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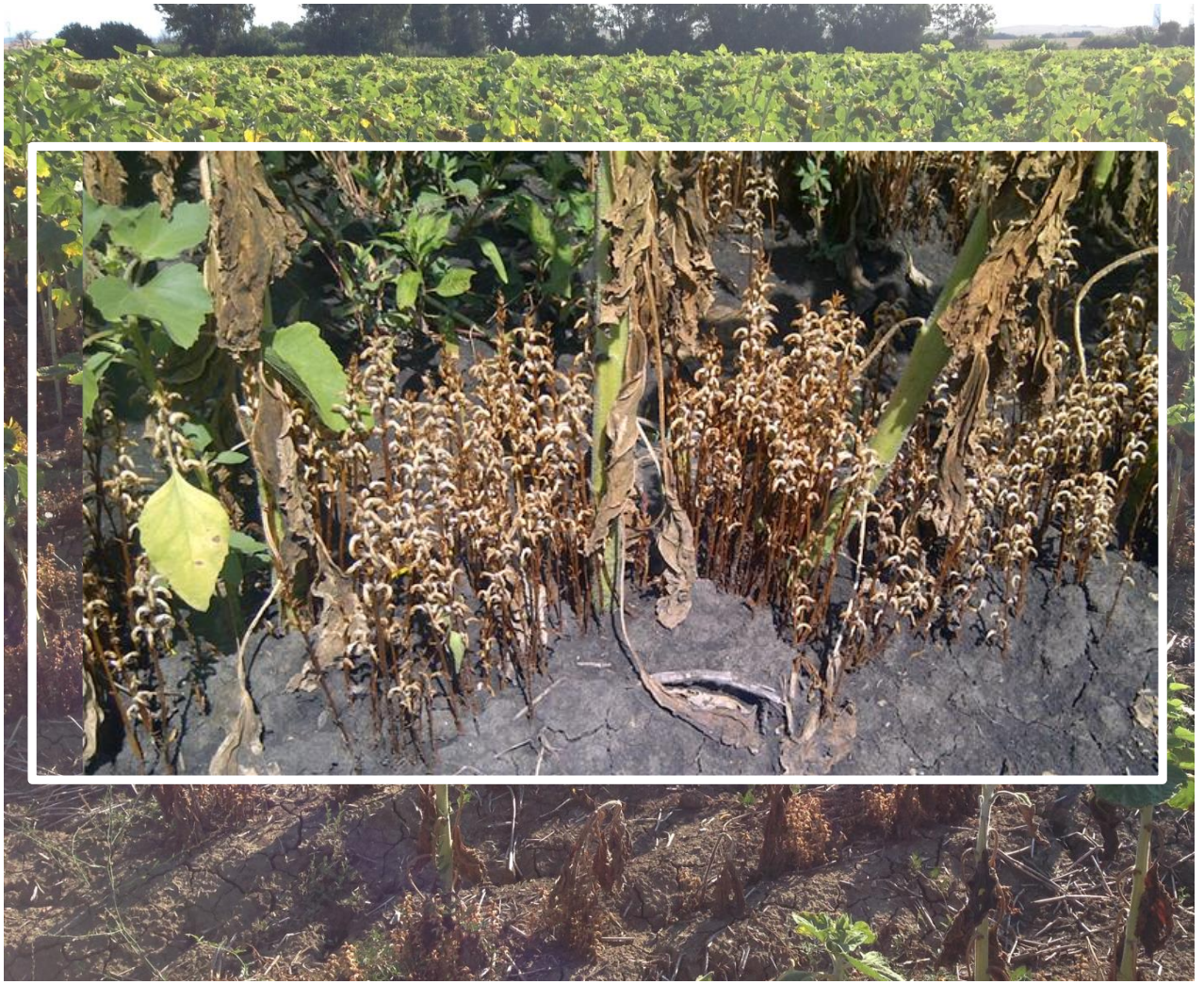
Resistance mechanisms to *Orobanche cumana* in sunflower

Alberto Martin-Sanz, Sunflower Discovery Pathologist

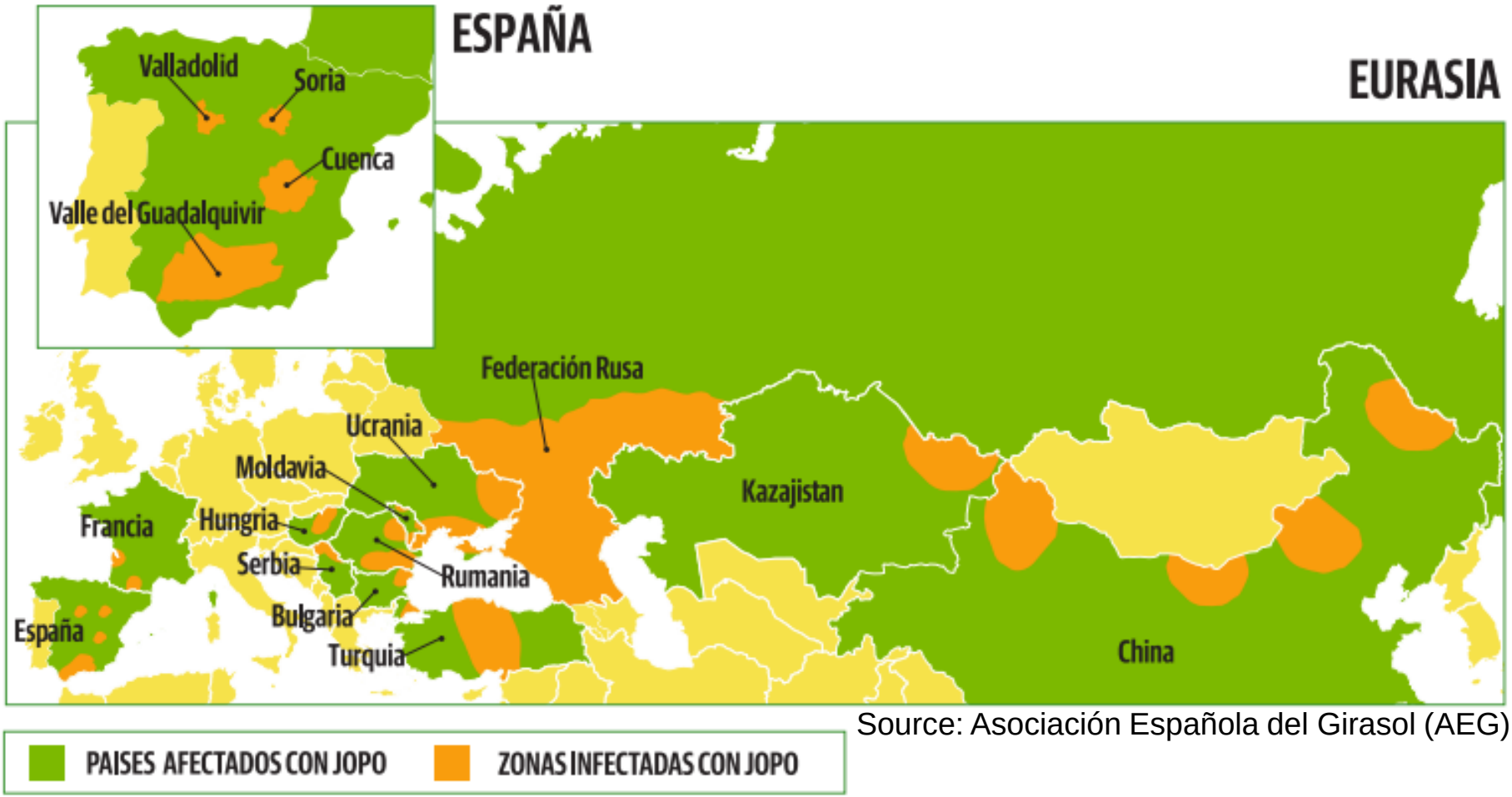
4th International Symposium on Broomrape, Bucharest, July 2018.

