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Studies on the Sporulation of Rice Blast Fungus, *Pyricularia oryzae* Cavara.

III. Relationship between the Development of Conidiophore Mother Hypha and the Sporulation.

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Summary

The cause of the difference in the potential for conidium production among four isolates of rice blast fungus, *Pyricularia oryzae*, was investigated. Two isolates of ken 60-19 and THU 61-33, which sporulated heavily, formed many conidiophores on each mycelial mat, and with the elapse of time, a large number of conidia were produced on the conidiophores under irradiation. On the other hand, the other two isolates of F 67-2 and kita 373, which sporulated sparsely, formed only a few conidiophores and aerial hyphae instead of conidiophores were formed abundantly. Many deeply pigmented hyphae were found in the mycelial mat of ken 60-19. On the contrary, the mycelial mat of F 67-2 consisted mostly of non-pigmented hyphae. A few pigmented hyphae were found on the surface only of the mycelial mat. Since the conidiphores might have originated from these pigmented hyphae, it was determined that the pigmented hyphae would be conidiophore mother hyphae.

The conidiophore mother hyphae was distinguished with the vegetative hyphae in the remarkable development of the cell wall and the septum of the hyphal cell. Consequently, the differences of sporulation among the four isolates of this fungus were closely related with the difference of the development of conidiophore mother hypha.

Usually, the number of condia which is produced under a controlled condition varies among each isolate in many fungi.

It was reproted that the number of of conidia of rice leaf spot disease fungus, *Helminthosporium oryzae*, varied because of the difference of light dependency of the sporulation among the strains (1). Although the number of conidia of rice blast fungus, *Pyricularia oryzae*, varied remarkably among the isolates (2, 3), it was considered that this fungus had the same light dependency for sporulation in all isolates used (4). Therefore, it was not suggested that the difference of the number of conidia of this fungus was a result of the difference in light dependency of the sporulation among the isolates as shown in the pathogen of the rice leaf spot

disease. The objective of this experiment is to investigate the cause of the difference in the potential for conidium formation among the isolates of *Pyricularia oryzae* Cavara.

Experimental methods

The isolates of *Pyricularia oryzae* used in this experiment were four isolates of ken 60–19, THU 61–33, F 67–2 and kita 373. These isolates were maintained on potato dextrose agar medium at room temperature. For investigation of the sporulation, oat meal decoction agar medium (oat meal 50 g, sucrose 20 g, agar 25 g, distilled water 1000 ml) was used.

Two ml of the spore suspension of each isolate was pourd onto the oat meal decoction agar medium in a petri dish and the medium was inoculated with spore and incubated at 25±1 C° in darkness for 10 days. Then the aerial hyphae which grew on the mycelial mat were removed by rubbing with a writing brush and washed away with tap water. After this treatment, the mycelial mat was placed in the chamber with three 20 W Black Light Bule fluorescent lamps (FL–20 BLB) as a light source, and was irradiated for each fixed period to accelerate sporulation. The chamber was placed in a dark room with controlled temperature (25±1 C°) and humidity (55~65 per cent).

The light source had a wavelength of from 290 to 410 nm and the light energy on the surface of the mycelial mat was about 1000 ergs/cm^2 per second. The light energy was measured with a thermopile (model 146, KIPP and ZONEN Netherland) and a DC μV meter (model DM 16A, TOA ELECTRISTICS Co. Tokyo).

Conidia which were produced on the mycelial mat were suspended in 10 ml of distilled water contained a trace of tween 80 and then spore counts were made with a heamacytometer.

In order to investigate the relationship between the sporulation and the amount of the mycelial mat formed on each isolate, the mycelial mat after incubation in the oat meal liquid medium for 10 days was harvested.

The mycelial mat was washed many times with distilled water and dried at 80°C. The dried mycelial mat was weightened.

For observation of the sporulation, the mycelial mat was cut vertically by a razor at 15 and 24 hr after irradiation, respectively and observed under microscope.

The mycelial mat was incubated for 10 days in darkness and then sampled to investigate the hyphae constitutency of the mycelial mat. The mycelial mat was fixed in FAA, dehydrated with ethanol-butanol, embedded in paraffin, cut at 25 μm by a microtome, and then stained with hematoxirin.

The observations of conidiophore formation were made as follows. The mycelial mat after incubation for 10 days was irradiated for 24 hr. After irradiation, a section of the mycelial mat was prepared by the same ways as mentioned above, and observed under a microscope.

Results

The relationship between the number of conidia and the growth of vegetative hyphae.

