Development of Sclerotia and Spermogonia in *Cercospora sesamicola* and *Ramularia carthami*

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Abstract. *Cercospora sesamicola* Mohanty and *Ramularia carthami* Zaprometov causing angular and brown leaf spot diseases of sesame (*Sesamum orientale* Linn.) and safflower (*Carthamus tinctorius* L.) respectively produce sclerotia, spermogonia and immature perithecia during their life cycle. The structure and development of these stages have been described. Maturation of the perfect stage was not observed in these fungi. It is considered that because of similarities with the *Mycosphaerella* species, *Cercospora sesamicola* and *Ramularia carthami* are probably species of the genus *Mycosphaerella* Johanson.

Cercospora sesamicola Mohanty (1958) and *Ramularia carthami* Zaprometov (1926) incite angular leaf spot and brown spot diseases of sesame (*Sesamum orientale* Linn.) and safflower (*Carthamus tinctorius* Linn.) respectively (Figs. 1, 2), causing heavy losses in the oilseed yield. No spore forms other than conidial stage have hitherto been observed to occur on these pathogens. Both *C. sesamicola* and *R. carthami* develop i) sclerotia, ii) spermogonia and iii) perithecial initials in the infection spots, but no mature perithecia were observed. The sclerotia probably serve the primary source of inoculum for fresh infection. It was, therefore, considered essential to investigate their structure and mode of development.

Material and Methods

Morphological development of the sclerotia, spermogonia and perithecial initials have been studied in microtomed serial sections (8—12 μ). Infected leaf tissues in various stages of development were sampled from material collected and stored in the previous season. Pieces of leaf tissues were fixed in FAA soln. Sections 10 μ thick were cut from paraffin blocks (Johansen 1940).

In an attempt to obtain mature perithecia, the infected leaves of sesame (*Sesamum orientale* Linn.) and safflower (*Carthamus tinctorius* Linn.) with well-developed sclerotia were exposed outdoors as i) infected leaves were placed in pairs facing their lower surfaces in between 2 wire gauzes, ii) placed in the surface of the ground and protected from winds by a wire gauze cage, iii) buried in potted field soil at 1, 2 and 3 inches depth, iv) stored at room temperature (26—30° C) in the laboratory and kept moistened by an atomized spray of water daily during the time of spermatial discharge from April through July. The leaves of all these samples were checked periodically for the presence and development of ascigerous stage.

Observations

The sclerotial stage of *Cercospora sesamicola* on sesame appears always towards the close of the grop-growing season late in September or in early October. The sclerotia are produced abundantly as dark pycnidia-like bodies on both surfaces on the leaves. Such leaves fall off readily on being disturbed. The sclerotia of *Ramularia carthami* were observed just prior to harvesting of the crop. Two types of symptoms of the sclerotial stage are observed on safflower. In one type a dark brown, well-defined ring of 1—3 mm diam. develops encircling the old spots, which were producing the conidiophores and conidia. Numerous black pin head-like structures appear on the lower surface in this peripheral zone. In the other type developing by fresh infections, chestnut brown, cicular spots, 5 mm in diam. develop with sclerotia on their lower surface.

Development of sclerotial stage:

The mode of sclerotial development in *C. sesamicola* and *R. carthami* was found similar. The conidial germ tubes enter the leaf by penetration of the stomata. On entering the substomatal chamber, hyphae begin to spread intercellularly. Hyphae are never seen to penetrate the living host cells, but they penetrate after collapse and death of host cells. The hyphae at this early stage are hyaline, septate and 2—3 μ in diam. They become dark brown, thick-walled and closely septate increasing to 7 μ (5—7 μ) in diam. prior to development of sclerotia. The fruiting bodies begin to form at this stromatic stage. The sclerotia develop by continued multiplication of cells of a single hyphal branch just below the epidermis resulting in a compact, dark brown, globose structure (Figs. 3—5). Enlargement by further multiplication of the cells pushes the epidermis upward. Subcylindrical cells are formed at the neck, which project out through the stomata.

