NUMBER 5

INSTITUTE FOR FERMENTATION
OSAKA

RESEARCH COMMUNICATIONS

(ANNUAL REPORT 1969-1970)

1971

財団法人 発酵研究所

RESEARCH COMMUNICATIONS

No. 5

(*Annual Report 1969-1970*)

1971

INSTITUTE FOR FERMENTATION, OSAKA

4-54, *Juso-Nishinocho, Higashiyodogawa-ku,*
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IFO Res. Comm. 5,
1–9, 1971 (*March*)

FURTHER STUDIES ON THE PATHOGENIC MECHANISM OF BACTERIAL DISEASES IN GNOTOBIOTIC SILKWORM LARVAE

Reijiro KODAMA and Yugoro NAKASUJI

As previously reported[2], *Streptococcus faecalis-Streptococcus faecium* intermediate E–5 and *Serratia piscatorum* E–15, both of which were isolated from dead silkworms, had great pathogenicity for silkworm larvae reared aseptically on an artificial diet, when each strain was inoculated to larvae by feeding. However, a marked difference was observed between the number of the viable cells of these two species required for accomplishing 100% lethality. And the death of larvae was hastened by mixed infection of these strains, exhibiting a synergistic effect in their pathogenicity.

S. faecalis-S. faecium intermediate E–5 of a relatively alkali-resistant character grew rapidly in the gut of larvae after inoculation by feeding, but *Serratia piscatorum* E–15 did not. In contrast with this, *Serratia piscatorum* E–15 could grow rapidly in the hemocoele of larvae when injected, but *S. faecalis-S. faecium* intermediate E–5 could not.

On the basis of these results, a mechanism of pathogenic effects of bacteria on silkworm larvae was discussed in the same preceding paper.

In this paper the results of further studies on the pathogenic mechanism of bacterial diseases in gnotobiotic silkworm larvae will be presented.

Materials and Methods

Bacterial pathogens. *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15.

Silkworm. Healthy silkworm larvae in the fifth instar which were reared aseptically on an artificial diet according to the method of Matsubara and his associates[3].

Inoculation with bacteria by feeding. This was conducted as described previously[2].

Measurement of number of viable cells in the gut contents and the blood. Medium A and B were used for measurement of number of the viable cells of *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 respectively.

Medium A consisted of yeast extract, 5g; peptone, 5g; glucose, 20 g; K_2HPO_4, 1 g; Salts B[5], 10 ml; agar, 20 g; distilled water, 1000 ml; pH 9.0.

Medium B consisted of meat extract, 10 g; peptone, 10 g; Nacl, 1 g; agar, 20 g; distilled water, 1000 ml; pH 8.0.

When these two strains were coexisting in the samples to be examined as a result

of mixed infection, medium A supplemented with 5 mg of neomycin and medium B supplemented with 5 mg of leucomycin were used respectively.

Determination of volatile and non-volatile acids in the gut contents. The gut contents vomited out of larvae with an electric shock were centrifuged out for 15 minutes at 3,000 r.p.m. The supernatants were distilled out under acidic condition. The residuals were submitted to extraction with ether. The distillate and the ether-soluble fraction were titrated with 0.1 N-NaOH, and the values were calculated as acetic and lactic acid respectively, because the Rf values of their chromatographic spots were identical with those of acetic and lactic acids.

Results

I. Multiplication of *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 in the gut and the hemocoele of larvae.

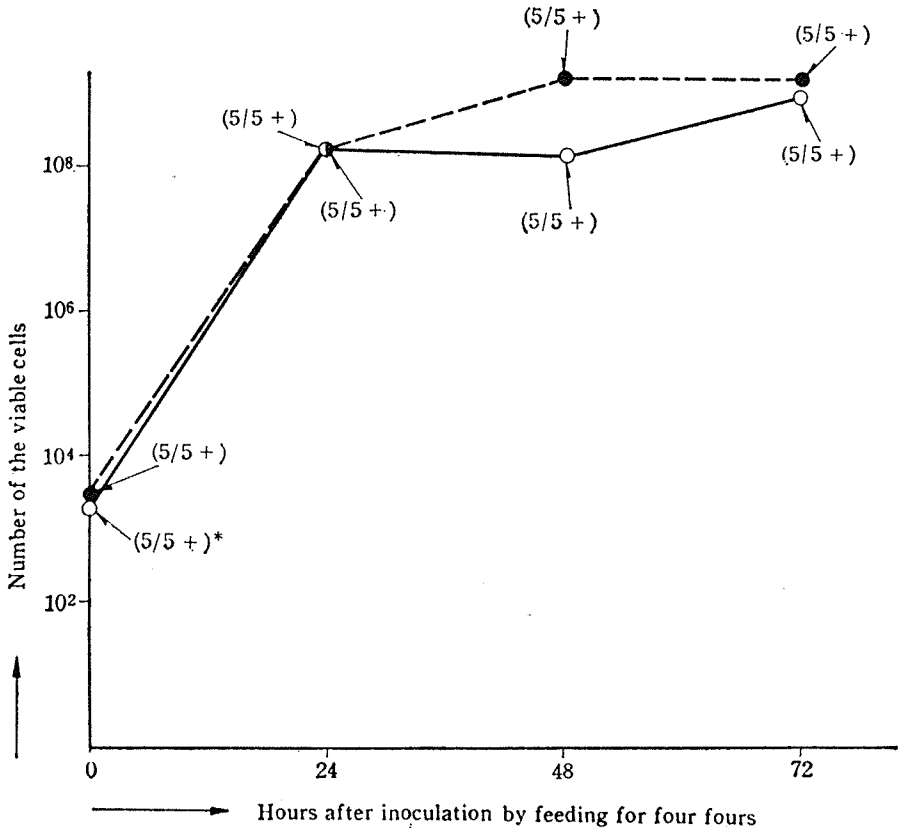

Fig. 1. Number of the viable cells of *S. faecalis-S. faecium* intermediate E–5 in the gut contents.

●···● Number of the viable cells when the streptococci were inoculated alone to larvae (an average of five larvae).

○—○ Number of the viable cells when the streptococci were inoculated to larvae together with *Serratia piscatorum* E–15 (an average of five larvae).

* 5/5+ indicates that the viable cells of the streptococci were recovered from five of five larvae. This is to be repeated in the following.

Judging from the maximum pH values for the growth[2], it is difficult to infer that *Serratia piscatorum* E–15 may be able to grow readily in the gut of larvae. When this strain is coexisting with *S. faecalis-S. faecium* intermediate E–5 in the gut, however, it is conceivable that *Serratia piscatorum* E–15 also may be able to grow there as a result of lowering of the pH values of the gut contents following the growth of the streptococci.

In order to substantiate the above inference, the behaviors of these two strains in the gut and the hemocoele of silkworm larvae were pursued, when both organisms were inoculated by feeding either alone or together.

Results obtained were as follows:

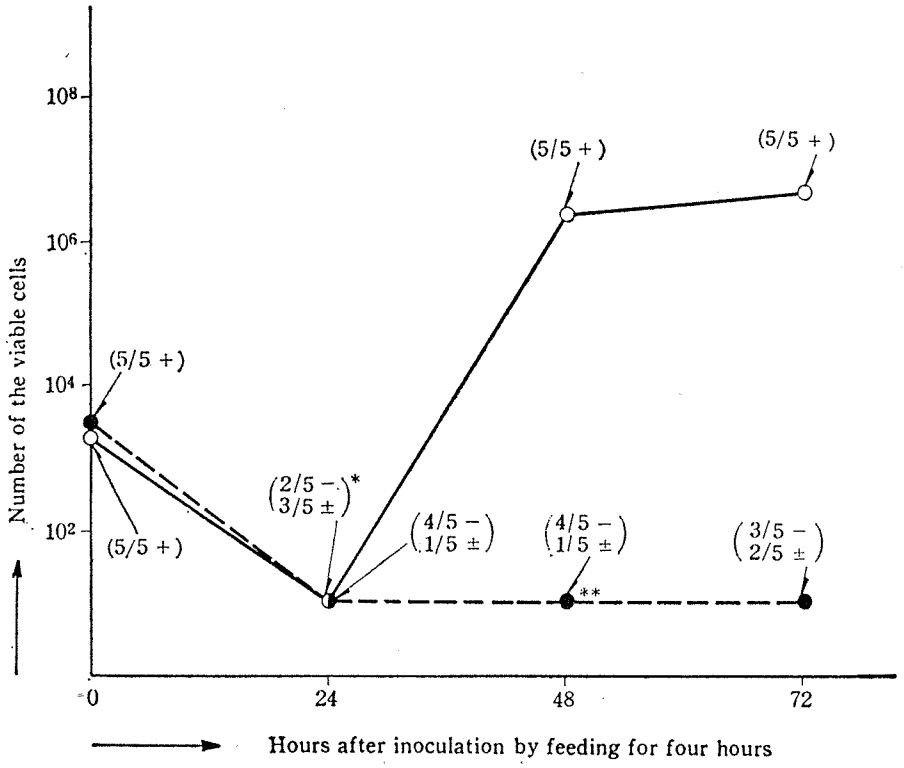

Fig. 2. Number of the viable cells of *Serratia piscatorum* E–15 in the gut contents.
●···● Number of the viable cells when *Serratia piscatorum* E–15 was inoculated alone to larvae (an averge of five larvae).
○—○ Number of the viable cells when *Serratia piscatorum* E–15 was inoculated together with *S. faecalis-S. faecium* intermediate E–5 (an average of five larvae).
* 2/5− indicates that the viable cells of *Serratia piscatorum* E–15 were not recovered from two of five larvae.
3/5± indicates that the viable cells of *Serratia piscatorum* E–15 less than 3×10^1 were recovered from three of five larvae. These are to be repeated in the following.
** 8.9×10^3 viable cells of *Serratia piscatorum* E–15 were recovered from one of five larvae.

(1) *S. faecalis-S. faecium* intermediate E–5 grew rapidly in the gut irrespective of existence of *Serratia piscatorum* E–15 (Fig. 1). The streptococci were recovered from the blood within 48 hours after the inoculation, but did not seem to grow in the hemocoele (Fig. 3).

(2) *Serratia piscatorum* E–15 could not grow in the gut except one of five larvae when inoculated alone (Fig. 2). When this strain was inoculated together with *S. faecalis-S. faecium* intermediate E–5, however, the former also could grow in the gut somewhat later than the maximum growth of the latter (Fig. 2), and then also invaded into the hemocoele following the streptococci (Fig. 4).

II. Determination of organic acids in the gut contents of silkworm larvae infected with *S. faecalis-S. faecium* intermediate E–5 by feeding.

As already reported, *S. faecalis-S. faecium* intermediate E–5 was a strain of homofermentative lactic acid bacteria, which produced acids from glucose vigorously. On the other hand, it was also reported that the pH values of the gut contents of silkworm larvae infected with the strain lowered gradually. From these facts, the vicissitudes of organic acids in the gut contents infected with the same strain were investigated. As

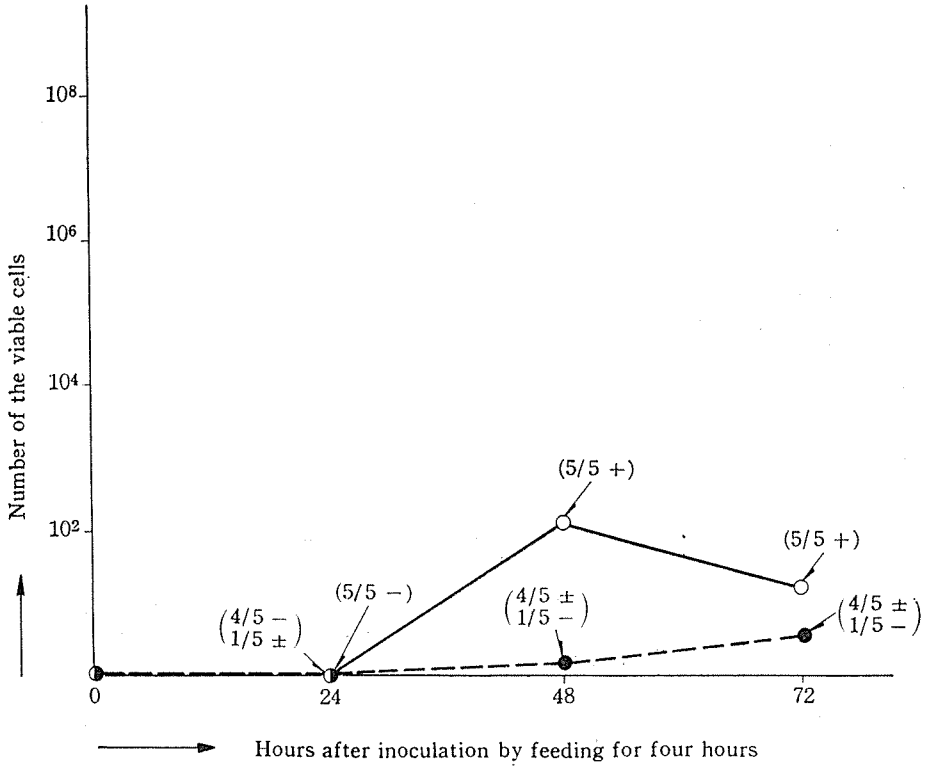

Fig. 3. Number of the viable cells of *S. faecalis-S. faecium* intermediate E–5 in the blood.

●···● Number of the viable cells when the streptococci were inoculated alone to larvae (an average of five larvae).

○—○ Number of the viable cells when the streptococci were inoculated to larvae together with *Serratia piscatorum* E–15 (an average of five larvae).

seen from Table 1, volatile as well as non-volatile acid in the gut contents increased gradually in course of time after inoculation by feeding. The Rf values of volatile acid (Rf 0.35–0.36 as sodium salt; solvent system, n-butanol saturated with aqueous 1.5 N-NH_3) and non-volatile acid (Rf 0.63–0.64; solvent system, n-butanol: formic acid: water=4:2:1) were indentical with those of acetic acid and lactic acid respectively.

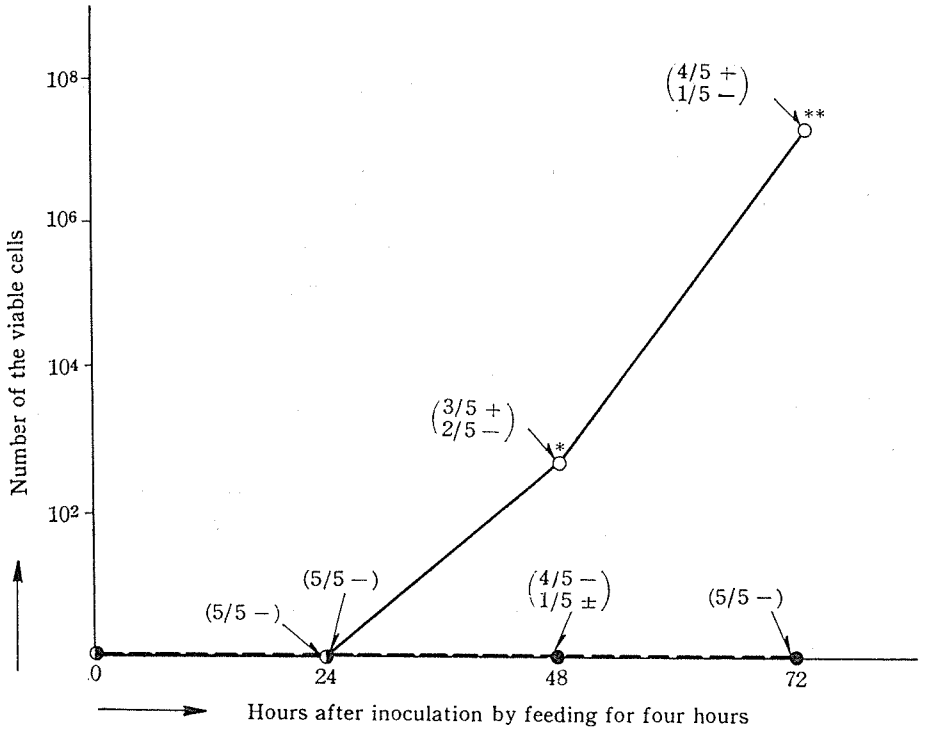

Fig. 4. Number of the viable cells of *Serratia piscatorum* E–15 in the blood.
●···● Number of the viable cells when *Serratia piscatorum* E–15 was inoculated alone to larvae (an average of five larvae).
○—○ Number of the viable cells when *Serratia piscatorum* E–15 was inoculated together with *S. faecalis-S. faecium* intermediate E–5 (an average of five larvae).
* An average of three larvae from which the viable cells were recovered.
** An average of four larvae from which the viable cells were recovered.

Table 1. Organic acids in the centrifugal supernatants of the gut contents which were vomitted electrically out of larvae infected with *S. faecalis-S. faecium* intermediate E–5 by feeding. (0.7×10^3 viable cells of the streptococci were inoculated to 1g of an artificial diet.)

Hours after infection (hr)	Centrifugal supernatant		Volatile acid per ml of the supernatants (as acetic acid) (mg)	Non-volatile acid per ml of the supernatants (as lactic acid) (mg)
	Total volume (ml)	pH		
Control (not infected)	42	9.74	0.22	1.30
24	54	8.14	0.58	1.96
48	46	7.12	0.74	3.42

Discussion

On the basis of our experimental results so far obtained by the use of two bacterial pathogens, *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15, a diagram to show the mechanism of bacterial diseases in aseptically reared silkworm larvae is tentatively shown in Figs. 5-a, 5-b and 5-c.

First, when silkworm larvae are infected with the streptococci by feeding, they present symptoms of shrinkage following diarrhoea. The streptococci grow rapidly in the gut (Fig. 1) and produce acetate and lactate (Table 1), resulting in lowering of the pH values of the gut contents. Then, the streptococci invade into the hemocoele, but do not grow readily there (Fig. 3), the fact being also substantiated by the results obtained previously from injection experiments[2]. These are diagrammed in Fig. 5-a.

Second, *Serratia piscatorum* E–15 do not grow readily in the gut of silkworm larvae, but do so rapidly in the hemocoele, bringing about a lethal septicemia to larvae. This fact indicates that the strain E–15 is to be included in a group of "potential pathogens" in the classification by Bucher[1]. When an artificial diet is supplied continually, however, the strain E–15 grows occasionally also in the gut, indisregard of the light inoculum (Fig. 2), and then invade into the hemocoele, resulting in rapid growth there (Fig. 4). As seen from Figs. 2 and 4, the strain E–15 grows in the gut of one of five larvae at the period of 48 hours after inoculation by feeding, and recovered from the blood of one of five larvae at the same period. In other words, "potential pathogens" act as "facultative pathogens" under the conditions of circumstances. It is possibly inferred that much more increase such larvae, when increased amounts of the viable cells of the strain E–15 are inoculated by feeding. These are diagrammed in Fig. 5-b.

Third, under the conditions of coexistence of two species of above-mentioned pathogenic bacteria in the gut, which exhibit the different types of pathogenic effects,

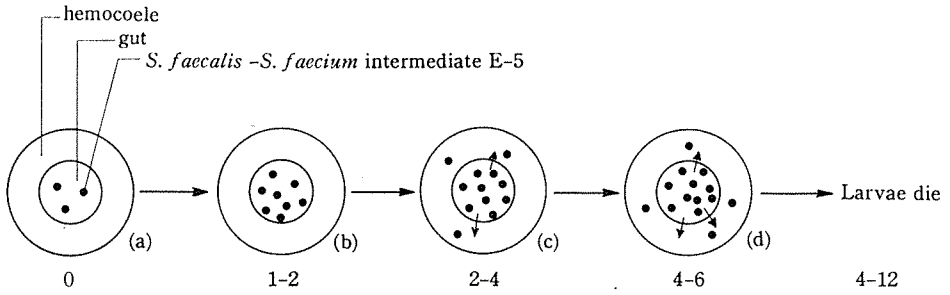

Fig. 5–a. Diagram to show the progress of silkworm larvae infected with *S. faecalis-S. faecium* intermediate E–5 alone. The mumerals indicate days after inoculation with the strain.
(a) The streptococci invade into the gut.
(b) The streptococci grow in the gut; larvae begin to suffer from diarrhoea and to lose their appetite.
(c) The streptococci begin to invade into the hemocoele, but do not grow there; the growth of larvae cease.
(d) The streptococci continue to invade into the hemocoele; larvae shrink.

what correlation should be considered between the roles to be played by these two pathogens? As seen from Figs. 1 and 2, *S. faecalis-S. faecium* intermediate E–5 to be capable of growing at relatively high pH values grows first in the gut prior to the growth of *Serratia piscatorum* E–15. Now, it is conceivable that a canal into the hemocoele is formed as a result of the growth of the streptococci, because the streptococci are recovered from the blood after their maximum growth in the gut. Consequently, *Serratia piscatorum* E–15 also should have an opportunity to invade into the hemocoele through the canal. An environmental condition favorable for the growth of *Serratia piscatorum* E–15 such as lowering of the pH values of the gut, which is prepared by the streptococci,

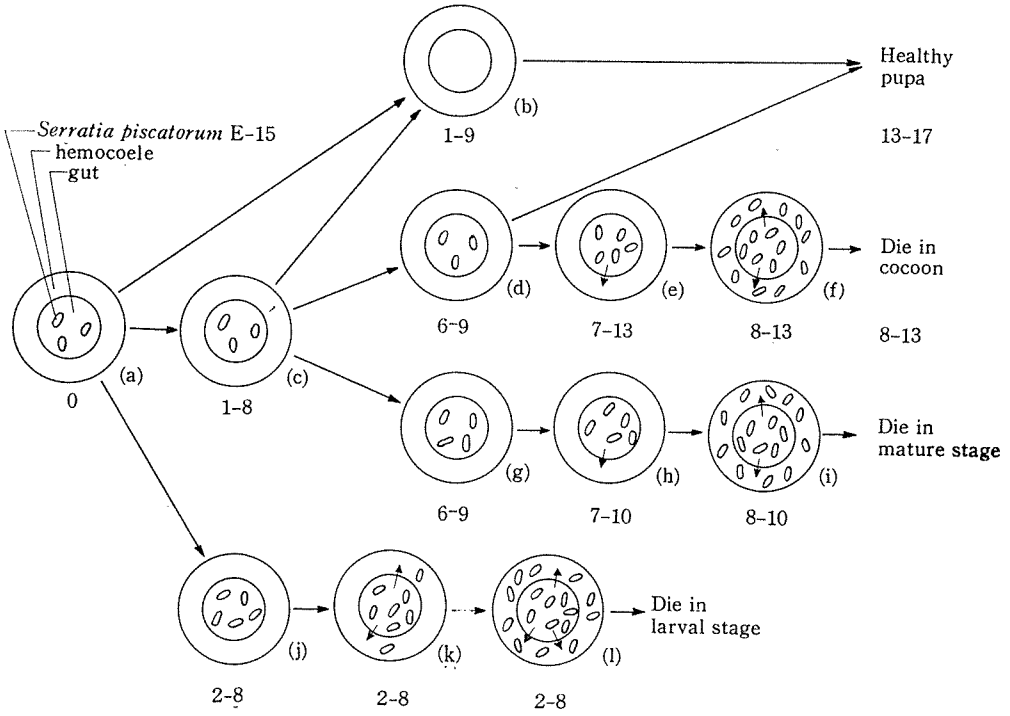

Fig. 5–b. Diagram to show the progress of silkworm larvae infected with *Serratia piscatorum* E–15 alone. The numerals indicate days after inoculation with the bacteria.

(a) The bacteria invade into the gut.
(b) Both the gut contents and the blood are bacteria-free; maturing and cocooning.
(c) The bacteria are recovered from the gut contents without multiplication; larvae continue to grow.
(d) The bacteria are recovered from the gut contents without multiplication; larvae continue to grow; maturing and cocooning.
(e) The bacteria grow in the gut, and then begin to invade into the hemocoele.
(f) The bacteria grow rapidly in the hemocoele; matured silkworms die in cocoon, causing a lethal septicemia.
(g) The bacteria grow slightly in the gut; maturing.
(h) The bacteria grow in the gut, and then begin to invade into the hemocoele.
(i) The bacteria grow rapidly in the hemocoele; matured silkworms die before cocooning, causing a lethal septicemia.
(j) The bacteria begin to multiply in the gut; larvae continue to grow.
(k) The bacteria begin to invade into the hemocoele of larvae.
(l) The bacteria grow rapidly in the hemocoele; larvae fall, causing a lethal septicemia.

should be regarded as increasing the opportunity for invasion. These are diagrammed in Fig. 5-c. Moreover, such a property observed by Ono and Ichikawa[4] that some strains belonging to the genera *Serratia, Aeromonas* and *Pseudomonas* are able to dissolve the peritrophic membrane of silkworm larvae, may be closely related to the invasion of the bacteria into the hemocoele.

Thus, the present authors hold the view that *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15 act in principle as primary and secondary invadors respectively. The view put a tentative interpretation upon a synergistic effect in the pathogenicity of these two bacterial pathogens for aseptically reared silkworm larvae. Further, the same view does not contradict with the old observations that, in case of occurrence of flacherie of silkworm larvae fed with mulberry leaves, streptococci usually appear in the gut prior to multiplication of gram-negative bacteria and the pH values of the gut contents lower considerably.

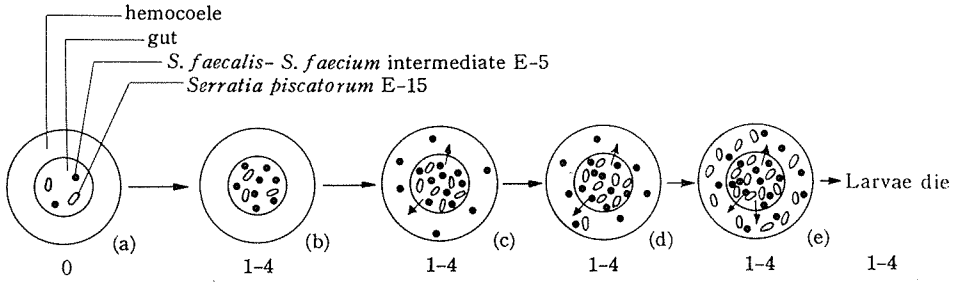

Fig. 5–c. Diagram to show the progress of silkworm larvae infected together with *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15. The numerals indicate days after inoculation with both strains.
(a) Both strains invade into the gut.
(b) The streptococci grow first in the gut, and then *Serratia piscatorum* E–15 begin to grow; larvae lose their appetite.
(c) The streptococci invade into the hemocoele; *Serratia piscatorum* E–15 grow in the gut.
(d) *Serratia piscatorum* E–15 begin to invade into the hemocoele.
(e) *Serratia piscatorum* E–15 grow rapidly in the hemocoele; larvae fall, causing a lethal septicemia.

Summary

Further studies were carried out on the pathogenicity of bacteria for aseptically reared silkworm larvae by the use of two species of pathogenic bacteria, *S. faecalis-S. faecium* intermediate E–5 and *Serratia piscatorum* E–15. The results obtained were:

(1) *S. faecalis-S. faecium* intermediate E–5 grew rapidly in the gut of larvae irrespective of the existence of *Serratia piscatorum* E–15, and was recovered from the blood within 48 hours after inoculation by feeding, but did not grow readily in the hemocoele.

(2) Accompanied by the growth of *S. faecalis-S. faecium* intermediate E–5 in the gut, lactate and acetate in the gut contents increased gradually, resulting in lowering of the pH values of the gut contents.

(3) *Serratia piscatorum* E–15 did not grow readily in the gut of larvae both fed on an artificial diet and starved. When this strain was coexisting with *S. faecalis-S. faecium* intermediate E–5 in the gut, however, the former could multiply in the gut somewhat later than the maximum growth of the latter.

(4) The pathogenic mechanism of bacterial diseases in aseptically reared silkworm larvae was discussed. The mechanism was postulated as follows: *S. faecalis-S. faecium* intermediate E–5 (in general, pathogenic streptococci) was associated with the bacterial diseases as the primary invador, producing an intestinal disease of chronic character and also playing an inductive role for the secondary invador, while *Serratia piscatorum* E–15 (in general, gram-negative and, presumably, gram-positive bacterial pathogens) was associated with bacterial diseases as the secondary invador, producing a septicemic disease by the rapid growth in the hemocoele.

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IFO Res. Comm. 5,
10–19, 1971 (*March*)

FORMATION AND SEGREGATION OF DOUBLE LYSOGENS OF λ-RELATED PHAGES

Teiji IIJIMA and Yutaka SAKAMOTO

Temperate phage φ170 which can attack *Escherichia coli* K12 was found in 1961 from a strain of *E. coli* in faeces which were brought to the Research Institute for Microbial Diseases, Osaka University. The characteristics of φ170 and interaction between φ170 and other related phages (lambdoids) have been revealed in this laboratory. The present paper reviews results obtained in recent years and discusses some problems related to lambdoids.

Actively growing cells of the original strain were resuspended in a medium. After induction and incubation at 37C, the cells lysed and produced a number of phage particles which form plaques on W3110, a nonlysogenic strain of *Escherichia coli* K12. Burst size of the phage is about 100 PFU per infected cell. These plaques have a turbid center, that is, characteristics of temperate phage, and it is possible to obtain a stable lysogenic strain of φ170 from the center of the plaque. Clear plaque mutants were isolated from the wild type phage.

The lysogenic strain produced φ170 by treating an inducing agent, such as ultraviolet or Mitomycin C. The immune specificity of φ170 is different from known phages, such as λ, 434, 82, φ80 and φ81, but the host range is analogous to that of 434 and 82, that is, 434 or 82 does not adsorb on φ170 resistant cell. Some of the characteristics of φ170 are shown in Table 1. Immune specificity (*i*) and host range (*h*) are highly convenient as genetic markers for the following experiments, because these markers are easily tested by replica plating method, and locate at the separate locus on the phage

Table 1. Characteristics of phages

indicator	characteristics	φ170	434	82	λ	φ80	φ170hy	φ434hy	82hy	λhy
F1	W3110	+	+	+	+	+	+	+	+	+
F1539	F1/λ	+	+	+	−	+	−	−	−	+
F1467	F1/φ170	−	−	−	+	+	+	+	+	−
F1541	F1/φ 80	+	+	+	+	−	+	+	+	+
F563	F1 (φ170)	−	+	+	+	+	−	+	+	+
F1018	F1 (434)	+	−	+	+	+	+	−	+	+
F1381	F1 (82)	+	+	−	+	+	+	+	−	+
F1192	F1 (λ)	+	+	+	−	+	+	+	+	−
F1039	F1(φ80)	+	+	+	+	−	+	+	+	+

Phage lysates which were prepared from lysogenic strains, are spotted on various indicators. +: lytic zone appeared after overnight incubation at 37C. − : no lytic zone appeared. /: resistant strain. ( ) : lysogenic strain. φ170 hy : $i^{\phi 170}$ h^{λ} 434hy : i^{434} h^{λ}, λhy : i^{λ} $h^{\phi 170}$, 82hy : i^{82} h^{λ}.

genome. When transducing ability of a lysate prepared from a lysogenic strain F563; W3110(φ170), was examined, it was found that the lysate specifically transduced the galactose markers to galactose negative mutants of *E. coli* K12 at a low frequency (Iijima 1966). Using a low frequency transducing (Lft) lysate, heterogenotic transductants were obtained. Furthermore, the heterogenotes produced high frequency transducing (Hft) lysates (Iijima 1967). The Hft lysates transduced the galactose markers at a higher rate about 10^{-3} to 10^{-4} per PFU. In transduction using an Hft lysate, defective transductants designated as *gal*⁻(φ170dg) were obtained.

From the findings mentioned above, one would assume that 1) the locus of prophage φ170 is in the region neighboring the *gal* locus. Other experiment using F_1-*gal* and F_8-*gal* episomes, shows that prophage φ170 locates on F_1-*gal* but not on F_8-*gal* episome (Iijima and Sakamoto 1969). This is consistent with the assumption mentioned above. 2) Transduction of the *gal* markers by φ170 are mediated by a defective phage φ170dg. Characteristics of the defective transductant, *gal*⁻(φ170 dg) are shown in Table 2.