The number of conidia of the four isolates formed on oat meal decoction agar are shown as table 1. Ken 60–19 isolate of all the four isolates used sporulated most, and THU 61–33 isolate followed. But both the isolates of kita 373 and F 67–2 sporulated sparsely. The number of conidia of ken 60–19 isolate was about 15 times that of F 67–2 isolate. The relationship between the number of conidia and the dry weight of vegetative hyphae in the four isolates was shown as Fig. 1. The dry weight of vegetative hyphae of the four isolates were almost equalled. Accordingly, it was apparent that the difference of the number of conidia among the isolates was not related with the amount of each vegetative hyphae.

TABLE 1. *The difference of sporulation among 4 isolates of Pyricularia oryzae.*

Expt. No.	Number of spores ($\times 10^4$/petri dish)			
	ken 60–19	THU61–33	F 67–2	kita–373
I	3759	2568	230	416
II	3864	2732	245	367
III	3516	2515	258	387

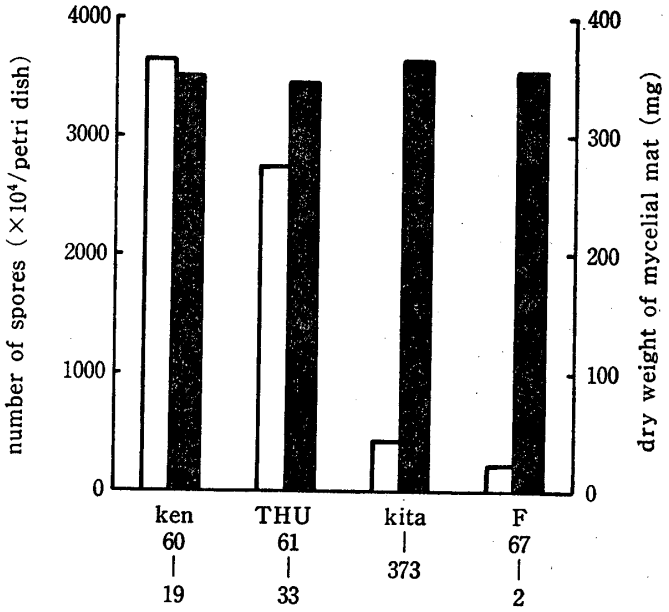

FIG. 1. The relationship between sporulation and amount of mycelial mat.
□: number of spores ■: dry weight of mycelial mat.

The sporulation on the mycelial mat.

The result of the sporulation on the mycelial mat of four isolates are shown on Plates 1 and 2. Both the isolates of ken 60–19 and THU 61–33 formed many

conidiophores on the mycelial mat at 15 hr after irradiation. At 24 hr after irradiation, a large number of conidia were produced on the conidiophores. On the other hand, both the isolates of F 67–2 and kita 373 formed only a few conidiophores on the mycelial mat, rather aerial hyphae instead of conidiophores developed at 15 hr after irradiation. At 24 hr after irradiation, aerial hyphae formed abundantly.

Observation of the hyphae within the mycelial mat.

As described above, the difference of the number of conidia formed by each isolate was a result of the difference of the number of conidiophores formed on the mycelial mat. Thus, the isolates which produced a large number of conidia were capable of differentiating many conidiophores from the mycelial mat by irradiation, and the isolates which produced only a few conidia were not able to differentiate the conidiophores by irradiation. Then, the hyphae within the mycelial mat of both the isolates of ken 60–19 which sporulated heavily and F 67–2 which sporulated sparsely were observed and compared. The results by this observation are shown on Plate 3. In the mycelial mat of ken 60–19 isolate, many pigmented hyphae were observed at both the surface and inside of the medium. On the contrary, a very few pigmented hyphae were recognized only on the surface of the mycelial mat in the isolate of F 67–2, and the mycelial mat of this isolate was almost consisted of non-pigmented hyphae. It seemed that pigmented hyphae is able to form conidiophore by irradiation. Thus, it was apparent that the number of pigmented hyphae was related to the conidiophore. As shown in plate 4, the pigmented hyphae was charactrized by having a brown color and forming a developed cell wall.

Observation of the conidiophore formation from the pigmented hyphae.

The results of the observation are shown on Plate 4. Conidiophore originated from pigmented hyphae in both the isolates of ken 60–19 and F 67–2. It was apparent that this pigmented hypha had a capacity for conidiophore formation by irradiation. The isolate which developed only a few pigmented hyphae formed conidiophore scarsely, but aerial hyphae were formed abundantly.

