Physalospora paulowniae sp. nov. causing a Die-back of the Paulownia Tree and its Conidial Stage, *Macrophoma*.

By Kazuo ITÔ and Takao KOBAYASHI

With two plates

Introduction

The die-back or canker caused by *Valsa paulowniae* MIYABE et HEMMI is well known to be one of the most destructive diseases of paulownia trees (*Paulownia tomentosa* STEUD.) in the northern districts of Japan. Some aetiological and pathological studies on this disease were already made by HEMMI (1916) and KITAJIMA (1916).

In the recent years another die-back disease of the paulownia tree has drawn the writers' attention in the Kantô district. In the course of detecting the causal organisms of this die-back, the writers have collected many fungi belonging to the several genera. Among them, the genera *Phomopsis*, *Fusarium*, *Macrophoma* and *Physalospora* are considered to be more important.

The writers observed frequently the constant association of two forms of fungi, *Physalospora* and *Macrophoma*, with the lesions. Since some species of *Physalospora* have been known to possess a *Macrophoma* as their conidial stages, the writers were led to the presumption that there would be a possible connection between these two fungous forms. Recently this assumption has been verified by culture experiments and inoculation tests.

It is the purpose of the present paper to report new *Physalospora* and *Macrophoma* collected on the diseased twigs with the special reference to the genetic relation between the two spore stages.

The writers wish to express their heartiest thanks to Mr. Rokuya IMAZEKI, Chief of Forest Protection Division, of the Government Forest Experiment Station, for helpful suggestions during the progress of the work and for critically editing the Latin diagnosis, and to Mr. Michio NAKAGAWA for the assistance in preparing the illustrations.

Morphology of the fungus

Dead twigs of the 6-year-old trees collected in May, 1950 were examined thoroughly. Free-hand sections showed commonly the pycnidial stage of the genus *Macrophoma* and immature perithecia of unknown species in the majority of cases. Occasionally, however, mature ascomycetous fungus belonging to the

genus *Physalospora* was observed. The superficial macroscopic appearances of the sporocarps of the two stages were so similar as to make them readily confused.

Morphological characters of each of the two sporocarps examined under the microscope are briefly noted as follows:

1. Conidial stage, *Macrophoma*

Pycnidia, solitary or aggregate, occur usually in a stromatic tissue. The size of the stromata vary with the thickness of the bark and other conditions. Pycnidia are globose, subglobose or oblong-ellipsoidal, 111–172 × 103–153 μ in size. Walls of pycnidia are deep brown, 21–36 μ in width. Conidiophores are clavate, 10–13 μ in length, and pycnospores ellipsoidal, granular in content, almost colorless, 19–23 × 5–7 μ, averaging 21.1 × 6.0 μ (Pl. Ⅰ, C, D; Pl. Ⅱ, D, E).

2. Ascigerous stage, *Physalospora*

Perithecia are usually solitary and formed on stromatic mycelial layers. They are at first buried in the cortical tissues, then protruding at maturity by a short, papillate ostiole, 13–19 μ in width. Their form is globose to subglobose, 249–297 × 237–267 μ, averaging 274.3–256.8 μ in size. Thickness of the wall is 21–29 μ, averaging 24.8 μ.

Asci are usually clavate or fusiformis, 80–109×17–23 μ, averaging 94.0×20.5 μ, 8-spored. Ascospores are one-celled, ellipsoidal, granular, hyaline, 21–27×6–11 μ, averaging 24.1 × 8.1 μ in size. Paraphyses are distinct, 0- or 1-septate, not branched, showing sometimes to be clavate at the apex, 92–111 × 1.7–2.5 μ, averaging 104.2×2.0 μ (Pl. Ⅰ, A, B,; Pl. Ⅱ, A, B, C).

Genetic relation between the *Macrophoma* and the *Physalospora*

Pycnospores of the *Macrophoma* collected on the lesion germinated in a few hours on 2 per cent glucose agar at 25°C., and counting nearly 100 per cent germination in 24 hours. The germination started usually at both ends of the conidium (Pl. Ⅱ, F).

Another germination test of conidia was employed by Van Tieghem-cell method using sterile distilled water. Conidia produced on potato-glucose agar were used in this experiment. Effects of various temperatures and times passed upon the germination are briefly shown in Table 1.

