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# **Update on transferring *Sclerotinia* resistance genes from wild *Helianthus* species into cultivated sunflower**

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# Introduction

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- ▶ Cultivated sunflower lacks sufficient genes for Sclerotinia resistance
- ▶ Perennial *Helianthus* species are **highly resistant** to Sclerotinia
- ▶ Crosses and backcrosses have been made to introgress the resistant genes from **hexaploid**, **diploid** wild species and interspecific **amphiploids**, using HA 441 or HA 410 as the recurrent parents
- ▶ Continue to **incorporate resistance genes** from diverse wild species into cultivated backgrounds
- ▶ **Field evaluation** to identify **new** resistance materials



# Progress-Phase 1

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- ▶ Seed increase and field test for stalk and head rots for the materials obtained from 11 different sources
  - ❖ Hexaploids backcrossed with HA 410  
*H. californicus* and *H. schweinitzii*
  - ❖ Diploids backcrossed with HA 410  
*H. maximiliani*, *H. giganteus*, and *H. grosseserratus*
  - ❖ Diploids backcrossed with HA 441  
*H. nuttallii* and *H. maximiliani*
  - ❖ Amphiploids backcrossed with HA 410  
*H. nuttallii*/P21, *H. maximiliani*/P21, *H. gracilenatus*/P21, *H. grosseserratus*/P21, and *H. strumosus*/P21



## Seed increase (SI) and Field test (FT) in 2009-2012

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Sources	2009 SI	2009 FT	2010 SI	2010 FT	2011 SI	2011 FT	2012 SI	2012 FT
<b>Hexaploids</b>	160	72 (SR)	112	92 (SR) 51 (HR)	37	85 (SR) 52 (HR)	32	65 (SR) 27 (HR)
<b>Diploids- HA410</b>	153	32 (SR)	164	71 (SR) 56 (HR)	43	58 (SR) 56 (HR)	55	56 (SR) 45 (HR)
<b>Diploids- HA441</b>	70	163 (HR)	181	169 (SR) 179 (HR)	99	122 (SR) 110 (HR)	120	121 (SR) 90 (HR)
<b>Amphiploids</b>	124	77 (SR)	75	81 (SR) 23 (HR)	40	59 (SR) 22 (HR)	34	42 (SR) 11 (HR)
<b>Total</b>	507	181 (SR) 163 (HR)	532	413 (SR) 309 (HR)	219	324 (SR) 240 (HR)	241	284 (SR) 173 (HR)



