sporophore collected at Hutomi-mura, Akaiwagun, Okayama-Ken. Grown at 24°C.

No.	Species name	Growth after 10 days	Growth after 20 days	Order after 20 days
2	*Cryptomeria japonica* D. DON.	8 mm	15 mm	21
3	*Pinus densiflora* SIEB. et ZUCC.	9	18.5	18
4	*Abies firma* SIEB. et ZUCC.	10.8	16.8	20
5	*Tsuga Sieboldii* CARR.	16	21.7	14
6	*Petrophiloides strobilacea* REID et CHANDLER	15	25	7
7	*Betula carpinifolia* SIEB. et ZUCC.	13	25	8
8	„ „	13.4	26	5
9	*Carpinus carpinoides* MAKINO	16.6	26	6
10	*Fagus crenata* BLUME.	20	28.5	3
11	„ „	20	29.6	2
13	*Castanea crenata* SIEB. et ZUCC. (mainly heartwood)	10	12.5	22
15	*Quercus serrata* THUNB.	15.6	24.6	9
17	*Zelkowa serrata* MAKINO	5	7.6	23
18	*Cercidiphyllum japonicum* SIEB. et ZUCC.	20	27.3	4
19	*Prunus serrulata* LINDL. var. *spontanea* MAKINO	4.3	6	24
20	*Phellodendron amurense* RUPR.	12.5	22.5	11
21	*Aesculus turbinata* BLUME.	15.3	23.3	10
22	„ „	20	33.3	1
23	*Acanthopanax sciadophylloides* FR. et SAV.	7.5	18	19
24	*Kalopanax ricinifolium* MIG. var. *typicum* NAKAI	10	18.7	17
25	„ „	12	20	16
26	*Cornus controversa* HEMSL.	13.5	22.5	12
27	*Fraxinus japonica* BLUME.	15	22.5	13
28	„ „	12	21	15

second experiment, observation was made after 20 and 30 days' incubation, and the results are shown in Table IV. Table V gives the average growth for 20 days' period, arranged in order from the fastest to the slowest.

Table III shows that the Siitake mycelium made better growth in the culture media prepared from the following sawdusts, arranged in the order of *Aesculus turbinata* BLUME, *Fagus crenata* BLUME and *Cercidiphyllum japonicum* SIEB. et ZUCC.

Table IV shows that *Fagus crenata* BLUME, *Carpinus carpinoides* MAKINO, and the sapwood of *Castanea crenata* SIEB. et ZUCC. were the most suitable trees. The unsuitable trees in the first experiment, were *Prunus serrulata* LINDL. var. *spontanea* MAKINO, *Zelkowa serrata* MAKINO heartwood of *Castanea crenata* SIEB. et ZUCC. and *Cryptomeria japonica* D. DON.; and in the second experiment, the heartwood of *Castanea crenata* SIEB. et ZUCC. *Phellodendron amurense* RUPR., *Zelkowa serrata* MAKINO and *Prunus serrulata* LINDL. var *spontanea*.

The order arranged after averaging the growths for a period of 20 days in each kind of sawdust was as given in Table V; *Carpinus carpinoides* MAKINO, *Fagus*

Table IV.

Growth of Siitake Mycelium on Various Kinds of Sawdust. Experiment II.

The culture medium prepared by adding 2% sugar solution to sun-dried sawdust. Siitake fungus, strain No. 434. Grown at 24°C.

No.	Species name	Growth after 20 days	Growth after 30 days	Order after 20 days
1	*Cryptomeria japonica* D. DON.	30.7 mm	46.6 mm	14
3	*Pinus densiflora* SIEB. et ZUCC.	36.6	60.4	10
4	*Abies firma* SIEB. et ZUCC.	31.3	42.6	13
5	*Tsuga Sieboldii* CARR.	52.3	61.0	4
6	*Petrophiloides strobilacea* REID et CHANDLER	43.6	58.0	6
7	*Betula carpinifolia* SIEB. et ZUCC.	36.9	61.3	9
9	*Carpinus carpinoides* MAKINO	58.4	75	2
10	*Fagus crenata* BLUME.	60.4	75	1
12	*Castanea crenata* SIEB. et ZUCC. (mainly sapwood)	52.4	75	3
13	„ „ (mainly heartwood)	8.2	13.2	21
14	*Quercus serrata* THUNB.	33.9	63.4	11
16	*Zelkowa serrata* MAKINO	15.7	26.6	19
18	*Cercidiphyllum japonicum* SIEB. et ZUCC.	32.5	53.6	12
19	*Prunus serrulata* LINDL. var. *spontanea* MAKINO	17.8	28.6	18
20	*Phellodendron amurense* RUPR.	12.2	19.2	20
21	*Aesculus turbinata* BLUME.	42.3	62.3	7
23	*Acanthopanax sciadophylloides* FR. et SAV.	20.8	34.1	17
24	*Kalopanax ricinifolium* MIG. var. *typicum* NAKAI	27.2	43.9 } 41.1	15
25	„ „	22.3	38.3 }	16
26	*Cornus controversa* HEMSL.	40.2	59.0	8
27	*Praxinus japonica* BLUME.	49.9	67.0	15

crenata BLUME, *Tsuga Sieboldii* CARR, *Petrophiloides strobilacea* REID et CHANDER and *Aesculus turbinata* BLUME and *Cornus controversa* HEMSL. being highly suitable, whereas *Zelkowa serrata* MAKINO, *Prunus serrulata* LINDL. var. *spontanea* MAKINO, *Phellodendron amurense* RUPR., *Acanthopanax sciadophylloides* FR. et SAV. and *Kalopanax ricinifolium* MIG. var. *typicum* NAKAI, very unsuitable.

It is of interest to note in this experiment that, in *Castanea crenata* SIEB. et ZUCC. Siitake mycelium made a beautiful growth on the sawdust prepared from the sapwood; but on the heartwood, it was extremely poor. This fact was also true when the fungus was inoculated in the log. It did not grow into the heatwood, but made a rapid growth in the region of sapwood. Logs from *Quercus serrata* THUNB. are highly prized in Siitake production, but as a sawdust medium, it was found to be of intermediate in value, much inferior than *Carpinus carpinoides* MAKINO or *Fagus crenata* BLUME; moreover, although *Carpinus carpinoides* MAKINO is highly suitable for the growth of Siitake mycelium, others such as *Fagus crenata* BLUME, *Petrophiloides strobilacea* REID et CHANDLER, *Aesculus turbinata* BLUME or *Cornus controversa* HEMSL. were found to be equally suitable by this experiment.

Table V.

The Relation of Growth of Siitake Mycelium to the Kinds of Sawdust used as Culturing Medium. (Summarized Result on the Experiments using Large Mouth Glass Bottles.)

The average growth of Experiments I and II arranged in order after 20 days of incubation.

Order	Species name	Growth
1	*Carpinus carpinoides* MAKINO	42.2 mm
2	*Fagus crenata* BLUME	39.5
3	*Tsuga Sieboldii* CARR.	37.0
4	*Petrophiloides strobilacea* REID et CHANDLER	34.3
5	*Aesculus turbinata* BLUME	33.0
6	*Cornus controversa* HEMSL.	31.4
7	*Fraxinus japonica* BLUME	31.1
8	*Cercidiphyllum japoncum* SIEB. et ZUCC.	29.9
9	*Quercus serrata* THUNB.	29.3
10	*Betula carpinifolia* SIEB. et ZUCC.	29.0
11	*Pinus densiflora* SIEB. et ZUCC.	27.6
12	*Abies firma* SIEB. ZUCC.	26.5
13	*Castanea crenata* SIEB. et ZUCC.	24.4
14	*Cryptomeria japonica* D. DON.	22.9
15	*Kalopanax ricinifolium* MIG. var. *typicum* NAKAI	22.1
16	*Acanthopanax sciadophylloides* FR. et SAV.	19.4
17	*Phellodendron amurense* RUPR.	17.4
18	*Prunus serrulata* LINDL. var. *spontanea* MAKINO	11.9
19	*Zelkowa serrata* MAKINO	11.7

IV. Cultural Experiments on Sawdust Decoction Agar Media.

A. Methods.

In this experiment a comparison on the growth of fungus was made on the sawdust decoction agar media prepared from various kinds of sawdust. 20 grams

Table VI.