From Table 1, it is seen that the germination occurs within 3 hours at 25°–30°C. even in distilled water, and the optimum temperature for the germination lies between 25°C and 30°C., especially near 30°C.

However, germination of ascospores of the *Physalospora* was very sparse, and even after 48 hours the percentage of it was about 10 per cent.

Monoascosporic isolations of the *Physalospora* were made by a modification of Yoshii's method (Yoshii 1933, Itô 1950). Single perithecia were removed,

Table 1. Showing the temperature-effects upon the germination of conidia.

Temperature (°C)	Germination	Lapsed period (hour) 3	6	10	24
0	G. P. (%)	0	0	0	0
	M. L. G. (μ)	—	—	—	—
10	G. P. (%)	0	0	0	17.6
	M. L. G. (μ)	—	—	—	24.4
15	G. P. (%)	0	22.2	88.1	80.0
	M. L. G. (μ)	—	12.2	42.7	73.2
20	G. P. (%)	0	79.1	89.6	89.4
	M. L. G. (μ)	—	54.9	134.2	146.2
25	G. P. (%)	30.8	89.0	99.5	99.5
	M. L. G. (μ)	14.6	134.2	231.8	512.4
30	G. P. (%)	92.0	97.0	98.8	99.3
	M. L. G. (μ)	34.1	158.6	256.2	585.6
35	G. P. (%)	0	59.8	71.7	84.5
	M. L. G. (μ)	—	43.8	85.4	170,8

Notes: G. P.·····Germination percentage,
M. L. G.·····Maximum length of germ-tube.

under the hand lens, with a flamed scalpel. They were crushed in a drop of 2 per cent aqueous solution of copper sulphate on flamed slide and then smeared streakly on 2 per cent glucose agar in Petri dishes. Single ascospores were marked by aid of the low power of the microscope. Germination followed in a few hours. Monospores were transferred to sterile tubes of potato-glucose agar and allowed to grow. By the same manners, monosporous isolates were also gained from conidia of *Macrophoma* form.

1. Culture characters

The mycelium of the fungus grew very luxuriously on potato-glucose agar. When young it was white and fluffy, later becomming abundant in aerial mycelium and dark gray in color.

After about 5 weeks at 25°C., fruit-bodies of *Macrophoma* stage were produced on the marginal part of the colony near the wall of tubes. The cultures have been kept for eight months in both dark and light conditions, but no ascigerous structures have developed (Pl. I, E).

Pycnidia produced on the ascosporous culture were proved to be similar to those obtained in culture from pycnospores. There have been recognized no differences between the culture started from ascospore of *Physalospora* and that of *Macrophoma*.

2. Inoculation experiments

During the past summer, several inoculations have been made with cultures from both *Physalospora* and *Macrophoma* forms. The current year's shoots and 1-year-old twigs of the paulownia trees were inoculated, in each case wound being made to serve as infection courts.

The methods of inoculation employed by the writers are the same as those applied by TOGASHI (1931) and ITÔ (1950) in the studies of *Valsa* cankers and *Pestalotia* shoot blight. The surface of the twigs were carefully treated with 80 per cent alcohol, sterilized with 0.1 per cent mercuric chloride, washed several times with sterile distilled water, and then a slit was incised with a burning hot scalpel on each twig.

Bits of agar bearing mycelium from pure cultures were placed in slits in the bark of twigs. Check twigs were inoculated in the same way, using sterile agar. The wounds were covered with moist absorbent cotton and paraffin paper for 10 days. Until the covering was taken off the cotton was moistened with sterile water once or twice every day.

On August 21, 1950, 14 twigs were inoculated with fungi from two different sources, one of which was isolated from ascospore of *Physalospora* form and the other was the isolate from pycnospore of *Macrophoma* stage.

On the 11th day when the covering was removed, the resulting lesion was visible as a discolored sunken area on each of the twigs. Afterwards, the lesions gradually enlarged in size, but very slowly.

Table 2. Showing the result of inoculation experiments.

