Decreased fitness of herbicide resistant weeds suggests options for management.
Case study: *Echinochloa crus-galli*

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SEVENTH FRAMEWORK PROGRAMME

Background

- ***Echinochloa crus-galli***

  - Annual weed, grows well in organic soils; hexaploid, reproduces by seed, predominantly self-pollinating, has a C4 photosynthetic cycle;

  - very successful competitor and a prolific seed producer which often result in important soil seed bank, i.e. a healthy plant can produce up to more than 400,000 seeds;

  - particularly troublesome when infesting monoculture cropping systems (i.e. maize, rice)

- **Resistance to acetolactate synthase (ALS) inhibitors:**

  - widely used since they were first introduced in 1982;

  - their recurrent use has resulted in a rapid resistance evolution identified in populations of many weed species, both monocots and dicots.

Rationale and aim

In several areas of Europe herbicide resistance is widespread and seed banks mostly contain “resistant” seeds.

To be able to reverse this situation would significantly contribute to increase the sustainability of the affected cropping systems.

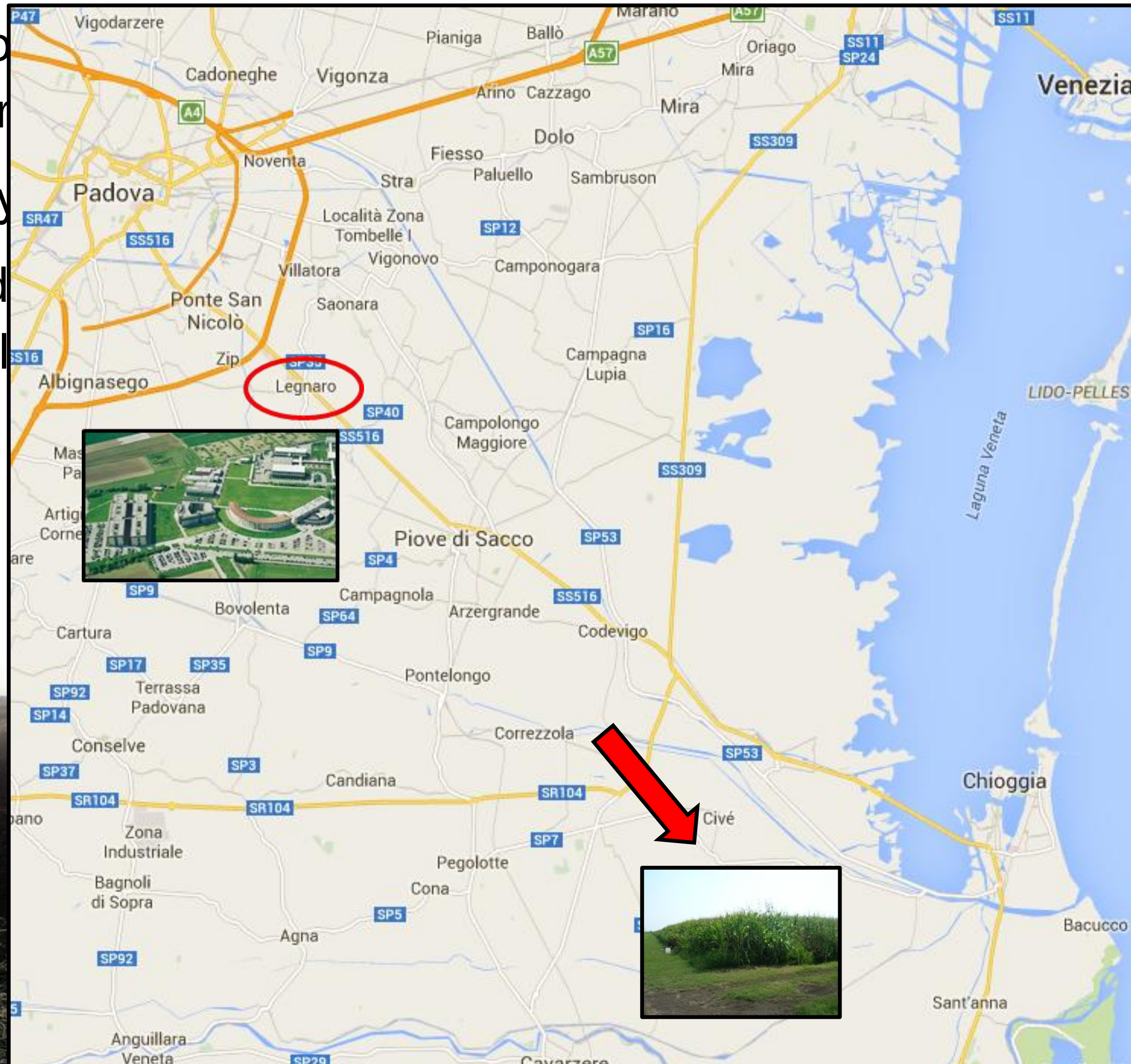
To check whether the herbicide resistance evolution process may be reversed

