Behavior of the enantiomers of the herbicide imazaquin in agricultural soils under different application regimes

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1   **Abstract**

2   Sorption, persistence, and leaching of the enantiomers of the herbicide imazaquin, *S*-imazaquin

3   and *R*-imazaquin, in two soils under different application regimes were studied. Imazaquin

4   dissipation was evaluated after a single application of the racemic herbicide and the pure

5   enantiomers, and after a repeated application of the racemic herbicide. The effects of adding two

6   olive-mill wastes (ALP and ALPc), biochar (BC), and organoclay (SA-HDTMA) on sorption,

7   dissipation, and leaching of imazaquin enantiomers applied as racemic herbicide to the soils was

8   also evaluated. For all treatments, sorption, dissipation, and leaching of imazaquin in the soils

9   were either non-enantioselective or scarcely enantioselective. No interconversion of *R*-

10   imazaquin to *S*-imazaquin or vice-versa was observed in the experiments with the pure isomers.

11   Addition of ALP, ALPc, or BC did not enhance the negligible sorption of imazaquin enantiomers

12   by the soils, but accelerated their dissipation. Addition of SA-HDTMA increased sorption of both

13   enantiomers and delayed their dissipation and leaching. The results illustrate how agricultural

14   practices can impact the behavior of imazaquin enantiomers in soils and support the suitability

15   of replacing currently used racemic formulations of imazaquin with formulations based on the

16   single biologically-active (*R*) enantiomer to reduce the environmental impact of this chiral

17   pesticide.

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20   *Keywords:* amendments; chiral pesticides; imidazolinones; organic residues; organoclays; soils

21

22   *Abbreviations:* ALP, fresh olive-mill waste; ALPc, composted olive-mill waste; BC, biochar; rac,

23   racemic; SA-HDTMA, hexadecyltrimethylammonium-modified Arizona montmorillonite

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1. Introduction

Imazaquin is an imidazolinone herbicide used at low rates to control a wide spectrum of weed species by inhibiting the enzyme acetohydroxyacid synthase, mainly in soybean crop, turf, and ornamentals (Tan et al., 2005). This pesticide has a chiral carbon in its structure, and consequently can exist as two enantiomers: R-imazaquin and S-imazaquin (Figure 1). Even though it has been reported that the herbicidal activity of the R enantiomer is greater than that of the S enantiomer (Lao and Gan, 2005; Ramezani et al., 2010), imazaquin is marketed and used as formulations consisting of 1:1 mixtures of its enantiomers, i.e. as racemic mixtures. As a consequence, the toxicity of the non-active enantiomer to non-target organisms is a matter of concern.

Imazaquin is an amphoteric compound with several functional groups with acid-base character. Values of pKa are: pKa$_1$= 1.8 (NH$^+$), pKa$_2$= 3.8 (COOH), and pKa$_3$= 10.5 (NH) (Figure S1) (Weber et al., 2003). Because of this structure, one of the most relevant factors in the sorption of imazaquin in soils is the pH. At common agricultural soil pH levels (pH > 5), the anionic form of imazaquin predominates (Aichele and Penner, 2005; Lee et al., 1990), and repulsive interactions with soil colloids lead to low or negative sorption and high leaching potential (Che et al., 1992; Loux et al., 1989a; Porfiri et al., 2015; Regitano et al., 2000; Stougaard et al., 1990). Another important factor in imazaquin sorption on soil is the soil organic matter content. Che et al. (1992) suggested that imazethapyr and imazaquin have higher affinity for organic matter than for clay. Other soil characteristics that can influence imazaquin sorption and leaching are texture (Weber et al., 2003), water retention (Regitano et al., 2002), and iron and aluminum oxides contents, particularly in soils with low amount of organic matter (Gennari et al., 1998; Rocha et al., 2002).

An important feature of the behavior of imazaquin in soils is its relatively long persistence (Basham et al., 1987). Degradation of imazaquin in soils is mainly because of photodegradation and biodegradation. Imazaquin hydrolysis is also possible, but this degradation process appears to be very slow at pH< 9 compared with the other two aforementioned (Barkani et al., 2005;

55 Ramezani et al., 2008). Biodegradation of imazaquin has been related to soil pH and soil organic

56 matter. Aichele and Penner (2005) found that the degradation of imazaquin in soils at pH= 7 was

57 slightly faster than that at pH= 5. This was attributed to a greater herbicide sorption at lower pH

58 that meant less amount of herbicide available to be degraded. Only a few studies about the

59 enantioselectivity of biodegradation process of imazaquin have been conducted. Jarman et al.

60 (2005) concluded that the degradation of imazaquin in aqueous soil slurries was non-

61 enantioselective. Ramezani et al. (2010) found a slightly faster degradation of R-imazaquin

62 compared to S-imazaquin in several Australian soils that presented a wide range of physico-

63 chemical properties.

64   Several studies have evaluated the effects of organic amendments on the behavior of

65 imazaquin in soils. Wang et al. (2007a) amended a sandy loam soil with farm litters and

66 observed an increase in the degradation rate of imazaquin, which was attributed to the

67 incorporation of new microbial population and a large quantity of nutrients. Undabeytia et al.

