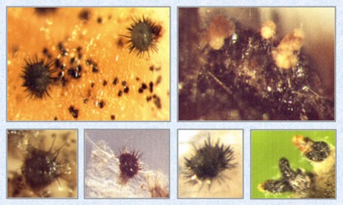

Identification and Pathogenic Analysis of *Colletotrichum* Species Causing Soybean Anthracnose

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Introduction

Soybean anthracnose seriously affects agricultural economics especially in wet, warm and humid areas. The most common pathogen that causes soybean anthracnose is the fungus *Colletotrichum truncatum*. Several other *Colletotrichum* species have also been reported, including *C. coccodes*, *C. destructivum* (teleomorph, *Glomerella glycines*), *C. gloeosporioides* (teleomorph, *G. cingulata*), and *C. graminicola* (teleomorph, *G. graminicola*).

Objectives

1. To isolate *Colletotrichum* species from soybean plants in the United States and identify species by morphological examination and molecular analysis.
2. To evaluate pathogenicity of the *Colletotrichum* isolates on the susceptible soybean cultivar Williams 82.

Methods

1. Isolation of *Colletotrichum* fungi: Infected petioles were surfaced sterilized, cultured in water agar until fungi developed. Pure cultures were obtained by single spore or hyphal tip isolation and kept in APDA plates.

2. Morphological identification: Isolates were initially classified by colors and features in the APDA plates, the formation of acervuli or perithecia, and their spore shapes and sizes.

3. Molecular analysis: PCRs were performed by amplifying ITS region and partial mitochondrial cox1 gene. Phylogenetic trees were constructed by MEGA 5 using maximum likelihood with 500 bootstrap replicates.

Primers	Sequences
ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
ITS4	5'-TCCTCCGCTTATTGATATGC-3'
coxu1	5'-ACAAATGCTAAAGATATAGG-3'
coxr1	5'-GTATTAAAGTTTCTATCTGTT-3'

4. Pathogenicity analysis: Mycelial plugs were used to inoculate detached leaves of the soybean cultivar Williams 82. Disease severity was assessed 14 days post inoculation.

Acknowledgements

1. NRES Excellence in Research Fellowship, University of Illinois
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Results

Table 1. Summary of 25 *Colletotrichum* isolates collected in Illinois

Isolate Name	Location	Year	Conidial Shape	Perithecia	Pathogenecity Rating (Fig.3)
IL-CFAR-1A	Urbana, IL	2009	curved	X	1
IL-CFAR-2A	Urbana, IL	2009	curved	X	2-3
IL-GREIN-3A	Urbana, IL	2009	curved	X	1
IL-MON-4A	Monmouth, IL	2009	curved	X	1
IL-MON-6A	Monmouth, IL	2009	curved	X	1
IL-MON-7A	Monmouth, IL	2009	curved	X	1
IL-MON-8A	Monmouth, IL	2009	curved	X	1
IL-CFAR-9A	Urbana, IL	2009	curved	X	1
IL-MON-10A	Monmouth, IL	2009	curved	X	1
IL-MON-11A	Monmouth, IL	2009	curved	X	1
IL-MON-12A	Monmouth, IL	2009	curved	X	1-2
IL-CFAR-13A	Urbana, IL	2009	curved	X	1
IL-MON-14A	Monmouth, IL	2009	curved	X	1
IL-CFAR-15B	Urbana, IL	2009	curved	X	4
IL-MON-16D	Monmouth, IL	2009	curved	X	4
IL-MON-18A	Monmouth, IL	2009	straight	√	0
IL-MON-19A	Monmouth, IL	2009	straight	√	0
IL-MON-20A	Monmouth, IL	2009	straight	√	0-1
IL-MON-21A	Monmouth, IL	2009	straight	√	0
IL-MON-22A	Monmouth, IL	2009	straight	√	2-3
IL-MON-23A	Monmouth, IL	2009	straight	√	0-1
IL-MON-24A	Monmouth, IL	2009	straight	√	0-1
IL-MON-25A	Monmouth, IL	2009	straight	√	0-1
IL-CFAR-26A	Urbana, IL	2009	straight	√	0-1
IL-MON-27B	Monmouth, IL	2009	straight	√	0-1

Figure 2. Phylogenetic analysis of *Colletotrichum* isolates using ITS region and cox1 gene

