OBSERVATION ON SPORE GERMINATION AND PLASMODIUM OF TWO SPECIES OF MYXOMYCETES[1]

BAO-YU YANG[2]

INTRODUCTION

The purpose of this study attempts to investigate the plasmodial behavior which may lead to a better understanding of the role and activities of the protoplasm of living organisms.

The observations made from this study are three folds: (1) the germination of spores (2) the formation of plasmodium and (3) the developing of fruiting bodies. Consequently, the spore to spore life cycle of *Stemonitis splendens* Rostof. and *S. flavogenita* Jahn have been successfully completed in culture. The time required for completing such a cycle is about two or three weeks.

The details of the events which took place during the course of their growth and devolopment are summarized in Table I.

MATERIALS AND METHODS

Stemonitis splendens Rostof. and *S. flavogenita* Jahn were collected from the field and brought to laboratory for immediate use.

Spores collected from a mature unopened sporangium were sown in distilled water in stender dishes. Close observation was made for their germination and the rate of germination was approximately estimated with a counting chamber.

The spores after being sown for about half an hour were then poured on agar medium in petri dishes. Various media have been used by different workers but in this study the weak agar medium was used and found to be very desirable. Plasmodium gradually appeared on the surface of the medium in one to two days.

Amoeboid swarm cells both with and without flagella were seen under a phase contrast microscope and micrographic-work was done with a Pm 7 camera.

Techniques used in bacteriology or mycology were also applied here during the processes of transferring or examining of the plasmodia in order to avoid contamination from the cultures.

All the cultures in petri dishes, thus prepared as indicated above were placed in a dark place approximately at 25°C.

(1) This study is assisted by Miss Ming Ting who is in our botany department. She has been keeping the culture alive and observed the first complete cycle. Misses F. M. Hsu, C. H. Liu have been responsible for the micrographic work. Four mycology students, Misses Ning-ning Kao, Choong-noag Chen, Ni-ning King and Mr. Kwa-shi Tsu, each has repeated the spore to spore life cycle of *S. splendens* and *S. flavogenita*.

(2) Professor of Botany, National Taiwan University. (楊寶玉)

Table I. Showing characteristics and events occurred in the life cycles of *Stemonitis splendens* Rost., *S. flavogenita* Jahn

Name of Species	Date of Collecting	Habitat and Locality	Characteristics				Germination time required	Germination rate	Emergence-flagellated stage	Life Cycle Completed in days (from spere to spore)
			Sporangia	Spores	Swarm cells	Plasmodium				
Stemonitis splendens Rostòfionski (IX)	July 15, 1968	On bark of fallen wood, shaded, damp, private garden, Taipei	Brown, cylindric 12-16 mm long stipitate, stipe black, polished, shining, rising from silvery film hypothallus	Brown, warted 8-9μ	(flagella-stage) 12μ	Yellow, veins prominent	3 days	13%	1 day	24
Stemonitis flavogenita Jahn (VII)	July 6, 1968	On oxalis leaves, on wet garden soil	Brown, cylindric obtuse, fascicled, stipitate, stipe short, black	Pale ferrugi-nous 7-9μ		Whitish	3 days	13%	1 day	17

All the collections of myxomycetes studied in this paper were made by the author herself.

OBSERVATIONS AND RESULTS

I. *Stemonitis splendens* R. (IX) was found on bark of a decaying log and also on the leaves of Oxalis.

A. Spores—Brown, minutely warted, about 8–9μ in diam.

B. Sporangia—Irregularly clustered, purple brown in mass, cylindric, long, stipitate; stipe black, polished, shining, arising from a common hypothallus. (Plate I, Fig. 3)

C. Spore germination—Spores germinated about three days after sowing, at a germination rate of 24%. Protoplasts emerge through a minute pore as described by Gilbert (8). The emergence of protoplasts from spore walls to flagellated stage occurred in one day.

D. Swarm cells and myxamoebae—Generally swarm cells in this species are spherical, uniflagellate, each about 12μ in diam. Marked protoplasmic processes extending from the cell proper are seen at one end, opposite to the end that bears the flagellum (Plate II, Figs. 3–4). Fusion of two or more swarm cells or one large cell with one small cell to form myxamoebae was observed (Plate II, Fig. 6). Some swarm cells lose their flagella forming cysts; some continued to move about with flagella.

