STUDIES ON PHYTOPHTHORA DISEASE OF ECONOMIC PLANTS, 1*

Phytophthora Disease of Pumpkin and Squash

By

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1 INTRODUCTION

In the later part of June, 1946, it came to the attention of the writer that a certain disease had been causing considerable damage to the cultivated squash fruits (*Cucurbita moschata* DUCH) on the farm of Shimogamo, Kyoto. A preliminary examination revealed the presence of a species of Phytophthora mixed with a species of Fusarium on every specimens gathered. However it could not make clear which of them was the primary infection agent, in those days. Then on June 13, of the following year, a member of our laboratory brought to me a diseased stem and fruit of pumpkin (*Cucurbita maxima* DUCH.) cultivated at a private house nea the Ginkakuji-temple in Kyoto. Under close investigation, it was found to be a species of Phytophthora observed in the previous year with regard to its shape.

This disease has caused considerable damage to pumpkin and squash fruits and the infected area were extended over the neighborhood of Kyoto. And also the squash fruits (*C. moschata*), displayed at the central market of Kyoto city from Tottori prefecture, were found to be deteriorated in large number under the effect of the same fungus.

On the disease of pumpkin and squash fruit caused by the species of Phytophthora, TANAKA (9) was the first to report its occurrence in Shizuoka prefecture, and named it in Japanease as a disease because it was new to Japan.

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His record involved the morphological study on the causal fungus favoring infection and it is *Phytophthora citrophthora* (R. et SMITH) LEONIAN or a similar species. In April of the next year, KAWAI (2) reported that the identical disease occurred in Yamagata and Shizuoka districts, and the factors favoring infection, *Phytophthora capsici* LEONIAN, are very much alike. But formerly, NAKATA (6) eported that *Phytophthora Melongenae* was the factors favoring infection to pumpkin. Positive results were obtained by TOMPKINS and TUKER (12) who inoculated on *Cucurbita maxima* DUCH. and *C. pepo* L. *var. condensa* BELL. with *P. capsici* found on honeydew melon.

Among the investigation reported those of TANAKA (9) and KAWAI (2) seem to be of the same nature with the writer's study which incidentally was done just at the same time. This paper deals with an investigation on the disease, with special reference to cause, factors favoring infection, and host range.

Before going further the writer wishes to express his sincere thanks to Dr. T. HEMMI, emeritus professor of Kyoto Univ., Dr. T. ABE, professor of Saikyo Univ., Dr. S. AKAI, professor of Kyoto Univ. and professor H. ASUYAMA of Tokyo Univ., for affording him various facilities such as access to their libraries and precious guidance. Also thanks are due to the members of our laboratory for valuable technical assistances.

2 SYMPTOMS OF THE PRESENT DISEASE

In the neighborhood of Kyoto city, the present disease appears early in June and continues until the end of cultivation on leaves, stems and fruits of pumpkin and squash. The damage is most severe from the later part of June to the end of July. A characteristic of this disease is that sometimes fruits do not reveal further evidence of the disease but after harvest when they are likely rot off on thir storing, transporting and even on the shops. Consequently it could be regarded as the market disease.

Symptoms on fruit

Although the coloring of lesion somewhat differs from the kind of pumpkins and squashes and on the degree of ripening, at first it appears as a little damp,

sunken circular spot about 1 cm in diameter, and then rapidly spreads to form the extent of a large water-soaked, dull green, slightly concave circular area. The water-soaked lesions are covered with a dense, velvety, grayish-white, frosty-like, nonzonate mycelium containing sporangia. Wrinkle arise with the progress of disease (Plate I., Fig. 1). Numerous fleecy aerial mycelia occur under a high degree of humidity. In a immature fruit of *C. maxima* light green in color, the rim of the lesion exhibited a reddish- or purplish-brown. The border between the affected part and the healthy one is generally distinct with some exception. The affected part gradually rots off and issues nasty smell. On inspecting the transversal section of the lesion, it was found that while spreading on the surface of the fruit, the disease penetrates deeper and deeper until it reaches the seeds (Plate I., Fig. 2). In many cases the disease begins from where the fruit touches the soil on the field. Ultimately different lesions may fuse and the affected fruits rot off within a few days.

Symptoms on leaf

With regard to the leaf, in general, the disease appears on the edge of the leaf, and the lesions are water-soaked, dull green and have a circular or irregular shape. With dampness the disease codition increases very rapidly and slight gray aerial mycelium (including sporangia) arise on the surface and the back of lesions. In case of dryness the affected leaf turns dark green then brown, and finally withers and becomes brittle. A rather clear distinction exists between the lesion and the healthy part of the leaf.

Symptoms on stem

The incipient indication of the disease appears on the stem as a water-soaked spot. It girdles them rapidly, thus causing the wilting and withering of the upper part of the affected shoot. The white sorus of the causal fungus arise slightly on the surface of the affected part. The disease begins from the parts of the stem where it touches the ground, but mostly it attacks the stem between the top of the root and the 5th or 6th node and also at the foot of branches. In many cases, when this disease is in progress, a species of Fusarium and bacteria secondarily grow forth.

3 ISOLATION OF THE CAUSAL FUNGUS

As has been stated, a secondary species of Fusarium and Lecteria would make their appearance on the lesion, consequently it was quite difficult to isolate the causal fungus. On August 10, 1948, the writer collects the diseased pumpkin fruits (*C. maxima*) in incipient stage, cultivated on the farm in Kamigamo, Kyoto, and cut away the tissue into small blocks near the boundary of the diseased and healthy part of the fruit and sterilize the surface of each tissue by soaking them in 80% alcohol for 30 to 60 seconds and then washing them thoroughly with sterilized water, and finally transfered into culture medium of pumpkin extract agar. The writer at last has succeeded in isolating this causal fungus from the mycelium beginning to grow up thereafter for the first time. This isolation experiment was done from 25 little pieces of diseased fruit tissue and it was recognized to be the same fungus throughout the investigation.

Accordingly, the fungus was called the causal fungus No. I of pumpkin and squash in this study. On June 11, 1949, the writer again succeeded in isolating another causal fungus from diseased stems of young plant collected at Kibune, Kyoto, and named it causal fungus No. 2. However, it seemed to be the same with the above-mentioned fungus No. 1. As a consequence, the experiment was carried on with the fungus No. 1. except in some cases where for special comparative studies, fungus No. 2, was used.