BROOMRAPE IMPACT ON SUNFLOWER CROP



Pictures: Corteva Agriscience™

BROOMRAPE AFFECTED AREAS



Source: Asociación Española del Girasol (AEG)

SUNFLOWER BROOMRAPE IS SPREADING TO NEW AREAS

1990's

2018

- Spread to new areas
- Populations with higher aggressiveness

- In-depth knowledge of the parasite diversity and race distribution
- Define strategies for sustainable genetic resistance and integrated control

Data and pictures: Corteva Agriscience™

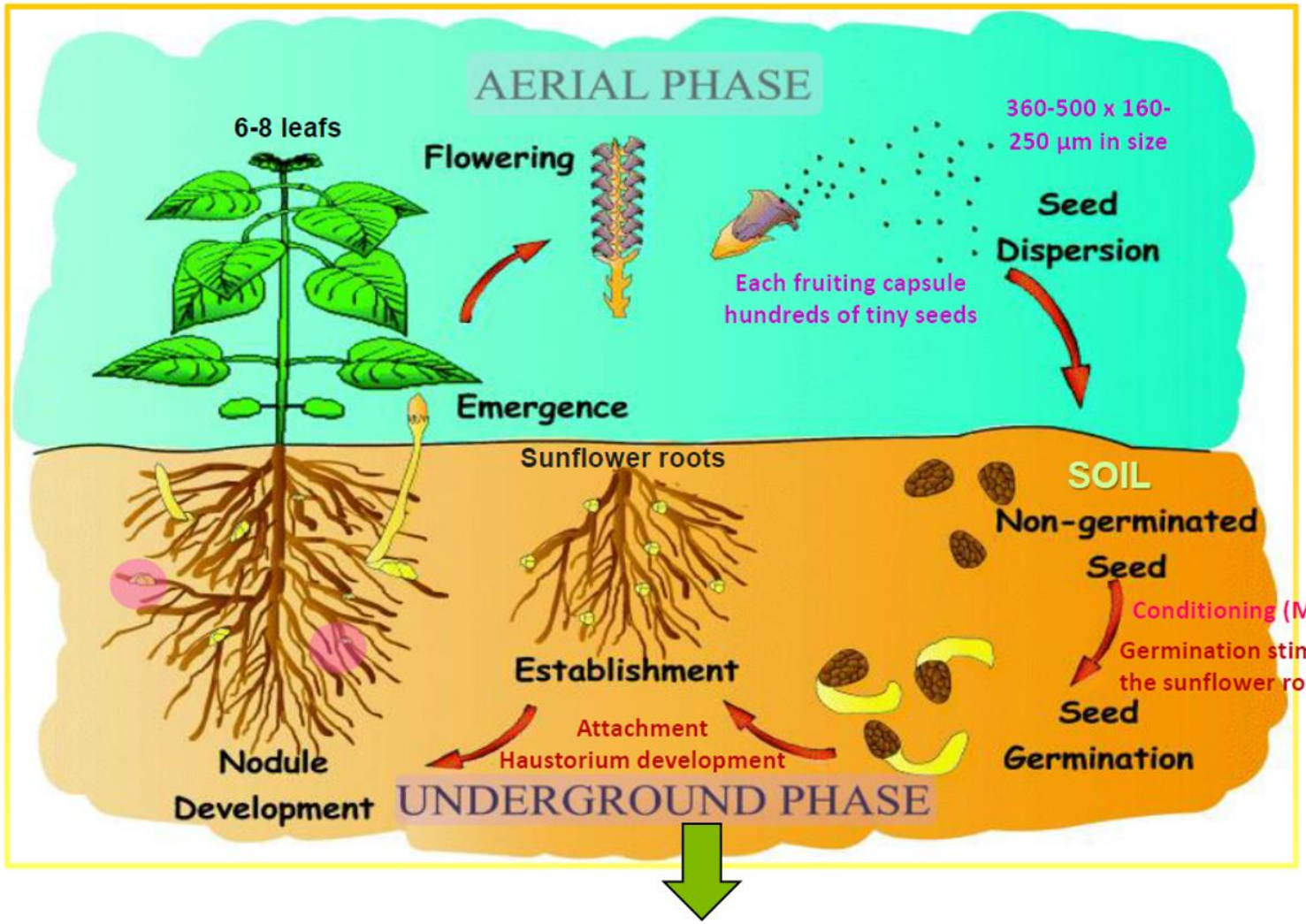
BROOMRAPE RACIAL DISTRIBUTION

Race E: NR5 (Or5):
Race F: P96 and hybrids
Race G: hybrids, DEB2

Racial situation of sunflower broomrape is complex

Race G+ populations are increasing dramatically in all Europe

Orobanche cumana life cylcle



Based on Rispail *et al.*, 2007

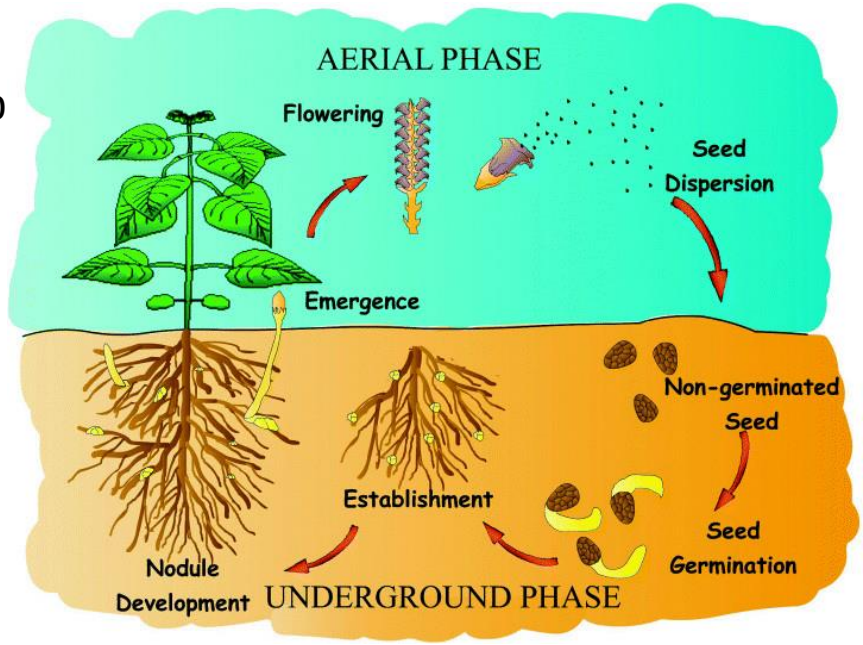
Key stages for resistance traits

MECHANISMS OF RESISTANCE TO PARASITIC PLANTS

- **Pre-attachment** → prior to parasite contact with the host root

  Low induction of seed germination (↓ germination stimulants, inhibitors of germination), chemotropism

- **Post-attachment** → attached parasites fail to develop



Pre-haustorial

Reinforcement of the cell walls (lignine)

Production of toxic compounds

(phytoalexines, peroxidases, etc)

Post-haustorial

Delivery of toxic compounds such as phenolics or peroxidases

Swalling of host vessels by gel or gum-like substances and blocking the flux of nutrients from the host to parasites

MECHANISMS OF RESISTANCE TO SUNFLOWER BROOMRAPE

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BROOMRAPE RESISTANCE MAJOR GENES IN SUNFLOWER

SOURCE	R GENE	LG	RACE	GENETICS	MECHANISM	REFERENCE
P1380	*Or5*	3	A-E	Dominant	Pre-haustorial	Lu et al (2000); Tang et al (2003)
Breeding line	Or_{SII}	4	A-G	Partial dominant	Post-haustorial	Sayed et al (2006)