Inocul. No.	Fungus	Part of host inoculated	Size of lesion (mm)	Pycnidium formation
1	*Macrophoma*	Green shoot	20 × 12	+
2	do.	do.	15 × 19	+
3	do.	do.	18 × 10	+
4	do.	do.	16 × 11	+
5	do.	1-year-old twig	21 × 18	+
6	do.	do.	20 × 12	+
7	do.	do.	16 × 11	+
8	*Physalospora*	Green-shoot	16 × 13	+
9	do.	do.	18 × 9	+
10	do.	do.	18 × 13	+
11	do.	do.	24 × 15	+
12	do.	1-year-old twig	21 × 13	+
13	do.	do.	20 × 13	+
14	do.	do.	21 × 13	+
15	Check	Green-shoot	—	—
16	do.	do.	—	—
17	do.	1-year old twig	—	—
18	do.	do.	—	—

About a month after inoculation, the pycnidia of *Macrophoma* form, being solitary mostly, appeared on the lesions near the slits. In check, the incisions healed over and no sign of the disease was observed on any of twigs even after 3 months (Pl. I, F).

Re-isolations were made from the conidia produced on the lesions resulting from inoculations. The mycelial colonies and conidia produced were of the same characters as monoconidial and monoascosporous cultures used as inocula.

The results of the experiments at the end of 6 weeks are briefly given in Table 2.

It is evident from Table 2 that there are no difference in the parasitism between the two isolates, from the *Macrophoma* and *Physalospora*, and the pathogenicity is very weak.

From the foregoing it is proved that the *Macrophoma* is the conidial stage of the *Physalospora*, an ascomycetous fungus.

Taxonomy of the fungus

According to the writers' search ascomycetous fungi inhabiting on twigs or branches of the living paulownia tree described hitherto are as follows:

Diatrype microstoma Syd. (Hara 1913-a),
Hypoxylon nectorioides Speg. (Hara 1913-b),
Ceratostoma Paulowniae Hara (Hara 1918-a),
Calosphaeria Imperiales Hara (Hara 1918-b),
Valsa paulowniae Miyabe et Hemmi (Hemmi 1916, Kitajima 1916),
Chaetosphaeria tristis (Tode) Schroet. (Shirai & Hara 1927).

So far as the writers can ascertain there is no account concerning the occurrence of any species of *Physalospora* on the paulownia tree.

The causal fungus of the apple black rot, *Physalospora obtusa* (Schw.) Cooke (Syn. *P. Cydoniae* Arnaud, *P. malorum* (Peck) Schear), was colleted by Shear et *al.* (1925) on the following broadleaved hosts: *Acer*, *Alnus*, *Hicoria*, *Liriodendron*, *Platanus*, *Prunus*, *Quercus*, and *Salix*, etc. By the studies of Hesler (1913), Shear (1914) and Shear et *al.* (1925), the conidial form of *P. obtusa* was proved to be *Sphaeropsis malorum* Peck with a brown conidium.

On *Citrus* and other hosts in America, Stevens (1926) colleted two species of *Physalospora*, *P. fusca* Stevens and *P. rhodina* (Berk. et Curt.) Cooke, bearing brown conidia in the pycnidial stages.

The above three species of *Physalospora* are distinctly different from the present fungus in many characters, namely shape and size of asci, ascospores, paraphyses and furthermore pycnidial stages.

European species of *Physalospora* inhabiting on broadleaved trees, namely *P. Corni* Sacc. on *Cornus*, *P. pustulata* Sacc. on *Aucuba*, *P. Salicis* (Fuck.) Sacc. and *P. protuberans* (Fuck.) Sacc. on *Alnus*, etc. are not similar to the

fungus in question.

Besides the fungi mentioned above, several species of *Physalospora* parasitic on broadleaved trees have been studied by Japanese workers.

According to MIYAKE (1916) and TANAKA (1918) *Physalospora minuta* Miyake inhabiting on living twigs of *Morus alba* may be the ascigerous stage of *Macrophoma minuta* BERL., though lacking in the experimental proof of the genetic relationship between the two. The morphological characters of *Physalospora minuta* do not coincide with those of the writers' *Physalospora*.

FUKUSHI (1921) described a new species of *Physalospora* causing a willow canker under the name of *Physalospora Miyabeana*, and he noted a *Gloeosporium* as the conidial form of this fungus.

In the studies of grape diseases, NISHIKADO (1921, 1923) reported the genetic relation between *Physalospora baccae* CAVARA and *Macrophoma reniformis* (VIALA et RAV.) CAVARA. *Physalospora baccae* differs from the present writers' fungus from the shape of perithecia and the chickness of the apex of asci.