4 MORPHOLOGICAL STUDIES OF THE CAUSAL FUNGUS

Mycelium

The mycelium was found to be well distributed throughout diseased areas but was confined to the intercellular space. There was no indication of intracellular invasion by the haustrium. The gnarled mycelium of this fungus is often very distinctive in diseased tissue and becomes densly tuberous under certain cultural conditions, branching off actively. LEONIAN (4) often recognized the egg-formed called "tuberous outgrowth" in his studies on *P. capsici*. This was also observed by the writer on old medium of potato and apricot hard agar

but was not as remarkable. Young mycelium has plenty of granules but lack the septum. The width of mycelium is between 3μ and 10μ, with an average of 6.03μ. No chlamydospore was observed.

Sporangium

Sporangiophore is single or branched and put a sporangium apically. It seems that sporangiophore varies in size under different temperatures and degrees of humidity. It moreover tends to reach a remarkable length in cases where the degree of humidity is high, generally its length is about 54-380μ, its width, 3.6 -10μ. It is rich in granule but lack in septum.

Sporangia are hyaline and ovoid or oval with a remarkable apical papilla. The basal part of sporangium shows wedge-shaped or round and frequently is connected with the sporangiophore by a short handle (Plate II, A). An investigation on the form of the basal part reveals that there were 176 wedge-shaped, 54 round and 20 with short handle. In many cases the top part of the sporangiophore is rather expanded and is connected with the wedge-shaped base of the sporangium. This seems to be a peculiarity of this fungus as it could be found but rarely in other species of Phytophthora. The size of sporangium is as varied as seen in other similar fungus and after the measurement upon 1321 individuals, the area of size variation is 20.48-106.00×13.25-53.00μ, with a mode of 43.05×23.85μ; the average being 48.04×26.96μ; the papilla 2.64-9.80×4.77-6.63μ, the mode 5.30×5.30μ and 6.20×5.43μ in average. Accordingly, we can, from the data obtained, surmise that the papilla is slim in form. Figure I shows the size variation of sporangium.

The sporangia, in water, germinate with the zoospore, or germ-tube. In a case of germination by zoospores, the contents of sporangium begin to undergo division and show somewhat larger granulous condition. The zoospoes are formed within 30 minutes to 5 hours and escape through the ruptured apical plug, they at once more actively. The zoospores are reniform with two long cillia attached near the middle or the upper part of their concave side. The head part of the zoospore is somewhat small. It seems that by moving one of the cilia bends forward while the other, backward. The zoospores have an

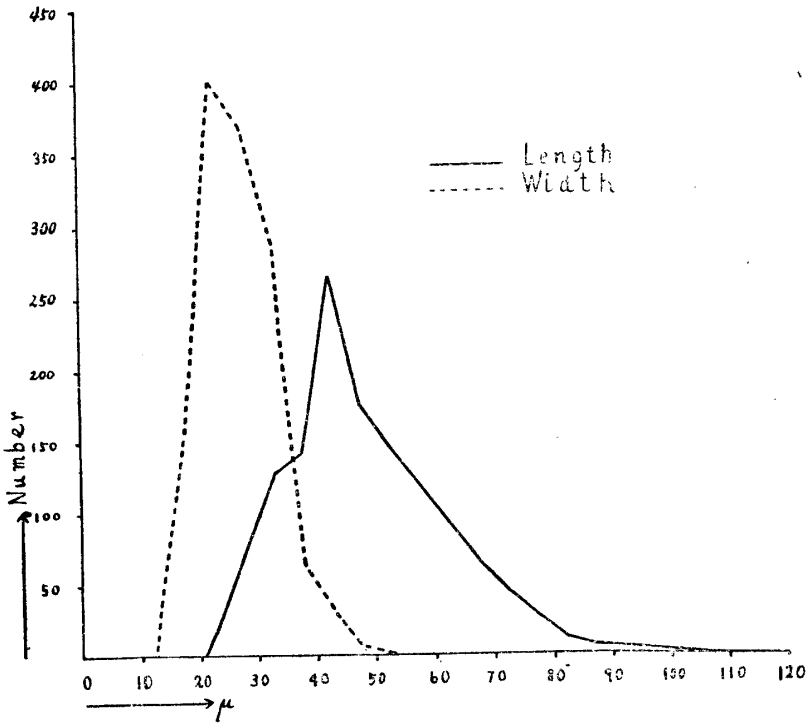

Fig. 1.—Graph showing the variation in length and width of zoosporangia.

approximate size of 12.4×9.7μ and after a short time of active swimming, they then lose their cilia and come to rest, assuming a spherical shape, the so-called cystospore (diam. 11.19-13.25 μ). This cystospores produce the germtubes. In some cases, the sporangia either produce germtubes directly through the apex, or laterally ; occasionally the germ-tubes produce secondary new apical sporangium.

Sexual organ

This causal fungus forms the oogonium and antheridium as sexual organ. In the later part of autumn where the atomospheric temperature is below 15°C, 45 days after inoculation, oogonia and antheridia are formed on submerged mycelium as terminal swellings of branches from the same hyphae in the diseased tissue. The same phenomenon also takes place on corn-meal agar and kidney-bean pod medium as well.

Light yellow or colorless, the oogonia are spherical in form and are covered with thin membrane. Results obtained from the measurement of 1014 individuals reveal that the diameter of the oogonia fluctuates between 19.98-44.00μ, with a mode of 30.95μ; the average being 31.70μ. With regard to the thickness of the membrane it was found to be 0.6-1.3μ with an average of 0.89μ.

After fertilization, a single oospore is formed in each oogonium. Many oogonia are somewhat ovoid in shape but some of them are spherical, light yellow colored and have thick membrane.

Concerning the cospores, data obtained from 1014 measurements show that the diameter ranges from 19.98-29.15×13.25-23.85μ, with a mode of 17.95×22. 95μ and the average being 19.2 ×23.78μ. The antheridia are hyaline, ellipsoidal or bowl-shape and seem to have an amphigynous relation to each oogonium. Accoding to the classification of ROSENBAUM (7), it falls into the Phaseoli group because of its basal antheridium. The antheridia measure in diameter 7.90-22.53×7.90-26.50μ the mode 14.95×13.25μ and 14.74×13.35μ in average.

5 PHYSIOLOGICAL STUDIES OF THE CAUSAL FUNGUS

(1) The growth of mycelium on culture media

Comparative studies on the growth of mycelium on different culture media were made by transferring pieces of colony previously cultured on potato agar medium at 28°C to Petri dishes each containing 10 cc of culture media of the following descriptions:

a.	Corn-meal agar	corn-meal 50gr., agar 17gr., distilled water 1000cc.
b.	Potato extract agar	potato 200gr., sugar 20gr., agar 17gr., distilled water 1000 cc.
c.	Apricot extract agar	apricot 25gr., agar 35gr., distilled water 1000 cc.
d.	Kidney-bean pod extract agar	bean-pod (Dolichos Lablab) 200gr., sugar 20gr., agar 17gr., distilled water 1000 cc.
e.	Pumpkin fruit extract agar	pumpkin fruit (C. maxima) 200gr., sugar 20gr., agar 17gr., distilled water 1000 cc.