Growth of Siitake Mycelium on Sawdust Decoction Agar Medium Prepared from Various Kinds of Tree. (Result of Experiment 1.)

Fungus: Strains 434 and 439, produced by mating single-spores taken from a sporophore collected at Hutomi-mura, Akaiwa-gun, Okayama-ken. Incubation temperature: 24°C.

No.	Name of tree	Strain No. 434			Strain No. 439		
		Diameter of colony in mm.		Order after 20 days	Diameter of colony in mm.		Order after 20 days
		After 11 days	After 20 days (Average)		After 11 days	After 20 days (Average)	
1	*Cryptomeria japonica*	8.0	24.5 } 18.8	12	11.5	11.5 } 8.8	13
2	„ „	4.5	13.0 }		3.0	6.0 }	
3	*Pinus densiflora*	29.6	60.1	4	19.5	33.7	10
4	*Abies firma*	7.5	14.8	13	8.0	8.6	14
5	*Tsuga Sieboldii*	31.0	59·3	6	21.3	48.5	8
6	*Petrophiloides strobilacea*	40.1	71.8	3	32.0	56.1	6
7	*Betula carpinifolia*	38.1	62.6 } 57.9	7	26.7	41.0 } 48.8	7
8	„ „	26.7	53.2 }		28.3	56.5 }	
9	*Carpinus carpinoides*	17.5	43.6	9	35.6	76.0	3
10	*Fagus crenata*	36.4	62.7 } 55.2	8	35.0	63.1 } 58.9	5
11	„ „	27.6	47.6 }		20.5	54.6 }	
12	*Castanea crenata* (Sapwood)	24.0	41.0 } 26.8	11	22.5	47.6 } 32.0	12
13	„ „ (Heartwood)	2.8	12.5 }		7.8	16.3 }	
14	*Quercus serrata*	35.1	58.0 } 60.1	5	30.5	36.7 } 40.0	9
15	„ „	38.8	62.1 }		32.8	43.3 }	
16	*Zelkowa serrata*	—	3.0 } 1.5	16	3	— } 0	18
17	„ „	—	— }		—	— }	
18	*Cercidiphyllum japonicum*	40.0	76.7	1	40.5	76.1	2
19	*Prunus serrulata*	3	—	17	—	3	17
20	*Phellodendron amurense*	3	—	18	—	—	19
21	*Aesculus turbinata*	21.2	30.8 } 43.0	10	23.1	43.5 } 61.8	4
22	„ „	32.5	55.1 }		41.3	80.0 }	
23	*Acanthopanax sciadophylloides*	4	7.7	14	3	5.6	15
24	*Kalopanax ricinifolium*	7.3	10.1 } 7.55	15	5	6 } 5.5	16
25	„ „	3	5 }		3	5 }	
26	*Cornus controversa*	39.1	73.5	2	49.5	80.0	1
27	*Fraxinus japonica*	39.6	72.8 } 64.3	3	25.3	33.8 } 33.4	11
28	„ „	29.3	55.8 }		24.8	33.0 }	

of previously sun-dried sawdust was cooked in 200 cc. of distilled water for one hour in a steamer. After filtering and making up to the original volume of 200 cc., 2% agar was added, and was completed by following the usual procedure. The Siitake mycelia (strains No. 434 and No. 439) were inoculated on to these media poured in Petri dishes, and their growth was measured. The diameter of colonies was measured after 5, 11, 15 and 20 days of inocubation at a temperature of 24 °C.

B. The results of the Experiments.

The results are given in Table VI. It shows that the growth of two strains of Siitake fungus was not entirely identical in the same sawdust medium, nor was it in the case where the sawdusts were from different trees, but of the same species. The latter is parhaps due to the difference that may exists between the

Table VII.

Growth of Siitake Mycelium on Sawdust Decoction Agar Medium Prepared from Various Kinds of Tree. (Result of Experiment 2.)

Fungus: Strains 434 and 439, produced by mating single-spores taken from a sporophore collected at Hutomi-mura, Akaiwa-gun, Okayama-ken.

Fungus strain	No.	Name of tree	Diameter of Colony in mm.		Order after 21 days
			After 14 days	After 21 days	
No. 434	1	*Cryptomeria japonica*	9.2	20.6	15
	3	*Pinus densiflora*	23.6	25.5	14
	4	*Abies firma*	10.0	18.0	16
	7	*Betula carpinifolia*	22.0	37.0	10
	9	*Carpinus carpinoides*	38.6	52.2	3
	10	*Fagus crenata*	33.4	44.2	7
	11	„ „	22.0	37.0 (40.6)	
	12	*Castanea crenata* (Sapwood)	25.0	52.0	12
	13	„ „ (Heartwood)	4.0	10.5 (31.3)	
	15	*Quercus serrata*	38.0	51.5	4
	17	*Zelkowa serrata*	5.0	10.0	18
	18	*Cercidiphyllum japonicum*	17.5	37.0	9
	20	*Phellodendron amurense*	19.5	31.7	11
	21	*Aesculus turbinata*	30.0	42.6	6
	22	„ „	29.0	40.6 (41.6)	
	26	*Cornus controversa*	39.6	53.8	1
	28	*Fraxinus japonica*	24.6	38.8	8
No. 439	5	*Tsuga Sieboldii*	17.6	29.5	13
	6	*Petrophiloides strobilacea*	37.6	52.6	2
	14	*Quercus serrata*	24.2	43.8	5
	16	*Zelkowa serrata*	8.3	12.2	17
	27	*Fraxinus japonica*	7·0	10.0	19

heartwood and the sapwood. The names of the woods that showed favorable growth of fungus strain No. 434 were in the order, *Cercidiphyllum japonicum* SIEB. et ZUCC., *Cornus controversa* HEMSL., *Fraxinus japonica* BLUME, *Petrophiloides strobilacea* REID et CHANDLER, *Fagus crenata* BLUME, *Betula carpinifolia* SIEB. et ZUCC. and *Quercus serrata* THUNB. This relationship is generally true in the strain No. 439. The growth of both strains of fungi was poor in *Zelkowa serrata* MAKINO, *Prunus serrulata* LINDL. var. *spontanea* MAKINO and *Phellodendron amurense* RUPR.; followed by *Kalopanax ricinifolium* MIG. var. *typicum* NAKAI and *Cryptomeria japonica* D. DON.

The results of similarly repeated experiments are shown in Table VII. It follows closely with that of Table VI. The growth on the decoctions of *Cornus controversa* HEMSL., *Carpinus carpinoides* MAKINO and *Quercus serrata* THUNB. was found to be greater than that of all the others. In contrast, the growth on the decoction of *Zelkowa serrata* MAKINO was extremely poor.

V. Experiments on Sawdust Culture Medium.
(2) Culture in Test Tubes.

A. Methods.

The sawdusts used in this experiment were received in May, 1936, from the Tuyama Forestry Station. The trees consisting of 21 families, including 30 species, are listed in Table II. Three strains of Siitake fungus employed in these experiments are listed below. One or two strains were used at a time in each experiment.

Strain No. 4, (A 6 × D 1), medium size sporophore and fast growing, strain No. 23, (A 6 × N 2), medium to large size and strain No. 56, (K 6 × N 1), medium to large size. All of these are improved strains producing abundant sporophores developed at this Institute.