Mature sclerotia are chestnut brown, spherical to globose, markedly raised above the level of the epidermis (Fig. 6). They are composed of dark brown pseudoparenchyma with larger and thinner-walled inner cells surrounded by thick-walled cells. The cells in the 2 outer layers are darker than the inner cells. The sclerotia of *C. sesamicola* and *R. carthami* measure 35—70 × 30—85 μ and 40—80 × 50—70 μ respectively. Many Ramularias have been reported to develop similar sclerotia (Dring 1961, Gregory 1939, Hughes 1949). No reports were noted of the species of *Cercospora* producing sclerotia as described in *C. sesamicola*.

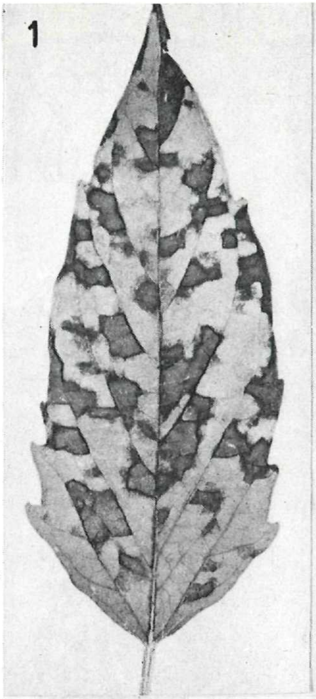
Structure and development of spermogonia:

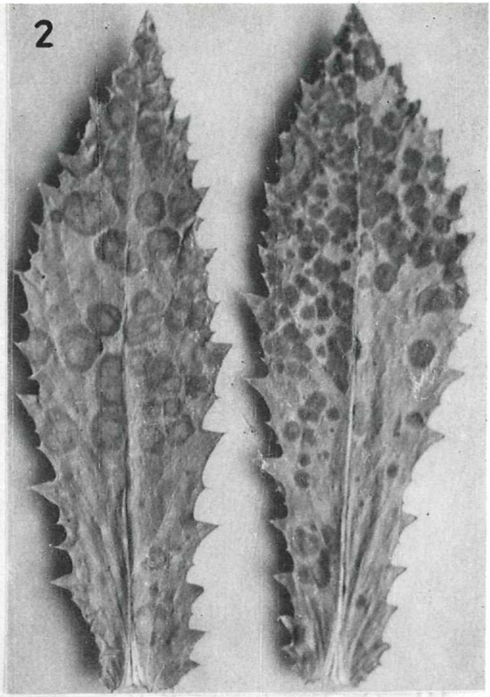
The structure and development of the spermogonia of both the pathogens was found similar. The spermogonia may develop within the old conidial stromata (Fig. 15), and/or originate independently in separate fruit bodies below the epidermis. The spermogonia begin to appear in a similar manner as the sclerotia and all its earlier stages of development are similar to that described earlier for sclerotia. Both sclerotial and spermogonial initials are found mixed and are indistinguishable in their earlier stages. Mature spermogonia are ovate to globose, dark brown to black, at first embedded subepidermally in the leaf tissues later become erumpent, ostiolate and measure $45-110 \times 40-150$ μ (Fig. 16).

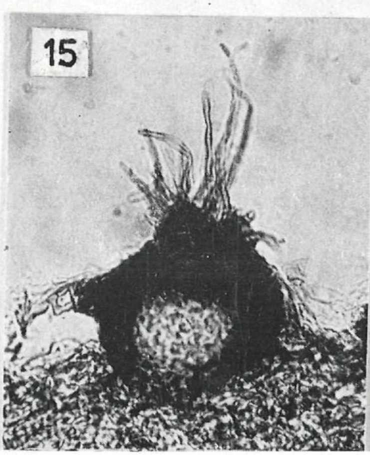
As the development progresses, the cells in the central region of the pseudoparenchymatous fruit body become lighter, hyaline, thin-walled and dense in protoplasm (Fig. 7). These cells gradually become enlarged and differentiated (Fig. 8). Each of them begins to give rise to spermatia endogenously (Fig. 9). These spermatial mother cells are hyaline, thin-walled and flask-shaped. The spermatia are small, hyaline, rod- to bone-shaped and discharged from the mother cells through an apical pore into the cavity of the spermogonium (Fig. 11). The spermatia of *C. sesamicola* and *R. carthami* measure $2.5 \times 0.8-1$ μ and $3.5-4 \times 1$ μ respectively. The empty mother cells after discharging the spermatia collapse and eventually disintegrate. In the mature ostiolate spermogonium the original outer thick layer of cells becomes reduced to a 2-layered wall of thick-walled cells lined with a thin layer of fertile cells (Fig. 10). The discharged spermatia accumulate in the developing cavity in the spermogonium and become mixed with mucilage. The contents begin to exude through the ostiole at maturity. The spermogonium finally becomes exhausted and begins to collapse. Further development was not observed.