The culture were kept at 28°C in incubator and observations were made at the third and fifth day after incubation. Tabulated data on the growth of mycelium on different culture media are given in Table 1.

From Table 1 it can be seen that, next to corn-meal agar, mycelium grows best on pumpkin extract agar. On the other hand, in general, the growth of the colony is dense and cottony on potato agar. The fungus spreads over the medium rapidly in fan-like fashion to produce the divergent type of growth.

Table 1.—The growth of the fungus on culture media

Culture media	Average diameter of colony (in cm)		Aerial mycelium	Divergent type of colony
	3 days	5 days		
Corn-meal	4.37	7.47	++	++
Potato	3.43	5.35	+++	+++
Apricot	3.06	5.28	+	−
Kidney-bean pod	3.01	5.35	+	−
Pumpkin	4.08	7.25	++	+++

The so-called "divergent type" of growth pointed out by TOMPKINS and TUCKER (11), appears remakably on potato and pumpkin agar media but less on corn-meal media. The colony is fairly tough especially when old and cannot be easily cut off with a needle. Aerial mycelium gradually adheres to somewhat old, hard medium and gives it the appearance of leather.

(2) Effect f temperature on the growth of the fungus

Observation were made on the growth of isolates on agar plates. In this experiment small pieces of colony, about 2mm in diameter, taken from fungus cultured on kidney-bean extract and maintained at 26°C for one week were transplanted to pumpkin and barley-corn meal agar plates in Petri dishes. At the following temperatures namely, room-temperature, 16°, 20°. 22°, 24°, 26°, 28°, 30°, 32°, 36° and 40°C, 5 dishes each were incubated for a period of 5 days. The same experiment was repeated three times. The prescriptions of pumpkin fruit and barley-corn meal extract agar medium were as the follows.

Pumpkin extract agar	The same as in p.57
Barley-corn meal agar	barley-corn meal 25gr., agar 17gr., distilled water 1000 cc.

Results obtained from the measurements of the colony incubated at different maintained temperatures are given in Table 2.

Table 2.—Effect of temperature upon the growth of the fungus

Temp. °C	Diameter of colony in mm							
	On pumpkin extract agar				On barley-corn meal agar			
	I	II	III	Average	I	II	III	Average
R.T. * (9°-12°C)	19	20	20	19.7	16	17	13	15.3
16	32	33	33	32.7	34	34	36	34.7
20	46	47	46	46.3	47	45	47	46.3
22	49	52	50	50.3	52	51	52	51.7
24	52	54	51	52.3	55	56	59	56.7
26	62	65	63	63.3	68	68	68	68.0
28	69	72	66	69.0	82	82	81	81.7
30	77	77	76	76.7	90	86	85	87.0
32	72	74	74	73.3	73	74	74	73.7
36	20	21	20	20.3	15	15	12	14.0
40	—	—	—	—	—	—	—	—

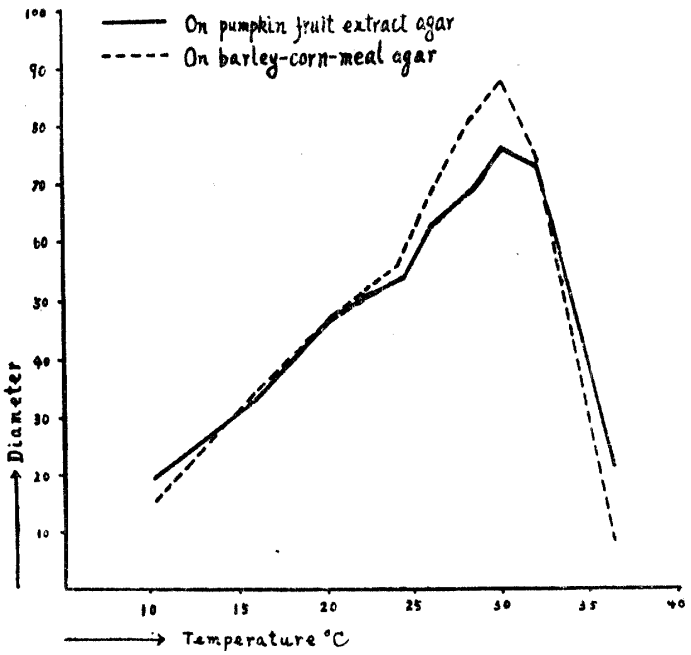

Fig. 2.—Effect of temperature upon the growth of the fungus

* R. T. : means room temperature ; maximum being 12°C and minimum, 9°C.

Depicted in Figure 2 are graphs representing the data given in Table 2.

Results obtained show that the present fungus can grow at temperatures ranging from 10° to 36°C, the optimum temperature being probably slightly under 30°C. Though temperatures below 10°C were

not attempted it could be surmised that the minimum growth temperature would be somewhat lower than 10°C. As regard to maximum temperature for growth, it might be a little over 36°C (Table 2 and Fig. 2), as no sign of the growth is observed at 40°C. In the genus Phytophthora, for instance, *P. capsici*, *P. drechsleri*, *P. meadii*, *P. palmivora* and *P. parasitica* etc. are these which prefer comparatively high temperature, as for the present investigating fungus it seems to resemble the above-mentioned group of fungi with regard to the optimum temperature. Sporangium was found only at 32°C and 5 days after incubation on both media.

(3) Effect of H-ion concentations of culture media on the growth of the fungus

To determine the effect of H-ion concentrations of culture media on the growth of the fungus, hard and liquid media with various H-ion concentrations (Table 3 and 4) containing in Petri dish and flask respectively were used. Liquid medium consisted of potato 200gr and sugar 20gr; in the case of hard medium 17gr of agar were addes. Kept in incubator under a constant temperature of 25°C, and observations were made at the 6th day after incubation. For hard media measurements were carried out on the diameter of the grown colony; in the case of liquid media, the dry weight of the grown colony collected on filter paper were weighted. This experiment was repeated 5 times with 7 cultures at each H-ion concentration. The results obtained are given in Tables 3 and 4.