The defective phage, φ170 dg is able to transduce the *gal* markers, but some part of the genome would be replaced by the bacterial *gal* region, because no active particle produced from the defective lysogen, nevertheless *gal*⁻ (φ170dg) is immune to the superinfection of homoimmune phage and produced a lytic zone on an indicator which is lysogenic for λ or another related lambdoid. Formation of lytic zone on a λ lysogenic strain is due to a lytic action of recombinant phages liberated from *gal*⁻(φ170dg) by superinfection of λ (Iijima and Sakamoto 1968). The defective transductants, *gal*⁻(φ170dg), are unstable in respect of *gal* marker and segregate *gal*⁻ nonlysogenic cells. As *gal*⁻(φ170dg) produces a lytic zone on W3110 (λ), we can clearly distinguish a nonlysogenic colony from that of defective lysogen by replica plating

Table 2. Characteristics of lysogenic strains

strain	gal	lytic zone[1] on W3110	W3110(λ)	W3110 (φ170)	sensitivity[2] to λ	φ 170	[3] segregation
gal⁻(λ)	−	+	−	+	R	S	−
gal⁺(λ)	+	+	−	+	R	S	−
gal⁻(φ170)	−	+	+	−	S	R	−
gal⁻(λdg)	+	−	−	+	R	S	+
gal⁻(φ170dg)	+	−	+	−	S	R	+
gal⁻(λdg) (λ)	+	+	−	+	R	S	+
gal⁻(φ170dg)(φ)	+	+	+	−	S	R	+
gal⁻(λdg) (φ170)	+	+	+	+	R	R	+
gal⁻(φ170dg)(λ)	+	+	+	+	R	R	+

[1] Culture of an Indicator strain W3110, W3110(λ) or W3110(φ170) was mixed with soft agar and layered on a nutrient agar plate. Strains to be tested were spotted on the indicators and examined formation of lytic zone after overnight incubation at 37C.

[2] Sensitivity to phage was tested by cross-streaking against phage lysate.

[3] An aliquot of culture of a bacterial strain was plated on EMB-galactose agar plate. Unstable strains produce some galactose negative colonies.

method. In a large number of colonies produced from a strain *gal*$^-$(ϕ170dg), it was found that a few *gal*$^+$(non) and *gal*$^-$ defective lysogenic cells exist with *gal*$^-$(non) and *gal*$^-$(ϕ170 dg) cells.. The galactose negative defective lysogen, designated as *gal*$^-$(ϕ170dg-n), is immune to the superinfection of ϕ170 and segregates gal$^-$ nonlysogenic colonies. Transduction experiment using an Hft lysate prepared from *gal*$^-$(ϕ170)(ϕ170 dg-n) showed that the galactose marker of ϕ170dg-n is identical to that of the host. So the defective lysogen is represented as $\cdots gal_a^- \cdots i^{\phi 170} \cdots gal_a^- \cdots bio$. The mechanism of production of gal$^+$ nonlysogenic or gal$^-$ nonlysogenic cell is an excisive recombination between *gal*$^-$marker of the host and *gal*$^+$marker of prophage, by intervention of Rec system, because we cannot find gal$^+$ nonlysogenic segregant when a host has a *rec*$^-$ mutation.

Phage ϕ170 recombines with λ at a high frequency and produces all the possible 4 kinds of particles; λ (i^λ, h^λ), λhy (i^λ, $h^{\phi 170}$), ϕ170 ($i^{\phi 170}$, $h^{\phi 170}$), and ϕ170hy ($i^{\phi 170}$, h^λ). On the other hand, ϕ170 recombines at a low frequency with ϕ80 which lysogenizes near the *trp* locus (Matsushiro 1961). Recombination of the phages at high frequency suggest that arrangement and function of cistrons of ϕ170 are analogous to those of λ.

Similarly, recombinant type phages are produced from a defective double lysogen such as *gal*$^-$(ϕ170dg) (λ). When the active particles liberated from *gal*$^-$ (ϕ170 dg) (λ) were examined, it was revealed that they contained hybrid particles along with the parental type. For example, both λ and ϕ170hy ($i^{\phi 170}$, h^λ) particles were liberated from *gal*$^-$(ϕ170dg) (λ) and both ϕ170 and λhy particles are liberated from gal$^-$ (λdg) (ϕ170). Generally we can find only one host range of the active phages among the liberated particles from *gal*$^-$(λdg) (ϕ170) or *gal*$^-$(ϕ170dg) (λ), while the lysates of double lysogens, such as (λ) (ϕ170) and (ϕ170) (λ) always contained phage particles having each of the two host ranges.

This clear distinction suggest that dg-particles do not possess of gene (or genes) controlling host range.

Table 3. Complementation test with λ sus

	λ sus phages spotted														No. of ϕ170 dg obtained
	N_7	O_8	P_3	Q_{21}	R_5	A_{11}	B_{10}	C_{20}	E_4	G_9	M_{87}	L_{63}	I_2	J_6	
Su$^-$(ϕ170 dg-a)	—	—	+	+	+	—	—	—	—	—	—	—	—	—	4
Su$^-$(ϕ170 dg-b)	—	—	+	+	+	+	—	—	—	—	—	—	—	—	6
Su$^-$(ϕ170 dg-c)	—	—	+	+	+	+	+	+	—	—	—	—	—	—	14
Su$^-$(ϕ170 dg-d)	—	—	+	+	+	+	+	+	+	—	—	—	—	—	1
Su$^-$(ϕ170)	—	—	+	+	+	+	+	+	+	+	+	+	+	+	
Su$^-$	—	—	—	—	—	—	—	—	—	—	—	—	—	—	
Su$^+{}_2$	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

Lystes of various λ sus phage mutants are spotted on defective lysogens *Su*$^-$(ϕ170dg). + : lytic zone appeared after incubation. — : lytic zone did not appeared. *Su*$^-$: non-permissive host, W3110, *Su*$_2^+$: permissive host, C 600

It was found that most of λ sus phages except for mutants having mutation on *N* or *O* cistron, can grow on *Su*⁻(φ170), while they cannot grow on nonlysogenic *Su*⁻ strains. So it is possible to examine the defective region of φ170dg by this complementation test (Iijima and Sakamoto 1968). When a lysate of λsus is spotted on a *Su*⁻(φ170dg) indicator in a soft agar layer, and incubated overnight, a lytic zone was appeared, if the λ sus was complemented with φ170dg prophage. Table 3 shows that independent isolates of *gal*⁻(φ170dg) were divided into some groups according to their complementation pattern.

The result shows that the order of cistrons is similar in those two related phages, but the defective region of each φ170dg is different from each other: some lose cistrons from *A* to *J* some *G* to *J*. If the order of cistrons is different from each other, we will obtain a somewhat irregular pattern, such as $A^+ B^- C^+ E^-$ or $A^- B^+ C^+ E^-$.

When the φ170dg or φ170 is integrated into the host chromosome, cistrons of prophage are arranged in a definite order on the host chromosome. By isolation of deletion mutants from a lysogenic strain we would infer the order of cistrons. Adhya et al. (1968) reported that chlorate resistant mutants were isolated by incubating cells in anaerobic condition in the presence of potassium chlorate and glucose, and that most of the chlorate resistant mutants are deletion mutants. This method was applied to a φ170 lysogenic strain, and a number of deleted mutants which lost some part of prophage genome along with the *chl* genes were isolated. The absolute order of φ170 prophage cistrons on the chromosome can be assumed from analysis of the deleted region of the host chromosome. According to the chromosome map of *E. coli* K12 (Taylor 1970), chlorate resistant mutation *chlA* locates at the right side of *bio* and *chlD* locates between *gal* and *attλ* (Fig. 1). If a deletion proceeds from *chlD* to prophage φ170, the left side part of the prophage genome should be deleted first. Examining a number of deleted mutants, we found that host range locus was deleted with *bio*, *uvrB* and *chlA*. Similarly, immunity locus was deleted with *pgl* and *chlD* (Table 4). From the result in Table 4 and the result of complementation test in Table 3, it is concluded that the order of φ170 prophage cistrons on the host chromosome is *gal*···*pgl*···*chlD*···*N*···*O*···*R*···*A*···*J*···*bio*···*uvrB*···*chlA*. This conclusion exclude the possibility that φ170 is inserted into the host chromosome in reversed direction.

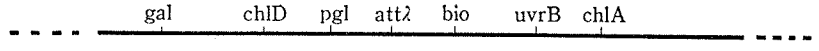

Fig. 1. Chromesome map of Escherichia coli K12.

Fig. 1 shows the partial chromosome map of E. coli K12, in the neighbor of *gal* and *bio*. *gal*: glactose utilization *chl* : chlorate resistant. *attλ*: attachment site of prophage λ (and lambdoid) *bio*: biotin requiring *uvrB*: ultraviolet sensitivity. pgl: 6-phosphogluconolactonase

Table 4. Deleted regions of chlorate resistant mutants of ϕ170 lysogen

Lytic zone on W3110	Lytic zone on W3110(λ)	bio	uvr	pgl	No. of mutants obtained	deleted region gal chlD pgl i h bio uvr chlA
−	−	−	S	+	1	←————————
−	±	−	S	+	25	←————————
∓	±	−	S	+	7	←————————
−	−	+	R	−	1	———→
∓	±	+	R	~	2	———→

A number of chlorate resistant mutants were tested their lysogeny, requirement for biotin (bio), UV sensitivity (uvr) and maltose blue gene (pgl, Kupor and Frenkel 1969). Table shows the presumed deleted region of these mutants

It was reported earlier that a double lysogenic strain for λ and ϕ170 was unstable and segregated off stable single lysogens (Iijima 1966). Lysogeny, is a stable characteristics, therefore, spontaneous elimination of prophage is extremely rare, except in special cases. Elimination of prophage occurs in the following cases 1) selection of non-lysogenic strain after treatment with mutagen. 2) heteroimmune superinfection 3) segregation of transductants such as *gal*$^-$(ϕ170dg) 4) segregation of double- or poly-lysogens for related temperate phages.

We supposed that two independent phenomena; segregation of double lysogens and curing of prophage by heteroimmune superinfection, are due to an interference of the two related phages of the same *att* site. Calef (1967) reported that the structure of the double lysogenic strain of λ was able to deduce by examining their monolysogenic segregants and it was linearly inserted in a tandem structure. Similarly from an analysis of segregational pattern of a heteroimmune double lysogen, we can assume the structure of the original double lysogen (Iijima and Sakamoto 1969). In the experiment for analysis of segregation, all strains are resistant to the both phages to exclude the possibility of reinfection and heteroimmune curing by superinfection of external phages, because the superinfected phage may result *trans* activation of prophage genome (Thomas 1966). In the previous report three possible types of integration of the second superinfecting phage were designated as α-, β- and γ- type. Guerrini (1969) and Signer et al. (1969) represented the attachement site of bacterial chromosome as BB' and that of phage genome as PP'. Using this notation, α-type integration is represented as an integrative recombination between BP' and PP' (abbreviated as $BP' \times PP'$). Similarly γ-type integration is represented as $PP' \times PB'$. β-type integration proceeds without intervention of *att* sites (Fig. 2 A).

As the segregation proceeds in a reciprocal way of integration, three possible types of excision of one complete set of phage genome were proposed in a previous paper, α'-, β'- and γ'-type excision. According to the present notation, α'-type excision is represented as an excisive recombination between BP' and PP' ($BP' \times PP'$). Similarly γ'-type excision is represented as $PP' \times PB'$, and β'-type excision again

does not involve a recombination of *att* sites (Fig. 2 B).

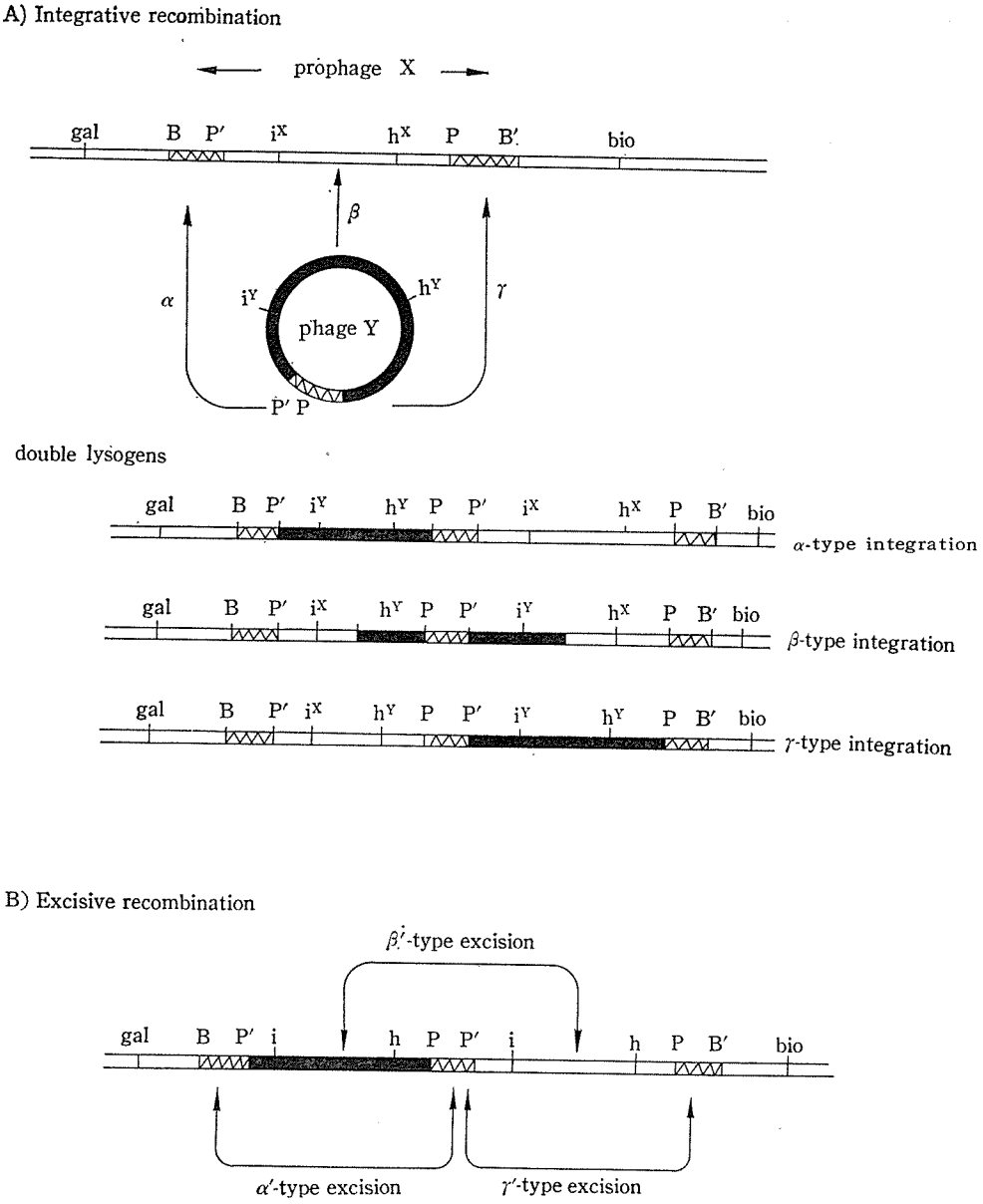

Fig. 2. Integrative and excisive recombination

The results in Table 5 are represented as the followings using the present notation. 1) When a nonlysogenic strain was superinfected by a temperate phage, the *att* site of phage PP' recombines with bacterial *att* site BB', and linearly inserted into the host chromosome (Campbell 1962). Thus we obtained a monolysogenic strain having two *att* sites with a sturcture of $BP'\cdots N\cdots R\cdots A\cdots J\cdots PB'$ on the host chromosome. 2) When heteroimmune phage particles are superinfected to this monolysogen, the *att* site PP' of the superinfected phage (second phage) recombines with two possible *att* sites BP' (left) and PB' (right).

3) The results of Table 5 suggest that most of the second phages recombine with BP' (left). The resulted double lysogens have a structure of $BP'\cdots N\cdots R\cdots A\cdots J\cdots PP'\cdots N\cdots R\cdots A\cdots J\cdots PB'$ (Fig. 2).

4) In excisive recombination from the double lysogens of α- or γ-type, α'-type excision occurs most frequently. As shown in Fig. 2, α-type double lysogens are represented by $BP'\cdots PP'\cdots PB'$ and the γ-type double lysogens are also $BP'\cdots PP'\cdots PB'$. The result in Table 5 shows that $BP' \times PP'$ (α'-type excision) occurs more frequent than $PP' \times PB'$ (γ'-type excision), and nonlysogenic strain ($BP' \times PB'$) is not found.

5) Double lysogens for $\phi 80$ and other lambdoids do not segregate under the condition of the experiment. It suggests that segregation occurs when the two prophages locate on the chromosome in a tandem structure. The phenomena of segregation of double lysogens and curing the prophage by heteroimmune superinfection suggest that the two prophages lysogenize at the same locus and have a tandem structure.

6) Stable double lysogens possessing $\phi 170$ and λ prophage were isolated. The structure of this double lysogen is not clear at present. Parhaps it may be different in structure.

Table 5. Segregational pattern of double lysogens

Strains used (double lysogen)	No. of colonies tested	No. of double lysogen	type of segregation α'	β'	γ'	No. of segregant (%)	type * of integration
F2320- 3 : $(\lambda)/\lambda$ $(\phi 170)/\phi 170$	648	626	16	4	2	22 (3.4)	α (7)
F2320-15 : $(\lambda)/\lambda$ $(\phi 170)/\phi 170$	648	423	190	1	34	225 (35)	γ (2)
F1911-27 : $(\phi 170)/\phi 170$ $(\lambda)/\lambda$	647	282	328	3	34	365 (56)	α (17)
F1911-29 : $(\phi 170)/\phi 170$ $(\lambda)/\lambda$	648	621	20	5	2	27 (4.1)	γ (4)
F1911-21 : $(\phi 170)/\phi 170$ $(\lambda)/\lambda$	6480	6480	0	0	0	0 (0)	(1)
F2631 : $(434)/\phi 170$ $(\lambda)/\lambda$	2968	2801	141	12	9	162 (5.5)	α (2)
F2632 : $(434)/\phi 170$ $(\lambda)/\lambda$	7420	7336	54	29	1	84 (1.1)	γ (1)
F2619 : (82) $/\phi 170$ $(\lambda)/\lambda$	3180	2620	338	193	29	560 (17.5)	α (1)
F2621 : $(\phi 80)/\phi 80$ $(\lambda)/\lambda$	2590	2590	0	0	0	0 (0)	
F2623 : $(\phi 80)/\phi 80$ $(\phi 170)/\phi 170$	3240	3240	0	0	0	0 (0)	

* Figures in parenthesis shows the number of strains defined to each type.

When a temperate phage is linearly inserted into the host chromosome a site specific recombination enzyme (*int*) of phage, and a specific site of the phage PP' and that of bacteria BB' are concerned in the process (Signer and Beckwith 1966, Zissler 1967, Gingery and Echols 1967, Weil and Signer 1968, Gottesman and Yarmolinsky 1968). Guarneros and Echols (1970), Kaiser and Masuda (1970) isolated a new mutant *xis* from bacteriophage λ. The phage mutant *xis* is able to ingterate into the host

chromosome but unable to excise from the host chromosome of the single lysogen. Echols (1970) analysed the specificity of integrative and excisive recombination using phages with specific *att* sites. The result showed that *int* protein was essential for both integrative and excisive recombination but products of *xis* gene was essential only for excisive recombination. He also showed that $ba' \times ab'$ ($BP' \times PB'$) was effectively carried out by both *int* and *xis*, while $bb' \times aa'$ ($BB' \times PP'$) was carried out by *int* only.

From an analysis of segregational pattern in Table 5, we can assume not only the structure of double lysogens but also the efficiency of recombination between the specific site on the host chromosome and that on the phage genome. The result in Table 5 are summarized that integrative recombination between $BP' \times PP'$ is more frequent than that of $PP' \times PB'$. Excisive recombination between $BP' \times PP'$ is more frequent than that of $PP' \times PB'$. Similarly an analysis of segregational pattern from gal$^-$(ϕ170dg) (λ)/ λ or gal$^-$ (λdg) (ϕ170)/ ϕ170, it was concluded that integrative recombination between PP' and BB' is more frequent than that of $PP' \times BP'$, where the *att* site of active superinfecting phage is PP' and those of the (ϕ170 dg) on (λdg) are $BP' \cdots BB'$. At the same time, excisive recombination from $BP' \cdots BP' \cdots PB'$ proceeds more frequently between BP' and BP'. Thus the frequency of integrative recombination is the following order $PP' \times BB' > PP' \times BP' > PP' \times PB'$.

In the above section, it is shown that phage ϕ170 has a different immunity specificity and host range, but its genetic structure; arrangement and function of cistrons are analogous to those of λ phage. It is also suggest that prophage ϕ170 lysogenize *att* λ, because segregation occurs in double lysogens for ϕ170 and other lambdoids, except ϕ80, and curing of prophage ϕ170 also occurs by heteroimmune superinfection by λ and other lambdoids.

The mechanism of the segregation is not clear at present. However, a site specific recombination system might be activated at a low level, even in a state without heteroimmune superinfection or without induction of prophage (Signer 1970). In the analysis of segregants from double lysogens, (λ)/λ (ϕ170)/ϕ170 or (ϕ170)/ϕ170 (λ)/λ, we can find the definite three types of segregants. But when a recombination negative mutant (*rec*$^-$) was used as a host, segregational pattern from *rec*$^-$ double lysogen is different from that of *rec*$^+$ double lysogen. In the case of *rec*$^-$ host, β'-type excision was not found, although α'- and γ'-type excision is remained. Therefore, it is assumed that β'-excision proceeds by Rec system, as in the case of segregation of defective transductants, such as *gal*$^-$ (ϕ170dg) and *gal*$^-$(λdg), but α'- and γ'-excision involving attachment sites proceed by other system, presumably by Int and Xis. As mentioned above, the result obtained from analysis of segregants coincide with that of Echols (1970) obtained by phage recombination, although a little difference is noticed.

Viruses are independent genetic system which are subject to evolutionary changes and appear to be close relations of the genetic materials of the host. The genome of a temperate phage may integrate into and excise from the genome of a host by a elegantly

evolved systems. The integrated phage genomes endow the host with new characteristics. The analysis of site specific recombination system implies to elucidate a general feature of recombination between homologous sites and also between nonidentical specific sites on the chromosome. It is also implies to understand the interaction of host genome and external genetic materials, and evolutionary changes in temperate phages.

Summary

1) Phage ϕ170 is different from λ and λ-related phage in immune specificity. 2) Phage ϕ170 can transduce *gal* markers to *gal*$^-$ mutants of *E. coli* K12, the transduction of *gal* marker mediated by a defective phage ϕ170dg. 3) Arrangement and function of cistrons of ϕ170 is analogous to λ except for *N*—*O* region. 4) Attachment site of ϕ170 prophage is the same site as that of λ (*att* λ), so the curing of prophage and segregation of double lysogens occurred. 5) Analysis of segregational pattern of various double lysogens, we can assume not only the structures of double lysogens but also the efficiency of integrative and excisive recombination of *att* sites. 6) Segregation of double lysogens and defective lysogens, such as *gal*$^-$(ϕ170 dg), occurs in intervention of Rec system. It may occur also in intervation of product (*s*) of Int and other genes.

Acknowledgement

The authors wish to thank to Dr. A. Campbell, Dr. T. Fukasawa, Prof. A. Matsushiro, Dr. N. Otsuji and Prof. H. Ozeki for their supply of bacteria and phage strains. The author also indebted to Dr. T. Hasegawa, the Director of this institue for his active interest, and Miss H. Ootsuji for her technical assistance. This work was supported in part by the Research Grant from the Ministry of Education.

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IFO Res. Comm. 5,
20–23, 1971 (*March*)

A NATURAL VARIANT OF STREPTOMYCES LAVENDULAE

Kôiti NAKAZAWA

It was already reported that the *Actinomyces* sp. 700 (*Streptomyces* sp. 700) isolated by the author produced a streptothricin-group substance (Nakazawa, 1950).[1] This suggested that *Streptomyces* sp. 700 could be concluded to be a strain of *Streptomyces lavendulae* (Waksman et Curtis) Waksman et Henrici. The identity of *Streptomyces lavendulae* has been recognized by the production of a characteristic lavender-colored aerial mycelium on various media and of a soluble brown pigment on protein media.[4] During a continued observation of *Streptomyces* sp. 700, the author isolated a natural variant from a slant culture of this strain in 1952 and assigned the number, 700A to it. The variant had blue color aerial mycelium on various media but had quite the same morphological and physiological characteristics as *Streptomyces* sp. 700. These characters are unchanged to this day, and at present, it seems worthy to describe such an unusual natural variant. The present note deals with the description of the taxonomical characteristics of both strains of *Streptomyces* sp. 700 and the variant, 700A.

The color of sporulating aerial mycelium at maturity is one of the significant criterion in the streptomycete taxonomy. Pridham et al.[5] divided the species of Streptomyces into six series based on the color of their aerial mycelium; namely, white, olive-buff, yellow, blue, red, and gray series. As shown in the plate, *Streptomyces lavendulae*

Table 1, Utilization of carbon sources[2]

	St. 700	St. 700A
Control	—	—
Glucose	++	++
Maltose	++	++
Sucrose	—	—
Fructose	+	+
L-Arabinose	—	—
D-Mannitol	—	—
Raffinose	—	—
Xylose	—	—
Lactose	—	—
Rhamnose	—	—
Mannose	++	++
Inositol	—	—

—: no growth, +: scant growth, ++: good growth.

Table 2. Cultural characteristics of *Streptomyces* 700 and 700A

	St. 700	St. 700A
Aerial mycelium Spore surface	Straight Smooth	Straight Smooth
Czapek's agar	Moderate growth Am: Scant Reverse: Colorless	Moderate growth Am: Scant Reverse: Colorless
Glucose asparagine agar	Good growth Am: Pale Brownish Vinaceous (Rdg. XXXIX, 5‴ -f)* Reverse: Tilleul-Buff (Rdg. XL, 17‴ -f)	Good growth Am: Deep Bluish Glaucous (Rdg. XLII, 27‴ -d) Reverse: Olive Buff (Rdg. XL, 21‴ -d)
Inorganic salt starch agar	Good growth Am: Tilleul Buff (Rdg. XL, 17‴ –f) Reverse: Pale Olive-Buff (Rdg. XL, 21‴ -f)	Good growth Am: White Reverse: Pale Olive Buff (Rdg. XL, 21‴ -f)
Yeast starch agar	Good growth Am: Scant, Pale Brownish Vinaceous (Rdg. XXXIX, 5‴ -f) Reverse: Colorless	Good growth Am: None Reverse: Colorless
Bennett's agar	Good growth Am: Light Grayish Vinaceous (Rdg. XXXIX, 9‴ -d) Soluble pigment: Faint Brown Reverse: Deep Brownish Drab (Rdg. XLV, 9‴ -i)	Good growth Am: Dark Bluish Glaucous (Rdg. XLII, 37‴ -b) Reverse: Dark Greenish Olive (Rdg. XXX, 23″ -m) Soluble pigment: Brown
Yeast extract malt extract agar	Good growth Am: Light Brownish Vinaceous (Rdg. XXXIX, 5‴ -d) Reverse: Clay color (Rdg. XXIX, 17″) Soluble pigment: Faint Brown	Good growth Am: Deep Bluish Glaucous (Rdg. XLII, 37‴ -d) Reverse: Deep Olive Buff (Rdg. XL, 21‴ -b) Soluble pigment: Brown
Glycerin asparagine agar	Moderate growth. Am: Pale Brownish Vinaceous (Rdg. XXXIX, 5‴ -f) Reverse: Colorless	Good growth, Am: Scant white. Reverse: Vinaceous Buff (Rdg. XL, 17‴ -d)
Ca malate agar	Growth colorless. Am: White Soluble pigment: None	Growth colorless to black. Am: None Soluble pigment: Purple
Bouillon agar	Good growth. Am: None Soluble pigment: Army Brown (Rdg. XL, 13‴ -i)	Good growth. Am: None Soluble pigment: Army Brown (Rdg. XL, 13‴ -i)
Glucose bouillon agar	Good growth. Am: None Soluble pigment: Army Brown (Rdg. XL, 13‴ -i)	Good growth. Am: None scluble pigment: Army Brown (Rdg. XL, 13‴ -i)
Litmus milk	Surface Ring. Peptonization weak.	Surface Ring. Complete peptonization after 14 days.
Potato plug	Good growth. Am: Scant, Pale Brownish Vinaceous (Rdg. XXXIX, 5‴ -f) Darkend the plug. Brown soluble pigment.	Good growth, black. Am:None. Darkend the plug. Brown soluble pigment.
Carrot plug	Am: Light Grayish Vinaceous (Rdg. XXXIX, 9‴ -d)	Am: Dark Bluish Glaucous (Rdg. XLII, 37‴ -b)
Tyrosin agar	Melanoid pigment produced.	Melanoid pigment produced.
Nutrient nitrate broth	Nitrit from nitrate	Nitrit from nitrate
Gelatin	Liquefied	Liquefied

Am: Aerial mycelium. * Ridgway: Color Standard and Color Nomenclature (1912).

Table 3. Maximal NaCl tolerance of Streptomyces 700 and 700A[3].

NaCl %	St. 700	St. 700A
1	++	++
2	++	++
3	++	++
4	++	+
5	+	±
7	−	−

700 belongs to the red series while the natural variant 700 A stated here belongs to the blue series. Tresner et al.[3] reported the relationship of NaCl tolerance to various taxonomic features and suggested the usefulness of this property as a taxonomic criterion. The streptomycetes belonging to blue series were pointed to have rather higher tolerance to NaCl whereas red series to have lesser tolerance. The maximum NaCl tolerance of the blue-spored variant 700A is the same as that of original strain 700, and is not that in the species of blue series. As far as the properties investigated the both strains, 700 and 700 A still now agreed well with each other except for the color of their aerial mycelia.

Summary

The description of a blue color natural variant 700 A of *Streptomyces lavendulae* 700 has been made. The variant was isolated in 1952. Its characteristics are unchanged until today.

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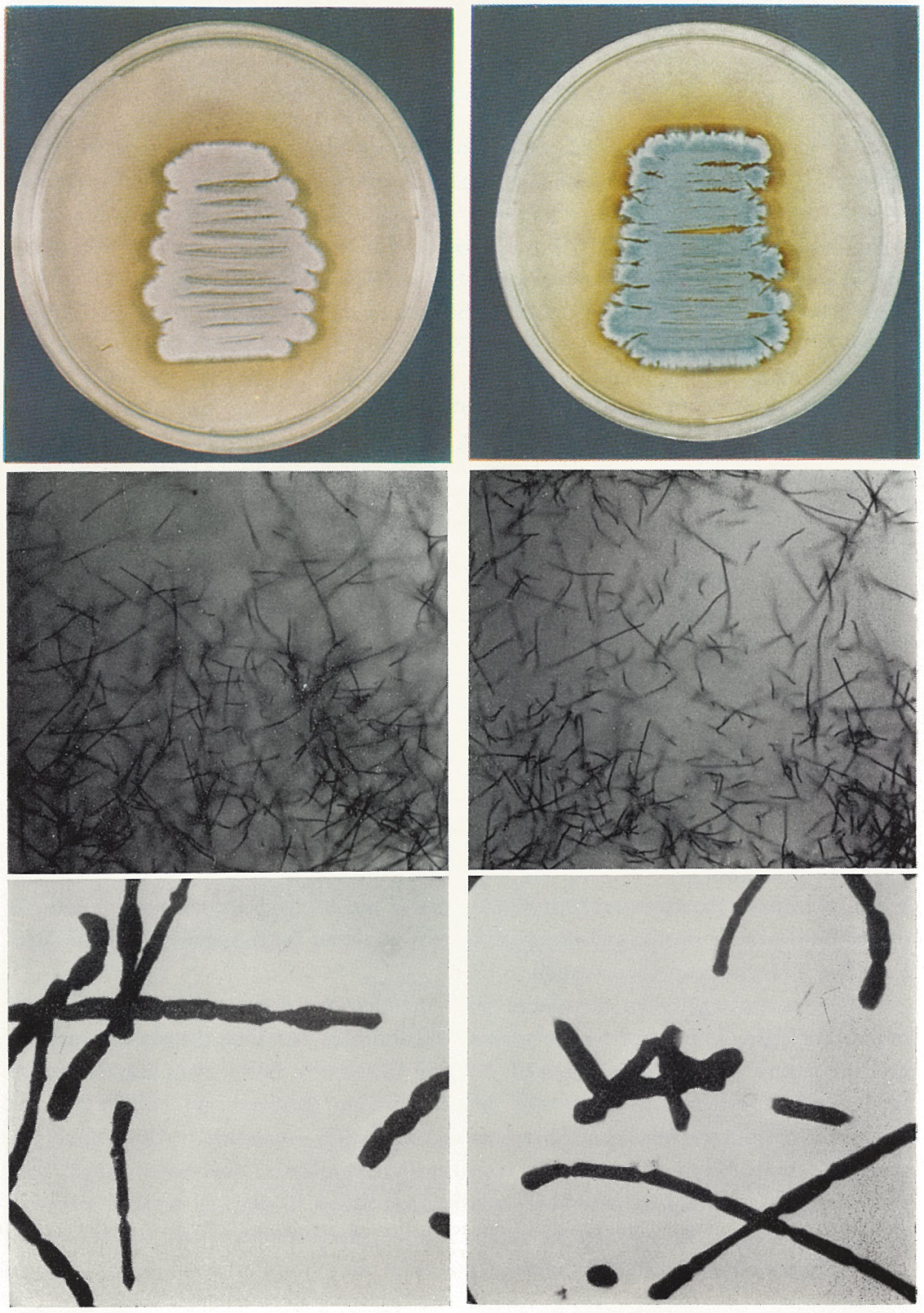
Photo. 1. Colonial and microscopical characteristics of *Streptomyces* sp. no. 700 (left side) and no. 700A (right side). Upper: Aerial mycelia on agar media: Middle: Morphology of aerial mycelium (×200); Lower: Spore surface under electron microscope (×6000).