AB-VL-8	$Or_{ab\text{-}vl\text{-}8}$	13			?	Imerovski et al (2016)
DEB2	Or_{DEB2}	?	A-G	?	?	Velasco et al (2012)
Breeding line	*Or7*	7	A-G	Dominant	Pre-haustorial	Duriez et al (2018)
LC-1093	Or_6	?	A-F	Dominant	?	Pacureanu et al (2004)
J1	*Or6 Or7*	?	A-F	Partially dominant	?	Velasco et al (2007)
P-96	*Or5 or6 or7*		A-F	Recessive	?	Akhtouch et al (2002)

PRE-HAUSTORIAL RESISTANCE

Take place from the first contact of the parasite seedling with the host root up to the establishment of vascular connections through haustorium development

- Formation of an encapsulation layer
  - *H. debilis* ssp *debilis* (Labrouse et al, 2001)
  - *H. annuus* (Dörr et al, 1994)

- Suberization and protein cross-linking in HE-39999 (Echevarría-Zomeño et al, 2006)

- Callose deposition in LR1 (Letousey et al, 2007)

- Lignification and suberization for cell wall fortification
  - Linked to peroxidases: Antonova et al (2004)
  - *Or7* resistance gene (Duriez et al 2018): lignine/suberine wall

- Chemical barriers: phytoalexins (phenolics) acting around broomrape infection area (Echevarría-Zomeño et al, 2006)

POST-HAUSTORIAL RESISTANCE (Once the parasite develops vascular connections to the host)

- Sealing sunflower xylem vessels with gel or gum-like substances to block the flux of wáter and nutrients from the host to the parasite → LR1 (Labrouse et al, 2001)

- *Or_SII* (Sayed et al, 2006; Martín-Sanz et al, 2017)

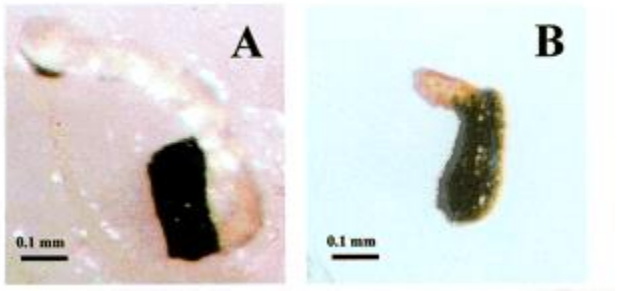
PRE-ATACHMENT RESISTANCE

► **Low pH values in the root promotes susceptibility (Morozov, 1947)**

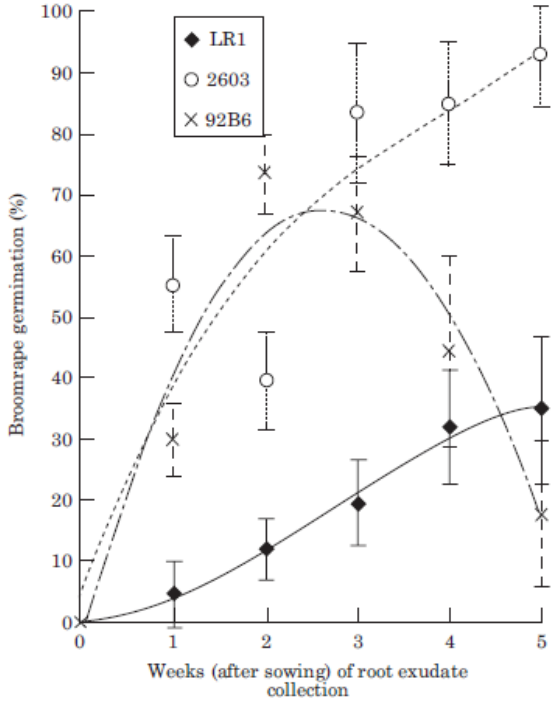
► **Broomrape germination stimulants:**

Strigolactones (Yoneyama et al, 2011) and other metabolites (Sesquiterpene lactones, dehydrocostus lactone, heliolactone) stimulate broomrape germination (Macias et al, 2009; Joel et al, 2011; Ueno et al, 2014).