To remove the herbicide selection pressure ← This means to be able to manipulate R/S plants ratio → To exploit any possible fitness penalty of R biotypes

The cropping system

Continuous maize since end ‘50s, located in the Veneto region, NE Italy (N 45° 09’ 27.2”, E 12° 09’ 12.7”, -1.6 m b.s.l.)

- no sp… …eping the water…
- highly…
- weed… …tant site, mainl…



The case study

***Echinochloa crus-galli* resistant to ALS inhibitors:**

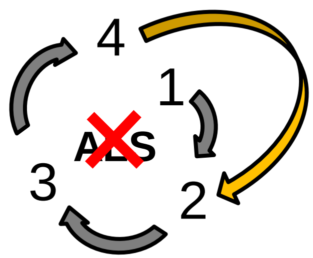
- first problems in the farm in 2002; treated two times per year with nicosulfuron 60 g a.i. ha^{-1} at least since 2000;
- outdoor and pot experiments confirmed that the pop. is highly cross-resistant to all ALS inhibitors (SU, IMI, TP, PTB) with RI ranging from 15 to >32

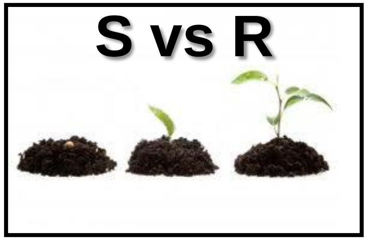


Experimental framework

Three-year field experiments

Two topics were investigated

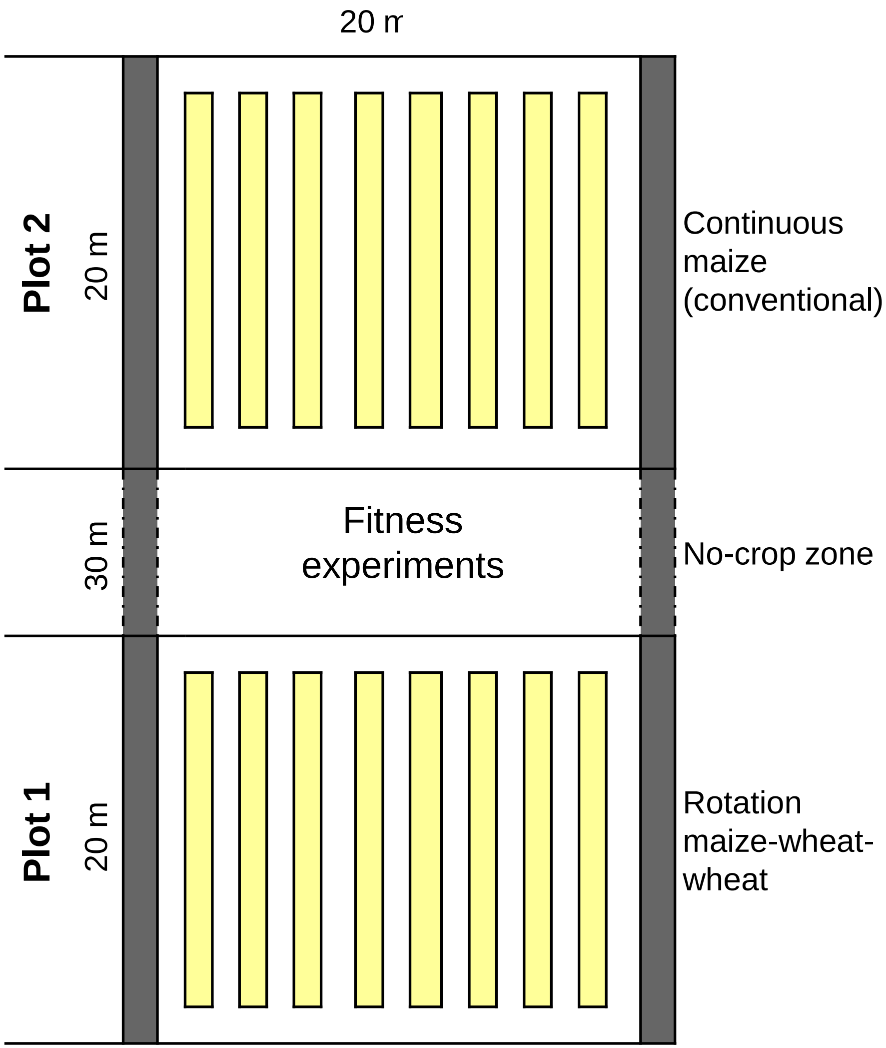




1. Rotation related experiments: to follow the evolution of R/S alleles ratio removing ALS herbicides and introducing a crop rotation