IFO Res. Comm. **5**,
24～42, 1971 (*March*)

SUCCESSIVE FUNGAL FLORA ON STERILIZED LEAVES IN THE LITTER OF FORESTS. I.

Keisuke TUBAKI and Tatsuo YOKOYAMA

In recent years the succession of microfungi which colonize on a plant debris while it is still in situ on the plant and later during the process of its incorporation into soil has been dealt by many scientists. The application of the microhabitat concept has provided the knowledge about the fungi on leaf, stem and other tissue during the period that the identity of these microhabitats is retained. Fungal population in a litter of herbaceous plants has been extensively examined by such scientists as Webster (1956, 1957, 1960), Hudson & Webster (1958), Pugh (1958), Hudson (1962) and others. On the fungi in a litter of *Pinus sylvestris*, studies have been made intensively by investigators including Gremmen (1957), Kendrick (1958 a, b), Kendrick & Burges (1962) and Hays (1965); that of *P. densiflora*, by Ishii (1967) and Tubaki & Saitô (1969). On the other plant organs including the leaves of the trees such as *Alnus, Quercus* or beech, the results have been less frequently reported by Wiltkamp (1960), Chesters (1950), Cadwell (1963) and Carré (1964), Dickinson & Pugh (1965) and others.

From these previous studies, many individual species have been encountered in these succession on the leaves and constant patterns of decomposition of the organs by microfungi have been recently presented by Dickinson & Morgan-Jones (1966). Dickinson (1965) shows that three groups can be delimited among the phylloplane fungi associated with *Halimione*-leaves: those that the fungi present only as propagules on the leaves, those growing actively and sporing on the leaves, and thirdly a group which only produces vegetative mycelium while the leaves remain green. At the onset of senescence, fungi in the latter group may sporulate and the leaf is also invaded by a number of saprophytes not actively associated with the phylloplane. However, not much has been known about the patterns of access on the initial fungal colonists which eventually form a major part of the flora of the decaying leaves and also patterns of the first invador during the decay of the leaves either on the plant or on the litter surface are not investigated as much.

The microfungal flora associated with the decomposition process of the plant organs are highly influenced by environmental conditions in which the plant is growing and the nature of the organs obtained. Even in the case of fallen plant leaves, various degrees of decomposition viz. brown, blackish brown, decayed and heavily decayed are results of both the natural environment and the fungal succession. Above concepts, therefore, have followed the present experimental studies on successive fungal flora continuously from that on moribund, newly dead leaves to that on decayed leaves in the litter. Hence, we attempt to obtain a new interpretation about the successive colonization of the embedded sterilized leaves in the naturally developing litter by

fungi which may include saprobic and facultative parasites. These can exist saprobically on the remains of their host plants. Many of them naturally consist of ubiquitous species. Such a situation is free from the artificial influence of cultivation and is of interest that it is subject to environmental influences including various microorganisms.

Materials and Method

The plant stands examined were in three naturally developed forests in the vicinity of Ootsu, Shiga Pref. The selected plants are *Castanopsis cuspidata* Schottky and *Quercus phillyraeoides* A. Gray. They are evergreen plants growing dominantly in the temperate forest in Japan; leaf formation and fall occur throughout the year, but the rates of formation and fall differ from season to season. Green and newly detached leaves of both plants were collected in sterile polyethylene bags. The former, fully expanded and undamaged and still on the plant, were taken directly from the branches falling to the litter surface; the latter were picked up at random from the litter surface.

Two methods were employed for the sterilization of the collected leaves. The leaves were completely dried by air current in the laboratory for two days and then sterilized in the screw-capped bottles saturated with propylene-oxide gas for over 24 hours. This procedure is suitable only for the surface sterilization of thin dried leaves, however, as it is often insufficient for killing all propagules already emerged below the cuticle which covers the leaves. When we used this procedure practically, bacteria or fungus-mycelia sometimes developed from the gas-treated *Castanopsis*-leaves on the agar medium. In practice, therefore, irregular may occur due to the insufficient sterilization.

The second method applied was an ordinal sterilization of the leaves by autoclave. After trials, the method was standardized as follows. Green and newly detached leaves were dried for one day by air current at the room temperature. Then each thirteen leaves of both plants were sandwiched between a square stainless-wire net (10×15 cm)(Pl. 2 C) which was then wrapped by the alminium-foil and autoclaved for 20 minutes at 120°C. As immediately as possible, these traps were embedded in the litter of three stations (Pl. 1): forest of Togakushi Shrine (A), of Ishiyama Temple (B) and of Arato Shrine (C). The forests have Laurilignosa-Laurisilva, typically of temperate zone which are mixed with two plants above and other evergreen trees, and the ground is covered with the litter up to 5–10 cm in depth. (Pl. 2 A). The upper layers of the litter was gently taken off partially and the twelve prepared traps (green leaves trap, 6; dead leaves trap, 6) were put in order in a plane over the lower bed of the litter (Pl. 2 B), the traps were covered completely with the removed leaves again. Hence, the traps were sandwiched between the upper and the lower layers of the litter. The sterilized leaves were surrounded by the natural litter communities and were easily colonized by a large number of microorganisms occurring in this zone. The actively growing saprophytes may invade the leaves through meshes of the wire-net

and inactive propagules may be carried in by minute animals, rain or even by other microorganisms. Each trap was then collected to the laboratory once a month during the period of October, 1969 to March, 1970. The traps were opened in the laboratory and then the leaves were put in the sterile petri dishes with a small amount of sterile water, then incubated under moist-condition for at least one month at the room temperature. During the incubation, every leaf-surface was examined every day under the binocular disecting microscope and all fungi were isolated or mounted for detailed observation. For the isolation, a malt extract agar with added aureomycin (50 γ per ml) was employed. The isolates except ubiquitous fungi viz. *Penicillium, Cladosporium, Trichoderma*, etc. were re-inoculated onto the gas-sterilized leaves of *Castanopsis* or *Quercus* which were previously put on the plain agar plates. By this method of cultivation, many sterile fungi or less-sporulating fungi may come to develop the fruiting structures or typical sporulation, and even the perfect states may be developed from some imperfect isolates.

Results

A list of genera observed is given in Table 1. The species of fungi isolated from the leaves together with their frequency of occurrence on the various leaf condition are listed in Table 2.

Autoecology and Species

Phycomycetes: Mucorales is generally known as not colonizing initially on herbaceous debris but can develop on plant materials after they are incorporated into soil and decomposed. In the present study, however, five genera were found on the leaves of rather early decay. *Absidia* was found only from Station B and all are *Ab. glauca* Hagem. *Backusella circina* Ellis & Hesseltine (+)* was found only once in Station A. This species has been so far found from the soil of Florida and New Jersey (Ellis & Hesseltine, 1969), and might be introduced from the soil during the decay. *Cunninghamella echinulata* (Matr.) Thaxter was found only from six-month-decayed leaves and might be introduced from the soil. *Mortierella* has been fairly commonly encountered throughout the study, but seems to be much frequent during the process of early decay. As generally known, most of *Mortierella* are originated from soil. Therefore, those found in the present study would be encountered from soil habitats. It is interesting, however, that they are less frequently found on the leaves of the longer decay, more than four months, although they are not so important colonizers of the leaves. Among *Mortierella, M. isabellina*-series was most common. *Mucor* was mainly limited to the leaves of the early decay and completely absent after three months. Most species belong to *M. genevensis* Lendner, *M. hiemalis* Wehmer, *M. mucedo* Bref.

* Identification was kindly carried out by Drs. Hesseltine and Ellis, Northern Research & Development Division, Peoria, USA, to whom we owe many thanks.

Table 1. List of genera of fungi in the three communities examined.

Phycomycetes	
Absidia	*Mortierella*
Backusella	*Mucor*
Cunninghamella	
Ascomycetes	
*Arachniotus**	*Mollisia*
Ceratocystis	*Toxotrichum*
Fungi Imperfecti	
*Actinopelte**	*Harpographium**
Alternaria	*Hyalodendron*
*Arthrinium**	*Idriella*
*Beltrania**	*Illosporium**
Botrytis	*Macrophoma**
Calcarisporium	*Monacrosporium*
Catenularia	*Myrothecium**
Cephalosporium	*Oidiodendron*
*Chaetopsina**	*Paecilomyces*
Chalara	*Penicillium*
Chloridiella	*Pestalotia*
Chloridium	*Phialophora*
Chrysosporium	*Phoma*
Cladosporium	*Polyscytalum*
Clathrosphaerina	*Ramularia*
Codinaea	*Rhinocladiella*
Crinula	*Spondylocladiella**
*Cryptophiale**	*Sporothrix*
Dactylaria	*Stilbum*
*Dendrophoma**	*Subulispora*
Dendryphiella	*Sympodiella**
*Dinemasporium**	*Thysanophora*
Fusarium	*Trichoderma*
*Gonytrichum**	*Verticillium*
Basidiomycetes	
*Marasmius**	
Myxomycetes	
Stemonitis	

* .... observed only on the naturally decayed leaves.

Table 2. Seasonal occurrence of fungi on the sterilized leaves in three stations (A, B & C).
Growth extent was symbolized by ○ and ●. ○～ growth is apparent, but less intensive, not appearing on entire surface. ●～ growing actively or often covering entire surface. 1 & 2=the sterilized green & dead leaves of *Castanopsis*. 3=sterilized leaves of *Quercus*.

Station A

genus	Oct.			Nov.			Dec.			Jan.			Feb.			March		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Backusella	○																	
Mortierella		○			○						○			○				
Mucor	○	○	○	○	○													
Ceratocystis	○	●	○		○		○	●	○		○	○	○	○	○		○	○
Mollisia												○			○			
Toxotrichum								○						○			○	
Calcarisporium			●					○						○				
Cephalosporium	○	○			○								○					
Chalara			●								○				○			
Chloridium			○												○			
Cladosporium	○	○	○	○	○	○					○		○	○	○	○	○	○
Codinaea					○			○			○			○			○	
Crinula																○		
Dactylaria								○		○		○		○				○
Dendryphiella															○			
Hyalodendron									●					○				○
Idriella					○					○								
Monacrosporium														○				
Oidiodendron		●			○			●	○	○	○	○	○	○	○		○	○
Paecilomyces	○	○					○											
Penicillium	●	●	●	●	●	●	○	○	○	○	○	○	○	○	○	○	○	○
Pestalotia	○																	
Phoma												○						
Ramularia				●		○	○		○		○		○			○		
Rhinocladiella	○						○	○										
Sporothrix		○																
Subulispora	●	○	●	●	○	○	●		○	●		●	○		○	○	○	○
Sympodiella			○					●	○		○	○		○			○	○
Thysanophora										○								
Trichoderma	○	○	●		○	○	○	○	○		○	○	○	○	○		○	○
Verticillium					○					○								

Station B

genus	Oct.			Nov.			Dec.			Jan.			Feb.			March		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Absidia				○	○		○				○							
Mortierella					○			○										
Mucor	○	○				○												
Ceratocystis	○		○	○	○	○	○			○		○				○	○	
Toxotrichum				○														
Alternaria			○									○						
Botrytis												○						
Calcarisporium		○						○	○		○	○		○		○	○	
Catenularia								○			○							
Cephalosporium		○	○	○									○	○				
Chalara										○								
Chloridium																	○	
Cladosporium	○	○	○			○						○			○	○		
Codinaea					○	●	○				○					○		
Fusarium															○			
Idriella	○		●						○	○		○	○			○		
Monocillium														○			○	
Oidiodendron				○	○		○	●	○		●			○	○	○	○	
Paecilomyces	○			○													○	
Penicillium	○	●	●	○	○	○	○	○	○	○	○	○	○	○	○	○	○	○
Pestalotia	○		○	○								○						
Polyscytalum										○	○	●						
Ramularia				○						○			○					
Rhinocladiella			○															
Sporothrix													○					
Subulispora	○		●	○	○		○			○	○		○					
Sympodiella	○						○									○		
Thysanophora							○											
Trichoderma	○			○	○	○	○	○	○	○	○	○	○	○	○	○	○	
Verticillium								○			○					○		
MYXOMYCETES(*Stemonitis*)										○		○					○	

Station C

genus	Oct.			Nov.			Dec.			Jan.			Feb.			March		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Cunninghamella																		○
Mortierella	○			○	○	○			○		○	○						○
Mucor	○	○			○													
Ceratocystis		●	○	○	○		○	○	○			○	○	○			○	○
Toxotrichum											○			○	○		○	
Botrytis							○									○		○
Calcarisporium		○					○	●	○					○			○	
Catenularia										○		○						
Cephalosporium	○	○			○				○									
Chalara				●	○	●	○		●	○		○	○	○	○	○	○	○
Chloridium		○											○			○		
Cladosporium	○		○	○	○	○					○		○	○		○		
Clathrosphaerina																	○	○
Codinaea				○					●		○			○	○		○	
Crinula												○				○		○
Idriella	○	○	○	○	○	○	○			○		○	○				○	
Oidiodendron		○			○			○		○	○	○	○	○		○	○	○
Paecilomyces					○				○		○							
Penicillium	○	●	○	○	○	○	○	○	○	○								
Phialophora																		○
Polyscytalum				○														
Ramularia	○			●														
Stilbum													○					
Subulispora	○		●	●		○	○		●	○		○	○		○	○		○
Sympodiella	●	●												○				
Thysanophora	○			○	○	○	○	○										
Trichoderma	○	○		○	○		○	○	○	○	○	○		○		○	○	○
Verticillium											○						○	
MYXOMYCETES		○	○															

and *M. varians* Povah, and they are considered to be introduced from soil.

Ascomycetes: *Ceratocystis* is known as an economically important fungus and several of the genus are responsible for blue stain or plant diseases. It is significant that *Ceratocystis* is not uncommon or often dominant on the leaves throughout the study. Perithecia of *Ceratocystis* developed solitarily or widely scattered on the leaves of even one-month decay. *Sporothrix*-state of *Ceratocystis* (Barron, 1968) is also common. Species of *Ceratocystis* on the leaves belongs to a single species.

Mollisia, a genus of the Discomycetes, was first encountered after three-month decay limiting to one microhabitat. Species of *Mollisia* is generally not uncommon to the well decayed leaves, barks, twigs or other plant debris and is considered to be able to grow on these materials after decomposition proceeds greatly.

Toxotrichum first developed the cleistothecia in abundance on the leaves of the early decay and was then encountered on seven occasions in all stations during the study. Six of the eight collections have been made on the newly detached leaves and one on the green leaves of *Castanopsis*; only one record was made on the dead leaves of *Quercus* as shown in Table 2. All isolates belong to a single species, *Tox. cancellatum* (Ph.) Orr & Kuehn. The conidial state of it is *Oidiodendron* as shown by these authors (1964) which is rather slow growing, restricted and grayish blue under culture. The fungus has been recorded historically from plant debris or soil.

Fungi Imperfecti: *Alternaria*, an ubiquitous fungus, has been found only twice on the detached leaves of *Quercus* in Station B, although it is one of the commonest fungi on forest leaves and also of airborne contaminants. This shows to some extent that *Alternaria* can live saprophytically on materials derived from the break-down of plant remains but are more likely develop the food materials naturally from the living plant and then produces fruiting structure after the death of their hosts.

Botrytis is also ubiquitous like *Alternaria* and the all isolates belong to *B. cinerea*-type.

Calcarisporium is generally associated with decaying fruitbodies of higher fungi, particularly Basidiomycetes. Therefore, it is significant that this fungus has been found on the leaves throughout the study fairly commonly. All isolates belong to *C. arbuscula* Preuss and produce sclerotium-like bodies under culture. The present result seems to indicate that this fungus exists saprophytically on plant remains in litter and is capable of parasiting upon such host materials when it has contact with them in a proper condition.

Catenularia has been found on four occasions on the leaves of both plants only of three- and four-month decay in Station B and C. In Station B, the collections were restricted on those of detached *Castanopsis*-leaves. Most of the isolates may belong to a single species (unidentified).

Chalara is not one of the uncommon colonizers developed during the decay and was most common on the leaves in Station C and did not reduce in frequency even in the latest process during the present experiment. Its growth is white and often spread widely on the surface of the leaves. Though it is difficult to identify all isolates with

species, a single species is particularly dominant and is identified with that described by Barron (1968). Phialophores are slightly inflated in the lower half and long and tubular in the upper half; small and oblong phialospores, truncate at both ends, produced in a long fragile chains. Its growth is especially marked by the veins of the leaf.

Chloridium is not uncommon under the study in all leaves and all isolates belong to *Chl. chlamydosporis* (v. Beyma) Hughes which is a common soil-borne fungus, and this record indicates that the fungus was introduced from the surrounding soil.

Cladosporium is known as the commonest primary saprophyte and, in the present study, many isolates were obtained, belonging mostly to *C. herbarum* (Pers.) Link ex Fr., *C. cladosporioides* (Fres.) de Vries and *C. sphaerospermum* Penzig.

Clathrosphaerina has been found twice on detached leaves of *Castanopsis* and *Quercus* of the only six-month decay. This occurrence is reasonable because *C. zalewskii* v. Bew. develops only on the dead, decayed leaves (Tubaki, 1958).

Codinaea is one of the common fungi on the leaves of more than two-months' decay, sometimes spreading widely on the leaves. Dominant species are *C. simplex* Hughes and probably conidial state of *Chaetosphaeria dingleyae* Hughes, Kendrick & Shoemaker. Almost all the collections were made on the detached leaves; only two small growths were observed on the green leaves.

Crinula, a characteristic synnematous fungus, developed a few fresh-gelatinous, yellow or dark brown synnemata on the leaves mostly during the latter half of the decay; species is unidentified.

Dactylaria is known as a common leaf-fungus. However, only one species, *D. purpurella* Sacc. has been found on the leaves of three- to six-month decay only in Station A; its growth is white and powdery.

It should be noticed that *Fusarium* was extremely rare in the present study. *Fusarium* is a typical pathogenic fungus and the commonest in the cultivated field. However, it appeared that the fungus is rather rare in the natural, not-cultivated soil and may increases in number as the soil is cultivated. *Fusarium* can remain as mycelia or chlamydospores in the soil after the host tissues are decomposed and can parasitize when associated with the plant surface, while the three present stations are not in a cultivated condition and therefore, they might be unsuitable communities for *Fusarium* itself.

Hyalodendron developed white fragile growth on different leaves in Station A only and is considered to be *H. lignicola* Diddens so far.

Idriella and allied genera are not uncommon during the decay of leaves, especially common in Station C. Those found on the leaves of early decay, two- and three-month, is Stations A and C are *Chloridiella* sp. I (Kiffer & Reisinger, 1970) which is close to *Chloridiella leucopoda* (Bon. ?) Arnaud.

Monacrosporium ellipsosporum (Grove) Cooke & Dickinson was found only once on the detached leaves of *Castanopsis* of the five-month decay and is expected to be not uncommon on more decayed leaves so far as other microflora on the natural litter leaves are concerned. This is a only predacious fungus in this study.

Oidiodendron is one of the common leaf-fungi in this study in all stations and mainly consists of the two groups; one is yellowish and the other is blue-grayish. The yellowish group, probably *O. citrinum* Barron, over-grew even on the leaves of one-month decay and was dominant more or less on the detached *Castanopsis* leaves. The blue-grayish group, a conidial state of *Toxotrichum cancellatum* as described previously, is also common but not restricted to one kind of host leaves. In considering the gross flora of *Oidiodendron*-species, there is a noticeable succession on the leaves. Yellowish *Oidiodendron* is present at first on the leaves of very early decay and has a tendency to decrease over a long period of time, while blue-grayish *Oidiodendron* is inclined to appear rather in a latter half of the decay which has become suitable also for the perfect state for their formation.

Paecilomyces is a ubiquitous member, but appeared in a rather early period of the decay. Dominant species are *P. farinosus* (Dick. & Fr.) Brown & Smith, *P. bacillisporum* Onions & Brown, *P. elegans* (Cda.) Mason & Hughes and *P. varioti* Bain.

Penicillium, generally known as ubiquitous and omnivorous, has been very commonly recorded on all leaves in all the stations. But the ecological distribution of the penicillia during the decay within each community showed a remarkable inequality. The leaves of the one month decay were colonized extensively by the *Penicillium*-species often overgrew the whole surface and, in this case, dominant species are *P. implicatum* Biourge, *P. oxalicum* Currie & Thom, *P. janthinellum* Biourge, *P. thomii* Maire and *P. expansum* Link. *P. implicatum* was first present even on the leaves of one-month decay. *P. thomii* was dominant and characteristic on the leaves throughout the study, developing many pinkish sclerotia on the aerial parts of the leaves; later, conidial fructifications grew on top of the sclerotia.

Pestalotia has been found mostly on the sterilized green leaves only during the very early decay of the process. This result indicates that this fungus prefers rather the green, functional leaves. However, the traces of the spots were uncertain on those on the later decay because many other fungi grew since then covering these older spots. Much more data are needed on the leaf ecology in the case of *Pestalotia*.

Polyscytalum was sometimes not infrequent, especially on three kinds of leaf-samples of the four-month decay in Station B. The species is uncertain. Conidia are hyaline, cylindrical and two celled, catenulate in a fragile chain.

Phialophora, a common soil fungi, was recognized only once at the stage of the last decay process of the *Quercus*-leaves supporting the ordinal distribution which has been made mostly on a heavily decayed plant debris.

Ramularia sp. has appeared significantly on the sterilized green leaves in all stations throughout the decay and its growth was often very luxuriant. All isolates belong to a single species with unseptated biconic conidia in a single or branched chain.

Rhinocladiella appeared during the first half of the decay and some of them have *Cladosporium*-stage in addition to the sympoduloconidial state.

Subulispora, a newly established genus (in press), is significant in that it grows in a

very early stage of decay process and continues to colonize throughout the process. The genus consists of two species with characteristic sharply subulate conidia. Taking the gross flora of the leaves with consideration, the members of this genus are quite clear because of the characteristic conidia even under a disecting microscope. These species colonized very frequently accompanying with *Ramularia*-species which was described previously, and they are apparently a first colonizer during the process and disposed to decrease in number with the decay.

An undescribed species of *Sympodiella* (in press) was also one of the commonest fungi in this study. It appeared at first on the leaves of the one month decay in abundance and it continued to grow to the latter half of the period. Conidial development of it is similar to *S. acicola* Kendrick, a type of the genus, but is significant in the multiseptate conidia and habitat. *S. acicola* which has been found on decaying needles of *Pinus sylvestris* (Kendrick, 1968), *P. densiflora* (Tubaki & Saitō, 1969) or rarely from soil (Barron, 1968), developed at the last stage of the decay and probably is originated in *Pinus*, which grows in the present forests.

Thysanophora penicilloides (Roum.) Kendrick is the only species of the genus found during the decay and appeared rather at the early to middle processes of the decay mostly on the *Castanopsis*-leaves. The genus is associated with the decay of the coniferous needle (Kendrick, 1961) and this species is not uncommon in the soil from coniferous woods (Barron, 1968). The present record on the *Castanopsis*-leaves, most abundantly in Station C, indicates that this fungus is not only transient but is able to invade the leaves of a non-coniferous tree.

Trichoderma is a ubiquitous and global fungus appearing very commonly in the present study. All isolates consist of the two groups, viz. green *Trichoderma* and white *Pachybasium*-type. The former is *Tr. viride*-complex and spreads widely on the leaf, and the latter grows at first as white mycelial mass. The difference of the two groups in preference of the leaf is uncertain.

Verticillium has been found several times rather at the middle and later processes of the decay.

Myxomycetes: One species has been found on the leaves of four-month decay producing many sporangia which are at first glistering orange-colored and dried purplish brown when matured. It belongs to *Stemonitis** sp. The same species developed as an exception on the dead *Castanopsis*- and *Quercus*-leaves of the very early decay, only one-month, in the Station C.

In order to compare the gross flora of the leaves naturally fallen with those of the sterilized leaves, natural leaf samples of *Castanopsis* were collected in the three communities every month at the same time. Fungal flora of these naturally decayed leaves, some of which were early decayed and some were completely decayed or nearly blackened, has been examined at the same time by the same method. Of course, the the same fungi that have been found on the sterilized leaves also developed on them, but

* Identification was kindly carried out by Dr. Y. Emoto to whom we owe many thanks.

the following fungi were observed only on the naturally decayed leaves (Table 3).

From the Table 3, it is quite clear that the colonization of *Beltrania* is rather limited to completely decayed or nearly blackened leaves and other species are probably of little importance in the early decay. *Marasmius* spp, Basidiomycetes, also prefer rather well decayed leaves.

Table 3. List of fungi found only on the naturally decayed leaves.

	(frequency)
Ascomycetes: *Arachniotus* sp.	○
Fungi Imperfecti:	
Actinopelte castanopsidis Yokoyama et Tubaki	○
Arthrinium phaeospermum (Cda.) Ellis	○
Beltrania rhombica Penzig	●
Chaetopsina fulva Rambelli	○
Chrysosporium sp.	○
Cryptophiale udagawae Pirozynski	○
Dendrophoma sp.	○
Dinemasporium sp.	○
Gonytrichum macrocladum (Sacc.) Hughes	○
Harpographium fasciculatum Sacc.	○
Illosporium sp.	○
Macrophoma sp.	○
Myrothecium sp.	○
Spondylocladiella sp.	○
Basidiomycetes:	
Marasmius spp.	○

Symbols (○, ●) are same with Table 2.

Discussion

Generally the fungal flora of the dead leaves has been considered to alter as the leaf material decomposes and the fungal succession may occur. The present studies of the fungal flora of the sterilized leaves which were previously set in the natural litter suggest that the flora include three groups of fungi. The first group comprises of the transient fungi present on the leaf surface only as detachable propagules which have germinated in the moist condition. Most of them probably have been transported to the leaf from the soil. As postulated by Chesters (1949), soil fungi can grow upon particles of organic matter. However, these fungi, especially of Mucorales viz. *Absidia, Backusella, Cunninghamella, Mortierella* and *Mucor*, are probably of little importance in the decay processes.

The second group of fungi, of which *Penicillium* is the best example, grew actively and sporulated on the leaf surface throughout the decay. The majority of *Cladosporium*,

Penicillium and *Trichoderma* are included in this group. The widespread occurrence of such may be accounted for the efficient dispersal of the spores or the the extensive growth of the fungi over other neighbouring plant materials. The present observation on this second group is nearly identical with that given by Pugh (1958). He observed that species of *Penicillium* which provided the bulk of these isolations, appeared to be adversely affected by a high-water content in the substrata, especially when the temperature was low. Hence, in the present study, high frequency of *Penicillium* species is considered probably due to the higher humidity and the lower temperature in a moist chamber during the isolation procedure. In any case, the members of this second group can be considered to be the first invador of the newly dead leaves and can continue to be such degree. A notable infrequency of *Penicillium* on the naturally fallen leaves of the longer decay which were collected at the same time around the stations as a control indicates that such fungi prefer the moribund or the newly dead leaves as substrates. Fungi of this second group are, if anything, plurivorous tolerating a wide range of conditions, and the process of colonization on the leaf is rather rapid.

The third group includes those found during the early decay process but they have a tendency to reduce later. They may also be the first invador on the newly dead leaves. *Ceratocystis* is included in this group as discussed already. *Subulispora* and *Sympodiella* are the initial colonists of the newly dead leaves. Those of the second and the third groups usually show an extensive growth over nearly the whole surface and efficiently disperse the spores which may invade a new leaf. Within a four- to five-month decay, these fungi decreased in frequency and were followed by the inhabitants of the residual products of decomposition.

The fourth group includes those which are frequently recorded on the leaves during the period of latter half of the decay but are uncommon on those of the very early decay. They probably do not attach the newly dead leaves as substrates but prefer those decomposed already by previous colonists. *Toxotrichum, Calcarisporium, Chalara, Codinaea, Crinula, Dactylaria, Oidiodendron, Thysanophora* and *Verticillium* may be representatives of this group.

Fifteen genera, described above, are encountered only on the natural, unsterilized decayed leaves. A successive change of the flora to these inhabitants may attribute to the more progressive breakdown of the leaves as already pointed out by Newman & Norman (1943) that the nature of the available energy materials largely determines the fungal flora.

Much of the fungi recorded in the present study are also known as the so-called "soil fungi", but such a distribution between the two groups, viz. soil vs. leaf litter fungi is to a certain extent theoritical. As stated by Chesters (1949) and Pugh (1958), many soil fungi are found in the leaf litter, and the new leaf is possibly invaded first by the fungi growing on debris already in the soil and later contributes available energy materials to either soil fungi or leaf litter fungi. Although such common soil fungi may be encountered on the leaf, it is striking that none of the members of *Aspergillus, Aureo-*

basidium, Curvularia or other extremely common soil fungi were found in this study. This is probably due to their nutritive preferances.

Effects of the variation in a host leaf: Species showing preferance for one type of the substrate are often more limited. But since the majority of the fungi are able to colonize many different substrates, nutritional habits are of little importance as limiting factors. Once a fungus reaches a new leaf it can survive and establish itself if it can find a suitable ecological niche. In the present study, we employed two kinds of leaves viz. *Castanopsis cuspidata* and *Quercus phillyraeoides*. Concerning the effect of the variation on the host leaf, it is considered that there is no remarkable influence by the kind of leaves. Only the following two exceptions have been observed. *Alternaria* has been found only on *Quercus*-leaves, though the colonization was restricted only in the Station B. The same was true with *Hyalodendron* in Station A.

The effect of the leaf condition: Pugh (1958) segregated the fungal flora of *Carex paniculata*, basing on a distinction given by Thom & Morrow (1957), into the initial colonists of moribund & newly dead leaves and into the inhabitants of the residual products of decomposition. Among the dominant phylloplane fungi on the *Halimione*-leaves, however, no marked reduction took place even when the leaves become moribund as described by Dickinson & Pugh (1965). In conjunction with a related problem, we employed, in this study, two kinds of sterilized leaves of *Castanopsis*, namely green, functional, and newly dead leaves. As might be expected, there appeared no marked floral difference between these different conditions of the leaf except for the following cases. *Calcarisporium, Oidiodendron, Pestalotia* and *Subulispora* are most prominant. The results are summarised in Table 4.

As shown in this table, *Calcarisporium* prefers the dead leaves. This fungus is known as associated with decaying fruitbodies of higher fungi, but is not uncommon on the leaves, and has a tendency to keep away from the sterilized green, functional leaves. *Oidiodendron* is significant in that its active growth occurred only on the dead leaves. This fungus colonized extensively even in the very early decay of the process and decreased in frequency to some extent as decomposition continued. *O. citrinum* was especially prominent as the first invador. On the contrary, both species of *Oidiodendron* were notably infrequent on the sterilized green leaves and appeared poorly at last when the leaves were decomposed. *Pestalotia* is also significant appearing on the sterilized green leaves only of the very early decay and is completely absent in the latter half of the decay, and no record was obtained on the sterilized dead leaves. However, the trace of the spots was uncertain on those of the latter decay because many other fungi grew since then covering these old spots. *Subulispora* is prominent in its active growth only on the sterilized green leaves of the very early decay, contrasting sharply with that on the dead leaves. This fungus, like *Oidiodendron*, decreased in number as the leaves decomposed and became even less frequent later. Differences above on the occurrence of the two kinds of leaf conditions may be explained on the basis that *Pestalotia* and *Subulispora* have a competitive advantage because of their abilities to grow

Table 4. Occurrence of four fungi on different conditions of the leaves of *Castanopsis*.

species	leaf condition	station	Oct.	Nov.	Dec.	Jan.	Feb.	March
Calcarisporium arbuscula	G	A	—	—	—	—	—	—
		B	—	—	—	—	—	○
		C	—	—	○	—	—	—
	D	A	—	—	○	—	○	—
		B	○	—	○	○	○	○
		C	○	○	●	—	○	—
Oidiodendron citrinum	G	A	—	—	—	○	○	—
		B	—	○	○	—	—	○
		C	—	—	—	○	○	○
	D	A	●	○	●	○	○	○
		B	—	○	●	●	○	○
		C	○	○	○	○	○	○
Pestalotia sp.	G	A	○	—	—	—	—	○
		B	○	○	—	—	—	○
		C	—	—	—	—	—	○
	D	A	—	—	—	—	—	—
		B	—	—	—	—	—	—
		C	—	—	—	—	—	—
Subulispora spp.	G	A	●	●	●	●	○	○
		B	○	○	○	○	○	—
		C	○	●	○	○	○	○
	D	A	○	○	—	—	—	○
		B	—	○	—	○	—	—
		C	—	—	—	—	—	—

G ... green, functional leaves; D ... newly dead leaves; — ... growth absent; other symbols are same with Table 2.

on the leaves before the leaves become moribund or dead. On the other hand, the surface decay or deterioration and energy materials derived by the processes are conductive to other fungi, viz. *Calcarisporium* and *Oidiodendron*.

