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Susceptibility to imazamox in Italian weedy rice populations and Clearfield® rice varieties

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Summary

The introduction of imidazolinone-tolerant rice varieties has made selective *Oryza sativa* (weedy rice) control possible. We hypothesise that Italian weedy rice populations have variable degrees of susceptibility to imazamox prior to imidazolinone-tolerant variety introduction. To this end, 149 Italian weedy rice populations collected from fields never before cultivated with imidazolinone-tolerant varieties were tested in a glasshouse-based, whole-plant response screening study. Imazamox was applied to all populations post-emergence at a rate of 70 g a.i. ha^{-1}, resulting in 70-90% shoot biomass reduction in the majority of cases. The results prompted a second study of the seedling dose-response of four weedy rice populations from the initial study group. Three imidazolinone-tolerant and one conventional rice variety were also included. The seedling roots were cut six days after germination and exposed to different concentrations of imazamox. The root re-growth associated with each concentration-exposure was then measured. Imazamox concentrations to inhibit weedy rice root growth by 50% varied by about two orders of magnitude, or between 0.0018 mM and 0.12 mM. Even with this result, imidazolinone-tolerant varieties were at least 31.8 times less susceptible than weedy rice populations, suggesting that Italian weedy rice populations were not tolerant to imazamox before introduction of these varieties.

Keywords: *Oryza sativa*, red rice, herbicide sensitivity, root bioassay

Introduction

Rice is a crop that is key to meeting the present and future demands for high-energy foods, mainly in underdeveloped countries. Weedy rice (*Oryza sativa* L.) is a major threat to rice crop ecosystems throughout much of the world. It is still unclear as to whether this weedy form of rice was a problem during the entire history of rice cultivation. However, documented reports exist in both Europe (Biroli, 1807) and the USA (Craigmiles, 1978) that date to the early 19th century. Reports indicate that as rice cropping systems intensified in these areas, so did weedy rice infestations. Weedy rice also spread significantly when the direct seeding method for rice superseded transplanting after 1960 (Ferrero & Vidotto, 1998). Weedy rice infestations also spread with the planting of rice crop seed contaminated with weedy rice seeds (Agostinetto, 2001). Weedy rice infestations reduce yields, particularly when plant density is high. Direct loss estimates from competition caused by weedy rice in rice fields are about 20% (Fleck *et al*., 2008).

Weedy rice control is difficult because of its genetic and physiologic similarities to cultivated rice (Gealy *et al.*, 2009), which handicaps development of herbicide and weed management practices that are both effective and selective to cultivated rice. For a farmer to avoid weedy rice seedbank enrichment and persistence that enhances weed populations, several preventive measures are at his disposal during planting: use rice seed free of weedy rice grains, improve water management and utilise stale seedbed and crop rotation (Agostinetto, 2001). Post-planting interventions aimed at preventing further dissemination (Dunand, 1993) include using cut- or wipe-bars wetted with non-selective systemic herbicides. However, these interventions may not be effective in large or highly-infested fields.

The use of herbicides for selective control of weedy rice plants in rice post-emergence has become possible with the introduction of imidazolinone-tolerant rice cultivars (Croughan, 1994). Imidazolinones (IMIs) are a class of herbicides that have been used effectively in pre- or post-emergence in rice fields in the Americas and Europe during the past decade. The primary target site of IMIs is the enzyme acetohydroxyacid synthase (AHAS), also called acetolactacte synthase (ALS). It catalyses the first common step in the amino acid biosynthetic pathways of leucine, isoleucine and valine (Tan *et al.*, 2005). Rice cultivars were developed from mutated rice plants with imidazolinone herbicide resistance by classic breeding methods (Croughan, 1994). Later, the technique that used imidazolinone-tolerant rice varieties (IMI-tolerant varieties) in association with imidazolinone herbicides, was patented as the “Clearfield®” rice technology by BASF Agrochemical Products. It was introduced in the mid-1990s and it is now widely adopted for rice weed control.

The approximate area cultivated with IMI-tolerant rice varieties in the USA is 736200 ha (Sudianto *et al.*, 2013) or about 68% of the total USA rice area (USDA, 2013). About 50% of rice varieties planted in southern Brazil (Kalsing, 2012) are IMI-tolerant. Whereas in Italy, the group of IMI-tolerant varieties planted in 2013 represented about 20% of the rice area (Ente Nazionale Risi, 2013).

