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SHORT NOTES

Morphological modifications in wheat seedlings infected by *Fusarium culmorum* examined at SEM

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Summary. *Fusarium culmorum* is one of the most important pathogens of winter cereals in Italy and is typical of cereals grown in dry soils of temperate areas throughout the world. The fungus causes a range of diseases such as seedling blight, brown foot rot and ear blight. When tissue portions from the crown areas of durum wheat seedlings, at decimal growth stage (GS) 13, grown in *F. culmorum* inoculated compost were examined at the scanning electron microscope (SEM), they showed a damaged epidermal cell layer that exposed the parenchyma which was characterised by high cell proliferation, with widely spaced, irregularly shaped and loosely arranged cells. No damage was observed at root level.

Key words*: Fusarium culmorum,* seedling blight, ultrastructure.

Introduction

Fusarium culmorum (W.G.Sm.) Sacc. is one of the five main *Fusarium* species attacking cereals in the temperate cereal-growing areas of the world (Parry *et al*., 1994). It is commonly associated with warm dry conditions and is greatly influenced by temperature and rainfall (Wiese, 1987). *Fusarium culmorum* can cause extensive yield losses, especially in durum wheat, with over 50% reductions in grain yield being common (Jones and Clifford, 1983). It causes a succession of diseases: seedling blight, brown foot rot and ear blight, on a wide range of cereals (Parry *et al.,* 1995). Its typical symptoms are a brown discoloration or more pronounced rotting of infected areas. In Italy it is one of the most important components of the foot and root disease complex of durum wheat (Frisullo and Rossi, 1991; Rossi *et al*., 1995; Balmas *et al*, 2000; Innocenti *et al*., 2000).

The pathogen, mainly soil-borne, is characterised by the production of 3-5 septate macroconidia, oval to globose chlamydospores and an absence

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of microconidia; the perithecial state is still unknown. Infection at the seedling stage occurs in the hypocotyl, coleoptile and crown regions of cereal plants. The fungus enters through stomata, wounds made by crown root emergence and/or between epidermal cells (Wiese, 1987). Detailed information on fungus-plant interaction at the early stages of growth is quite limited and the mode of transmission of the fungus from soil level to ear is also still unclear (Clement and Parry, 1998). The aim of this work was to analyse by scanning electron microscopy (SEM) the effect of early infection with *F. culmorum*, and to study the relationship between the fungus and wheat plants at decimal growth stage (GS) 13.

Materials and methods

Fungal cultures and preparation of inoculum

The virulent strain used was *F. culmorum* LM 3.97, which is deposited in the collection belonging to the Dipartimento di Protezione e Valorizzazione Agroalimentare of the University of Bologna, Italy. The culture was from single-spore lines from naturally infected durum wheat plants collected in an area near Bologna. The fungus was stored at 5°C in the dark in Difco potato dextrose agar (PDA) tubes, under mineral oil.

Inoculum was prepared with 20 g of millet and wheat kernels (1:1, v:v) soaked in tap water for 12 h, drained on gauze by pressing, dispensed to 250-ml Erlenmeyer flasks and autoclaved at 120°C, 1 h a day for 2 days. Each flask was then inoculated with 5 agar discs (6-mm diameter) excised from the margins of actively growing PDA cultures of *F. culmorum*. The inoculated kernels were maintained for three weeks at 20°C (± 2°C) in the dark, air-dried and then stored at 5°C until the beginning of the experiment.

Glasshouse experiment

Kernels of durum wheat cv. Creso, susceptible to *F. culmorum*, were surface-sterilised with 1% NaOCl for 10 min, rinsed several times in sterile distilled water and dried at room temperature on sterile filter paper. Thirty kernels were sown in a plastic tray (20x10x10 cm) containing 400 g of autoclaved compost consisting of soil-sand-peat (2:1:1, v:v:v) to which *F. culmorum* inoculum (1% w) was added 24 h before planting. Kernels grown in the same conditions without fungal propagules were tested in the same way as controls. The trays were maintained in a glasshouse under controlled conditions at 23°C (±2°C), 70% RH and a 12-h-day. Durum wheat seedlings four weeks old, at decimal growth stage (GS) 13 (Zadoks *et al.,* 1974), were harvested to determine the effect of the fungus. Tissue portions of these wheat seedlings, with the typical browning symptoms, were plated on PDA to isolate any fungus and confirm its responsibility for the symptoms caused.

Scanning electron microscopy

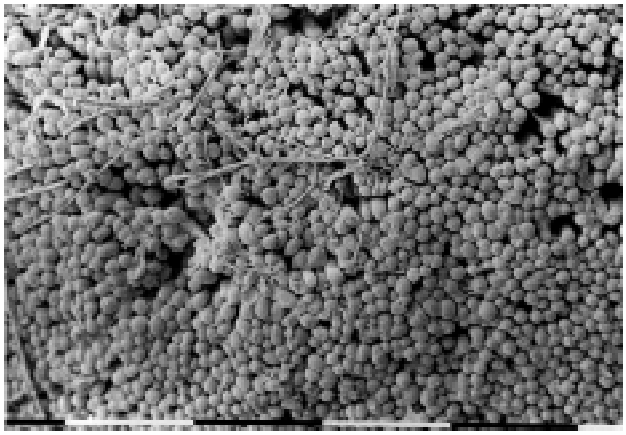
Roots and crown-level stems from *F. culmorum*-infected and healthy plants, were processed for SEM to study the effect of the fungus.