Having had some difficulties in obtaining a uniform growth in the previous experiment with large mouth glass bottles, in the present experiment the ordinary glass test tubes of 18 mm. in diameter were used. 20 g. of dried sawdust was poured into each of these test tubes, and was moistened with 10 cc. of tap water. The moistened sawdust in the test tube was lightly packed with a glass rod, and the whole was autoclave for 30 minutes at 15 lbs. pressure. When the sawdust was found to have forced its way up and have adhere to the side of the test tube, it was carefully scraped down and packed in place with a sterile glass rod. Test tubes were inoculated with well developed sawdust cultures of the Shiitake fungus. To insure a perfect contact, the introduced inoculum was lightly pressed down against the substratum with a sterile glass rod. The inoculated test tubes were incubated at 24 °C., and the extent of growth was measured at the ends of 10 and 15 days.

B. Results: The Effect of Size of Sawdust Particle and Moisture to the Development of the Fungous Mycelium.

The growth of the fungous mycelium on sawdust medium was not only influeced by the kind of tree from which the sawdust was made, but also it seems to

be effected by the size of the sawdust particles. Furthermore, the amount of water that can be added in the preparation of the sawdust medium should vary accordingly. Thus to clarfly these points the following experiment was performed.

Sawdust prepared from the following six kinds of trees were used: *Quercus serrata* THUNB., *Cornus controversa* HEMSL., *Machilus Thunbergii* S. et Z., *Acer pictum* THUNB. var. *Paxii* SCHW. subvar. *eupictum* PAX, *Albizzia Julibrissin* DURAZZ var. *speciosa* KOIZ., and *Petrophiloides strobilacea* REID et CHANDLER. The sawdust particles that passed through the 3 mm. sieve but remained in the 2 mm. was designated as "large". In other words the large particles had a diameter of 2–3 mm. By a similar method of preparation, the "medium" size particles had 1.5–2 mm., and the "small" particles had a diameter less than 1.5 mm. 20 cc. of sawdust was placed in a test tube having an inner diameter of 18 mm. and to this, 7, 10 and 12 cc. of tap water was added. Fungus strain No. 4 was inoculated into the prepared sawdust media and was incubated at a temperature of 24 °C. Observations were made at the end of 10 and 15 days of incubation by measuring the extent of growth of the mycelium along the wall of the test tube. The results are shown in Table VIII.

Table VIII.

Growth of Siitake Mycelium on Sawdust Medium: Effects of the Size of the Sawdust Particle and the Moisture Content of the Medium.

Experiment began on January 10, 1940.

Name of tree	Size of particle (Diameter in mm.)	Growth in mm. after 10 days			Growth in mm. after 15 days		
		* 7 cc	10 cc	12 cc	7 cc	10 cc	12 cc
Quercus serrata	Large (2–3)	34.0	40.5		46.0	48.0	
	Medium (1.5–2)	27.6	29.0		40.3	41.0	
	Small (below 1.5)		31.3	31.6		45.0	42.7
Cornus controversa	Large (2–3)	43.0	41.0		52.3	54.0	
	Medium (1.5–2)	40.3	41.0		57.7	52.2	
	Small (below 1.5)		34.3	32.3		47.3	45.5
Machilus Thunbergii	Large (2–3)	41.6	42.0		49.0	47.0	
	Medium (1.5–2)	32.6	36.0		44.3	48.0	
	Small (below 1.5)		28.3	26.6		40.0	40.7
Acer palmatum	Large (2–3)	41.6	38.0		55.7	50.0	
	Medium (1.5–2)	30.6	30.0		45.3	44.7	
	Small (below 1.5)		32.0	33.6		47.5	48.0
Albizzia Julibrissin	Large (2–3)	24.5	30.3		37.0	41.7	
	Medium (1.5–2)	24.3	32.3		34.3	38.3	
	Small (below 1.5)		22.5	26.6		32.7	40.7
Petrophiloides strobilacea	Large (2–3)	37.3	32.3		57.3	51.7	
	Medium (1.5–2)	38.6	36.6		56.3	52.7	
	Small (below 1.5)		33.0	34.6		51.7	52.7

* The volume of water added to 20 cc of dry sawdust.

Although in view of the fact that the growth of Shiitake fungus is influenced by the size of sawdust particle, the amount of moisture and the kind of tree from which the sawdust is made, further made it difficult to make all the conditions optimum. The present experiment was conducted as outlined above, and the results were compared among those that showed best mycelial growths. The comparison is given in Table IX.

Table IX.

Relation of Size of Sawdust Particle in Sawdust Medium to the Growth of Siitake Mycelium.

Size of sawdust particle: Large, 2 - 3 mm.; medium, 1.5 - 2 mm.; small, below 1.5 mm.

Name of tree	Growth in mm. after 10 days			Growth in mm. after 15 days		
	Large	Medium	Small	Large	Medium	Small
Quercus serrata	40.5	29	31.6	**48.0**	41.0	45.0
Cornus controversa	43.0	41.0	34.3	54.0	**57.7**	47.3
Machilus Thunbergii	42.0	36.0	28.3	**49.0**	48.0	48.0
Acer pictum	41.6	30.6	33.6	**55.7**	45.3	48.0
Albizzia Julibrissin	30.3	28.3	26.6	**41.7**	38.3	40.7
Petrophiloides strobilacea	37.3	**38.6**	34.6	**57.3**	56.3	52.7

Note: The figures in bold type indicate the greatest growth.

According to this experiment, the Shiitake mycelium grew best when the sawdusts were of large particles — 2 - 3 mm. in diameter. The medium sized particles — 1.5 - 2 mm.— were next in order and the small particles showed the least growth.

If the moisture content is taken into consideration, and compared among the six different trees, *Quercus serrata* Thunb., *Machilus Thunbergii* S. et Z., *Albizzia Julibrissin* Durazz. var. *speciosa* Koiz. supported the fungus better when 10 cc. of water was added rather than 7 cc. This was true in the media composed of large as well as medium sized particles. With *Cornus controversa* Hemsl. better result was obtained by the addition of 10 cc. water to the large particled sawdust medium, while the opposite was true in the medium sized sawdust. In *Acer pictum* Thunb. var. *Paxii* Schw. subvar. *eupictum* Pax and *Albizzia Julibrissin* Durazz var. *speciosa* Koiz, slightly better growth was observed with 7 cc. water. There was but a very little difference between 10 and 12 cc.

Further experiment was conducted using 30 different kinds of wood. In view of the fact that it was difficult to have variations in the moisture content, all the media were made containing 10 cc. of water per 20 cc. of sawdust. The experimental results are shown in Table X.

Table X.

Relation of Kind of Sawdust and their Size to the Growth of Siitake Mycelium. I. Amount of Growth and Aerial Mycelium Formed after 9 and 15 days of Incubation at 24°C. Average of 3 Petri-dish Cultures.

Size of particles: Large, 2–3 mm.; medium, 1.5–2 mm.; and small, below 1.5 mm. Fungus used: Strain No. 4.