The present communication has father extended the information concerning the nature of the leaf fungal flora, demonstrating its similar character in different seasons in different ecological habitats in widely separated localities. At the time of this writing, we are investigating the similar fungal flora under the same procedure not only in same stations, but in two other subtropical stations which are set up on

Yakushima and Tanegashima Islands, Kagoshima Pref.

The main environmental factors which appeared to influence the seasonal activities of the fungi are the temperature and the water content of the substrates. The succession of fungal flcra on the same substrates in the rainy season and in the summer month is being continued in our laboratory and will be presented in the next paper of this serial study, and a discussion on the succession throughout the year shall be given.

We are extremely grateful to Dr. T. Hasegawa, the Director, for his encouragement and also to Mr. T. Ito for isolation and identification of fungi. Most of the penicillia were identified by Mr. I. Asano to whom we owe many thanks.

Summary

Attemps were made to obtain a new interpretation about the successive colonization by fungi on the embedded sterilized leaves of *Castanopsis cuspidata* Schottky and *Quercus phillyraeoides* A. Gray in the naturally developing litter of the forests. Embedded leaves were returned from the forests to the laboratory once a month during the period of Oct. 1969 to March, 1970 and the fungi developed on the leaves were isolated and determined. Fifty eight genera of fungi were encountered and discussions were given on succession, autoecology, effect of variation in a host leaf and effect of leaf-condition.

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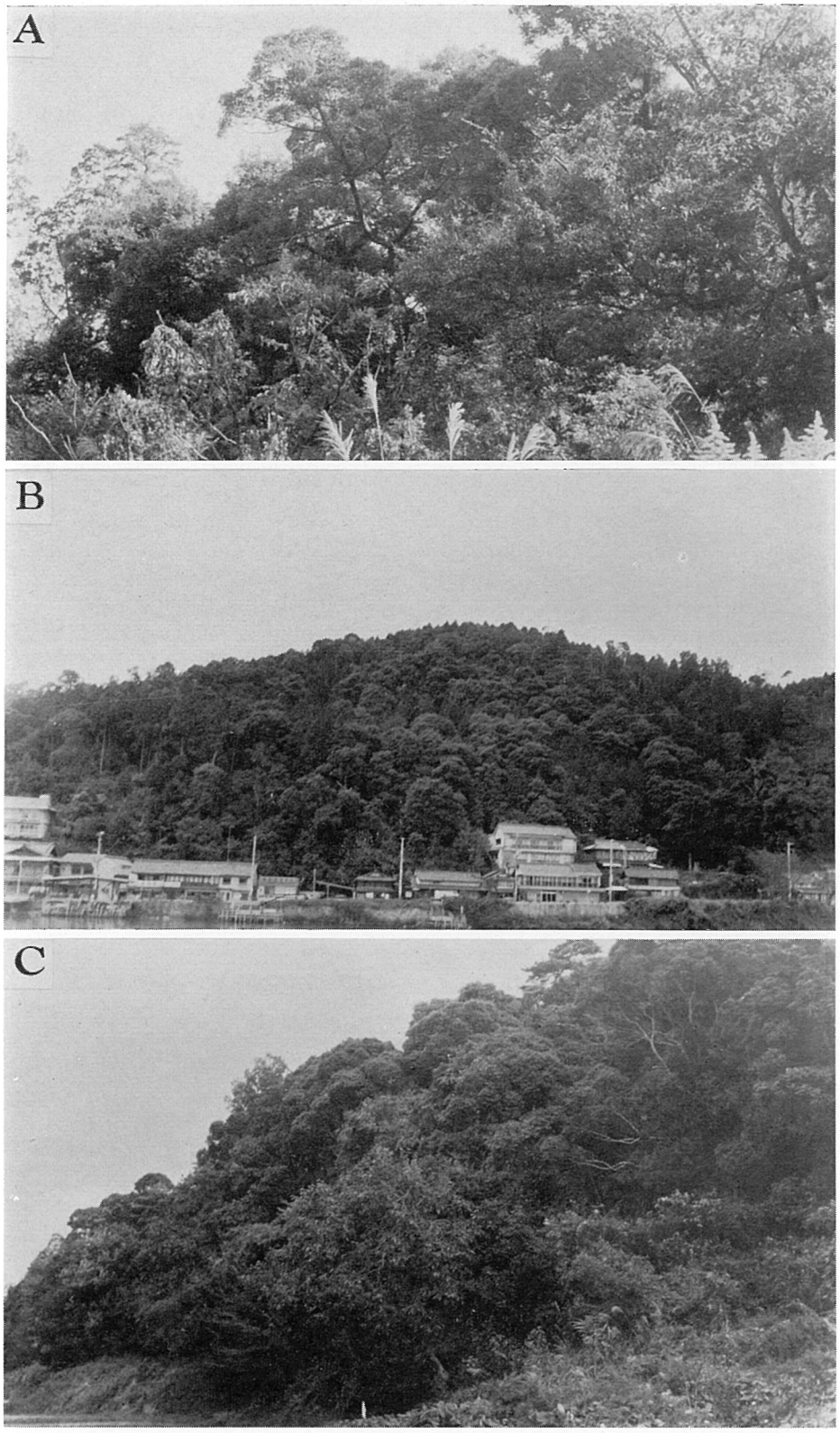

Plate 1. Outside views of three natural forests, mainly consists of evergreen trees. A. Forest of Togakushi Shrine, B. Forest of Ishiyama Temple, C. Forest of Arato Shrine.

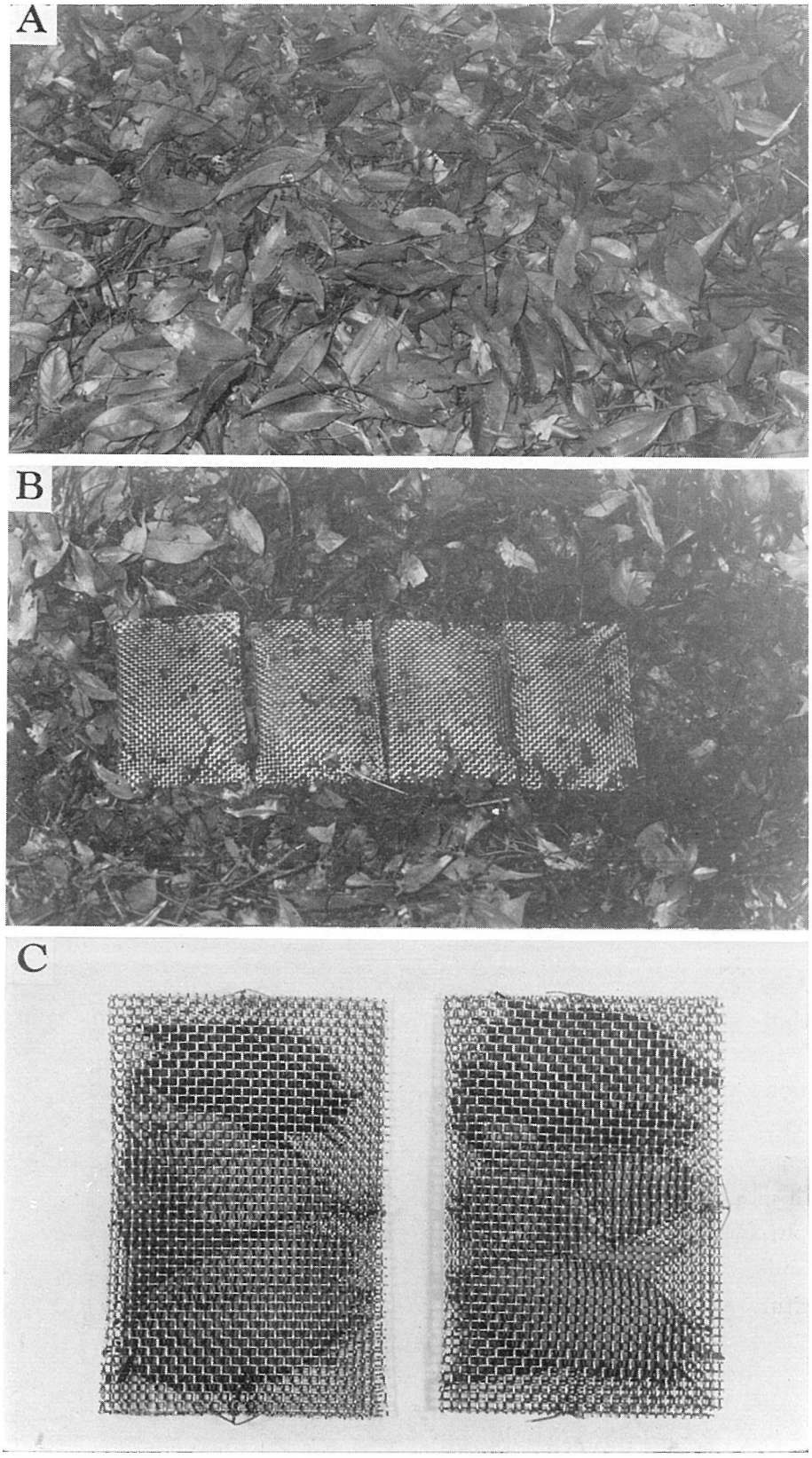

Plate 2. A. The litter surface of Station A. B. Setting of the leaf-traps on the lower bed of the litter. C. Stainless-wire nets, each containing sterilized leaves.

IFO Res. Comm. 5,
43–77, 1971 (*March*)

CULTURAL AND TAXONOMICAL STUDIES ON THE GENUS ACTINOPELTE

Tatsuo YOKOYAMA and Keisuke TUBAKI

Introduction

The genus *Actinopelte* was established in 1913 by Saccardo based on a single species, *A. japonica* Sacc., and is unique in its characteristic structure of the scutellum developed on the leaves. Three species have been hitherto known, namely *A. japonica* Sacc., *A. dryina* (Sacc.) Hoehnel and *A. stellata* Farr. *A. japonica*, a type of the genus, was first reported in Japan by Hara (1914) as a causal parasite of the leaf spot disease of cultivated chestnut trees, but, in a modern plantation of the trees in Japan, the fungus is nothing more serious pathogen at the present time. Hara's material was sent to Saccardo previously and the genus was made by the latter (1913), however, it is unfortunately not clear if the type specimen is still in existence. Therefore an exiccati specimen of *A. japonica,* preserved in the National Fungus Collection, Beltsville, was examined throughly by the courtesy of Dr. C. R. Benjamin, and was compared with those of our recent collections.

During the collection and isolation of *A. japonica*, additional 22 fresh materials of other *Actinopelte*-species have been obtained. These materials have been collected mostly from the leaves of various deciduous trees, especially of Fagaceous trees in Japan. Eventually they became assignable to *A. dryina* (Sacc.) Hoehn. and to three taxa which are clearly distinguishable from the hitherto described species of *Actinopelte* by morphological and physiological behaviors and host preferances. In addition to the above fresh materials collected during the study, an isolate of *A. dryina* was examined which was sent from Centraalbureau voor Schimmelcultures, Baarn.

Isolation was made by picking up of a single conidium from fresh fruiting bodies onto malt extract agar and potato sucrose agar with added lactic acid or tetracycline as bacteriostatic agents. Germination was observed in all conidia on the agar medium at 25°C and several monosporic-isolates have been obtained.

The purpose of the present investigation is to know the genus in more detail under cultural condition on the revision of the morphology and taxonomy of it. Report of this type of cultural study of all species of the genus have apparently not been published to date.

Histological Survey

Saccardo gave following generic diagnosis for the genus *Actinopelte* in 1913, "Perithecia superficialia, dimidiata, plano-convexa, membranacea, atra, poro pertusa ambitu

subcircularia, contextu eximie radiato, margine profunde fimbriato-fisso, cellulis furcato-ramosis, marginalibus liberis et spinuliformibus, appressis. Asci pauci (6–9) breviter, ovato-ellipsoidei, monospori, breviter crassiuscule stipitati. Sporidia ascum implentia, continua, nubilosa, subhyalina." It is apparent that Saccardo had erroneously considered the fungus as to be of ascomycetous as described below. *A. japonica* was originally described on the specimen collected by K. Hara on October, 1910 at Kawauye-mura, Prov. Mino, now Gifu Prefecture, in Japan, parasiting on the leaves of *Castanea pubinervis* Schneid. Since the size of the conidia of this fungus was exceptionally large and the globose shape of them was very close to the common fungus ascus, Saccardo misconceived conidium as a monosporic ascus. In the same year, 1910, Theissen showed that the large globose bodies are not asci, but truly conidia. This was also confirmed by Petrak (1924).

A. dryina, the second species of the genus, was originally described by Saccardo in 1878 as *Leptothyrium dryinum* Sacc. According to Limber and Cash (1945), the specimen cited was issued in 1876 as Mycotheca Veneta no. 555 under the name of " ?*Stigmella dryina* Lév." Based on the extensive studies on the morphology, taxonomy and synonymy of the fungus from various sources of hosts as current collections and herbarium specimen, Limber and Cash have given a detailed and emended description for the fungus and the additional accounts concerning its structure, particularly with reference to the attachment of the fruiting body to the leaf surface of the hosts were given. *A. drynia* would seem to be a common and ubiquitous species and it is quite probable that this species distributes widely in both new and old continents.

The third species was found on the leaves of Brasilian *Brysonima coriacea* DC. by Farr (1967) who proposed a new specific name, *A. stellata*, to the fungus. This fungus exclusively developed on the raised blotches caused by an undetermined, internal pyrenomycete on the same host. According to the original description and author's remarks, the fungus concerned more or less deviates from the generic concept of the *Actinopelte* in possessing of a distinct basal membrane on the pycnothyrium. However, Farr did not show a detailed drawing of the basal membrane in the text-figure nor gave sufficient interpretation for the function of it.

Description

Actinopelte japonica Saccardo (Pl. 1 A-D, 2A, 3A, 4 A-H, 8 A-B)
Ann. Mycol. **11:** 312, 1913.

Parasitic on living leaves of *Castanea pubinervis* Schneid. Spot 2–6 mm in diameter, circular to subcircular, or oblong, sometimes angular or irregular in shape, pale tan color to ochraceous, border definite, reddish brown or fuscous. Fructifications usually epiphyllous, very rarely hypophyllous, solitary to abundant, unevenly scattered or gregarious to crowded, sometimes coalescing, blackish brown or almost black, suborbicular, convex, adpresso-campanulate, umbilicate at the center; margin attached to the epidermis of the host plant when young, then becoming flatten or recurved by the pressure of the

mass of growing conidia beneath when matured; 50–180 (–200) μ in diameter, composed of scutellum and columella supporting the former. Scutellum consisting of thick-walled, chestnut brown hyphae radiating from a central portion, 4–6 μ wide, 1–several times bifurcate, septate, free as sharp-pointed horns at each end, showing somewhat brick-layering pattern at the middle zone, composed of small, spherical to irregular shaped cells to make a parenchymatous tissue around a single central cell; membraneous except their free ends in general appearance when young, then the hyphal strands of the scutellum tend to separate, presumably by the pressure of the mass of successively discharged conidia beneath, so that the membraneous texture become more looser and sometimes becoming almost completely disintegrated into setae-like appendage on the fertile tissuees. Central cell hyaline or pale yellow, thick walled, stuffed or empty, 10–20 μ in diameter, scutellum borne on a columella just beneath the central area. Columella cylindrical, 20–50 μ in diameter, 20–30 μ in height, reaching up to 200 μ in both dimension in culture, consisting of fertile tissue of small, parenchymatous, hyaline cells surrounding a single large central cell. Fertile tissue also arising from the under side of the scutellum near the columella. Conidiophores develop on the growing end of the fertile tissue extending sidewards around the columella and both downwards and outwards beneath the scutellum, subglobose, ellipsoid, clavate, swollen at the base, tapering to a slender neck about 2–3 μ wide when the conidia are borne, 10–20 μ long, 5–10 μ wide, hyaline. Conidia acrogenous, blastic, globose, subglobose or broadly ellipsoidal, connected to conidiophores by a prominent neck, liberate by cutting of the neck just 2–3 μ below the base leaving a distinct frill, hyaline to pale ochraceous, double walled, wall hyaline, germinate from any part of conidial surface, 40–55 $\times$ 35–45 μ. Microconidia present, spermatia-like, bacilliform, botuliform, narrowly naviculate, 5–10 $\times$ 1–2μ, hyaline, of phialospore-type, produced in a specialized rhizothyrium homologous with that produces the conidia, but usually small, suborbicular, conical or campanulate, umbonate, 60–80 μ in diameter, 15–30 μ in height; scutellum consist of thick-walled, 3–5 μ in thick, brown hypha radiating from a single central cell, membraneous except for margin, supporting by a columella which measuring 5–10 μ in thickness and 10–20 μ in height; central cell thick walled. Germination of microconidia uncertain.

Hab. On the necrotic spot on living leaves of *Castanea pubinervis* Schneid. (Kuri).

Specimens examined: On *Castanea pubinervis* Schneid. (Kuri). Kawauye-mura, Prov. Mino (Gifu Pref.), Aug. 1920, K. Hara (Sydow's Fungi exotici exsiccati no. 526, deposited in National Fungus Collection, Beltsville); Tomobe-cho, Ibaragi Pref., Oct. 25, 1948, K. Ito (HGFXS* No. 00042 and No. 00200); Kushigata-mura, Ibaragi Pref., Nov. 9, 1950, O. Chiba (HGFXS No. 00271); Kamagaya-cho, Chiba Pref., Oct. 29, 1948. K. Ito (HGFXS No. 00485); Morioka, Iwate Pref., Oct. 9, 1962, T. Kobayashi (HGFXS No. 00674); Wada-mura, Nagano Pref., Sept. 9, 1958, Y. Zinno (HGFXS No. 01945); Chiyoda-mura, Ibaragi Pref., Aug. 13, 1969, K. Uchida (IFOH** -No. 11611)

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* Herbarium of Government Forest Experiment Station, Tokyo.

** Herbarium specimen preserved in the IFO.

and a culture made by T. Yokoyama (44081301=IFO–9268); Kasama, Ibaragi Pref., Sept. 17, 1969, Y. Kobayashi (IFOH-No. 11612) and cultures made by T. Yokoyama (44091701=IFO–9269 and 44091704=IFO–9270); Ootsu, Shiga Pref., Sept. 2, 1970, T. Yokoyama(45090206=IFOH-No. 11613 and IFO–9340; 45090209=IFOH-No. 116 14 and IFO–9431); Ootsu, Shiga Pref., Oct. 30, 1970, T. Yokoyama (45103003=IFOH-No. 11615 and IFO–9342).

Actinopelte dryina (Sacc.) Hoehnel (Pl. 1 E, 2B, 3B, 5 A-G, 9 A-F)
Mitt. Bot. Inst. Tech. Hochsch. Wien **2:** 69, 1925.

Fructifications superficial, usually epiphyllous, rarely hypophyllous, gregarious to crowded, sometimes coalescent, black to blackish brown, suborbicular to convex with irregular margin, flattened or depressed at the center, composed of scutellum and columella; margin of fructification adheres to epidermis of the host plant when immature, then recurved or arched by the pressure of the mass of growing conidia beneath; usually immersed in the successively discharged conidia at its periphery leaving a exposed central area, 30–50 μ in diameter. Scutellum consisting of thick-walled, sooty-brown to olivaceous-brown hyphae radiating from a central cell, septate, 1–3 bifurcate, each hyphal strand free as rounded or sharply pointed spines at the marginal portion, 2–4 μ thick. Columella a single cell, thick-walled, 3–6 μ in diameter, 5–10 μ in height, surrounded by fertile tissue of small parenchymatous, hyaline to subhyaline cells at the upper portion, fertile tisse with conidiophores up to 10–20 μ in diameter, connected to the mycelium within the leaf by a slender strand at the base. Conidiophores produced on the growing periphery of the fertile tissue arising from upper portion of columella and also under side of the scutellum near the central cell, clavate, tapering to a slender neck about 1 μ wide on which a single conidium is borne, 2–4 μ wide, 5–10 μ long. Conidia acrogenous, borne on the tapering end of condiophores with narrow, 1 μ thick neck, blastic, ellipsoidal to ovate, subcylindric in some isolates, double walled, sooty, fuligineous to olivaceous fuscous, becoming pale blackish brown before germination, 12–15 $\times$ 5–8 μ, rounded at the distal end, with distinct frill at the base showing the remain of neck. Microconidia of phialospore-type, clavate, curved-cylindrical, 5–7$\times$1.5–2 μ, hyaline, produced within the fruiting structure which is usually smaller than that produces the conidia, membraneous, composed of thick-walled radiating hyphal strands, up to 4 μ in wide, pale sooty brown; germination not observed.

Hab. On both living and fallen leaves of *Quercus* spp. and *Castanea pubinervis* in Japan and also in living leaves of *Quercus* spp. and other broadleaved trees in North America and Europe.

Specimens examined: On *Castanea pubinervis* Schneid. (Kuri). Ikeda, Osaka Pref., Nov. 11, 1968, T. Yokoyama (43111102=IFOH-No. 11616 and IFO-9265); Minoo, Osaka Pref., Oct. 8, 1969, T. Yokoyama (43121806=IFOH-No. 11617 and IFO–9266).

On *Quercus phillyraeoides* A. Gray (Ubamegashi). Suita, Osaka Pref., Oct. 8, 1969, T.

Yokoyama (44100802=IFOH-No. 11618 and IFO-9267).
On *Quercus glauca* Thunb. (Arakashi). Ootsu, Shiga Pref., Feb. 27, 1970, T. Yokoyama (45022703=IFOH-No. 11619 and IFO-9343).
Culture of an isolate from CBS, The Netherland (213-66, from leafspots of *Quercus robur*, van der Aa, in List of cultures, 1968=IFO-9101).

Remarks: The fungus is uncommon in our country. Whether or not the present fungus gives the pathogenicity to living plants has not as yet been determined so far. In the case of the present Japanese collections, leaf spots on which the fungus were borne would seem to be caused by other primary invadors. Moreover, inoculation tests done in our laboratory on the seedlings of such host plants as *Quercus phillyraeoides, Castanea pubinervis* and *Quercus glauca* resulted in a negative evidence in causing no recognizable sign of spot.

Because the type specimen of *A. dryina* and other reference specimens cited by Limber and Cash (1945) has not been examined, we can not certify the strict conspecificity of our isolates with that emended by them. Many characters, however, of the fungus concerned would seem to fit fundamentally to those indicated by them and also later by Tehon (1948). The shape and size of the conidia of *A. dryina* as well as its pigmentation may vary to great extent in each samples from different sources as already mentioned by many authors.

Actinopelte rubra Yokoyama et Tubaki, sp. nov.
(Pl. 1 F, 2 D, 3 D, 6 A-G, 10 A-H)

Rhizothyria hypophylla vel raro epiphylla, superficialia, gregaria vel laxe caespitosa, interdum coalescentia, punctiformibus, scutellariformibus, dimidiato-convexa vel campanulata, in centro umbonata vel fere plana. Scutella membranacea, radiata, fibris absolute continuis, pallide brunneis, tenui parietales, marginem continuatum et involutum formantibus, 60–100 μ in diametro. Columella unicellulatia et hyalina, demum dilute ochracea, cellulae globosae fertiles in apice columellarum fasciculatae. Conidiophora unicellulata, cylindracea, clavata, hyalina, 6–10 μ longa, 3–4 μ crassa, desuperiter lateraliterque nascentibus. Conidia lato-ellipsoidea vel ovata, continua, teres, hyalina, deinde subrubescentibus, apice utrinque rotundatis sed ad basim brevissimopedicellatis, crassiparietalia, levia, 12–15 $\times$ 10–13 μ. Microconidia bacilliformibus, cylindracea, recta vel curvata, saepe sigmoidea, hyalina, 8–10 $\times$ 1 μ.

Parasitic on living leaves of *Quercus phillyraeoides* A. Gray, usually without any remarkable symptom, sometimes giving reddish tint on the leaf surface. Fructifications abundant, mainly hypophyllous, often epiphyllous, densely gregarious to caespitose, sometimes coalescent, suborbicular, convex to campanulate, dimidiate, umbonate or plane, ochracous to orangish brown, consisting of scutellum and columella. Scutellum usually membraneous, never free from nor splitting into each individual hyphal strand radiating from a central cell and adhering each other to make membraneous circular mat; margin continuous, more or less undulated, distinctly involved; 60–100 μ in diameter; hyphal strands yellowish brown to light orange brown, thick-walled, septate, rarely

branched, 3–5 μ thick. Columella a single cell, thick-walled, yellowish to pale ochraceous, 5–6 μ thick, 10–20 μ high, oblong to cylindrical, truncated at the base, connected to the epidermis of host cell by a slender intracellular mycelium; fertile tissue of small spherical, hyaline, 5–6 μ thick, parenchymatous cells develop around the columella but originated from under surface of the scutellum. Conidiophores clavate to cylindrical, 3–4 μ thick, 6–10 μ long, hyaline, produced around the growing end of fertile tissue downwards near the columella and outwards at the periphery, tapering to a slender neck of 1 μ thick on which a conidium is borne. Conidia acrogenous, blastic, broadly ellipsoid to ovate, hyaline at first, then pale yellowish brown to finally light orange yellow, 12–15 $\times$ 10–13 μ, with a distinct frill at base, double-walled, hyaline, smooth. Microconidia produced in abundance, usually on separate fructifications, in some cases on the same fructification that produces the conidia, of phialospore-type, elongated bacilliform, cylindrical, straight, curved or sigmoid, hyaline, 8–10 $\times$ 1 μ, ejected successively from the conidiophores. Conidiophores elongated clavate, 6–10 μ long, 2–3 μ thick, arranged downwards and outwards on the fertile tissue beneath the scutellum. Germination of the microconidia uncertain.

Hab. Parasitic on the living leaves of *Quercus phillyraeoides* A. Gray. (Ubamegashi).

Specimens examined: On *Quercus phillyraeoides* A. Gray. (Ubamegashi). Kyoto, Kyoto Pref., June 7, 1969, T. Yokoyama (44060716=IFOH-No. 11620 and IFO–9271; 44060718=IFOH-No. 11621 and IFO–9272); Oct. 28, 1969, T. Yokoyama (44102801= IFOH-No. 11622, **TYPE** and IFO–9273); Ootsu, Shiga Pref., Jan. 27,1970, T. Yokoyama (45012702=IFOH-No. 11623 and IFO–9274; 45012703=IFOH-No. 11624 and IFO–9275); Apr. 14, 1970, T. Yokoyama (45041401=IFOH-No. 11625 and IFO–9276); Minabe, Wakayama Pref., March 1, 1970, T. Yokoyama (45030102=IFOH-No. 11626) ; Yakushima, Kagoshima Pref., May 29, 1970, T. Yokoyama (45052912=IFOH-No. 11627 and IFO–9277); Miyagawa-mura, Mie Pref., Aug. 2, 1970, T. Yokoyama (450 80210=IFO–No. 11634 and IFO–9371); Mugi, Kochi Pref., Aug. 26, 1970, T. Yokoyama (45082601=IFOH-No. 11628).

The type specimen was deposited in the herbarium of IFO. A culture derived from the type is preseved in the IFO culture collection.

Remarks: The present fungus is very characteristic in entirely membraneous scutellum and reddish color of the vegetative mycelium as well as of the rhizothyrium. The conidia of this species are also characteristic in being broadly ellipsoidal and in turning to pale orange brown when matured. In Japan, the present fungus may widely distributed along with the distribution of the host plants, *Q. phillyraeoides*. Materials collected indicate the continuous distribution of this species from Mie Prefecture to Kagoshima Prefecture, western- or southern-half areas of the Japan Islands. This does not mean, of course, negative evidence of the distribution of this fungus in the other half area of Japan. Further survey will be necessary to give more detailed distribution map of this fungus.

Pathogenicity to host plant is not as yet completely determined, though the fungus concerned have been isolated mainly on either living or senescent leaves and sometimes

on freshly fallen leaves. No case has appeared any detectable symptom caused by this fungus. The green living and senescent leaves, sometimes even the dead leaves still on the trees, even though infected by the fungus, provide no fruiting structure in nature. These infected leaves, however, produce numerous rhizothyria with conidia in abundance only when they are maintained in a moisture chamber in the laboratory. In some cases, senescent leaves still on the plants give a distinct blush of irregular pattern which is probably caused by the vegetative mycelium of the fungus developed within the host tissues.

Inoculation tests by an isolate of the present species on the healthy seedlings of 6– to 8–leaves stage gave the positive result indicating that this fungus may invade the host tissue and subsequently produce new fructification on the inoculated leaves and even on uninoculated latest leaves when they are maintained in a moisture chamber. Further inoculation test is necessary to show strict pathogenicity to this host plant and another possible host plants of different species as well as its infection process.

Actinopelte subglobosa Yokoyama et Tubaki, sp. nov.
(Pl. 1 G, 2 E, 3 E, 7 E-H, 8 E-F, 11 A-F)

Rhizothyria epiphylla sed raro hypophylla, superficialia, gregaria vel subconferta, interdum plus minusve coalescentia, punctiformibus, scutellariformibus, in primo dimidiato-convexa, deinde plana vel recurvata, fere atra. Scutella membrancea, radiata, contextu cellulis atro-brunneis, furcato-ramosis, percrassiparietales, 1–3 bifurcatis, apicibus liberis, acuminatis, 80–150 μ in diametro. Columella parenchymatico-sporodochiiformibus, cum cellulae fertiles globosae fasciculatae. Conidiophora elongato-clavata, subcylindracea, cum apice elongato-angustissimae, hyalina, 8–12 μ longa, 2–3 μ crassa, desuperiter et laterliter nascentibus. Conidia globosa vel subglobosa, hyalina, demum flavo-ochracea vel pallide brunnea, apice utrinque rotundatis sed base brevisissime pedicellatis, crassiparietalia, levia, 10–13 $\times$ 9–11 μ. Microconidia non.

Doubtfully parasitic producing distinctly bordered spot of irregular pattern on living leaves as well as similar spot on fallen leaves of *Quercus glauca* Thunb. Fructifications usually epiphyllous, rarely hypophyllous, superficial, gregarious to subcrowded, almost black, dotty, flat, dimidiate, consisting of scutellum and columella. Scutellum convex to flat, often recurved at the margin because of the pressure of the mass of successive conidia beneath when matured, membraneous, composed of thick-walled fuscous hyphal strands of 3–5 μ thick radiating from a central cell, septate, 1–3 bifurcate; each hyphal strand free as sharply pointed spines or horns at the marginal area where branching predominant, but becoming more looser and sometimes becoming easy to separate each other even near the central area; 80–150 μ in diameter. Columella a single cell of 8–10 μ wide, thick-walled, hollow or stuffed, usually surrounded by fertile tissue of small, spherical, 5–10 μ thick, parenchymatous, hyaline cells near the upper portion, connected to the epidermis of host cell with a slender intracellular mycelium. Coni-

diophores elongated clavate, subcylindric, 8–12 μ long, 2–3 μ thick, tapering to a narrow neck of 0.5–1 μ thick, produced on growing periphery of fertile tissue near the central portion and also beneath the radiating hyphal strands. Conidia borne on the conidiophores blastically, connected by a narrow neck from which each conidium is liberated leaving a distinct frill at the basal end, globose to subglose, hyaline to pale yellowish ochraceous, double-walled, 10–13 × 9–11 μ. Microconidia not yet observed.

Hab. On the leaves of *Quercus glauca* Thunb. (Arakashi).

Specimens examined: On *Quercus glauca* Thunb. (Arakashi). Kyoto, Kyoto Pref., Jan. 30, 1968, T. Yokoyama (43013001 = IFOH-No. 11629, **TYPE** and IFO–8931); Ootsu, Shiga Pref., Feb. 27, 1970, T. Yokoyama (45022702=IFOH-No. 11630 and IFO–9344).

The type specimen was deposited in the herbarium of IFO. A culture derived from the type is preserved in the IFO culture collection.

Remarks: The fungus can easily be distinguished from *A. dryina* by large, dark colored rhizothyria and hyaline, subglobose conidia. Cultural feature of this fungus is very characteristic in having cinnamoneous to reddish fuscous, concentrically zonate, viscid colony and also in abundant and even sporulation on the whole surface of the colony.

Actinopelte castanopsidis Yokoyama et Tubaki, sp. nov.
(Pl. 1 H, 2 F, 3 F,7 A-D)

Rhizothyria epiphylla vel raro hypophylla, superficialis, sparsa, gregaria, caespitosa, interdum plus minusve coalescentia, punctiformibus, scutellariformibus, dimidiata, convexa vel campanulata, deinde plana, fere atro-brunnea. Scutella membranacea, radiata, fibris furcato-ramosis, brunneis, crassiparietales, 1–2 bifurcatis, apecibus liberis, obtusis vel acutis, 100–150 μ in diametro. Columella unicellulatia vel parenchymatico-sporodochiiformibus, cum cellulae fertiles fasciculatae, 10–20 × 5–15 μ. Conidiophora clavata, cum apice elongato, hyalina, 8–10 μ longa, 2–4 μ crassa, desuperiter et lateraliter nascentibus. Conidia oblongo-ellipsoidea vel oblonga, apice utrinque rotundatis sed ad basim brevisissimo-pedicellatis, crassiparietalis, levia, hyalina, 12–13 × 7–8 μ. Microconidia non.