68 (2004) added two organic amendments to four soils with different properties. The amendments

69 generally led to a decrease in the sorption of imazaquin on the soils due to blocking of sorptive

70 surfaces and equilibrium pH rises (Undabeytia et al., 2004). The effect depended on the nature of

71 the organic amendment and the properties of the soils, such as pH, amount of organic matter,

72 and amorphous iron oxide content. Clay materials, including pillared clays and organoclays, have

73 also been studied as sorbents of imazaquin (Polubesova et al., 2002; Undabeytia et al., 2013).

74 Park et al. (2014) developed a study using hexadecyltrimethylammonium-treated Arizona

75 montmorillonite (SA-HDTMA) to remove imazaquin from aqueous solution and concluded that

76 this organoclay was an excellent material to sorb imazaquin.

77   With very few exceptions (Jarman et al., 2005; Ramezani et al., 2010; Yi et al., 2007), previous

78 studies on the behavior of imazaquin in soils did not explicitly account for the behavior of its

79 individual enantiomers, treating the herbicide as if it was an achiral compound. However,

80 considering the enantioselective behavior of chiral pesticides in the environment and possible

81 differences between enantiomers is necessary to develop accurate environmental risk

82 assessment, design successful remediation/prevention pollution strategies, and assess the

83 suitability of using single (biologically-active) enantiomers for a more sustainable pesticide use

84 (Celis et al., 2015; Gámiz et al., 2016; Magrans et al., 2002). In this work, we conducted an

85 enantiomer-selective study to: i) evaluate the enantioselectivity of the sorption, dissipation, and

86 leaching processes of imazaquin in two Mediterranean agricultural soils, ii) assess how the

87 repeated application of the herbicide and its co-application with different soil amendments

88 altered the individual behavior of imazaquin enantiomers, and iii) address the question of

89 whether imazaquin enantiomers were configurationally stable in the soils.

90

91 **2. Materials and methods**

92 *2.1. Herbicide*

93 Imazaquin has a molecular weight of 331.3 g/mol, an aqueous solubility of 60 mg/L (25°C),

94 and a vapor pressure < 0.0013 mPa (60 °C) (Tomlin, 2006). Analytical standard-grade racemic-

95 imazaquin (purity > 99.9%) provided by Sigma-Aldrich (Spain) was used in this study. Pure *S*-

96 imazaquin and *R*-imazaquin aqueous solutions were obtained by semi-preparative HPLC using

97 the same equipment and chromatographic conditions as those described in section 2.7, but with

98 0.001 M HCl:acetonitrile (60:40) as mobile phase instead of 0.01 M H_3PO_4:acetonitrile (60:40) to

99 avoid the presence of the strongly competing phosphate anion in the purified enantiomer

100 solutions. After injecting 50 µL of a 500 mg/L *rac*-imazaquin solution prepared in mobile phase

101 into the HPLC analytical column, the peaks eluting at about 6.4 min (*S*-imazaquin) and 7.3 min

102 (*R*-imazaquin) were collected and neutralized with 0.1 M NaOH to a pH ~ 6. Next, the

103 acetonitrile was evaporated from the neutralized collected fractions using a gentle N_2 stream.

104 The resulting aqueous solutions contained the individual enantiomers of imazaquin at a

105 concentration of ~30 mg/L with an enantiomeric purity > 99%.

106

107 *2.2. Soils*

108   Two agricultural soils from Seville (SW Spain) were collected (0-20 cm), air-dried, and then

109   passed through a 2 mm sieve and stored at 4 °C. The selected soils had different texture and

110   mineralogy, but presented similar alkaline pH values and low organic matter and Fe oxide

111   contents. Soil 1 was a clay soil with 6% sand, 26% silt, 68% clay (20% montmorillonite, 38%

112   illite, 10% kaolinite), 0.49% organic carbon, and a pH of 8.6. Soil 2 was a sandy loam soil with

113   74% sand, 6% silt, 20% clay (10% montmorillonite, 4% illite, 6% kaolinite), 0.50% organic

114   carbon, and a pH of 8.3. The oxalate-extractable Fe_2O_3 content of both soils was < 1%.

115

116   *2.3. Amendments*

117   Three organic amendments derived from the olive-oil processing industry were used. The

118   first one was a fresh (uncomposted) solid byproduct, called "alperujo" (ALP), generated during

119   the two-phase olive-oil extraction process. The second organic amendment was a stabilized

120   alperujo (ALPc) which was obtained by subjecting a fresh ALP to a composting process for 5

121   months. Both amendments, ALP and ALPc, were ground, sieved (2 mm), and stored at 4 °C. The

122   third organic amendment was a biochar (BC) that was made by heating ALPc at 400 °C for 4

123   hours under a flow of N_2 at 1.5 L/min. The most important characteristics of ALP, ALPc, and BC

124   are summarized in Table S1. The rate of ALP, ALPc and BC applied to the soils (2% w/w) was

125   equivalent to 60 t/ha, considering a soil bulk density of 1.5 g/cm^3 and a soil depth of 0-20 cm.

126   The fourth amendment used was hexadecyltrimethylammonium-saturated Arizona

127   montmorillonite (SA-HDTMA) which was added to the soils at a rate of 2% or 5% (w/w). This

128   organoclay was prepared by a cation exchange reaction according to Celis et al. (2007) and its

129   characterization is detailed in Gámiz et al. (2015). It had 22.1% C, 1.26% N, and a basal spacing

130   value of 2.25 nm, which indicated the successful intercalation of HDTMA cations adopting a

131   paraffinic structure in the interlayer of the clay (Gámiz et al., 2015).