No.	Name of tree	Growth in mm. and amount of aerial mycelium after 9 days				Growth in mm. and amount of aerial mycelium after 15 days			
		Large	Medium	Small	Average	Large	Medium	Small	Average
1	*Petrophiloides strobilacea*	31.0卅	32 7卄	27.3卄	30.3	57.7卅	50.3卄	52.3卅	53.4
2	*Betula ulmifolia*	33.0卄	31.5卄	28.0卄	30.8	55.0卅	46.0卅	51.7卄	50.9
3	*Carpinus carpinoides*	26.6+	27.0+	20.3+	24.6	51.6卄	55.7卄	49.7卄	52.3
4	*Castanea crenata*	21.3卄	15.3+	18.0+	18.2	32.3卄	25.3卄	25.0+	27.5
5	*Quercus acuta*	17.5卄	14.0+	14.0+	15.2	37.0卅	28.0卄	30.7卄	31.9
6	*Quercus serrata*	24.3卅	27.0卄	29.0卄	26.8	46.7卅	41.3卅	45.3卄	44.4
7	*Zelkowa serrata*	±	4.0	±	2.0	±	5,3±	6.7±	6.0
8	*Euptela polyandra*	33.5+	29.3+	30·3+	31.0	59.0卅	45.7卄	50.0卄	51.6
9	*Magnolia obovata*	22.0卄	18.3+	17.0卄	19.1	29.3卄	22.7+	17.0+	23.0
10	*Actinodaphne loncifolia*	27.3卅	33.7卄	28.7+	29.9	53.0卅	49.7卄	47.7卄	50.1
11	*Cinnamomum japonicum*	33.3卅	32.7卄	30.3卄	32.1	62.7卅	53.3卅	57.0卄	57.7
12	*Machilus Thunbergii*	31.5卅	32.0卄	29.7卄	31.1	49.0卅	56.0卅	54.3卄	53.1
13	*Prunus serrulata*	12.6+	7.0+	11.3+	10.3	24.3卄	16.0卄	17.7卄	19.3
14	*Albizzia Julibrissin*	20.0卄	20.7卄	20.3卄	20.3	38.0卅	35.7卅	41.3卄	38.3
15	*Cladrastis platycarpa*	21.0卄	22.0卄	18.0+	20.3	36.3卄	29.0卄	34.3卄	33.2
16	*Picrasma quassioides*	21.0卅	24.0+	20.7卄	21.6	42.5卅	50.3卄	40.0卄	44.3
17	*Acer rufinerve*	23.7卄	29.7+	28.0+	27.1	44.3卄	44.3卄	45.7卄	44.8
18	*Acer palmatum*	20.0+	26.5+	28.3+	24.9	43.0卄	49.0+	47.3卄	46.4
19	*Acer pictum*	25.3卄	23.3+	26.0+	24.9	51.3卅	37.6+	50.3卄	46.4
20	*Meliosma myriantha*	13.3卅	9.7+	11.5+	11.5	27.7卄	22.0+	19.5+	23.1
21	*Camellia japonica*	29.0卄	29.0+	24.0+	27.3	50.0卅	46.7卄	36.7卄	44.5
22	*Idesia polycarpa*	15.0+	19.0+	22.7+	18.9	35.0+	39.0卄	38.3卄	37.4
23	*Aralia elata*	13.7卄	17.7+	15.3+	15.6	28.6卄	27.0+	27.0卄	27.5
24	*Aucuba japonica*	20.0卄	23.3+	23.7+	22.3	39.3卄	34.0+	30.0卄	34.4
25	*Cornus controversa*	33.0卅	29.7卄	26.6+	29.8	59.3卅	48.7卄	52.3卄	53.4
26	*Clethra barbinervis*	22.3+	19.5+	22.3+	21.4	40.7+	32.5+	40.3+	37.8
27	*Pieris japonica*	27.7卅	29.5卄	26.3卄	27.8	49.3卅	33.3卄	42.3卅	41.6
28	*Diospyros kaki*	17.0卄	22.0卄	18.7+	19.2	38.7卄	39.5卄	38.3+	38.8
29	*Pterostyrax corymbosum*	16.7卄	24.3卄	23.0+	21.3	39.3卅	36.7卄	37.7卄	37.9
30	*Styrax japonica*	25.3卄	24.7+	23.7+	24.6	43.5卄	41.3卄	44.7卄	43.2

According to Table X, there were differences in the amount of growth depending upon the size of the sawdust particle. If the woods that showed the best

growth were classified according to the size of the particle the frequency will be as follows :

		After 9 days	After 14 days
Large particle	(2 – 3 mm.)	11 kinds	19 kinds
Medium „	(1.5 – 2 mm.)	14 „	5 „
Small „	(below 1.5 mm.)	5 „	6 „

Table XI.

Relation of Kind of Sawdust to the Growth of Siitake Mycelium.
II. Amount of Growth and Aerial Mycelium Formed after 10 and 15 Days of Incubation at 24°C. Average of 3 Petri-dish Cultures.

Fungus used : Strains No. 4 and 23.

No.	Name of tree	Growth in mm. and amount of aerial mycelium after 10 days			Growth in mm. and amount of aerial mycelium after 15 days		
		No. 4	No. 23	Average	No. 4	No. 23	Average
1	*Petrophiloides strobilacea*	33.5 ⧺	29.5 ⧺	31.5	56.5 ⧺	54.0 ⧺	55.3
2	*Betula ulmifolia*	30.3 ⧺	30.5 ⧺	30.4	50.0 ⧺	51.5 ⧺	50.8
3	*Carpinus carpinoides*	36.7 ⧺	30.0 ⧺	33.4	59.0 ⧺	55.0 ⧺	57.0
4	*Castanea crenata*	24.0 ⧺	25.5 ⧺	24.8	33.0 ⧺	38.0 ⧺	35.5
5	*Quercus acuta*	22.0 ⧺	22.0 ⧺	22.0	39.0 ⧺	34.5 ⧺	36.8
6	*Quercus serrata*	29.3 ⧺	27.5 ⧺	28.4	46.3 ⧺	44.0 ⧺	45.2
7	*Zelkowa serrata*	6.6 —	8.5 +	7.6	8.7 +	14.5 ⧺	11.6
8	*Euptela polyandra*	33.7 ⧺	27.0 ⧺	30.4	55.0 ⧺	48.5 ⧺	51.8
9	*Magnolia obovata*	11.0 ⧺	25.0 ⧺	18.0	12.3 ⧺	40.5 ⧺	26.4
10	*Actinodaphne loncifolia*	32.0 ⧺	26.5 ⧺	29.3	55.0 ⧺	49.0 ⧺	50.2
11	*Cinnamomum japonicum*	35.0 ⧺	31.0 ⧺	33.0	58.0 ⧺	53.0 ⧺	55.5
12	*Machilus Thunbergii*	32.7 ⧺	23.5 ⧺	28.1	61.3 ⧺	48.0 ⧺	54.7
13	*Prunus serrulata*	10.7 ⧺	12.0 ⧺	11.4	20.7 ⧺	21.0 ⧺	20.9
14	*Albizzia Julibrissin*	28.3 ⧺	26.5 ⧺	27.4	41.0 ⧺	46.0 ⧺	43.5
15	*Cladrastis platycarpa*	25.3 ⧺	23.5 ⧺	24.4	40.3 ⧺	41.5 ⧺	40.9
16	*Picrasma quassioides*	23.7 ⧺	25.0 ⧺	24.4	55.7 ⧺	51.5 ⧺	53.6
17	*Acer rufinerve*	29.7 +	28.0 +	28.9	53.3 +	49.5 +	51.4
18	*Acer palmatum*	33.3 ⧺	26.0 ⧺	29.7	54.0 ⧺	49.5 ⧺	51.8
19	*Acer pictum*	31.0 ⧺	29.0 ⧺	30.0	52.3 ⧺	50.0 ⧺	51.2
20	*Meliosma myriantha*	16.7 ⧺	9.5 ⧺	13.1	28.3 ⧺	17.0 ⧺	22.7
21	*Camellia japonica*	27.3 ⧺	25.0 ⧺	26.2	46.7 ⧺	47.0 ⧺	46.9
22	*Idesia polycarpa*	29.3 ⧺	25.5 ⧺	27.4	46.3 ⧺	44.5 ⧺	45.4
23	*Aralia elata*	14.3 ⧺	15.5 ⧺	14.9	28.0 ⧺	30.0 ⧺	29.0
24	*Aucuba japonica*	26.0 +	21.0 +	23.5	43.3 +	36.5 +	39.9
25	*Cornus controversa*	38.0 ⧺	33.0 ⧺	35.5	61.0 ⧺	54.0 ⧺	53.5
26	*Clethra barbinervis*	25.0 ⧺	21.0 ⧺	23.0	43.7 ⧺	28.5 ⧺	36.1
27	*Pieris japonica*	28.7 ⧺	22.0 ⧺	25.4	48.0 ⧺	45.0 ⧺	46.5
28	*Diospyros kaki*	33.0 ⧺	27.0 ⧺	30.0	55.7 ⧺	52.0 ⧺	53.9
29	*Pterostyrax corymbosum*	22.0 ⧺	28.0 ⧺	25.0	44.7 ⧺	53.5 ⧺	49.1
30	*Styrax japonica*	25.0 +	25.0 +	25.0	41.7 ⧺	35.0 ⧺	38.4

The result shows that when the sawdust particles are below 1.5 mm. in diameter, the growth of the fungus was found to be extremely poor. In the medium sized sawdusts, the fungus grew better up till 9 days' time, but was overtaken later by the large particled media. In general, there was but a slight difference in the growth between the large and the medium sized particles.