Table 3.—Effect of pH of potato agar media upon the growth of fungus

pH of media	Diameter of colony after 6 days (in mm)						Zoosporangium formation
	I	II	III	IV	V	Average	
2.8	0	0	0	0	0	0	−
3.4	34.5	37.0	38.4	41.2	31.1	36.4	−
4.7	67.0	60.6	52.2	57.8	61.9	59.9	+
5.3	79 0	65.0	61.5	65.4	69 0	67.9	++

5.8	70.0	68.0	66.2	67.8	71.4	68.7	+++
6.4	80.0	70.0	67.1	69 0	74.8	72.2	+++
7.0	60.0	73.2	63.8	66.4	79.0	68.5	+++
7.7	55.0	72.8	64.6	65.6	80.2	67.7	+++
8.4	57.0	66.2	69.0	59.4	66.2	59.6	+++
9.4	60.0	68.0	47.8	60.0	72.4	61.6	++
11.1	50.0	26.0	60.4	55.9	62.9	51.0	+
12.0	0	0	0	0	0	0	−

Table 4.—Effect of pH of potato decoction media upon the growth of the fungus

pH of media	Dry weight of colony after 7 days (in mg)						pH media after experiment
	I	II	III	IV	V	Average	
2.8	30.2	30.4	28.8	32.2	31.6	30.4	2.3
3.4	36.3	33.3	34.8	51.9	106.9	52.6	3.6
4.7	183.2	205.7	136.5	189.9	190.0	181.6	5.6
5.3	204.2	327.3	141.2	193.0	188.4	210.8	5.6
5.8	223.8	350.5	161.5	216.0	185.0	227.4	5.9
6.4	196.2	349.1	186.2	195.0	238.6	233.0	5.7
7.0	184.9	310.4	161.1	178.0	214.3	209.9	5.6
7.7	192.1	268.1	128.6	158.4	207.2	190.8	5.8
8.4	131.6	87.7	107.8	147.8	162.9	127.6	5.8
9.4	59.7	67.5	71.2	71.2	126.8	79.3	6.2
11.1	64.2	50.0	32.9	40.6	62.2	49.9	8.6
12.0	31.5	31.0	30.5	31.5	30.2	30.9	9.2

Remarks : Dry weights of colony shown in Table 4 include weight of the piece of colony transferred to liquid medium at the beginning of experiment.

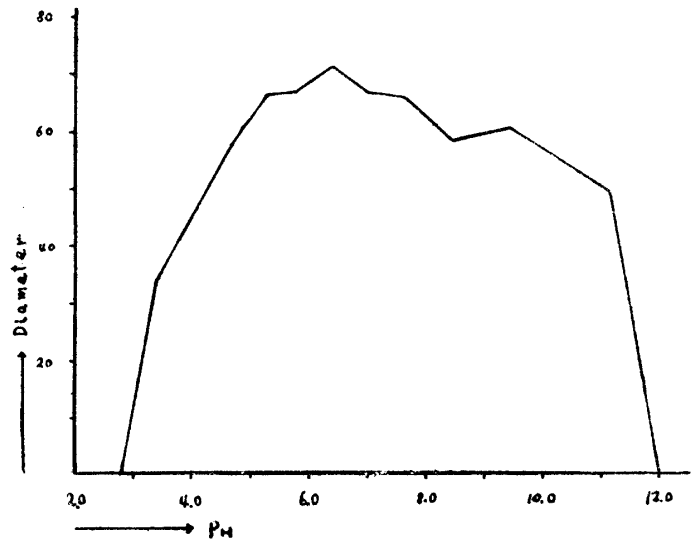

Fig. 3.—Effect of pH of potato agar media upon the growth of fungus

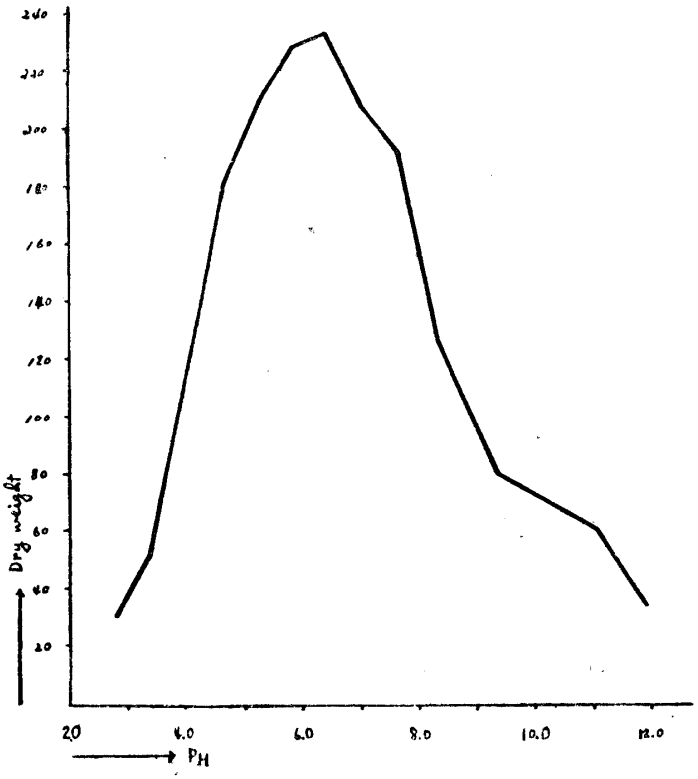

Fig. 4.—Effect of pH of potato decoction media upon the growth of the fungus

Figure 3 and 4 show graphs drawn from data given in Table 3 and 4 respectively.

According to Tables 3, 4 and Figs. 3, 4, it is clear that the fungus is able to grow up on the potato medium with pH between 3.4–11.1. The growth of the fungus is most vigorous at pH 5.3 to 7.0. From these experiments, it might be assumed that media having pH 6.4 is most favorable for the growth of this fungus. However, no growth was observed in medium with pH 2.8 and 12.0. It has to be pointed out here that the dry weight of the colony in Table 4 (consequently Fig. 4) includes the weight of the transplanted colony piece which were put in each liquid medium at the beginning of this experiment. The formation of sporangia can be recog-

nized at the fifth day of incubation. Sporangia are noticed most on media with pH between 5.8 and 8.4. With pH under 5.3 and above 9.4 only a few are formed ; whereas they were entirely absent at 3.4 and 12.0. It seems that aerial mycelium tend to grow densely in the acid side, but as a rule not so dense in the basic side.

6 INOCULATION EXPERIMENTS

(1) On pumpkin and squash

A pure culture (included sporangia) of the causal fungus was used in all infection experiments. The fungus were grown on kidney-bean extract agar in Petri dishes, incubated at room temperature, and used for inoculum when 10 days old.

a) Leaves

Inoculation experiments were carried out on both young and old leaves, the former from young seedlings having about 3 leaves, grown in pots with autclaved soil, the latter from healthy plants selected from fields. Old leaves were washed in running tap water and carefully rinsed in distilled water. Both young and old leaves were put in flask and a small piece of inoculum was placed on the injured and uninjured epidermis of the leaves. The whole thing was kept in the wetting room for 24 hours (temperature : max. 24.5°, mim. 20.5°C). Observations were made every day on the desk.

