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A LIFE-CYCLE OF SPHAEROTHECA FULIGINEA  
(SCHLECHT.) POLLACCI PARASITIC  
ON TARAXACUM CERATOPHORUM DC.

BY

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(With Plate VI)

Up to the present time, the conidial and perithecial formation of *Sphaerotheca* have been studied by several authors.

The ground work on the development and structure of the ascocarp of *Sphaerotheca* was done by A. DE BARY, in 1863 (1). The oogonium and antheridium of *Sph. Humuli* are formed at the crossing or touching point of two special hyphae. In 1895, R. A. HARPER (2) reported on the development of the perithecium of *Sph. Castagnei* on *Humulus*. In 1905, V. H. BLACKMAN and H. C. L. FRASER (3) reported on the ascocarp formation of *Sph. Humuli* on *Humulus*. In 1907, P. A. DANGEARD (4) published a paper under the title of "L'origine du périthèce chez les Ascomycètes", in which he described the perithecial formation of *Sph. Humuli* parasitic on *Humulus*. In 1911, Ö. WINGE (5) reported on that of *Sph. Castagnei* parasitic on *Melampyrum*. In 1914, N. BEZSSONOFF (6) reported on that of *Sph. Mors-Uvae* and *Sph. Humuli*.

In 1913, E. FOËX (7) reported on the development of the conidia of *Sphaerotheca Humuli* var. *Humuli* parasitic on *Erodium malacoides* and also of *Sphaerotheca Humuli* var. *fuliginea* on *Calendula arvensis*. According to him, the basal

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- (1) A. DE BARY: *Entwicklungsgeschichte der Ascomyceten*. Leipzig. 1863.
  - (2) R. A. HARPER: *Die Entwicklung des Peritheciums bei Sphaerotheca Castagnei*. (Ber. d. Deutsch. Bot. Gesell. XIII. 475-481, 1895).
  - (3) V. H. BLACKMAN and H. C. L. FRASER: *Fertilization in Sphaerotheca*. (Ann. Bot. XIX. 567-569, 1905).
  - (4) P. A. DANGEARD: *L'origine de périthèce chez les Ascomycètes*. 226-243, 1907.
  - (5) Ö. WINGE: *Encore le Sphaerotheca Castagnei*. (Bull. Soc. Myc. France, XXVII. 211, 1911).
  - (6) N. BEZSSONOFF: *Quelques nouveaux faits concernant la formation du périthèce et la délimitation des ascospores chez les Erysiphacées*. (Bull. Soc. Myc. France, XXX. 406-415, 1914).
  - (7) E. FOËX: *Evolution du conidiophore de Sphaerotheca Humuli*. (Bull. Soc. Myc. France, XXIX. 251-252, 1913).

cell (conidiophore) is unceasingly divided, forming the spore-mother cells. The spore-mother cell divides into two cells, each cell developing to a normal conidium. Besides, he noted that this mode of formation belongs to the first series of the four types of the conidial formation in Erysiphaceae. In *Sphaerotheca pannosa* on Rosa, the conidial formation coincides with that of *Sph. Humuli*. In 1925 (1), the same author published again on the formation of the conidia of *Sph. pannosa* parasitic on Rosa.

In the present paper, the writer has paid her special attention on the following three points in regard to the perithecial formation which have been left undetermined up to the present time, and also on the conidial formation.

1. On the origin of the antheridial and ascogonial hyphae.
2. On the migration of the nucleus of the antheridium.
3. On the chromosome number.

The writer wishes to express her heartiest thanks to Prof. S. ITO for his valuable advices.

### I. Single spore inoculation with the conidia

In the present study, *Sphaerotheca fuliginea* parasitic on *Taraxacum ceratophorum* was used for the material.

The young shoots of *Taraxacum ceratophorum* raised from the cuttings of the root and planted in pots were covered with large glass-tubes whose upper openings were closed with cotton plugs.