Phytoalexins (7-hydroxylated simple coumarins) with a deffensive role preventing broomrape germination (Serghini et al, 2001)



Low exudation of germination stimulants by sunflower roots (Labrousse et al, 2001)



PRE-ATACHMENT RESISTANCE IN OTHER PARASITIC PLANTS

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Low Strigolactone Root Exudation: A Novel Mechanism of Broomrape (*Orobanche* and *Phelipanche* spp.) Resistance Available for Faba Bean Breeding

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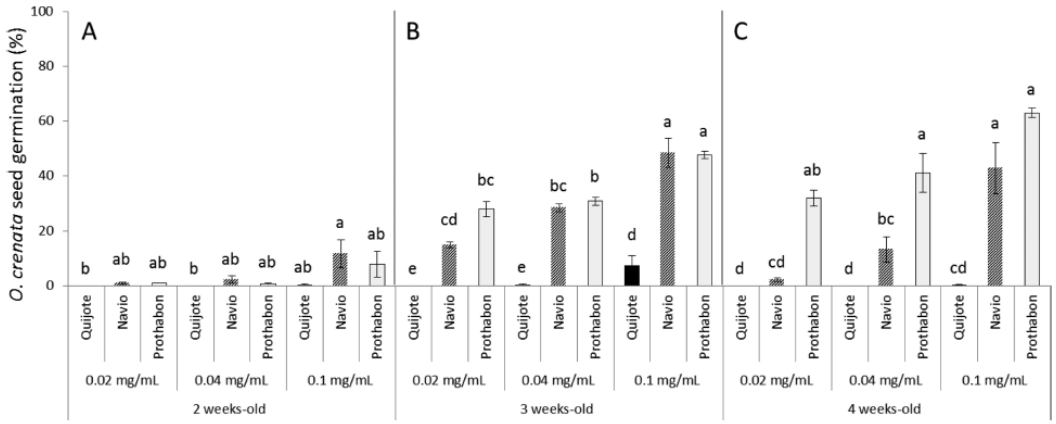

Figure 2. Effect of plant age, accession, and concentration of root exudates on germination of seeds of *Orobanche crenata*. Vertical bars represent standard error for $n = 4$. Analysis of variance was applied to transformed replicate data. For each plant age, bars with different letters are significantly different according to the Tukey test ($p = 0.05$). Negative control (distilled water) = 0%. Positive control (GR24, 10^{-6} M) = 61%.

Mutation in sorghum *LOW GERMINATION STIMULANT 1* alters strigolactones and causes *Striga* resistance

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Edited by Ronald L. Phillips, University of Minnesota, St. Paul, MN, and approved March 16, 2017 (received for review November 17, 2016)

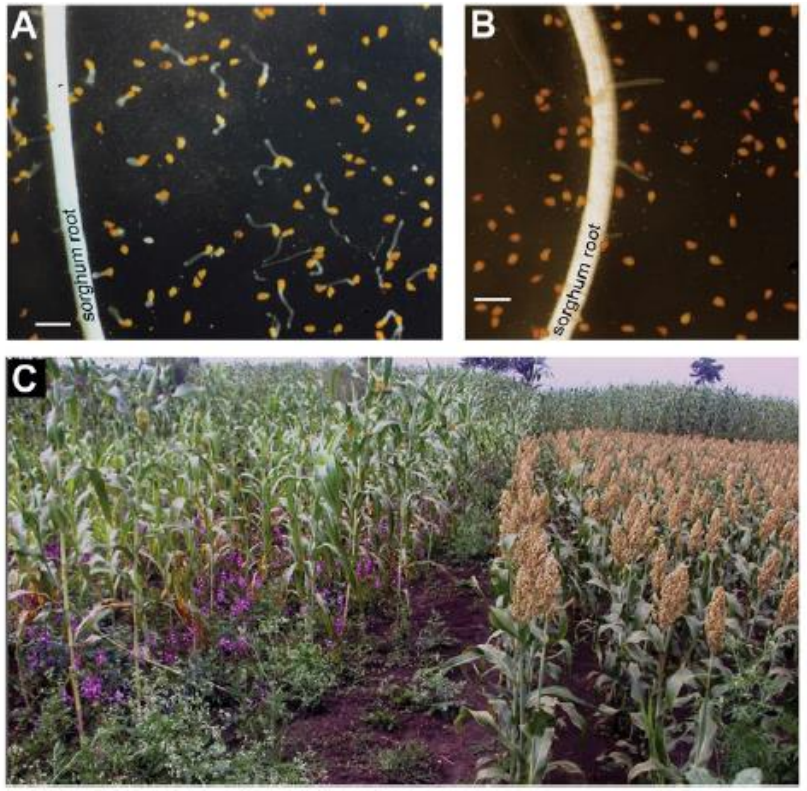
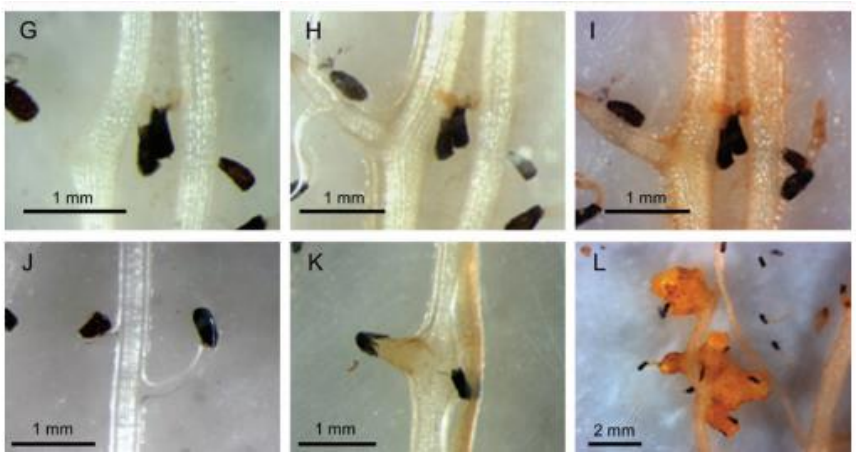
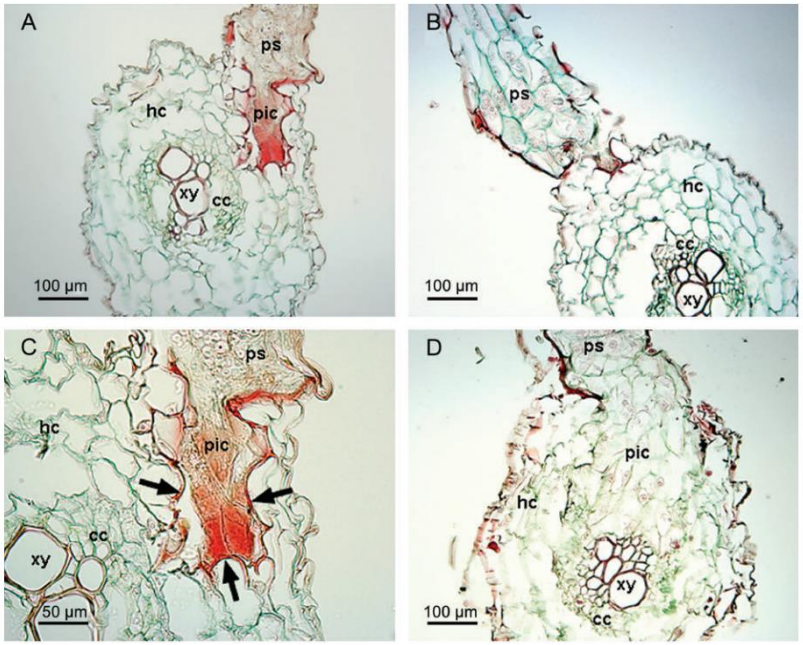