Following inoculation, the disease appeared on both young and old leaves. In injured leaves the water-soaked, dull green lesion of about 1 cm in diameter was noticed after two days of inoculation on young leaves, and three days on old ones. The disease was more severe in young than old leaves.

In general the lesion is circular at the incipient stage and becomes irregular or rhombic-like in the later stage. In old leaves, however, the border of the lesion is usually yellowish making out a halo, and the lesion begins to change color into brown in a few days after the appearance of the disease. Then the size of the lesion increases rapidly and at last affected leaves de-

cay after about 7 days of inoculation. No sign of disease was observed on controls where leaves were handled in the same manner except that sterile agar was substituted for the inoculum.

Showing the difference of the progressive lesion in comparison with the young and the old leaves of *C. maxima*, as this following Table 5.

Table 5.—Difference of the progressive lesion in comparison with the young and the old leaf of *C. maxima*

Lesion on	Average diameter of the lesions (in cm)					
	1 day	2 days	3 days	4 days	5 days	6 days
young leaf	–	1.5–1.5	2.0–1.5	3.5–3.0	4.0–3.5	6.0–4.0
old leaf	–	±	1.5–1.0	2.0–1.2	2.3–1.3	3.0–1.5

As the additional remarks, the leaf injured with carbo-rumdum met with more bad patient than it injured with knife.

b) Stems

Inoculation tests were made upon stem of seedlings and also somewhat old cutting stems. For inoculation tests, inoculum crushed in sterilized mortar and mixed with sterilized water was sprayed to the stems of seedlings or cuttings. Stems of seedlings and cuttings from older plants were prepared in the same manner as in the case of inoculation on leaves. Kept in wetting room (20° to 2 .5°C) observations were made every day.

In the case of seedling stems, the infection appeared after two days of inoculation. After 3 days, all the 33 individua's tested were observed to be infected and then wilted. The incipient sign of disease in the seedling stem is a small, water-soaked, dull green, sunken spot which girdles the stem and without exception, resulted in the so-called "damping off". Results obtained seems to indicate that the present fungus has an agressive pathogenicity for the seedlings of pumpkin and squash.

With regard to old stems, healthy cutting of about 40 cm long with two or three leaves, selected from a field where the disease was absent, were washed in running tap water, rinsed in distilled water, and put in flask. Inoculum prepared in the same manner as in the previous case was placed on the injured epidermis of the regulated stems in 3 places, at intervals of 10 cm from the top part.

Symptoms of the disease could be noticed within two days after inoculation. The lesion was almost rhombic, sunken and water-soaked dull green spot, in appearance, and though every place inoculated was affected, the development of the lesion was not rapid. As the affected stem became worse, one or two cracks in the lesion could be seen. In appearance, it was somewhat similar to that caused by a fungus of *Fusarium niveum*. Anyhow, it seems that the present fungus has also a pathogenicity to the old stem. Neverthless, the pathogenicity of the present fungus seems to be more severe on the stem of seedlings than on old cuttings.

c) Fruits

In this experiment, healthy immature and mature pumpkin fruit (*C. maxima*) serected from a field where the disease was absent, were washed in running tap water, rinsed in distilled water, and dried. Small pieces of inoculum were placed on the injured and uninjured epidermis of 5 immature and 5 mature fruits and all of them were kept in the wetting room (27.5° to 30°C). For controls, fruits were handled in the same manner except that sterile agar was substituted for the inoculum. The fungus (included sporangia) was grown previously on corn-meal agar in Petri dishes, incubated at room temperature. In immature pumpkin fruit inoculated, injured and uninjured parts were infected within 24 hours and 2 days respectively. Incipient indication of the disease was a water-soaked, slightly sunken spot averaging 1 cm in diameter. These artificially induced lesions were identical in colour and consistency with those resulting from natural infection. The non-inoculated controls continued to be healthy.

As regards to mature fruits, infection occurred without exception on injured parts. Inoculation made on uninjured epidermis resulted in the infection of 7

individuals among the 15 tested, as the experiment was repeated 3 times. According to the special inoculation by sporangial suspension from corn-meal agar media, no mature fruits were infected in the case of uninjured ; however, immature fruits were infected without exception even if the fruits were uninjured.

After these experiments, it can be said that though the present causal fungus is an agressive parasite in the case of immature fruits, mature fruits seem to be somewhat more resistant owing probably to layer of wax developed on their epidermis. As a matter of fact, the disease found in mature fruits had been probably infected through wounds caused by the carelessness in their handlings or by insects and soforth.

Table 6 shows the average extension of lesion occasioned by inoculation on injured epidermis of pumpkin fruits (*C. moschata* and *G. maxima*).

Table 6.—Diameter of the progressive lesion on the fruit of *C. maxima* and *C. moschata*

Fruit	Average diameter of the lesion (in cm)						
	1 day	2 days	3 days	4 days	5 days	6 days	7 days
C. maxima	–	1.5–1.5	4–4	6–6	9—11	entire surface	spoiled
C. moschata	–	0.5–0.5	2–2	5–5.5	9.5–11	entire surface	spoiled

According to Table 6, the *C. maxima* fruit shows a more remarkable lesion in the incipient stage of the disease and progresses quite rapidly but reaches approximately the same size as that in *C. moschata* in the 5th day.

(2) On various other pla ts

Grown on potato extract agar in Petri dish, incubated at room temperature, the fungus was used for inoculum when 7 days old. Only fruits and stems were artificially injured before inoculation tests. A small piece of inoculum was placed on the wounded point of fruits and stems and on the intact epidermis of leaves. The inoculated plants was kept in wetting room for 24 hours then transfered to room temperature.

a) Pepper (*Capsicum annum* L.)

The results of inoculation were uniformly positive. Under the climatic conditions of Kyoto in August the first visible lesions on the leaf and fruit appear within 24 hours and also on the woody stem within about 5 days after inoculation. Most of the pod becomes infected within a period of 3 days after inoculation. After 5 days no sound tissue remains on the pod which is at first soft and pulpy. It gradually dries off, shrivels, and finally becomes parchment-like. Meanwhile, if the drying does not proceed rapidly, the sporangial masses are borne on the surface of the pods. The lesion on the leaf is at first water-soaked and dull green but gradually dries and turns into brown. The lesion on the stem is at first water-soaked and sunken but soon becomes brown.

b) Egg-plant (*Solanum Melongena* L. *var. esculentum* NEES.)