E. Plasmodium—At first, it appeared whitish, later it became delicate yellow and transparent. Veins appeared in twelve days after sowing. Active streaming of protoplasm was observed reversibly but soon slowed down in 30 to 40 minutes. (Plate III, Figs. 1–2).

F. Fruiting body—The plasmodium, after being exposed to light (about 500 lux) began to collect into heavy masses which gradually lost their whitish appearance (Plate III, Figs. 3–6). At first, the white color changed to a light pink then through delicate lilac to deep rose and finally to purple before the brown color of the mature fruiting body was produced. It takes approximately 24 days to complete the spore to spore cycle of *Stemonitis splendens* in our laboratory.

This experiment has been repeated more than 6 times, and each time with the same results. When the spores of the F_2 sporangia were used they also produced a complete cycle from spore to spore in our laboratory.

All the fruiting bodies produced in antiseptic culture as described above were smaller in size as compared with those collected from the field.

II. *Stemonitis flavogenita* Jahn (VII)—Growing on the leaves of Oxalis, collected on July 6, 1968. (Plate I, Figs. 1–2)

A. Sporangia—Cylindric, obtuse, closely fasciculate, brown in color, stipitate 5–7μ; stipe short, black.

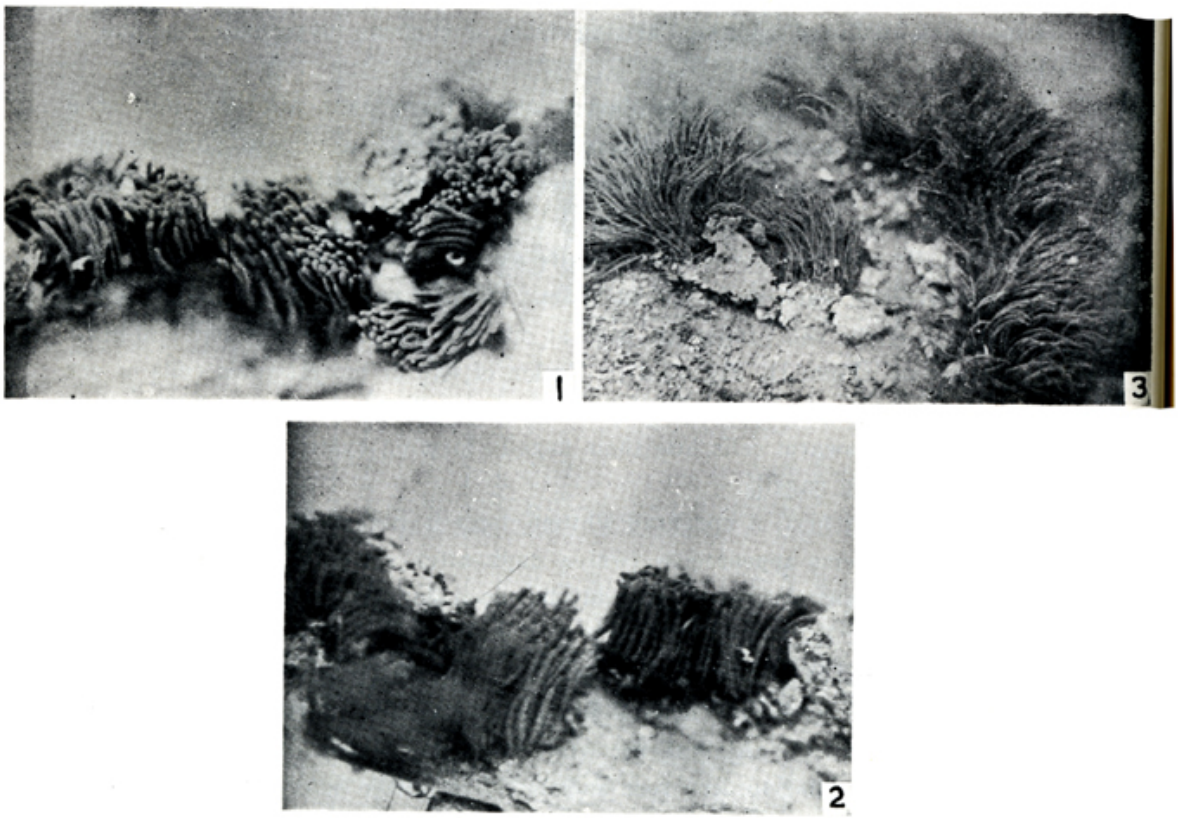


Plate I

Fig. 1-2. *Stemonitis flavogenita* Jahn (Habit) ×1
Fig. 3. *Stemonitis splendens* Rostofionski (Habit) ×1

B. Spore—Pale ferruginous, 7–9μ, time for germination is about 3 days, at a rate of 13%.

C. Plasmodium—Whitish in color. It took about one day from emergent protoplast to the flagellate stage. Plasmodium, delicate and transparent even when mature and has little stationary protoplasm external to the streaming inner mass, very similar to *S. fusca* (15).

