BERGERAC SEED & BREEDING

New progress in breeding and biocontrol dual approaches for tobacco broomrapes management

Anna Malpica, AP30, 13th October
CORESTA 2022

Progress in breeding and agronomical approaches for control of tobacco broomrapes

1. Parasitical plants affecting tobacco crops:
   Broomrapes and Striga
   Physiological cycles and control strategies

2. Broomrape/Striga tolerance breeding

3. Agronomical trials 2016-2022:
   Broomrape, *P. ramosa* biological control with mycoherbicide

4. Conclusion and next steps

Phelipanche ramosa on Tobacco, France 2018

Striga gesnerioides on Tobacco, South Africa 2022, A.Scholtz

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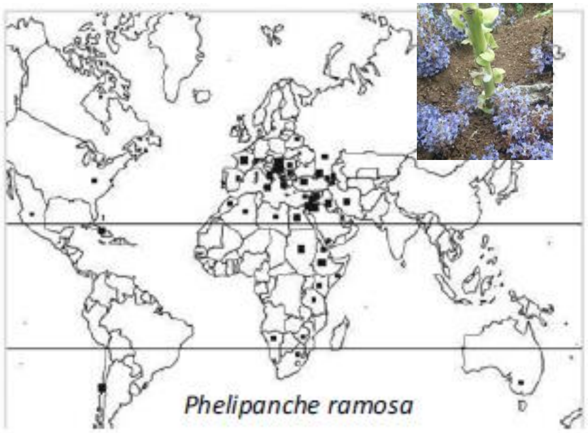
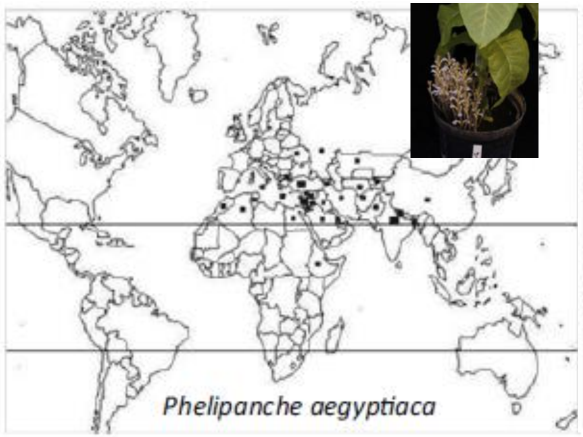
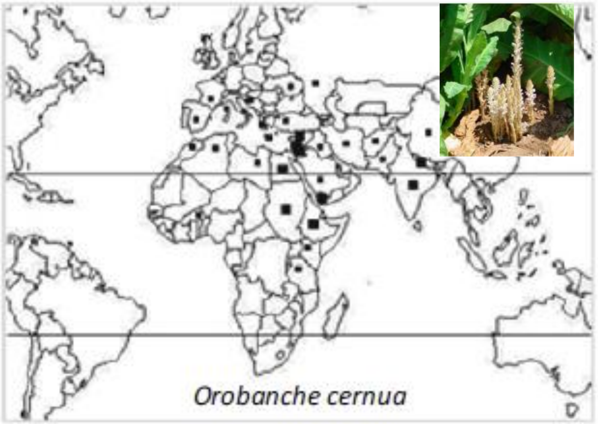
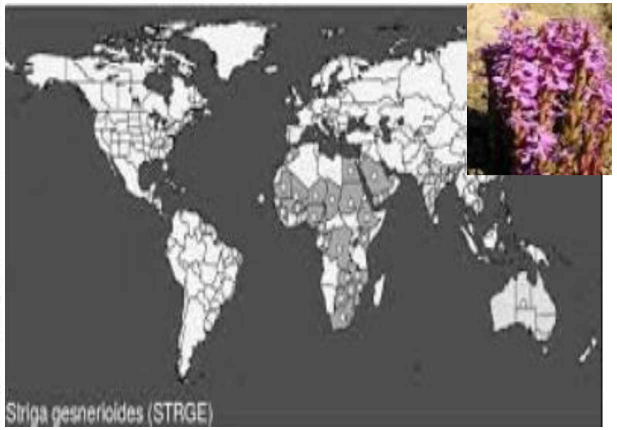
Broomrapes affecting tobacco crops