24 hours after inoculation, the lesion appears as a small, circular, somewhat sunken and brown spot, which rapidly increases in size to form a large brown area. Meanwhile, the sporangial masses are borne frosty on the surface of the lesion. The infected fruit rots off at last, sometimes looks like mummy if it were left in dry weather.

c) Tomato (*Lycopersicon esculentum* MILL.)

The incubation period on the stem and leaf is about 2 days. The lesion on the leaf is at first water-soaked dull green but gradually dries and changes in brown. The water-soaked lesion on the fruit appears within 24 hours after inoculation and forms rough aerial mycelium and bears sporangia on surface of the lesion only after 6 days after inoculation. The infected fruit rots off softly soon after.

d) Cucumber (*Cucumis sativus* L.)

Fruit, shows a remarkable sunken, circular, and water-soaked lesion about 1 cm in diameter after about 30 hours after inoculation, and repidly increases in size to form a large sunken area. Meanwhile, the sporangial masses are borne frosty on the surface of the lesion after 3 days but almost no aerial mycelium can be recognized in dry weather and the infected fruit rots off rapidly.

e) Water melon (*Citrullus vulgaris* SCHRAD.)

Inoculated fruit shows the same symptoms as in cucumber.

f) Makuwa-melon (*Cucumis Melo* L. *var. Makuwa* MAKINO)

Inoculating on stem of seedlings occasions the so-called damping off within 2 days after inoculation. The infected fruit has the same symptoms as in cucumber or water melon.

g) Kidney-bean (*Dolicos Lablab* L.)

Lesion with indistinct border of water-soaked, dull green appears within 2 days and generally spreads out over the surface of the fruit. Meanwhile, sporangial and mycelial masses are borne numerously on its surface. The colour of the lesion is generally brownish. The inoculated fruit rots off gradually but takes the appearance of being dried by dry weather.

h) Citron (*Citrus Natsudaidai* HAYAMA)

No immature fruits were infected in any of the inoculation tests.

i) Orange (*Citrus deliciosa* TENORE.)

Twenty eight mature orange were inoculated, and within 5 days one fruit were infected. The lesion on the infected fruit is at first water-soaked, dull brown, and irregular. No sporaugia appear on the lesion in the present tests. The present fungus seems to have no aggressive nature on artificially injured orange.

j) Fig (*Ficus Carica* L.)

Inoculated fruit shows at first an indistinct bordered, water-soaked lesion after 2 pays. The lesion spreads out rapidly over the surface of the fruit, and meanwhile numerous, frosty-like sporangial masses cover it entirely. At last, the infected fruit is dried off.

k) Persimmon (*Diospyros Kaki* THUNB.)

Inoculated fruit shows a water soaked, circular shape and dark colored lesion after 6 days. Though the progress of the disease is rather slow, it gradually rot off.

l) Potato (*Solanum tuberosum* L.)

Inoculated tuber shows a water-soaked, dull lesion after 24 hours but it is rather difficult to recognize its colour, though the lesion spreads and is circular in shape. Neither mycelium nor sporangium forms on the lesion.

As has been already stated, the present fungus is an aggressive parasite for the pumpkin and squash, yet it has somewhat less effect on uninjured mature fruits. Also it seems to have an aggressive nature on artificially injured red pepper, egg-plant, tomato, cucumber, water melon, makuwa-melon kidney-bean, fig, persimmon and potato. Nevertheless the present fungus have no infectional nature on immature citron and besides scarcely orange.

7 DISCUSSION

The writer carried out the experiments chiefly on the No. 1,—numbered by this laboratory—fungus of pumpkin and sqush. According to the results obtained, it reems that the present fungus is identical to *Phytophthora capsici* LEONIAN on pepper, a name proposed by L. H. LEONIAN (4) in 1922. On the disease of pepper, TAKIMOTO (8) for the first time reported in 1940 as the disease was new to Japan. However, the disea e of pepper causing by *P. capsici* has also been commonly observed in Kyoto, and the writer has the fungus isolated purely. So far as the writer's investigations are concerned the disease and its fungus on pepper, the description of which has been made by LEONIAN (4) and TAKIMOTO (8) agree with that observed by the writer in many respects. In the writer's opinion the causal fungus of Phytophthora disease on pepper mentioned-above is unquestionable *P. capsici*. Though the writer did not compare the fugus of pepper with that of pumpkin and squash in this paper, there is no doubt that both fungus are nevertheless the same species.

In connection with *Phytophthora capsici*, TOMPKINS and TUCKER (12) reported on honeydew melon, WIANT and TUCKER (14) on winter queen melon and KREUTZER and BRYANT (3) on tomato. Later, in 1931, TUCKER (13) made a detailed study on the classification of the Genus Phytophthora. The descriptions made on *P. capsici* in his report agree very well with that of the present fungus with regard to morphology, physiology and symptomatology. Judging from the results obtained by TOMPKINS and TUCKER (12) from inoculation test of *P. capsici* on pumpkin and squash, it could be assumed that similar results could be obtained with Cucurbita sp.

According to TANAKA (9), the fungus of pumpkin and squash was probably *P. citrophthora*, however results obtained by the writer show that this fungus has no aggressive nature, at least to immature citron and also to mature. Though similar to *P. citrophthora* in morphological aspects, the presence of its sexual organs and the difference in the point of pathogenicity, speaks well for the different nature of these two fungi. In spite of the fact that KAWAI (2) reported to the effect that the fungus of pumpkin and squash was *P. capsici*, the writer (1) do not think that KAWAI's fungus was *P. capsici*. KAWAI wrote that the antheridium of his fungus have a paragynous relation to the oogonium, but if the fungus is *P. capsici* that relation is certainly amphigyncus. As a consequence, accorhing to the classification of ROSENBAUM (7) it must belong to the Pheseoli group because of its amphigynous antheridium. As the fungus observed by the writer always shows amphigyny, then if KAWAI's fungus shows paragyny, it is clear that it could not be *P. capsici*.

Concerning the fungus observed by the writer, the mycelium grows out exhibiting the divergent type (TOMPKINS and TUCKER (12)) on extract agar media of both potato and pumpkin. The growing formation is pretty remarkable on the corn-meal agar medium. As regard to the divergent type of colony on agar medium, the writer also observed the same phenomenon on *P. capsici* which had been isolated from pepper and also on *P. parasitica* which had been isolated from cucumber and water melon. TASUGI and KUMAZAWA(10) reported that *P. paeoniae* also shows the star-like arowth of colony when 5% d-glucose is added to potato extract agar medium.

LEONIAN's (4) illustrate shows that the mycelium of *P. capsici* showed the clearly tuberous outgrowth on the potato extract agar medium. Though tuberous outgrowth was not remarkable with regard to the fungus observed by the writer, cases similar to those described by LEONIAN were often found.