Weed resistance to ALS-inhibiting herbicides has increased steadily since the mid-1980s (Heap, 2012). To limit selection of resistant weedy rice populations during introduction of IMI-tolerant varieties, several complementary and restrictive stewardship guidelines (Anonymous, 2012) are available for use: purchase only certified seed, use residual herbicides to increase grass control, control all weedy rice escapes, rotate herbicides with alternative modes of action and suspend consecutive-year use of Clearfield® rice in the same field. Despite these tactics, reports of tolerance to imidazolinone herbicides in weedy rice populations shortly after Clearfield® variety introduction have occurred in several regions: Arkansas/USA (Sales *et al.*, 2008), southern Brazil (Roso *et al.*, 2010a; Goulart *et al.*, 2012), northern Italy (Busconi *et al.*, 2012; Scarabel *et al.*, 2012), and northern Greece (Kaloumenos *et al.*, 2013). In Italy, Brazil and Greece, successive cultivation of resistant varieties in most rice fields has encouraged IMI-tolerant weedy rice population development. The problem has been made particularly worse in areas with rich soil seed banks of weedy rice (Roso *et al.*, 2010b; Busconi *et al.*, 2012; Kaloumenos *et al.*, 2013).

Previous studies have focused on weedy rice susceptibility to two non-selective herbicides, imazethapyr and glyphosate (Kuk *et al.*, 2008; Burgos *et al.*, 2011). Roso *et al.* (2010b) described methods to identify imidazolinone-tolerant rice varieties at distinct rice plant growth stages and showed that seed, seedling and tiller bioassays can distinguish tolerant and susceptible plants efficiently. Other authors (Seefeldt *et al.*, 1995; Tind *et al.*, 2009) have demonstrated the efficacy of dose-response studies in determining susceptibility to a given herbicide among a large number of weed populations. The technique can be useful to assess selectivity indices, as is done when comparing the tolerance of IMI-tolerant rice to the susceptibility of non-tolerant rice (Roso *et al.*, 2010b) and weedy rice populations.

Our interest lies in determining if there is a natural tolerance to ALS-inhibitors herbicides among Italian weedy rice populations harvested prior to introduction of IMI-tolerant rice varieties (Fogliatto *et al.*, 2012). We hypothesise that Italian weedy rice populations can be grouped according to their differential susceptibility to ALS-inhibiting herbicides, using imazamox as the screening herbicide. This study had two distinct aims: a) to estimate the natural susceptibility to imazamox of Italian weedy rice populations collected in rice fields with no history of IMI-tolerant variety adoption and b) to compare the response of three IMI-tolerant and one IMI-susceptible varieties to imazamox.

Material and methods

In 2008, 149 weedy rice populations were selected based on awn presence and sampling location (northwest, southwest, east) from a territory including about 90% of the total Italian rice field area (Fogliatto *et al.*, 2012). The chosen areas had no history of cultivation with IMI-tolerant rice varieties. The collected populations were about 56% awned, 17% mucronate, and 27% awnless. The following year, all populations were grown under identical conditions in a rice field (also with no history of IMI-tolerant rice varieties) located in Vercelli, Italy. Seeds from these populations were harvested and stored at room temperature until study initiation.

Susceptibility tests of the Italian weedy rice populations and rice varieties to imazamox were performed at the University of Torino, Italy (45° 3.998' N; 7° 35.567' E– WGS84) in 2011. The first of two response studies was to assess the range of imazamox sensitivity across the largest Italian weedy rice population set available (Fogliatto *et al.*, 2012) and to inform a second investigation. The follow-on study utilised four populations, randomly selected from among those of the first study found to be most susceptible (two populations) and least susceptible (two populations), to undergo a seedling dose-response study to imazamox.

Whole-plant response screening

Modular planting trays, with 60 cells each, were used to seed all weedy rice populations. Each population was sown in 12 cells randomly distributed in 4 trays (3 cells/tray) by planting 5 seeds per cell, such that each tray hosted one replicate of all populations. Each tray was filled with sandy loam soil and placed in the glasshouse in benches containing a 2-3 cm water layer to maintain the soil at field capacity. Plants were kept in the glasshouse at an average air temperature of 24°C and relative humidity of about 40%. Natural light was supplemented by metal halide lamps adjusted to produce 16 h day length delivering about 55 μmol s^{-1} m^{-2}. When plants reached growth stage BBCH 12-13, seedlings were thinned to 3 plants per cell. One day later, 6 of the 12 alveolar trays were sprayed with 70 g a.i. ha^{-1} of imazamox (Beyond, 40 g a.i. L^{-1}, SL, BASF Italia S.p.A.). The manufacturer’s label recommended two treatments (2-3 weeks apart) at 35 g a.i. ha^{-1} of imazamox. We used a rate two times that recommended by the label in a single treatment as suggested by Bond and Walker (2011) for better differentiation among rice varieties. The herbicide was applied using a cabinet track sprayer equipped with a single flat fan nozzle (Teejeet DG8002-VS), calibrated to deliver 260 L ha^{-1} of spray solution in a single pass with a pressure of 200 kPa. The remaining six trays represented the untreated control and were sprayed with water only. After treatment, water was regularly supplied to maintain the soil at field capacity until 29 days after treatment (DAT). At 30 DAT, the weedy rice plant aboveground fresh-weights were measured.