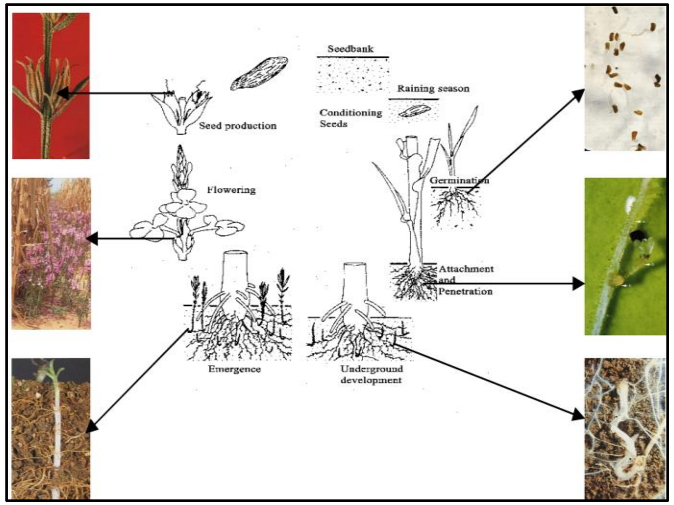


World distribution of broomrape species. Larger symbols indicate countries in which it causes significant crop losses. Smaller symbols indicate countries in which it occurs mainly on wild hosts,
Parasitic Orobanchaceae, Joel & al, 2013

World distribution of *Striga gesneriodes*. EPPO global database

Host range: tomato, eggplant, tobacco, pepper, rapeseed, hep, lentli, pea, carrot, celery, lettuce + wild hosts **Economic importance:** tobacco seriously affected in Europ, Cuba. 70-80 % biomass loss.	**Host range:** same than P. raosa + Brassicas (mustard in India) + Cucurbitaceous Distribution of P. aegyptiaca overlaps with P. ramosa **Economic importance:** severe damages (not quantified) in Turley and Israel.	**Host range:** Solanaceae crops (tomato, eggplant, tobacco) **Economic importance:** it has become a severe problem in India and Pakistan. About 40000ha devoted to tobacco in Andra Pradesh state have been infested.	**Host range:** cowpea, tobacco, sweet potatoe, wild hosts **Economic importance:** it occurs very locally on tobacco in South Africa, Ethiopia, Zimbabwe

Broomrapes and Striga parasitic cycles



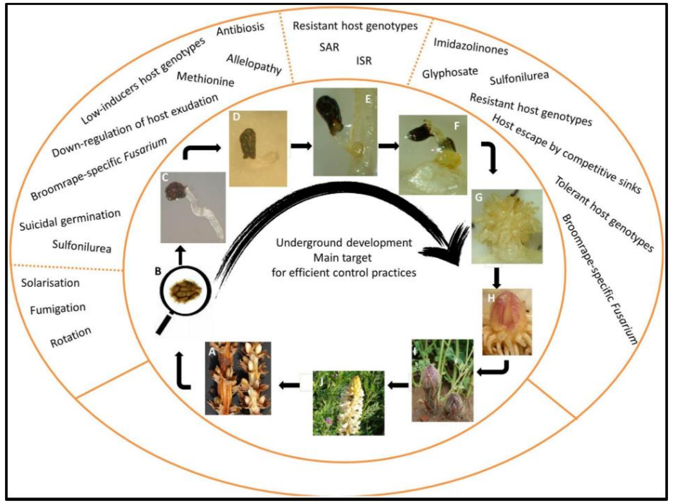
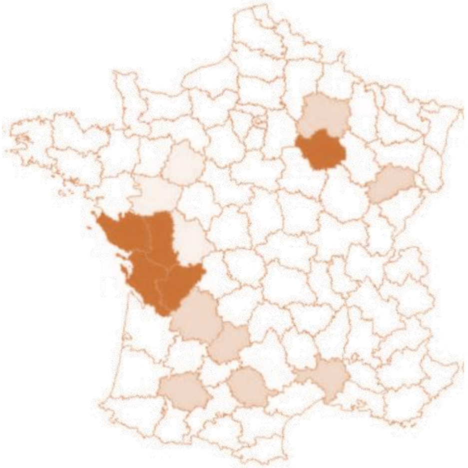