Fig. 2. *Striga* resistance phenotypes of *LGS1* variants. Sorghum seedlings with high *Striga* germination stimulant activity (*A*) will germinate conditioned *S. asiatica* seeds cocultured in agar, a centimeter or more from its root as the germination stimulant, 5-deoxystrigol, diffuses through the medium. Low-stimulant sorghum that exudes orobanchol instead of 5-deoxystrigol will not cause *S. asiatica* seeds to germinate in the agar gel assay, even very near its roots (*B*). (Scale bars, 1 mm.) The photograph (*C*) shows an *LGS1* WT high-stimulant sorghum (left) growing next to a line (right) carrying the *lgs1-1* allele in a field infested with *S. hermonthica* (purple flowers) in Ethiopia.

EXAMPLES OF PREHAUSTORIAL RESISTANCE

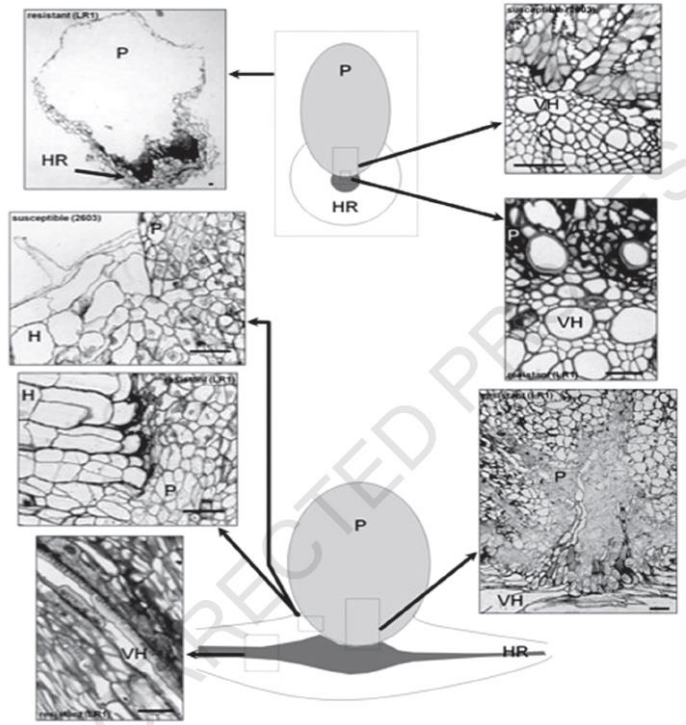
Echevarría-Zomeño et al (2006)