Biomass reduction in treated plants compared with untreated ones was calculated for each weedy rice population. The study consisted of two identical experiments (Exp. 1 and Exp. 2) conducted two months apart. The populations were grouped into three classes according to their relative biomass reduction compared with untreated plants: 1) <70%, 2) from 70 to 90% and 3) >90% (Table 1). The three groups corresponded to relatively resistant, intermediate, and susceptible populations, respectively. Two populations from class 1 (<70% biomass reduction), one from the high end of class 2 (with 90% biomass reduction) and one from class 3 (>90%) were randomly selected for the seedling dose-response study.

Seedling dose-response study

The seedling dose-response study was carried out on four Italian weedy rice populations and four rice varieties (Sirio CL, CL 26, Luna CL and Selenio). The varieties Sirio CL, CL26 and Luna CL were included as representative IMI-tolerant varieties; the Selenio variety was included as it is a well-known IMI-susceptible comparison. Seeds of both weedy rice and rice were germinated on filter paper saturated with distilled water and incubated in the light at 25°C for 6 days. Afterwards, the primary roots of five seedlings per population/variety were cut (Roso *et al*., 2010b) and the seedlings were transferred into the glasshouse and placed in plastic pots (50 mL) containing about 15 mL of expanded vermiculite and 25 mL of solution with different imazamox concentrations.

Seven concentrations in a log-base were chosen for this study: 0, 0.01 mM, 0.1 mM, 1.0 mM, 10 mM, 100 mM, and 1 M. Concentration 0 was included as an untreated control. The concentrations selected were based on those adopted by Roso *et al.* (2010b) in a similar study. Preliminary assays indicated that concentrations higher than 1 M resulted in complete growth inhibition, even in the IMI-tolerant variety (data not shown). Six days after placing the seedlings in the pots, the length of the longest newly formed root was measured. A completely randomised design with three replicates was adopted, with a pot containing five seedlings being the experimental unit. The study consisted of two identical experiments (Exp. 1 and Exp. 2) carried out with an interval of 1 month.

Statistical analysis

One-way ANOVA performed on shoot biomass data in the whole-plant response screening indicated that there were no differences attributable to the experiment. For this reason, the results of the two experiments were reported as averaged values.

In the seedling dose-response study, root length of each population/variety was expressed as a percentage of the untreated control (seedlings of the same population/variety kept at concentration 0), and the resulting data were fitted to a 3-parameter log-logistic regression model (Seefeldt *et al.*, 1995; Knezevic *et al.*, 2007):

$$y = \frac{d}{1 + \exp[b(\log x - \log EC_{50})]} \quad (1)$$

where y is the root length as a percentage of the untreated control at the herbicide concentration x (imazamox concentration, millimolar), d is the upper limit and b denotes the steepness of the curve around its point of inflexion, EC_{50}.

Model fitting was performed using the function *drm* of the add-on package *drc* (Ritz *et al.*, 2006) of the open source programme and environment R (R Development Core Team, 2012). Data from Exp. 1 and Exp. 2 were first analysed separately and then pooled to fit into a single model. An *F* test was performed using the *anova* function of R to check if the data were better described by a single model fitting the pooled data of the two experiments, instead of two models fitting separately Exp. 1 and Exp. 2. Two separate curves explained Exp. 1 and Exp. 2 data in the majority of weedy rice populations and rice varieties significantly better than did a single curve.The function *SI* of the package *drc* was used to test for differences between EC_{50} calculated from Exp. 1 and Exp. 2. The values of EC_{50} were used to compare weedy rice populations and rice varieties by calculating a Resistance Index (RI):

$$RI = \frac{EC_{50(\mathrm{A})}}{EC_{50(\mathrm{B})}} \quad (2)$$

where A and B refer to the two varieties/populations under comparison.

Results

Whole-plant response screening

The 149 Italian weedy rice populations treated with imazamox at 70 g a.i. ha^{-1} were aggregated into three classes of biomass reduction (<70%, 70-90% and >90%). Even though the majority of the collected weedy rice populations were awned, these were distributed across all classes of biomass reduction (Table 1).