Discussion

The difference of the number of conidia formed by each isolate of rice blast fungus, *Pyricularia oryzae*, was a result of the difference of the number of conidiophores formed on the mycelial mat. As the isolates which sporulated abundantly formed a great number of conidophores by irradiation, many conidia were produced. However, the isolates which sporulated sparsely produced only a few conidia even when irradiated because of the slight conidiophore formation. In such a case, many aerial hyphae instead of conidiophores were formed. Thus, the difference of the conidial production formed by the isolates was closely related with the capacity

of conidiophore formation on the mycelial mat of each isolate. A large portion of the mycelial mats of the isolates which sporulated abundantly consisted of pigmented hyphae. On the other hand, there were only a few pigmented hyphae in the mycelial mats of the isolates which sporulated sparsely. Furthermore, the result of the observation that the conidophores originated from the pigmented hyphae in both the isolates of ken 60–19 and F 67–2 suggested that these pigmented hyphae were able to form conidophore by irradiation. As the pigmented hyphae had a capacity to form conidiophore, it was determined that these pigmented hyphae would be conidiophore mother hypha. It was reproted that the conidiophore had formed from the conidiophore mother cell in the diseased rice tissue infected with the rice blast fungus, but no difference between conidophore mother cell and other hyphae was suggsted (5). The conidiophore mother hyphae was charactrized by a brownish pigmentation and a development of cell wall on the artificial medium. But, it seemed that the conidiophore mother hypha should be identified with the condiophore mother cell at the point of having a capacity to form conidiophore.

Consequently, the difference of the number of conidia among the isolates was closely related with the number of the condiophore mother hypha in the isolates of *P. oryzae.*

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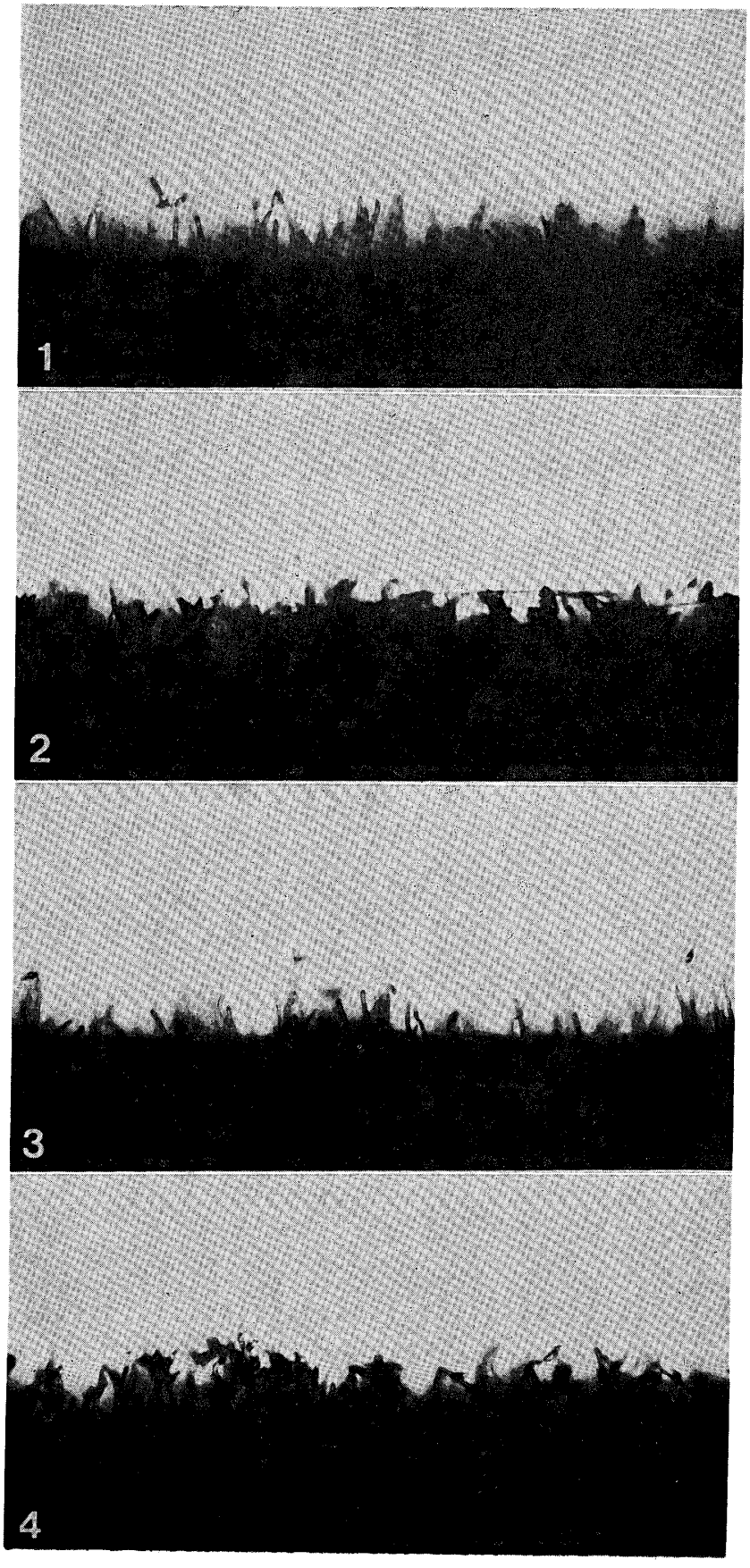
Explanation of plate

