Selection of resistance to multiple pathogens in tobacco assisted by markers and greenhouse screenings

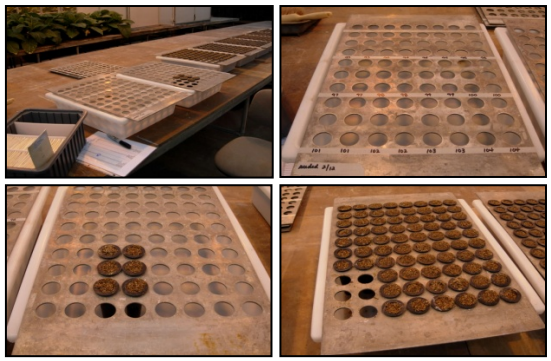
Natalia Martinez
Robert D. Miller, Dandan Li, and Glen Weinberger

Tobacco Breeding Program
Department of Plant & Soils
University of Kentucky

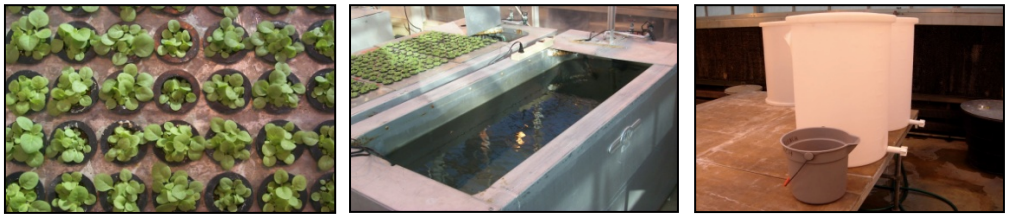
Screening for pathogen resistance in tobacco

DISEASE	MARKER	GREENHOUSE	FIELD
Black Shank race 0	✓	✓	✓
Black Root Rot	✓	✓	
Potyviruses	✓ (PVY)		✓ (TVMV)
TMV	✓	✓	
Blue Mold	✓		

Screening for pathogen resistance in the greenhouse



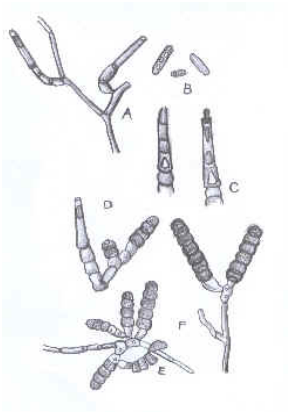
- Tobacco lines are carefully seeded in individual tubes (10 seeds ea.) in clean vermiculite soaking in 1/3 Hoagland's solution.
- Replicate at least 3 times
- Include susceptible and resistant check lines in each replication



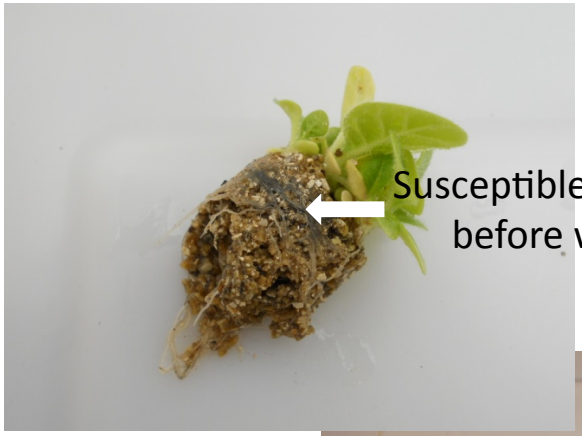
Four week-old seedlings are ready to be challenged with pathogen

Black Root Rot

Thielaviopsis basicola



Typical spores of *T. basicola*



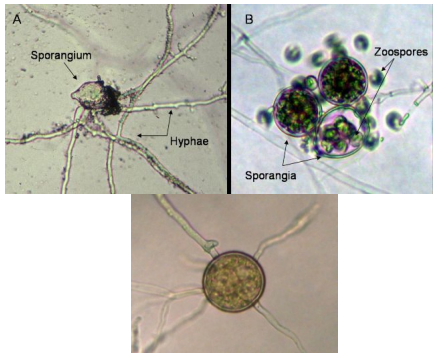
Susceptible dark roots before washing

Check lines for screening:

Casey:	Susceptible
B 21:	Low resistance from *N. tabacum*
KY 14:	Medium to low resistance from *N. tabacum*
KY 15:	High resistance from *N. debneyi*
KT 17:	High resistance from *N. debneyi*