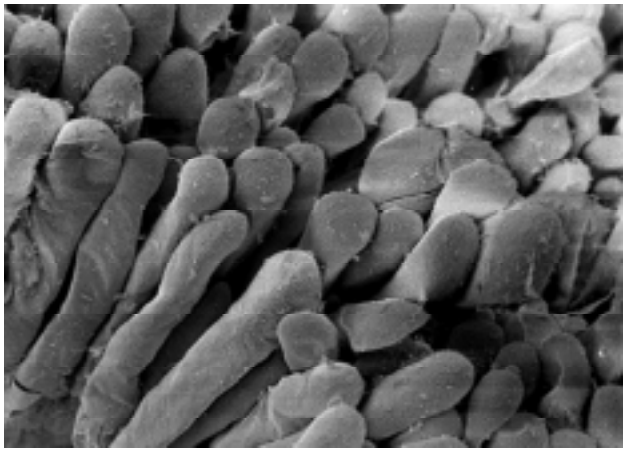
Segments (2-3 mm long) were fixed by immersion in 5% glutaraldehyde in 0.1 M phosphate buffer pH 7.2, dehydrated through a series of aqueous ethanol solutions (10, 30, 50, 75, 95%) and then placed in 100% ethanol for a few min at room temperature. All the fixation and dehydration steps except the last were carried out at 4°C. The specimens were dried in a critical point drier, mounted on aluminium stubs with silver glue, coated with gold-palladium film and observed under a Philips 515 scanning electron microscope at 7Kv.

Results and discussion

Macroscopic examination of 60 wheat seedlings grown in *F. culmorum*-inoculated compost revealed brownish spots at crown level, while the roots did not show any symptoms. Colonies of *F. culmorum* were re-isolated from the infected seedlings plated on PDA, confirming the association symptoms-*F. culmorum*. The crowns and roots of plants grown in non-inoculated compost were symptomless. Clement and Parry (1998) in a study on wheat plants at decimal growth stages 33 (stem elongation), 59 (inflorescence emergence), 77-87 (milk development) and 95 (ripening) recovered the fungus only from stem tissues above soil level. This further demonstrates that the fungus, even though it is soil-borne, may transfer its infection from the crown level to the head, leaving the roots healthy for the entire life cycle of the plant.

The SEM examination of root samples from plants grown in compost contaminated with *F. cul-*





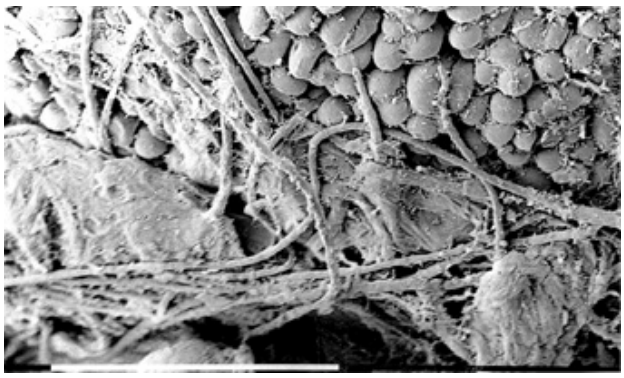


Fig. 1–3. SEM micrographs of stem tissue at crown level of wheat plants at decimal growth stage (GS) 13 grown in *Fusarium culmorum*-infected soil. Fig. 1. Completely exposed parenchyma showing high cell proliferation. Fig. 2. High magnification of loosely arranged parenchymatic cells with wide spaces in between. Fig. 3. Damaged parenchymatic tissue colonised by *F. culmorum* hyphae (bar=0.1 mm).

morum propagules confirmed that at the time of sampling the pathogen did not infect the roots and was localised only at crown level, where pronounced alteration of the host tissue was observed. Here some areas of the parenchyma were now completely exposed due to the absence of any cuticular and epidermal layers, and showed high cell proliferation with irregularly shaped, loosely arranged and widely spaced cells (Fig. 1 and 2). This damage occurred both where there was colonisation by pathogen hyphae (Fig. 3) and where the mycelium was absent. Healthy tissue treated in the same way and at the same time as diseased tissue did not show any damage to the outer cell layers.

Various studies have shown that *F. culmorum* produces the phytotoxic trichothecene toxins: deoxynivalenol, 3- and 15- acetyldeoxynivalenols and nivalenol in infected host tissues, inhibiting protein synthesis (Wong *et al*., 1995). A recent study by Kang and Buchenauer (2000) also found that wheat spikes infected with *F. culmorum* exhibited degeneration of the cytoplasm and organelles, collapse of the parenchyma cells and disintegration or digestion of the cell walls. These authors hypothesised that the damage they recorded was associated with the above phytotoxins.

To our knowledge there are no reports on morphological modifications caused by *F. culmorum* on wheat plants at the seedling growth stage. The data suggest that the alterations observed by SEM on the parenchyma are caused by the pathogen and might be associated with the phytotoxins it produces. Further studies on wheat seedlings are in progress to explore the pathogenic mechanisms that cause changes to the host cells in the presence of *F. culmorum*.

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