PLATE 1. The sporulation on the mycelial mat of the isolates which sporulated abundantly.
1, 2: ken 60–19 3, 4: THU 61–33 1, 3: at 15 hr after irradiation 2, 4: at 24 hr after irradiation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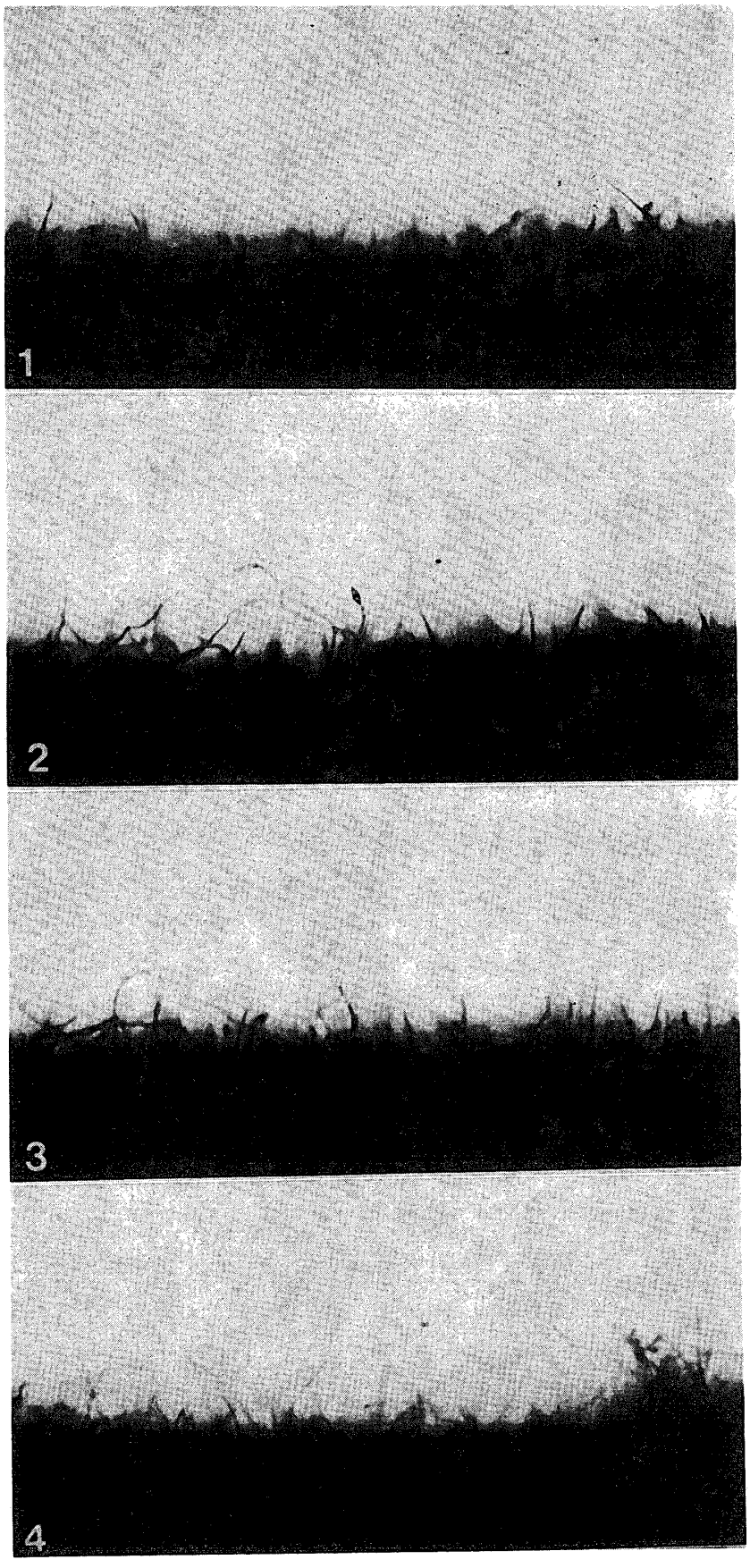