Illustration of broomrapes and Striga life stages



20% of cultivated surface contaminated in France (rapeseed, sunflower, hemp, tobacco)

- broomrape is an obligate parasitic plant
- Striga is an obligate hemi-parasitic plant (partially capable of photosynthetic activity)
- both parasitical crop cycles overlap the host plant cycle
- underground parasitism
- small, long-lasting, and hardly destructible seeds

→ Not controlled by management strategies designed for nonparasitic weeds (i.e., use of agro-chemicals)

→ Points of vulnerability (after germination) are reviewed as inhibition targets of the broomrape-tobacco association

Broomrape, control strategies

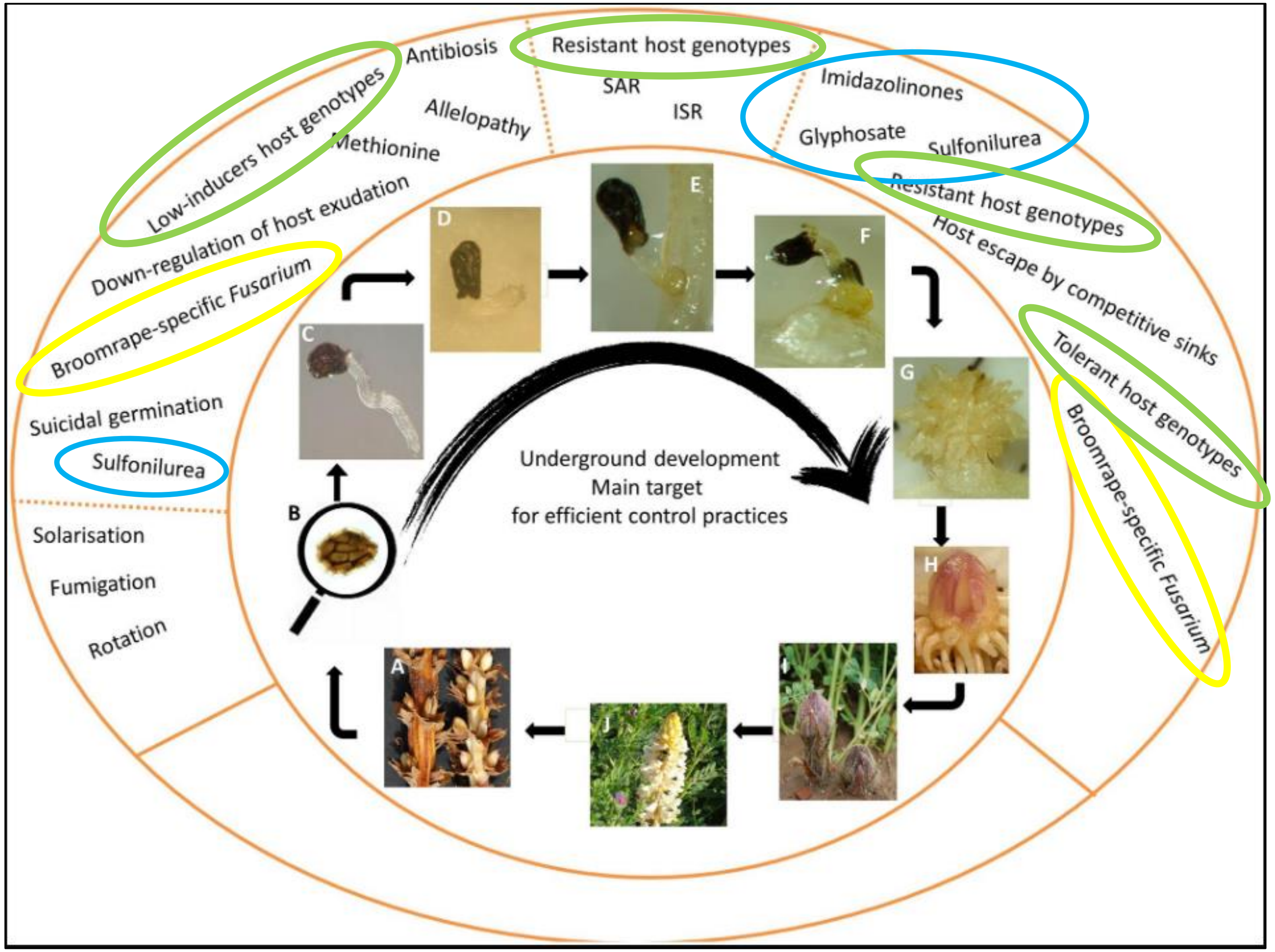


Illustration of broomrape life stages and mechanisms of control. Fernandez-Aparicio et al. 2016

Chemical control of post attached parasites

- in 2015: soil acidification, menthol oil application, imazamox, MH
- 2016: rimsulfuron, sulfosulfuron, Pilot (quizalofop-P-éthyl), Foly R (clethodim)
- 2017: rimsulfuron, sulfosulfuron, methionine, laminarine
- in 2018: rimsulfuron, amidosulfuron, Fresco (metobromuron), Bion, Laminarine

→No fully satisfactory results obtained
(Results available on demand)