Table XII.

Relation of Kind of Sawdust to the Growth of Siitake Mycelium. III. Amount of Growth and Aerial Mycelium Formed after 10 and 15 Days of Incubation at 24°C.

Fungus used: Strains No. 4, 23 and 56.

No.	Name of tree	Growth in mm. and amount of aerial mycelium after 10 days				Growth in mm. and amount of aerial mycelium after 15 days			
		No. 4	No. 23	No. 56	Average	No. 4	No. 23	No. 56	Average
1	*Petrophiloides strobilacea*	41.3卅	38.7卅	43.3卅	41.1	56.0卅	61.3卅	66.3卅	61.2
2	*Betula ulmifolia*	32.7卅	33.7卅	37.7卅	34.7	48.3卅	49.0卅	51.7卅	49.6
3	*Carpinus carpinoides*	35.3卄	36.0卄	41.0卄	37.4	51.3卅	54.3卄	64.7卅	56.7
4	*Castanea crenata*	24.3卄	25.7卄	26.0卅	25.3	33.0卄	37.3卄	36.7卄	35.6
5	*Quercus acuta*	25.0卄	25.0卄	24.0卄	24.6	39.6卄	38.0卄	38.0卄	38.5
6	*Quercus serrata*	35.7卄	36.7卄	40.0卄	37.5	54.0卄	53.0卅	60.0卄	55.9
7	*Zelkowa serrata*	5.2±	5.5±	6.0+	5.6	5.7±	7.0±	12.0±	8.2
8	*Euptela polyandra*	36.0+	37.7+	41.7卄	38.5	52.0+	58.6卄	59.3卄	56.6
9	*Magnolia obovata*	15.7+	28.7卅	32.0卅	25.4	14.7卄	35.7卄	38.7卄	29.7
10	*Actinodaphne loncifolia*	40.7卄	34.7卄	33.0卄	36.1	54.0卄	54.0卄	52.7卄	53.6
11	*Cinnamomum japonicum*	42.0卅	41.0卅	43.3卅	42.1	60.0卅	58.6卅	58.0卅	58.8
12	*Machilus Thunbergii*	39.3卅	33.0卄	38.3卅	36.9	59.3卅	52.0卅	58.0卅	56.5
13	*Prunus serrulata*	15.7卅	16.3卄	16.0卄	16.0	22.0卄	25.0卄	25.0卄	24.0
14	*Albizzia Julibrissin*	27.3卅	27.7卅	27.0卅	27.3	40.0卅	37.3卅	38.3卅	38.5
15	*Cladrastis platycarpa*	28.7卅	26.0卄	30.3卅	28.3	43.3卄	45.7卅	49.3卅	46.1
16	*Picrasma quassioides*	40.0卄	36.5卄	40.7卄	35.7	56.8卄	56.0卄	58.7卅	57.0
17	*Acer rufinerve*	36.3卄	32.3+	37.7+	35.4	55.7+	51.3+	56.7+	54.6
18	*Acer palmatum*	38.6+	33.7卄	39.7卄	37.3	60.8卄	53.3卄	63.0卄	59.6
19	*Acer pictum*	35.7卄	34.0卄	39.3卅	36.3	54.3卄	53.0卅	52.7卄	53.3
20	*Meliosma myriantha*	17.0卄	18.0卅	9.0+	14.7	25.7卄	24.0卄	17.0卄	22.2
21	*Camellia japonica*	28.7卄	25.7卄	26.3+	26.9	42.0+	42.3+	38.7卄	31.1
22	*Idesia polycarpa*	30.3+	29.7卄	33.7卄	31.2	45.7卄	45.0卄	50.5卄	47.1
23	*Aralia elata*	20.7+	21.3卄	26.7卄	22.9	34.0卄	33.7卄	39.3卅	35.7
24	*Acuba japonica*	35.3+	32.3+	34.0+	33.9	52.3+	47.7+	51.7+	50.6
25	*Cornus controversa*	41.7+	37.5卅	41.0卅	40.1	59.4卅	55.0卅	58.6卅	57.7
26	*Clethra barbinervis*	34.7卅	29.3卄	32.3卄	31.2	50.3卄	47.0卄	50.0卄	49.1
27	*Pieris japonica*	33.7卄	34.7卅	34.3卅	34.2	50.3卄	54.0卄	55.6卄	53.3
28	*Diospyros kaki*	40.0卄	35.0+	40.0+	38.3	61.3卄	57.0卄	60.0卄	59.4
29	*Pterostyrax corymbosum*	32.3+	31.7卅	38.0卅	34.0	45.0卅	45.0卅	53.7卅	47.9
30	*Styrax japonica*	33.0卄	34.0	34.3	33.8	52.0	53.7	52.7	52.6

C. Results, (cont.) — *The Kind of Sawdust in Relation to Fungous Growth.*

Having found that the sawdusts of medium and large size particles were more favorable for fungous growth, the medium sized sawdust were used in this experiment. The amount of moisture to be applied to the sawdust must very with the size and the kind of wood from which the sawdust was prepared, but in this experiment, water was applied in all cases at the rate of 10cc. per 20cc. of dried sawdust. The material was incubated at 24°C. for 10 and 15days, after which the extent

Table XIII.

Relation of Kind of Sawdust to the Growth of Siitake Mycelium. (Average of Experiments I, II, and III).