Alexopoulos 1959 observed that the plasmodium of *S. flavogenita* became yellow before fruiting. This agrees with what I have found in the present culture.

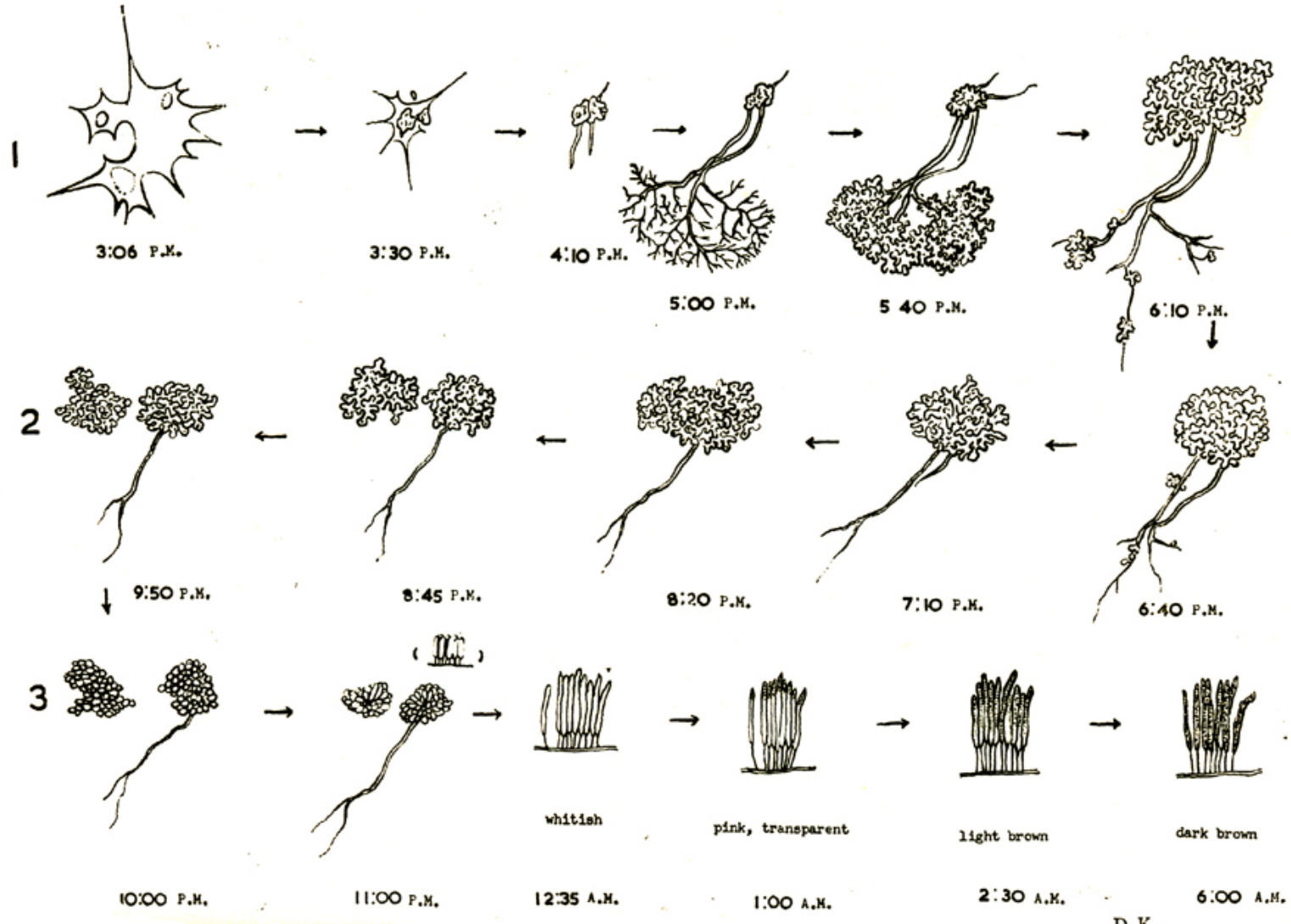
D. After the preparation of this paper it was found that the spore to spore cycle of *S. flavogenita* had been previously studied by Alexopoulos, so that part of our observations are not repeated here in this paper.