132

133   *2.4. Sorption experiments*

134   Firstly, imazaquin sorption on the unamended and amended soils was determined at a single

135   initial concentration (C_{ini}) of *rac*-imazaquin (1 mg/L). Triplicate aliquots of each soil (1 g), either

136   unamended or amended with ALP (2% w/w), ALPc (2% w/w), BC (2% w/w) or SA-HDTMA (2%

137   or 5% w/w), were treated with a 1 mg/L aqueous solution (8 mL) of *rac*-imazaquin and then

138   shaken at 20 ± 2 °C for 24 h. A preliminary kinetic study had revealed that differences in

139   sorption of imazaquin enantiomers after equilibration periods of 24, 48, and 72 h were not

140   statistically significant, so that 24 h was considered to be sufficient to reach the sorption

141   equilibrium for imazaquin. After equilibration, the suspensions were centrifuged (15 min at

142   8000 rpm) and the supernatant solutions were removed, filtered (0.45 µm pore size GHP

143   membrane disk filters), and analyzed by chiral HPLC to determine the solution concentration of

144   each imazaquin enantiomer in the equilibrated suspension (C_e). The amount of *S*- and *R*-

145   enantiomer sorbed (C_s) was calculated from the difference between the initial (C_{ini}) and

146   equilibrium (C_e) solution concentrations of each enantiomer. Triplicate initial imazaquin

147   solutions without soils served as controls. Distribution coefficients, K_d (L/kg), were determined

148   from the expression: $K_d = C_s/C_e$. The percentage of each enantiomer sorbed (% sorbed) was

149   calculated as: % sorbed= $[(C_{ini} - C_e)/C_{ini}] \times 100$.

150   In the treatments where significant sorption of imazaquin was observed, sorption-desorption

151   isotherms were measured by repeating the procedure described above with several *rac*-

152   imazaquin solutions with C_{ini} ranging from 0.5 to 5 mg/L. Immediately after the sorption

153   experiment, desorption was measured from the highest initial concentration point of the

154   isotherm ($C_{ini} = 5$ mg/L). After removing 4 mL of supernatant for the sorption analysis, 4 mL of

155   distilled water were added and the soil suspensions were shaken again for 24 hours at 20 ± 2 °C.

156   Then, the suspensions were centrifuged, and 4 mL of the supernatant were removed and

157   analyzed by chiral HPLC. This desorption procedure was repeated three times for each sample.

158   Sorption-desorption isotherms were fitted to the Freundlich equation:

159

160                         $$\log C_s = \log K_f + N_f \log C_e \qquad (1)$$

162 where C_s (mg/kg) is the amount of enantiomer sorbed at the equilibrium concentration C_e

163 (mg/L), and K_f ($mg^{1-Nf} kg^{-1} L^{Nf}$) and N_f (unitless) are the empirical Freundlich constants. The

164 thermodynamic index of irreversibility (TII) was obtained from the expression:

165

166 $$TII = 1-(N_{fd}/N_f) \qquad\qquad (2)$$

167

168 where N_f and N_{fd} are the Freundlich constants obtained from the sorption and desorption

169 isotherm, respectively (Sander et al., 2005). TII ranges from 0 to 1, where TII= 0 denotes

170 completely reversible sorption and TII= 1 indicates irreversible sorption.

171

172 *2.5. Dissipation experiments*

173   Dissipation experiments were carried out by soil incubations at 20 ± 2 °C under aerobic and

174 dark conditions. Samples (100 g) of unamended soils or of soils amended with ALP (2% w/w),

175 ALPc (2% w/w), BC (2% w/w), or SA-HDTMA (5% w/w) were moisturized (40% for soil 1 and

176 30% for soil 2), spiked with 3.3 mL of an aqueous solution of *rac*-imazaquin (60 mg/L), to give

177 an application rate of 2 mg/kg, and homogenized with a sterilized spatula. For the unamended

178 soils, the dissipation of imazaquin enantiomers upon a second application of *rac*-imazaquin at 2

179 mg/kg and after a single application of the separate enantiomers, *S*-imazaquin or *R*-imazaquin,

180 at a rate of 1 mg/kg was also investigated, the latter to address the question of whether

181 imazaquin enantiomers were configurationally stable in the soils (Li et al., 2009). Regularly,

182 triplicate aliquots of soil (3 g) were sampled and immediately frozen until their extraction and

183 analysis. Extraction was conducted by addition of a mixture (8 mL) of 0.01 M H_3PO_4 aqueous

184 solution:acetonitrile (60:40), followed by shaking for 24 h. Then, the suspensions were

185 centrifuged and the supernatants were filtered and analyzed by chiral HPLC. This extraction

186 procedure recovered more than 95% of the imazaquin freshly added to the soils.

187   Imazaquin dissipation data were fitted to first-order kinetics:

188

$$C = C_0 \cdot e^{-k \cdot t} \tag{3}$$

190

191 where C (mg/kg) is the enantiomer concentration in the soil at time t (days), C_0 (mg/kg) is the

192 concentration in the soil at t= 0, and k (days^{-1}) is the first-order dissipation constant. The half-life

193 ($t_{1/2}$) of each enantiomer was determined as $t_{1/2}$= 0.693/k.