Immature perithecia:

During the early developmental stages, the sclerotia, spermogonia and immature perithecia appear identical and are indistinguishable. Perithecial initials of both the pathogens develop similar to the sclerotia and spermogonia. As the development from a sclerotium continues, the thick-walled pseudoparenchymatous cells in the central region become gradually less compact, thin-walled and hyaline, containing dense protoplasm. Finally the fruit body becomes differentiated into inner loose, thin-walled cells surrounded by 2 layers of thin-walled, dark brown cells (Fig. 12). Several densely protoplasmic, hyaline archicarps with slightly bulged pitcher-like base and long trichogynes develop in the central region of the immature perithecia (Figs. 13, 14). Sections showing a complete archicarp with an entire trichogyne could not be







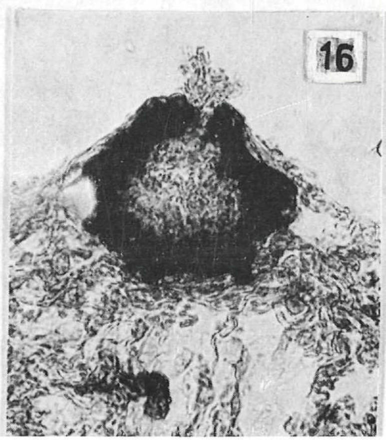
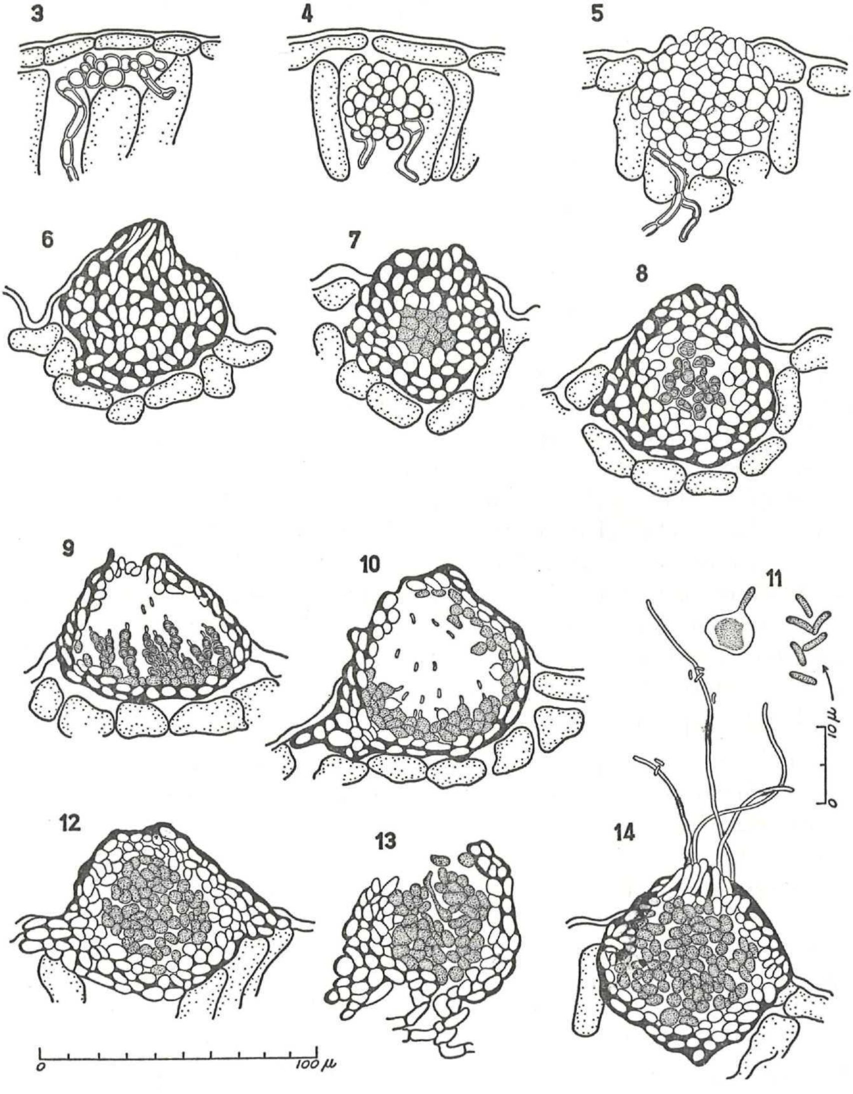