However, a brief account of our observation of specimens growing on garden soil, on July 12, 1968, are given here which may add some knowledge to our understanding of the life cycle of slime mold. Plate V with the attached explanation will show a sequence of the events leading to the production of the fruiting bodies of *Stemonitis flavogenita* Jahn.

At first whitish, watery plasmodium spread over the soil surface. This was observed at about 3:00 p.m. It was only a thin sheet of protoplasm, but soon it contracted into a thick, smaller central mass. Instantly it sent out veins and formed a network and finally a big irregular mass with protruberances and an uneven surface. Then in the next three or four hours (6:40–9:50 P.M.) the disappearance of the veins and clumping of the protoplasm into a rough surfaced heap was observed. From 10:00 P.M.–6:00 A.M. the initiation of sporangia took place. In the beginning, only the top view was seen, which resemble the surface of a strawberry, but it was whitish and transparent, at 11:00 P.M., it formed into cylindric short tubes accompanied by the change of color from white to pink, light brown, and finally dark brown, as the sporangia become mature. And by that time, the fruiting bodies which had been in a cluster, were getting separated from each other; this was around day break, the next morning. From the above account one may draw the following conclusions.

1. From the streaming of the plasmodium to the formation of sporangia it took about 15 hours in *S. flavogenita.*
2. During the course of sporangia initiation, it is always associated with the change of color—from white to pink, light brown and then to the characteristic, deep brown of the species.
3. The formation of the sporangia usually began in the evening and continued until the early hours of the next morning. The appearance of the plasmodium was first observed in the late afternoon. Another cycle from plasmodium to the formation of sporangia occurred in the next day, July 13, almost at the same

Plate V


1
3:06 P.M.
3:30 P.M.
4:10 P.M.
5:00 P.M.
5 40 P.M.
6:10 P.M.
2
9:50 P.M.
8:45 P.M.
8:20 P.M.
7:10 P.M.
6:40 P.M.
3
10:00 P.M.
11:00 P.M.
12:35 A.M.
whitish
1:00 A.M.
pink, transparent
2:30 A.M.
light brown
6:00 A.M.
dark brown

D. K.

Explanation of Plate V. *Stemonitis flavogenita* J.

Changes occurred in 15 hrs. and observed from outdoor on soil near a banana stump, from plasmodium stage through the formation of sporangia.

1. From 3:06 p.m.-6:10 p.m.
   Plasmodium, white and thin, observed at 3:06 p.m. (July 12, 1968) gradually disappeared, veins and nits began to form until finally migrating into the top and became minute clumps united in groups.
2. From 6:40-9:50 p.m.
   Protoplasm gathered at the top, forming a mass which began to divide and finally separate into cylindrical individuals, remained in creamy white color.
3. 10:00 p.m. Top view of the mass.
   11:00 p.m. Short tubes could be seen from a side view but still grouping together.
   12:35 a.m. Elongation of the cylindric whitish tubes.
   1:00 a.m. (July 13, 1968) Columns began to separate and now pink in color as white color disappeared.
   2:30 a.m. Light brown, finally at 6:00 a.m. a group of sporangia, now in dark brown are well formed.

(All the changes described above were observed July 12, 1968. Another cycle was found going through exactly the same processes in the next afternoon, July 13)

hours of the day, at the same area and reaching the same result as that of the previous one. This second observation confirmed all the processes that occurred in the life of *Stemonitis flavogenita* Jahn as studied in nature.

4. The spore to spore cycle developed in culture took about 17 days. It seems that the swarm cell stage, amoeboid stage, and the plasmodium formation took a longer time than the final stages toward the formation of sporangia. This takes only over night and usually during the dark—no matter whether it is in culture or in nature.

CONCLUSION AND SUMMARY

1. The spore to spore cycle of *Stemonitis splendens* R. developed in culture is the first time reported in science. It takes about 24 days to complete the cycle. The species is also the first record in Taiwan.
2. A spore to spore cycle of *S. flavogenita* Jahn was previously reported by Alexopoulos. The author has also succeeded in cultivating the Taiwan species in laboratory. This species is another first record to be added to the Taiwan flora.
3. The field observations on the formation of fruiting bodies of *S. flavogenita* Jahn have been made twice, each showed the fruiting bodies were formed in the late evening until the early hours of the next morning.