194

195 *2.6. Leaching experiment*

196   The leaching study was conducted in triplicate using glass columns of 30 cm length and 3.1

197 cm i.d. In the bottom of the columns, glass wood and sea sand (10 g) was placed to avoid loss of

198 soil particles. Then, the columns were filled up with 20 cm of unamended soil or with soil

199 amended with ALPc, BC (at the rate of 2% w/w along the entire column) or SA-HDTMA (at the

200 rate of 5% for the top 0-2.5 cm of soil). Finally, sea sand (10 g) was added on the surface of the

201 columns. The soil columns were saturated with distilled water (100 mL) and allowed to drain for

202 24 hours. The pore volume of each soil column was calculated from the gravimetric mass

203 balance (V_p= 65 ± 1 mL for soil 1 and V_p= 61 ± 1 mL for soil 2). Leachate pH values (pH= 7.8-8.2)

204 were similar to those reported in Table S2 for the equilibrated suspensions of the unamended

205 and amended soils during the batch sorption experiments. Next, *rac*-imazaquin (0.015 mg) was

206 applied on the column surface as 2.5 mL of a 6 mg/L *rac*-imazaquin aqueous solution (200 g/ha).

207 Daily, distiller water (15 mL) were added to the columns and, after 24 hours, the leachates were

208 collected, filtered, and analyzed by chiral HPLC to determine the concentration of each

209 imazaquin enantiomer.

210

211 *2.7. Analysis of imazaquin enantiomers*

212   The analysis of imazaquin enantiomers was performed by reversed-phase chiral HPLC using

213 a Waters 600E chromatograph coupled to a Waters 996 diode-array detector. The conditions

214 used were: Chiralcel OD-3R analytical column (150 mm length x 4.6 mm i.d., 3 μm particle size)

215 from Chiral Technologies Europe, 0.01 M H_3PO_4 aqueous solution (pH= 2.2):acetonitrile (60:40)

216 mobile phase at a flow rate of 1 mL/min, and UV detection at 250 nm. Five external standard

217 solutions with concentrations between 0.1 and 6 mg/L of analytical standard-grade *rac-*

218 imazaquin were used in the calculations. Instrumental LOQ, calculated as the concentration

219 resulting in a signal to noise ratio of 10:1 (Camacho-Muñoz et al., 2009), was 0.02 mg/L for both

220 enantiomers. The retention times for *S*- and *R*-imazaquin were 6.4 and 7.3 min, respectively

221 (Figure S2).

222

223 **3. Results and discussion**

224 *3.1. Sorption of imazaquin enantiomers by the unamended and amended soils*

225   Imazaquin enantiomers did not sorb on the soils used in this study (%Ads < 1%, K_d < 0.1

226 L/kg) (Table S2). This was attributed to the presence of the herbicide in its anionic form (Figure

227 S1) at the pH of the tested soils (pH > 8). The negative charge of the herbicide would be repelled

228 by soil colloidal particles, which are also negatively charged at alkaline pH levels (Lee et al.,

229 1990; Oliveira et al., 2006; Rocha et al., 2002). Other soil properties that may have contributed to

230 the lack of sorption of imazaquin on the soils are their low organic matter and iron oxides

231 contents (Regitano et al., 1997; Rocha et al., 2002). Very little or no sorption of imazaquin has

232 previously been observed for other alkaline soils with low organic carbon contents (Gennari et

233 al., 1998).

234   The addition of organic amendments (ALP, ALPc, and BC) did not increase the sorption of

235 imazaquin by the soils. Undabeytia et al. (2004) observed a similar effect in soils amended with

236 two organic residues. The residues even decreased imazaquin sorption in some soils, because

237 they competed with the herbicide for sorption sites on certain soil surfaces (Undabeytia et al.,

238 2004). However, data in Table S2 show that imazaquin sorption increased when the soils were

239 treated with the organoclay SA-HDTMA and that increasing the amendment rate from 2% to 5%

240 was accompanied by a further increase in imazaquin sorption. Incorporation of HDTMA cations

241 into SAz-1 montmorillonite leads to a paraffinic structure with a wide organic interlayer phase

242 that displays a high affinity for neutral and even anionic pesticides (Celis et al., 2007; Cornejo et

243 al., 2008; Cruz-Guzmán et al., 2005; Gámiz et al., 2015). The sorption of imazaquin on the soils

244 amended with SA-HDTMA can presumably be attributed to hydrophobic interactions between

245 the hydrocarbon chains of HDTMA cation and the quinoline group present in the imazaquin

246 structure (Celis et al., 2007; Cruz-Guzmán et al., 2005; Gámiz et al., 2015; Park et al., 2014).