Use of intercropping solutions:
Trifolium squarrosum, Lotus corniculatus
→Not easy to integrate to tobacco rotations in France

Breeding for tolerant/resistant varieties to broomrape invasion: 2016-2022

Development of mycoherbicides as biocontrol agents: 2017-2022

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Broomrape, tolerance breeding

P. ramosa on Tobacco, France 2018

Broomrape tolerance breeding: *P. ramosa*

- Wika, shows later/lower stimulation of broomrape (*Pelipanche ramosa*) seeds and capacity to grow in presence of broomrape, this tolerance is recessively inherited (Cailleteau et al., CORESTA 2006)
- Breeding lines were developed by conventional breeding from Wika tolerance.
- Development of a molecular marker, on chm 14, by Imperial team, TWC-2018

ITB 31612

Wika

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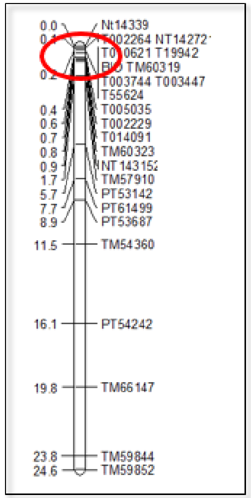
www.imperialbrandsscience.com



Imperial Tobacco

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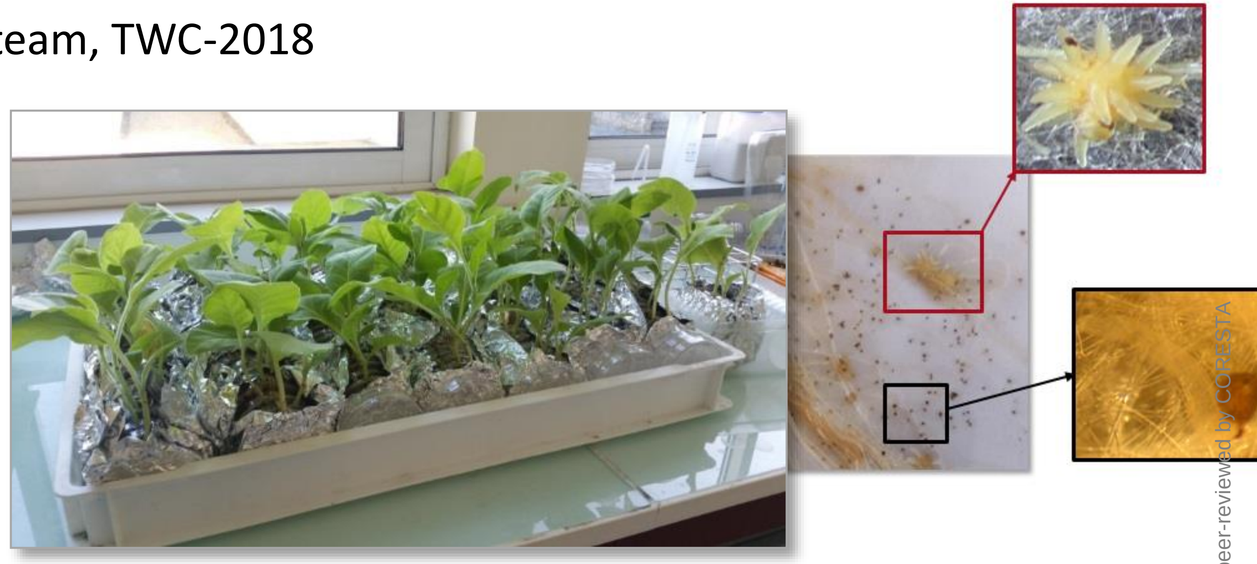
RNA-Seq analysis of Orobanche resistance in tobacco: development of molecular markers for breeding recessive resistance from Wika tobacco variety.