Table 1 near here

The majority of tested weedy rice populations responded with a 70 to 90% reduction in biomass to imazamox application at 70 g a.i. ha^{-1} (Table 1). As a consequence of treatment, seedling shoots yellowed and showed signs of stunting and desiccation. Symptoms were first observed about one week after herbicide treatment in all populations; however, only the awned populations showed biomass reductions of less than 70% or more than 90%. The mucronate and awnless populations were distributed principally among only a single class of biomass reduction (70%-90%). Among the populations exhibiting biomass reduction of at least 90%, populations 116 (mucronate) and 53 (awnless) were randomly selected to represent highly sensitive populations in the seedling dose-response study. The same selection was done for populations with a biomass reduction of less than 70%; in this case, populations 37 (awned) and 109 (awned) were selected.

Seedling dose-response study

The dose-response curves for imazamox concentrations on weedy rice populations and rice varieties are shown in Fig. 1. The 3-parameter log-logistic regression model provided an adequate fit to the data. The summary of estimated parameter values of the model is included in Table 2.

Figs 1 near here

Table 2 near here

In this study, root growth of four weedy rice populations in response to increased imazamox concentration resulted in highly variable EC_{50} values, which ranged from 0.0018 mM to 0.12 mM (Table 2). In all weedy rice populations, seedling root regrowth after cutting demonstrated inhibition at the lowest imazamox concentration (0.001 mM) (Fig. 1). At higher concentrations, weedy rice showed a much steeper root length reduction compared to that of the IMI-tolerant rice varieties, which started to decrease their root length only at concentrations above1 mM (Fig. 1). At the highest concentration (1 M), the seedlings of all weedy rice populations and rice varieties, including the IMI-tolerant ones, did not produce any new roots after cutting. As a consequence of the imazamox treatment, seedling shoots also displayed yellowing and desiccation signs. Shoot length reduction that occurred after treatment was considerably less (visual assessment) than that of the roots (data not shown).

Root lengths of weedy rice populations 37, 109, and 116, and rice varieties Sirio CL, CL 26 and Luna CL, of Exp. 1 and Exp. 2, were significantly better explained by two separate curves than one single curve fitting the pooled data (Table 2). Significant differences between EC_{50} values estimated in Exp. 1 and Exp. 2 were found only for weedy rice populations 37 and 116 (Table 2). The least susceptible population was mucronate 116, which had EC_{50} values of 0.0546 mM (Exp. 1) and 0.12 mM (Exp. 2). The most sensitive was population 53, with EC_{50} of 0.0018 mM (Exp. 1) and 0.0028 mM (Exp. 2). A comparison of EC_{50} values indicated that population 116 was 30.3 to 42.9 times more tolerant to imazamox than was the most sensitive population 53 (Table 2).

The EC_{50} values calculated for IMI-tolerant rice varieties were at least 3.82 mM (variety CL26, Exp. 2), which is orders of magnitude higher than those of weedy rice. The EC_{50} value of IMI-tolerant rice variety Sirio CL was > 21.07 mM, or at least 175 times more resistant than least-susceptible weedy rice population 116. Sirio CL compared with the most susceptible weedy rice population (53) proved to be about 7500-fold more resistant in Exp. 1 and about 14000 times more in Exp. 2. The IMI-tolerant rice variety CL26 was 31 and 1362 times more tolerant to imazamox than weedy rice populations 116 and 53, respectively (Table 3).

Table 3 near here

The EC_{50} value of Luna CL was > 7.44 mM; this variety showed itself to be > 99 and > 4000 times more tolerant to imazamox than weedy rice populations 116 and 53, respectively. Variety Sirio CL yielded RI values from 1.8 to 5.5 higher than those obtained in varieties Luna CL and CL26 (Table 3). According to RI values, rice variety Luna CL was at least 1.2 times more tolerant to imazamox than variety CL26.

Discussion

This work demonstrated the distinct susceptibility to imazamox, an ALS-inhibiting herbicide registered in Europe to control weedy rice in IMI-tolerant rice varieties, in the Italian weedy rice populations and rice varieties examined. Furthermore, the whole-plant response study supplied evidence that awned weedy rice populations differ from mucronated and awnless populations in the amount of biomass reduction caused by imazamox application. This work was, however, unable to confirm a clear association between awn presence and herbicide sensitivity, because awned populations were represented principally among the populations tested. In fact, only awned populations were not within the 70-90% biomass reduction interval, which included all mucronated and awnless populations (Table 1).