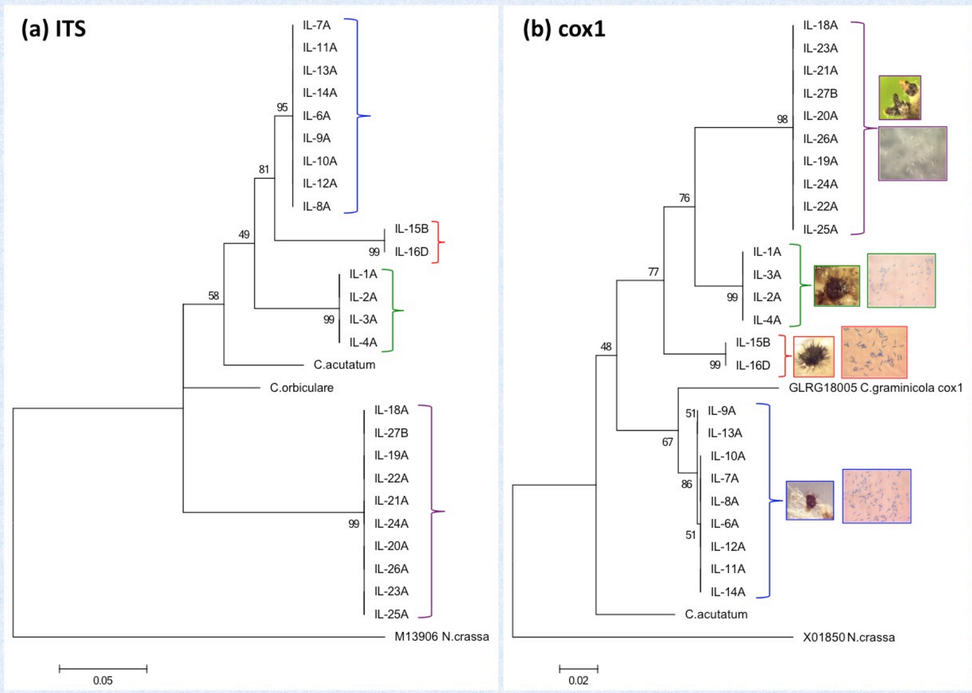

Figure 1. Diversity of fungal cultures and spore sizes and shapes

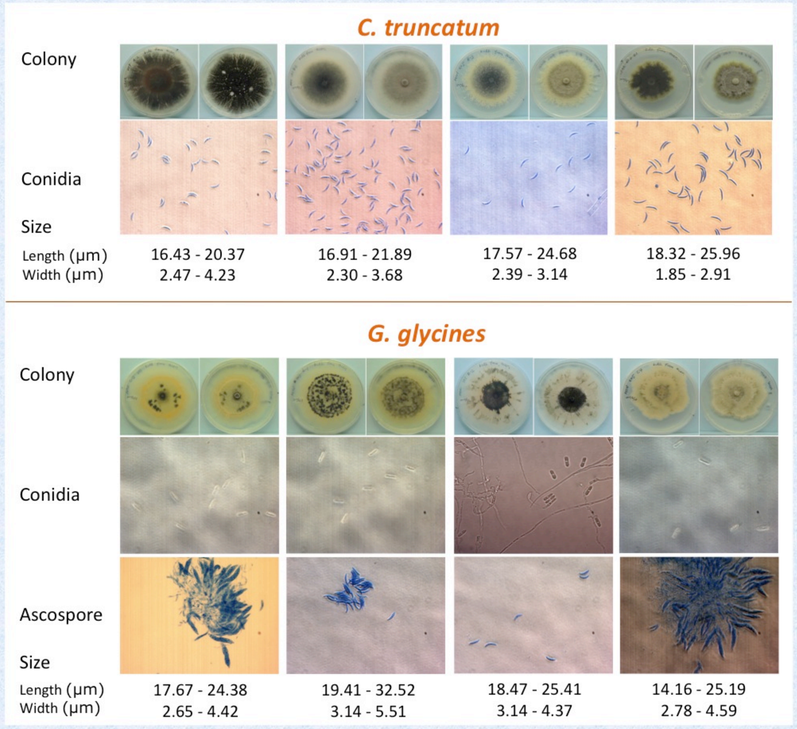

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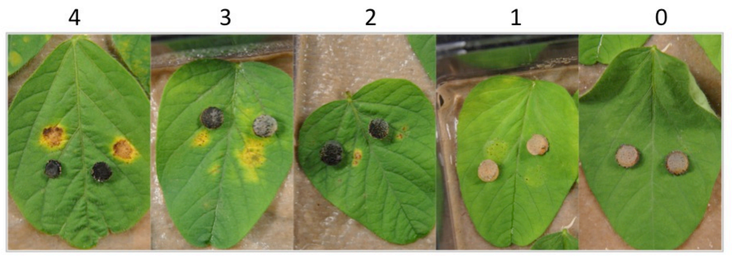
Figure 3. Pathogenicity test



The lesion scale used to assess the disease severity
Lesion size (severity) : 4 > 3 > 2 > 1; 0 = no lesion

Conclusions

1. More than 80 *Colletotrichum* isolates from infected soybean petioles were collected. Among them, 57 were curved-spored types and the rest were straight-spored types.
2. 25 *Colletotrichum* pure isolates were further analyzed their spore sizes and the formation of perithecia. The curved-spored type without perithecia formation were identified as *C. truncatum*. The straight-spored type with perithecia containing ascospores were *G. glycines.*
3. *Colletotrichum* spp. associated with soybean anthracnose could classified into 4 major genotypes by multi-gene phylogenetic analysis. The classification could match to the morphological observation.
4. There were differences in isolates in their capacity to produce symptoms on soybean. In general, curved-spored *Colletotrichum* caused more severe symptoms on soybean leaves than straight-spored *Colletotrichum*.