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Endophytic association of the pine pathogen *Fusarium circinatum* with corn (*Zea mays*)

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ABSTRACT

Recent work has shown that *Fusarium circinatum*, previously known only as a pathogen of pines (*Pinus*) and Douglas fir (*Picea abies*), can also infect species in the grass family Poaceae. The present study characterized the ability of *F. circinatum* to colonize corn (*Zea mays*), in comparison to closely related *Fusarium* species known to have endophytic associations with *Z. mays*. When inoculum was applied to seed, *F. circinatum* colonized roots, stems and developing ears of *Z. mays*, roots were colonized on plants grown in infested soil, and corn ears became infected when inoculum was applied to husk wounds or through silk channels without wounding. Colonization was restricted to intercellular growth in the cortical and epidermal tissue. No negative effects on growth or emergence were detected. These results constitute the first description of an endophytic association of *F. circinatum* with a grass species and are illustrative of ecological activities that may remain undetected where fungi have no visible impact on their plant host.

Introduction

Research on plant associated fungi has emphasized relationships that cause conspicuous effects on plant growth and productivity. This includes both plant pathogens and mutualists, such as endo- and ectomycorrhizal fungi (Carroll, 1988; Porras-Alfaro and Bayman, 2011; Saikkonen et al., 1998). However, many fungi colonize plants extensively without inducing symptoms (Carroll, 1988; Saikkonen et al., 1998) and, in some cases a fungus that causes disease on one host may be a symptomless colonizer of another (Gordon et al., 1989; Redman et al., 2001; Swett and Gordon, 2012). Infections that cause little or no damage often escape notice, and thus offer a means by which pathogens can be moved into uninfested areas. This mode of dispersal can expose a naïve host to an exotic pathogen and likely explains the establishment of destructive diseases such as chestnut blight, which effectively eliminated the American chestnut (*Castanea dentata*) as a dominant tree species in North America (Anagnostakis, 1987). Consequently, there is value in recognizing the extent to which the host range of a pathogen includes plants that do not show symptoms. This information also contributes to a more comprehensive view of a pathogen's ecological activities, which can help us to better appreciate the relative importance of pathogenesis in fungal life histories.

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It is now generally accepted that most if not all plants are internally colonized by fungi, most of which induce no symptoms (Partida-Martinez and Heil, 2011). The impact of these relationships on the host plant is variable and contingent on environmental conditions (Schulz and Boyle, 2005). Fungi that reside within plants may benefit their host by inducing resistance to disease (Arnold et al., 2003) or enhancing growth (Jumpponen and Trappe, 1998), whereas others are latent pathogens (Carroll, 1988; Stone, 1987). Some endophytic fungi are of concern in agriculture because they produce secondary metabolites known as mycotoxins that pose a threat to human health (Munkvold et al., 1997).

Mycotoxin producing fungi include several species of *Fusarium* that commonly colonize corn (*Zea mays*). These fungi typically induce no symptoms, but may cause disease when plants are stressed or injured (Bacon et al., 2008; Bacon and Hinton, 1996; Bacon and Yates, 2006; Kedera et al., 1992; Yates et al., 1997). Studies conducted by Desjardins et al. (2000) documented that two of the most important mycotoxin producers found in corn, *Fusarium verticillioides* (=*F. moniliforme*) and *F. subglutinans*, were also commensal associates of teosinte (*Z. mays* ssp. *mexicana*), the wild progenitor of corn (Doebley, 1994).

Unexpectedly, an isolate of *F. subglutinans* that was recovered from teosinte in Mexico, proved to be interfertile with *F. circinatum* (Desjardins et al., 2000), the cause of pitch canker, a disease affecting pines (*Pinus*). Although this close relationship was suggestive of recent divergence and thus a similar host range, *F. subglutinans* was found to be nonpathogenic on pines (Friel et al., 2007). On the other hand, *F. circinatum*, previously known only as a pathogen of conifers, was shown to be capable of colonizing corn (Swett and Gordon, 2009) and other grass species (Swett and Gordon, 2012) without inducing any symptoms. These findings call into question the relative importance of pines and grasses in the life history of *F. circinatum*; is this fungus primarily a pathogen of pines with a limited capacity to colonize grasses, or is it principally a commensal associate of grasses that incidentally infects and causes disease on susceptible pines?