247   Sorption-desorption isotherms of imazaquin enantiomers on the soils amended with SA-

248 HDTMA at the rate of 5% are shown in Figure 2. The isotherms reveal that the sorption of

249 imazaquin enantiomers was non-enantioselective (*S*-imazaquin and *R*-imazaquin sorbed to the

250 same extent) and was higher on SA-HDTMA-amended soil 2 (K_f= 0.96-0.97) than on SA-HDTMA-

251 amended soil 1 (K_f= 0.26-0.29) (Table S3). This difference in sorption may be a consequence of

252 the high clay content of soil 1, which should have exacerbated the repulsions between the

253 anionic form of imazaquin enantiomers and the negatively charged soil particles. Alternatively,

254 soluble components of soil 1 may have blocked imazaquin sorption sites on SA-HDTMA to a

255 greater extent than soluble components of soil 2 (Gámiz et al., 2010). The values of

256 thermodynamic index of irreversibility (TII), calculated according to Sander et al. (2005),

257 indicated that the sorption of imazaquin enantiomers on SA-HDTMA-amended soil 1 (TII= 0.65-

258 0.70) was more irreversible than on SA-HDTMA-amended soil 2 (TII= 0.25-0.34) (Table S3).

259

260 *3.2 Dissipation experiments*

261 *3.2.1 Dissipation in unamended soils*

262   Figure 3 shows the *S*- and *R*-imazaquin dissipation curves in soils obtained after a single

263 application of the herbicide as racemic mixture and as pure enantiomers. Imazaquin dissipation

264 proceeded slowly in both soils, as previously observed in other soils (Basham and Lavy, 1987;

265 Cantwell et al., 1989; Loux et al., 1989b). Half-lives ranged between 48 and 144 days (Table 1).

266 The herbicide dissipated slightly faster in the sandy loam soil (soil 2) than in the clay soil (soil 1)

267 (Figure 3, Table 1). The degradation of imazaquin in soils has been mainly attributed to

268 photodegradation and biological degradation (Ramezani et al., 2008; Wang et al., 2007b). In this

work, the dissipation experiments were carried out in the dark, so that biodegradation may have

been the major dissipation route for imazaquin. The analytical methodology we used in this

study to determine imazaquin enantiomers did not allow us to identify imazaquin degradation

products. However, previous studies have reported that, under aerobic conditions in the dark,

imazaquin degrades in soil to the moderately persistent major metabolite 2-(1-carbamoyl-1,2-

dimethyl-propylcarbamoyl)-quinoline-3-carboxylic acid and several minor metabolites,

including 2-(4-hydroxyl-5-oxo-2-imidazolin-2-yl) quinolone acid and quinoline-2,3-dicarboxylic

anhydride (EFSA, 2011; Wang et al., 2007a).

Dissipation of imazaquin was not enantioselective in either soil, that is, the two enantiomers

dissipated at approximately the same rate. Lack of enantioselectivity in imazaquin dissipation in

soil was also observed by Jarman et al. (2005), who suggested the possibility of the presence of a

microbial population in soil that transformed both enantiomers at approximately the same rate.

Ramezani et al. (2010), however, found some enantioselectivity in the dissipation of imazaquin

in several soils; the herbicidally-active (R) enantiomer was found to be dissipated faster than the

inactive (S) enantiomer. Nevertheless, this effect was observed mainly at long incubation times

and for soils where imazaquin enantiomers dissipated faster than in the present study.

An interesting observation in our study was that in both soils the dissipation of imazaquin

enantiomers occurred faster when they were added as pure isomers than when they were

applied as a racemic mixture of enantiomers (Figure 3, Table 1). This effect was much more

pronounced in soil 2, where the pure S- and R-enantiomers had a $t_{1/2}$ of 48 days, compared to the

values of 105-110 days obtained for the enantiomers applied as a racemic mixture (Table 1). In

the experiments with the pure isomers, the total amount of imazaquin added was half of that

added in the experiments with rac-imazaquin. This reduction in the total amount of imazaquin

added appeared to affect the enantiomers dissipation rate, as previously observed for other

chiral and achiral pesticides (Beigel et al., 1999; Liang et al., 2016; Qin et al., 2016). Accordingly,

application of the pure biologically-active enantiomer is expected to result in shorter half-life for

imazaquin as compared to the application of the racemic mixture of enantiomers. No

296   interconversion of *R*-imazaquin to *S*-imazaquin or vice-versa was observed during the

297   experiments with the pure isomers, i.e. the enantiomers were configurationally stable in the

298   soils. This is an important prerequisite for the application of biologically-active enantiopure

299   compounds, which otherwise would be pointless (Buerge et al., 2003: Li et al., 2009).

300   The dissipation of imazaquin enantiomers upon a second application of the racemic herbicide

301   to soil 2 was also evaluated. The differences in dissipation rate between the first application and

302   the second one were insignificant for both enantiomers (Figure S3). The insignificant effect of

303   repeated applications on imazaquin dissipation observed in this study agrees with the results

304   reported by Bundt et al. (2015). These authors conducted a non-enantioselective study on the

305   dissipation of five imidazolinone herbicides in soils with a two-year application history and

306   concluded that the prior application of imidazolinones did not stimulate microbial degradation

307   of herbicides from the same chemical group. Our results further indicate that this behavior

308   applies to both enantiomers of imazaquin.