It should be noted that the awned group included 84 out of 149 populations. The low count of awnless or mucronate populations (40 and 25, respectively) may, indeed, be associated with the narrow response range found in these groups. High variability has been found in previous studies for a number of biological traits in awned populations compared with awnless and mucronate ones. Morphologic and genetic characterisation studies conducted on the same Italian weedy rice populations included in this study have indicated wider differentiation in plant morphology, dormancy and growth behaviour within awned, than mucronated and awnless populations (Fogliatto *et al*., 2011; Fogliatto *et al*., 2012). Similarly, different glyphosate tolerance has been documented among blackhull, brownhull and strawhull weedy rice populations from Arkansas. Specifically, blackhull and brownhull biotypes, which are typically awned, exhibit higher response variability (Burgos *et al*., 2011). This result might relate to the fact that in the USA blackhull awned biotypes have higher genetic variability than do strawhull awnless types (Gealy, 2013).

The seedling dose-response study made it possible to classify tolerance to imazamox for the rice varieties tested, which all carried the same gene mutation (serine-asparagine at the 653 amino acid position) (Table 2). Tolerance to imazamox, in terms of resistance index, among these varieties, varied between 1.2 and 4.2 (Table 3). A study conducted on the germplasm of two IMI-tolerant rice lines in the USA has established that the PWC-16 line (derived from mutagenesis with EMS of USA rice variety Cypress) is 4.9-fold more tolerant than the 93AS3510 line (Wenefrida *et al.,* 2007). A change to the size of the amino acid side-chain may be the cause of insufficient herbicide binding, which may affect resistance level (Lee *et al.,* 1999). Moreover, the location differences of the gene mutation responsible for tolerance to ALS-inhibiting herbicides in IMI-tolerant varieties may be the cause for distinct levels of resistance (Roso *et al.,* 2010a).

Foreknowledge of variety tolerance is important. In Brazil, for example, the differential susceptibility level between IMI-tolerant rice varieties caused problems in early rice cultivar technology, which showed greater initial susceptibility to imazethapyr+imazapic herbicides (Avila *et al*., 2010). In addition, inbred Clearfield® rice varieties in the USA show delayed maturity when imazamox was applied at incorrect doses or times (Bond & Walker, 2011). It has been suggested that this differential susceptibility results from a combination of the parent lines used to develop the new variety and the type of amino acid substitution. For example, serine-asparagine substitution in the AHAS gene in rice line PWC-16 has determined more herbicide-tolerant rice than has glycine for glutamic acid substitution in this same gene in rice line 93AS3510 (Wenefrida *et al.,* 2007). In fact, this line was the progenitor of the first IMI-tolerant rice variety in Brazil, and the glycine for glutamic acid substitution in the line was also found in weedy rice populations that escaped herbicide control with imazethapyr + imazapic in Brazilian rice fields (Roso *et al*., 2010a).

In our study, the IMI-susceptible variety Selenio behaved similarly to weedy rice populations and it was at least 64 times more susceptible to imazamox than IMI-tolerant varieties. This difference in tolerance level between resistant and susceptible varieties was also found in another study to a different degree. In fact, Sirio CL (IMI-resistant variety from 93AS3510 line) was found to be 91.1 times more resistant to imazamox than was a non-IMI-tolerant rice variety (Kaloumenos *et al.,* 2013), as opposed to the RI between IRGA 422 CL (IMI-tolerant rice variety from 93AS3510) and IRGA 417 (non-IMI-tolerant variety) which was >31 (Roso *et al.*, 2010b).

In all the IMI-tolerant varieties, we found a slight increase in root length at sub-inhibitory imazamox concentrations, which might indicate a hormesis effect (Brain & Cousens, 1989). This phenomenon could be verified with the Brain-Cousens's model (Brain & Cousens, 1989). However, this work utilised fewer than four or five imazamox concentrations less than 25% $EC_{50,}$ which is the minimum number of herbicide doses (Cedergreen *et al.,* 2005) to describe well the shape of the hormesis curve. Additionally, the EC_{50} value for IMI-susceptible rice variety Selenio was > 0.042 mM and the calculated resistance index revealed this variety to be >353, >64, and 175 times more sensitive to imazamox compared with the IMI-tolerant rice Sirio CL, CL26, and Luna CL, respectively (Table 3).

For the weedy rice populations included in this study, the imazamox concentration necessary to inhibit root growth by 50% (between 0.0018 mM and 0.12 mM) varied by about two orders of magnitude. Even though the seedling dose-response study was carried out on only four of 149 weedy rice populations, the other populations are expected to behave within a similar response range. The populations tested in the seedling dose-response study were, in fact, selected from among the least and the most susceptible to a whole-plant application of imazamox. Sensitivity variability to imidazolinone herbicides among different weedy rice population has also been found in both Greece (Vasilakoglou & Dhima, 2005) and the USA (Kuk *et al.*, 2008).