# Comparison of resistant and susceptible checks among field tests

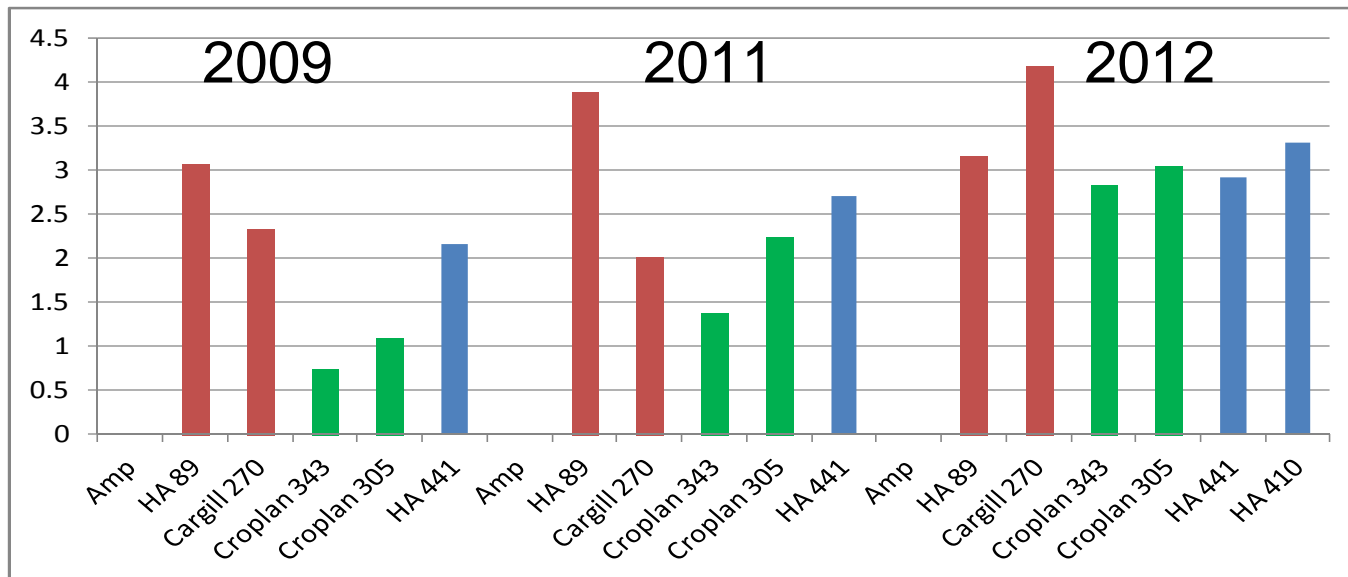
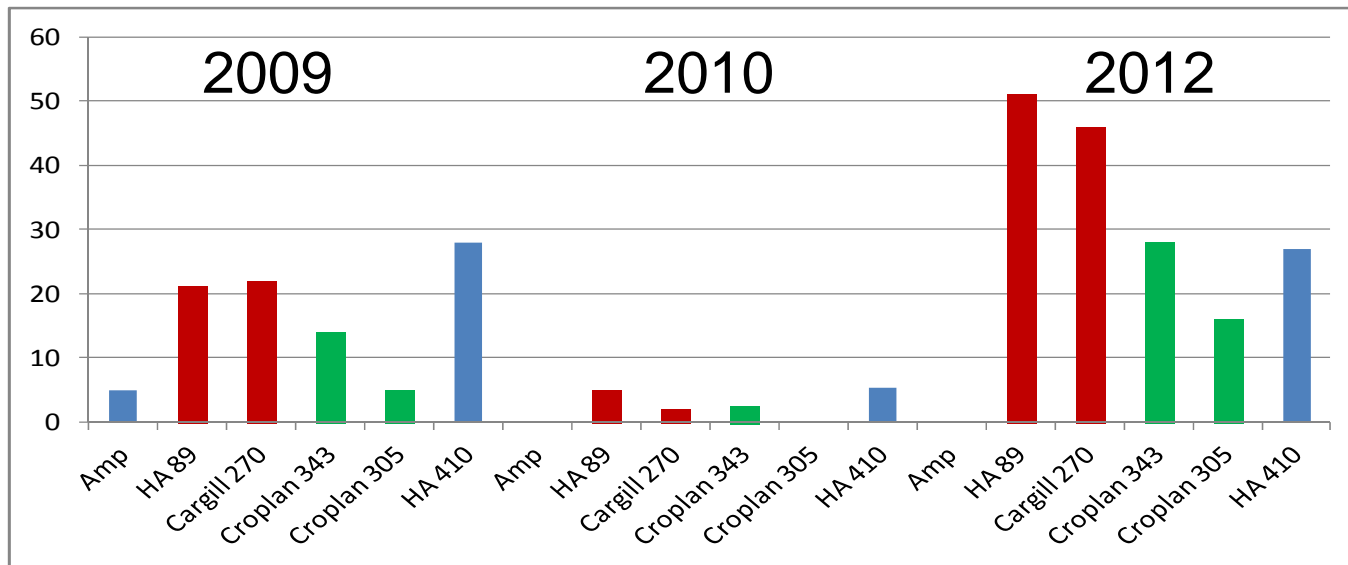