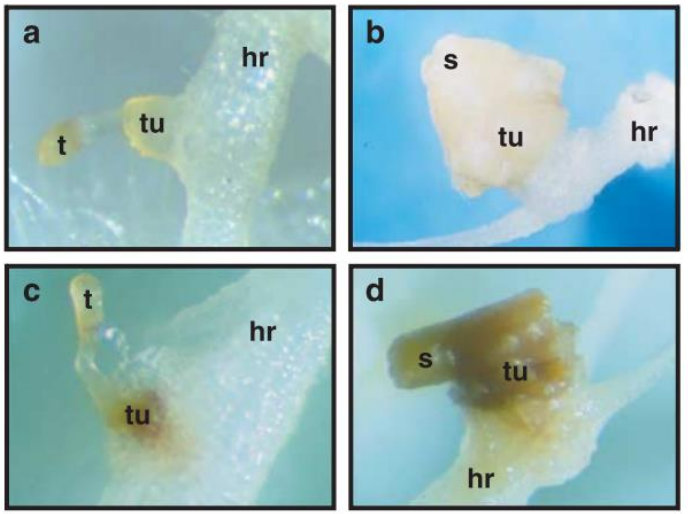




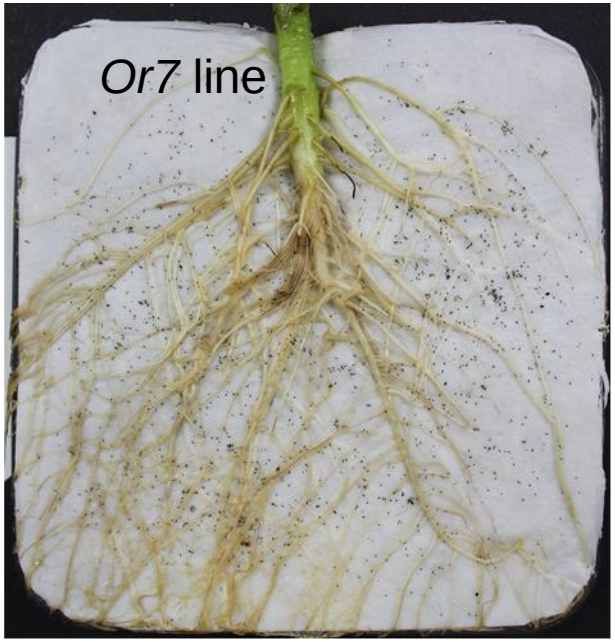
LR1

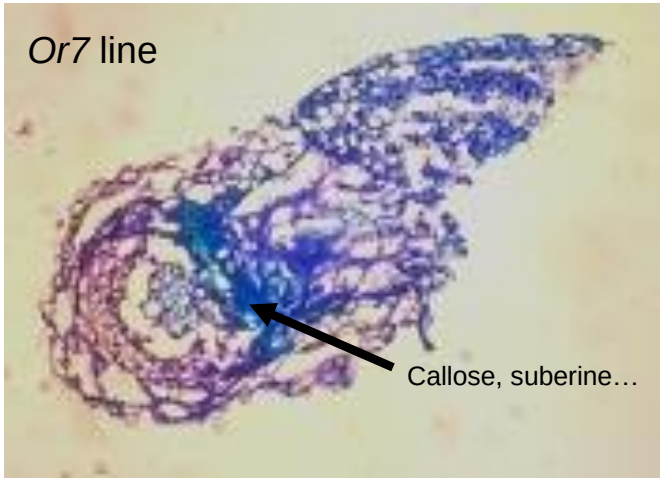
Labrouse et al (2001)
Letousey et al (2007)
Louarn et al (2016)





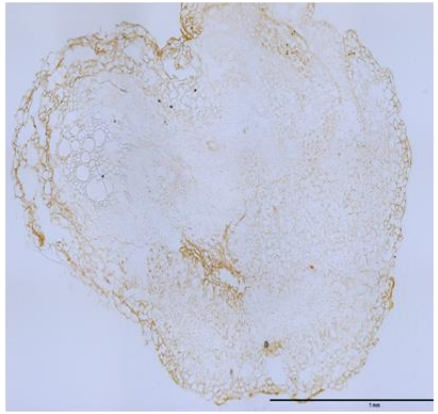
EXAMPLES OF PREHAUSTORIAL RESISTANCE: *Or7*



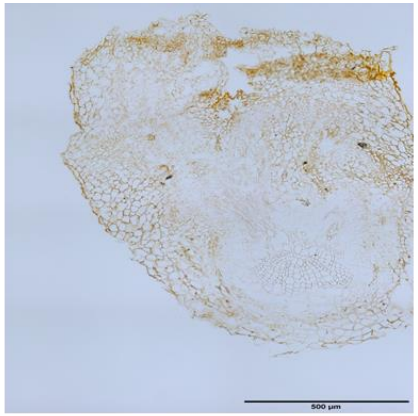


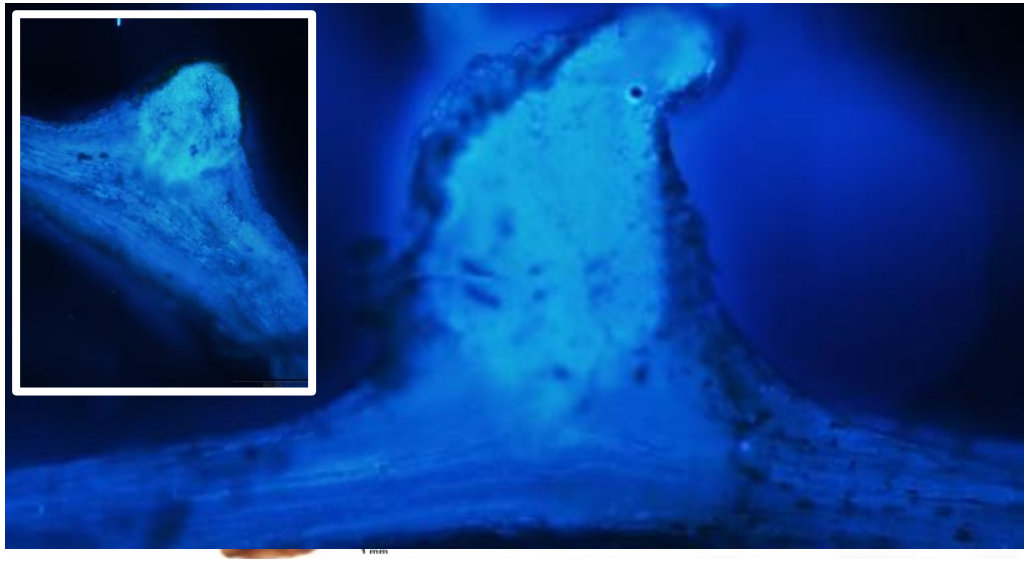
EXAMPLES OF POSTHAUSTORIAL RESISTANCE: *Or_SII*