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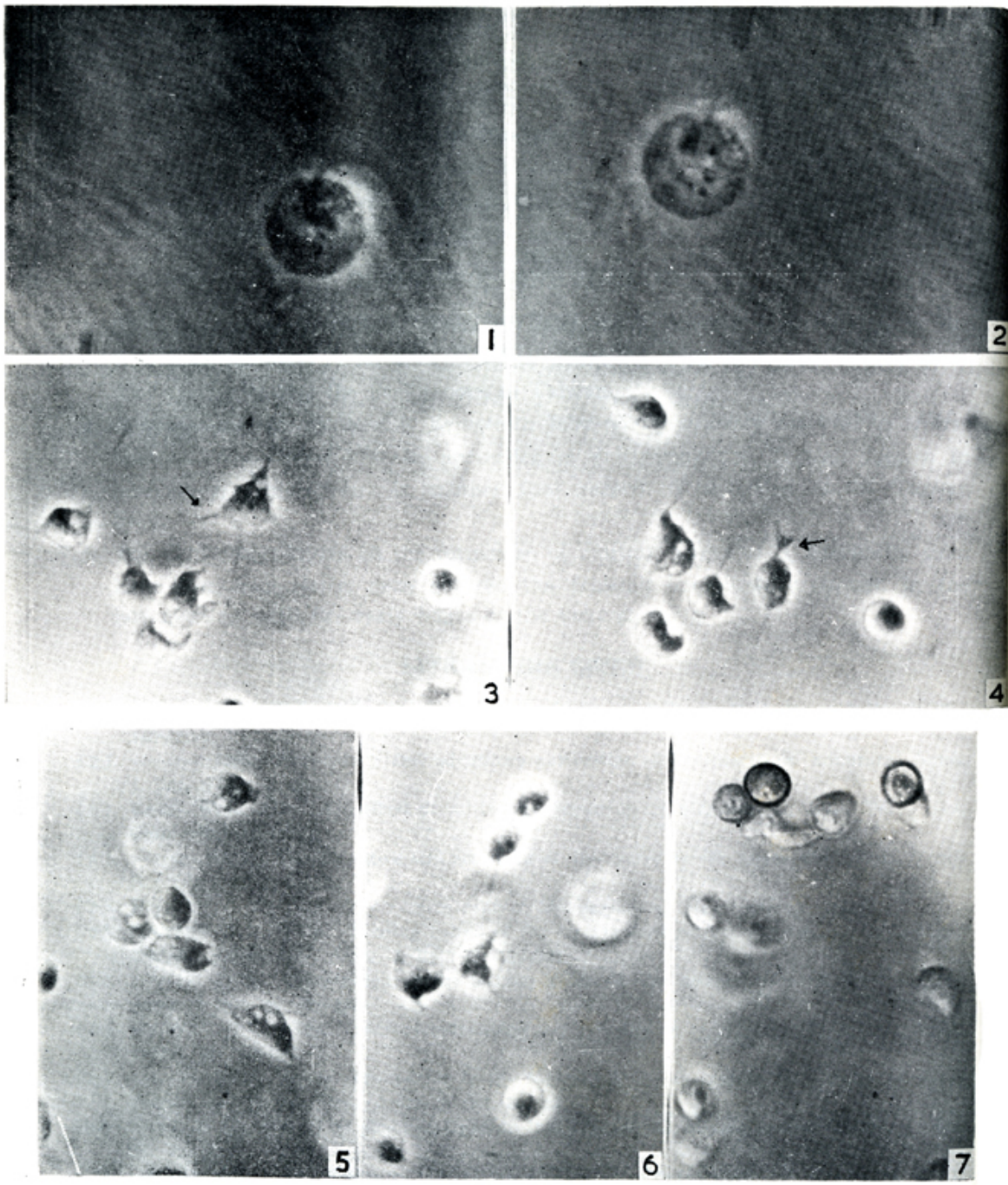

Plate II

Fig. 1-2. Biflagellated swarm cells of *Stemonitis flavogenita* J. (XVI, Hsin Tien). ×1500 Fig. 3-5. Uniflagellated swarm cells and myxamoebae of *S. splendens* R. ×600 Fig. 3-4. Fusion of flagellated myxamoebae. ↗ showing protoplasmic processes. Fig. 5. Myxamoebae showing a pair of vacuoles. Fig. 6. Pairing between swarmers, upper; between flagellated myxamoebae below ×600 Fig. 7. Emerging of swarm cells ×600