Black Shank

Phytophthora nicotianae



Fungal reproductive structures: sporangia, zoosporangia and chlamydospores

Typical Black Shank symptoms in mature tobacco

<u>Check lines for screening</u>:

B21:	Susceptible
KY 14:	Susceptible
Coker371:	Resistance to Race 0 from *N. plumbagnifolia* and little Race 1 resistance
KY 14 X L8:	Resistance to Race 0 from *N. longiflora*
KT 200:	Resistance to Race 0 & 1 from Florida301
Beinhart:	Resistance to Race 0 & 1

Marker-assisted selection in tobacco pathogen resistance breeding

DISEASE	SCAR* MARKER
Black Shank race 0	BS770 (Johnson et al. 2002)
Black Root Rot	Chall-2 (Julio et al. 2006)
Potyviruses	PVYME1 (Julio et al. 2006)
TMV	E1E2 (Whitham et al 1994)
Blue Mold	$SOPR06_{268}$ (Milla et al. 2005)

*SCAR= Sequence Characterized Amplified Region

2010-11 Greenhouse No.	GREENHOUSE SCREENING 2010-2011			2010 FIELD MARKER DATA				
	Black Root Rot	Black Shank (Race 0)						
	T. basicola conidia (score 0-5)	*P. nicotiana* zoospores (% disease)	*P. nicotiana* chlamydospores (% disease)	BM	BS	BRR	PVY	TMV
5	0.19	0.0	0.0	R	R	R	R	
6	0.27	0.0	0.0	R	R	R	R	
14	0.31	0.0	0.0	R	R	R	R	
15	0.14	0.0	0.0	R	R	R	R	
26	1.03	5.9	0.0	R	R	R	R	
27	3.04	0.0	8.3	R	R	R	R	
28	3.07	0.0	0.0	R	R	R	R	
30	1.67	0.0	0.0	R	R	R	R	
31	0.52	0.0	0.0	R	R	R	R	
46	0.21	0.0	0.0		R	R	R	
58	2.84	0.0	0.0		R	R	R	
59	0.31	0.0	0.0		R	R	R	
74	2.64	46.2	18.8			R	R	
76	0.07	0.0	0.0		R	R	R	
77	0.14	0.0	0.0			R	R	
78	1.27	0.0	0.0		R	R	R	
79	0.51	0.0	0.0		R	R	R	
80	3.58	0.0	0.0		R	R	R	
81	3.43	0.0	0.0		R	R	R	
82	2.93	0.0	0.0			R	R	
83	2.00	0.0	3.8		R	R	R	
84	2.57	0.0	0.0		R	R	R	
85	1.02	0.0	0.0		R	R	R	
86	0.00	0.0	4.3		R	R	R	
89	0.08	20.0	0.0		R	R	R	
90	0.03	19.0	10.0			R	R	
91	0.02	0.0	0.0		R	R	R	
92	0.32	0.0	0.0		R	R	R	
93	0.27	0.0	0.0		R	R	R	
94	0.11	0.0	0.0		R	R	R	
95	0.43	0.0	0.0		R	R	R	
96	0.32	0.0	0.0		R	R	R	
153	2.94	0.0	0.0		R	R		S
154	1.68	0.0	0.0		R	R		S
155	3.24	0.0	0.0		S	R		S
156	2.60	0.0	0.0		R	R		S
KY14	3.49	88.5	83.5					
B21	4.41	61.9	66.9					
KY15	0.32							
KY17	0.19							
KY14xL8		0.0	0.8					
Beinhart		11.3	0.0					
Coker 371		0.0	0.0					

Features of each screening method

- **MARKER-ASSISTED SELECTION (MAS)**
  - Fast results (1-2 days)
  - Able to use one tissue sample to check resistance genes for different pathogens
  - Test resistance for non-culturable pathogens (ex. *Peronospora tabacina* - Blue Mold)

- **GREENHOUSE**
  - Results in 3-4 weeks
  - Test resistance for a selected pathogen with chosen environmental conditions
  - Able to detect segregation of recombinants by partial resistance phenotypes

- **FIELD**
  - Results in 6-8 weeks
  - Test if resistance is expressed in natural occurring conditions and pathogen levels
  - Able to detect segregation of recombinants and additional sources of resistance
  - Need to maintain throughout the growing season and could lose data due to inclement weather