Physalospora piricola, the perfect stage of *Macrophoma Kuwatsukai* HARA, described by NOSE (1934) is clearly different from the fungus in question in the characteristics of asci and paraphyses.

TOGASHI (1926) found a new *Physalospora* parasitic on the leaves of *Camellia japonica* and named it *Physalospora Japonica* TOGASHI. *Physalospora Kaki* HARA causing leaf curl of Japanese persimmon was noted by HARA (1930).

A very similar species to the present fungus may be *Physalospora Juglandis* SYD. et HARA occurring on the twig of *Juglans Sieboldiana* (*J. regia* var. *sinensis*) which was first described by SYDOW (1913) and then HARA (1927). Accordingly a morphological comparison of the two species in the ascigerous stage, lacking in the description of the conidial form of *Physalospora Juglandis*, will be noted in Table 3.

Table 3. A comparison of *Physalospora Juglandis* and the present writers' fungus

Fruit-body	*Physalospora Juglandis* SYD. et HARA	*Physalospora* of the writers
Perithecium	Dense gregariis, globosis, brevissime papillatis, 140–170 μ diam.	Solitary, globose to subglobose, with a short papillate ostiole, 237–267 μ in diam.
Ascus	Clavatis, apice rotundatis, crasse tunicatis, breviter stipitatis, 80–100 × 16–22 μ. Octosporis.	Usually clavate and sometimes fusiformis. 80–109 × 17–23 μ. 8-spored.
Ascospore	Distichis, ovoideo-oblongis vel oblongo-fusoideis, continuis, intus guttulatis, hyalinis, 17–26 × 7–8 μ.	One-celled, granular, ellipsoidal, hyaline, 21–27 × 6–11 μ.
Paraphysis	—	0- or 1-septate, not branched, filiformis, 92–111 × 1.7–2.5 μ.

Although, as shown in Table 3, the morphological characters of *Physalospora Juglandis* closely resemble those of the present fungus, the perithecia of the latter is about twice as large as those of the former and the width of the ascospores of the latter is larger than that of the former. Furthermore, there are seen some other differences in detailed characters between the two species.

Unfortunately the writers have never made direct comparison with the type specimen of *Physalospora Juglandis*, but it seems to be differ from the fungus under consideration, and therefore the writers wish to treat it as a new species, proposing the following name:

Physalospora paulowniae sp. nov.

Peritheciis sparsis, globosis vel subglobosis, epidermide tectis demum erumpentibus, extus fusicis, brevissme papillatis, 237-267 μ diam.; ascis clavatis vel fusiformibus, rectis raro leniter curvatis, 80-109×17-23 μ, 8-sporis; paraphysibus filiformibus, 0- raro-1-septatis, 92-111 μ longis; sporidiis 1-cellularibus, ellipsoideis, intus guttulatis, hyalinis, 21-27 × 6-11 μ.—Pycnidiis (Macrophoma) sparsis vel 2-3 congregatis, conidiis ellipsoideis, guttulatis, hyalinis, 19-23×5-7 μ.

Hab. in ramis corticatis Paulowniae tomentosae, Tokio Japonae, leg. K. Itô et T. Kobayashi (May 31, 1950).[1)]

Summary

On dead twigs of *Paulownia tomentosa* the writers found the constant association of two forms of fungi, *Macrophoma* and *Physalospora* with the lesions. The genetic relation between these two fungous form were verified by the cultural and inoculation experiments.

The fungus seems to be different from all the hitherto known species of *Physalospora* and was described by the writers as a new fungus to science under the name of *Physalospora paulowniae* sp. nov.

The pathogenicity of the fungus was proved by inoculation experiments, being weak in its virulence.

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1) The type specimen has been deposited in the Harbarium of the Government Forest Experiment Station, Tokyo, Japan.

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Explanation of plates

Plate I.

A. A part of the twig of *Paulownia tomentosa* attacked by *Physalospora paulowniae* sp. nov. ×0.9.

B. A perithecium of *Physalospora paulowniae* sp. nov. ×135.

C-D. Pycnidia of *Macrophoma* stage of *Physalospora paulowniae* sp. nov. ×135.