2. Fitness related experiments: to investigate fitness differences in resistant (R) and susceptible (S) plants of *E. crus-galli* with similar genetic background

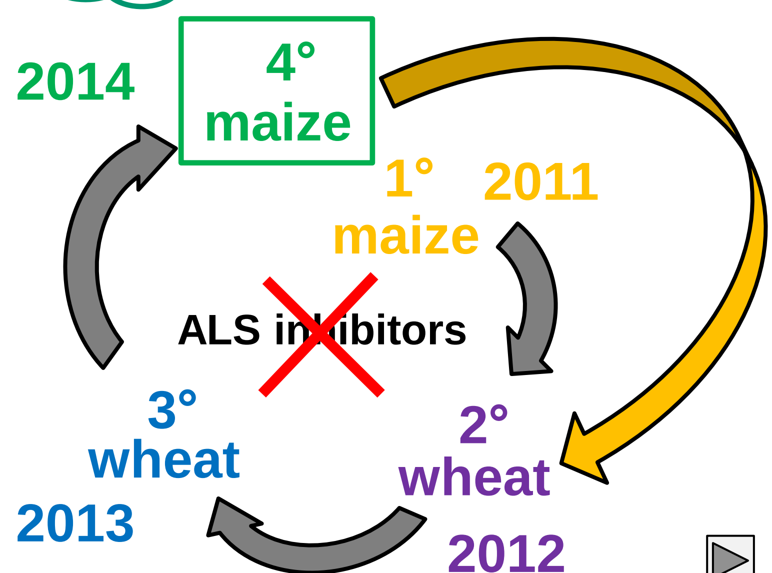
1. Rotation experiment



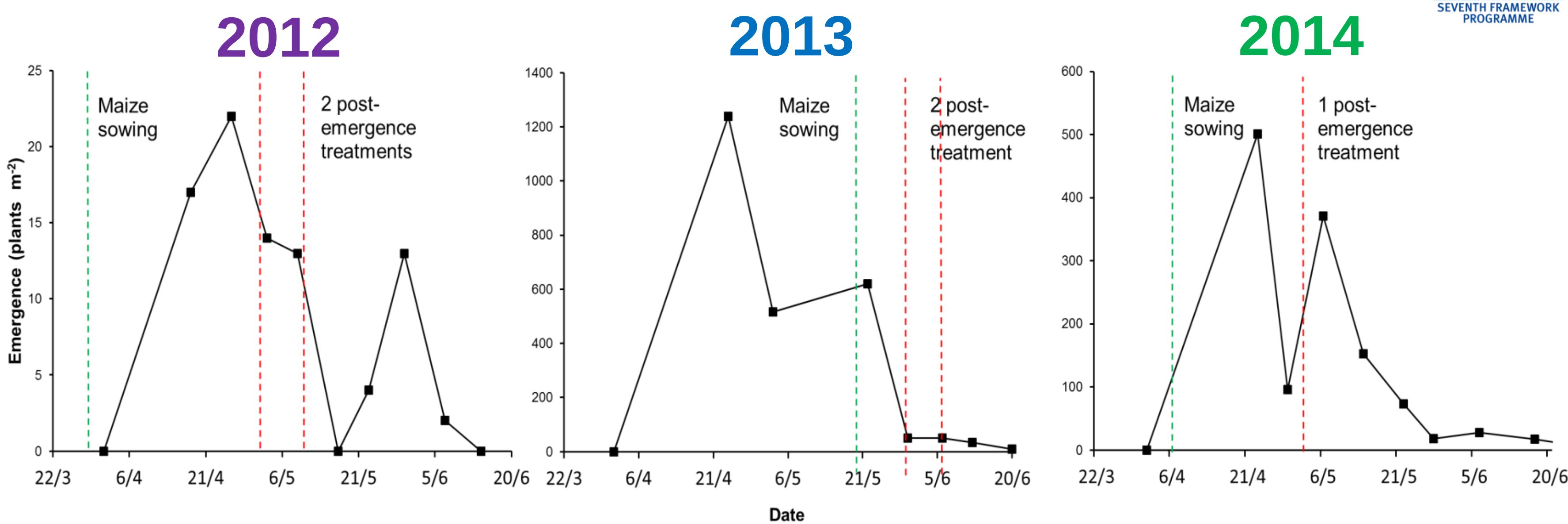
Plot 2

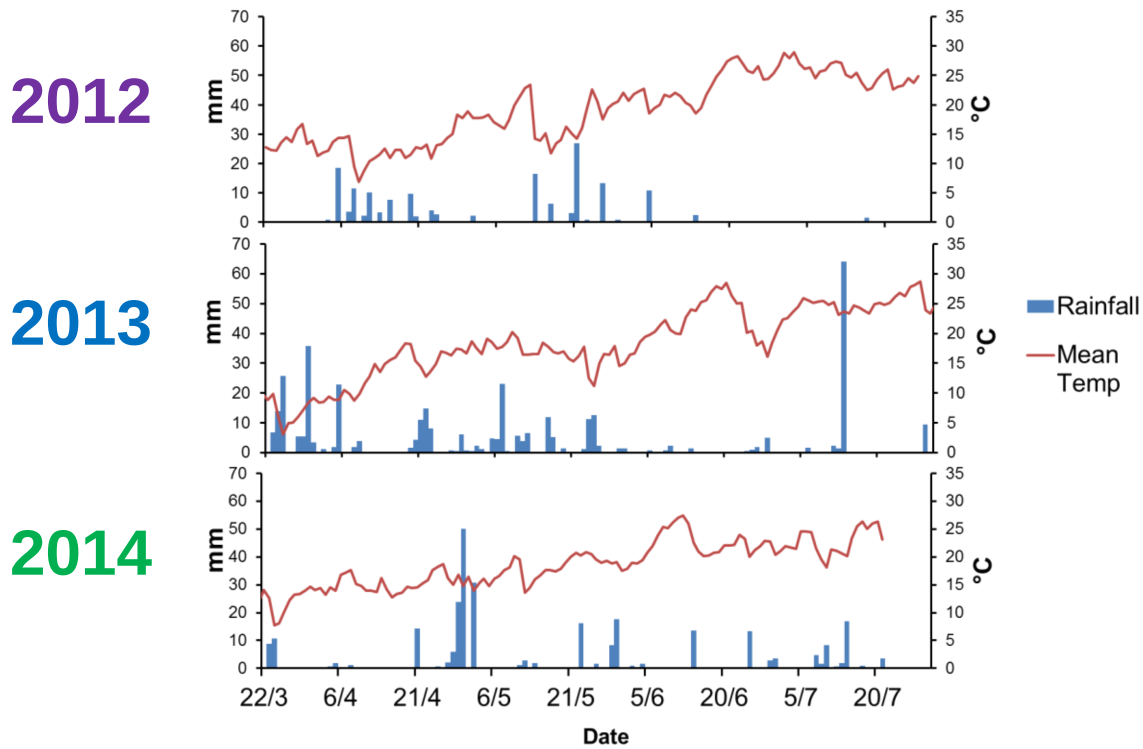
Continuous maize → best herbicide resistance management implemented by the farm

Plot 1



Echinochloa emergence dynamics





Weather conditions were very different in the three years:

- Maize sown more than 1 month later in the 2013
- No emergence of *Echinochloa* after herbicide treatments in 2013
- Cumulate rainfall: 256 mm in 2012, 520 mm in 2013 and 260 in 2014

Monitoring of weed flora

Scouting method: 13 rectangles (total 1 m^2) were randomly positioned and then monitored inside the plot 2 (continuous maize) in 2012 and 2013 and in both plots in 2014, before and 4 weeks after the last weed control operation

	2012			2013		
	Echinochloa crus-galli	Other weeds	TOTAL	*Echinochloa crus-galli*	Other weeds	TOTAL
Before treatments (plants m^{-2})	77 (61%)	50	**127**	232 (79%)	3	**295**
After 2 post-emergence treatments (plants m^{-2})	52 (63%)	31	**83**	11 (58%)	8	**19**

2014

	Continuous maize			Rotation (maize)		
	Echinochloa crus-galli	Other weeds	TOTAL	*Echinochloa crus-galli*	Other weeds	TOTAL
Before treatments (plants m^{-2})	178 (89%)	22	**200**	243 (29%)	600	**843**
After 1 post-emergence treatment (plants m^{-2})	68 (80%)	17	**85**	27 (21%)	101	**128**