Insight into these questions may be gained through a better understanding of the relationship between *F. circinatum* and a grass host. To this end, the present study was undertaken to characterize the capabilities of *F. circinatum* as a colonizer of corn, relative to known associates of this species, *F. subglutinans* and *F. verticillioides*. Colonization was evaluated following exposure to the test fungus through: (1) inoculation of seed; (2) growth in the presence of soilborne inoculum; and (3) inoculation of ears on mature plants. In addition, histological studies were undertaken to visualize patterns of tissue colonization.

Materials and methods

Plant material

All tests used the hybrid sweet white corn variety 'Silver Queen' (Lockhart seeds, Stockton, CA). This variety is reported to be highly susceptible to colonization by endophytic *Fusarium* species (Bacon and Hinton, 1996). Prior to sowing, corn seed (which had not been treated with fungicide) was imbibed for 24 hr in sterile 0.5 % KCl, or a spore suspension, as described below.

Seedling colonization from inoculated seed

Seeds were inoculated by soaking for 24 hr in a suspension of 10^5 or 10^7 spores ml^{-1} of one of the following isolates: *F. circinatum* (isolate GL 17), *F. subglutinans* (isolate GL 52) or *F. verticillioides* (isolate GL 1093). GL 17 is a known pathogen of pines (Aegerter and Gordon, 2006), GL 52 was originally isolated from teosinte in Mexico and is interfertile with *F. circinatum* (Desjardins et al., 2000), and GL 1093 was isolated from a healthy looking corn seedling (Swett and Gordon, unpublished). To prepare inoculum, spores and mycelium of 5–7 d old cultures were suspended in sterile 0.5 % KCl and filtered through two layers of cheesecloth. Spore densities were quantified using a hemocytometer and adjusted to the desired concentrations by addition of sterile 0.5 % KCl. Inoculum was stored at 4 °C and used within 24 hr of preparation. For controls, seeds were soaked in sterile 0.5 % KCl for 24 hr. All seeds were sown at a depth of approximately 2.5 cm in conical pots (6 cm diameter × 25 cm deep) containing Sunshine Professional Growing Mix (SunGro Horticulture Canada Ltd), with one seed per pot. Plants were maintained in a greenhouse wherein temperatures ranged from 27 to 37 °C, irrigated with de-ionized (DI) water as needed, and fertilized weekly with MiracleGro All Purpose Plant Food® at the rate recommended for potted plants (3.7 $g\,l^{-1}$). The experiment was arranged in a randomized complete block design, with five replications of four plants of each treatment. Treatment effects on root and shoot biomass (fresh weight) were evaluated for all four plants in each replication, and colonization was assayed for two plants per replication, as described below. The full experiment was conducted twice.

Emergence was monitored weekly, and fresh weight and extent of colonization were evaluated 21 d after planting. Plants were harvested by replication over a 5 d period, with all plants in a replication harvested and processed on the same day. Roots were placed under running water to remove potting mix and blotted dry with paper towels. The root system and stem of each plant were weighed separately. The primary (tap) root and the de-leafed stem were washed in 0.1 % Tween 20. The primary root was briefly dipped in 70 % ETOH and placed for 10 s in 1.0 % NaClO, whereas the stem was immersed for 30 s in 70 % ETOH followed by 1 min in 1.0 % NaClO. Tissue was cut into 1 cm sections, placed on a *Fusarium* selective medium (FSM) (15.0 g peptone, 20.0 g agar, 1.0 g KH_2PO_4, 0.5 g $MgSO_47H_2O$, 0.2 g PCNB and 0.3 g of streptomycin sulfate in 1 l DI water) as described by Aegerter and Gordon (2006) and incubated at 24–26 °C under ambient lighting. Each tissue segment was scored as positive or negative for emergence of the isolate with which seeds were inoculated. Cultures were identified as *F. subglutinans*, *F. circinatum* or *F. verticillioides* based on colony morphology on FSM, and when necessary, microscopic characteristics on 0.6 % KCL, using the criteria described by Leslie et al. (2006). Colonization incidence was quantified as the percentage of plants for which at least one tissue segment (shoot or root) was colonized. Conditional intensity of colonization was quantified as the proportion of