Table 1. Replicated field Sclerotinia stalk rot evaluations of 2011 retests (Test 1) and new interspecific crosses (Test 2) at Carrington, ND in 2012

Pedigree*	Percent Infected Plants and Number of Entries					
	0%	1-5%	6-10%	11-15%	16-20%	>20%
<b>TEST 1</b>						
<b>CAL 2376 x HA410, BC4F2- BC5F4</b>	<b>23</b>	<b>2</b>	<b>8</b>	<b>4</b>	<b>6</b>	<b>4</b>
((NMS HA89 x MAX=PI586892) x HA410), BC1F4	0	0	0	1	0	1
(NMS HA89 x GIG=PI547182) x HA410*2, BC2F3	0	0	0	1	0	1
((NMS HA89 x GRO=PI613793) x HA410*2, BC2F2	3	0	0	2	0	5
(DIV (68) x GRO (68)) x HA410*4, BC3F1	1	0	1	0	0	3
MAX (68) sib x HA410*3, BC2F2-BC2F3	3	2	0	0	0	0
STR (68) x HA410*3, BC2F2-BC2F2	3	0	0	3	0	1
(GRO (68) sib x HA410*4), BC3F1	1	1	1	0	0	1
(NMS HA89 x 1008(N)) x HA441, BC1F5- BC2F3	0	4	4	1	0	6
(NMS HA89 x 1018(M)) x HA441, BC1F5- BC3F3	2	3	1	2	0	12
(NMS HA89 x 1314(M)) x HA441, BC2F4- BC3F3	1	2	2	1	0	3
(NMS HA89 x 1323(M)) x HA441, BC1F4- BC3F4	2	2	3	2	5	6
(NMS HA89 x 1324(N)) x HA441, BC1F5- BC2F4	2	7	2	1	0	7
<b>TOTAL NUMBER</b>	<b>41</b>	<b>23</b>	<b>22</b>	<b>18</b>	<b>11</b>	<b>50</b>
<b>TEST 2</b>						
CAL 2376 x HA410*5, BC4F4	3	3	0	2	3	4
STR (68) x HA410*3, BC2F4	0	1	0	0	0	0
(NMS HA89 x MAX=PI586892) HA410, BC2F3	0	0	0	0	3	3
(NMS HA89 x GIG=PI547182) HA410*2, BC2F3	2	0	1	1	1	3
(NMS HA89 x GRO=PI613793) HA410, BC1F4	7	4	1	1	5	9
NUT (68) x HA410*3, BC2F3	0	1	0	1	0	3
STR (68) x HA410*3, BC2F3	0	0	2	0	0	5
MAX (68) sib x HA410*3, BC2F4	1	0	0	0	1	1
(NMS HA89 x 1323(M)) x HA441, BC1F5	1	2	1	0	0	1
(NMS HA89 x 1324(N)) x HA441, BC1F5	1	1	0	0	2	2
<b>TOTAL NUMBER</b>	<b>15</b>	<b>12</b>	<b>5</b>	<b>5</b>	<b>15</b>	<b>31</b>
<b>GRAND TOTAL ALL TESTS</b>	<b>56</b>	<b>35</b>	<b>27</b>	<b>23</b>	<b>26</b>	<b>81</b>



Croplan 305: 16%

85

Table 2. Replicated field Sclerotinia head rot evaluations of 2011 retests (Test 1) and new interspecific crosses (Test 2) at Staples, MN in 2012