Fructifications superficial, usually epiphyllous, rarely hypophyllous, scattered, sometimes coalescent, gregarious to caespitose, suborbicular, convex to almost plane, dimidiate; consisting of scutellum and columella. Scutellum composed of thick-walled, pale brown, 3–4 μ thick, septate, 1–2 bifurcate, hyphal strands radiating from a central cell, membraneous, free at the margin as rounded tip, 100–150 μ in diameter. Columella composed of the fertile tissue of small, hyaline, parenchymatous cell, especially at the upper portion, fertile tissue also develops beneath the scutellum, 10–20 × 5–15 μ. Conidiophores develop around the periphery of the fertile tissue both downwards and outwards, clavate, tapering to a slender neck of 1 μ thick on which a conidium is borne, 8–10 μ long, 2–4 μ thick, hyaline. Conidia acrogenous, blastic, oblong to oblong-

elliptical, hyaline, double-walled, 12–13 × 7–8 μ, with a distinct frill at the basal end indicating the separation from conidiophores. Microconidia not yet observed.

Hab. On the leaves of *Castanopsis cuspidata* Schottky. (Tsuburajii).

Specimens examined: On *Castanopsis cuspidata* Schottky. (Tsuburajii). Ootsu, Shiga Pref., Jan. 27, 1970, T. Yokoyama (45012701=IFOH-No. 11631, **TYPE** and IFO-9263); March 26, 1970, T. Yokoyama (B-572=IFOH-No. 11632 and IFO-9262).

The type specimen was deposited in the herbarium of IFO. A culture derived from the type is preserved in the IFO culture collection.

Remarks: The present fungus differs from other species in the oblong or elliptical hyaline conidia and blunt tip of hyphal strand of the scutellum as well as the restricted occurrance on the specific host plant, *Castanopsis cuspidata*. Cultural character also differs significantly from previous species described.

General remarks: Morphological features of the individual species previously described can be easily differentiated each other by the shape of the fructification, by the conidia, by the presence or absence of microconidial stage and by the host preference. These are summarized in table 1. The shape of the scutellum is the most remarkable morphological character of the fungi belonging to the present genus. The scutellum of *A. rubra* is most peculiar, composed of tightly arranged, membraneous hyphal strands

Table 1. Comparison of the main morphological characteristics and the host plants of *Actinopelte*

Species	Scutellum	Columella	Conidia	Microconidia	Host plants
A. japonica	50–180μ (up to 200 μ) irregular at margin	20–50 × 20–30μ (up to 200μ) parenchymatous	40–55 × 35–45μ subglobose hyaline to pale ochraceous	5–10 × 1–2μ bacilliform to naviculate hyaline	*Castanea pubinervis* epiphyllous, very rarely hypophyllous
A. dryina	30–50μ irregular at margin	3–6 × 5–10μ single cell	12–15 × 5–8μ ellipsoidal fuliginous to olivaceous brown	5–7 × 1.5–2μ clavate to botuliform hyaline	*C. pubinervis* *Quercus phillyraeoides* *Q. glauca* epiphyllous, rarely hypophyllous
A. rubra	60–100μ regular but strongly involved at margin	5–6 × 10–20μ single cell	12–15 × 10–13μ broadly obovate orangish brown	8–10 × 1μ elongated bacilliform to cylindrical hyaline	*Q. phillyraeoides* hypophyllous, rarely epiphyllous
A. subglobosa	80–150μ irregular at margin	15–20 × 5–10μ parenchymatous	10–13 × 9–11μ subglobose hyaline to fulvescent	unknown	*Q. glauca* epiphyllous, rarely hypophyllous
A. castanopsidis	100–150μ irregular at margin	10–20 × 5–15μ parenchymatous	12–13 × 7–8μ oblong to cylindrical hyaline	unknown	*Castanopsis cuspidata* epiphyllous, rarely hypophyllous

radiating from a central cell, unfreed at their margin, apparently involving at the margin, though such a type of the scutellum is largely deviated from a typical type of the genus (Pl. 6 C). The scutella of the other species are more or less free at the margin, giving a sharply pointed or blunted apex and composed of distinctly thick-walled, dark-colored radiating hyphal strands (Pl. 4 C, 5 C, 7 B, 7 F). The conidia of *A. japonica* is exceptionally large measuring up to 55 μ in diameter, but the dimension of the conidia of the other four species lies between 10 to 15 μ in the longest diameter. *A. dryina* is characteristic in having grayish to blackish brown conidia and A. rubra is also characteristic in the orange yellow colored conidia. On the other hand, conidia of the other three species remain colorless or, if any, very pale yellowish. It is worth to mention that whether the columella is composed of a single central cell or composed of parenchymatous tissues of small spherical cells. So far as examined, the columella of *A. japonica* is certainly composed of a fertile tissues of small, spherical to irregular sized, parenchymatous cells surrounding a single central cell, giving a structural similarity to sporodochia, whereas the columella of *A. dryina* as well as *A. rubra* are composed of a single central cell without surrounding parenchymatous fertile tissues (Pl. 8 A-D). Arrangement of conidiophores of all species clearly shows the inverse sporulation pattern, characteristic to the present genus, indicating all the fungi described here should be included into the genus *Actinopelte*.

Cultural Characteristics

All isolates from the materials collected of above five species were presented to the cultural study so as to determine the comparative activities for radial growth on the various cultural media. Petri dishes of 9 cm diameter which provided with crossing boundary within them were prepared to compare individual growth response of the isolates on four kinds of media at the same condition. Cultural media used are malt extract agar, potato sucrose agar, oatmeal agar and Czapek agar.

A. japonica: Colonies on malt extract agar grow rapidly, white at first, becoming pale yellow to pale ochraceous; aerial mycelium compact, silky or velvety, white to creamy yellow; immersed mycelium grows rapidly; reverse yellow to ochraceous; sporulation moderate, but produced on abnormal sporodochia-like structure. Colonies on potato sucrose agar grow rapidly, pale yellowish brown to chestnut brown; aerial mycelium compact, silky, white to pale yellow; immersed mycelium grows rapidly; reverse yellowish brown to blackish brown. Sporulation very rare. Colonies on oatmeal agar grow rapidly, ochraceous to pale yellowish brown; aerial mycelium compact, finely floccose, white to cream; immersed mycelium grows rapidly; reverse concolor. Colonies on Czapek agar grow restrictedly, grayish brown to pale blackish brown; aerial mycelium very poor; immersed mycelium scarcely developed; reverse concolor. (Pl. 2 A)

A. dryina: Colonies on malt extract agar grow rapidly, white at first, becoming soon fuliginous to fuscous, then grayish fuscous; aerial mycelium grows very rapidly, concolor,

floccose, surface irregular and rough, waved, concentric; immersed mycelium grows rapidly, almost black; reverse black. Sporulation abundant on both immersed and aerial mycelium, ejected as the mucous mass among the concentric ring. Colonies on potato sucrose agar grow rapidly, white, dingy white, then grayish brown; aerial mycelium white to pale fuscous, floccose, surface rough and irregularly waved, concentric; immersed mycelium grows rapidly, fuliginous brown; reverse black. Sporulation abundant, in mucilagenous masses. Colonies on oatmeal agar grow rapidly, pale brown to grayish brown; aerial mycelium concolor; immersed mycelium concolor; reverse blackish brown. Sporulation abundant as mucous mass. Colonies on Czapek agar grow very restrictedly, compact with minute hyphal tips; aerial mycelium none; immersed mycelium scarsely expanded, almost black; reverse concolor. Sporulation none. (Pl. 2 B)

A. rubra: Colonies on malt extract agar grow rapidly, creamy yellow, ochraceous, yellowish brown, finally reddish brown, compact, smooth, viscid; aerial mycelium poor, concolor; immersed mycelium grows very rapidly, white, creamy to ochraceous, turning to reddish. Sporulation dense at the growing center, evenly at other area, discharging as pale yellowish brown, viscid, yeast-like mass. Colonies on potato sucrose agar grow rapidly, ochraceous to yellowish brown, turning to reddish brown to deeply reddish fuscous; aerial mycelium grows moderately, compact, viscid; immersed mycelium grows rapidly, pale ochraceous to reddish brown; reverse concolor. Sporulation abundant, especially at the central zone, conidial mass yeast like. Colonies on oatmeal agar grow moderately, creamy to pale ochraceous, turning to reddish brown, smooth, viscid; aerial mycelium poor; immersed mycelium moderate, ochraceous to red dish brown; reverse concolor. Sporulation abundant and evenly, conidial mass yeast-like. Colonies on Czapek agar grow restrictedly or absent, reddish brown. (Pl. 2D)

A. subglobosa: Colonies on malt extract agar grow moderately to rapidly, dingy white, pale ochraceous, pale tan color, becoming cinnamoneous with reddish tint on zonte bands, viscid; aerial mycelium effuse, finely floccose, varing from thin to thick by concentric zonation, whitish to pale grayish brown; immersed mycelium well develop, pale ochraceous to pale grayish brown, grows rapidly; reverse concolor. Sporulation abundant and evenly. Colonies on potato sucrose agar grow moderately, white, clay-colored, blackish-brown, viscid, zonate; aerial mycelium effuse, finely floccose; immersed mycelium moderate, concolor; reverse grayish fuscous. Colonies on oatmeal agar grow rapidly, clay-colored, olive brown to dark brown, with more or less reddish tint, distinctly zonate, viscid; aerial mycelium effuse, finely floccose, concolor; immersed mycelium grows rapidly, concolor; reverse concolor. Sporulation abundant and evenly. Colonies on Czapek agar grow in very reduced or almost absent (Pl. 2 E)

A. castanopsidis: Colonies on malt extract agar grow rapidly, dingy white to clay-colored, more or less viscid; aerial mycelium effuse, floccose, white; immersed mycelium grows rapidly, dingy white to clay-colored; reverse concolor. Sporulation moderate, fructifications scattered as blackish brown dots composed of several rhizothyria from which white to creamy, mucous mass of the conidia eject. Colonies on potato sucrose agar

grow moderately, white to pale clay-color, zonate, cartilaginous; aerial mycelium poor, white; immersed mycelium moderate; reverse concolor. Sporulation abundant, creamy-white, yeast-like in mass. Colonies on oatmeal agar grow very rapidly, white to pale clay-colored; aerial mycelium poor or none, white; immersed mycelium luxuriant, pale ochraceous to clay-colored; reverse concolor. Sporulation abundant, dotted by numerous blackish brown, complicated groups of rhizothyria from which the conidia eject as creamy, viscid, mucous mass. Colonies on Czapek agar absent or very restrictedly if any. (Pl. 2 F)

In general, all cultures grow moderately or rapidly on malt extract agar, potato sucrose agar and oatmeal agar, while growth on Czapek agar usually very reduced and is completely absent in a single species, *A. castanopsidis*. Gross appearance of each colony is very characteristic and distinguishable each other into a respective species. *A. japonica* produces a swollen, compact, silky to velvety, dry, uniformly white to creamy white colony, whereas *A. dryina* produces a colony of grayish to blackish brown, zonate, viscid, and its superficial mycelium consists of white to grayish, roughly floccose, luxuriant, dry aerial hyphae. *A. rubra* develops aerial mycelium scarcely, but produces immersed mycelium vigorously and its colony usually becomes reddish. In *A. subglobosa*, the immersed mycelium is predominant, but the colony cinnamoneous, vinaceous brown to dark brown, not reddish, differing from *A. rubra*. Sporulating surface area is also not mucoid as in *A. rubra* and *A. castanopsidis*, but viscid and distinctly zonate. *A. castanopsidis* produces more or less cartilaginous, uniformly pale clay-colored colony. This species, exceptionally, never develop on Cazpek agar. Fruiting structures produced by each culture on agar media are similar or same to those found on the natural hosts. However, the ability of producing the fructification differs greatly in each species. *A. japonica* less produces fructification and conidia on agar media, while *A. rubra*, *A. subglobosa* and *A. castanopsidis* produce both fructification and conidia in abundance. On the other hand, the colony of *A. dryina* predominantly consists of conidia, and no typical fruiting structure developes on such agar media.

Scutellum development under culture: Particularly, in the case of *A. japonica*, the shape and size of the fructification differ from that on its natural host plants. It should be noted that the parenchymatous fertile tissue around the central cell develops rapidly which becomes sporodochia-like fruiting structure eventually on which many typical conidia are borne. In this case, development of the scutellum is poor and irregular, sometimes only provides serveal setae-like hyphal strands on upper portion of the sporodochia-like structures. Morphologically, these hyphal strands are fundamentally identical with those of the normal scutellum, developing as thick-walled, irregularly sinuous, sometimes bifurcated, sharp-pointed, septate mycelium. Each mycelium develops individually and independently and hardly construct a scutellum.

Another case of variation in the sporulating method on the agar media in which *A. dryina* involved is that the conidia are produced mainly on the sporodochia-like

structure or on more simple fertile tissue like acervuli, without a scutellum nor setae-like hyphal strand (Pl. 9 A, 9 B). This type of sporulating pattern is very similar to that found in the *Gloeosporium*. Development of the scutellum, if any, is very poor and limited in its extension forming a discal hyphal mat composed of tightly arranged thick-walled septate radiating hyphae.

The shape and size of the fruiting structures produced on the agar media by *A. rubra, A. subglobosa* and *A. castanopsidis* are quite identical with those produced on the natural hosts by a respective species.

Development of the columella is irregular within a given species when the fungus concerned grows on a cultural medium, while they are usually constant in its shape and size on their natural hosts. One of the main reasons of such variation in the structure of the columella is considered that the columella originally has a function to contact onto the surface of its natural host whose physical or mechanical conditions are no more present in the laboratory conditions. The presence or absence of the central cell and accompanying fertile tissue around or upper the central cell have been regarded as one of the critical taxonomic character of the genus concerned. The evidence derived from the present cultural experiment, however, clearly suggests that the structure of columella is not responsible for the strict concept of the genus.

There is no difference in the shape and size of the conidia which developed under cultural condition either on the normal fructification or on the sporodochia-like structure and even on a separate conidiophore arising directly from the somatic hypha, as well as those developed on the natural substrates.

Conidial development and their nature: The conidia of the present genus are of the blastic-type, developing acrogenously and solitarily from the elongate apex of the conidiophore. Each conidiophore develops a papillate or round body at the tip which soon later transforms to a conidium with a minute connection to the conidiophore. Outer wall of both conidium and conidiophore are continuous and a cytoplasmic connection between both cells may present within the thick-walled neck. When the conidium matures, it liberates from the conidiophore by cutting off the neck leaving a cylindrical, distinct frill on the basal end of the conidium. Conidial wall of all species is usually double and hyaline even if the content of the conidia is pigmented. The color of the conidia are variable depend to the cultural coditions, especially to the cultural age, as already pointed out in the case of *A. dryina* (Limber and Cash, 1945; Tehon, 1948). For a best example, *A. rubra* produces almost hyaline conidia of normal size which later change in color from pale yellowish brown to orange yellow and finally to dark brown, particularly before germination. The same is true in *A. subglobosa*. Immature conidia on the conidiophores are uniformly colorless. As the conidia mature, however, they become pale yellowish ochraceous and finally become pale grayish fuscous before germination. Conidia of both *A. japonica* and *castanopsidis* are almost colorless and scarcely change in color after the maturation, although the conidia of the former species more or less tinged with yellowish or pale ochraceous color. Color of

the conidia of *A. dryina* as well as its shape and size is very variable in the different isolates and also with the age and the conditions. Normally the color of this species varies from hyaline to grayish-, greenish-, or almost blackish-brown with age and the isolates. Variability of the color of the conidia of this species have also been discussed by Limber and Cash (1945).

Process of fruiting structure formation: Sporulating process and progressive formation of the fruiting structures were observed microscopically using the slide culture mehtods. Under the slide culture, *A. rubra* and *A. subglobosa* as well as *A. dryina* usually produce the similar fruiting structures and conidia to those developed on the natural host. In *A. dryina*, two types of the sporulating process were observed: one is a normal sporulating process producing fruiting structures such as rhizothyria and its reducible sporodochia-like structures (Pl. 9 F), and the other is a quite different type of a sporulating process commonly found in the hyphomyceteous fungi with branched dendroid conidiophores which bear terminal blastic conidia acrogenously (Pl. 9 A, 9 B). The second type of sporulation method is also common in the other species examined. Such kind of sporulating process is not normal in the order Sphaeropsidales, although it is common in the Hyphomycetes and even in the Melanconiales of the Coelomycetes.

Progressive formation of the fruiting structures was also examined under the slide culture using three species mentioned above. In the case of *A. subglobosa*, a best example, each conidiophore at first appears as a short and simple branch derived directly from the somatic mycelium and soon bears a single conidium acrogeneously in blastic manner. After the conidium matured and liberated from it, a new conidiophore may grow out from the tip of the old conidiophore just below the apical neck (Pl. 11 A-D). Repeated and successive growth of the subsequent conidiophores may occur and eventually a fertile tissue consisting of small spherical parenchymatous cells develops (Pl. 11 E, 11 F). It should be noted that the original conidiophore might has been transformed into a central cell, generally refered to as columella, and most part of the other senescent conidiophores become pigmented and thick-walled which later grow up into the elements of radiating hyphal strands composing the scutellum.

The very similar pattern of formation process of the fruiting structure can be observed in *A. rubra*. In this species, branched or dendroid conidiophore is very rare and usually a separate, branched, short conidiophore develops on which a single conidium is borne (Pl. 10 B). When the conidium matured and discharged from the conidiophore, initial conidiophore then transforms into a central cell from which a new conidiophore may grow downwards (Pl. 10 A). Secondary and, probably, subsequent conidiomay transform into either constructing elements of the scutellum or fertile tissue beneath which subsequent conidiophores may develop later. After a serial production of the conidiophores, a remarkable circular mat consisting of several irregular shaped parenchymatous cells surrounding a central cell develops on a short branch (Pl. 10 C-E). Fresh conidiophores may develop in abundance radially beneath the scutellum (Pl.

10 F-H). Such type of the successive sporulation and subsequent development of the fruiting structures were clearly recognized under the slide culture condition.

Generally each element of either scutellum or fertile tissue may derive from the residual mycelium of the senescent conidiophores that had been already discharged the initial conidia and contributed to induce a new conidiophore. In other words, sporulating process of the present genus is characteristic in producing the conidia not within the accomplished fruiting structure such as pycnidia which is typical and usual sporulating process in the order Sphaeropsidales, but within the particular type of the fruiting structure generally refered to as rhizothyrium in the ways that the production of the conidia proceeds successively during the process of the formation of the fruiting structure.

Cultural condition for production of fruit bodies: Since the standard formula of potato sucrose agar and malt extract agar media may induce the excessive vegetative growth and abnormal fruiting structures with conidia in abundance, weak media or water agar with plant materials is generally recommended for normal formation of the fructification. Plant materials sterilized by propylene oxide treatment or by autoclave is particularly useful in inducing the normal sporulation. Therefore, each isolate was inoculated on the autoclaved leaves of the following four kinds of host plants which previously put on water agar plate: *Quercus phillyraeoides, Q. glauca, Castanea pubinervis, Castanopsis cuspidata.* Each plate provided with five leaf-pieces (1.5×1.5 cm) of a given plant was inoculated by an individual isolate. The plates inoculated were incubated at 25°C for 2 weeks. Gross observation was made under a desecting microscope and closer examination was carried out under a microscope by transfering the fructification and conidia on the slide glass when they appeared. Results obtained are following.

A. japonica may produce the fructification abundantly but exclusively on the leaves of *Castanea pubinervis* and rarely produce them on the leaves of *Q. phillyraeoides.* Neither *Q. glauca* nor *C. cuspidata* is effective to produce the fructification. The shape and size of the fructification thus induced more or less differ from that produced on the natural host leaves in being elongated upwards and povide well-developed parenchymatous fertile tissue around a central cell. Such type of the structure is very similar to so-called sporodochium. Rich in supplying nourishment and high humidity in a plate would probably induce such a fruiting structure. In this case, as well as those produced both in plate and in slant culture, development of the scutellum is usually poor.

A. dryina may produce fructifications in abundance on the sterilized leaves of any kinds. This fungus, however, has tendency to develop more fructifications and conidia on the leaves of *Q. phillyraeoides* and *C. pubinervis* than on those of *C. cuspidata* and *Q. glauca.* The shape and size of the fructifications and conidia are almost the same with those produced on the natural hosts. Microconidial stage presents on any kinds of the plant materials, but seems to be predominent on *Q. glauca* and *C. pubinervis.*

A. rubra may develop the fructifications selectively on the leaves of *Q. phillyraeoides.*

Developed structure of the fructifications and conidia are not distinguishable from those on the natural host plants. Microconidial stage also developed on the same host leaves. This fungus did not sporulate on the remaining species of the host plants tested.

A. subglobosa produces the fruiting structures predominantly on the leaves of *Q. glauca* from which the fungus was isolated originally. This species also forms the fructifications and sporulates rarely on the leaves of *Q. phillyraeoides* and *C. pubinervis*. The shape and size of the fructification and the conidia are quite the same with that found on the natural hosts.

The sporulating process is very similar to or indistinguishable from that developed on the natural hosts and may exclusively occur when *A. castanopsidis* was allowed to transfer on the leaves of *C. cuspidata.*

There is some variations in each species in the substratum suitable for the growth and the normal sporulation. In general, except for only *A. dryina*, the most suitable substratum for the normal sporulation seems to be their original host plants from which each fungus was isolated. Such fungi should be generally considered as the facultative parasites with limited substratum preference or ecological obligate parasite. Conversely *A. dryina* which may produce numerous fruiting structures and conidia on yan kinds of host leaves tested, should be better considered as a obligate saprophyte with high inoculum potential until its parasitic ability is clearly demonstrated.

Again, fruiting structures of *A. japonica* are variable and abnormal on the sterilized leaves in agar plate. Differences in sporulating patterns between the natural and the artificial conditions may well interprete by considering that this species has significantly high affinity to the green living leaves of host plants rather than to the dead leaves of the same species. This species, in other words, has higher differentiated pathogenicity to the living chestnut leaves than that of other species. This is a remarkable character of *A. japonica* differing from the other species, previously refered to as the facultative parasite.

Host preference: The phenomena of host preference shown under culture on the sterilized leaves on the water agar plate may also be interpreted by a factor refering as a substratum resistance. It is generally accepted that not only living hosts but also dead substrata, can exert a resistance to colonization of the fungi inoculated. The substratum resistance is considered to be equivalent, al least in its ecological effects, to the host resistance of a living plants. Although the mechanisms of this substratum resistance may be attributed by several possible factors, what factor of them gives actively an effect on the respective species of the present genus remains as yet uncertain.

It is considered that there are at least three possible sources of substratum resistance. First, it may be a residual host resistance carried over from the living host into moribund tissue for a limited period, in which case it will have its highest level initially and subsequently decline. Second, it may be a result of the production of external toxic metabolites by microorganisms already active in earlier stages of substratum succession,

in which case it may fluctuate in intensity with time. Last, it may be due to an initial refractoriness in the substrate materials, in which case it may be necessary to have a higher inoculum potential which will permit the colonization of more difficult substrata in order to gain enter into them. However, the second factor should be neglected in the present case where the leaves used for inoculation had been previously aged and completely sterilized by an autoclave.

First source of substratum resistance may act a part against *A. dryina* and probably against *A. rubra* and also *A. subglobosa* to gain enter into and induce conidial stage on its respective hosts, though lacking in the accurate evidence. Since it is confirmed that *A. rubra* invade a green living leaves of *Q. phillyraeoides* still on the plants without permission of production of conidial state on the leaf surface while *A. dryina* do not gain enter into a living green leaves of any kinds of host plants tested, the degree of the substratum resistance differ quantitatively as well as qualitatively in according to the combination of the host species and its corresponding fungal invador. As mentioned already, *A. dryina* may gain enter into leaves of various kinds of host species if they are dead or possibly moribund. In these cases host plants should be considered to decline or finally lose their substratum resistance during the senescence and succession of the leaves.

The third source of substratum resistance will be of important factor for any fungi to gain enter into their substratum. The greater the inoculum potential of a given species the wider the range of available substrata species. In this respect, the inoculum potential of *A. dryina* exceed the degree of substratum resistance of many possible host plants, if they are dead, on which the present fungus can colonize and sporulate. On the other hand, inoculum potential of the other species is considered to be in comparatively lower level and much more restricted to colonize in a given host species. Of course, nutritional factors affecting the induction of fruiting structure and subsequent sporulation on it should be considered in addition to these factors.

Effects of temperature on the growth: Attempts were then made to determine the effect of temperature upon the radial growth of the fungi. Each isolate was inoculated on malt extract agar slant and was cultivated at 15, 20, 25, 30, 35 and 45°C, respectively, for 2 weeks. Pairs of the slant were tested for each temperature and all the tests were repeated twice. Results obtained are shown in table 2 and in plate 3. All cultures of the isolates grew gradually even at 15°C, but the more suitable temperature for rapid linear growth may be lie between 20 and 25°, 20 and 30°, 25 and 30°, respectively for *A. japonica* and *A. rubra,* for *A. dyrina,* and for *A. subglobosa* and *A. castanopsidis.* It is worth to mention that *A. rubra* did not grow at 30°, whereas *A. dryina* grows rapidly even at 35°. *A. rubra* was not killed at 30°, sometimes even at 35°, under which temperature no growth was recognized. The same is true in the case of *A. subglobosa* and *A. dryina* at 35°. All the isolates incubated at 45° for 2 weeks did not survive even if they were then transferred to suitable temperature for growth. An optimum temperature for the sporulation was not neccesarily the same as that for radial growth, though accurate measurement have not been given. Generally, however, the sporulation

decreases as the rate of radial growth decreases. That is, higher and lower temperatures of incubation decreased the number of conidia produced. An increase of temperature higher than the optimum markedly reduced in the sporulation than that in lower temperature below the optimum. *A. dryina* is particularly interesting in production of the microconidia in abundance and also in the absence of the conidia when cultivated at higher temperature than the optimum.

Table 2. Growth responce to temperature

Species		Temperature (°C)					
		15	20	25	30	35	45
A. japonica	44081301	++*	++	++	+	—	—
	44091701	++	++	++	+	—	—
	44091704	+	++	++	+	—	—
A. dryina	43111102	++	++	++	++	+	—
	43121806	++	++	++	++	+	—
	44100802	++	++	++	++	+	—
	CBS213-66	+	++	++	++	—	—
A. rubra	44060716	++	++	++	—	—	—
	44060718	++	++	++	—	—	—
	44102801	++	++	++	—	—	—
	45012703	+	++	++	—	—	—
A. subglobosa	43013001	+	+	++	++	—	—
A. castanopsidis	45012701	+	+	++	++	—	—

* - no growth, + moderate growth and ++ good growth

Discussion on Taxonomy

According to Ainsworth (1961), the genus *Actinopelte* have temporally been classified into form-order Sphaeropsidales, form-family Pycnothyriaceae. *Actinopelte* was originally established as the fungal genera belonging to Microthyriaceae of Hemisphaeriales by Saccardo in 1913, who erroneously considered the type species of the genus as an ascomycete. Soon later, Theissen (1913) pointed out that this fungus was not an ascomycete but should be a conidial state of some fungus. He could not, however, give any indication concerning to the family to which the fungus should be included. In 1915, Naoumoff has erected a new genus *Rhizothyrium* which belongs to his new family Pycnothyriaceae. The genus *Actinopelte* has been included by him in his new genus. Later in 1923, von Hoehnel has erected another family, Actinothyriaceae, in which he includes *Actinothyrium* G. Kunze and *Actinopelte* Sacc. In addition, he added a third genus *Columnothyrium* Bubak to this family two years later (1925). He did not give detailed description regarding the differences between Actinothyriaceae and Pyc-

nothyriaceae. On the other hand, Petrak (1924) has proposed to erect a new family Actinopeltaceae, based on the genus *Actinopelte* including only a single species, *A. japonica*, but he gave no citation about Hoehnel's Actinothyriaceae. Consequently Actinopeltaceae Petrak includes only a single monotypic genus at any rate.

A new family Rhizothyriaceae has been erected by Tehon in 1940. It should be noted that he included *Rhizothyrium* Naoum. and *Actinothyrium* G. Kunze in the Rhizothyriaceae but did not list the genus *Actinopelte* in it. He clearly defined the differences by which he distinguished the Rhizothyriaceae from Pycnothyriaceae and proposed a term "rhizothyrium" for the fruiting body produced by the fungi belonging to the Rhizothyriaceae. Differences between these two families are the following. In the Rhizothyriaceae a superficial mycelium or subiculum are devoided and the fruiting body (rhizothyrium) usually attached to mycelium within the host tissue by a columella, whereas in the Pycnothyriaceae the fruiting body (pycnothyrium) can be considered to be developed in connecting with an external mycelium or subiculum and no recognizable structure like a columella develops. Both families are characterized by superficial, radiate fructification and by its inverse sporulation. By the above reasons Tehon separated the genera usually included in the Leptostromataceae into the two families, Rhizothyriaceae and Pycnothyriaceae, and combines in a distinct order Pycnothyriales. These families differ from the Leptostromataceae in bearing the conidia on the under side of the pycnidial cover, not of the basally.

According to Limber and Cash's citation, the Pycnothyriaceae are placed as pycnidial forms near the Leptostromataceae of Sphaeropsidales in Hoehnel's key, whereas the Actinothyriaceae are classed with the Tuburculariaceae of Moniliales on the basis of lacking of the true pycnidia. They, however, considered that the family Rhizothyriaceae, like Petrak's Actinopeltaceae, seems to be indistinguishable from von Hoehnel's Actinothyriaceae. Consequently, according to the numerous previous knowledges concerning to the taxonomy of these groups of fungi, Limber and Cash claimed that Tehon's Rhizothyriaceae (Actinothyriaceae Hoehn.) and the Pycnothyriaceae in the order Pycnothyriales Tehon appears to be more logical than that in the von Hoehnel's classification. They recognized both the differences between these two families and the common characters which distinguish them from the Leptostromataceae.

The order Pycnothyriales, however, is not currently accepted and the division of the imperfect fungi into four orders is still used at present. They are the Sphaeropsidales, the Melanconiales, the Moniliales and the Mycelia Sterilia, respectively. Grove has devided the Deuteromycetes into two groups, the Hyphomycetes and the Coelomycetes: the former includes the Moniliales and the Mycelia Sterilia, and the later includes the Sphaeropsidales and the Melanconiales. The fungi classed into the Sphaeropsidales may produce the conidia within the pycnidia, whereas those grouped into the Melanconiales may produce the conidia within the acervuli. It is quite evident that the fungi belonging to the genus *Actinopelte* do not produce the true pycnidia, but produce

rhizothyria as previously mentioned. The conidia are borne inversely underbeneath the radiate scutellum supported by the columella. Such type of the open, inverse sporulating process is unusual in the coelomyceteous fungi, but is better to be considered to have some similarities with those of the sporodochia-forming groups of the hyphomyceteous fungi. This will support Hoehnel's classification in which he placed the Actinothyriaceae together with the Tuberculariaceae in the Moniliales on the basis of the lacking of true pycnidia. It is significantly important to note that the conidia of coelomyceteous fungi may produce and mature as a result of accomplishment of pycnidial formation, while in the present genus the fruiting structure may accomplish as a result of conidial formation or in accompaning by a successive formation of conidia. Another type of sporulating process which can be found under the slide culture condition where the conidia are produced on either simple or much branched and sometimes dendroid conidiophores, also suggests that the present genus behaves like the hyphomyceteous fungi in this sporulating process. In either cases, the type of sporulation in cultural condition is quite comparable to those of the hyphomyceteous fungi. It is obvious that the genus *Actinopelte* would possess a particular intermediate character between the coelomyceteous and the hyphomyceteous fungi in its sporulating process, although their appreciable taxonomic position still remain to determine.

Up to the present time, no available knowledge of possible perfect stage of the present genus is presented so far. Temporally, von Hoehnel have stated about the possible relationship to microthyriaceous fungi with his actinothyriaceous fungi and also to the genus *Dasyschypha* or *Lachnum* with *A. dryina*, without any appreciable evidence. Rhizothyriaceae has also been considered as the imperfect stage of the Polystomellaceae by Tehon only based on its morphological feature. Cultural evidence of relationship between the present genus and its perfect stage would be one of the indispensable factors for the authentic classification of *Actinopelte* and works on this problem are now in progress.