It is said that in general the size variation of the sporangium is extensive. This also applies with the fungus studied in this paper. About the fungus of the writer, its average size is $48.04 \times 26.96 \mu$, and this size of the sporangium shows that the present fungus belongs to a largest one in Phytophthora species

hitherto reported. Concernig the size of sporangium formed under the same condition, and LEONIAN (5) showed that *P. capsici* and *P. citrophthora* belong to the group D, and the average length of sporangium is about 40–48μ, the largest after *P. maxicana*.

About the physiological character of fhe writer's fungus, the mycelium on medium growth very well at 30°C, a fact that matches very well with investigations hitherto reported. According to TOGASHI (11), the most suitable temperature of growth is about 30°C for *P. capsici*, *P. colocasiae*, *P. drechsleri*, *P. fagopyri*, *P. meadii*, *P. omnivora*, *P. palmivora* and *P. parasitica* etc. It would be quite impossible to make a comparison between these and that observed by the writer, yet, *P. colocasiae*, *P. fagopyri* and *P. parasitica* which are in the writer's possession are quite different from it in many aspects. Particularly, when the fungus of the writer is compared with *P. parasitica*, it is diffe ent in such point that the sporangium of writer's fungus is generally elliptical and the base part shows almost the wedge-shape and the papillating is rather slender.

Following inoculation test, it is clear that the fungus of the writer has an aggressive nature on many plants. From the results obtai ed the fungus observed in this paper can be regarded as *Phytophthora capsici* LE NIAN found on the first time on pepper by LEONIAN (4) in 1922.

8 SUMMARY

(1) Since 1946, a disease which attacks the fruits, stems and leaves of pumpkin and squash (*Cucurbita maxima* DUCH.and *C. moschata* DUCH.) in Kyoto city was found to be caused by a species of Phytophthora. And also the pumpkin fruits (*C. moschata*), displayed at the central market of Kyoto city from Tottori prefecture, were found to be deteriorated in large number under the effect of the same fungus.

(2) The disease generally appears any time after June and continues until the end of cultivation. From the later part of June to the end of July the disease appears to be most severe. The rot of fruits appears further in storage

season and in the market.

(3) The first indication of the disease on the fruit is a small circular, about 1 cm in diameter, water-soaked, dull green and sunken spot which rapidly increases in size to form a large water-soaked area. The water-soaked lesion is covered with a dense, frosty, grayish white, nonzonate, velvety mycelium which may contains sporangia.

(4) Sporangia are usually oval-shaped and hyaline with apical papilla. Each of them measures 20.48-106.00×13.25-53.00μ and 48.04×26.96μ in average. The germination of the sporangia is either direct with germ-tubes or indirect with zoospores. The oogonia and antheridia arise as terminal swellings of branches from the same hyphae. The antheridium seems to have an amphigynous relation to the oogonium. The oogonia mesure 19.98-44.00μ in diameter and 31.70μ in average; the antheridia measure 7.90-22.53μ in diameter and 14.74×13.35μ in average. After fertilization, a single oospore is formed in each oogonium. Oospores are globose and somewhat yellowish with thin walls, and measured 19.98-29.15×13.25-23.85μ in diameter and 19.28×23.78μ in average. No chlamydospore observed.

(5) The fungus grows rapidly on corn-meal, potato, apricot, kidney-bean pod and pumpkin—agar, and best on the corn-meal agar. Colony of the fungus shows plainly a divergent type in appearance on potato and pumpkin extract agar and somewhat on corn-meal agar. Colony is generally dense, leather-like and strong in old culture media.

(6) The growth of the fungus was observed at about 10°C to 36°C, and no sign of the growth at 40°C on either hard or liquid media. The optimum temperature for growth of the fungus is about 30°C, the minimum, somewhat below 10°C and the maximum about 36°C.

(7) At pH 5.3 to 7.7, the growth was most vigorous and sporangia were produced abundantly. At pH 3.4 and 11.1 the fungus formed colony but very slightly. From these experiments, it might be assumed that media having pH 5.3 to 7.0 are most favorable for growth; the optimum being pH 6.4.

(8) When healthy pumpkins and squashes are inoculated with the mycelium

or the sporangium obtained from pure culture, the infection takes place readily. But on uninjured mature fruits, sometimes no sign of infec ion was observed to take place. The inoculation were also made upon some other plant by the injured method. From these experiments, it seems that the fungus has an aggressive nature on the following plants:

Pepper (*Capsicum annum* L.)·····fruit, stem and leaf
Fgg-plant (*Solanum Melongenae* L.)·····fruit, stem and leaf
Tomato (*Lycopercicon esculentum* MILL.)·····fruit, stem and leaf
Cucumber (*Cucumis sativus* L.) ·····fruit
Water melon (*Citrullus vulgaris* SCHRAD.) ·····fruit
Makuwa-melon(*Cucumis Melo* L. *var. Makuwa* MAKINO) ·····fruit and young plant
Kidney-bean (*Dolichos Lablab* L.) ·····pod
Fig (*Ficus Carica* L.)·····fruit
Persimmon (*Diospyros Kaki* THUNB.) ·····fruit
Potato (*Solanum tuberosum* L.)·····tuber

No infection was observed on inoculated fruits of Citron (*Citrus Natsudaidai* HAYATA). Twenty eight mature orange (*Citrus deliciosa* TENORE) were inoculated and only one fruit were infected.

(9) The writer intends to apply the specific name *Phytophthora capsici* LEONIAN to the present fungus.

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Explanation of Plates

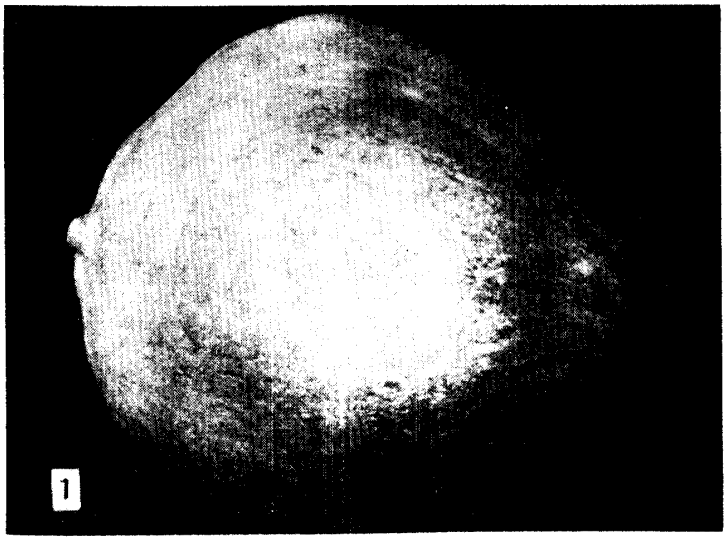
Plate I

1. A infected pumpkin fruit.
2. A cross section of the infected pumpkin fruit.
3. The growth of the mycelium on potato extract agar, showing the "Divergent type".
4. Photomicrograph of oogonium, antheridium and zoosprangium.