Susceptible – Fresh observation

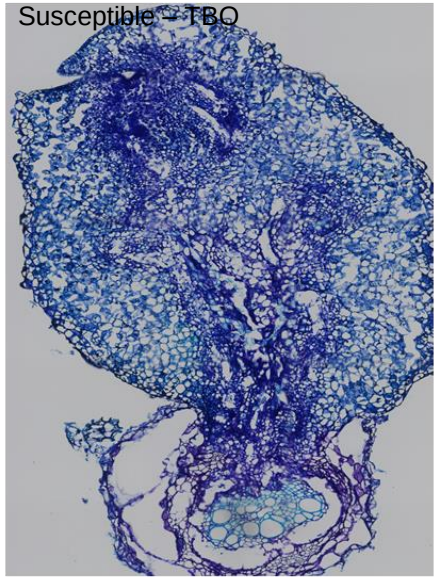


Resistant – Fresh observation

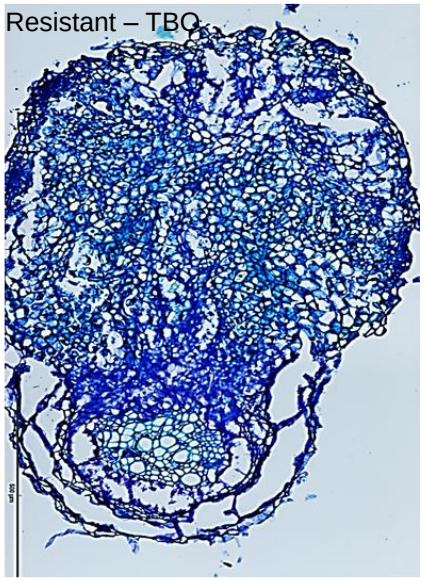




Susceptible – TBO

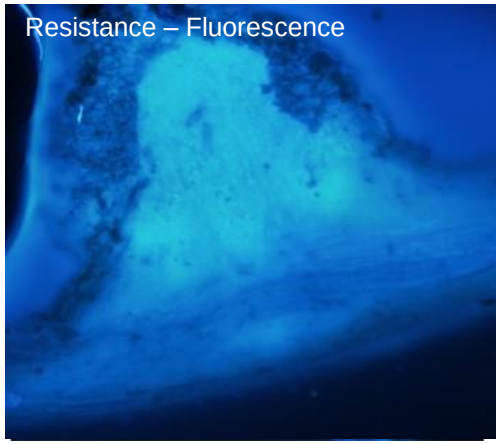


Resistant – TBO



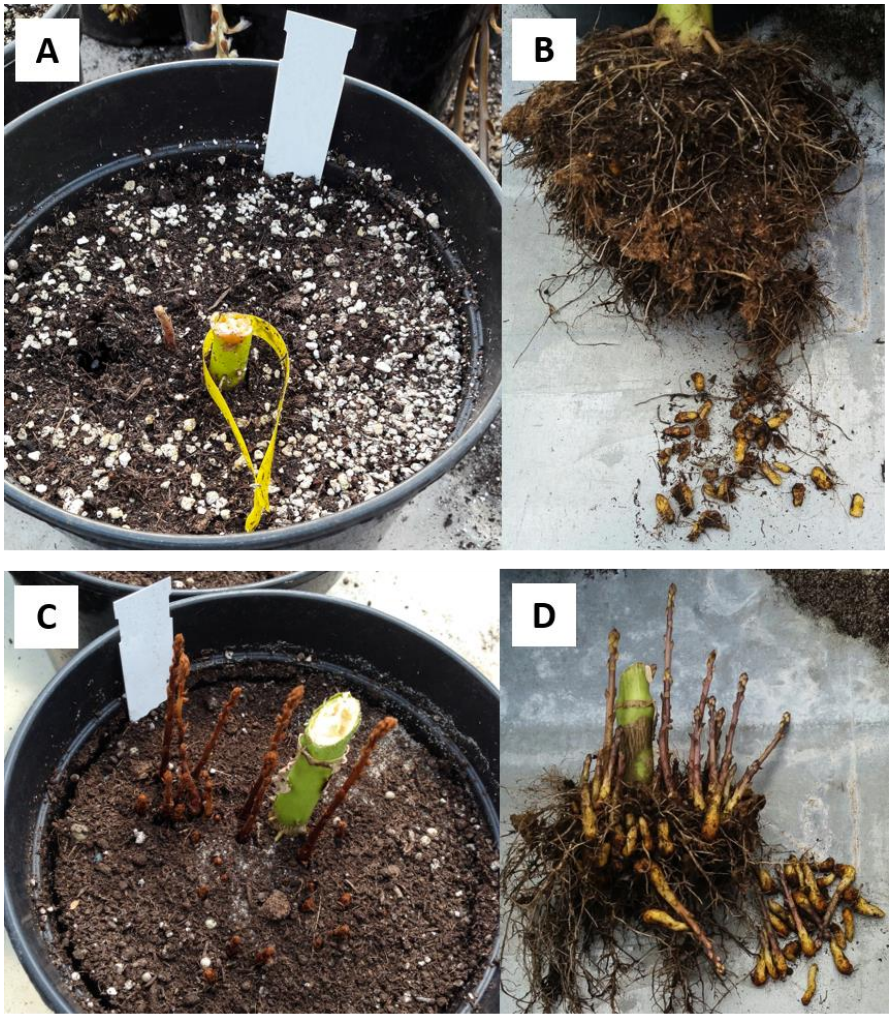
Susceptible –
Fluorescence

Resistance – Fluorescence



Strong fluorescence in host and parasite tissues, indicating accumulation of phenolic compounds in in the parasite produced by the SII genotype. Thus, SII provides resistance by “poisoning” the broomrape shoot

Or_SII: PHENOTYPIC CHARACTERIZATION



Phenotypic responses of SII resistance gene in homozygous (A and B) and heterozygous stages (C and D) in pot experiments. Figure E shows the phenotype of the susceptible inbred line used in the mapping population. Figures 1A and 1C show the emerged broomrapes while Figures 1B and D illustrate the size of the subterranean broomrape nodules. These pictures correspond to plants infested in the greenhouse with the broomrape population GT 90 days after infestation.