Pedigree*	Plant Disease Rating and Number of Entries				
	0-1.0	1.1-2.0	2.1-3.0	3.1-4.0	4.1-5.0
<b>TEST 1</b>					
(NMS HA89 x 1008 (NUT)) x HA441*3, BC3F2-BC2F4	4	3	5	6	0
((NMS HA89 x 1018 (MAX)) x HA441, BC1F4-BC1F6	1	0	2	0	0
((NMS HA89 x 1018 (MAX)) x HA441, BC1F2) x HA441,BC2F2-BC2F4	5	6	5	1	0
(NMS HA89 x 1314 (MAX)) x HA441*3, BC2F4-BC3F2	1	8	1	1	0
(NMS HA89 x 1323 (MAX)) x HA441, BC1F5	0	1	4	0	0
(NMS HA89 x 1323 (MAX)) x HA441*3, BC3F3-BC3F4	2	4	2	0	0
(NMS HA89 x 1324 (NUT)) x HA441*2, BC1F5-BC2F2	3	3	5	3	5
<b>TOTAL NUMBER</b>	<b>16</b>	<b>25</b>	<b>24</b>	<b>11</b>	<b>5</b>
<b>TEST 2</b>					
(NMS HAR9 x GIG=PI547182) x HA410*2, BC2F3	1	0	1	1	4
(NMS HA89 x GRO=PI613793) x HA410 ,BC1F4	1	2	0	8	2
(NMS HA89 x GRO=PI613793) x HA410*3, BC3F2	0	0	2	3	2
((NMS HA89 x GRO=PI613793) x HA410*2, BC2F3	0	1	5	4	5
{NMS HA89 x MAX=PI586892) x HA410},BC2F3	0	0	0	3	6
CAL 2376 x HA410*5, BC4F4	0	0	0	5	16
CAL 2376 x HA410*5, BC4F5	0	1	0	0	3
(DIV (68) x GRO(68)) x HA410*3, BC2F4	0	0	0	2	0
MAX (68) SIB x HA410*3, BC2F4	0	0	0	0	2
NUT (68) x HA410*3, BC2F4	0	0	0	2	1
STR (68) x HA410*3, BC2F4	0	0	0	1	3
(NMS HA89 x 1008 (NUT)) x HA441*2, BC2F4	2	2	3	0	0
(NMS HA89 x 1018 (MAX)) x HA441, BC1F6	1	0	1	0	1
(NMS HA89 x 1323 (MAX)) x HA441, BC1F5	0	0	1	0	0
(NMS HA89 x 1324 (NUT)) x HA441, BC1F5	2	0	0	1	0
<b>TOTAL NUMBER</b>	<b>7</b>	<b>6</b>	<b>13</b>	<b>30</b>	<b>45</b>
<b>GRAND TOTAL</b>	<b>23</b>	<b>31</b>	<b>37</b>	<b>41</b>	<b>50</b>

Croplan 343: 2.83

# Summary

- ▶ Over **200 advanced backcross progeny families** were grown in Fargo in 2012 to provide a continuous supply of materials for field evaluation
- ▶ Among the three years of field evaluations, **2012** was unquestionably **the best year** allowing us to make adequate comparisons and selections for further confirmation in 2013
- ▶ The families showing disease rating better than the resistant checks will be confirmed in 2013
- ▶ The families with good resistance will be prepared for germplasm release, further characterization, and the initiation of QTL mapping populations



## Progress-Phase 2

### ▶ New interspecific crosses initiated in 2011-2012

No.	Accession	Species	2n	Cross
1	PI 547171	<i>H. hirsutus</i>	68	2011
2	PI 547174	<i>H. hirsutus</i>	68	2011
3	Ames 30340	<i>H. salicifolius</i>	34	2011
4	Ames 30348	<i>H. salicifolius</i>	34	2011
5	PI 494594	<i>H. occidentalis</i> subsp. <i>plantagineus</i>	34	2011
6	Ames 30317	<i>H. occidentalis</i> subsp. <i>plantagineus</i>	34	2011
7	Ames 30356	<i>H. silphioides</i>	34	2011
8	Ames 30354	<i>H. silphioides</i>	34	2011
9		<i>H. resinosus</i>	102	2012
10	PI 503209	<i>H. divaricatus</i>	34	2012
11	PI 503216	<i>H. divaricatus</i>	34	2012
12	PI 503218	<i>H. divaricatus</i>	34	2012