Plate II

A. Zoosporangia B. Tubercurous mycelium
C. Germination of the zoosporangium with zoospores,
   a. Zoospores b. Cystospores
   c. Germination of encysted zoospores
   d. An empty zoosporangium after germination with zoospore.
D. Germination of the zoosporangium with germ-tubes,
   a. Secondary young zoosporanginm.

Plate I



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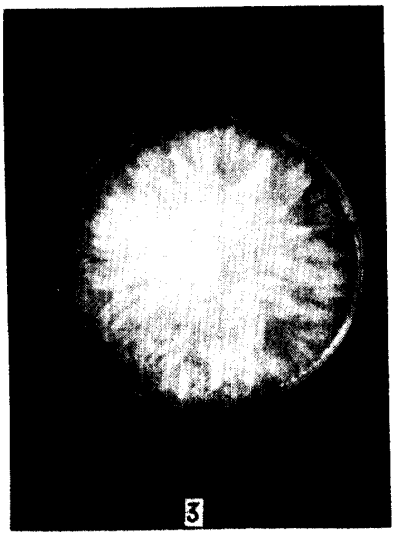

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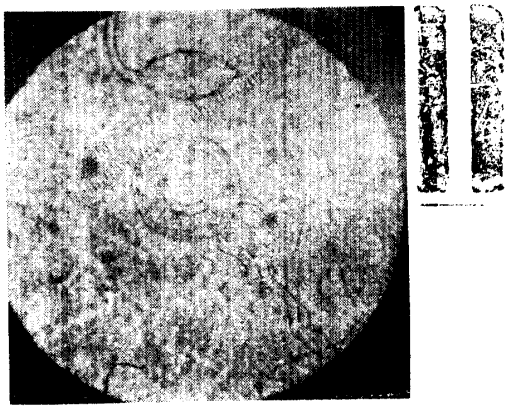

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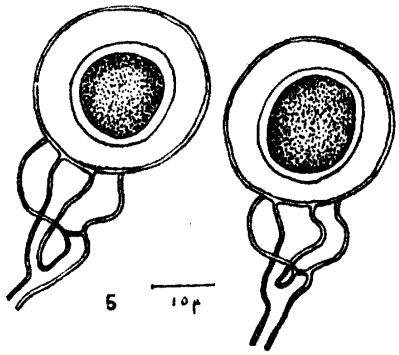

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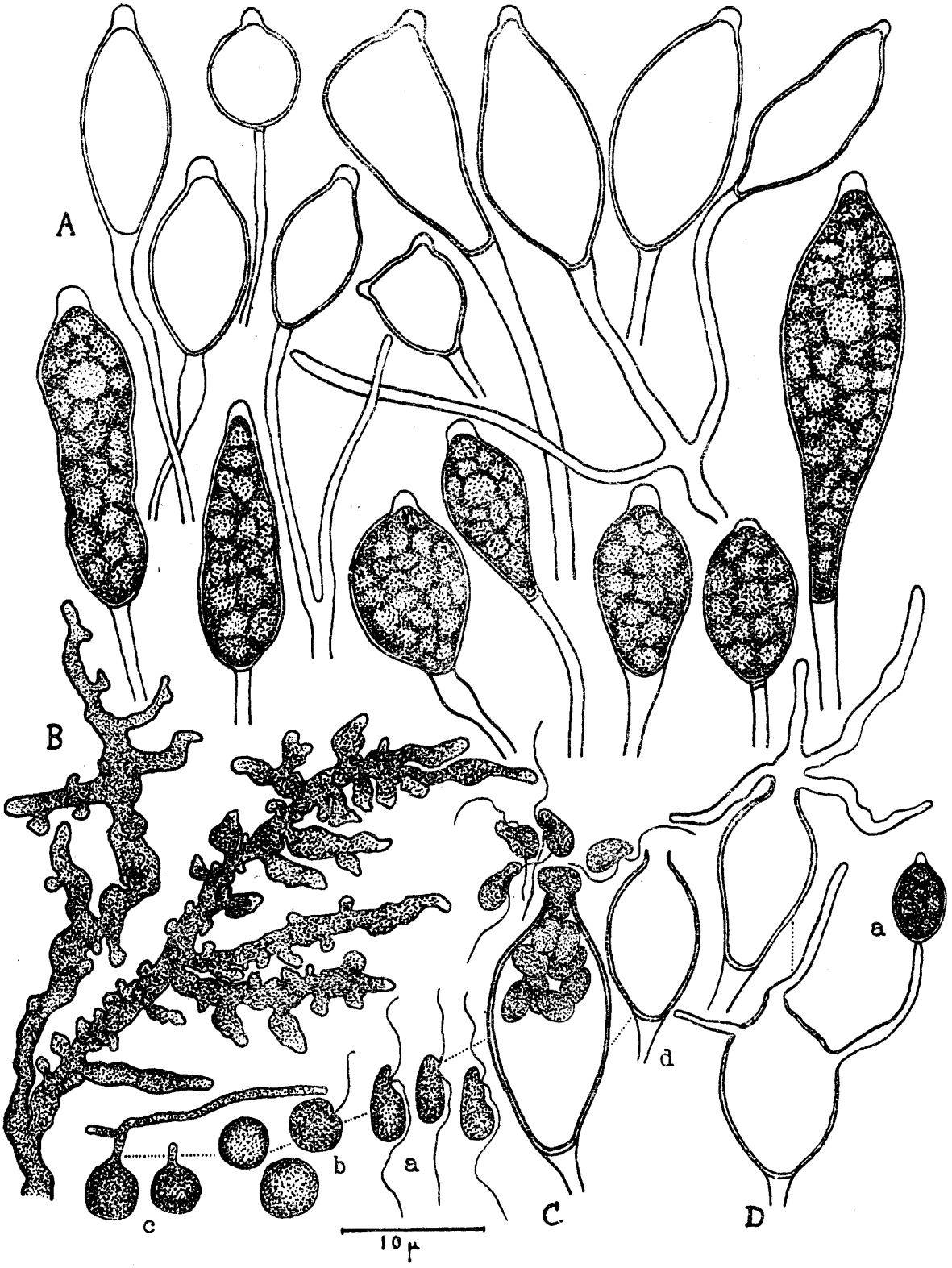
K. KATSURA del. a d photo.

Plate II



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