# Colonization of maize roots by *Colletotrichum graminicola* leads to symptomless systemic colonization of above ground plant tissue

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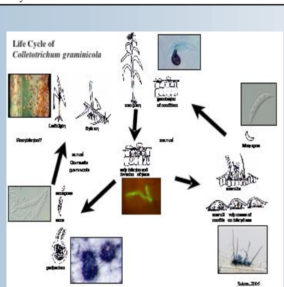
## Summary

*Colletotrichum graminicola* (Ces.) G. W. Wils. causes anthracnose stalk rot, top die-back and leaf blight of corn. The pathogen survives in residue on the soil surface, in buried residue, and as stromata on the soil (Bailey *et al*. 1992; Stack, 2003). Infected plant residue on the soil surface has always been considered the most important source of inoculum. Little is known about its role as a root pathogen though it has been suggested that *C. graminicola* can infect corn roots in the field (Bergstrom and Nicholson, 1992). Recent literature suggests that many fungi that are commonly regarded as causal agents of foliar diseases can also cause systemic infection of their hosts by invading roots. We are investigating the importance of root infections of *C. graminicola* on the anthracnose disease cycle. Corn seeds were grown in vermiculite that had been inoculated with mycelial agar plugs of *C. graminicola* isolate M1.001BH. Seedling roots were collected at various time points, washed, sectioned, and visualized with light and fluorescent microscopy. Inoculations of 3 day old roots were also done *in vitro* with agar plugs or spore suspensions of a M1.001-GFP strain of *C.graminicola*. Lesions on the roots were not observed, however fungal hyphae could be found colonizing the surface of the roots and invading epidermis, cortex and vascular tissues. Structures, typically formed by root pathogens but not previously reported for *C. graminicola* on roots, including runner hyphae, hyphopodia and microsclerotia were observed. Certain epidermal and cortical cells become infected from intercellular hyphae while surrounding cells are uninfected, resulting in a mosaic pattern of infection. Interestingly, conidia were formed in acervuli on the root surfaces but were also found filling epidermal cells and root hairs. The microsclerotia produced in culture were able to infect plant roots, demonstrating that the structures were viable and could serve as a source of inoculum. Twenty-eight percent of plants challenged with soil-borne inoculum became infected in above ground plant parts (stem and/or leaves) indicating that root infection can lead to asymptomatic systemic colonization of the plants. These observations suggest that root infection may be an important component of the maize anthracnose disease cycle.




## Methods

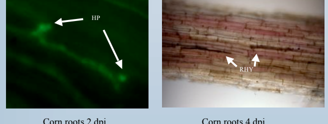
**Construction of a GFP-containing strain of *C. graminicola***

- A GFP transformant of *C. graminicola* was made by transforming strain M1.001BH with plasmid gGFP. The GFP plasmid vector (gGFP) contains the *A. nidulans Pgdp* promoter and contains the *hph* gene for resistance to hygromycin B. (Maor et al., 1998).
- Random transformants were assayed for GFP activity and pathogenicity. A single isolate that shows strong fluorescence and caused wild-type disease development was selected for further experiments.

**Pathogenicity Assays and Microscopy**

- Maize inbred line B73 was used for pathogenicity assays.
- *C. graminicola* strain M1.001BH or the GFP strain described above were used for pathogenicity assays.
- Agar plugs were mixed with autoclaved vermiculite and placed in 50 mL centrifuge tubes (Sesma and Osbourn, 2004). Seeds were surface sterilized, treated with the fungicide trifloxystrobin (commercial name Flint 50), placed in the tubes and incubated under fluorescent lights.
- Time points studies to study the progress of colonization were done *in vitro* with agar plugs or spore suspensions of a M1.001-GFP *C.graminicola*.
- Roots infected with the GFP strain were visualized with the appropriate light source and filters.

## Runner hyphae form on root surface

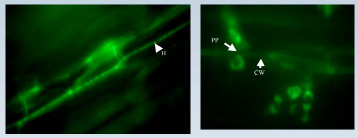



Corn roots 2 dpi. Corn roots 4 dpi.

Viewed with fluorescent and bright field microscopy.

- Thick melanized hyphae or runner hyphae (RHY) extensively colonize the root surface.
- Runner hyphae grow parallel to the root surface within the epidermal layer.
- The hyphae form some lateral swellings resembling hyphopodia (HP) at the junction between cells. No appressoria, typical foliar infection structures, were observed on the root surface when spores or vegetative hyphae were used as inoculum.

## Inter and Intracellular colonization

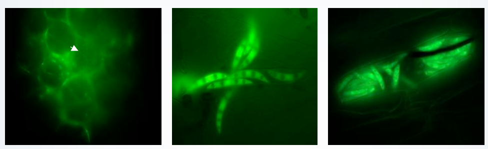



Corn roots 3 dpi using a GFP-tagged strain of *C. graminicola*.


Corn roots 10 dpi..

- *C. graminicola* hyphae (H) colonize intercellular spaces of epidermal roots cells.
- Hyphae swell and spread from cell to cell through specific points of contact with other cells. Note the constriction of the hyphae when crossing through the penetration pore (PP) on the cell wall (CW).
- Fungal hyphae were able to colonize large areas of the root cortex and vascular bundle.
- Cells become infected from intercellular hyphae (IH) in a mosaic pattern.
- Infected epidermal and cortical cells become packed with hyphae. Surrounding cells may not be infected.

