Resistance Improvement of Bean Through Multi-site Screening and Pathogen Characterization

J. R. Steadman, L. K. Otto-Hanson and K. Powers (University of Nebraska-Lincoln). Disease reaction data supplied by: P. Griffiths (New York), H. Schwartz (Colorado), P. Miklas (Washington), K. Kmiecik (Wisconsin), K. Grafton (North Dakota), J. Kelly (Michigan), J. Myers (Oregon), S. Singh (Idaho), R. Mainz (Minnesota), and C. Kurowski (California).

Introduction:

No complete resistance to *Sclerotinia sclerotiorum,* cause of white mold, has been found in common bean, *Phaseolus vulgaris*. The development of bean cultivars with partial physiological resistance and architectural avoidance to white mold would reduce disease losses and require no input costs to growers. At present, putative sources of resistance have shown variable responses in different sites. To find the most consistent lines with resistance, putative resistant lines were tested at multiple sites located in most of the major bean production areas in the USA. In addition to effects of different locations on variation of the disease reaction, *S. sclerotiorum* may vary in pathogenicity from site to site. Thus, pathogen isolates were collected from collaborators from various locations within US bean production areas. Pathogen variation was determined by mycelial compatibility groupings (MCGs), test of aggressiveness measured by the detached leaf test (DLT), and molecular genotyping.

Goal One:
Identify sources of resistance to Sclerotinia sclerotiorum in adapted common bean lines.

Goal Two:
Assess the variation in aggressiveness, mycelial compatibility groupings (MCGs) and intraspecific genetic variation.

Materials and Methods:

Sources of partial resistance incorporated into bean breeding lines were evaluated by greenhouse and field screening methods in 11 different US locations. Field tests consisted of two rows of each entry and a common susceptible bean line/cultivar, resulting in a three-row plot 4.6 m (15 ft.) long replicated three times in a randomized complete block design. The disease severity rating was determined by the percent of above ground plant canopy with white mold symptoms/signs. A visual rating was recorded for each entry. Each collaborator was asked to collect two sclerotia of *S. sclerotiorum* from a plant in each of the three reps of each of the following three entries: Ex Rico 23 (Bunsi), G122, and Beryl and send them to UNL.

The greenhouse tests utilized in this study were the straw (Petzoldt and Dickson, 1996), the oxalate (Kolkman and Kelly, 2000), and the detached leaf (Steadman et al, 1997). Each collaborator used their normal protocols for screening in the lab or greenhouse. Results of the screening tests were sent to UNL for analysis. Five sclerotia of each *S. sclerotiorum* isolate used for screening in each lab were also sent to UNL.

Results of the field and greenhouse/lab screening for 2003

Table 1. Comparison of rankings of 12 bean lines for white mold reaction at nine locations

Cultivar	Field						Greenhouse/Laboratory					
	ND	WA	MN	OR	CA	MI	WA	NY	MI	CO[1]	CO[2]	NE
N02 302	3	3	5	3	5	2	13	12	10	6	9	8
G122	4	1	6	2	3	5	3	4	3	2	7	5
Cornell 501	2	4	4	1	11	3	6	2	5	6	4	11
AN 37	7	2	7	4	7	1	4	9	7	6	3	4
Cornell 601	1	6	8	10	4	4	5	1	6	1	2	1
Bunsi	8	7	1	6	1	7	8.5	5	11	4	10.5	6
IO1892-115M	6	8	3	11	6	8	10	6	2	10.5	5.5	10
Dwarf Bees	-	-	-	-	-	-	1	7	1	8	1	-
AN 69	10	10	9	9	8	10	2	11	4	9	5.5	3
CO75944	9	11	11	7	9	-	7	-	-	-	-	-
USWA 6	5	5	10	5	10	6	11	8	9	3	8	9
AN 1	11	9	2	8	2	9	12	3	12	10.5	10.5	7
Beryl	12	12	12	12	12	11	8.5	10	8	12	12	2

(-) = No Data
[1]Most resistant (1) to most susceptible (12).
[1]Colorado oxalate test and [2]Colorado straw test

Table 2. Mean ranking of bean lines (1=most resistant) for white mold reaction in 12 separate greenhouse/lab and field tests in 2003

Entry	Mean Ranking	T Grouping				Seed Class
Beryl	10.292			A		GN
CO75944	9.000	B		A		PTO
AN 1	8.042	B		A	C	GN
AN 69	7.542	B	D	A	C	PTO
USWA 6	7.417	B	D		C	SR
IO1892-115M	7.167	B	D		C	BLK
N02 302	6.583	B	D	E	C	NAVY
Bunsi	6.208	F	D	E		NAVY
AN 37	5.083	F	D	E		PTO
Cornell 501	4.917	F	D	E		SNAP
G122	4.167	F		E		CRAN
Cornell 601	4.083	F		E		RK
Dwarf Bees	3.6	F				*P. coccineus*