On Nov. 19, 1931, the seven pots were prepared, and No. 1 was left as a control; No. 2 was inoculated with numerous conidia; No. 3 was inoculated with three spores; Nos. 4, 5, 6 and 7 were inoculated with a single spore.

The single spore inoculation was made by the following method. The matured conidia were quickly transferred in as small a quantity as possible to slide-glass from the leaf of the affected host plant. A single spore was picked up by means of a fine glass needle under the microscope, and it was gently inoculated on the leaf in the glass-tube.

In No. 2, on Nov. 26, the white patches of the conidia appeared on the leaves, and on Dec. 3, the formation of the perithecia began to take place. In No. 3, on Nov. 28, the infection spots appeared on the leaf, and on Dec. 6, the formation of the perithecia began to take place.

In No. 4, on Dec. 1, a small white spot appeared on the infected portion of the leaf, and on Dec. 12, the perithecia began to be formed. After a week normal perithecia were abundantly found on the mycelium.

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(1) E. FOËX: Notes sur quelques Erysiphacées. (Bull. Soc. Myc. France, XLI 417-438, 1925).

In Nos. 5, 6 and 7, the spots did not appear on the leaf, while in the control plant no signs of the disease were to be seen.

Besides, 3 out of the 18 pots which were inoculated with a single spore were successfully infected, and the perithecia appeared on the mycelial patches.

By the above experiments, the writer recognized that the male and female sexual hyphae originate from a single conidium in *Sphaerotheca fuliginea*, that is homothallism takes place in the fungus (1).

## II. Conidial formation

The incipient conidiophore is formed as a branch from the creeping mycelium on the surface of the host plant, and it is easily distinguished from the mycelial hyphae by its larger size. The young conidiophore assumes a cylindrical shape, and when it reaches about  $50 \mu$  in length it is delimited from the mycelium by a septum. (Fig. 1) The young conidiophore is divided into two cells by a septum at its upper portion, forming a new spore-mother cell. (Fig. 2) The nucleus of the young conidiophore divides into two, one of which is included in the new cell while the other remains in the conidiophore. The spore-mother cell divides into two cells, the apical one becoming a normal conidium when matured, and the lower one divides again into two cells. Similar divisions are repeated forming a chain of conidiospores. (Fig. 3-5) In this species, the matured conidia form a chain of spores 2 or 3 in number and the immatured conidia about 2 in number are found beneath them. The spore-mother cell is always found remaining between the immatured conidium and the conidiophore.

In *Erysiphe graminis* and *Podosphaera tridactyla*, when the conidia are abundantly produced under favorable conditions, the conidiophore sometimes divides again into two cells. In this case, the apical cell becomes a new spore-mother cell. But under the normal condition, the spore-mother cell is formed only once on the conidiophore.

## III. Perithecial formation

The young perithecia are formed from the center of the mycelial patch which was originated from a single spore. The ascogonium and antheridium are formed at the place where the ascogonial and antheridial hyphae cross each other and touch. The antheridial hypha is more slender than the ascogonial. Both hyphae always run side by side or coil about each other or cross each other. The ascogonium and antheridium seem to arise simultaneously;

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(1) Y. HOMMA: Homothallism in *Sphaerotheca fuliginea* (SCHLECHT.) POLLACCI. (Proc. Imp. Acad. IX, 186-187, 1933).

the former is larger than the latter from the start, also, the ascogonium grows more rapidly. (Fig. 6) Sometimes both organs swell up at the base, and adhere closely to each other and become more or less spirally twisted. At first, they are single cells which are delimited from the sexual hyphae with a septum. Later they divide into two cells. The apical cell is the ascogonium or antheridium, and the under one is the stalk cell. (Fig. 7) The ascogonium soon becomes larger than its stalk cell, while the antheridium remains smaller. The ascogonium grows straight up into the subglobose body, while the antheridium clinging to the side of the ascogonium adheres at its upper part. The antheridial nucleus divides into two nuclei, one of which goes into the ascogonium. When both organs become matured, the nucleus of the antheridium seems to have migrated into the ascogonium as two or rarely three nuclei are found enclosed in the ascogonium and one of them is always more or less smaller than the other.

