


## Improvement in screening for resistance to *Sclerotinia sclerotiorum* in common bean through characterization of the pathogen

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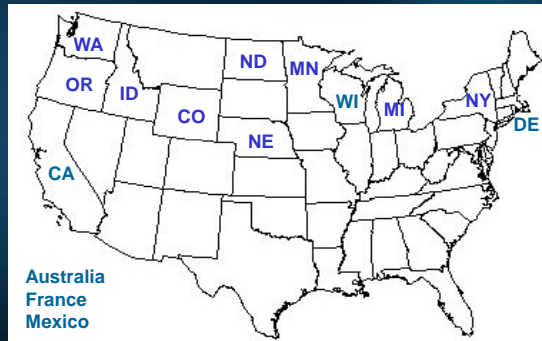


## Purpose of research

- Assess the variation in common bean isolates of *Sclerotinia sclerotiorum* from major bean production areas.
  - Collect isolates from major bean production areas
  - Test for genetic diversity with mycelial compatibility groups (MCG's)
  - Test aggressiveness of isolates
  - Characterize isolates using Kohn microsatellite markers

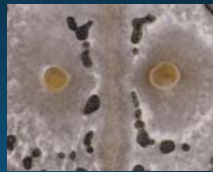
## Isolates Background

- *Sclerotinia sclerotium* isolates (279)
  - First collected isolates used in screening bean lines in greenhouse tests from 9 main bean production areas (2003)
  - Next collected isolates from bean nurseries in the same areas (2003-2005)
  - Now collecting from bean production fields ( 2007, 2008, hopefully 2009)

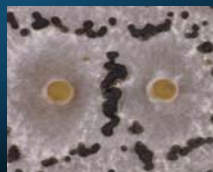


## Mycelial Compatibility Groups

- Previously reported:
  - 156 isolates collected in 2003, 2004 and 2005
  - 65 MCG formed



37 single isolate MCG



7 MCG with isolates from different locations

21 MCG with isolates from same location

## MCGs from isolates collected in 2007 from producer fields in 4 locations

Location	# Isolates	Total MCG	Clonal MCG's *	Unique MCG's
ND	21	11	8 isolates = 2 MCGs	13 isolates = 9 MCGs
NE	10	4	9 isolates = 3 MCGs	= 1 MCG
CO	20	6	18 isolates = 4 MCGs	= 2 MCGs
WA	22	13	8 isolates = 3 MCGs	14 isolates = 10 MCGs

\* Formed MCG with characterized isolates from same location

## Test for Aggressiveness

- **Straw test utilized – more sensitive**
  - Inoculate bean plants 21 days after planting



-Inoculate 4<sup>th</sup> node with plugs of mycelia grown for 2 days on PDA plates

-Disease progression rated 8 days after inoculation

-Modified Petzoldt and Dickson scale (1-9) (Teran et al, 2006)

## Aggressiveness Results

- Straw test conducted on same 156 isolates
  - Highly significant differences in aggressiveness were found between MCGs of *Sclerotinia sclerotiorum*
  - Isolates within the MCGs did not differ in aggressiveness from each other whether one host or multiple hosts were used
  - This data supports the hypothesis that the differences in *S. sclerotiorum* aggressiveness are due to the clonal group, not the individual isolate

## Aggressiveness Results - isolates collected in 2007 from producer fields in 4 locations

Location	Sig. differences between isolates	Sig. differences between MCG aggressiveness*	Sig. differences among isolates within MCG
North Dakota	Yes, p=0.0040	Yes, p=0.0034	No, p=0.1003
Nebraska	Yes, p=0.0027	Yes, p<0.0001	No, p=0.5319
Colorado	Yes, p<0.001	Yes, p=0.0002	No, p=0.5299
Washington	No, p=0.1156	No, p=0.0623	No, p=0.5424

\* Would like to get more isolates from ND, NE, and CO

## Isolate molecular characterization

- 2001 – Sirjusingh and Kohn published set of 25 primers for amplifying products containing microsatellites
- Z.K. Atallah et al 2004 reported 11 of the 25 to be polymorphic with isolates from potato
- Selected same 11 microsatellite loci to further investigate molecular characterization of collected isolates



## Isolate DNA

- DNA from each isolate extracted from sclerotia initiated mycelia
  - Used fungal DNA mini-prep from Jeff Rollins
- Isolates varied in polysaccharide content
  - Select young mycelia mats in PD broth
  - Lyophilize tissue
  - Added 2M NaCl clean up step



## Greenhouse Screening Isolates

- Selected 9 greenhouse isolates to test 11 microsatellite primers
- Sequenced forward and reverse – most products between 150-570 bp
- Analyzed using Phylogeny.fr Robust Phylogenetic Analysis For The Non-Specialist

ID	Microsatellite	Alleles
5-2	(GT) <sub>8</sub>	2
7-2	(GA) <sub>14</sub>	2
12-2	(CA) <sub>9</sub>	3
13-2*	(GTGGT) <sub>6</sub>	3
7-3	(GT) <sub>10</sub>	2
17-3*	(TTA) <sub>19</sub>	3
55-4	(TACA) <sub>10</sub>	4
92-4	(CT) <sub>12</sub>	4
106-4	(CATA) <sub>25</sub>	4
110-4	(TATG) <sub>9</sub>	1
114-4	(AGAT) <sub>14</sub> (AAGC) <sub>4</sub>	4 & 1

\*microsatellite too close to primers = poor sequence

## Examples of Un-rooted Phylogenetic Trees\*

Primer 7-2

Primer 92-4

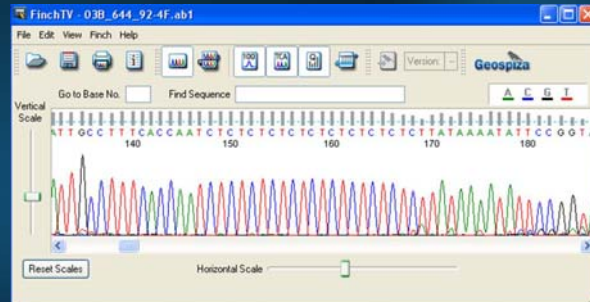
Primer 106-4

Primer 114-4

\* Trees created using TreeDyn(v198.3).

## Nebraska isolates from 2 locations and 3 years - primer 92-4 (CT)<sub>12</sub>

- Sequenced PCR product from 27 isolates (2004-2007)
  - (CT)<sub>11</sub> = all have the same allele



→ (CT)<sub>12</sub> = greenhouse screening isolate (2003)

## Allele results from 7-2 & 92-4 primers...

- Primer 92-4  
46 isolates from  
5 locations  
Alleles =  
(CT)<sub>11, 12, 13</sub>

Location	# isolates	# alleles
Delaware	3	2
Minnesota	10	2
Michigan	10	3
Oregon	7	2
Washington	16	3

- Primer 7-2  
91 isolates from 9 locations = 5 alleles - (GA)<sub>14</sub> and (GA)<sub>13</sub>  
most common forms
- Continuing to sequence 156+ isolates with primer 7-2, 92-4, 106-4 and 110-4

Thank you for your attention.