E. Mycelial colonies of *Physalospora paulowniae* sp. nov. on potato-glucose agar. ×0.9.

a. Isolate from the ascospore of *Physalospora* stage.

b. Isolate from the conidium of *Macrophoma* stage.

F. Inoculations with *Physalospora paulowniae* sp. nov. ×0.9.

a. Check.

b. Inoculated with the isolate from *Physalospora* stage.

c. Inoculated with the isolate from *Macrophoma* stage.

Plate II.

A. Asci and ascospores of *Physalospora paulowniae* sp. nov.

B. Paraphyses of *Physalospora paulowniae* sp. nov.

C. Ascospores of *Physalospora paulowniae* sp. nov.

D. Conidiophores in the pycnidium of *Macrophoma* stage of *Physalospora paulowniae* sp. nov.

E. Conidia of *Macrophoma* stage of *Physalospora paulowniae* sp. nov.

F. Germinating conidia of *Macrophoma* stage of *Physalospora paulowniae* sp. nov.

キリの枝枯病を基因する *Physalospora paulowniae* sp. nov. 及びその不完全時代 *Macrophoma*

（摘　要）

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Valsa paulowniae Miyabe et Hemmi に因るキリの腐爛病（立枯病）は本邦北部に於て激害を及ぼしているものであるが，著者等はこれとは異る枝枯病を関東地方で屢々認めている。目下この病原を研究中であるが患部に見出される菌類の主なものは *Phomopsis*, *Fusarium*, *Macrophoma* 及び *Physalospora* である。

本報文は *Macrophoma* と *Physalospora* についてだけ述べたもので，これらは病斑上に近接して存在することが多く，種々の点から著者等は両者の同根関係を予想した。

Macrophoma の柄胞子及び *Physalospora* の子嚢胞子から夫々単箇培養によつて培養基上の性質の比較並に接種試験を行い，両者は同一菌の異る胞子型で，*Macrophoma* は *Physalospora* の不完全時代であることを実験的に明かにした。

接種試験の結果本菌はキリの生枝に対して病原性はあるがその程度は強烈なものではないことがわかつた。

従来キリの枝に寄生する *Physalospora* 菌に関する記録はなく，他の広葉樹及び果樹に寄生する本邦及び外国産の菌と比較したがこれに該当するものが見当らないので，未記載の菌と認め完全時代に対して次のように命名する。

Physalospora paulowniae sp. nov.

枝に寄生し子嚢殻は孤生し，球形乃至類球形，初め表皮下に形成され後にこれを破つて小乳頭を突出す。殻壁の外部は暗色，直径 237–267 μ。子嚢は棍棒状又は紡錘形を呈し，多くは直立，稀に僅かに彎曲し，大さ 80–109×17–23 μ，各々8 箇の子嚢胞子を有する。側糸は糸状で稀に 1 箇の隔膜があり，長さ 92–111 μ。子嚢胞子は単胞，楕円形を呈し殆ど無色，大さ 21–27×6–11 μ。

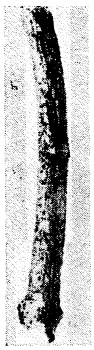
Plate I

Bull. Gov. Forest Exp. Sta. No. 49.

B

D

A



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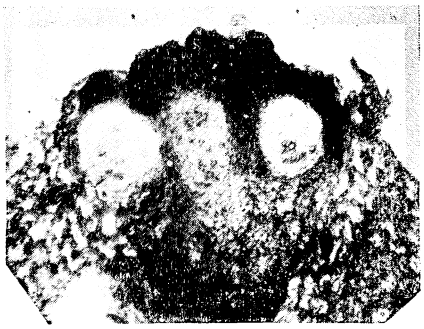
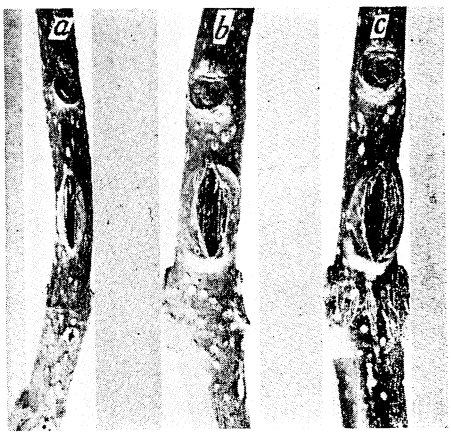