} Difficult to make crosses

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- Interspecific crosses were established between four perennial *Helianthus* species and cultivated sunflower in 2011
  - Initiated cross between three *H. divaricatus* accessions and *H. resinosus* with cultivated sunflower
  - Backcrosses and embryo rescue for F1 or BC1 progenies for 12 accessions of six perennial *Helianthus* species
  - Mitotic chromosome counting for F1 and BC1F1 plants
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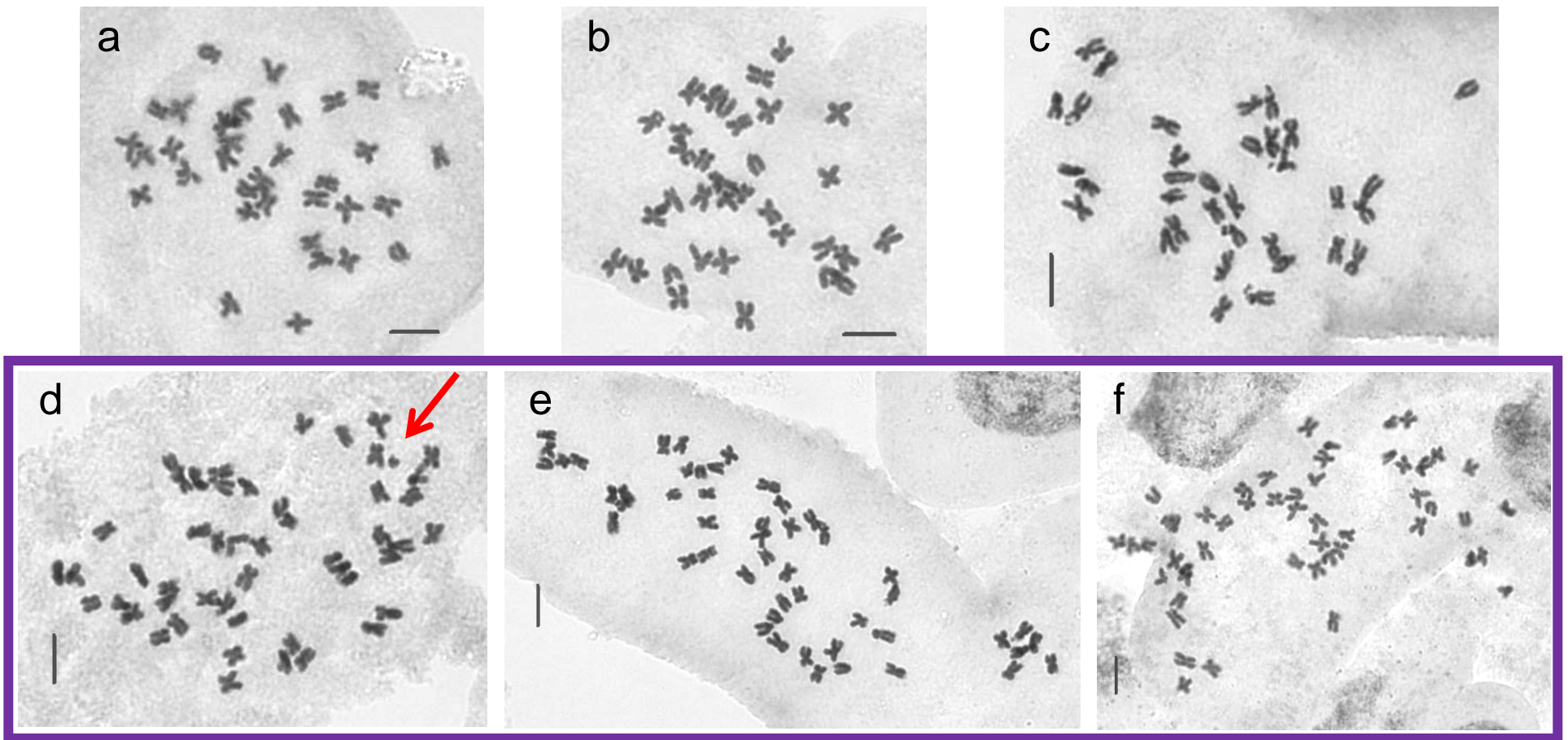
Table 3. Pollen stainability of F<sub>1</sub> between HA 410, HA 451, and NMS HA 89 and wild *H. hirsutus*, *H. salicifolius*, *H. occidentalis*, and backcross seed set with HA 410 or HA 451

