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Histology of Carpel Infection by *Claviceps fusiformis* in Pearl Millet

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Claviceps fusiformis conidial infection enters the carpel of *Pennisetum typhoides* through stigma, style or ovary wall. The hyphae of the fungus were located below the ovary wall 3 days after inoculation and subsequently the fungus showed preferential growth towards ovary wall. When once the ovary wall was replaced by plectenchymatous fungal hyphae the fungus started invading interior cells of the ovary. The whole ovary was replaced by interwoven fungal hyphae by 10th day after infection. In all these cases the fungal spread was intercellular. The honeydew production started 5 days after inoculation and persisted for about a week.

Key Words: Pearl millet, Ergot disease, *Claviceps fusiformis*, **Carpel infection, Histology**

Introduction

Ergot disease caused, by *Claviceps fusiformis* Lov. (Loveless 1967, Siddiqui & Khan 1973 and Bhat 1977) is a limiting factor in pearl millet (*Pennisetum typhoides* (Burm. Stapf. & Hubb.) seed production. The disease spreads in the field by conidia produced in honeydew (Bhide & Hegde 1957, Nene & Singh 1975). The pathogen is specific to ovary of floret, conidia reach ovary either through stigma, style or ovary wall to bring about infection. There are reports (Reddy et al. 1969, Sundaram 1975) that infection takes place through the stigma and ovary wall. However, well documented report on histology of infection is lacking. Such studies have been made in dallisgrass (*Paspalum dilatatum* Poir.) ergot caused by *Claviceps paspali* Stevens and Hall (Luttrell 1977) and ergot of cereals caused by *Claviceps purpurea* (Campbell 1956, 1958, Dickerson et al. 1978). The present paper deals with histology of infection and establishment of the pathogen in the pearl millet carpel.

Materials and Methods

To study the entry point of infection, the carpel was carefully separated from pre-

viously bagged fresh protogynous florets and such carpels were kept on clean glass slides. The conidial suspension was just allowed to touch different parts of carpel viz., ovary, style and stigma. Sampling was done after 36 hr and squashes were prepared for microscopic observations. To study the path of infection under natural conditions protogynous florets of previously bagged earheads were sprayed with aqueous suspension of conidia, covered for 36 hr and florets were sampled after 36 hr and squashes were prepared. For squash preparations dissected ovaries were suspended in 5% sodium hydroxide for 12 hr. The florets were thoroughly washed with tap water to remove sodium hydroxide completely. Staining was done with cotton blue (0.5%) in lactophenol and destained with anhydrous lactophenol. The florets were mounted in lactophenol.

For preparing permanent slides, florets were collected from artificially inoculated earheads. Artificial inoculation was done by spraying an aqueous suspension of *C. fusiformis* conidia, using a spray pump, containing 10×10^{-4} conidia/ml, onto florets of previously bagged earheads of pearl millet (HB-3). Such sprayed earheads were bagged using polythene covers. Samples were drawn at daily intervals until matured sclerotia appeared. The florets were fixed in Farmers' fluid (Acetic acid, Absolute alcohol 1:3 v/v) for 24 hr and dehydrated using alcohol series (Johansen 1940). The material was embedded in wax using standard histological techniques and a complete series of longitudinal as well cross sections of 12 μ to 15 μ thickness were obtained using rotory microtome. The sections were mordanted in 4% iron alum (2 hr), washed in distilled water for 30 min, stained in 0.5% aqueous haematoxylin (2 hr) and differentiated in a saturated aqueous solution of picric acid (15-20 min). The experiment was conducted in December, 1979.