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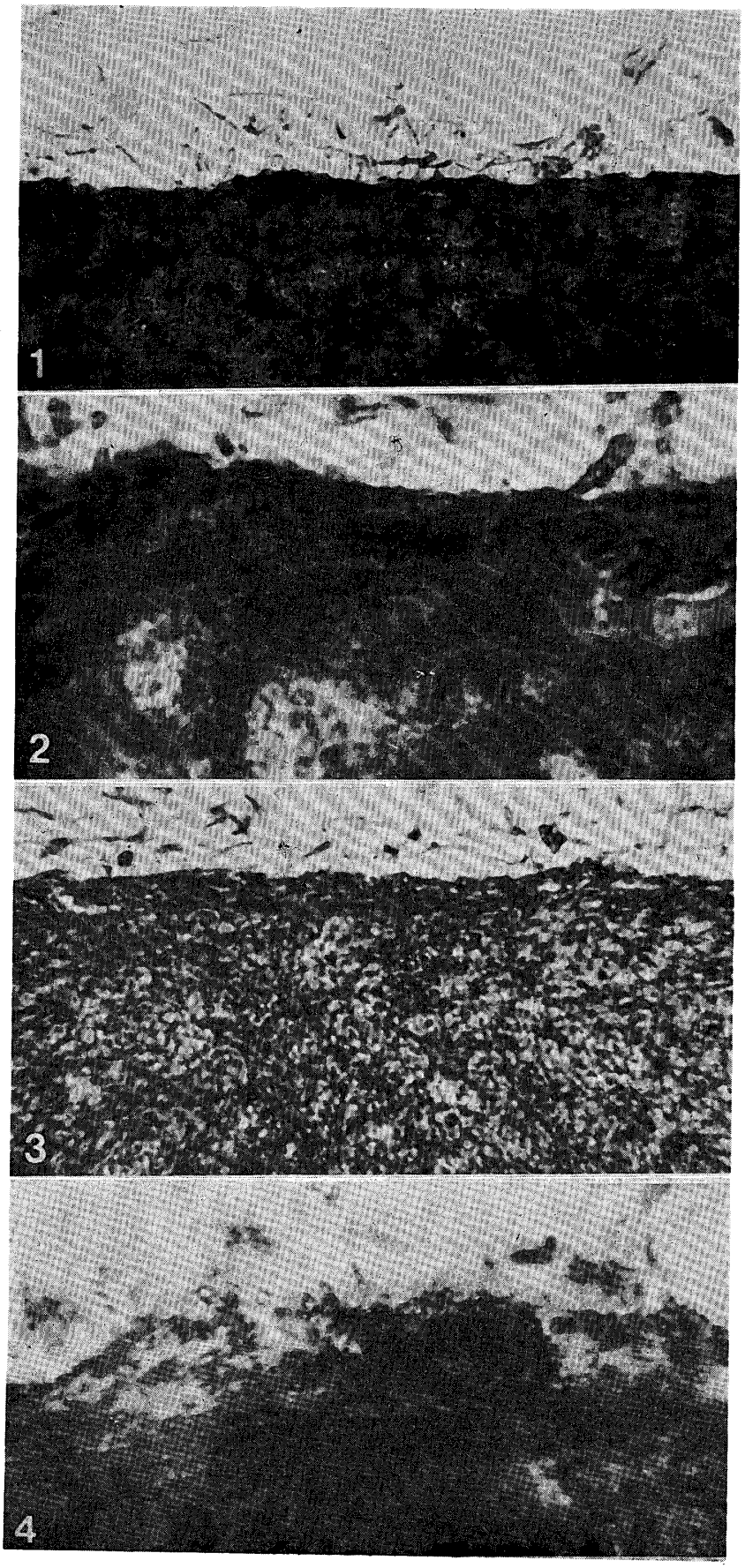
PLATE 2. The sporulation on the mycelial mat of the isolates which sporulated sparsely. 1, 2: F 67–2 3, 4: kita 373 1, 3: at 15 hr after irradiation 2, 4: at 24 hr after irradiation