Fig. 1. Angular leaf spot on sesame by *Cercospora sesamicola*. Fig. 2. Brown leaf spot of safflower by *Ramularia carthami*. (Nat. size).

Figs. 15–16. Development of sclerotium and spermogonium in *Cercospora sesamicola* (contd.). Fig. 15. A developing spermogonium with remnants of withering conidiophores. Fig. 16. A mature spermogonium. (All ×300).



Figs. 3—14. Development of sclerotium and spermogonium in *Cercospora sesamicola*. Fig. 3,4 . Multiplication and thickening of cells of the hyphae developing into a subepidermal young sclerotium. Fig. 5. Young immature sclerotium. Fig. 6. A mature sclerotium with developing ostiole. Fig. 7. Developing spermogonium differentiating thin-walled, densely protoplasmic cells in the center. Fig. 8. Young spermogonium forming loose cells in the center. Fig. 9. Spermogonium with spermatial mother cells at the base, producing spermatia. Fig. 10. Mature spermogonium containing spermatia awaiting escape through ostiole. Fig. 11. Spermatial mother cell and spermatia. Fig. 12. A young perithecium with loose thin-walled cells in the center. Fig. 13. Young perithecium with archicarps. Fig. 14. Young perithecium showing long filiform, projecting trichogynes with few spermatia attached.

observed. The exterior portion of the trichogyne is long, slender, unbranched and measures 50—120×1.3—1.7 μ. As many as 8—12 trichogynes were observed to protrude from the young initials of perithecium indicating an equal number of archicarps. Often 2—3 spermatia are observed adhering to the tip of the trichogynes. Later stages of development were not observed.

Discussion

The perithecial stage is known for less than a dozen species out of approximately 400 species of *Ramularia* (Ehrlich and Wolf 1932). Some species produce normally all the 3 stages viz. sclerotial, spermogonial and perithecial stages, which are considered as the complete cycle forms, while other species may develop the sclerotia and/or spermogonia during the cycles as the pathogens under study. The life cycles of *C. sesamicola* and *R. carthami* resemble that of *Ramularia armoraciae* Fuckel (Dring 1961), which develops only the sclerotial and spermogonial stages. Some of the sclerotia of *C. sesamicola* and *R. carthami* transform into spermogonia and immature perithecia, while the remaining undergo no morphogenic changes and serve the function of oversummering the pathogens. The sclerotia giving rise to the spermogonia and the immature perithecia are indistinguishable until later stages of development.

Earlier it was reported by Jenkins (1938) that failure of the perithecial development may be due to lack of free water during the spermatial discharge and they were produced only when the leaves were artificiually sprinkled or with rainfall during the spermatial discharge. The perithecial initials of *C. sesamicola* and *R. carthami* failed to mature in spite of water sprinkling and exposure to natural precipitation during their spermatial discharge. This indicates that moisture in a film deposit is not the limiting factor for perithecial development. Temperature possibly plays an important role in the perithecial production as in *Mycosphaerella arachidicola* Jenkins and *M. berkeleyii* Jenkins (Jenkins 1928). Similarity in the production of sclerotia, spermogonia and immature perithecia with those of species of *Mycosphaerella* described earlier (Dring 1961, Jenkins 1938) suggests possibility of *Mycosphaerella* as the perfect stage for *C. sesamicola* and *R. carthami*.

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