LSD=2.7514
Alpha=0.05

In 2003, 12 separate screening tests, six field and six greenhouse, were rated on a scale ranging from most resistant (1) to most susceptible (12). Spearman and Pearson correlations were used to compare entry rankings in each test. Each test included 11 common bean lines, the *P. coccineus,* cultivar Dwarf Bees, was added for greenhouse screening, and CO75944 replaced Dwarf Bees for field screening. From the field and greenhouse screening in 2003 the following partially resistant lines/sources were identified: Dwarf Bees, Cornell 601, G122, Cornell 501, AN 37, and Ex Rico (Bunsi).

Molecular characterization of greenhouse/lab isolates

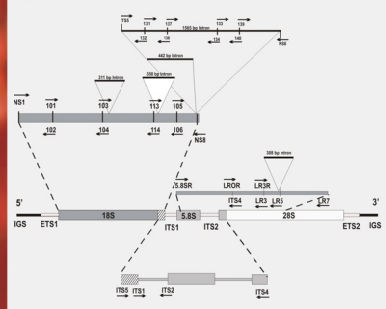

Fig. 1 Map of the nuclear ribosomal RNA gene repeat of *Sclerotinia* showing location of introns, primers and sequenced regions.

Analysis of the nrDNA used as an interspecific diagnostic tool. PCR amplification of the nuclear ribosomal repeat (nrDNA) reveals that fragment length polymorphism due to the presence or absence of introns within the small subunit (SSU) and large subunit (LSU) of the nrDNA can be used as an interspecific diagnostic tool (Figure 1). We also found that intraspecific fragment length polymorphism of the nrDNA repeat due to SSU or LSU introns occurs in *S. trifoliorum* but not in *S. sclerotiorum*.

Microsatellite primers find presence of multiple alleles. The DNA of the nine greenhouse/lab *S. sclerotiorum* isolates was amplified using the primers specific to microsatellite-containing loci (Sirjusingh and Kohn, 2001). Figure 2 illustrates the presence of multiple alleles, and thus a significant level of intraspecific polymorphism in *S. sclerotiorum* isolates from common bean.

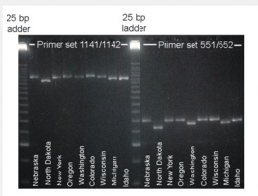

Figure 2. Presence of multiple alleles in the nine greenhouse/lab isolate population, amplified by primer set 1141/1142 and 551/552 and separated on a high resolution metaphor agarose gel.

References:
Kolkman, J.M. And Kelly, J.D. 2000. Crop Sci. 40: 281-285.
Petzoldt, R. and Dickson, M.H. 1996. Ann. Rep. Bean Improv. Coop. 39: 142-143.
Sirjusingh, C. and Kohn, L.M. 2001. Mol. Ecol. 1: 267-269.
Steadman, J.R., Powers, K. and B. Higgins. 1997. Ann. Rep. Bean Improv. Coop. 40: 140-141.

Nine greenhouse/lab isolates compared for aggressiveness using the DLT

Table 3. Mean lesion size of nine *S. sclerotiorum* isolates on bean line G122

Isolate-Source	Mean Lesion Size	t Grouping		
JRS 483-IDAHO	17.5	A		
JRS 274-N. DAKOTA	17.3	A		
JRS 478-WISCONSIN	17.0	A		
JRS 443-NEW YORK	15.8	A	B	
JRS 482-MICHIGAN	15.7	A	B	C
JRS 455-OREGON	14.2	A	B	C
JRS 152-NEBRASKA	12.8		B	C
JRS 467-COLORADO	12.0			C
JRS 456-WASHINGTON	8.3	D		

LSD=3.14, Alpha=0.05

Figure 3A.

Figure 3B.

In the DLT, a detached trifoliolate was inoculated with an agar plug containing *S. sclerotiorum* mycelium (Figure 3A). After 48 hours of incubation in high humidity, the area of the lesion caused by the fungus was measured by digital analysis (Figure 3B). The most aggressive isolates were from Idaho, North Dakota, and Wisconsin. The Idaho, North Dakota, and Wisconsin isolates were significantly more aggressive than the Nebraska, Colorado, and Washington isolates.

Characterization of 9 greenhouse/lab isolates by MCG

Mycelial Compatibility Grouping (MCG) of the nine greenhouse/lab isolates was determined. One plug of an isolate was placed on one side of a plate of red PDA (Potato Dextrose Agar), and another plug was placed 2.5 cm across from it. After 7-14 days, if the isolates formed a barrage line where the hyphae from the two different isolates met, then the isolates were incompatible (Figure 4B), and were considered to be unique isolates. If there was no evidence of mycelial interaction, the isolates were compatible (Figure 4C), and were considered clonal.
No two greenhouse isolates were compatible, each isolate was unique (Table 4).