Summary

Collections and isolation of *Actinopelte japonica* Sacc. (on *Castanea pubinervis*), a type of the genus, and *A. dryna* (Sacc.) Hoehn. (on *Castanea pubinervis*, on *Quercus phillyraeoides* and on *Q. glauca*) were made and the emmended descriptions based on the morphological and the cultural characteristics were given. In addition to them, many materials of undescribed species of *Actinopelte* were collected from leaves of various deciduous trees and the following three new taxa were presented: *Actinopelte rubra* (on *Q. phillyraeoides*), *A. subglobosa* (on *Q. glauca*) and *A. castanopsidis* (on *Castanopsis cuspidata*). Discussion on scutellum development, conidial development, process of frulting structure formation, cultural conditions for fruiting body production, host preference and effect of temperature for the growth were presented. A possible systematic position of the genus *Actinopelte* was also discussed.

Acknowledgement

The authors are pleased to acknowledge the sending of the specimens of the National Fungus Collection, Beltsville, to Dr. C. R. Benjamin, and are indebted to Dr. T. Hasegawa, the Director, for his generosity. The writers wish also to thank Dr. T. Kobayashi and Dr. K. Uchida for their kind cooperation in collecting many fresh maturials of *A. japonica* and for the privilege of examining herbarium specimens.

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Plate 1. Habits of the fructifications on the host plants. A-D. *A. japonica*. A. Necrotic spot on the host leaf. B. Rhizothyria within a spot (44091701). (Ultropak, ×38). C. Rhizothyria and conidia on the sterilized host leaf (44081301). (Ultropak, ×38). D. Rhizothyria within a spot (Specimen from Sydow's fungi exotici exsiccati no. 526). (Ultropak, ×38). E. *A. dryina* (43111102). (Ultropak, ×38). F. *A. rubra* (44102801). (Ultropak, ×38). G: *A. subglobosa* (43013001). (Ultropak, ×38). H. *A. castanopsidis* (45012701). (Ultropak, ×38).


A
B
C
D
E
F
G
H

Plate 2. A-F. Comparative growth on different media. Upper left, PSA; upper right, oatmeal agar; lower left, malt extract agar; lower right, Czapek agar. *A. Actinopelte japonica* (44091704). B. *A. dryina* (43111102). C. *A. dryina* (CBS 213–66). D. *A. rubra* (44102801). E. *A. subglobosa* (43013001). F. *A. castanopsidis* (45012701).

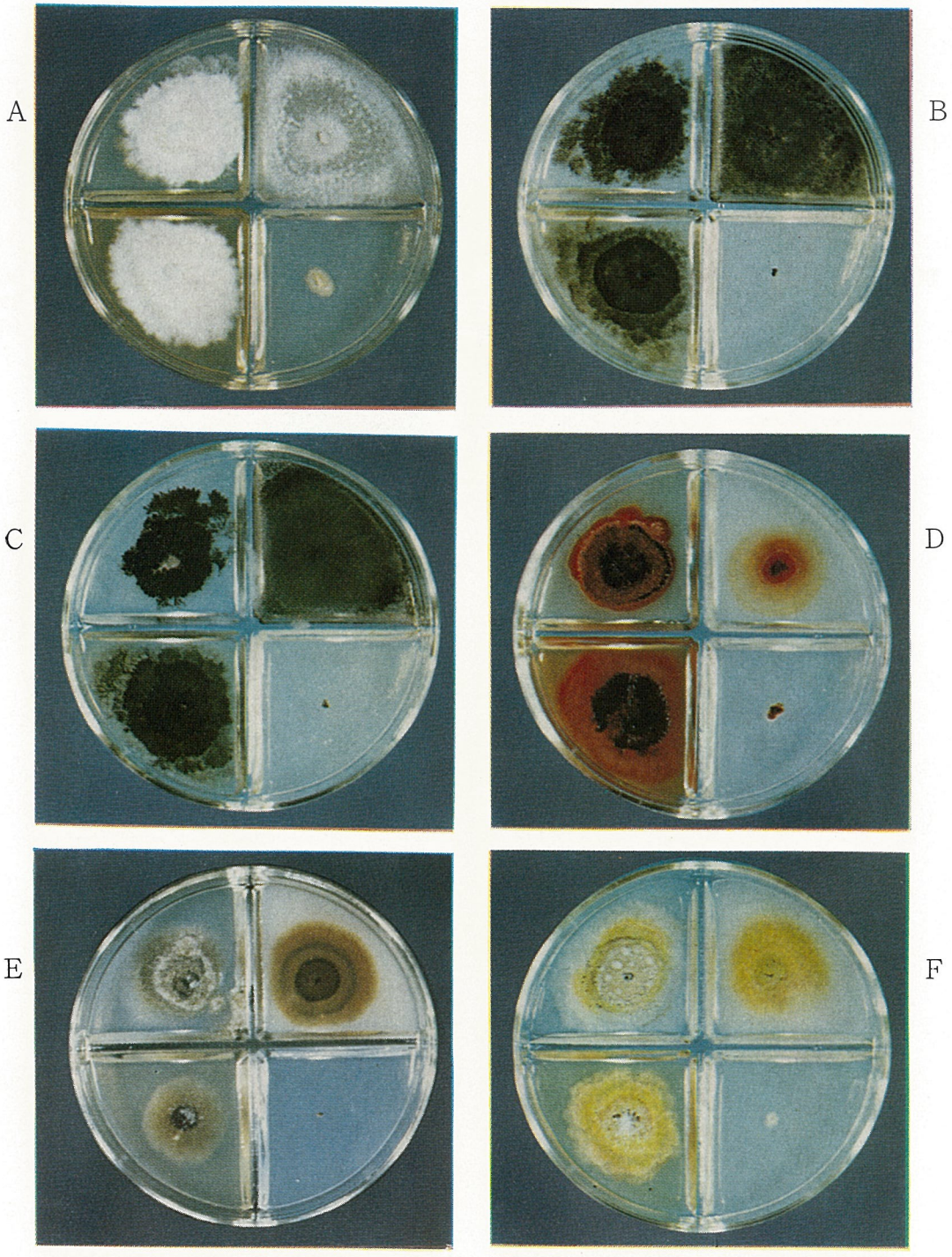
A
B
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Plate 3. A-F. Comparative growth on malt extract agar slant under different temperatures. A. *Actinopelte japonica* (44091701). B. *A. dryina* (43111102). C. *A. dryina* (CBS 213–66). D. *A. rubra* (44102801). E. *A. subglobosa* (43013001). F. *A. castanopsidis* (45012701).


A
B
C
D
E
F
15 20 25 30 35 45°C

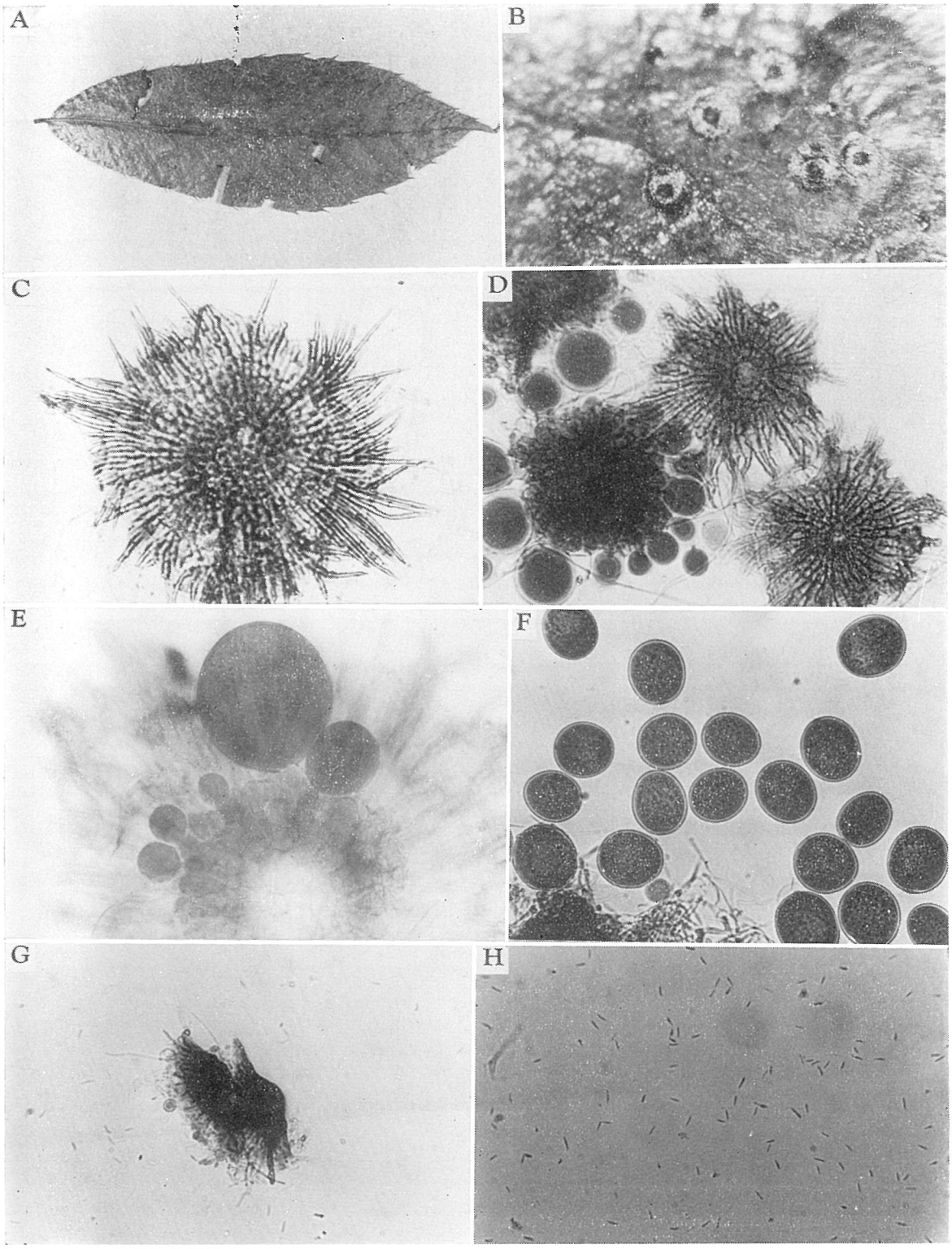

Plate 4. A-H. *Actinopelte japonica*. A. Specimen from the Sydow's fungi exotici exsiccati, No. 526. B. Habit of the fructifications on the host leaf. (Ultropak, ×38). C. Surface view of a rhizothyrium showing radiating scutellum. (44091701, ×250). D. Bottom view of a rhizothyrium showing the parenchymatous columella and the conidiophores surrounding it. (44091701, ×250). E. Pattern of the conidial formation in detail. (44091701, ×400). F. Conidia. (44081301, ×250). G. Microconidial rhizothyria. (44081301, ×250). H. Microconidia. (44081301, ×250).

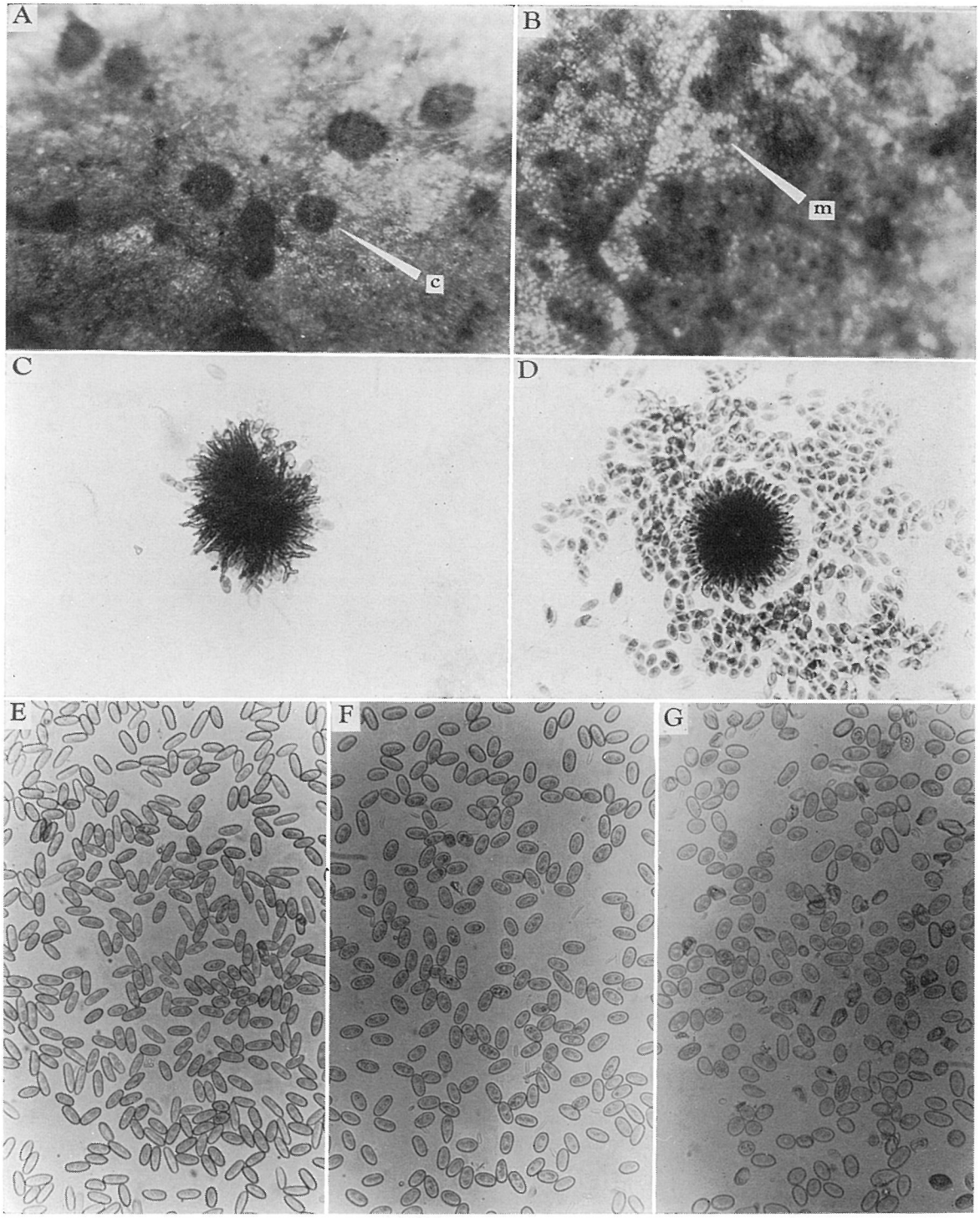

Plate 5. A-G. *Actinopelte dryina*. A. Habit of the fructifications; conidial rhizothyria (c) on the host leaf. (43111102, Ultropak, ×38). B. Ditto; microconidial rhizothyria (m) on the host leaf. (43111102, Ultropak, ×38). C. Surface view of a rhizothyrium showing the radiating scutellum. (43111102, ×250). D. Bottom view of a rhizothyrium showing the conidiophores surrounding the columella. (43111102, ×250). E-G. Conidia. (×250). E. 43111102. F. 44101806. G. CBS 213–66.

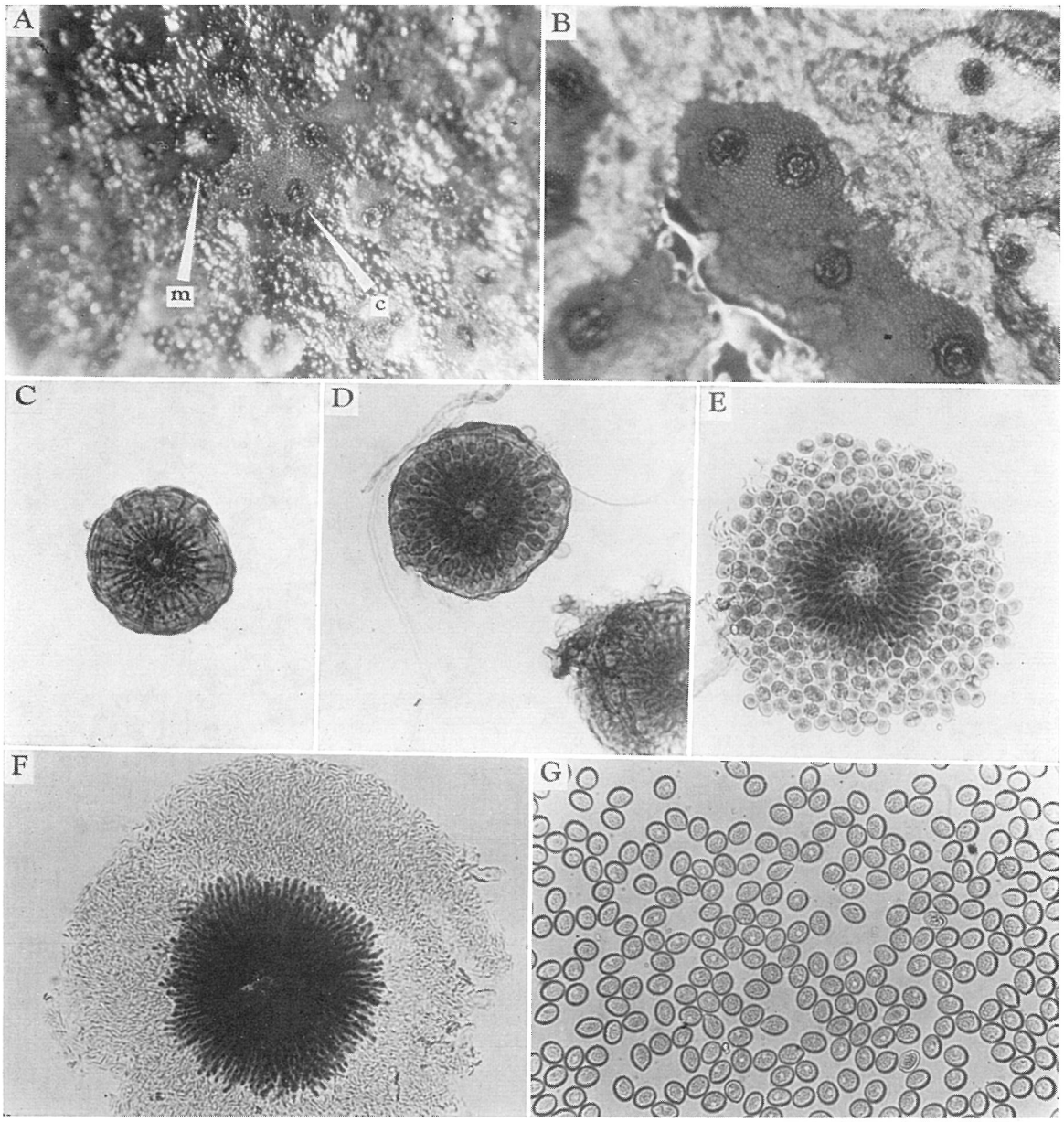

Plate 6. A-G. *Actinopelte rubra*. (44102801). A. Habit of both the conidial (c) and the microconidial rhizothyria (m) on the host leaf. (Ultropak, ×38). B. Ditto. (Ultropak, ×65). C. Surface view of a rhizothyrium with the waved and continuous periphery. (× 250). D. Bottom view of a rhizothyrium showing the fertile tissue arising from just beneath the scutellum. (×250). E. Ditto. (×250). F. Bottom view of a microconidial rhizothyrium. (×250). G. Conidia. (×250).

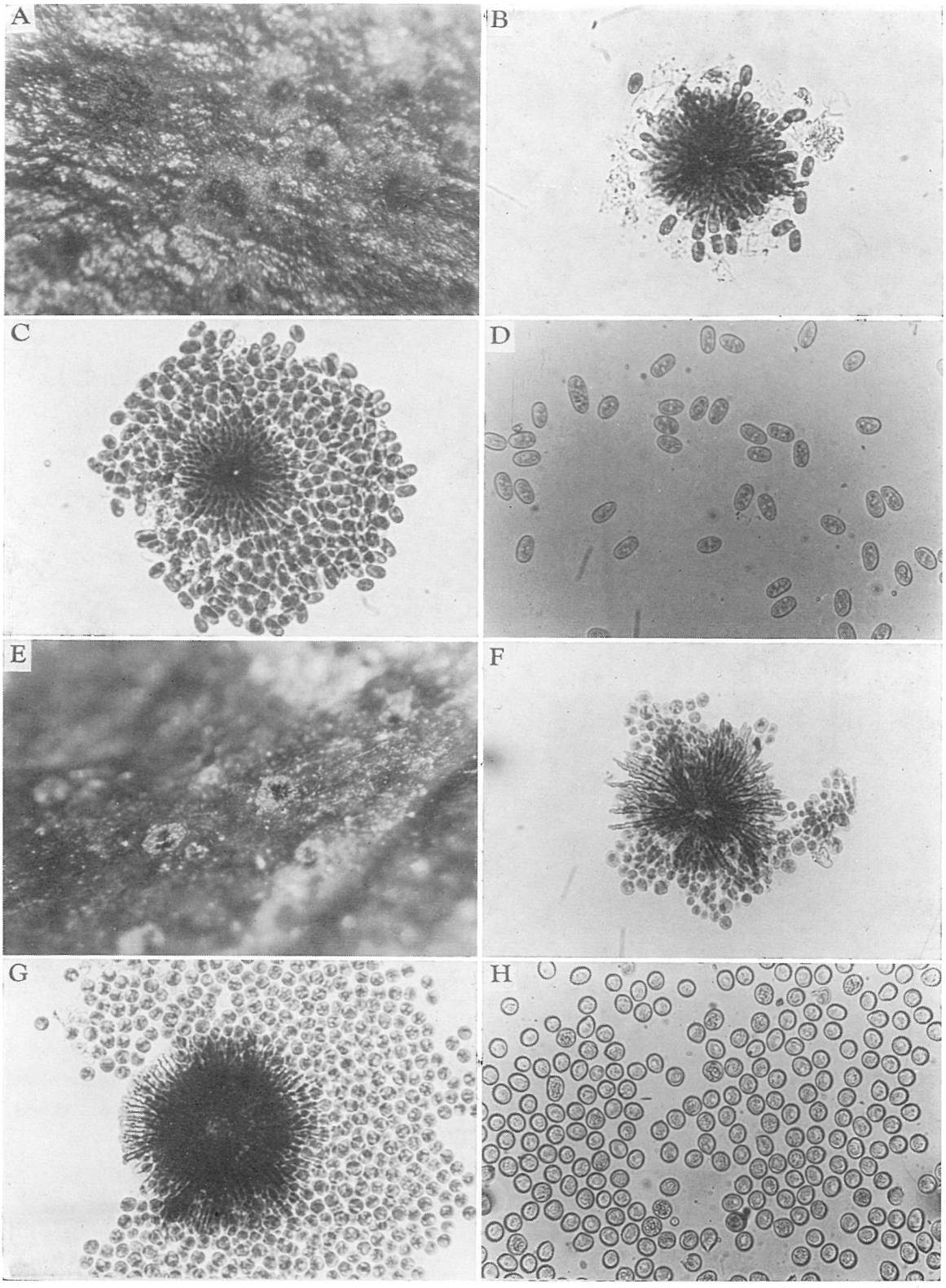

Plate 7. A-D. *Actinopelte castanopsidis* (45012701). A. Habit of the fructification. (Ultropak, ×38). B. Surface view of a rhizothyrium showing the radiating scutellum. (×250). C. Bottom view of a rhizothyrium showing the parenchymatous fertile tissue and conidial mass. (×250). D. Conidia. (×250). E-H. *Actinopelte subglobosa* (43013001). E. Habit of the fructification on the host leaf. (Ultropak, ×38). F. Surface view of a rhizothyrium showing the radiating scutellum and conidiophores underbeneath it. (×250). G. Bottom view of a rhizothyrium showing the parenchymatous fertile tissue and conidial mass. (×250). H. Conidia. (×250).

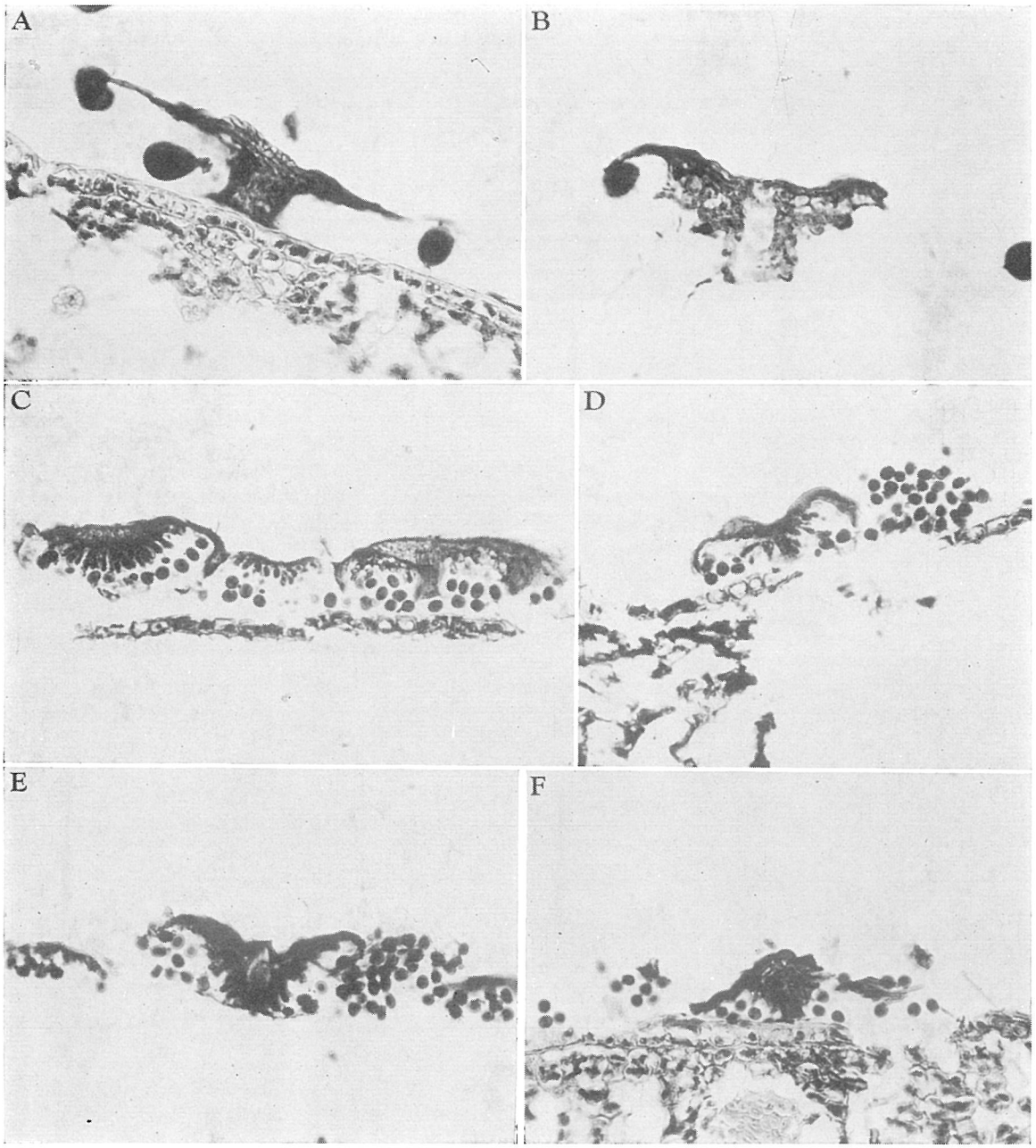

Plate 8. A-F. Cross sections of the rhizothyria. (×250). A and B. *Actinopelte japonica*. C and D. *Actinopelte rubra*. E and F. *Actinopelte subglobosa*.

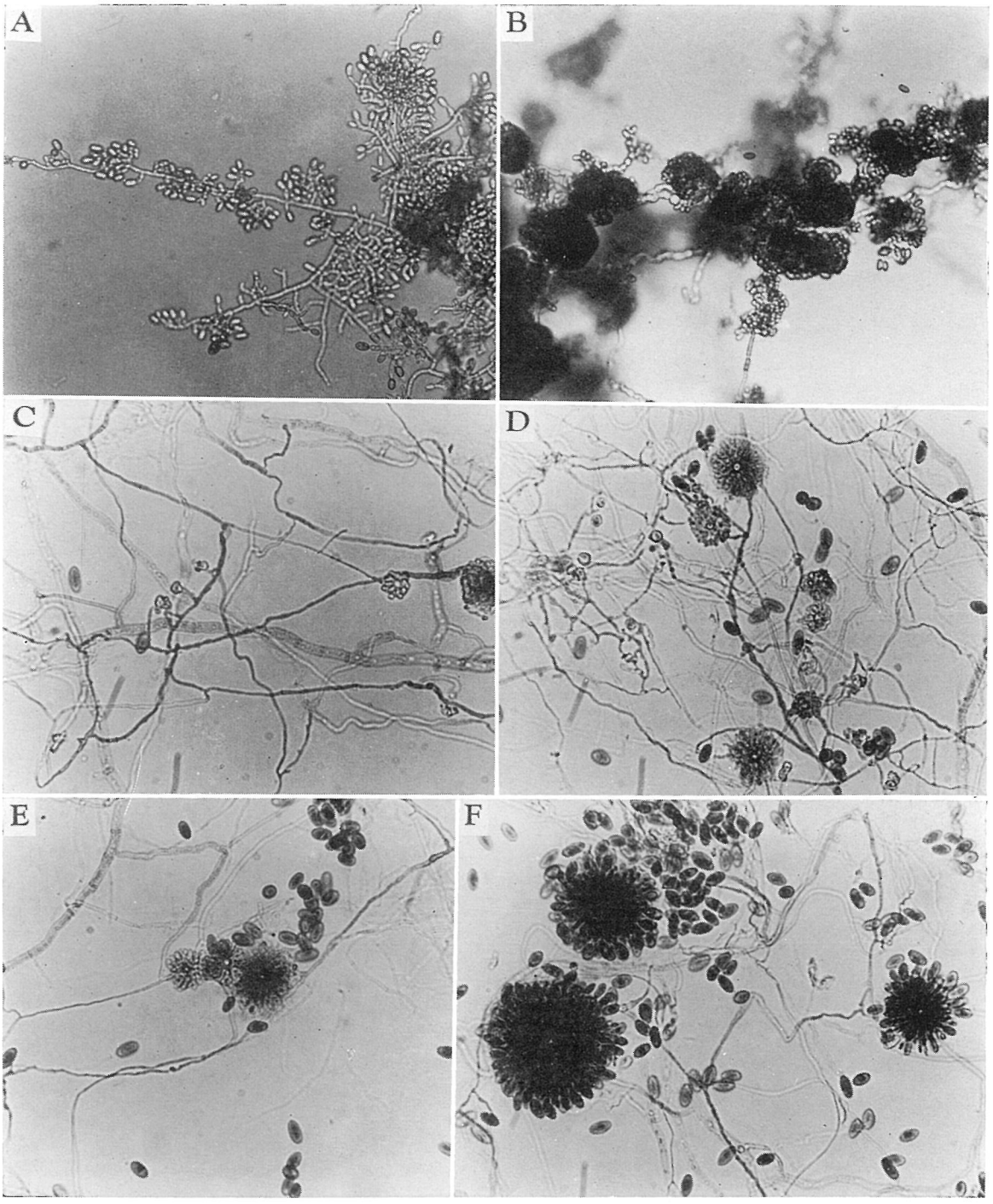

Plate 9. A-F. Two types of conidial formation of *Actinopelte dryina* under culture. (×250). A. Conidial development on the conidiophores, giving the hyphomyceteous sporulating pattern. (CBS 213–66). B. Ditto. (43111102). C-F. Sporulation by rhizothyrial development showing a series of the progressive formation of the conidia and rhizothyria. (43111102).