Observations

Entry points of infection

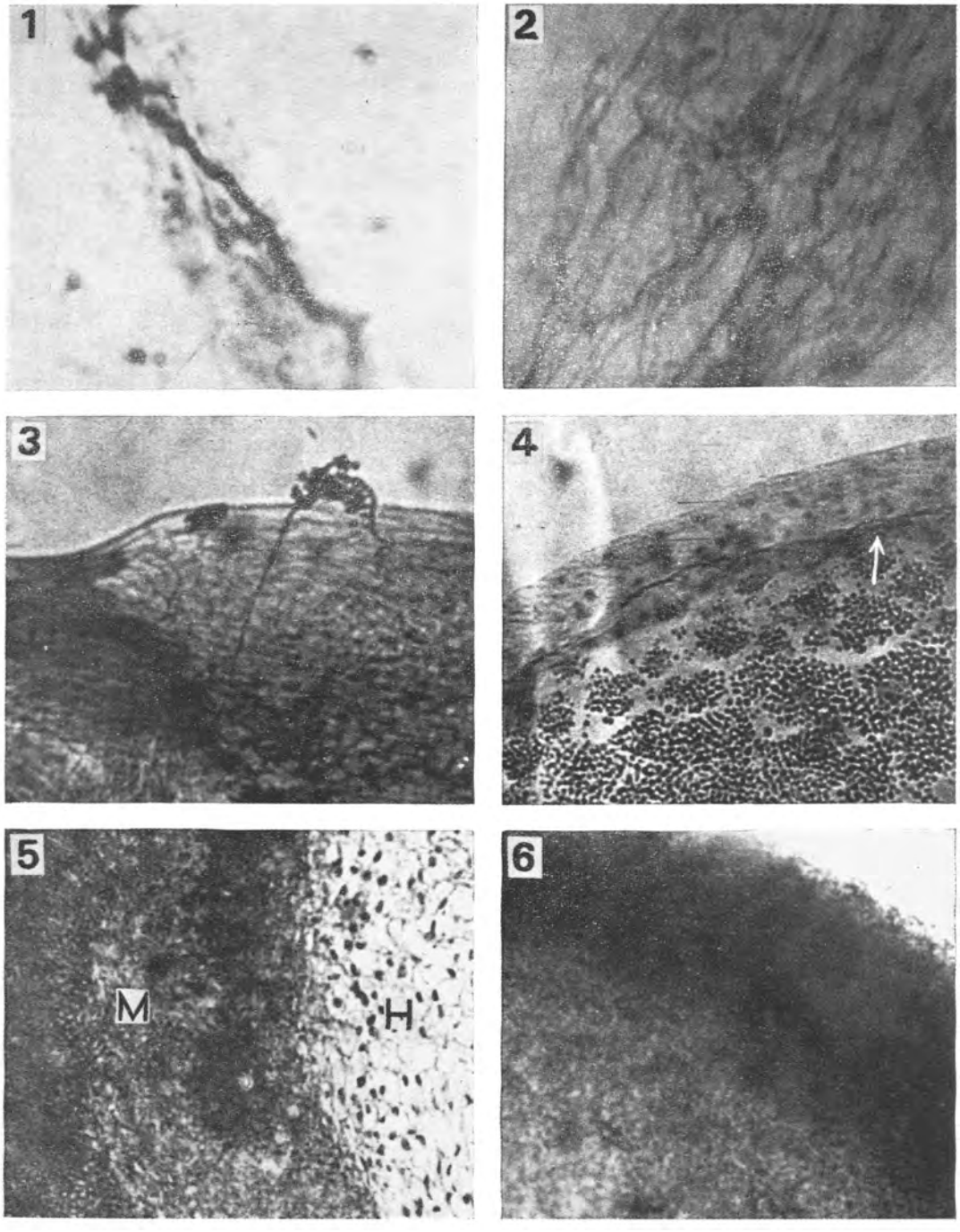
The penetration from germ tubes of conidia was direct without producing any appressoria. The germ tube was found entering the carpel through stigma, style and ovary wall (figures 1-3). But under natural conditions entry through ovary wall was less frequent.

The squash preparation of inoculated florets, collected on first day after inoculation have not shown any infective hyphae in them. On second day, the germ tubes of conidia were found entering through stigmatic lobes and style. The infection hyphae traversed through intercellular spaces and it proceeded down the stigma and style into ovary. All the conidia germinated did not find their way into the carpel. When once the conidial infection was through the style, the style withered away.

From style, the hyphae penetrated the inner layers of ovary wall and mycelia were located between the ovary wall and nucellus (figure 4). The spread of hyphae was intercellular and by third day after inoculation an intercellular mycelium occupied the ovary wall.