Monitoring of weed flora

Resistance status

Monitoring of % of resistant *Echinochloa crus-galli* plants at the begin and at the end of four-year project in the two situations

2011
2012

	Maize conventional	
	August 2011	**April 2012**
No. of plants sampled	369	513
Nicosulfuron-R plants (%)	98.4	97.2

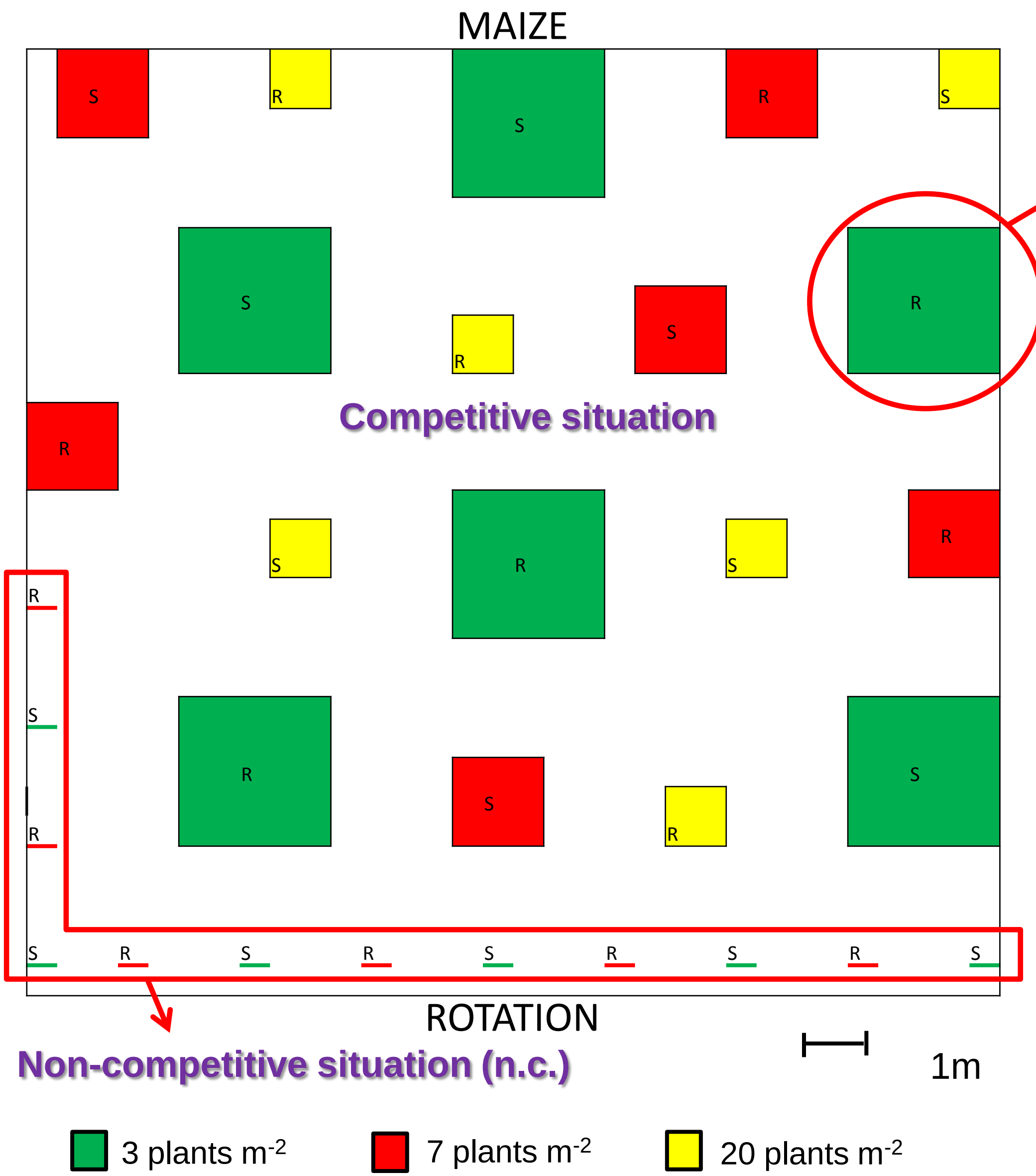
2014
(April)

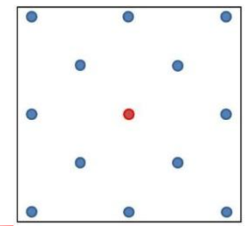
	Maize conventional	**Rotation (maize)**
No. of plants sampled	236	198
Nicosulfuron-R plants (%)	94.5	90.1

Most likely, only a rotation cycle is not enough to induce a significant shift in the ratio of R/S alleles

2. Fitness experiments

Experimental design





Layout of a thesis
In red the target plant and in blue the plants of the other biotype.