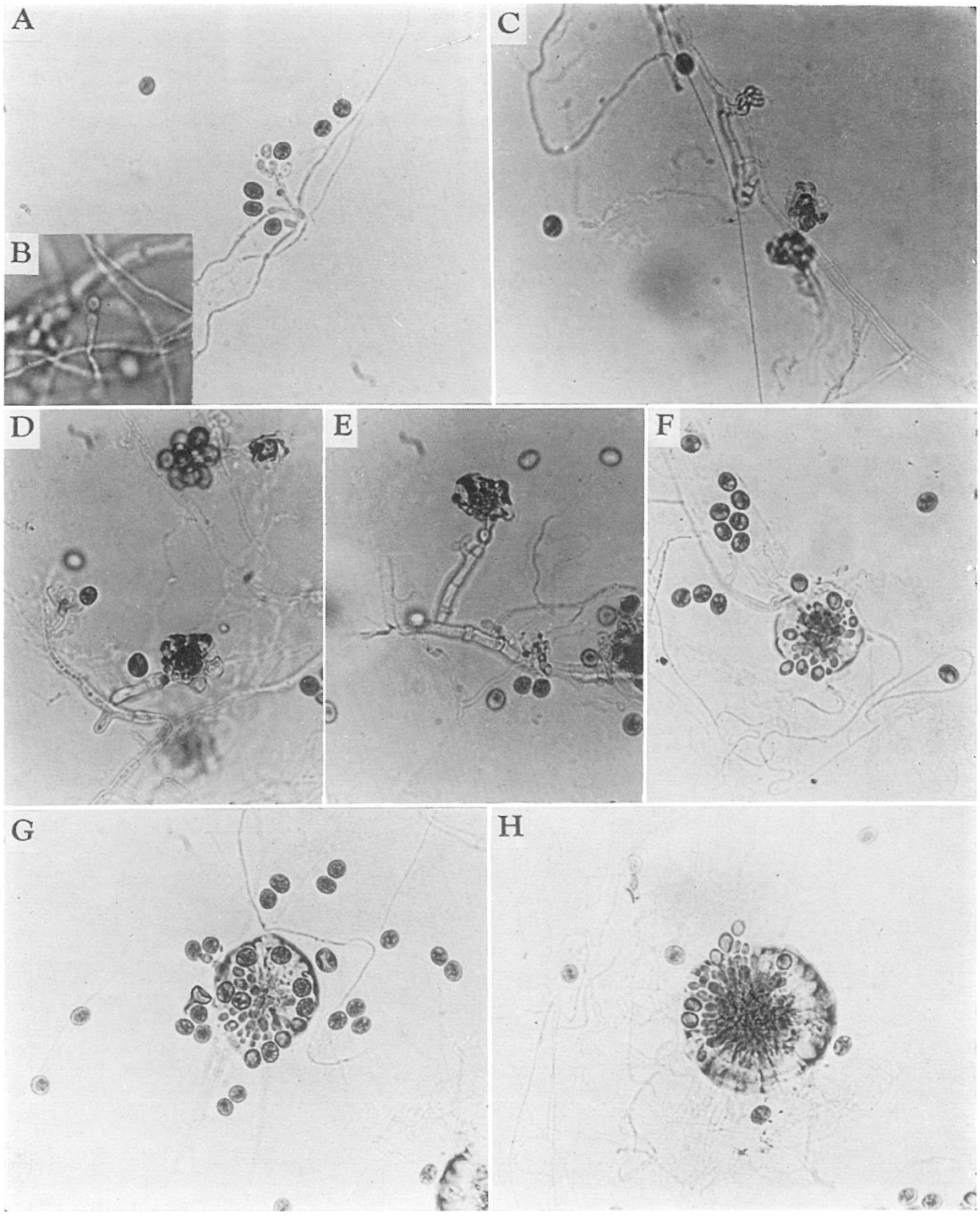

Plate 10. A-H. A series of the progressive formation of the conidia and rhizothyria of *Actinopelte rubra* under culture. (44102801, ×250). A. Secondary and subsequent formation of the conidiophore above the initial conidiophore. B. Primary formation of the conidium on the simple conidiophore arising from the somatic mycelium. Note the connecting neck between the conidium and the conidiophore. C-F. Early phases of the formation of the rhizothyria. F-H. Progressive development of the rhizothyria and continuous formation of the conidia.

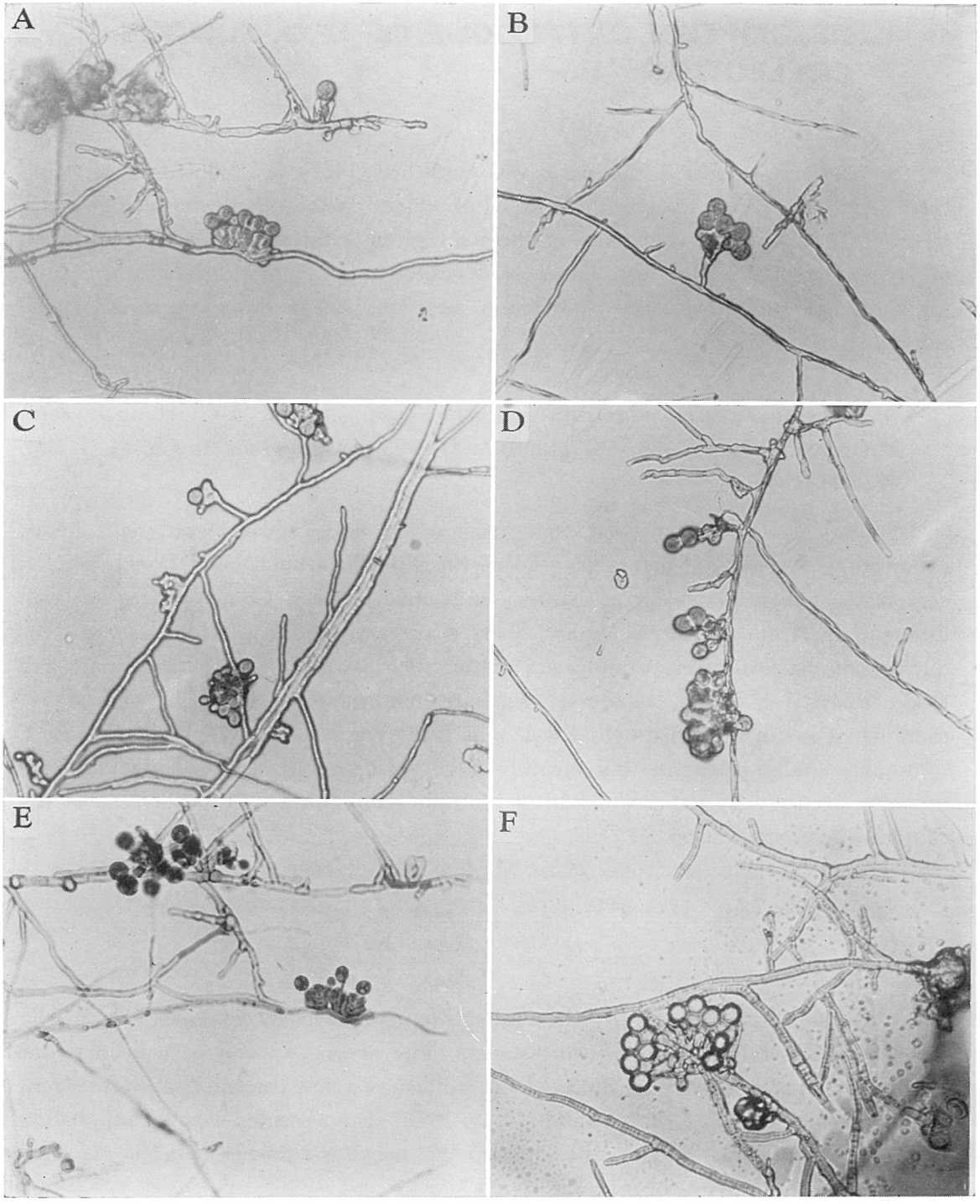

Plate 11. A-F. Serial patterns of the conidial and the rhizothyrial development of *Actinopelte subglobosa* under culture. (×250).

IFO Res. Comm. **5**,
78–90, 1971 (*March*)

DESCRIPTIVE CATALOGUE OF I.F.O. FUNGUS COLLECTION. II.

Since the first paper in this series was published (1969), the following interesting fungi have been added to our collection, all of which have been not yet or insufficiently recorded from Japan. Descriptions of them are given with explanatory notes. Unless otherwise stated, the dried materials were deposited in the Herbarium of the Institute for Fermentation. As described previously, new taxa will be described in other mycological periodicals. (K. TUBAKI)

12. **Berkleasmium concinum** (Berk.) Moore (Pl. 1, A-C) Hyphomyectes Moore, in Mycologia **50:** 687 (1958) & **51:** 734 (1959); Goos, in Canad. J. Bot. **47:** 503 (1969)

Sporodochia on rotten wood, superficial, sessile, gregarious or scattered, hemispherical or flattened, at first mucous, glistering and pale cream colored, then becoming compact, confluent and black at maturity as conidia develop. Conidiophores not well differentiated from vegetative hyphae, short if present, 2.5–3.5 μ wide, septate, producing conidia terminally. Conidia are of the aleuriospore-type, at first hyaline and long cylindrical or fusoid, transversely septate, then becoming darker and phaeodictyosporous at maturity; broad cylindrical, cells fairly regular, fuscus, 30–112$\times$22–26 μ, commonly 90–100 μ long, with a abruptly truncated base measuring 3–4 μ wide. Germination occurred by the production of multiple germ tubes from outer cells of the conidia on common media.

Hab.; On the rotten wood, Maze, Mashita-district, Gifu Pref., July, 1970.

Strain preserved; IFO–9334 Coll. K. Tubaki
Isol. K. Tubaki
Det. K. Tubaki

Cultural behaviour is nearly the same with that described by Goos (1969) who was first successful in culturing the fungus. The present species have been so far found only on the dead wood. Since Moore published a new combination (Sept.-Oct., 1958), Hughes (Canad. J. Bot. **36:** 740, Nov. 1958) also presented a new cominbation as *B. concinum* (Berk.) Hughes one month later, hence the former name is accepted according to the principle of priority.

13. **Candelabrum spinulosum** v. Beverwijk (Pl. 1 D-E) Hyphomycetes Antonie v. Leewenhoek **17:** 9 (1951); Bottomley, in Trans. Brit. Mycol. Soc. **37:** 234 (1954).

Vegetative hyphae slender, branched, septate, 1.5–2 μ, hyaline. Conidiophores short, straight, uneven at surface with a constriction at base, variable in length, commonly 14–15 μ long, 2.0–2.5 μ wide at base, tapering at point of conidial attachment, hyaline,

bearing simple terminal conidium. Conidia consist of H-shaped body of four smooth central cells and eight lateral cells which grow out from each of central cells; lateral cells irregularly oblong and characteristically densely covered with blunt spines. Diameter of conidia in top view, 12–14 μ, and in side view, 12–14$\times$9–10 μ, hyaline.

Growth on malt extract agar, slow, tough and leathery, irregularly furrowed, without prominent aerial mycelium, tufts of vegetative hyphae may develop at central part, pale cream colored. Conidial production often observed at outer part of colonies.

Hab.; On the decaying leaves in water, Botanical Garden of the Osaka City University, Kisaichi, Osaka, Feb., 1968 (166–6–2).

Strain preserved; IFO–9335 Isol. T. Ito
Det. K. Tubaki

The present species is not uncommon on the decaying, nearly blackened leaves, especially under water. Second species, *C. japonense* Tub. (J. Hattori Bot. Lab. **20:** 149, 1958) which has larger conidia is rarely found. In addition to them, an undescribed large spored species of *Candelabrum* is frequently encountered during the study on the fungal flora of the leaves; diameter of the conidia is more than 80 μ. Description will be given otherwise.

14. **Dactylaria purpurella** (Sacc.) Sacc. (Pl. 2 A-B) Hyphomycetes Michelia **2:** 20 (1880); Hering, in Trans. Brit. Mycol. Soc. **48:** 666 (1965).

Conidiophores erect from vegetative or aerial hyphae as side branches, often little differentiated from hyphae, septate, simple or sometimes branched, 68–130 μ long or more, 3.5–4 μ wide at base, tapering to apex measuring 2–2.5 μ wide, hyaline, denticulate at upper part, increase in length in acropetal succession. Conidia are of the sympodulospore type, borne singly on denticles, mostly in a loose cluster, cylindrical with a pointed base, 1–3–septate, 14–22$\times$3–4 μ, hyaline.

Growth on malt extract agar slow and more or less restrict, raised, gray-brown to pale pinkish purple; reverse and agar dark red-purple; sporulation occurs on the mycelium developed along the inside of test tubes or petri dishes, pure white in mass. On potato-sucrose agar, growth slightly reduced, same colored.

Hab.; On the dead leaves of *Castanopsis cuspidata* Schottky var. *sieboldii* Nakai embedded in the forest litter for three months, Ishiyama Temple, Shiga Pref., Dec. 1969 (A–3–4–7).

Strain preserved; IFO–9336 Isol. T. Ito
Det. K. Tubaki

Same fungus also developed on the dead leaves of *Quercus phillyraeoides* A. Gray in the same locality (in this journal*, p. 32). Presence of the present fungus, a type of the genus, in the litter was reported since Saccardo (1880) by Hering (1965) who isolated it from about one-third of the leaves collected in an oak litter.

* .. Tubaki, K. & Yokoyama, T.: Successive fungal flora on sterilized leaves in the litter of forests. I. *in this journal* p. 24, 1971

15. **Deightoniella torulosa** (Syd.) M.B. Ellis (Pl. 2 C–D) Hyphomycetes Mycol. Pap. Comm. Mycol. Inst. **66:** 7 (1957).

Conidiophores erect from immersed or creeping hyphae, single or in small groups, straight or flexuous, septate, simple or rarely branched, swollen into a subglobose bulb 12–13 μ wide at apex, with up to thirteen successive proliferations through each conidial scar, up to 300 μ long, 7–8 μ wide at base, 6–7 μ wide at apex just below uppermost bulb, dark reddish brown and paler apex. Conidia are of the aleuriospore-type, develop singly as blown-out ends of conidiophores, proliferate successively through previous conidial scars, straight or slightly curved, obpyriform or obclavate, pale brown to pale gray brown, dark brown around scars, 3–6–pseudoseptate, 35–60$\times$17–20 μ.

Growth on malt extract agar rapid, woolly, gray to gray-olive, with conidia in abundance; reverse dark olive brown. On potato-sucrose agar and on V–8 agar, growths are moderate with conidia. On Czapek agar, growth is more or less restrict.

Hab.; On decaying leaves of banana (*Musa* sp.), Miyanoura, Yaku Island, Kagoshima Pref., June, 1970.

Strain preserved; IFO–9333 Coll. T. Yokoyama
Isol. T. Yokoyama
Det. T. Yokoyama

The present fungus is unique in the conidiophore with many bulbs, and the conidium-development method is principally homologous with the development of annellation: successive elongation of the conidiophore through the scars left by the previous aleuriospores. This fungus is known as a causal one of the Jamaican Lacatan banana fruit-spots (Meredish, in Nature, Lond. **187:** 961, 1960; Trans. Brit. Mycol. Soc. **44:** 95, 1961; ibid **44:** 265 & 391, 1961), and is distributed widely in tropical area (Ellis, 1957; Meredish, 1960; Joly, in Bull. Soc. Mycol. Fr. **81:** 285, 1965), mostly on *Musa* sp. This fungus is also not uncommon on the banana-leaves in Japan although no description has been given so far.

16. **Monacrosporium ellipsosporum** (Grove) Cooke & Dickinson (Pl. 2 E-F) Hyphomycetes

Cooke & Dickinson, in Trans. Brit. Mycol. Soc. **48:** 621 (1965).
syn. *Dactylella ellipsospora* Grove, in J. Bot. Lond., **24:** 200 (1886).

Vegetative hyphae slender, septate, 1.5–2.5 μ wide, hyaline. Conidiophores erect from hyphae, hyaline, septate, straight, simple or often branched, 120–200 μ long, 2–3 (4) μ wide at base, tapering upward to 1.0–1.5 μ wide, bearing a single terminal conidium. Conidia symmetrically fusoid, acutely rounded at apex, truncate at base, typically 4–septate, central cell larger, 36–46$\times$9–15 μ, hyaline. Stalks bearing predacious adhesive knobs, unseptate, 3–10 μ long, 2–3 μ wide; knobs nearly globose, 4–6 μ wide, hyaline.

Growth on malt extract agar moderate, woolly, white to pale olive; reverse hyaline; conidial production reduced. On potato-sucrose agar, growth is similar, pale olive to gray; reverse olive; conidial production reduced. On oat-meal agar, growth is good, producing synnemata; gray green to dark olive; reverse same colored; conidia developed in abundance.

Hab.: On the dead leaves of *Castanopsis cuspidata* Schottky var. *sieboldii* Nakai, Ishiyama Temple, Shiga Pref., Feb. 1970 (A–2–5–4).

Strain preserved: IFO–9337 Isol. T. Ito
Det. K. Tubaki

The present species is unique in its symmetrically fusoid conidia. Growth on the leaves spreads and conidiophores erect separately from the creeping hyphae. The present species differs from *M. lysipagum* (Drechs.) Subramanian mainly in the absence of the predacious ring. Diameter of the conidia of both species is nearly identical or those of the latter species are somewhat smaller. Diameter of the knobs of the present strain is smaller than the description given by Drechsler (1937), however such difference not warrents to separate a species because the spores or other organs in pure culture are usually smaller than those in the presence of nematodes.

17. **Oidiodendron citrinum** Barron (Pl. 3 A) Hyphomycetes
Canad. J. Bot. **40:** 589 (1962).

Conidiophores arise from vegetative and aerial hyphae or from coremia, usually 50–150 μ long, 1.5–2 μ wide, tapering towards tips, septate, smooth, fuscous, hyaline in upper portions; fertile hyphae branched undulate, 1.5 μ wide. Conidia are of the arthrospore-type, developed basipetally on fertile region, ovoid or short cylindrical, 2.5–4.5 $\times$ 1.5–2.5 μ, smooth or finely roughened, yellow to orange; connectives persist between conidia.

Growth on malt extract agar rapid, quickly becomes green-yellow, with conidia in abundance, often funiculose in center, margin paler, velvety, with sterile margin; reverse olive-brown to dark brown.

Hab.: On the decayed leaves of *Castanopsis cuspidata* var. *sieboldii* Nakai, Ishiyama Temple, Ishiyama, Shiga Pref., Nov. 1969 (A–2–1).

Strain preserved: IFO–9338 Isol. T. Ito
Det. K. Tubaki

Collections have been made on the embedded sterilized leaves of the above trees and also of *Quercus phillyraeoides* A. Gray during October through March. This fungus has developed on the one-month embedded leaves in the natural forest litter from fall to spring (in this journal, p. 33). Growth on the leaves is readily distinguished because of its bright yellow to mustard-yellow color.

The present fungus is close to *O. flavum* Szilvinyi, but the undulate fertile hyphae are characteristic.

18. **Phialomyces macrosporus** Misra & Talbot (Pl. 3 B-E) Hyphomycetes Canad. J. Bot. **42:** 1287 (1964).

Conidiophores erect from vegetative hyphae, usually unbranched, straight, septate, up to 1 mm long, 5–6 (8) μ wide, hyaline; terminate in a fascicle of phialide which arranged by a monoverticillate manner, bearing a persistent chain of phialospores. Phialides subcylindrical to bottle shaped, 1–3 (4) in number, abruptly attenuated into a short neck at apex, 22–28 (30) $\times$6–9 μ, hyaline. Conidia are of the phialospore-type, fusiform-ellipsoid or lemon-shaped, biapiculate; distal end gradually apiculate and promixal end abruptly truncate; dark yellow-brown at maturity, verrucose, thick walled, 20–28 (30)$\times$16–20 μ.

Growth on malt extract agar rapid, spreading widely, velvety, with sterile marginal area, at first grayish olive, then becoming dark olive to almost black; reverse and agar yellow-brown to dark brown.

Hab.: Isolated from soil, Hiroshima, April, 1970 (Minoura, a–7 P).

Strain preserved: IFO–9323 Isol. K. Minoura, Hiroshima Univ.
Det. K. Minoura & K. Tubaki

The present fungus has been found only twice from the soil of India and New Zealand (Misra & Talbot, 1964), hence the present isolation is apparently the third record from nature. Morphological characteristics of the present isolate agree well with the description given by the authors.

Phialospore-formation is peculiar. As the first conidium develops, the next conidium-initial swells out from the phialide as an elliptical one pushing up the promixal end of the first conidium, then gradually transformed to lemon-shape; cross wall is evident between the two. Distal end of the conidium is apiculated adhering the minute pointed connective (Pl. 3 E) which attached to the promixal end of the previous conidium; promixal apiculus is shorter than the distal one and abruptly truncate at apex. Therefore, individual conidium is characteristically different in shape of top and bottum, especially in younger conidia although this difference may become less prominent in matured ones. This unequal shape of both ends may indicates that the connections of the conidia has not developed by usual manner but by a special wedge-like formation between the two.

19. **Sporotrichum thermale** Apinis (Pl. 3 F) Hyphomycetes Nova Hedwigia **5:** 74 (1963); Semenium & Carmichael, in Canad. J. Bot. **44:** 105 (1966).

Vegetative hyphae consist of septate, irregularly branched, more or less sinuous hyaline hyphae, 1.0–2.0 μ wide. Conidiophores not well differentiated. Conidia are of the aleuriospore-type, acropleurogenous, develop directly on hyphae or on short stalks, in a large, irregular, loose clusters, ovate, pyriform or clavate with a broad scar or pointed at base, thick walled, smooth or sometimes warted at maturity, 4–7 $\times$3–4 μ, hyaline or pale brown.

Growth on malt extract agar at 37–45°C, very rapid, broadly spreading, velvety or granular, at first white, then becoming pinkish buff, fulvous to light gray brown; reverse hyaline to brown or darker. Colonies at 25° for 5 days were 14 mm wide; 30°, 55 mm; 37° to 45°, luxuriant with conidia in abundance; 55°, growth absent and didn't yield when transfered to 30°C.

Hab.: Isolated from the heated portions of the imported rubber tree chips stored outdoors, Kyoto, Jan., 1970 (Ueyama, 3–3–45–a).

Strain preserved: IFO–9219. Isol. A. Ueyama, Kyoto Univ.
Det. K. Tubaki

Apinis isolated several strains of this species from the soil in the upper layers of dry and water-logged pastures and from plant debris in Nottinghams, England. Carmichael, then, isolated a same fungus from molding, open-stock alfalfa silage and from fir and spruce pulpwood chips in Canada. Therefore this fungus would be concerned to be widespread in nature, especially in self-heating organic materials. As Carmichael aptly pointed out, the present species is very close to *Chrysosporium* because of the aleuriospore-formation.

20. **Tetraploa ellisii** Cooke (Pl. 4 A-G) Hyphomycetes

Cooke & Ellis, in Grevillea **8:** 12 (1879); Shirai & Hara, A List of Jap. Fung. Hith. Known, ed. III, p. 387 (1927); M.B. Ellis, in Trans. Brit. Mycol. Soc. **32:** 249 (1949).

Colonies on host plant, irregular, spreading, dark brown. Vegetative hyphae slender, sinuous, septate, branched, 2–2.5 (3.5) μ in diam., pale to dark brown. Conidiophores absent or very short, 5–6 μ long, pale brown; often small and globose cell swells out which measuring 6–8 μ in diam. bearing conidium-primordium on it. Conidia borne directly on hyphae or on small basal swellings, each consisting of 2 or 4 verrucose columns, each of which gradually attenuated into a long septate appendage, dark brown; one of these columns connected to hyphae or to globose cell at base; body of conidia, 34–40 μ long, 14–16 μ wide, 1–5 septate; appendages, (8) 16–45 μ long, 4–8 μ wide at base, tapering to 2–3.5 μ at apex, 2–5–septate, brown.

Growth on malt extract agar, rapid, floccose, with conidia in abundance, pale olive brown; reverse same colored.

Hab.: On dead stalks of *Miscanthus sinensis* Anders., Suita, Osaka, June, 1969.

Strain preserved: IFO–9215. Coll. K. Tubaki
Isol. K. Tubaki
Det. K. Tubaki

The present fungus was already reported from Japan by Shirai & Hara (1927) on an unnamed host with neither description nor cultural data, therefore detailed observation under culture was presented here. The fungus produces conidia in abundance under culture, however, only one type of the conidium "a" (sensu M.B. Ellis, 1949)

has been seen in which the appendages are short and few-septated. Appendages of the conidia developed on the host were short, sometimes shorter than 8 μ and only 0–1–septated, but they become longer in culture up to 45 μ; still shorter than that described by Ellis. Development of the conidia is same with the description given by Ellis (1949) in *T. aristata* as shown in the plate, but the conidia-bearing globose cells swell out from the hyphae are newly described.

22. **Toxotrichum cancellatum** (Phillips) Orr & Kuehn

(Pl. 5 A-B) Ascomycetes

Orr & Kuehn, in Mycologia **56:** 473 (1964); Udagawa, Trans. Mycol. Soc. Japan **4** (4): 95 (1963).

syn. *Myxotrichum cancellatum* Phillips

Ascocarp spherical, at first white to pale yellow or greenish yellow, then becoming dark brown to dark olive brown as peridial hyphae develop, nearly blue-black in mass, 120–210 μ excluding appendages. Peridial hyphae 2–4 μ wide, coarsely tuberculate at maturity, anastomosed dichotomously or trichotomously forming a net-like structure; outermost peridial hyphae strongly arched bearing one or two elongate appendages outward per arch. Appendages elongate, 1–6–septate, rigid, smooth, tapering toward apex, straight or slightly curved, pale to dark brown or almost black, 80–315 μ, commonly 120–160 μ long, 3.5–4 μ wide at base. Centrum light brown at maturity. Asci globose to subglobose, 8–spored, 2.5–3 (4)$\times$1–2 μ, hyaline, pale yellow in mass. Homothallic. Conidial state is *Oidiodendron*, gray brown to dark gray. Conidiophores up to 50 μ long, 1.5–2.0 μ wide, branched repeatedly at upper part forming a cluster of sporogenous hyphae which segment to conidial chain at maturity. Conidia are of the arthrospore-type, globose, subglobose or ovoid, smooth, 2.3–3.5$\times$2.0–2.8 μ.

Growth on malt extract agar, restrict, uneven, tough, with granular surface texture, white to pale olive; reverse dark olive to almost black. On potato-sucrose agar, growth is close to the above. On oat-meal agar, growth is moderate with conidia in abundance, at first gray olive, then covered by abundant white to greenish yellow ascocarps; reverse and agar dark purplish olive.

Hab.: On the decaying packing paper, Tomikawa-cho, Hokkaido, Jan., 1968.

Strain preserved: IFO–8950 Coll. T. Yokoyama
Isol. T. Yokoyama
Det. T. Yokoyama

The fungus was collected afterward in every month on the decaying leaves in three nearby localities (in this journal, p. 31). The present species was already recorded in Japan by Udagawa under the name of *Myxotrichum spinosum* Massee & Salmon without the description on the conidial state. The present second record from Japan, therefore, is given adding the description of the *Oidiodendron*-state. Although Orr & Kuehn didn't give a specific name to the conidial state, the present isolate is very close to *Oidiodendron cerealis* (Thum.) Barron in many respects.

23. **Trochophora simplex** (Petch) Moore (Pl. 5 C-D) Hyphomycetes
Moore, in Mycologia **47:** 90 (1955).

syn. *Helicostylbe simplex* Petch. Linder, in Ann. Missouri Bot. Gard. **16:** 334 (1929).

Colonies hypophyllous, circular to angular, usually vein-limited, dark brown. Synnemata scattered, long, commonly 200–500 μ high, 40–55 μ wide at base, gradually narrowed at middle to 15–20 (30) μ, slightly expanded at apex, dark brown. Conidiophores fuscous, 2–3 μ wide., terminate in slightly swollen club-shaped apices which may be simple or branched, erect or recurved, with or without septa, measuring 6–7 μ wide. Conidia are of the aleuriospore-type, acrogenous, solitary, 1–1¹/4 times coiled, commonly 4–celled, rarely up to 6–celled, diameter of coiled conidia 10–18 μ, commonly 12–15 μ, filament 4–5 μ wide with 3–3.5 μ wide bluntly rounded base, fuscous.

Growth on malt extract agar slow, restrict, elevated and tufted, with woolly texture, margin irregular, dark olive to almost black; reverse same colored.

Hab.: On the under side of living leaves of *Daphniphyllum macropodum* Miq., Miyagawa-mura, Mie Pref., Aug., 1970 (4505–3–4).

Strain preserved: IFO–9339 Coll. T. Yokoyama
Isol. T. Yokoyama
Det. T. Yokoyama

The present species has been so far found only in Celyon on the under side of the living leaves of *Daphniphyllum glaucescens* and the present collection is apparently the second record obtained on the plant of same genus. From the description given by Linder (1929), the conidia are slightly smaller (8–10 μ, by Linder), but such difference seems not to be warrentable to separate the species because the coiled conidia may often vary in whole diameter depend to the mounting fluid or other conditions. Cultural observation has never been reported so far.

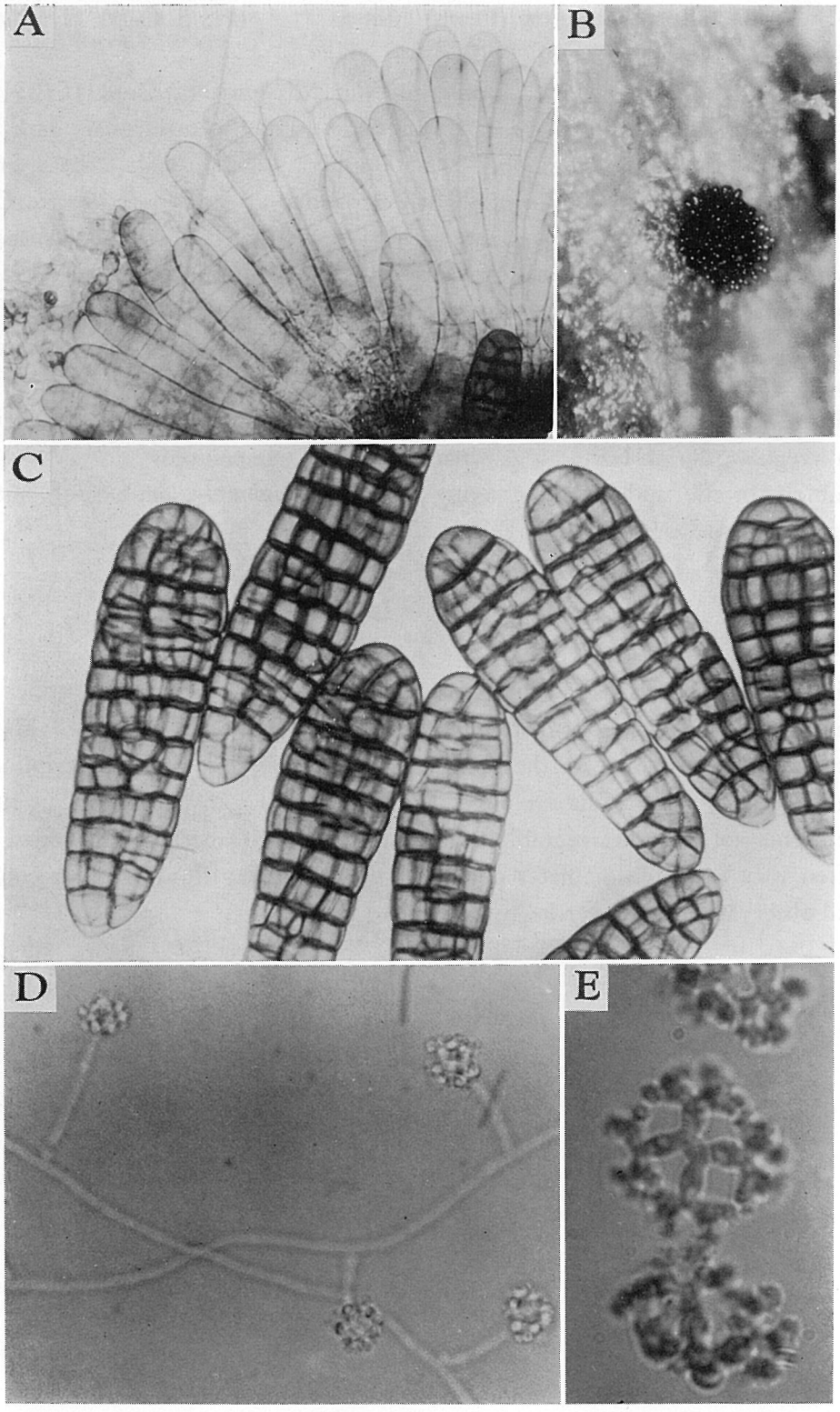

Plate 1. A-C. *Berkleasmium concinum* A. Young sporodochium, ×100, B. Sporodochium, Ultropak ×38, C. Conidia, ×400. D-E. *Candelabrum spinulosum* D. Habit in slide culture, ×400, E. Conidia, ×1000.

