Microscopic interactions between butternut trees and the butternut canker fungus

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Abstract

Butternut canker is a lethal disease of butternut (*Juglans cinerea*) trees caused by the fungus *Ophiognomonia clavigignenti-juglandacearum* (*Oc-j*). The disease has decimated populations of the species in many areas and is one of the main impediments to maintaining *J. cinerea* as a component of hardwood forests throughout its native range. Small elliptical cankers form at wound sites and natural openings on all woody tissues. Coalescence of multiple cankers kills branches and trees of all ages. In addition to complications from disease, *J. cinerea* readily hybridizes with non-native Japanese walnut (*J. ailantifolia*). Recently, *Oc-j* was found to cause leaf lesions on butternut and its hybrid. Prior research on the histological interactions between the fungus and host focused solely on canker progression within stem tissue. Little is known about surface interactions between the primary inoculum in the pathosystem (conidia) and leaf and stem tissues. In this study, detached leaflets and stem sections in moisture chambers were inoculated with deionized water suspensions of *Oc-j* conidia and interactions were analyzed by scanning electron microscopy. We report evidence that spore germination on plant surfaces readily occurs. Subsequent hyphal growth is apparently haphazard and no active penetration structures were observed. We further report the likely mode of infection through the surface of non-wounded leaflets, as well as infection development within foliar tissue.

Inoculation Methodology and Sample Collection

- Spore suspensions produced and adjusted between 10^5-10^6 spores/µl.
- For leaf tissue
  - Fully expanded, disease-free leaflets were collected from available trees and placed adaxial side down in a moist chamber.
  - 20µl droplet of spore suspension placed on abaxial surface without wounding.
- For stem tissue
  - Stem sections were collected from 12 week-old seedlings, split in half tangentially and placed in a moist chamber xylem side down.
  - 40µl of spore suspension placed on surface of outer bark.
- Material was sampled at varied time points from 24-96 hours post inoculation.

Conclusions and Future Directions

- *Oc-j* colonizes foliar tissue and grows readily on tissues in vitro.
- No direct penetration structures observed on foliar or stem tissues.
- Possible sites of infection identified.
- No observed preferential growth toward natural openings.
- Further research will confirm primary infection courts and colonization patterns.

Preparation of Plant Material

- For SEM
  - Leaf and stem samples were immediately fixed in 2.5% buffered glutaraldehyde and dehydrated through a graded ethanol (EtOH) series to 100% EtOH before a final wash with Hexamethyldisilazane (HMDS) as a substitute for critical point drying.
  - Leaf and stem samples were coated with gold/palladium and images were collected under high vacuum.
- For light microscopy
  - Individual leaflets collected from 14 week-old seedlings grown under greenhouse conditions were inoculated and sampled after lesion formation
  - Samples were immediately fixed in formalin-acetic acid-alcohol (FAA) before being dehydrated in a graded Tert-Butyl/Ethyl alcohol series and embedded in paraffin wax before sectioning and staining.

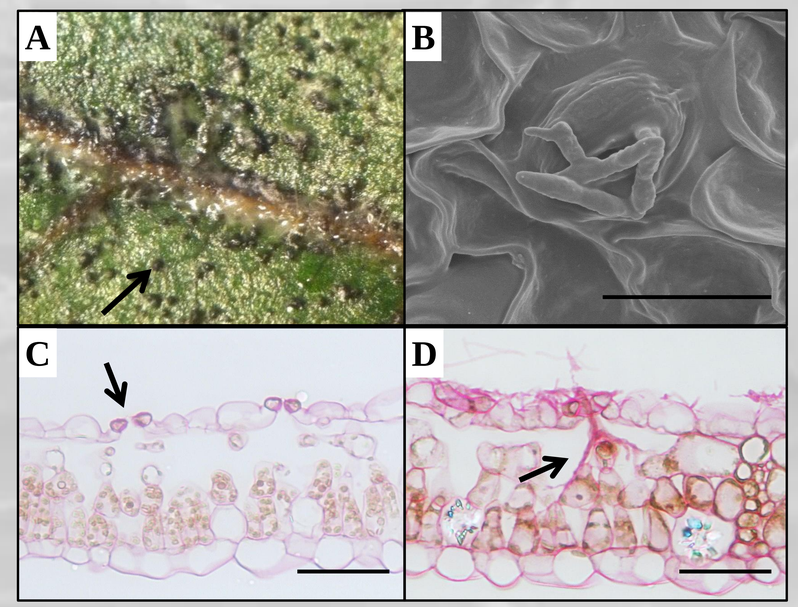

Figure 1 – Colonization of foliar tissues. A) Active sporulation of *Oc-j* when leaf tissue is colonized in vitro, arrow pointing to mature pycnidia. B) Spore germination on the surface of a butternut leaf directly over stomata. C) Open stomata in cross section. D) Inoculated leaflet with *Oc-j* growing through open stomata. Scale Bars = 20µm on image B; 50µm on images C and D