No.	Name of tree	Growth in mm. after 10days					Growth in mm. after 15days				
		Exp. I	Exp. II	Exp. III	Aver-age	Order	Exp. I	Exp. II	Exp. III	Aver-age	Order
1	*Petrophiloides strobilacea*	33.7	31.5	41.1	35.4	3	50.4	55.3	61.2	56.6	2
2	*Betula ulmifolia*	34.2	30.4	34.7	33.1	6	50.9	50.8	49.6	50.4	11
3	*Carpinus carpinoides*	27.3	33.4	37.4	32.7	8	52.3	57.0	56.7	55.4	3
4	*Castanea crenata*	20.2	24.8	25.3	23.4	24	27.5	35.5	35.6	32.9	25
5	*Quercus acuta*	16.9	22.0	24.6	21.2	26	31.9	36.8	38.5	35.7	24
6	*Qercus serrata*	29.8	28.4	37.5	31.9	9	44.4	45.2	55.9	48.5	14
7	*Zelkowa serrata*	22.2	7.6	5.6	5.1	30	6.0	11.6	8.2	8.6	30
8	*Euptela polyandra*	34.4	30.4	38.5	34.4	4	51.6	51.8	56.6	53.3	6
9	*Magnolia obovata*	21.2	18.0	25.4	21.5	25	23.0	26.4	29.7	26.4	27
10	*Actinodaphne loncilolia*	33.2	29.3	36.1	32.9	7	50.1	50.2	53.6	51·3	9
11	*Cinnamomum japonicum*	35.7	33.0	42.1	36.9	2	57.7	55.5	58.8	57.3	1
12	*Machilus Thunbergii*	34.5	28.1	36.9	33.2	5	53.1	54.7	56.5	54.8	5
13	*Prunus serrulata*	11.5	11.4	16.0	13.0	29	19.3	20.9	24.0	21·4	29
14	*Albizzia Julibrissin*	22.6	27.4	27.3	25.8	22	38.3	43.5	38.5	40.1	22
15	*Cladrastis platycarpa*	22.6	24.4	28.3	25.1	23	33.2	40.9	46.1	40.1	23
16	*Picrasma quassioides*	24.0	24.4	35.7	28.0	15	44.3	53.6	57.0	51.6	8
17	*Acer rufinerve*	30.1	28.9	35.4	31.5	11	44.8	51.4	54.6	50.3	12
18	*Acer palmatum*	27.7	29.7	37.3	31.6	10	46.4	51.8	59.6	22.6	7
19	*Acer pictum*	27.7	30.0	36.3	28.0	16	46.4	51.2	53.3	50.3	13
20	*Meliosma myriantha*	12.8	13.1	14.7	13.5	28	23.1	22.7	22.2	52.6	28
21	*Camellia japonica*	30.3	26.2	26.9	27.8	17	44.5	46.9	31.1	40.8	21
22	*Idesia polycarpa*	21.0	27.4	31.2	26.5	21	37.4	45.4	47.1	43.3	18
23	*Aralia elata*	17.3	14.9	22.9	18.4	27	27.5	29.0	35.7	30.7	26
24	*Aucuba japonica*	24.8	23.5	33.9	27.4	19	34.4	39.9	50.6	41.6	19
25	*Cornus controversa*	33.1	35.5	40.1	37.2	1	53.4	53.5	57.7	54.9	4
26	*Clethra barbinervis*	23.8	23.0	31.2	27.0	20	37.8	36.1	49.1	41.0	20
27	*Pieris japonica*	30.9	25.4	34.2	30.2	12	41.6	46.5	53.3	47.1	15
28	*Diospyros kaki*	21.3	30.0	38·3	29.9	13	38.8	53.9	59.4	50.7	10
29	*Pterostyrax corymbosum*	23.7	25.0	34.0	27.6	18	37.9	49.1	47.9	45.0	17
30	*Styrax japonica*	27.3	25.0	33.8	28.7	14	43.2	38.4	53.9	45.2	16

of mycelial growth and the occurrence of aerial mycelium were noted as shown in Tables XI and XII.

Figure I.

Relation of Kinds of Sawdust to the Growth of Siitake Mycelium.
(Grouped According to Natural Classification of Families.)
Results after 15 Days at 24°C.

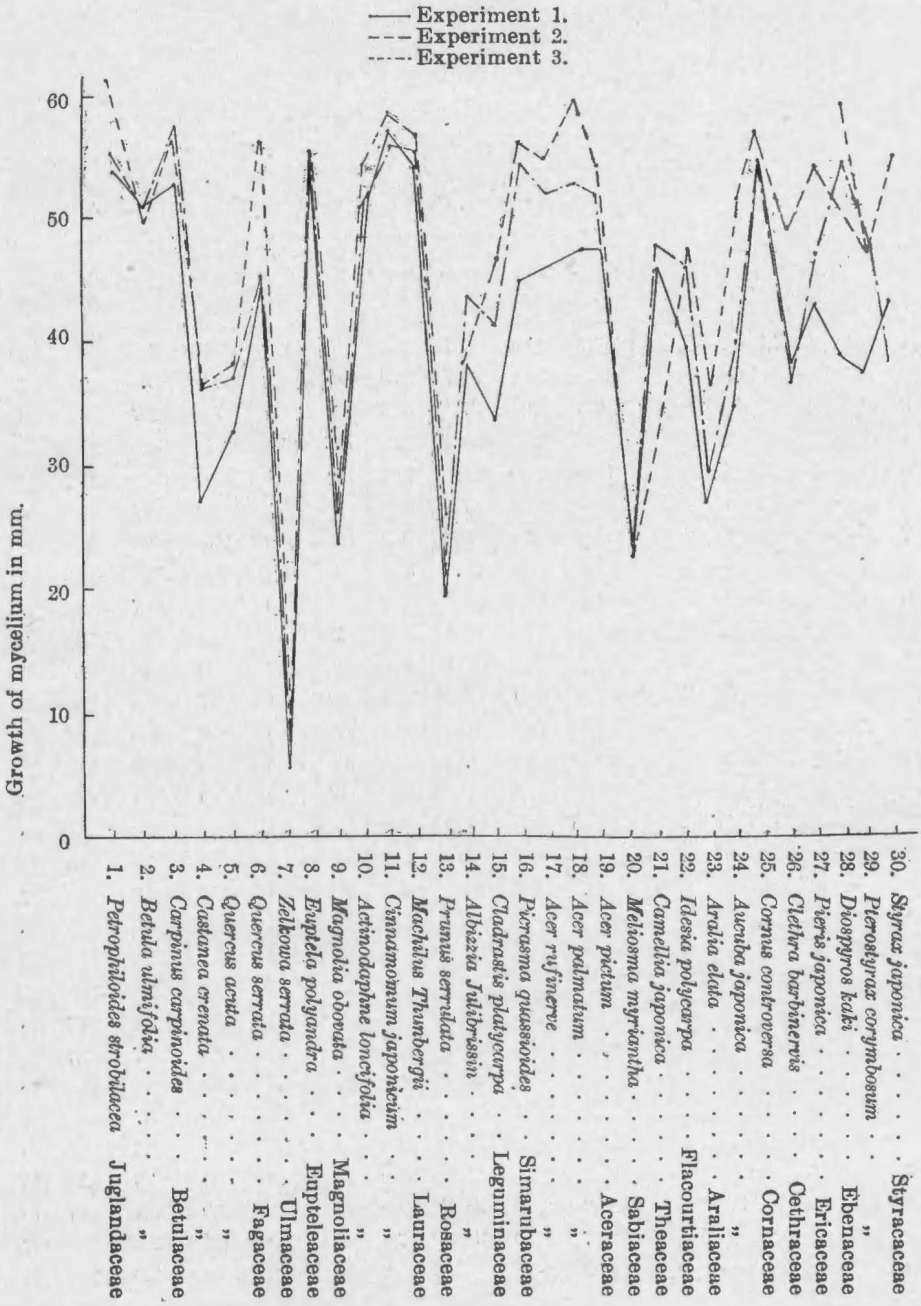

Table XIII presents the summarized results of Tables X to XII, and the results are expressed in curves of Figure I. After 15 days of incubation, the fungus was found to have made best growth in sawdust made from *Cinnamomum japonicum* SIEB. followed by *Petrophiloides strobilacea* REID et CHANDLER, and others in the order of *Carpinus carpinoides* MAKINO, *Cornus controversa* HEMSL., *Machilus Thunbergii* S. et Z., *Euptela polyandra* SIEB. et ZUCC., *Acer pictum* THUNB. var. *Paxii* SCHW. subvar. *eupictum* PAX, *Picrasma quassioides* BENN, *Actinodaphne lonci folia* MEISSN, *Diospyros kaki* THUNB. var. *silvestris* MAKINO, *Betula ulmifolia* SIEB. et ZUCC., *Acer palmatum* THUNB. and *Acer rufinerve* SIEB. et ZUCC. All of them have made more than 50 mm. of growth. Next in order were *Quercus serrata* THUNB., *Pieris japonica* D. DON, *Styrax japonicum* SIEB. et ZUCC., *Pterostyrax corymbosum* SIEB. et ZUCC, *Idesia polycarpa* MAXIM, *Aucuba japonica* THUNB., *Clethra barbinervis* SIEB. et ZUCC, *Camellia japonica* L. var. *hortensis* MAKINO, *Albizzia Julibrissin* DURAZZ var. *speciosa* KOIZ and *Cladrastis platycarpa* MAKINO, making more than 40 mm. of growth, and *Quercus acuta* THUNB., *Castanea crenata* SIEB. et ZUCC, and *Aralia elata* SEEM. with more than 30 mm. of growth; finally, those that made less than 30 mm. were *Magnolia obovata* THUNB., *Meliosma myriantha* SIEB. et ZUCC, *Prunus serrulata* LINDL var. *spontanea* MAKINO and *Zelkowa serrata* MAKINO, among which *Zelkowa serrata* MAKINO showed the poorest growth.