Parentage	F <sub>1</sub> (2011-2012)				BC <sub>1</sub> F <sub>1</sub> (2011-2012) (F <sub>1</sub> as female)				BC <sub>1</sub> F <sub>1</sub> (2012) (F <sub>1</sub> as male)		
	F <sub>1</sub> Embryo /florets	Plants	Fertile pollen %	BC seeds/florets	BC <sub>1</sub> Embryo /florets	Plants	2n	Seed set %	BC <sub>1</sub> Embryo /florets	Plants	2n
<i>H. hirsutus</i> × HA 410	140/1715	51	12.2 (0-47.3)	44/6418	55/3790	0	-	-	139/4000	45	34
<i>H. hirsutus</i> × HA 451	63/446	34	6.52 (0-36.5)	98/23750	16/2450	11	44-68	3.6	19/3200	11	34
NMS HA 89 × <i>H. hirsutus</i>	470/6044	162	13.1(0.0-43.6)	67/17954	38/4265	24	38-68	8.4	-	-	-
<i>H. salicifolius</i> × HA 410	241/4700	26	2.7 (0-6.2)	48/14244	88/6698	19	34-52	9.1	254/7200	172	34-51
NMS HA 89 × <i>H. salicifolius</i>	31/11300	5	1.4	2/1590	5/350	1	34	1.3	-	-	-
<i>H. occidentalis</i> × HA 410	217/1210	15	1.4 (0-2.9)	10/9225	23/3732	4	34-35	7.1	355/3200	216	34
<i>H. occidentalis</i> × HA 451	182/2060	28	1.2 (0.5-3.4)	21/5923	20/2580	3	35-46	0.6	64/4000	25	34
NMS HA 89 × <i>H. occidentalis</i>	4/3650	3	2.23	-	7/600	1	34	25.3	-	-	-