Development of the honeydew stage

Due to lateral and outward growth of hyphae the ovary wall was lifted and ruptured. By 4th day of inoculation the hyphae formed plectenchymatous extramatrical stroma in place of ovary wall and free hyphae were found covering the



Figures 1-6 *1*, Conidial germ tube of *C. fusiformis* penetrating through stigmatic lobe (×450); *2*, Conidial germ tubes traversing through the style towards ovary (×300); *3*, Germ tube from conidia penetrating ovary directly (×300); *4*, A portion of L.S. of infected ovary, 4 days after inoculation, with fungal mycelia just below the ovary wall (×280); *5*, A portion of L.S. of infected ovary, 4 days after infection, showing intact ovary cells in the centre (H) surrounded by plectenchymatous fungal tissue (M) (×280); *6*, Infected ovary completely malformed into fungal sclerotia showing two distinct portions; outer wall of sclerotia and inner core (×280)

stroma. The ovule had become an islet of intact host tissue surrounded by the fungus. The ovary collected on 5th day were almost pulpy but the deed-seated cells of ovary were still intact (figure 5). The stroma was found around the ovary closely appressed to palea and lemma. The size of such infected ovary increased considerably. At this stage the fungal hyphae penetrated into nucellus and then into embryosac. From underlying hymenial layer of stroma short, almost club-shaped conidiophores were produced. The conidia were produced at the tip of conidiophores. The honeydew appeared in the form of dew droplets on infected floret containing numerous conidia. On 6th day the honeydew production was in copious amount and it was colourless. The honeydew production persisted even upto 10 days and the colour changed from hyaline to pink. By this time the fungus invaded the entire ovary with closely knitted hyphal mat. But still the interface between the sclerotial wall and the cortex was clear (figure 6). In any case, the fungus hyphae was not found entering receptacle cells, which were just below the ovary.

Development of sclerotium

In this phase the ovary was disintegrated completely and was replaced by the fungal hyphae. Wall of the ovary was replaced by white, soft layer of the fungus. Even at this stage conidial production was seen on some points. By 15th day after infection, white smooth layer was found replaced by an irregular brownish cortex. The sclerotia had two distinctive layers, the inner core being thicker than the outer layer of cells. Even in mature sclerotia conidia were found on the surface.

Discussion

The histological changes that take place during infection and establishment of pathogen are particularly important in understanding host-parasite relationships. A fungus can make its entry into ovary through stigma, style, ovary wall or directly from mother plant (Neergaard 1975). But entry from mother plant has not been reported for any of the ergot fungus. Reddy et al. (1969) observed infection of *Claviceps microcephala* through ovary and Sundaram (1975) reported infection through stigma and tender ovary wall. Engelke (1902) reported growth of *Claviceps purpurea* downward through the style and stigma of pistil to the base of the ovary, which seems to be the more probable course of infection under natural conditions. But this is not the only course of infection as it was found that infection occurred even when stigma was removed from carpels. In case of rye ergot caused by *C. purpurea* infection of ovary started from the base (Tulasne 1853, Kirchhoff 1929, Campbell 1958) and mycelia spread intracellularly inside ovary (Campbell 1958). In case of *C. microcephala* also mycelial growth commences at the base of the ovary of pearl millet and penetrates into the inner tissues (Ramakrishnan 1963). This is true when infection takes place through ovary wall. But when infection is through stigma down the style, the growth of the fungal mycelia commences from the lower layers of ovary wall and spread intercellulary first outwards then into the nucellus and embryosac.

The sclerotial development of *C. fusiformis* closely agrees with *C. paspali*. The developmental process in these two fungi is simple when compared to the complex nature of sclerotial development

in *C. purpurea*. In *C. purpurea* the sclerotium differentiates from the base of the sphacelial stroma (Tulasne 1853, Dickson 1947).

The fungus daaws nutrition from the host for its development and establishes itself on the host in the form of sclerotia, thus replacing the seed. Such sclerotia become important source of primary inoculum.

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