Julio, E. (1) ; Malpica, A. (2) ; Cotucheau, J. (1) ; Bachet, S. (2); Volpatti, R. (1); Decorps, C. (1); Dorlhac de Borne, F. (1).

(1) Imperial Tobacco Limited, Leaf research, La Tour, 24100 Bergerac, France

(2) Bergerac Seed and Breeding, La Tour, 24100 Bergerac, France



Broomrape tolerance breeding:
P. ramosa

		Fixation count	germination %	conclusion
2021	S control	3,75	5	S
2021	ZZ100 tolerant candidate	0,33	0,6	R
2021	Wika tolerant control	0	0	R
2021	F1 S control x ZZ100	1,3	7,3	S
2021	F1 Wika x ZZ100	2,7	7,7	S

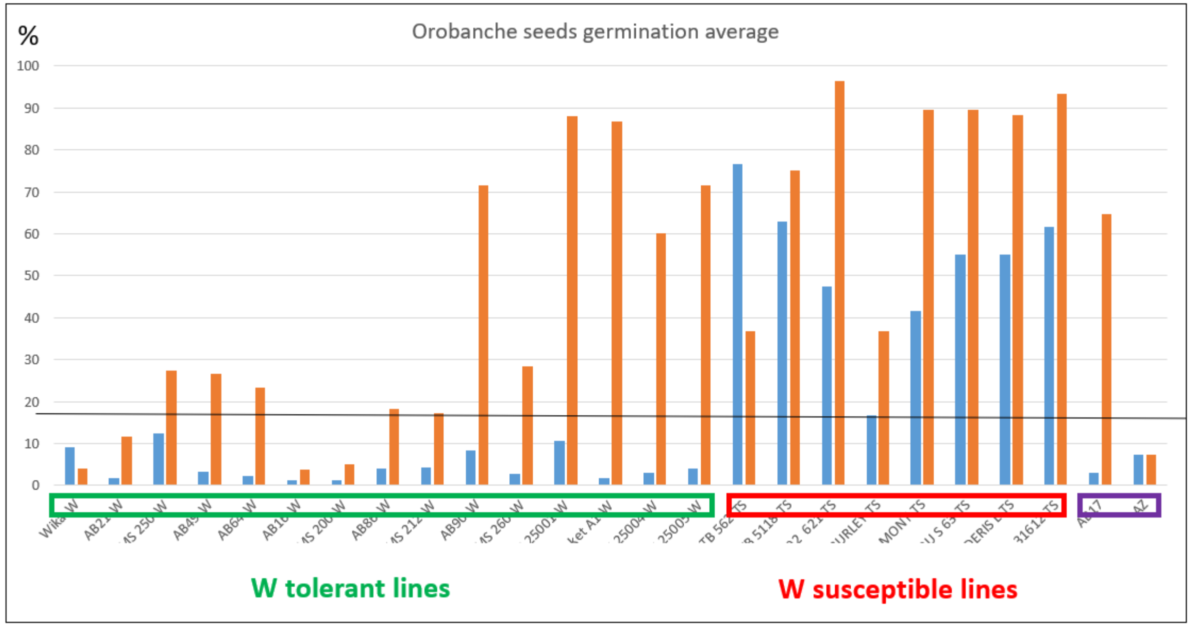
		R %	S %	conclusion
2021 test on 190 plants	F2 S control x ZZ100	25%	75%	1 recessive gene
2022 test on 200 plants	F2 Wika x ZZ100	37%	63%	2 indepedent genes?