Table 4. Results of the MCG test of the nine greenhouse/lab isolates

	NY	CO	WA	WI	ND	MI	OR	NE	ID
Blank	-	-	-	-	-	-	-	-	-
NY	O	X	X	X	X	X	X	X	X
CO		O	X	X	X	X	X	X	X
WA			O	X	X	X	X	X	X
WI				O	X	X	X	X	X
ND					O	X	X	X	X
MI						O	X	X	X
OR							O	X	X
NE								O	X
ID									O

O-Compatible MCG Reaction
X-Incompatible MCG Reaction
(-)-Cultures didn't grow or touch

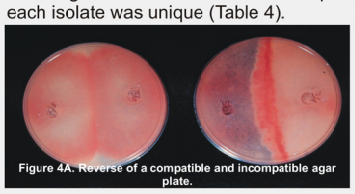
Figure 4A. Reverse of a compatible and incompatible agar plate.

Figure 4B. Incompatible MCG Reaction

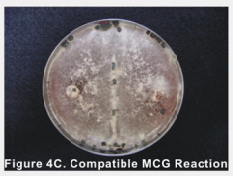
Figure 4C. Compatible MCG Reaction

Field sclerotia collection from 2003-2004

Table 5. Field Isolates 2003: 28 Total

Number of Sclerotia	Source (Location)
9	Michigan
9	Washington
7	Oregon
2	Minnesota
1	Wisconsin

Table 6. Field Isolates 2004: 49 Total

Number of Sclerotia	Source (Location)
9	Minnesota
4	Delaware
9	California
9	Nebraska
9	Oregon
6	Washington
3	Michigan

Isolate Collection
In 2005, we plan to complete the isolate collection from multi-site nurseries used to screen for white mold. This collection will give us representative samples from most of the major bean production areas in the US. The field sclerotia collection will provide the basis for isolate comparisons by MCG, aggressiveness, and molecular genetic markers. We also anticipate having some new breeding lines to test at the multiple locations.

Field white mold severity rankings of breeding lines tested in 2004

Table 7. Comparison of rankings of 12 bean lines for white mold reaction at eight field locations in 2004

Cultivar/Site	MN	CA	ND	NE	OR	MI	TAS*	FRA**
Cornell 601	1	3	5	10	6	3	11	2.5
G122	2	4	1	1	2.5	7	12	2.5
Cornell 501	3	7	6	3	2.5	5	10	8
AN 37	8.5	10	3	7	9	9.5	7	6
Ex Rico	6	2	9.5	5	4	7	5	5
NO2 302	10	1	2	2	5	1	2	10
IO1892-115M	4	5.5	4	8	7.5	7	1	7
USWA 6	8.5	9	9.5	6	1	3	6	2.5
AN 69	6	11	9.5	9	11	3	3	11
AN 1	11	5.5	9.5	4	7.5	9.5	9	2.5
CO75944	6	8	7	12	11	11	4	9
Beryl	12	12	12	11	11	12	8	12

TAS*-Tasmania
FRA**-France

Table 8. Mean rankings* of bean lines for white mold reaction in eight separate field tests in 2004

Entry	Mean Ranking	t Grouping				Seed Class	Source
Beryl	11.250			A		GN	*Check-Susceptible*
CO75944	8.500	B		A		PTO	M. Brick
AN 69	7.938	B		C		PTO	P. Miklas
AN 37	7.500	B		C		PTO	P. Miklas
AN 1	7.313	B		C		GN	P. Miklas
USWA 6	5.688	B		C	D	SR	P. Miklas
Cornell 501	5.563	B		C	D	SNAP	P. Griffiths
IO1892-115M	5.500	B		C	D	BLK	J. Kelly
Ex Rico	5.438	B		C	D	NAVY	*Check-Intermediate*
Cornell 601	5.188			C	D	RK	P. Griffiths
NO2 302	4.125				D	NAVY	J. Kelly
G122	4.000				D	CRAN	*Check-Resistant*

*Most resistant (1) to most susceptible (12)

LSD=3.09
Alpha=0.05

The 2004 field screening results were similar to the 2003 overall rank correlation. Beryl was again the most susceptible entry, while G122 was also one of the most resistant entries. However, AN 37 and Cornell 501 were ranked among the most susceptible, compared to their rankings in 2003, while NO2 302 appeared to be more resistant in the 2004 field screen, which we expect is due to architectural escape in the field.