- Two biotypes (S and R) pre-selected by herbicide treatment and reproduced;
- 12 S and 12 R target plants in four different densities (spaced, 3-7-20 plant m^{-2}), surrounded by 12 equally spaced plants of the other biotype, were considered;
- panicles of the target plants were counted weekly and covered just prior to onset of seed shatter (in 2012 and 2013);
- target plants were harvested and dry weight of different plant parts (stem, leaves and panicles) were recorded.

Molecular and chemical analyses

Dose-response results

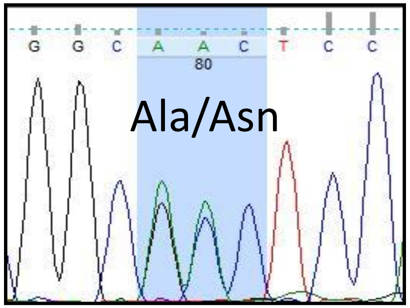
Pop	nicosulfuron LD$_{50}$ (g a.i. ha^{-1})	RI	penoxsulam LD$_{50}$	RI	bispyribac-Na LD$_{50}$	RI	imazamox LD$_{50}$	RI
	Plant survival							
S	20 (0.6)		12 (1.0)		11 (1.0)	-	7.5 (0.4)	-
R	383 (30)	19	>653	>56	>490	>42	>640	>85

Broad cross-resistance to all ALS inhibitors tested

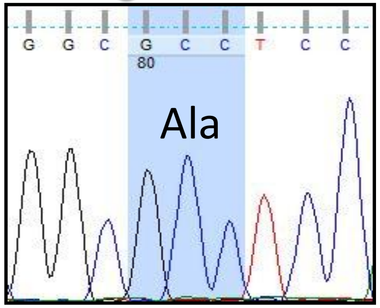
Position 122 of ALS gene

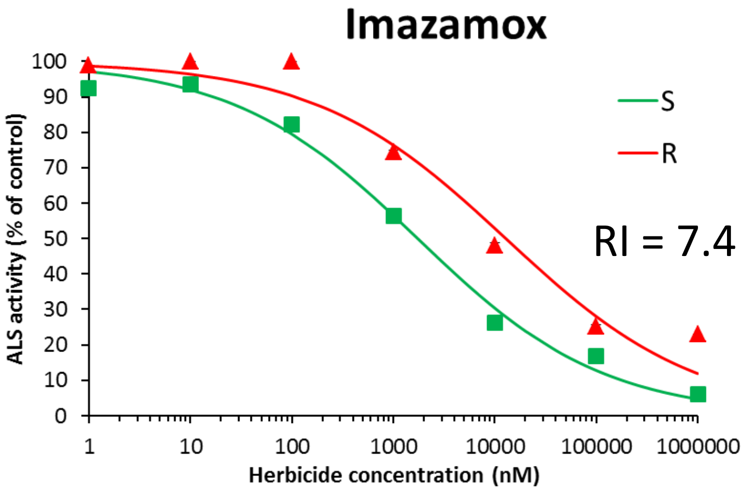
A target-site resistance mechanism is responsible for the high levels of resistance detected.
No non-target-site mechanisms of resistance seem to be involved.

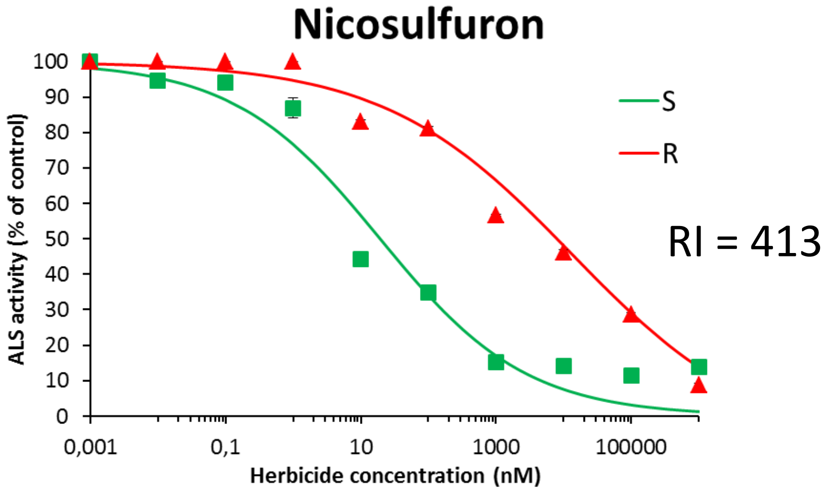
R



S







Phenology

Beginning of flowering:

2012

	Spaced	plants m^{-2} 3	7	20
S	10/7	12/7	12/7	25/7
R	17/7	20/7	30/7	30/7
Δ (d)	7	8	8	5

2013

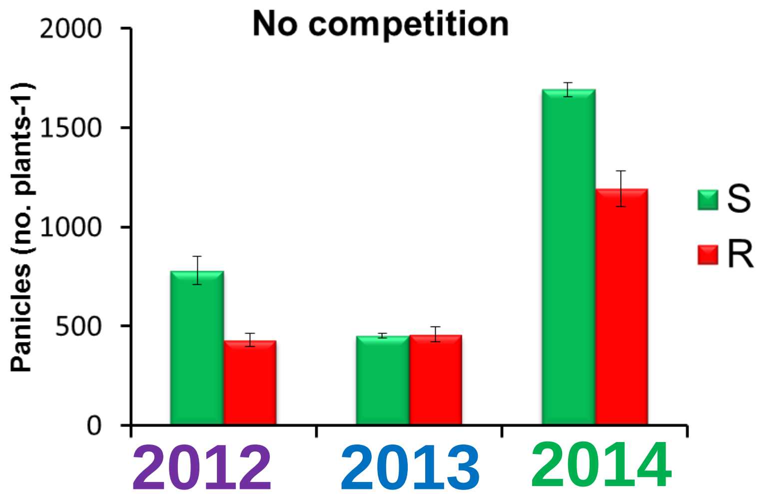
	Spaced	plants m^{-2} 3	7	20
S	05/8	01/8	05/8	01/8
R	13/8	08/8	13/8	13/8
Δ (d)	8	7	8	12