In spite of this large variability, the IMI-tolerant varieties considered in the study showed EC_{50} values at least 31.8 times higher than that of the tested weedy rice populations (Table 3). This result corroborates the hypothesis that Italian weedy rice populations did not present tolerance to imazamox prior to the introduction of IMI-tolerant varieties. The fact that imazamox was highly effective on the entire set of weedy rice populations included in the whole-plant response study is insufficient to exclude an *a priori* presence of tolerant populations. Yet, a recent study has suggested that IMI-tolerant weedy rice populations in Italy are likely the result of hybridisation events that occurred in the first years of IMI-tolerant variety use (Busconi *et al.*, 2012).

The dose-response methodology based on the seedling root growth evaluation adopted in this study proved able to discriminate imazamox susceptibility in weedy rice populations and rice varieties. In this method, the “dose” cannot easily translate to application rates at the field scale, as they actually represent herbicide solution concentrations in which the seedlings grow. Nevertheless, it should be considered a valuable tool for comparing populations/varieties in relative terms, as long as the study includes, as reference plant material, known sensitivity to certain herbicides. Both susceptible and tolerant/resistant plant material references should be included. For example, in the case of weedy rice sensitivity to imazamox, reference plant material can be represented by IMI-tolerant and IMI-susceptible varieties.

In comparison to the ordinary whole-plant dose response study, the seedling dose-response test adopted in this study has some practical advantages. One is the time required to carry out a test, which is usually shorter. In our case, twelve days was required *versus* at least twice that for a whole-plant bioassay. A second advantage is the smaller space required, both during germination and herbicide solution exposure. Third, there is no need for expensive cabinet sprayers or other equipment for precise spray application of the herbicide. Conversely, the cutting of primary roots is time-consuming, even though it generally equates to the amount of time required for plant thinning before herbicide application, which is an operation almost always required in whole-plant dose response studies.

References

AGOSTINETTO D, FLECK, NG, RIZZARDI, MA, MEROTTO JR A, VIDAL, RA (2001) Red rice: ecophysiology and strategies of control. *Ciência Rural* **31**, 341-349.

ANONYMOUS (2012) Clearfield® rice: stewardship guide. 2. BASF Available at: www.ricetec.com. (last accessed 25th August 2013).

AVILA LA, TELÓ GM, FERREIRA RB *et al.* (2010) Retorno da produção de arroz irrigado com cultivares convencionais após o uso do sistema Clearfield®. *Planta Daninha* **28**, 123-129.

BIROLI G (1807) *Del riso*. Tipografia Giovanni Silvestri, Milano, Italy.

BOND JA & WALKER TW (2011) Differential tolerance of Clearfield rice cultivars to imazamox. *Weed Technology* **25**, 192-197.

BRAIN P & COUSENS R (1989) An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Research* **29**, 93-96.

BURGOS NR, SHIVRAIN VK, SCOTT RC *et al.* (2011) Differential tolerance of weedy red rice (*Oryza sativa* L.) from Arkansas, USA to glyphosate. *Crop Protection* **30**, 986-994.

Busconi M, Rossi D, Lorenzoni C, Baldi G & Fogher C (2012) Spread of herbicide-resistant weedy rice (red rice, *Oryza sativa* L.) after 5 years of Clearfield rice cultivation in Italy. *Plant Biology* **14**, 751-759.

Cedergreen N, Ritz C & Streibig JC (2005) Improved empirical models describing hormesis. *Environmental Toxicology and Chemistry* **24**, 3166-3172.

Craigmiles JP (1978) Red Rice: research and control. In: *Introduction*. (ed Texas Agricultural Experiment Station), 1270 edn, 5-6. Texas Agricultural Experiment Station, Beaumont, USA.

Croughan TP (1994) Application of tissue culture techniques to the development of herbicide-resistant rice. *Louisiana agriculture* **37**, 25-26.

Dunand RT, Dilly Jr, RR, Meche, GA (1993) Maleic hydrazide and red rice control in rice. *Lousiana agriculture* **36**, 17.

Ente Nazionale Risi (2013) Ente Nazionale Risi. Available at: www.enterisi.it (last accessed 28th August 2013).

Ferrero A & Vidotto F (1998) Germinability after flowering, shattering ability and longevity of red rice seeds. In: *Proceedings of the 6th Mediterranean Symposium EWRS* (Ed. JM) (13-15 May 1998, Montpellier, France). 205-211.[1] Wageningen, The Netherlands.

Fleck NG, Agostinetto D, Galon L & Schaedler CE (2008) Competitividade relativa entre cultivares de arroz irrigado e biótipo de arroz-vermelho. *Planta Daninha* **26**, 101-111.