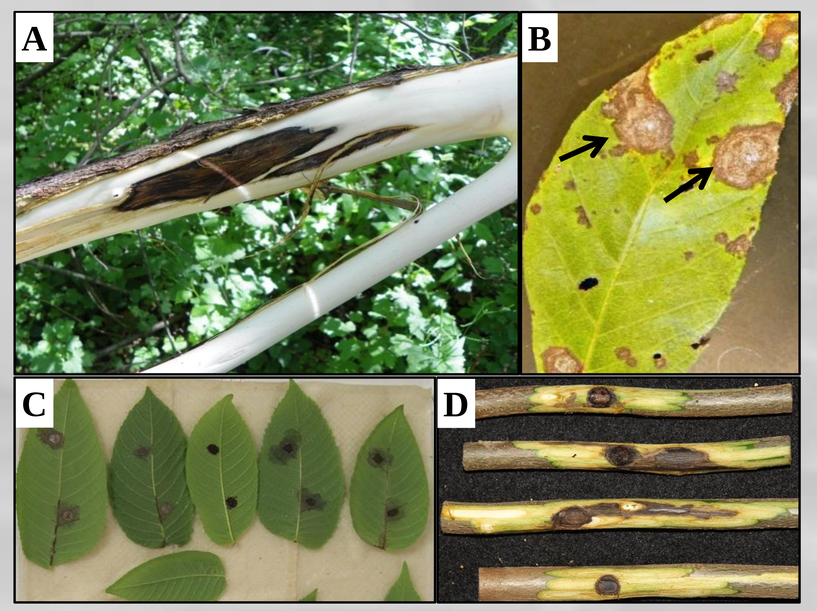

Figure 2 – Disease progression in various butternut tissues both natural and inoculated. A) Natural butternut canker after removal of bark tissue. B) Natural leaf spot that produced a pure culture of *Oc-j* after surface sterilization. C) Leaf lesions produced in vitro from spore suspension inoculation without wounding. D) Cankers produced by artificial inoculation of butternut stem tissue.

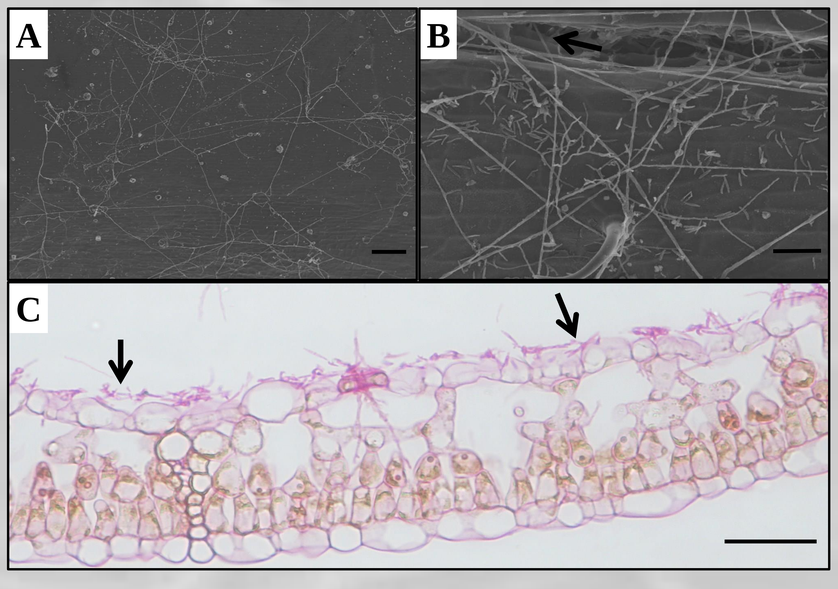

Figure 3 – Surface colonization of butternut tissue. A) *Oc-j* stem tissue (with bark) is colonized in vitro; mycelium spreading over tissue can be traced back to germinating *Oc-j* conidia. B) Spore germination and mycelial growth on outer bark surface near crack, arrow pointing to hyphal strand growing into crack. C) Inoculated leaflet in cross section, arrows indicating extent of surface mycelium growth after inoculation with spore suspension. Scale bars = 50µm for B and C; 200µm for A

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For more information about butternut research visit:
http://www.agriculture.purdue.edu/fnr/HTIRC/woeste.html