PLATE 3. Pigmented hyphae (p h) in the mycelial mat.
1, 2: ken 60–19 3, 4: F 67–2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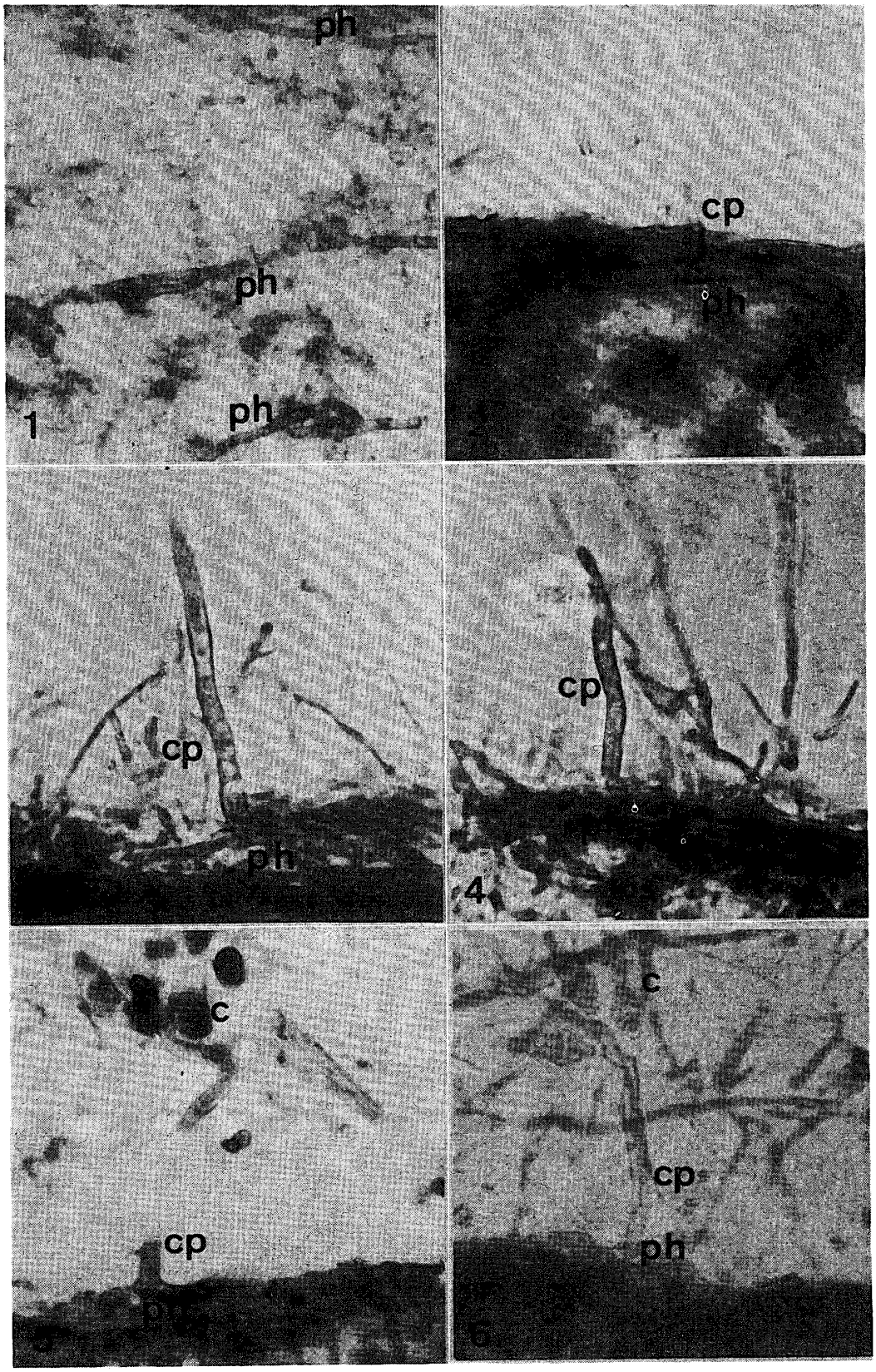
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PLATE 4. Pigmented hyphae and conidiophore formation from the pigmented hyphae. 1, 2, 3, 4: ken 60–19 5, 6: F 67–2 c: conidium cp: conidophore ph: pigmented hypha