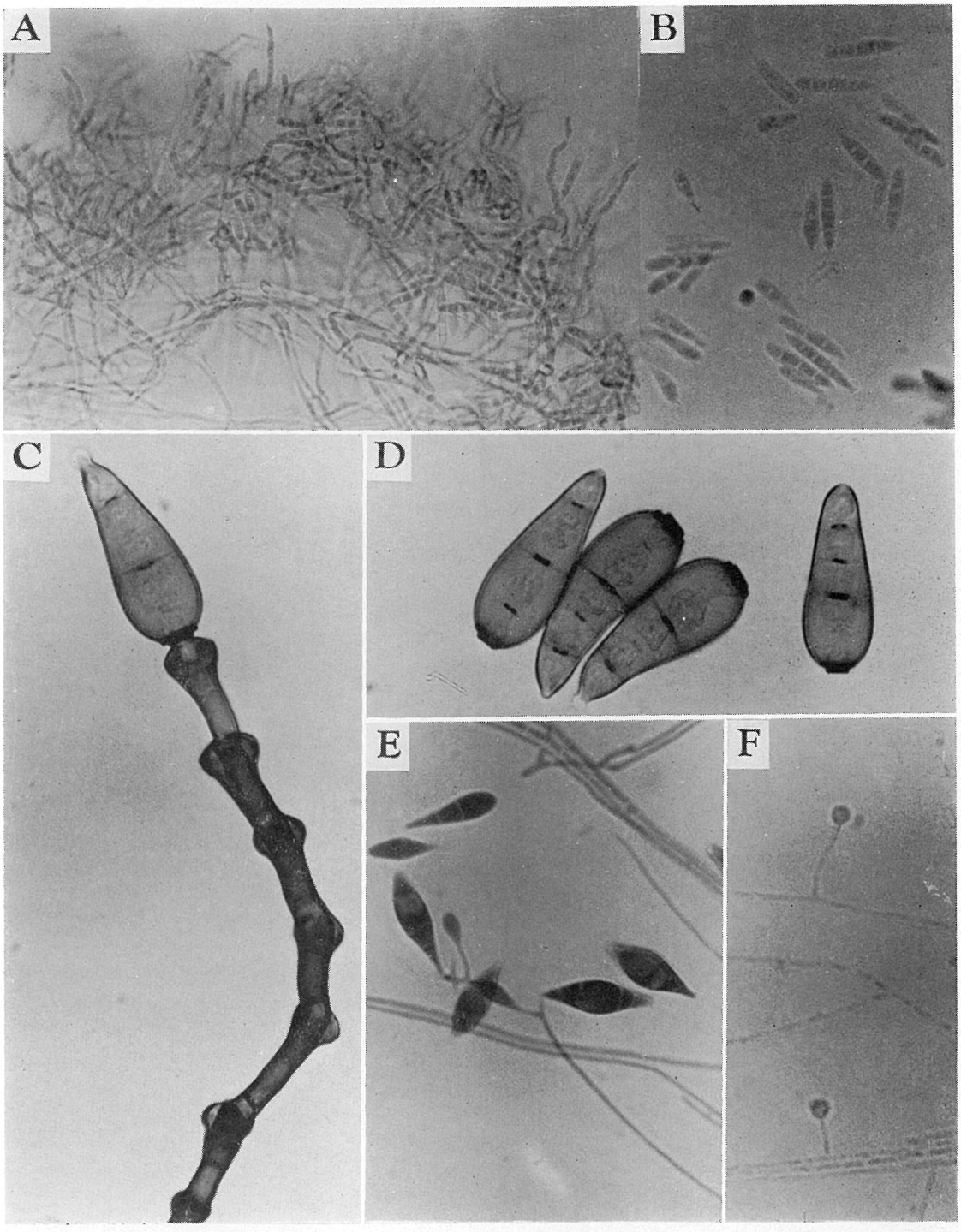

Plate 2. A-B. *Dactylaria purpurella*, ×400. Condiophores (A) and conidia (B). C-D. *Deightoniella torulosa*, ×400. Conidiophore (C) and conidia (D). E-F. *Monacrosporium ellipsosporum*, ×400. Conidiophore, conidia (E) and predacious knobs (F).

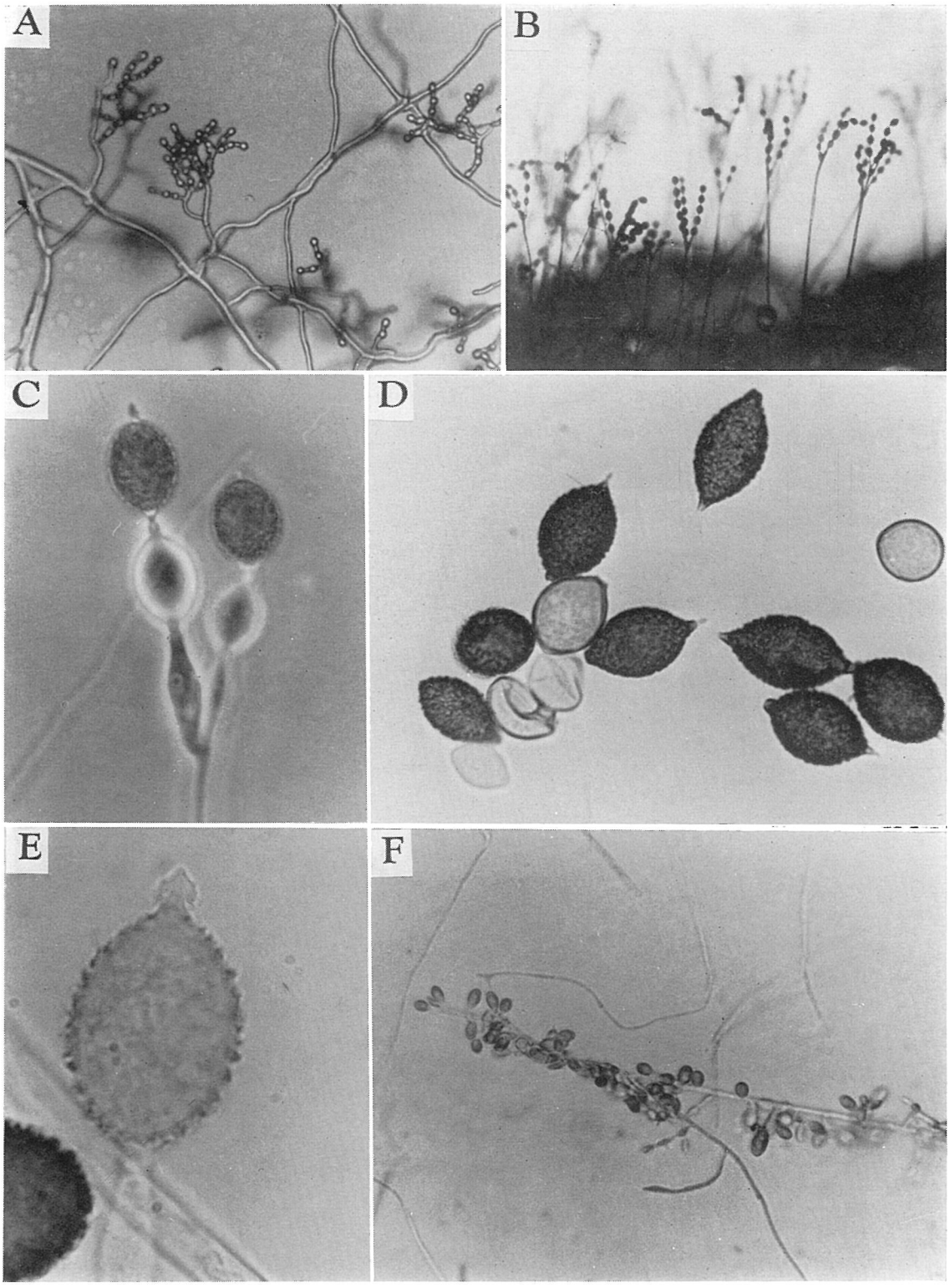

Plate 3. A. *Oidiodendron citrinum*, ×250. B-E. *Phialomyces macrosporus*. B. Habit, ×40, C-D. Conidiophore and conidia, ×400, E. Young conidium showing connective, ×1000. F. *Sporotrichum thermale*, ×400.

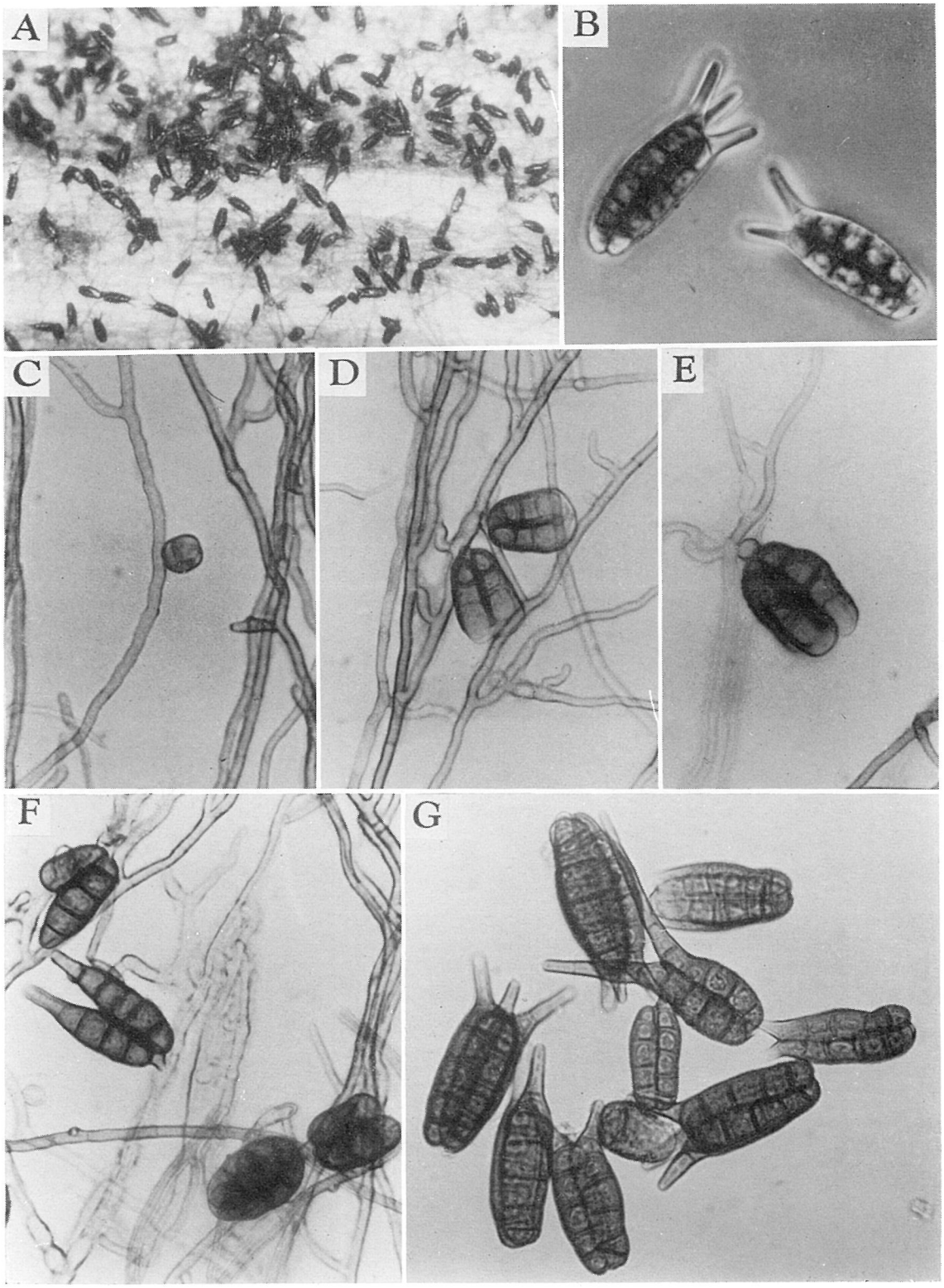

Plate 4. *Tetrapola ellisii* A. Habit, Ultropak ×65, B. Conidia in natural habitat, ×400, C-F. Several stages of conidial development, ×400, G. Conidia in culture, ×400.

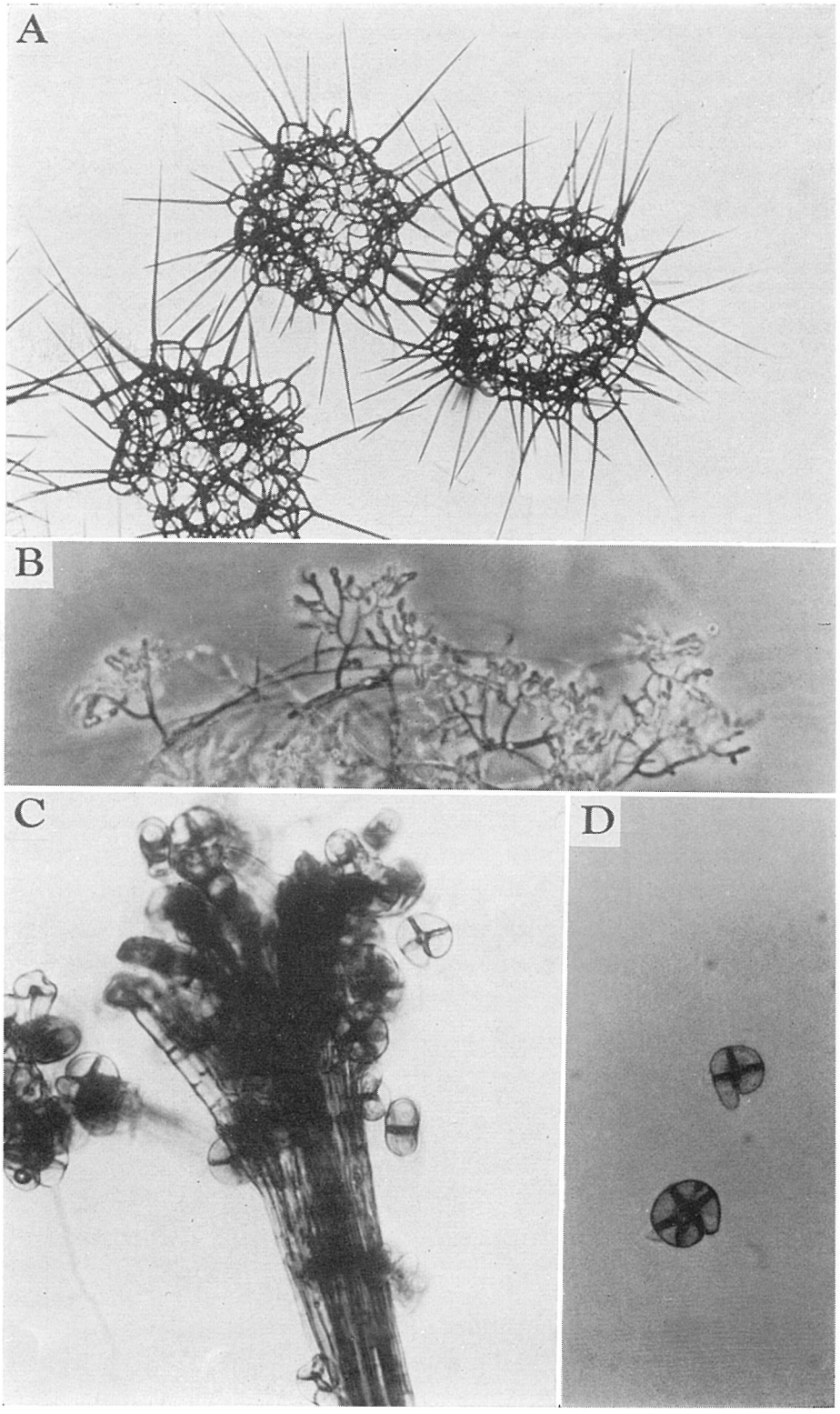

Plate 5. A-B. *Toxotrichum cancellatum*. Ascocarps (A), ×100 and *Oidiodendron*-state (B), ×400. C-D. *Trochophora simplex*. Synnemata (C) and conidia (D), ×100.

ABSTRACTS 1969-1970

Reijiro KODAMA and Yugoro NAKASUJI

Bacteria isolated from silkworm larvae

II. Taxonomical studies on two strains of bacteria, E-5 and E-15, which showed pathogenic effects on aseptically reared silkworm larvae

J. Sericult. Sci. Japan, **38** (2): 84–90 (1969)

Investigations were made on the taxonomy of the gram-positive cocci, strain E–5, and the gram-negative rods, strain E–15, both of which showed pathogenic effects on silkworm larvae reared aseptically on an artificial diet.

The strain E–5 was found to share physiological characters between two species of the genus *Streptococcus, Str. faecalis* and *Str. faecium,* while the strain E–15 was found to be closely related to *Serr. piscatorum* taxonomically.

Str. faecalis-Str. faecium intermediate E–5 was homofermentative lactic acid bacteria, and required calcium pantothenate, nicotinic acid, biotin, folic acid, B_6-group vitamin, arginine, glutamic acid, glycine, histidine, isoleucine, leucine, methionine, threonine, tryptophan and valine essentially and alanine, cysteine, purines and pyrimidines stimulatorily for the growth.

Reijiro KODAMA and Yugoro NAKASUJI

Bacteria isolated from silkworm larvae

III. Pathogenicity of lactic streptococci and *Serratia piscatorum* for aseptically reared silkworm larvae

J. Sericult. Sci. Japan, **38** (2): 103–109 (1969)

Further studies were made on pathogenic effects of *Str. faecalis-Str. faecium* intermediate E–5, *Serr. piscatorum* E–15 and various species of lactic acid bacteria on healthy silkworm larvae in 5th instar, which were reared aseptically on an artificial diet.

The results obtained were:

(1) In each case of *Str. faecalis-Str. faecium* intermediate E–5 and *Serr. piscatorum* E–15, the death of larvae was earlier with increasing amounts of living cells inoculated by feeding. However, a marked difference was observed between the number of living cells of these two species required for accomplishment of 100% mortality.

(2) *Str. faecalis-Str. faecium* intermediate E–5 grew rapidly in the gut of larvae after inoculation by feeding for four hours, the diet being withheld from the larvae thereafter, but *Serr. piscatorum* E–15 did not.

(3) Among lactic acid bacteria tested, only two strains of the genus *Streptococcus* produced pathogenic effects on larvae; those of the genera *Pediococcus, Leuconostoc* and *Lactobacillus* did not.

Reijiro KODAMA and Yugoro NAKASUJI

Bacteria isolated from silkworm larvae

IV. A study on the pathogenic mechanism of bacterial diseases in aseptically reared silkworm larvae

J. Sericult. Sci. Japan, **38** (5): 406–412 (1969)

Further studies were carried out on the pathogenicity of bacteria for aseptically reared silkworm larvae by the use of two species of pathogenic bacteria, *Str. faecalis-Str. faecium* intermediate E–5 and *Serratia piscatorum* E–15.

The results obtained were:

(1) *Str. faecalis-Str. faecium* intermediate E–5 grew rapidly in the gut of larvae, regardless of the coexistence with *Serratia piscatorum* E–15, and was recovered from the blood within 48 hours after feeding of the organisms, but could not grow rapidly in the hemocoele.

(2) Accompanied by the growth of *Str. faecalis-Str. faecium* intermediate E–5 in the gut, lactate and acetate in the gut contents increased gradually, resulting in lowering of the pH values of the gut contents.

(3) *Serratia piscatorum* E–15 could not grow readily in the gut of larvae both starved and fed. But when this strain was coexisting with *Str. faecalis-Str. faecium* intermediate E–5 in the gut, the former could multiply in the gut somewhat later than the maximum growth of the latter.

(4) The pathogenic mechanism of bacterial diseases in aseptically reared silkworm larvae was discussed. The mechanism was postulated as follows: *Str. faecalis-Str. faecium*

intermediate E–5 was associated with the bacterial diseases as the primary invader, producing an intestinal disease of chronic character and also playing an inductive role for the secondary invader, while *Serratia piscatorum* E–15 was associated with bacterial diseases as the secondary invader, producing a lethal septicemia by the rapid growth in the hemocoele.

Yugoro NAKASUJI and Reijiro KODAMA

Bacteria isolated from silkworm larvae

V. Identification of gram-negative bacteria and their pathogenic effects on aseptically reared silkworm larvae

J. Sericult. Sci. Japan, **38** (6): 471–480 (1969)

Taxonomical and pathological studies were carried out on twenty-six strains of gram-negative bacteria isolated from epizootics in population of silkworms in various parts of Japan. The results obtained were:

(1) These strains were classified into ten species: one strain of *Proteus vulgaris* HAUSER, one strain of *Proteus morganii* (WINSLOW *et al.*) RAUSS, two strains of *Proteus inconstans* (ORNSTEIN) SHAW *et* CLARKE, five strains of *Serratia piscatorum* (LEHMANN *et* NEUMANN) BREED, two strains of *Serratia marcescens* BIZIO (nonchromogenic), three strains of *Aerobacter aerogenes* (KRUSE) BEIJERINCK, six strains of *Aerobacter cloacae* (JORDON) BERGEY *et al.*, one strain related to *Alcaligenes bookeri* (FORD) BERGEY *et al.*, one strain related to *Achromobacter superficialis* (JORDAN) BERGEY *et al.*, and four strains related to *Pseudomonas ovalis* CHESTER.

(2) The pathogenic effects of these strains on healthy silkworm larvae in fifth instar, which were reared aseptically on an artificial diet, were examined. The results obtained were:

i. *Serratia piscatorum, Serratia marcescens* (nonchromogenic), *Proteus vulgaris, Proteus morganii* and *Proteus inconstans* exhibited pathogenic effects on larvae either by feeding or by injection, especially showing always 100% lethality by injection.

ii. One of three strains of *Aerobacter aerogenes* and five of six strains of *Aerobacter cloacae* exhibited pathogenic effects on larvae only by feeding, and *Achromobacter suprficialis*-related strain only by injection.

iii. *Pseudomonas ovalis*-related strains and *Alcaligenes bookeri*-related strain exhibited little or no pathogenic effects.

Yugoro NAKASUJI and Reijiro KODAMA

Bacteria isolated from silkworm larvae

VI. Relationship between the species of the genera *Micrococcus* and *Staphylococcus* and their pathogenicity for aseptically reared silkworm larvae

J. Sericult. Sci. Japan, **39** (3): 187–193 (1970)

A study was carried out on the relationship between the species of the genera *Micrococcus* and *Staphylococcus* and their pathogenicity for healthy silkworm larvae in 5th instar, which were reared aseptically on an artificial diet.

Twenty-four strains of both genera were used for the study. They consist of fourteen strains isolated from epizootics in population of silkworms in various parts of Japan and also of ten strains maintained in the culture collection in our institute. The results obtained were:

(1) Fourteen isolates were found to be closely related to the following species taxonomically: one to *M. freudenreichii*, one to *M. flavus*, four to *M. candidus*, five to *M. caseolyticus* and three to *S. epidermidis*.

(2) The pathogenicity of these isolates and the strains maintained in our culture collection for aseptically reared silkworm larvae were tested by feeding the organisms to larvae and also by injection into the hemocoele of larvae. The results obtained indicate that one of two strains of *M. flavus* and two of four strains of *M. candidus* were pathogenic only by injection. *M. freudenreichii, M. caseolyticus, M. aurantiacus, M. roseus, M. luteus, M. varians, M. rubens, M. afermentans, S. aureus* and *S. epidermidis* exhibited no pathogenicity either by feeding or by injection.

Yugoro NAKASUJI, Akira KOBAYASHI and Reijiro KODAMA

Bacteria isolated from silkworm larvae

VII. Two patterns of the pathogenic effect exhibited by the strains belonging to the genus *Streptococcus*

J. Sericult. Sci. Japan, **39** (5): 377–381 (1970)

Further investigation was carried out on the pathogenicity of twenty-two strains belonging to the genus *Streptococcus* isolated from epizootics in population of silkworms in various parts of Japan, employing healthy silkworm larvae in the 5th instar, which were reared aseptically on an artificial diet. The pathogenicity of these strains was tested

by feeding the organisms to the larvae, and by injection into hemocoele of the larvae. The results obtained were:

(1) It was found that there were two patterns—an intestinal disease and a septicemic disease—in the pathogenic effects exhibited by these strains except two non-pathogenic isolates.

(2) The strains producing an intestinal disease exhibited the pathogenicity only by feeding. They were capable of growing in media of relatively high pH values, compared with the strains producing a septicemic disease and could not liquefy gelatin.

(3) On the other hand, the strains producing a septicemic disease exhibited little or no pathogenicity by feeding, but exhibited a great pathogenicity by injection. They could liquefy gelatin and the maximum pH values for their growth were relatively lower than those of the strains producing an intestinal disease.

Keisuke TUBAKI and Toshi SAITÔ*

Endophragmia alternata sp. nov and other Hyphomycetes on *Pinus* leaves in Japan

Trans. Brit. Mycol. Soc. **52** (3): 477–482 (1969).

Three fungi isolated from fallen leaves of *Pinus densiflora* were described and one of them was assigned to a new species as *Endophragmia alternata*. This new species and two other fungi, *Chaetopsina fulva* and *Verticicladium trifidum*, are discussed with notes on their ecology.

* *Miyagi University of Education, Sendai, Japan.*

Keisuke TUBAKI and Natsuki NISHIHARA**

Alternaria helianthi (Hansf.) comb. nov.

Trans. Brit. Mycol. Soc. **53** (1): 147–149 (1969).

Helminthosporium helianthi Hansf., a fungus causing brown spots on *Helianthus annuus*, was examined and was concluded to be excluded from *Helminthosporium* because of the longitudinal septa of the conidia. Consequently, a new combination was proposed.

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Keisuke TUBAKI

Historical survey of the studies on microfungi in Japan

Proc. First. Intern. Cong. Culture Collection, p. 57–61 (1970).

Brief outline of the history of the studies of microfungi in Japan was given.

Keisuke TUBAKI

Notes on the Japanese Hyphomycetes. IV. *Bactridium*

Trans. Mycol. Soc. Japan **11** (2): 49–52 (1970).

Four species of *Bactridium*, *B. clavatum*, *B. fulvellum*, *B. flavum* and *Bactridium* sp. 11277 were described.

Ko IMAI, Isao BANNO and Teiji IIJIMA

Inhibition of Bacterial Growth by Citrate. I.

J. Gen. Appl. Microbiol. **16** (6) 479–487 (1970)

According to Bergey's Manual of Determinative Bacteriology, 7th edition, the genus *Arthrobacter* is divided into two groups, one of which utilizes citrate as a sole organic nutrient and the other does not utilize citrate. Among authentic strains of this genus, the growth of *Arthrobacter pascens* IFO 12139 and *Arthrobacter simplex* IFO 12069 was inhibited by citrate. It was found that *A. pascens* IFO 12139 required bivalent cation(s) for growth and failed to grow when the medium contained excess of citrate against bivalent cations. On the other hand, *A. simplex* IFO 12069 could not utilize citrate as a sole source of carbon, and citrate inhibited glucose utilization of the organism. Among many strains belonging to the genera *Corynebacterium*, *Brevibacterium*, and *Bacillus*, growth was inhibited by citrate.

MISCELLANEOUS SCIENTIFIC PAPERS

1. Takezi HASEGAWA 1969. Management of culture collections. Kagaku to Seibutsu 7 (11): 684–689. [In Japanese]
2. Takezi HASEGAWA 1970. Microbial systematics past and present. Technological Biology 1 (1): 69–71. [In Japanese]
3. Takezi HASEGAWA and Chikao TODA* 1970. Preservation of microorganisms, p. 244–283. In Teijiro Uemura and Ko Aida [ed.], Fermentation and microorganisms, vol. 2. Asakura Publ. Co. Ltd., Tokyo. [In Japanese]
4. Keisuke TUBAKI 1970. Historical survey of the studies on microfungi in Japan, p. 57–61. In Hiroshi IIZUKA and Takezi HASEGAWA [ed.], Proceedings of the Ist International Conference on Culture Collections. University of Tokyo Press, Tokyo.
5. Rokuya IMAZEKI**, Tsuguo HONGO*** and Keisuke TUBAKI 1970. Common fungi of Japan in color. 1–175. Hoikusha Publ. Co. Ltd., Osaka. [In Japanese]

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PRESENTATION OF PAPERS AT SCIENTIFIC MEETINGS, 1969-1970

Author(s)	Title	Scientific Meeting of
I. BANNO	An investigation of marine bacteria I. Isolation and classification of bacteria from coastal water of Osaka bay.	Agricultural Chemical Society of Japan Meeting in Tokyo (April, 1969)
Y. NAKASUJI & R. KODAMA	Identification of gram-negative bacteria and their pathogenic effects on aseptically reared silkworm larvae.	Society of Sericultural Sciences of Japan, Meeting in Tokyo (April, 1969)
K. IMAI	Inhibition of bacterial growth by citrate. I.	Agricultural Chemical Society of Japan, Meeting in Kyoto (September 1969)
K. TUBAKI	Basauxic conidiophore and other criteria of the Hyphomycetes.	Ist International conference on Fungi Imperfecti at Kananaskis in Canada (September, 1969)
K. TUBAKI & T. YOKOYAMA	Cultural stage of *Graphiola phoenicis*.	Mycological Society of Japan, Meeting in Sapporo (September, 1969)
T. HASEGAWA	Preservation and technical treatment of the ISP cultures of *Streptomyces*.	Society for Actinomycetes, Japan, Meeting in Sapporo (September, 1969)
T. IIJIMA & Y. SAKAMOTO	Formation and segregation of polylysogens of lambdoid. I.	Genetics Society of Japan, Meeting in Kanazawa (October, 1969)
Y. NAKASUJI & R. KODAMA	Relationship between the species of the genera *Micrococcus* and *Staphylococcus* and their pathogenicity for aseptically reared silkworm larvae.	Society of Sericultural Sciences of Japan, Meeting in Matsuyama (November, 1969)
I. BANNO	A taxonimic investigation of yeasts producing acids from hydrocarbons.	Society of Fermentation Technology Japan, Meeting in Osaka (November, 1969)
K. TUBAKI	Marine fungi in Japan.	Agricultural Chemical Society of Japan, Meeting in Osaka (December, 1969)
R. KODAMA, Y. NAKASUJI & M. NISHIO	Bacteria isolated from silkworm larvae VIII. Experiments to protect gnotobiotic silkworm larvae from bacterial diseases by oral administration of antibiotics.	Society of Sericultural Sciences of Japan, Meeting in Ueda (April, 1970)

R. KODAMA, Y. NAKASUJI & M. NISHIO	Bacteria isolated from silkworm larvae. IX. The behavior of antibiotics added to an artificial diet.	*ibid.*
I. BANNO	An investigation of marine bacteria. II. Classification of bacteria isolated from sea water by numerical technique	Agricultural Chemical Society of Japan, Meeting in Fukuoka (April, 1970)
T. IIJIMA & T. SAKANE	A modified method of lyophilization of bacteria. I.	Japanese Society for Research of Freezing and Drying. Meeting in Tokyo (April, 1970)
K. IMAI & T. IIJIMA	Inhibition of bacterial growth by citrate. II.	Agricultural Chemical Society of Japan, Meeting in Kyoto (May, 1970)
K. TUBAKI	On Japanese species of *Bactridium*.	Mycological Society of Japan, Meeting in Tokyo (May, 1970)
T. YOKOYAMA & K. TUBAKI	Studies on Japanese Coelomycetes. I. *Actinopelte*.	*ibid.*
K. TUBAKI	Studies on the marine fungi. IV. On brackish water fungi.	Botanical Society of Japan, Meeting in Matsuyama (October, 1970)
K. TUBAKI	Problems in the classification of Fungi Imperfecti.	Japanese Society for Medical Mycology, Meeting in Osaka (October, 1970)
T. IIJIMA & Y. SAKAMOTO	Segregation of *rec*$^-$ defective lysogens.	Genetics Society of Japan, Meeting in Tokyo (October, 1970)
Y. SAKAMOTO & T. IIJIMA	Formation and segregation of polylysogens of lambdoids. II.	*ibid.*
T. IIJIMA & T. SAKANE	A modified method of lyophilization of bacteria. II.	Japanese Society for Research of Freezing and Drying, Symposium in Tokyo (October 1970)

ANNUAL REPORT, 1969-1970

It is a great pleasure for us to publish the fifth report on the research activities of our institute. The serial containing our original papers had been issued biennially upto the fourth publication under the title of "Annual Report". The title has now been changed to "I.F.O. Research Communications" from the present issue.

The world Federation of Culture Collections made a good start at the 10th International Congress of Microbiology in Mexico and Dr. Stanley M. Martin took office as Chairman, Prof. Hiroshi Iizuka as Vice-chairman, Dr. Stephen P. Lapage as Secretary and Prof. Victor Bruce D. Skerman as Treasurer. We take this oppotunity to hope that the international network of culture collections will be brought up by the Federation.

The last extension work of the research laboratories and facilities for the IFO Culture Collection is making as much progress as can be fairly expected. It is scheduled to be completed in June of this year. Then a new laboratory for the bacterial taxonomy with an inoculation room, and a specimen room attached to Mycology Section are installed in addition to a new culture collection room, the temperature of which is regulated under 5°C. (The temperature of the collection room being in use is always adjusted at 8–9°C.)

Ever since 1969 the Collection has been taking charge of the culture center in Japan of the ISP standard strains of *Streptomyces* in compliance with request of the Society for Actinomycetes, Japan.

During the years of 1969 and 1970, 780 strains of fungi and bacteria were obtained either from natural sources or through the courtesy of other collections and research organizations, and 6373 subcultures were distributed in Japan and abroad. The total number of cultures in the Collection reached 7752 at the end of 1970. A new issue of "List of Cultures, Institute for Fermentation, Osaka" (5th ed.) is being compiled and will be made available at the beginning of 1972.

RESEARCH COMMUNICATIONS
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Annual Report No. 3, 1967

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発酵研究所研究報告　第５号

昭和46年３月15日　印刷
昭和46年３月25日　発行

編集兼
発行人　長　谷　川　武　治

発行所　財団法人 発 酵 研 究 所
大阪市東淀川区十三西之町４丁目54番地

印刷所　日 本 印 刷 出 版 株 式 会 社
大阪市福島区亀甲町２丁目62番地