The primary perithecial wall cells are formed from the twisting hyphal cells springing upward from the stalk cell of the ascogonium. (Fig. 8, 9) The enclosing wall thus formed is closed at the upper part of the ascogonium. (Fig. 10) At this time, the antheridium is seen adhering at the outer side of the wall. (Fig. 11) The second perithecial wall cells develop between the ascogonium and the first wall. (Fig. 12) In this stage, the antheridium withers and gradually disappears. Thus, when about 4 to 5 layers of the wall are developed, the appendages arise at the place nearer to the base of the perithecium, and the perithecial hyphae are sent out from its basal portion. (Fig. 16-21) The outer two layers are composed of large cells, whose walls gradually turn brown in color. These layers constitute the so-called outer wall. The inner two or three layers make up the inner wall, which is always hyaline and binucleate.

As the time, when two layers of the outer perithecial wall are developed, the male and female nuclei in the ascogonium conjugate to form a more or less large nucleus. (Fig. 13) Then the ascogonium divides into two cells. (Fig. 14, 15) The upper cell divides again into two cells. (Fig. 16, 17) The same process is repeated thus forming 4 to 5 cells. The penultimate cell is always more or less larger than the rest, and its character for the staining solution differs from the others. This cell gradually grows and forms the ascus. In the young ascus a large nucleus is formed. (Fig. 18) This nucleus divides three times, and eight free ascospores are formed. (Fig. 19, 20) Eight chromosomes could be counted in the first division, but it was difficult to detect them in the second and third division.

#### IV. The inoculation experiment with the ascospore

The perithecia on the leaves which had been kept dry in the green house were collected in early March. Experiments on germination of the ascospores were made in the laboratory every ten days. On July 5, the ascospores were put on the leaves of *Taraxacum ceratophorum* planted in pots which had been covered with large glass-tubes. On July 16, small white patches were observed on the inoculated portions. On the check plant, the mycelial hyphae did not appear on the leaves. The mycelial patches increased in number by the secondary infection, numerous conidia were produced. On July 22, young perithecia were produced on the first mycelial patch.

#### Summary

1. The conidia are successively produced from a single spore-mother cell formed at the end of the conidiophore, and the mode of conidial formation noted by FOËX seems to appear under a special condition.
2. The antheridial and ascogonial hyphae are derived from a single spore.
3. The nucleus in the antheridium seems to have migrated into the ascogonium, as two or rarely three nuclei were found in the young ascogonium.
4. In the first nuclear division in the ascospore formation, eight chromosomes were counted.

**Explanation of Plate**Fig. 1-5  $\times 550$ ; 6-21  $\times 815$ 

- Fig. 1. Young conidiophore.
- Fig. 2. Spore-mother cell on conidiophore.
- Fig. 3-5. Chain of spores on spore-mother cell.
- Fig. 6. Male and female branches.
- Fig. 7. Antheridium and ascogonium.
- Fig. 8, 9. Primary perithecial wall cells from stalk-cell of ascogonium.
- Fig. 10. Primary wall closed at upper part of ascogonium.
- Fig. 11, 12. Male and female nuclei in ascogonium.
- Fig. 13. Conjugated nucleus.
- Fig. 14. Two nuclei formed by division.
- Fig. 15. Two cells from ascogonium.
- Fig. 16, 17. Three cells from ascogonium.
- Fig. 18. Young ascus, including a large nucleus and binucleated perithecial wall cells.
- Fig. 19. Two nuclei in ascus.
- Fig. 20. Four nuclei in ascus.
- Fig. 21. Outer and inner perithecial walls and young appendage.