- ZZ100 tolerance seems linked to 1 recessive gene as Wika's
- ZZ100 and Wika tolerance genes are probably different

→ Tests will be repeated for validation in 2023

→ Breeding populations with ZZ100 are launched

Broomrape tolerance breeding:
O. cernua



Lines with and without Wika tolerance have been tested in Petri dishes tests with *P. ramosa* inoculum and *O. cernua* inoculum

- Susceptible lines *to P. ramosa* are also susceptible to *O. cernua*
- Within group of lines with Wika tolerance all are tolerant to *P. ramosa* but most of these are fully susceptible to *O. cernua*
- 1 new Burley accession from North Europe origin is showing tolerance to *O. cernua*

		Fixation count	germination %	conclusion
2021	**S control**	10,6	68,33	S
2021	**Zerlina, tolerant candidate**	0,5	4,3	R
2021	**F1 S control x Zerlina**	3,5	40,25	S

		R	S	
2021 test, on 145 plants	**F2 S control x Zerlina**	13%	88%	2 genes? 1

		Fixation count	germination %	conclusion
2022	**S control**	7,6	26,16	S
2022	**Zerlina, tolerant candidate**	3,1	9,3	R
2022	**F1 S control x Zerlina**	26,8	53,83	S

		R	S	
2022 test, on 150 plants	**F2 S control x Zerlina**	34%	66%	1 recessive?

- Zerlina seems tolerant to *O. cernua*, with a lower tolerance level than Wika front to *P. ramose*
→ Will this tolerance level be enough and useful in contaminated field conditions?

- Zerlina tolerance is recessive and may be controlled by 1 or 2 genes

Striga tolerance breeding

- Test of Wika and ZZ100 tolerant lines behavior under an in vitro test with Striga inoculum:

		ORO FR												
		Plant 1		Plant 2		Plant 3		Plant 4		Plant 5		Plant 6		
N° ordre	var	Nb fixation	% germination	Nb fixation	% germination	Nb fixation	% germination	Nb fixation	% germination	Nb fixation	% germination	Nb fixation	% germination	
STRIGA 2	WIKA	0	2	0	3	0	3	0	2	0	2	1	10	R
STRIGA 3	RUBY (Wika tol variety)	0	1	1	3	1	3	0	2	2	2	0	0	R
STRIGA 4	ZZ100	7	52	18	60	25	55	1	4	0	11	0	9	S-
STRIGA 5	(susceptible control)	52	85	51	80	42	70	54	95	60	90	22	75	S

→Future test for Zerlina?

→Future field tests?

Progress in breeding and agronomical approaches for control of tobacco broomrapes

Agronomical trials 2016-2022:
Biological control with mycoherbicide

INRA SCIENCE & IMPACT

Stéphanie Gibot Leclerc
Christian Steinberg
Carole Reibel
Lucie Guinchard
Nadine Gautheron
Veronique Eddel-Hermann

P. ramosa on Tobacco, France 2018

Biological control with mycoherbicides

Harvest of broomrape plants with fungus symptoms for creation of a collection of fungal candidates with potential mycoherbicide effect.