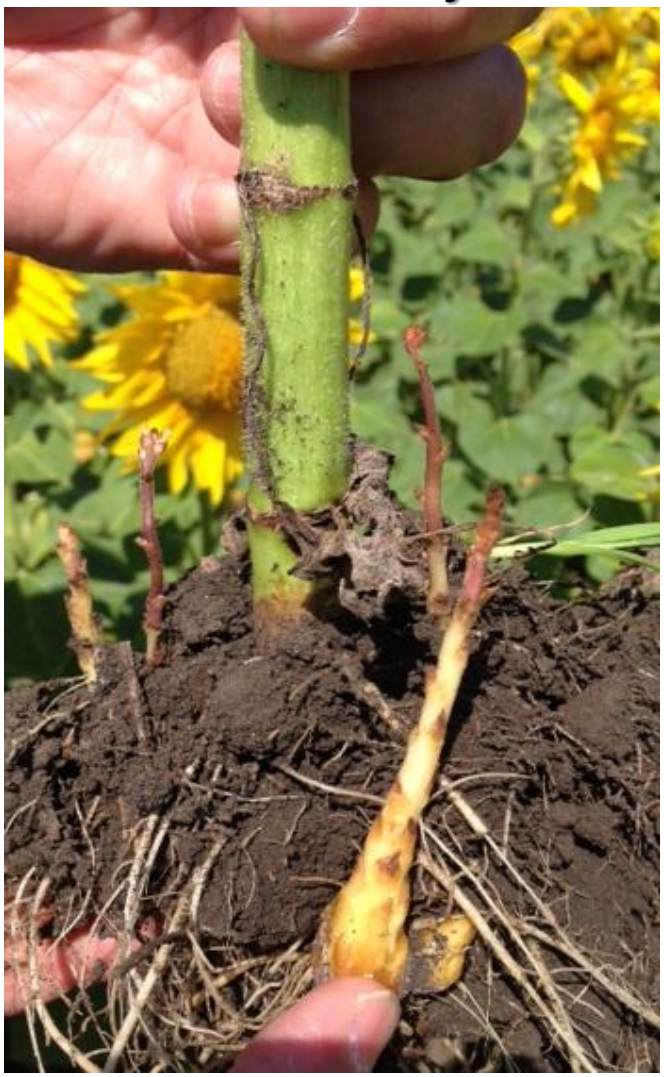
GENETIC RESISTANCE TO BROOMRAPE

System I Resistance

Prehaustorial resistance

Or5, Or7

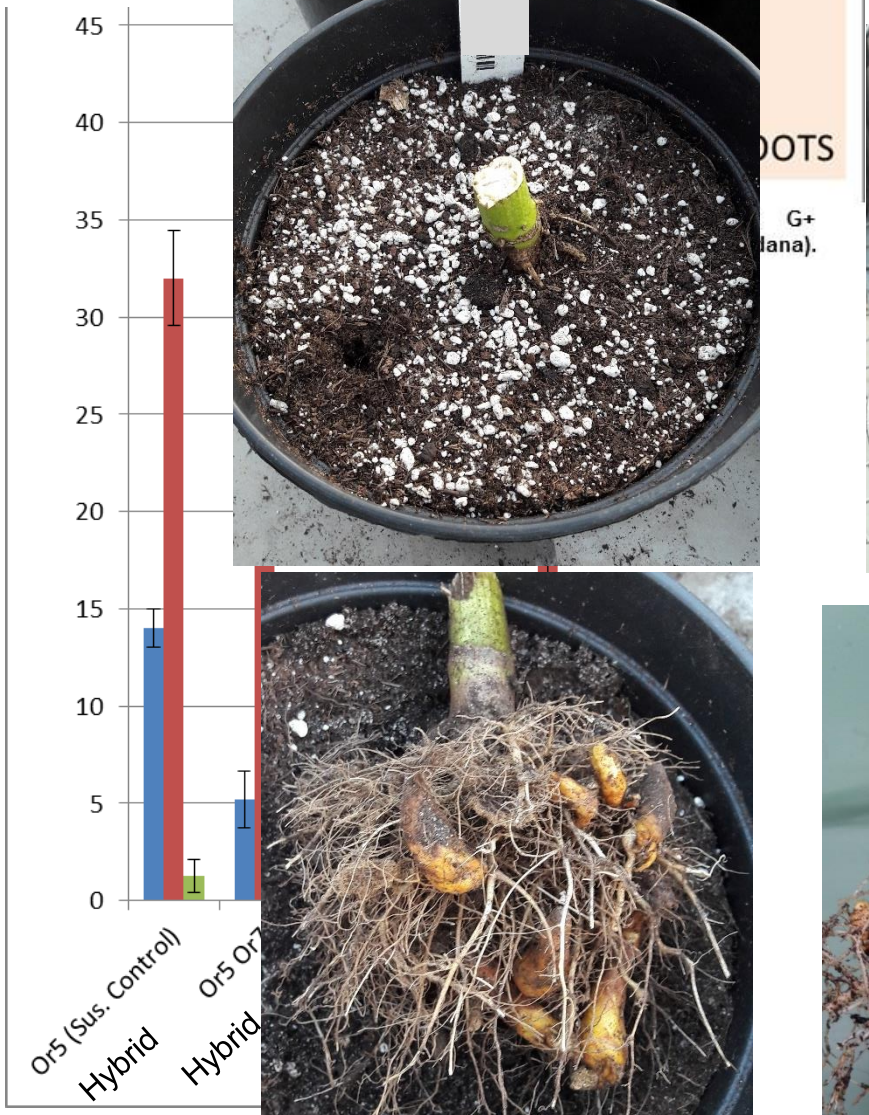
System II Resistance



Posthaustorial resistance

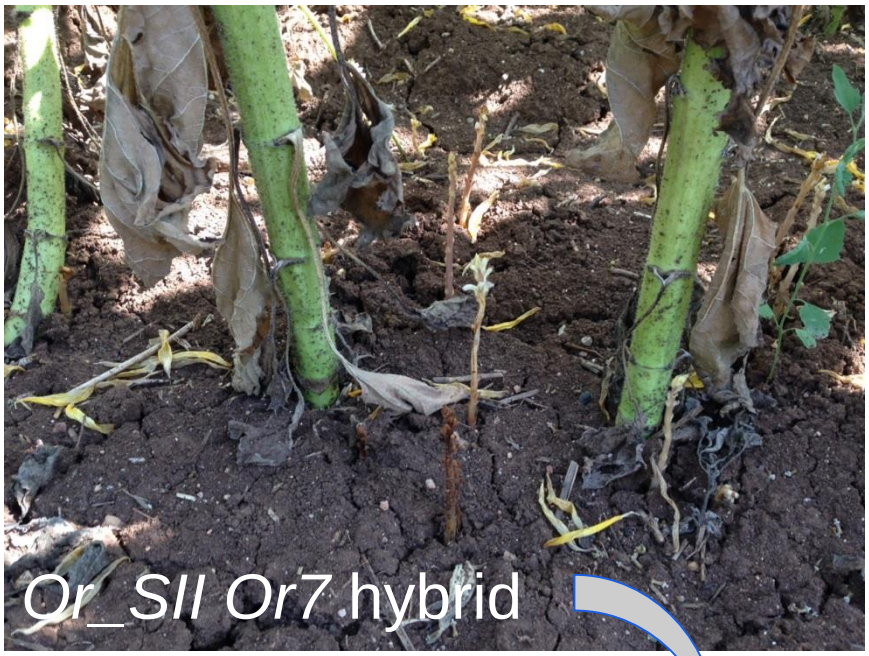
Or_SII

EFFECT OF THE Or_*SII* RESISTANCE GENE IN COMBINATION WITH OTHERS



Race G+ Volgograd

TOWARDS DURABLE GENETIC RESISTANCE: Race G+ scenario



THANK YOU FOR YOUR ATTENTION!