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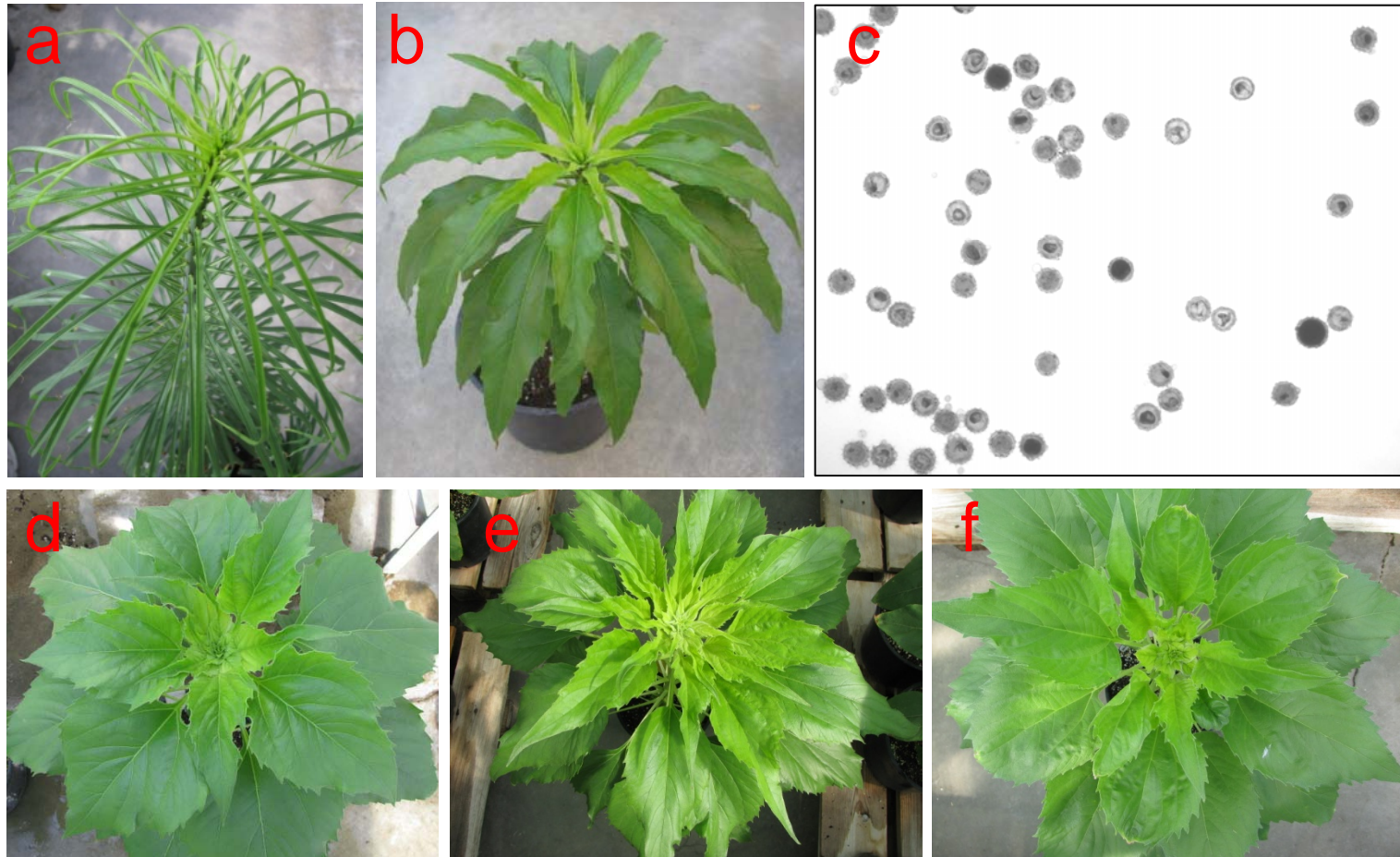
469

# Chromosome non-reduction - *H. salicifolius*



a: *H. salicifolius* Ames 30340; b: F1 derived from *H. salicifolius* x HA 410; c: NMSHA89 x (*H. salicifolius* x HA410), 2n=34; d: NMSHA89 x (*H. salicifolius* x HA410), 2n=49; e: NMSHA89 x (*H. salicifolius* x HA410), 2n=50; f: NMSHA89 x (*H. salicifolius* x HA410), 2n=51. The arrow shows a tiny chromosome. Bars=5  $\mu$ m.

# The wild parent, F1 and BC1F1 plants



a: *H. salicifolius*; b: G10/1129 x HA 410, F1; c: pollen fertility of F1; d: BC1F1 ( $2n=34$ ); e: BC1F1 ( $2n=36$ ); f: BC1F1 ( $2n=51$ ) .





# Frequency of abnormal chromosome number

Cross	( <i>H. salicifolius</i> x HA 410) x HA 410	%	NMS HA 89 x ( <i>H. salicifolius</i> x HA 410)	%
2n=34	12	63.16	147	85.47
2n=35	1	5.26	0	0.00
2n=36	1	5.26	1	0.58
2n=41	1	5.26	0	0.00
2n=48	0	0.00	1	0.58
2n=49	0	0.00	1	0.58
2n=50	0	0.00	14	8.14
2n=51	3	15.79	8	4.65
2n=52	1	5.26	0	0.00
Total	19		172	

21.05 %

13.95 %

Relatively high frequency of chromosome non-reduction was observed in the BC1 progeny when F1 was used as the female or male parent.