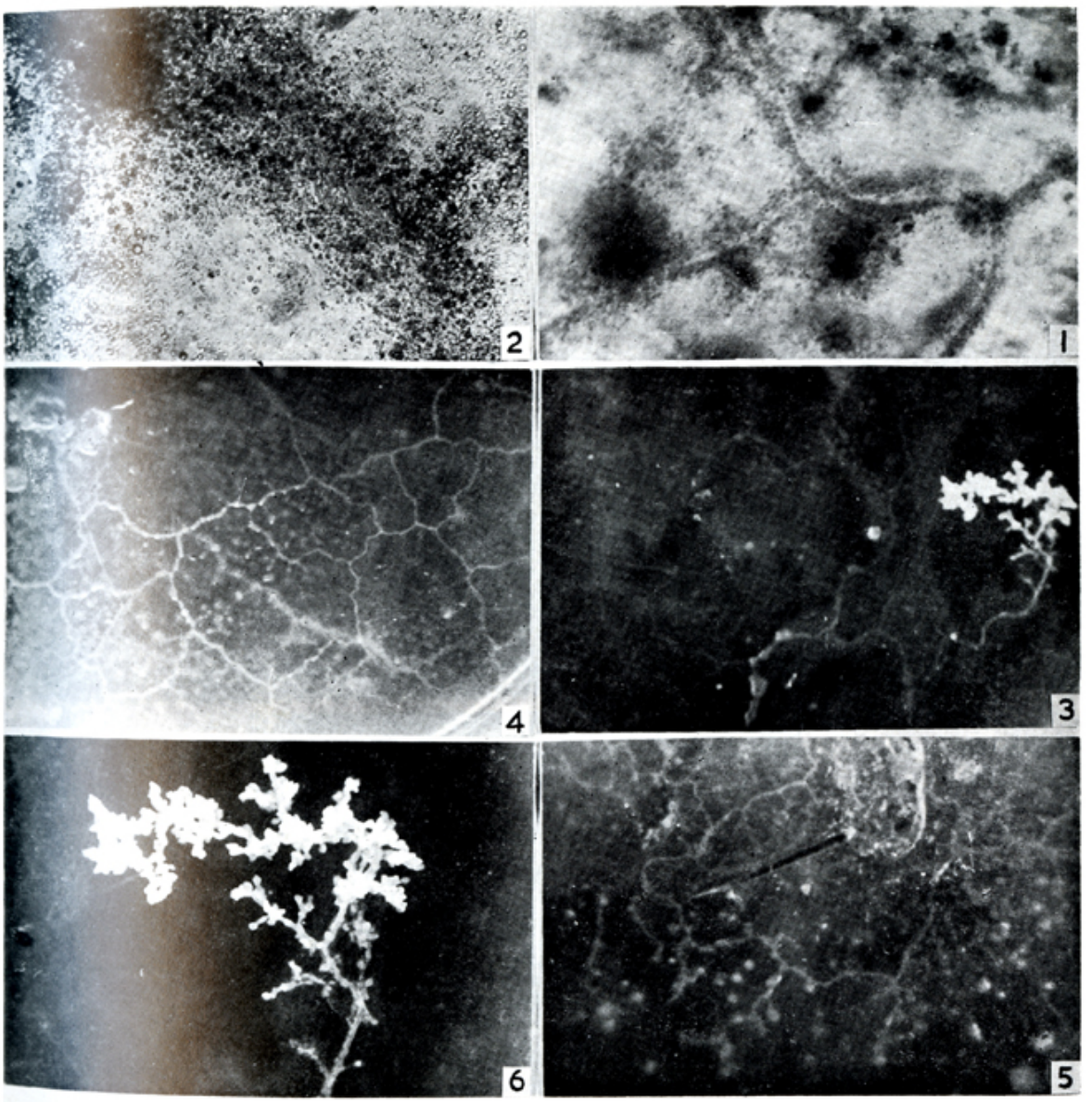

Plate III

Fig. 1. Plasmodium and Streaming of protoplasm in veins of *Stemonitis splendens* R. ×150
Fig. 2. Ditto, portion of vein ×600 Fig. 3. Plasmodium and the aggregated branches ×1.5
Fig. 6. Ditto ×6 Fig. 4-5. Network and veins ×1.5