309

310   *3.2.2 Dissipation in amended soils*

311   Addition of the organic amendments (ALP, ALPc and BC) to the soils did not alter the non-

312   enantioselective character of imazaquin dissipation, but led to an increase in the dissipation rate

313   of both enantiomers. This effect was particularly noticeable in soil 1 treated with ALP (Figure 4,

314   Table 1). The incorporation of organic residues into the soils, in particular fresh ALP, may have

315   introduced exogenous microorganisms that degraded the herbicide and/or fostered a co-

316   metabolic biodegradation with the indigenous microbes. Furthermore, addition of organic

317   matter could provide supplementary nutrients to the soil and stimulate the growth of

318   indigenous microorganisms, leading to accelerated degradation of imazaquin (Barriuso et al.,

319   1997; Ferreira et al., 2002; Wang et al., 2007a). Finally, even though the organic amendments did

320   not increase the sorption of imazaquin in the soils (Table S2), they could have impacted the soil-

321   microorganism interactions and in turn the ability of the soil microbiota to degrade imazaquin.

322   These effects were clearly soil- and amendment-dependent, leading to the different dissipation

323   patterns shown in Figure 4.

324   In contrast to the organic amendments, the organoclay (SA-HDTMA) was found to increase

325   the persistence of imazaquin enantiomers in both soils. This effect was attributed to the sorption

326   of imazaquin on the soils amended with the organoclay (Figure 2, Table S2), since sorption can

327   protect the herbicide from microbial degradation (Barriuso et al., 1997; Fernandes et al., 2006;

328   Loux and Reese, 1992) and the addition of SA-HDTMA is not expected to provide exogenous

329   microorganisms or nutrients. Conversely, quaternary alkylammonium ions such as HDTMA can

330   even exhibit some antimicrobial properties and delay the natural degradation of pollutants in

331   the environment (Gámiz et al., 2012; Nye et al., 1994; Sarkar et al., 2010). Imazaquin dissipation

332   was slower in soil 2 amended with SA-HDTMA than in SA-HDTMA-amended soil 1 (Table 1),

333   because soil 2 amended with the organoclay showed greater herbicide sorption compared to soil

334   1 (Figure 2, Table S2).

335

336   *3.3 Leaching experiments*

337   *3.3.1 Leaching in unamended soils*

338   Figure 5 depicts the breakthrough curves (BTCs) for *S*- and *R*-imazaquin in unamended and

339   amended soil columns. The fractions of *S* enantiomer in the leachates collected during the

340   experiment, EF= [S]/([S]+[R]), are included in the graphs. In the unamended soils, imazaquin

341   leaching was not enantioselective, i.e., the two enantiomers moved through the soils at equal

342   pace (EF ~ 0.5). In soil 1, the maximum concentration (C_{max}) of *S*- and *R*-imazaquin was 0.20

343   mg/L and appeared after adding 65 mL of water. In soil 2, C_{max} was 0.10 mg/L for the two

344   enantiomers and appeared after adding 90 mL of water (Table 2). The faster herbicide leaching

345   in soil 1 in comparison to soil 2 was attributed to the high clay content of soil 1, which probably

346   exacerbated repulsions between imazaquin anions and soil particles during leaching. The total

347   amounts leached showed that for both soils most of the herbicide applied to the columns was

348   recovered in the leachates (Table 2, Figure S4). The slight differences in the percentage of

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349   leached imazaquin between both soils was in agreement with the higher persistence of the

350   herbicide in soil 1 compared to soil 2.

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352   *3.3.2 Leaching in amended soils*

353   The treatment of the soils with organic residues (ALPc and BC) did not modify the non-

354   enantioselective behavior of imazaquin leaching in the soils tested. In soil 1, we did not observe

355   meaningful changes in the leaching of imazaquin after the addition of ALPc, whereas in BC-

356   amended soil 1, the relative BTCs were more oblate (C_{max}= 0.10 mg/L) than in unamended soil 1

357   (C_{max}= 0.20 mg/L) (Figure 5). This fact may reflect the faster dissipation of imazaquin

358   enantiomers in the soil amended with BC (Table 1), as confirmed by the total amount leached

359   where only 75 % of the added imazaquin was recovered in leachates (Table 2, Figure S4).

360   In soil 2, the effect of the organic amendments was less important. For all treatments, C_{max}

361   appeared after adding the same volume of water (90 mL). This fact is typical of cases in which

362   the addition of amendment does not affect sorption of pesticides. The slight differences in C_{max}

363   between the soil treated with the different organic amendments reflect small differences in the

364   rate of degradation during the leaching experiment.

365   In both soils, the addition of SA-HDTMA delayed the leaching of imazaquin. In soil 1 treated

366   with SA-HDTMA, C_{max} appeared after adding an amount of water of 115 mL and was reduced to

367   0.1 mg/L (Table 2). This slower imazaquin leaching was a consequence of the higher sorption of

368   the imazaquin enantiomers in the organoclay-amended soil layer (0-2.5 cm). Similar results

369   were observed in soil 2 amended with the organoclay, where imazaquin enantiomers displayed

370   the highest delay. These outcomes agree with the data of the sorption experiment, since soil 2

371   amended with SA-HDTMA had a higher sorption capacity than soil 1 amended with the

372   organoclay (Figure 2, Table S2).

