Imazamox-resistant red rice (*Oryza sativa* L., var. *sylvatica*) in Italian Clearfield rice crop: monitoring and risks

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Rice cultivation in Italy

- Relatively high value crop
- Largest rice producer in Europe, around 230,000 ha
- Mainly in the north-western Italy
- Average farm size around 50 ha
- Continuous rice cropping is predominant
- Intense herbicide use
- Direct seeded
  - 70% paddy rice
  - 30% sown in dry fields

Data from: [www.enterisi.it](http://www.enterisi.it)
Red rice (*Oryza sativa* var. *sylvatica*)

Weed infesting the cultivated rice (*Oryza sativa* L.)

Highly competitive:
- Tall plants
- High number of tillers
- High seed shattering ability
- Long seed dormancy
- Grains often have red pigmented pericarp
- More efficient N uptake

Lower grain quality, optical selection during processing

Lower yield
Herbicide-tolerant rice varieties

- Red rice infestation has increased significantly since 1990
  - contamination of commercial seed lots
  - introduction of less competitive semi-dwarf indica-type rice varieties

- Lack of selective herbicides

Herbicide-tolerant rice varieties: a tool to selectively control of red rice in post-emergence
Clearfield® technology in rice: Italian story

 ✓ 2003 agreement between BASF and Ente Nazionale Risi to develop/introduce this new technology in Italy

 ✓ 2004-2005 Experimental tests with variety CL161

 ✓ 2005 Registration in national list of varieties, LIBERO (31 December 2005)

 ✓ 2007 cultivated area around 17,000 ha

 ✓ Currently around 70,000 ha are cultivated with Clearfield® varieties in Italy, i.e. about 1/3 of the rice cultivated area
Clearfield® technology in Italy implies the use of imazamox (commercial name: Beyond®)

Mode of action: ALS inhibitor

High efficacy against several key weed species in rice: e.g. red rice, *Echinochloa* spp., *Alisma plantago-aquatica*, *Schoenoplectus mucronatus*, *Heteranthera* spp.
Major threat to the sustainability of the Clearfield® technology is that the IMI-tolerant trait might also be acquired by red rice through three possible mechanisms:

(a) selection of spontaneous mutations conferring IMI resistance

(b) cross-pollination between the Clearfield® variety and the wild relatives

(c) simple contamination of Clearfield® seed lots with IMI-resistant off-types originated and selected during the selection process of the variety and then favoured by the positive selection pressure imposed by the IMI herbicide
Guidelines for cultivation provided by BASF

They are attached to the contract that farmers have to sign to cultivate Clearfield® rice

✓ Rotation every two years with conventional rice
✓ Herbicide application: double treatment with Beyond® at equal rate 0.875 L ha⁻¹ (40 g imazamox L⁻¹)
✓ Red rice that escaped treatments should be totally removed

Complete control to avoid the evolution of IMI-resistant red rice populations
Red rice complaint monitoring

From 2010-2013
47 fields in Piedmont and Lombardy regions were sampled

✓ Collected field histories of agronomic practices
✓ Whole plant bioassay to assess the imazamox resistance
✓ Molecular analyses to identify the mutation responsible for the resistance
Resistance assessment in red rice populations collected in 2010

35 g imazamox ha$^{-1}$

Survival
VEB

Population

VEB = visual estimation biomass
010-1, 010-2 and 010-3 susceptible populations
Resistance assessment in red rice populations collected in 2011

35 g imazamox ha$^{-1}$
Molecular results

The 26 imazamox-resistant populations were analysed.

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<thead>
<tr>
<th>Phenotype</th>
<th>Codon 653</th>
<th>Amino acid</th>
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<tbody>
<tr>
<td>S</td>
<td>AGT</td>
<td>Ser</td>
</tr>
<tr>
<td>R</td>
<td>AAT</td>
<td>Asn</td>
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Most of the resistant plants were homozygous at codon 653.

The mutation found in the ALS gene is the same as that present in the IMI-R rice cultivar.

Hybridization events took place in the very first years of cultivation of Clearfield rice varieties.
Current situation of resistant red rice

- 37 imazamox-resistant populations out of 63 tested
- 27 municipalities are affected
Field histories

✓ the rotation of Clearfield® with conventional rice was implemented in 10 fields out of 43.

✓ Clearfield® rice was cultivated for at least 4 consecutive years in 11 fields, with 7 where continuous IMI-tolerant varieties were grown for at least 5 out of 6 years since 2006 and three where Clearfield® varieties had been cultivated continuously for six years.

✓ The latter fields therefore received 12 IMI treatments in 6 years.
Field histories

✓ Farmers did not always follow the guidelines for cultivation: several farms cultivated Clearfield® varieties for more than two consecutive years

✓ Simultaneous presence in some fields of *Echinochloa* spp. and sedges «difficult to control»

✓ Intense selection pressure imposed by ALS inhibitors in general, i.e. more than 95% of rice fields are treated at least once, with ALS inhibitors
Sustainable weed management for Clearfield® technology (1)

- New IMI-tolerant rice varieties and are further increasing the cultivated area
- Use certified seed batches
- Avoid cultivation of IMI tolerant varieties for more than 2 consecutive years
- Rotate where possible with soybean or maize
- Given the rapid evolution of IMI-resistant red rice, it would be worthwhile to re-evaluate the guidelines and recommended to rotate Clearfield® every year
Sustainable weed management for Clearfield® technology (2)

- Red rice that escaped treatments should be totally removed to avoid hybridization
- Prefer a stale seed-bed preparation and then control of seedlings with a burn-down herbicide
- Winter flooding can be used to reduce the red rice seed bank
- Proper weed management should be implemented at cropping system level, i.e. considering several years. It is estimated that more than 50% of the rice fields are affected by other ALS-resistant weeds (*Echinochloa* spp., Alismataceae and Cyperaceae).

**The selection pressure imposed by ALS inhibitors should be reduced**
Intense use of ALS inhibitors: let also not to forget the positive sides......

Thanks for your attention