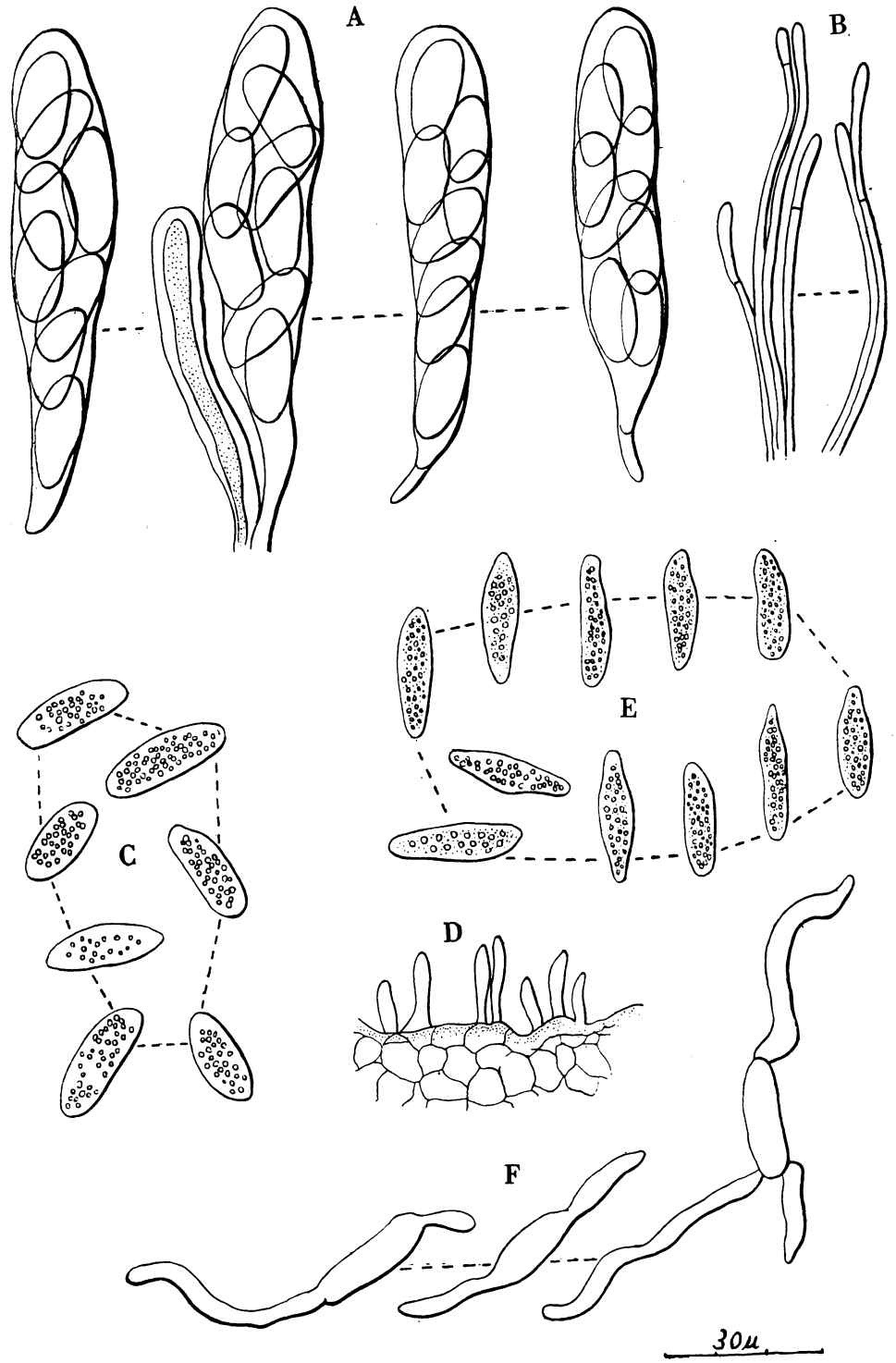
E

F



K. Itô & T. Kobayashi: ***Physalospora paulowniae*** sp. nov.

Plate II



K. Itô & T. Kobayashi: *Physalospora paulowniae* sp. nov.