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374   **4. Conclusions**

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375   The behavior of imazaquin in two alkaline soils with low organic carbon contents did not

376   reveal a marked enantioselectivity for any of the processes studied (sorption, dissipation, and

377   leaching). Imazaquin enantiomers did not appreciably sorb on the soils (K_d< 0.1 L/kg) and their

378   dissipation proceeded slowly. The dissipation of imazaquin enantiomers upon a second

379   application of racemic imazaquin did not differ from that observed under the first application;

380   however, dissipation was faster when the enantiomers were applied as pure isomers than when

381   they were applied as a racemic mixture. Addition of organic residues did not alter the negligible

382   sorption of imazaquin enantiomers observed in unamended soils, but the exogenous organic

383   matter accelerated the imazaquin enantiomers dissipation rate. Conversely, the addition of

384   organoclay (SA-HDTMA) to the soils increased the sorption of imazaquin enantiomers and, as a

385   consequence, delayed their dissipation and leaching. Accordingly, SA-HDTMA behaved as an

386   efficient amendment to immobilize the imazaquin enantiomers in the soils and to reduce their

387   high leaching potential. The long persistence of both enantiomers of imazaquin and lack of

388   isomer interconversion during their transformation in soil support the suitability of replacing

389   formulations based on imazaquin racemic mixtures with the single biologically-active

390   enantiomer (*R*-imazaquin) to reduce the environmental impact of this chiral pesticide.

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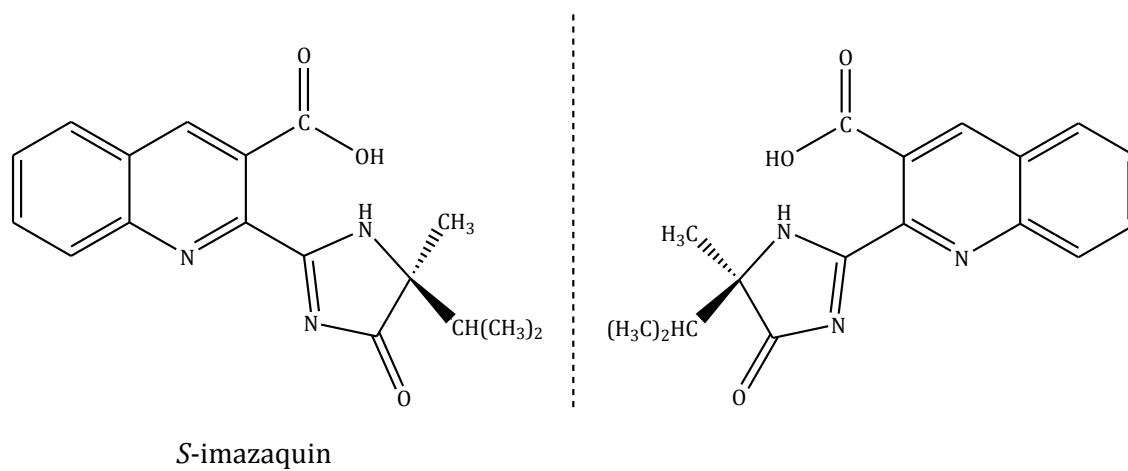546

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560   **Fig. 1.** Chemical structures of *S*-imazaquin and *R*-imazaquin.

561

562   **Fig. 2.** Sorption-desorption isotherms of *S*- and *R*-imazaquin on soils amended with SA-HDTMA

563   (5% w/w). Error bars correspond to standard errors of triplicate measurements.

564

565   **Fig. 3.** *S*- and *R*-imazaquin dissipation curves in soils after application of the herbicide as racemic

566   mixture (*rac*-imazaquin) and as pure enantiomers (*S*- and *R*-imazaquin). Symbols represent

567   experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error

568   bars correspond to standard errors of triplicate measurements.

569

570   **Fig. 4.** *S*- and *R*-imazaquin dissipation curves in unamended soils and in soils amended with ALP,

571   ALPc, BC and SA-HDTMA after application of the herbicide as racemic mixture (*rac*-imazaquin).

572   Symbols represent experimental data, whereas lines are the fittings to single first-order

573   dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

574

575   **Fig. 5.** Breakthrough curves (BTCs) of *S*- and *R*-imazaquin in unamended and amended soil

576   columns after application of the herbicide as racemic mixture. Enantiomer fractions (EF) in the

577   leachates are indicated in the relative BTCs. Error bars correspond to standard errors of

578   triplicate measurements.

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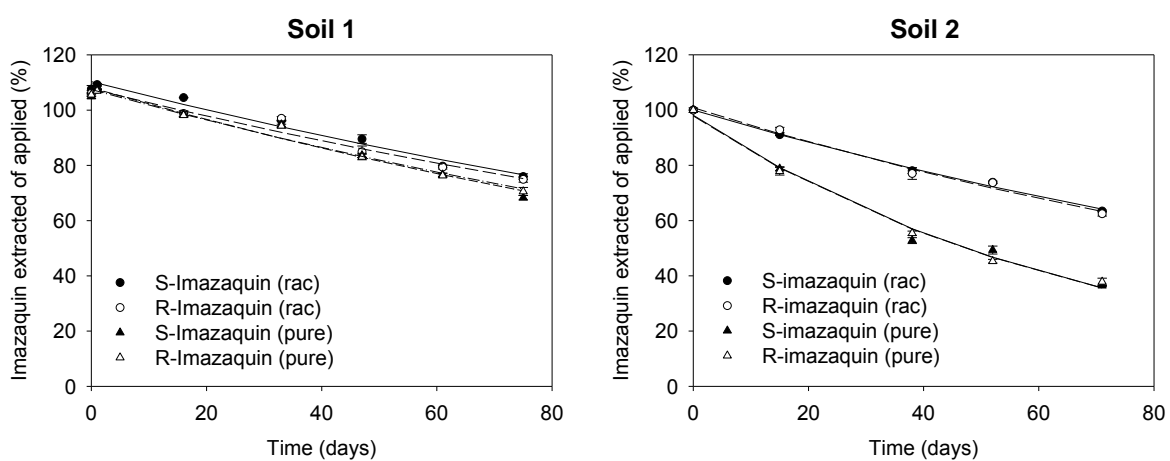
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S-imazaquin