Fogliatto S, Vidotto F & Ferrero A (2011) Germination of weedy rice in response to field conditions during winter. *Weed Technology* **25**, 252-261.

Fogliatto S, Vidotto F & Ferrero A (2012) Morphological characterisation of Italian weedy rice (*Oryza sativa*) populations. *Weed Research* **52**, 60-69.

Gealy DR, Agrama HA & Eizenga GC (2009) Exploring genetic and spatial structure of U.S. weedy red rice (*Oryza sativa*) in relation to rice relatives worldwide. *Weed Science* **57**, 627-643.

Gealy DR (2013) Diagnostic of the red rice problem in the U.S.A. Proceeding of the Weed Science Society of America Meeting. Available at: http://www.ncaur.usda.gov/research/publications.htm?SEQ_NO_115=299162. (last accessed 22 July 2013).

Goulart ICGR, Matzenbacher FO & Merotto A (2012) Differential germination pattern of rice cultivars resistant to imidazolinone herbicides carrying different acetolactate synthase gene mutations. *Weed Research* **52**, 224-232.

Heap IM (2012) The International Survey of Herbicide Resistant Weeds. Available at: http://www.weedscience.org (last accessed 22 July 2013).

Kaloumenos NS, Capote N, Aguado A & Eleftherohorinos IG (2013) Red rice (*Oryza sativa*) cross-resistance to imidazolinone herbicides used in resistant rice cultivars grown in northern Greece. *Pesticide Biochemistry and Physiology* **105**, 177-183.

Kalsing A (2012) Plantas daninhas resistentes a herbicidas. *Lavoura Arrozeira* **61**, 40-41.

Knezevic SZ, Streibig JC & Ritz C (2007) Utilizing R software package for dose-response studies: the concept and data analysis. *Weed Technology* **21**, 840-848.

Kuk YI, Burgos NR & Shivrain VK (2008) Natural tolerance to imazethapyr in red rice (*Oryza sativa*). *Weed Science* **56**, 1-11.

Lee Y-T, Chang AK & Duggleby RG (1999) Effect of mutagenesis at serine 653 of *Arabidopsis thaliana* acetohydroxyacid synthase on the sensitivity to imidazolinone and sulfonylurea herbicides. *FEBS Letters* **452**, 341-345.

R Development Core Team (2012) R: A language and environment for statistical computing. . R Foundation for Statistical Computing, Vienna, Austria.

Ritz C, Cedergreen N, Jensen JE & Streibig JC (2006) Relative potency in nonsimilar dose-response curves. *Weed Science* **54**, 407-412.

Roso AC, Merotto J, A., Delatorre CA & Menezes VG (2010a) Regional scale distribution of imidazolinone herbicide-resistant alleles in red rice (*Oryza sativa* L.) determined through SNP markers. *Field Crops Research* **119**, 175-182.

Roso AC, Merotto JR. A & Delatorre CA (2010b) Bioensaios para diagnóstico da resistência aos herbicidas imidazolinonas em arroz. *Planta Daninha* **28**, 411-419.

Sales MA, Shivrain VK, Burgos NR & Kuk YI (2008) Amino acid substitutions in the acetolactate synthase gene of red rice (*Oryza sativa*) confer resistance to imazethapyr. *Weed Science* **56**, 485-489.

Scarabel L, Cenghialta C, Manuello D & Sattin M (2012) Monitoring and management of imidazolinone-resistant red rice (*Oryza sativa* L., var. *sylvatica*) in Clearfield® Italian paddy rice. *Agronomy* **2**, 371-383.

Seefeldt SS, Jensen JE & Fuerst EP (1995) Log-logistic analysis of herbicide dose-response relationships. *Weed Technology* **9**, 218-227.

Sudianto E, Beng-Kah S, Ting-Xiang N, Saldain NE, Scott RC & Burgos NR (2013) Clearfield® rice: its development, success, and key challenges on a global perspective. *Crop Protection* **49**, 40-51.

Tan S, Evans RR, Dahmer ML, Singh BK & Shaner DL (2005) Imidazolinone-tolerant crops: history, current status and future. *Pest Management Science* **61**, 246-257.

TIND T, MATHIESEN TJ, JENSEN JE, RITZ C & STREIBIG JC (2009) Using a selectivity index to evaluate logarithmic spraying in grass seed crops. *Pest Management Science* **65**, 1257-1262.

USDA (2013) Foreign Agriculture Service. Available at: http://www.fas.usda.gov/psdonline/psdreport.aspx?hidReportRetrievalName=BVS&hidReportRetrievalID=893&hidReportRetrievalTemplateID=1 (last accessed 28 August 2013).

