

Fusarium longipes—a mycoparasite of *Sclerospora graminicola* on pearl millet*

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Abstract : A mycoparasite, *Fusarium longipes* Wollenw. and Reinking, parasitizing *Sclerospora graminicola*, that causes downy mildew of pearl millet is reported. The mycoparasite attacks only downy mildew-infected areas of the leaves. The mycoparasite produces brownish spots with variable sizes and may cover the entire infected leaf under humid conditions resulting in the complete inhibition of sporangial production. Such plants are killed immaturely. Attempts to selectively control the mycoparasite by the use of fungicide or to identify genotypes which can support *S. graminicola* but not *F. longipes* have been unsuccessful.

Keywords : *Fusarium longipes*, mycoparasite, oospores

Downy mildew (*Sclerospora graminicola*, (Sacc.) Schroet.) is the most important disease of pearl millet (*Pennisetum glaucum* (L.) R.Br.) in India and Western Africa. Severe epidemics of this disease have occurred frequently in India in the past resulting in tremendous reduction in grain yield (Safeeulla, 1977; Singh *et al.*, 1987). Utilization of host-plant resistance is the only feasible method for the control of this disease on pearl millet. To obtain resistant sources, a large scale field screening technique is used in routine that mainly utilizes sporangia as source of inoculum, supplied by earlier planted 'infector rows' (Williams *et al.*, 1981). For the past several years, we have observed a mycoparasitic infection on downy mildew infected leaves of cultivars HB 3 and 7042, which are used in infector rows. The infection occurs only during the rainy season; under frequent rains and overcast sky, the mycoparasite spreads quickly covering downy

mildew infected leaf areas within a few days. This adversely affects production of sporangia in the infector rows reducing the efficiency of the screening system. The purpose of this study was to identify the mycoparasite and how to control it without affecting the growth of the downy mildew pathogen.

MATERIALS AND METHODS

Isolation and identification of the mycoparasite

Downy mildew infected leaves containing leaf spots were excised from the interface of the downy mildew and mycoparasite infection. The leaf pieces (3 mm × 3 mm) were surface-disinfected in 0.1 per cent mercuric chloride for 1 min. followed by four rinses in sterile distilled water, transferred to freshly prepared potato dextrose agar (PDA) in petri plates and incubated in an incubator (Percival, Boone, Iowa) at 25°C with a 12 h photoperiod for 3 days. Stock cultures of the pathogen were grown on PDA slants in

test tubes and incubated as described previously. Conidia produced from the natural growth on infected leaf were measured for taxonomic comparisons.

Inoculation of the mycoparasite on downy mildew-infected plants

This test was conducted on a universal downy mildew susceptible cultivar, 7042. Seedlings of this cultivar were inoculated with sporangia of *S. graminicola* using a seedling inoculation technique (Singh and Gopinath, 1985). Thirty-days old systematically infected downy mildew plants were sprayed with a suspension of conidia (1×10^3 conidia ml^{-1}) of the mycoparasite. Each plant was inoculated with 50 ml of inoculum. Inoculated plants were kept in a humid chamber (RH 100, temperature 20-25°C) for 48 h and then kept in a greenhouse. Plants sprayed with water served as the control. The experiment was repeated three times with 10 plants each time.

Histopathological test

Downy mildew infected leaves showing clear mycoparasitic infection were selected. Free hand sections were taken through the mycoparasitized area on precleaned microscopic slides (75 mm \times 25 mm). The sections were mounted in lactophenol and covered with microscopic cover glass

(19 mm \times 19 mm) and observed under a microscope (250X, Olympus B071) to detect the parasitic effect of the mycoparasite on oospores of *S. graminicola*.

RESULTS

Symptoms

The symptoms of mycoparasitic infection are characterized by the presence of spots of variable shapes and sizes on systemically infected downy mildew leaves. The spots begin as small, round, brownish dots that enlarge in size with time. Fully developed spots have ash-coloured centres surrounded by a brownish margin (Fig. 1a). The spots measured 5-35 mm in length and 2-12 mm in width. However, under high humidity, the spots coalesce, covering the entire downy mildew-infected leaf areas, which are eventually killed. The growth of the fungus on the spots is not conspicuous.