Self-pollinated seed set of the 469 BC1F1 plants resulted from NMSHA 89 x F1s.

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Female	Male	Recurrent Parent	Seed set (%)	Note
<i>H. hirsutus</i>	HA451	NMSHA89	73.18	2n=34
<i>H. hirsutus</i>	HA410	NMSHA89	66.05	2n=34
<i>H. salicifolius</i>	HA410	NMSHA89	66.2	2n=34
<i>H. salicifolius</i>	HA410	NMSHA89	0.70	2n=34 (4 plants more like wild parent)
<i>H. salicifolius</i>	HA410	NMSHA89	0.30	2n>34 (25 plants)
<i>H. occidentalis</i>	HA410	NMSHA89	68.3	2n=34
<i>H. occidentalis</i>	HA451	NMSHA89	74.79	2n=34

Seeds of about 425 BC1F2 families (>30% seed set) will be increased in 2013.

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Table 4. Pollen stainability of F1 between HA 410, HA 451, and NMS HA 89 and wild *H. resinusus*, and *H. divaricatus*, and backcross seed set with HA 410 or HA 451

Parentage	F <sub>1</sub> (2011-2012)			
	F <sub>1</sub> Embryo /florets	Plants	Fertile pollen %	BC seeds/florets
<i>H. resinusus</i> × HA 451	8/1875	8	50.3(18.4-65.2)	37/16095
<i>H. divaricatus</i> × HA 410	-	9	-	24/4600
<i>H. divaricatus</i> × HA 451	-	4	-	12/1890
NMS HA 89 × <i>H. divaricatus</i>	35/9600	31	-	46/7210



Continue to backcross in greenhouse





# F<sub>1</sub> plants of *H. resinosus* x HA 451 highly resistant to powdery mildew

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a: *H. resinosus* x HA 451, F<sub>1</sub> (G11-691, resistant to powdery mildew);  
b: HA 89 x *H. nuttallii* 102, F<sub>1</sub> (G11-681, susceptible to powdery mildew)

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# Summary

- ▶ 469 BC1F1 plants were obtained via embryo rescue using F1 plants as the male parents. More than 400 BC1F2 families will be planted in the field in 2013 to increase seed
- ▶ Continue to backcross BC1F1 progenies having 2n chromosome numbers >34
- ▶ High frequency of abnormal chromosome number in the BC1F1 progenies of *H. salicifolius* was observed with either the F1 as female or male parent
- ▶ Among all the new crosses, the F1 plants of *H. resinosus* x HA 451 showed high resistance to powdery mildew

## Conclusion and Future work

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- ▶ Field evaluation showed progeny families with good stalk and head rot resistance, indicating **successful introgression** of resistance genes
- ▶ **The outstanding families** will be confirmed and prepared for germplasm release, continue cytogenetic and molecular studies, and used to initiate QTL mapping populations
- ▶ Extensive efforts has been made to **obtain F1 and BC1F1 plants for the new crosses**. In addition to the seed increase of the BC1F2 families in 2013, we will continue to backcross the progenies with  $2n>34$



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- ▶ Continue to identify addition lines, and alien chromosomes or fragments in cultivated background utilizing **GISH and FISH** for  $2n=35$  and  $2n=36$  plants
  - ▶ **Previously produced BC1F1 seed** of wild *H. decapetalus*, *H. laevigatus*, *H. smithii*, *H. giganteus*, *H. pauciflorus* (*rigidus*), and *H. resinosus* crossed with HA 89 will be added to the Sclerotinia project in 2013



# Acknowledgements

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## Funding: National Sclerotinia Initiatives

Lisa Brown

Sandra Tuinstra

Marjorie Olson

Leonard Cook

Megan Ramsett

Angelia Hogness

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