VASILAKOGLOU I & DHIMA K (2005) Red rice (*Oryza sativa* L.) and barnyardgrass (*Echinochloa* spp.) biotype susceptibility to postemergence-applied imazamox. *Weed Biology and Management* **5**, 46-52.

WENEFRIDA I, UTOMO HS, MECHE MM & NASH JL (2007) Inheritance of herbicide resistance in two germplasm lines of Clearfield® rice (*Oryza sativa* L.). *Canadian Journal of Plant Science* **87**, 659-669.

Figure legends

Fig. 1 Effect of imazamox concentration (molar) on root length expressed as a percentage of untreated control in weedy rice populations (37, 53, 109 and 116) and rice varieties (CL26, Luna, Sirio CL and Selenio).Symbols refer to Exp. 1 (■) and Exp. 2 (▲).

Table 1 Frequency of weedy rice populations in various classes of relative biomass reduction, separated by awnedness

[1]Biomass reduction in plants treated with imazamox (70 g a.i. ha^{-1}) compared with untreated ones.

% Biomass reduced by class[1]	Frequency of weedy rice populations			
	Awned	Mucronated	Awnless	Total
<70	6	-	-	6
70-90	75	25	40	140
>90	3	-	-	3
Total populations	84	25	40	149

Table 2 Estimates of model parameter values (*b*, *d*, EC_{50}), and their standard error, fitting root length reduction percentage data obtained in seedling dose-response study. *P*-overall values are the probabilities of that the two experiments can be described by a single model fitting all data from Exp. 1 and Exp. 2. *P*-EC_{50}values are the probabilities that EC_{50} calculated from Exp. 1 and Exp. 2 are estimates of the same value

Weedy rice population/ rice variety	Exp.	Parameters[1] and standard error (SE)						*P*-overall	*P*-EC_{50}
		b	SE	*d*	SE	EC_{50}[2]	SE		
37	1	1.373	0.2571	100.52	4.8166	0.0344	0.0000072	0.0133	<0.000
	2	0.9506	0.194	102.02	4.5186	0.0718	0.0000152		
53	1	0.3239	0.0459	100.22	3.1267	0.0018	0.0000007	0.9256	0.3567
	2	0.3417	0.0519	100.18	3.1346	0.0028	0.0000012		
109	1	0.5304	0.0745	100.61	4.1516	0.0485	0.0000139	0.0174	0.0781
	2	0.9481	0.1645	78	3.9947	0.0790	0.0000152		
116	1	0.5555	0.1029	100.37	5.4055	0.0546	0.0000198	0.0064	<0.000
	2	1.2486	0.3703	100.52	4.5943	0.1200	0.0000300		
Selenio	1	0.6691	0.1127	101.59	3.7747	0.0424	0.0000089	0.6662	0.1719
	2	0.6777	0.0807	98.85	3.931	0.0596	0.0000120		
Sirio CL	1	1.0197	0.2215	95.14	3.3582	25.159	0.00668	0.0238	0.6320
	2	1.3510	0.3185	107.32	3.2375	21.077	0.00438		
CL26	1	1.2791	0.2489	105.06	2.5933	6.0613	0.00095	0.0312	0.1544
	2	0.8011	0.1156	104.83	2.9072	3.8146	0.00075		
Luna CL	1	0.8513	0.1129	105.49	2.5179	7.4476	0.00129	0.0270	0.050
	2	0.5937	0.0652	101.58	2.7864	11.90	0.00284		

[1] Data were fitted to nonlinear regression three-parameter logistic model (Equation 1);[2] Herbicide concentration (mM) causing 50% root length reduction.

Table 3 Resistance index values, calculated as EC_{50} ratio between IMI-tolerant varieties over weedy rice populations or rice varieties

Weedy rice population/ rice variety	Exp.	Resistance Index (RI)		
		Sirio CL	CL26	Luna CL
37	1	731.4	176.2	216.5
	2	293.6	53.1	128.7
53	1	13,977.2	3,367.4	4,137.6
	2	7,527.5	1,362.4	4,250.0
109	1	518.7	125.0	153.6
	2	266.8	48.3	150.6
116	1	460.8	111.0	136.4
	2	175.6	31.8	99.2
Selenio	1	593.4	143.0	175.7
	2	353.6	64.0	200.0
Sirio CL	1	-	0.2	0.3
	2	-	0.2	0.6
CL26	1	4.2	-	1.2
	2	5.5	-	3.1
Luna CL	1	3.4	0.8	-
	2	1.8	0.3	-