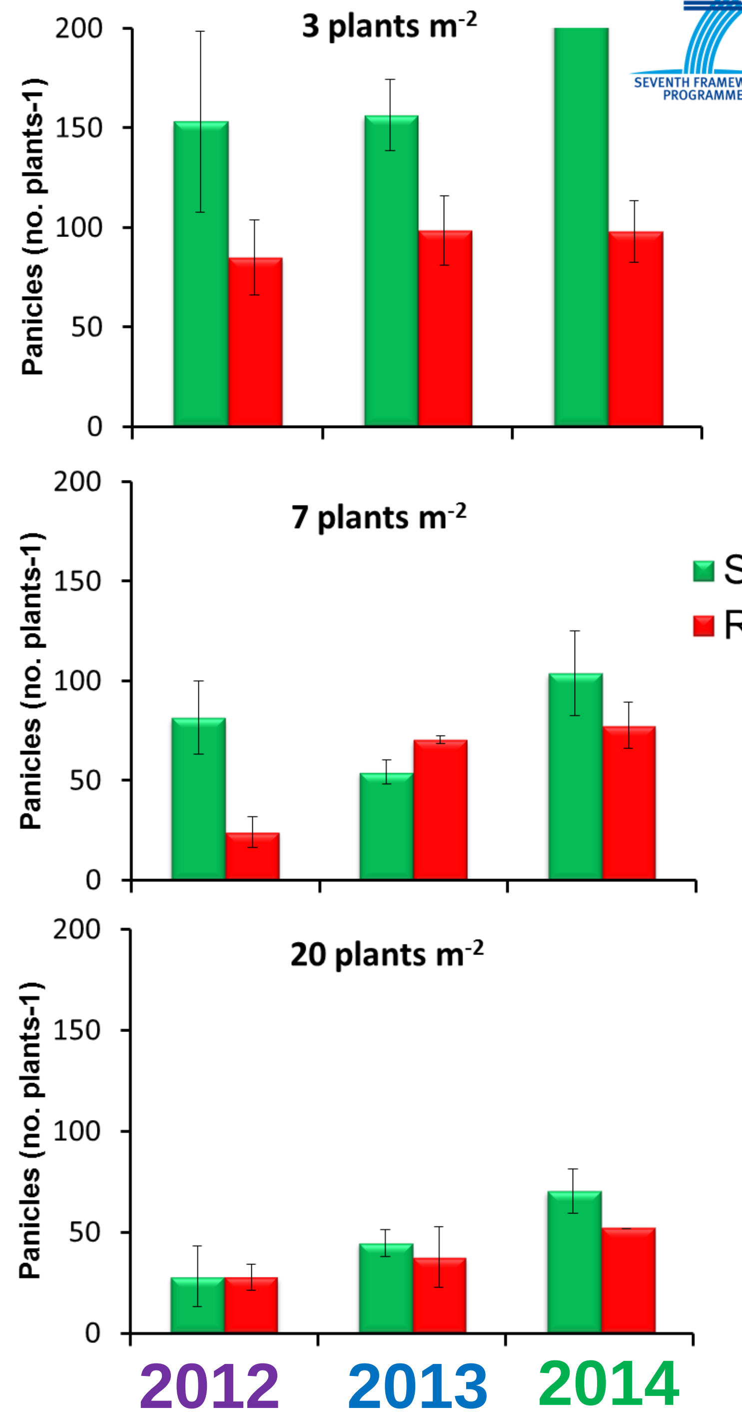
2014

	Spaced	plants m^{-2} 3	7	20
S	26/6	29/6	29/6	1/7
R	29/06	01/7	9/7	9/7
Δ (d)	6	2	10	8

Growth analysis

Panicles number at harvest





In 2012 and 2014, S plants produced a higher number of panicles than the R ones at all densities considered,
In 2013 results were different for n.c. and 7 plant m^{-2} thesis.

- A fitness cost is present in the R biotype, but it is strongly affected by weather conditions and, in particular, by rainfall and sowing time
- The fitness cost disappears at high plant densities

Summarizing

- *Echinochloa crus-galli* is the most problematic weed in the experimental field and its emergence and control are influenced by climatic conditions (rainfall and temperature);
- The high resistance levels are associated to a target-site resistance mechanism due to the double mutation GC-AA in position 122 of the ALS gene;
- A fitness cost was detected in the R biotype, but it is strongly affected by climatic conditions and influenced by competition level:
    - different time of plant development and flowering was confirmed for S and R biotypes,
    - differences in seed production for the two biotypes were linked to the seasonality,
    - the fitness cost phased out at high plant densities;
- Crop rotation, and the removal of the ALS selection pressure, only slightly affected the ratio between R and S biotypes.

Final considerations

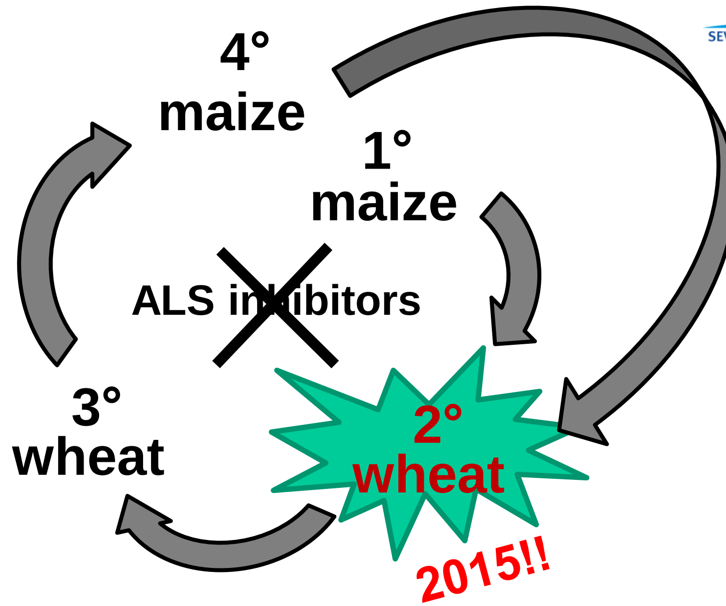
Good starting point to develop a management strategy based on population dynamic

Most likely, only a rotation cycle is not enough to induce a significant shift in the ratio of R/S alleles

How can the fitness penalty of R biotype be exploited?

- Use of a different crop rotation (es. maize-wheat-soybean)
- Delayed sowing of the summer crops
  → the use of a late-sown summer crop coupled with stale seed bed preparation could improve the depletion of the seed bank
- Early harvest: i.e. growing silage rather than grain maize
  → the anticipation of the harvest decrease the spreading of resistant seeds

THANK YOU FOR THE ATTENTION!



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