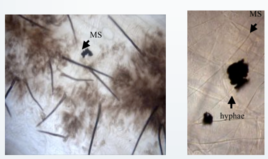
## Root hairs become filled with falcate conidia Root cells become filled with falcate and oval conidia



Corn root mesophyll cells 20 dpi. Corn root hairs cells 20 dpi. Corn root epidermal cells 20 dpi.

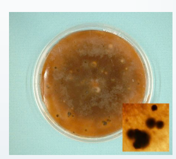
- At 10 dpi, some corn root hairs can been seen filled with falcate conidia.
- Some conidia can be observed germinating within the root hairs (not shown).
- At 20 dpi some root cells are filled with falcate conidia or oval conidia (arrow).

## Formation of acervuli and microsclerotia




Acervuli and microclerotia (MS).

Maize roots 8dpi viewed with bright field microscopy.



Microsclerotia (MS) produced *in vitro*

- Acervuli with visible setae can be found on root surface within 4 dpi.
- Microsclerotia (MS) tend to be formed near acervuli.
- MS produced in culture were able to infect plant roots (not shown)

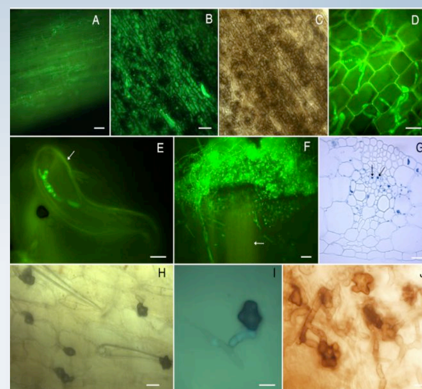
## Incidence of *C. graminicola* isolation from maize plants 8 weeks after infection

| Maize Inbred Line | No. of plants tested | Number (and percentage) of plants from which hygromycin resistant *C. gramincola* was recovered | | | |
|---|---|---|---|---|---|
| | | Roots | Stem | Leaves | Aerial Tissues (Stem or Leaves[1]) |
| B73 | 15 | 15 (100%) | 6 (40%) | 3 (20%) | 6 (40%) |
| Mo940 | 12 | 12 (100%) | 3 (25%) | 2 (16.6%) | 4 (33.3%) |
| H99 | 19 | 18 (94.7%) | 1 (5.2%) | 2 (10.5%) | 3 (15.8%) |
| Total | 46 | 45 (97.8%) | 10 (21.7%) | 7 (15.2%) | 13 (28.3%) |

[1]Plants with infected stem and leaves were only counted once.

•Twenty-eight percent of plants challenged with soil-borne inoculum became infected in their above ground plant parts

## Systemic colonization from infected roots to aerial plant parts




- Plants were from maize seeds grown in a sterile vermiculite system artificial inoculated with agar plugs containing vegetative mycelium of *C. graminicola* expressing GFP.
- (A) Adventitious root of maize systemically colonized by *C. graminicola,* 28 dpi.
- (B, C) Senescent coleoptile colonized by *C. graminicola*, 28 dpi. Viewed with fluorescent and bright field illumination. Some spores can be seen on the coleoptile surface.
- (D) Colonization of the 3rd leaf sheath from a root-infected plant, 28 dpi.
- (E) Hyphae growing inside of a leaf trichome (arrow).
- (F) *C. graminicola* growing from individual vascular bundles (arrow) of a stem cut in transverse section and cultured on isolation medium.
- (G) 5 µm cross section of a maize leaf from a root inoculated plant, 28 dpi. Hyphae are indicated by arrows.
- (H) Lobed hyphopodia on an infected, senescent leaf sheath, 28 dpi.
- (I) Hyphopodia begin to accumulate melanin though GFP fluorescence within the hyphae is still observed in the hypha leading to the hyphopodium.
- (J) Lobed hyphopodia produced in liquid culture.
- A, F, scale bar = 100 µm. B, C, G, scale bar = 20 µm. D, E, H, I, J, scale bar = 10 µm.

## Conclusions

- *C. graminicola* can infect root tissue. Lesions on the roots were not observed in this study, however fungal hyphae could be found colonizing the surface of the roots and invading epidermis, cortex and vascular tissues.
- Structures not previously reported for *C. graminicola* on roots, including runner hyphae, hyphopodia and microsclerotia were observed. These structures are commonly reported for root pathogens.
- Conidia were formed in acervuli on the root surfaces but were also found filling epidermal cells and root hairs. These observations suggest that root infection may be an important component of the corn anthracnose disease cycle.
- Twenty-eight percent of plants challenged with soil-borne inoculum became infected in above ground plant parts (stem and/or leaves) indicating that root infection can lead to asymptomatic systemic colonization of the plants.
- Many of the traits observed in *C. graminicola* have been previously reported in other root-pathogenic fungi, suggesting that these traits are evolutionally conserved in multiple fungal lineages. These observations suggest that root infection may be an important component of the maize anthracnose disease cycle.
- Soil borne inoculum could be a means for pathogen dissemination that has not been previously considered in the life cycle of *C. graminicola.* This study can help to improve our understanding of the maize anthracnose life cycle, the biology of *C. graminicola* and its interactions with its host.
- The root infection cycle may represent a prolonged biotrophic interaction and may be useful for the study of biotrophic pathogens.

## References

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