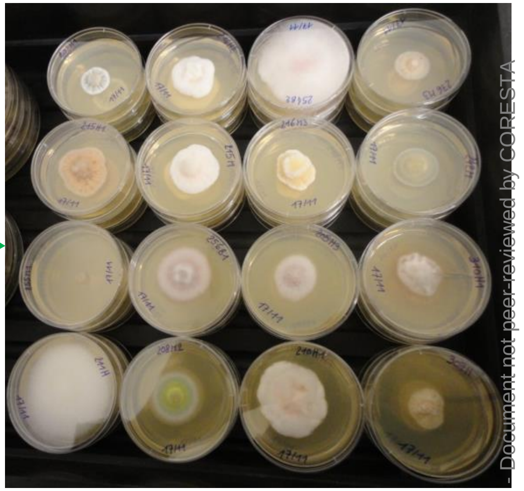
A total of 573 samples collected in 2017 and 54 samples collected in 2018

Isolation on acidic malt + antibiotic media of fungal population collected

Morphological and taxonomical characterization of isolated population

→ **525 fungal strains isolated**

→ **An important diversity dominated by genus *Fusarium***

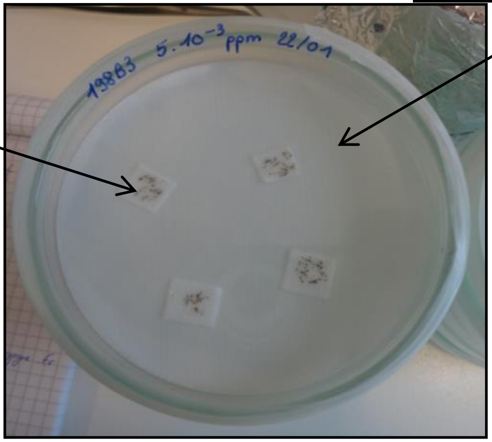


Biological control with mycoherbicides

Müller-Stöver method

Disinfected non dormant broomrape seeds

Filter with 3 mL fungal suspension



For each fungal strain: 2 Petri dishes with GR24 and 2 Petri dishes without GR24

Evaluation of germinated broomrape seeds after 3 weeks test
→Selection of the 20 most efficient strains (> 75 % of germination inhibition)

Koch pathogenicity test

Fo47 (souche témoin non pathogène)