VI. The Effect of the Addition of Various Nutrients to the Sawdust Culture Medium.

A. Materials and Methods.

For culturing the Shiitake fungus, besides pure sawdust, which was used as the basic material, other nutrients as rice- or wheat-bran, potato, soybean sauce, or decocction of onion bulb, were added, and their effect on the development of the fungus mycelium was studied.

The methods of preparation were as follows:

(1) Pure Sawdust Medium. To *Pinus densiflora* SIEB. et ZUCC sawdust, sufficient water was added and throughly mixed. The moisture content was such that no free water dripped when squeezed by hand.

(2) Sawdust-medium. Identical as in (1), but 1% sucrose solution was used in moistening the sawdust.

(3) Sawdust-Rice-bran-Sugar Medium. To 1 part of medium prepared as in (2), 3 parts of rice-bran was added.

(4) Sawdust-Wheat-bran-Sugar Medium. Wheat-bran was used in place of rice-bran as prepared in (3).

(5) Sawdust-Rice-bran-Potato-Sugar Medium. Macerated potato was added at the rate of 20 g. to 1 liter of the medium prepared as in (3).

(6) Sawdust-Wheat-bran-Potato-Sugar Medium. Wheat-bran was substituted in place of rice bran of (5).

(7) Sawdust-Soybean sauce-Medium. 5% soybean sauce was added in place of sugar solution of (2).

(8) Sawdust-Soybean Sauce-Sugar Medium. To the sugar solution of (2), soybean sauce was added to make up a 5% solution.

(9) Sawdust-Onion Decoction Medium. Decoction of onion bulb (200 g. per 100 cc. water) was used in place of sugar of (2).

(10) Sawdust-Onion Decoction-Sugar Medium. To the onion decoction, sugar was added making a 1% solution.

(11) Sawdust-Potato Starch Medium. Potato starch was added 3% by volume to sawdust and sufficient water was added as in (3).

B. Results.

The results on the growth of the Shiitake mycelium after inoculating the above prepared media with the pure growth of the fungus and incubating at 25°C. for 20 days, are given in Table XIV.

Table XIV.

Relation of Various Nutrients Added to the Sawdust Medium to the Growth of Siitake Mycelium.

Results after 20 days of incubation at 24°C. Each is an average of 4 cultures.

No.	Nutrients added to basic sawdust medium	Growth of mycelium in cm.	Density of mycelium
1	Basic sawdust medium alone	9.8	+
2	Sucrose	9.2	++
3	Sucrose + rice-bran	10.0	+++
4	Sucrose + wheat-bran	10.2	+++
5	Sucrose + rice-bran + potato	10.5	+++
6	Sucrose + wheat-bran + potato	10.4	+++
7	Soybean sauce	8.6	++
8	Sucrose + soybean sauce	9.2	++
9	Extract of onion	9.0	+++
10	Sucrose + extract of onion	9.0	+++
11	Potato starch	9.8	+++

Note: In the column of density of mycelium, the greater member of plus signs indicates the better or denser mycelial growth.

According to Table XIV, the fungus made best growth in the sawdust-rice-bran-potato-sugar medium and the sawdust-wheat-bran-potato-sugar medium; and was followed by similar media minus potato. There was a fair growth even with the addition of only potato starch. This was followed by the addition of sugar or sugar and soybean sauce. The use of soybean sauce alone produced very poor growth. The elongation of mycelia in pure sawdust medium was not very different from that occurred in the sawdust-rice-bran-sugar medium, but it lacked the density of the mycelium.

VII. Discussion.

The tests conducted on the growth of Shiitake mycelium on sawdusts prepared from various kinds of tree contained in large mouth glass bottles or test

tubes, and on sawdust decoction agar media in Petri dishes showed that the sawdusts can be divided into two large groups : suitable, and unsuitable for Siitake culture. The comparative results are shown in Table XV.

Of the six plants classed as suitable in Table XV, *Carpinus carpinoides* MAKINO, *Petrophiloides strobilacea* REID et CHANDLER and *Cornus controversa* HEMSL occurred as suitable in all three experiments, while *Fagus crenata* BLUME was classed as such in two of the experiments. In the unsuitable group, of the five trees, *Zelkowa serrata* MAKINO, and *Prunus serrulata* LINDL. var. *spontanea* MAKINO were common in all three experiments and *Kalopanax ricinifolium* MIG. var. *typicum* NAKAI, *Acanthopanax sciadophylloides* FR. et SAV. and *Phellodendron amurense* RUPR. in two of the experiments.

Considering from the above results, we can conclude that for the growth of the Siitake mycelium, *Carpinus carpinoides* MAKINO, *Petrophiloides strobilacea* REID et CHANDLER, *Cornus controversa* HEMSL. and *Fagus crenata* BLUME. can be regarded as being highly suitable trees ; and similarly, *Zelkowa serrata* MAKINO and *Prunus serrulata* LINDL. var. *spontanea* MAKINO can be classed as being very unsuitable trees, followed by *Phellodendron amurense* RUPR, *Acanthopanax sciadophylloides* FR. et SAV. and *Kalopanax ricinifolium* MIG. var. *typicum* NAKAI.

In actual practice, the culture medium is rarely composed of sawdust alone, but in most cases, rice-bran or wheat-bran, starch, or sugar

Table XV.

Names of Trees used for Sawdust Medium Suitable and Unsuitable to the Growth of Siitake Mycelium.

	Result of exp. I	Result on agar medium	Result of exp. II
Suitable trees	***Carpinus capinoides***	***Cornus controversa***	*Cinnamomum japonicum*
	Fagus crenata	*Cercidiphyllum japonicum*	***Petrophiloides strobilacea***
	Abies firma	***Petrophiloides strobilacea***	***Carpinus carpinoides***
	Petrophiloides strobilacea	***Carpinus carpinoides***	***Cornus controversa***
	Aesculus turbinata	*Fagus crenata*	*Machilus Thumbergii*
	Cornus controversa	*Betura carpinifolia*	*Euptela polyandra*
Unsuitable trees	*Kalopanax ricinifolium*	*Phellodendron amurense*	*Aralia elata*
	Acanthopanax sciadophylloides	*Acanthopanax sciadophylloides*	*Magnolia obovata*
	Phellodendron amurense	*Kalopanax ricinifolium*	*Meliosma myriantha*
	Prunus serrulata	***Zelkowa serrata***	***Prunus serrulata***
	Zelkowa serrata	***Prunus serrulata***	***Zelkowa serrata***

is supplemented as the added nutrients. Consequently, it is highly probable that the fungus obtains most of its nurishments from them, and the question of whether a certain sawdust is suitable or not seems to be of secondary importance. However, in as much as the results of practical experience showed the logs of *Petrophilloides strobilacea* REID, *Carpinus carpinoides* MAKINO and *Quercus serrata* THUNB., highly favorable, sawdusts from them should be used whenever they are available, and should avoid such wood as *Zelkowa serrata* MAKINO, *Prunus serrulata* LINDL. var. *spontanea* MAKINO and *Meliosma myriantha* SIEB. et ZUCC. Furthermore, as the size of the sawdust particles influences the rate of growth, particles smaller than 1.5 mm. in diameter should be avoided.