Isolation and identification of the mycoparasite

The organism isolated from the leaves produced pinkish mycelium on PDA at 25°C. The colonies were cottony white in the initial stage but gradually became light pink in colour. The conidiophores were hyaline and loosely



Fig. 1 : (a) Symptoms of *Fusarium longipes* on downy mildew infected pearl millet leaf. (b) Conidia of *F. longipes*, bar = 47 μm . (c) *F. longipes* parasitizing oospores of *S. graminicola*, bar = 47 μm .

branched, arising from aerial mycelium. Each conidiophore terminated in a conidiogenous cell that bore a solitary conidium on an apical pore. After the formation of conidium, second, third and adjacent pores were formed that, in turn, bore conidia, thus representing a polyblastic sympodial cell (Booth, 1971). Conidia were sickle-shaped and slightly curved at both ends with pointed apices and wedge-shaped basal cells with three to five septa. Intercalary chlamydospores were round to globose and occurred singly or in chains. The conidia measured 45-84 to 7-10 μ (Fig. 1b). The mycoparasite was identified as *Fusarium longipes* Wollenw. and Reinking by the CAB International Mycological Institute, Ferry Lane, Kew, England (herbarium IMI number 328737 and 328738). The culture was deposited with the above Institute.

Inoculation of the mycoparasite on downy mildew-infected plants

The inoculated plants developed symptoms typical of those observed in the field within 5-6 days after inoculation. The spots increased in size with time and within 10 days of inoculation, younger leaves were fully covered and dried. Older leaves also developed severe symptoms under humid conditions. When kept under a humid environment, a pinkish fungal growth developed in the centres of the spots. Re-isolations yielded *F. longipes*.

Histopathological study

Microscopic examination revealed that oospores were heavily parasitized with *F. longipes*, and conidiophores were seen emerging from the parasitized oospores (Fig. 1c). The walls of such oospores crumbled and the oospores were totally devoid of contents. The effects of the mycoparasite on the mycelium were not observed.

DISCUSSION

Under Patancheru conditions, the mycoparasite appears only during rainy season and it spreads quickly under high humidity (> 90% RH) and overcast sky. The spread is greatly reduced under

dry conditions (non-rainy days) even in the rainy season. The mycoparasite has not yet been observed during the post-rainy dry season. This shows a close relationship between relative humidity and parasitization which needs to be determined further. During the course of this study, attempts have also been made to control *F. longipes* without affecting the growth and spread of *S. graminicola* in the infector rows. Our efforts to achieve this using benomyl as seed dressing or spraying have been unsuccessful. We also evaluated about 3,000 downy mildew-susceptible genotypes for resistance to the mycoparasitic infection in the downy mildew nursery during 1990-91 rainy season. None showed freedom from the mycoparasite. Efforts are continuing to look for resistant material that can be used as an inoculum donor.

During rainy season, the mycoparasite is capable of killing downy mildew infected plants. This has two beneficial effects: (a) it reduces the production of sporangia which, in turn, can reduce secondary spread of the disease; and (b) it can also kill oospores in downy mildew infected plants, thereby reducing the source of primary inoculum for the coming season. If seedling death can be caused in the early stages of crop growth, then the incidence of downy mildew in pearl millet can be drastically reduced. In addition to *F. longipes*, *F. semitectum* Berk. and Rav., and *Drechslera setariae* (Sawada) Subram. and Jain have also been reported to parasitize *S. graminicola* on pearl millet (Rao and Pavgi, 1976; Balasubramanian, 1979). Although information on the parasitic effect of *D. setariae* is lacking, *F. semitectum* has been shown to cause extensive damage to the oospores similar to that done by *F. longipes* in the present study. With the availability of several mycoparasites, opportunities exist for testing the possibility of a biological control of downy mildew of pearl millet.

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