Strain 2876

Non-inoculated control

From the lab to the field

Preparation of an inoculum which can be brought to the field

→ liquid inoculum cultivated on liquid culture medium

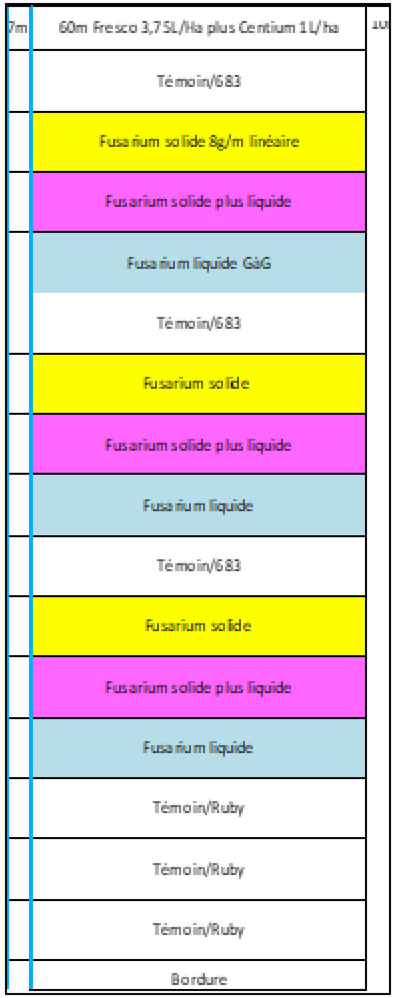
→ solid inoculum cultivated on millet

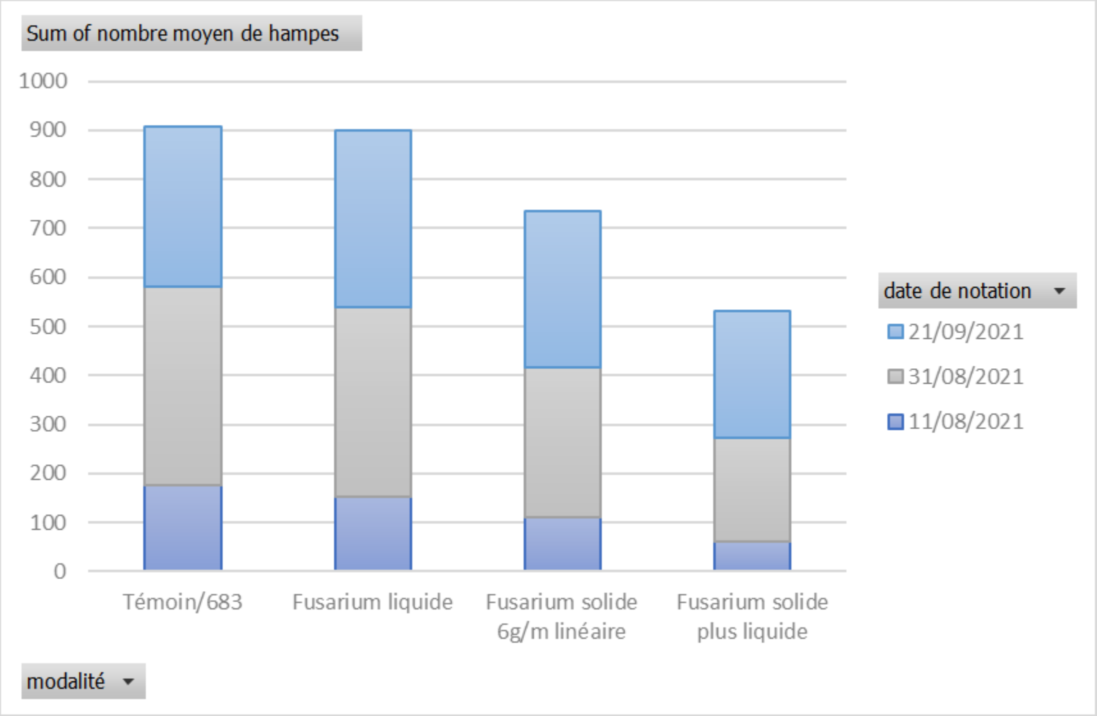
From the lab to the field

2021 trial design,
1 strain selected

3 modalities:

- solid inoculum
- liquid inoculum
- solid + liquid inoculum





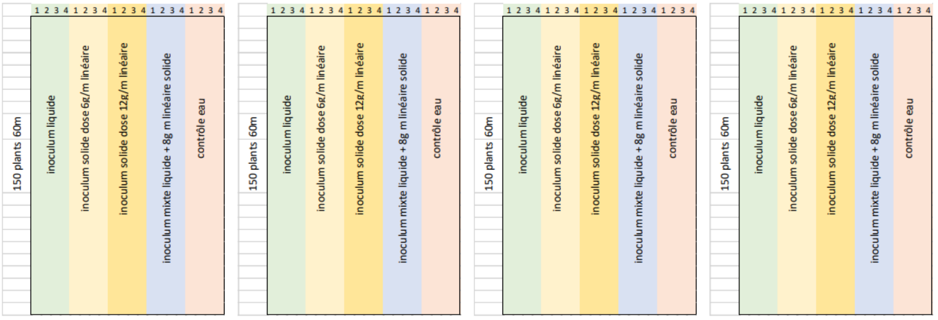
Conclusion

Parasitical plants represent a challenge for impacted growers as no traditional chemical control strategy appears fully efficient.

→breeding tolerances: active work for introduction within commercial varieties

parasitic plant	Breeding strategy	Breeding status	Next breeding step
P. ramosa	Introgress high level of tolerance in elite lines	Commercial varieties available for filler flu-cured tobaccos and aromatic burley tobaccos	Cumulate Wika tolerance and ZZ100 tolerance for a potential increase of stability and intensity of tolerance
O. cernua	Introgress high level of tolerance in elite lines	Selection of elite lines on going, no commerical variety available yet	Propose fixed varieties in Burley tobacco
Striga gesnerioides	Explore	First screening steps	Validate tolerance interest in local infected areas

→biocontrol myco-herbicide strategy, a hope for future, trialing intensification in 2022



Bloc 1 –souche 1 | Bloc 2 –souche 1 | Bloc 1 –souche 2 | Bloc 2 –souche 2

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