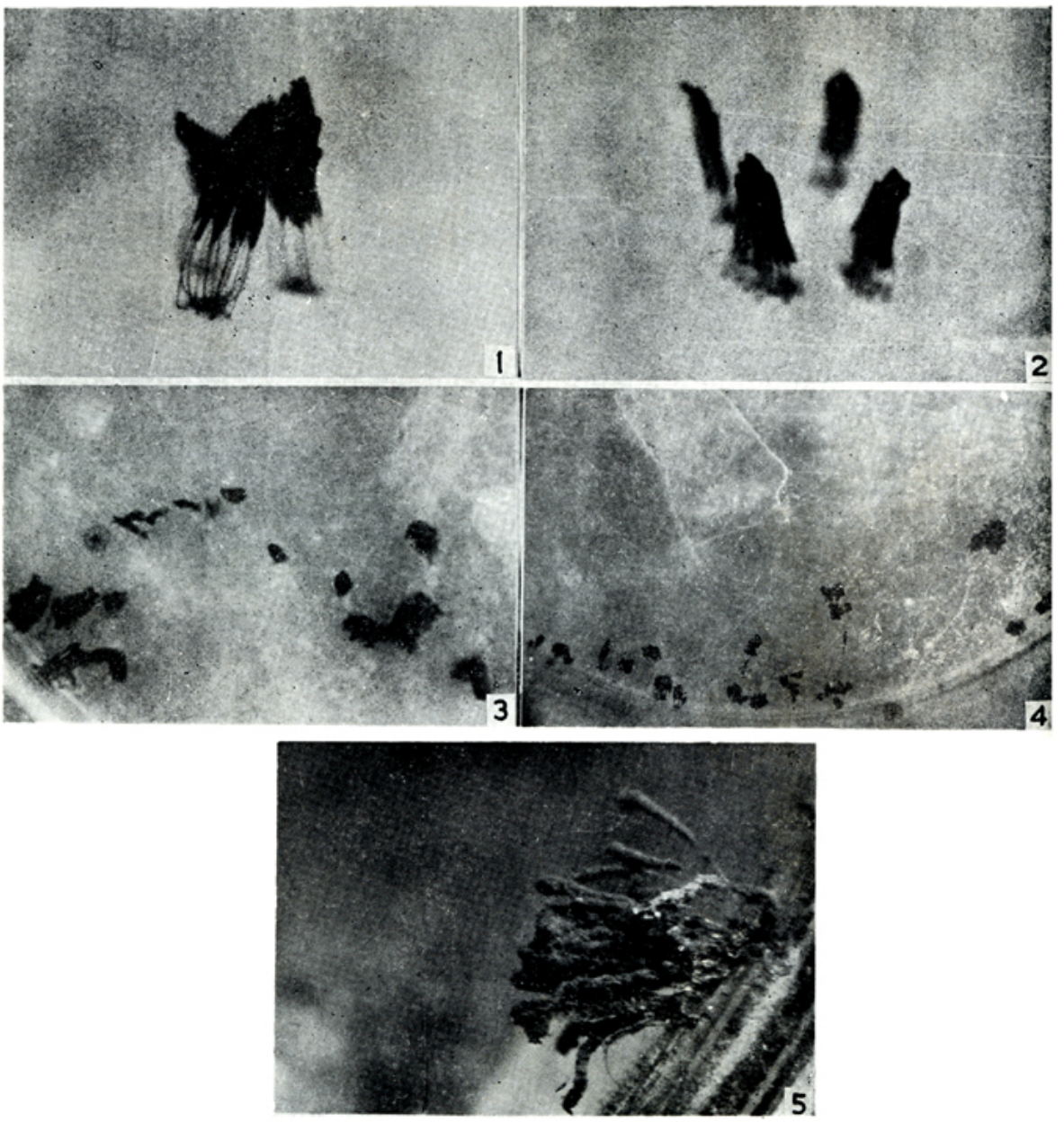

Plate IV

Fig. 1-4. *Stemonitis flavogenita* J. produced in petri dishes. Fig. 1. ×6. Fig. 4. ×1
Fig. 2-3. *Stemonitis splendens* R. produced in petri dishes. Fig. 2. ×1.5. Fig. 3. ×1
Fig. 3-4. Sporangia formed at the ridges of plates
Fig. 5. Portion of 4×6