The present investigation is not only applicable in making sawdust cultures of Siitake mycelium, but also in selecting logs for growing Siitake mushroom.

At the present time, among Fagaceae, *Quercus* spp. and *Castanea* spp.; Betulaceae, *Carpinus* spp.; and Juglandaceae, *Petrophiloides* spp. are being employed principally for Siitake production, but little consideration is given to other kinds of tree. This study, as shown in Fig. 1, brought out the facts that *Petrophiloides strobilacea* REID et CHANDER of Juglandaceae, *Betula ulmifolia* SIEB. et ZUCC., *Carpinus carpinoides* MAKINO of Betulaceae also make excellent logs. Among Fagaceae, sapwood of *Castanea crenata* SIEB. et ZUCC. and *Quercus serrata* THUNB., produced excellent growth; slightly inferior growth in *Quercus acuta* THUNB., while extremely poorer growth in the heartwood of *Castanea crenata* SIEB. et ZUCC.

The growth of the fungus in the sawdusts of *Quercus serrata* THUNB. and *Quercus acuta* THUNB. of Fagaceae were even more inferior than those found in Lauraceae, *(Actinodaphne loncifolia* MEISSN, *Cinnamomum japonicum* SIEB, *Machilus Thunbergii* S. et Z.*)*; Eupteleaceae, *(Euptela polyandra* SIDB. et ZUCC.*)*; Simarubaceae, *(Picrasma quassioides* BENN.*)*; Aceraceae, *(Acer palmatum* THUNB., *Acer pictum* THUNB. var. *Paxii* SCHW. subvar. *eupictum* PAX., *Acer rufinerve* SIEB. et ZUCC.*)*; Cornaceae, *(Aucuba japonica* THUNB., *Cornus controversa* HEMSL.*)*; and Ebenaceae, *(Diospyros kaki* THUNB. var. *silvestris* MAKINO*)* and approaching that found in Leguminaceae *(Albizzia Julibrissin* DURAZZ var. *speciosa* KOIZ, *Cladastis platycarpa* MAKINO*)*; Styracaceae, *(Styrax japonica* SIEB. et ZUCC., *Pterostyrax corymbosum* SIEB. et ZUCC.*)*.

The present study revealed that for Siitake mushroom production, logs should not be restricted to the several members of Fagaceae, Betulaceae nor Juglandaceae as traditionally thought, but they should include also some members of Lauraceae, Aceraceae and Cornaceae as well, since they make comparable or evens uperior growth than the former members.

KITAJIMA, (Shokubutu oyobi Dobutu, 8 : 1 : 210 – 214, 1940) says: "Siitake fungus develops its fruiting bodies after absorbing their nurishments from the substratum — consisting principally of cellulose and lignin contained in woody cell walls; hence, Siitake sporophores will develop on any deciduous tree provided the substratum does not contain poisonous materials to the fungus. The writer, in fact, has obtained abundant production of Siitake mushrooms on *Acer pictum* THUNB. var. *Paxii* SCHW. subvar. *eupictum* PAX. In times of shortage of standard materials, various miscellaneous woods occurring naturally in abundance in all

parts of Japan Proper should be substituted, and this will become an important factor in the development of our natural resource." Our results agree with his statement, for we also obtained good growths on several members of Aceraceae.

The mycelial growth was especially poor on only *Zelkowa serrata* MAKINO, *Prunus serrulata* LINDL. var. *spontanea* MAKINO, *Meliosma myriantha* SIEB. et ZUCC, and they should be avoided for the sake of safety. On the log of *Prunus Yedoensis* MATUMURA, (10 - 15 cm.) the authors, however, obtained Siitake mushroom — inoculated on spring, 1936 and produced fruiting bodies on autumn, 1938. In this case, there seemed to be a difference of growth in the heartwood and the sapwood. On *Prunus serrulata* LINDL. var. *spontanea* MAKINO, *Polystictus versicolor* usually invaded the log and caused much spoilage. A further report on the relation between the kind of wood and Siitake mushroom production shall be presented later under a separate title.

VIII. Summary.

1. The present study reports on the relation of kind of sawdust and the supplementary nutrients to the growth of the Siitake mycelium.

2. Sawdusts used in the present experiment were prepared from various trees grown in Hirosima, Nitihara and Tuyama Forestry Stations. They included a total of 24 families and 41 species : Pinaceae (4 spp.), Juglandaceae, Betulaceae (3 spp.), Fagaceae (4 spp.), Ulmaceae, Eupteleaceae, Cercidiphyllaceae, Magnoliaceae, Lauraceae (3 spp.), Rosaceae, Leguminaceae (2 spp.), Rutaceae, Simarubaceae, Aceraceae (3 spp.), Hippocastanaceae, Sabiaceae, Theaceae, Flacourtiaceae, Araliaceae (3 spp.), Cornaceae (2 spp.), Cethraceae, Ericaceae, Ebenaceae and Styracaceae (2 spp.).

3. Studies on the relation of kind of sawdust to the growth of the Siitake mycelium was conducted by (1) using only pure sawdust as the culturing medium — this experiment was divided into 2 parts, one using the large mouth glass bottles of one pound capacity, and the other using ordinary test tubes 18 mm. in diameter ; and (2) using sawdust decoction agar medium made by cooking one part of sawdust in 10 parts of water.

4. There was a slight discrepancy among the three experiments conducted above, but as a whole, Siitake mycelium made very favorable growth in the order of *Carpinus carpinoides* MAKINO, *Petrophiloides strobilacea* REID et CHANDLER, *Cornus controversa* HEMSL. followed by *Fagus crenata* BLUME. All of these are commonly used as logs for Siitake mushroom culture. Comparatively, good growths were obtained on *Tsuga Sieboldii* CARR., *Aesculus turbinata* BLUME, *Cercidiphyllum japonicum* SIEB. et ZUCC., *Cornus controversa* HEMSL., *Cinnamomum japonicum* SIEB. *Machilus Thunbergii* S. et Z. and *Euptela polyandra* SIEB. et ZUCC.

5. Mycelial growth was poorest on *Zelkowa serrata* MAKINO, *Prunus serrulata* LINDL. var. *spontanea* MAKINO and was followed by *Phellodendron amurense* RUPR., *Acanthopanax sciadophylloides* FR. et SAV., *Kalopanax ricinifolium* MIQ. var. *typicum*

NAKAI, *Aralia elata* SEEM., *Magnolia obovata* THUNB., and *Meliosma myriantha* SIEB. et ZUCC.

6. The sapwood of *Castanea crenata* SIEB. et ZUCC. was extremely suitable for mycelial growth, but on the heatwood the opposite was true.

7. Although *Quercus serrata* THUNB. and *Quercus acuta* THUNB. are used extensively in Siitake mushroom culture, logs need not be limited to the family of Fagaceae. Some members of the families of Lauraceae, Aceraceae and Cornaceae were found to be distinctly suitable. Their growth was not below that of *Carpinus* spp. *Quercus* spp. nor *Peterophiloides* spp.

8. The growth of the fungus mycelium was rapid on sawdusts that had a particle diameter of 1.5 – 3.0 mm., but was slower when the particles were smaller. Although the moisture content varied somewhat with the particle size and the kind of wood from which sawdust was made, the most favorable sawdust medium was prepared by using 2 parts of sawdust to 1 part of water.

9. The addition of rice- or wheat-bran and sugar to the sawdust distinctly improved the growth of the fungus. A further addition of a small amount of potato starch proved the medium most suitable.