**Fig. 1.** Chemical structures of *S*-imazaquin and *R*-imazaquin.

Fig. 2. Sorption-desorption isotherms of *S*- and *R*-imazaquin on soils amended with SA-HDTMA (5% w/w). Error bars correspond to standard errors of triplicate measurements.

Fig. 3. *S*- and *R*-imazaquin dissipation curves in soils after application of the herbicide as racemic mixture (*rac*-imazaquin) and as pure enantiomers (*S*- and *R*-imazaquin). Symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

Soil 1

Soil 2
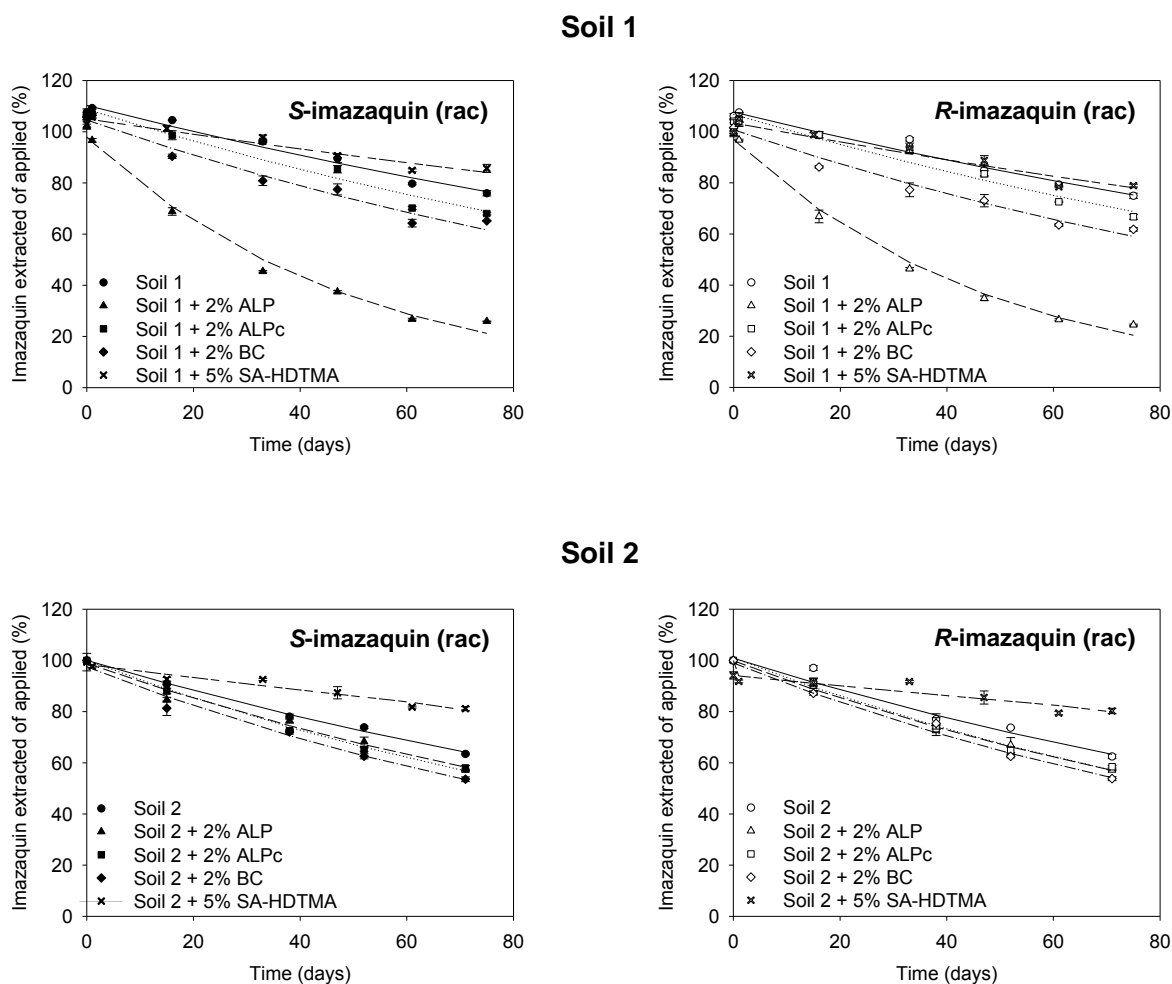
Fig. 4. *S*- and *R*-imazaquin dissipation curves in unamended soils and in soils amended with ALP, ALPc, BC and SA-HDTMA after application of the herbicide as racemic mixture (*rac*-imazaquin). Symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.
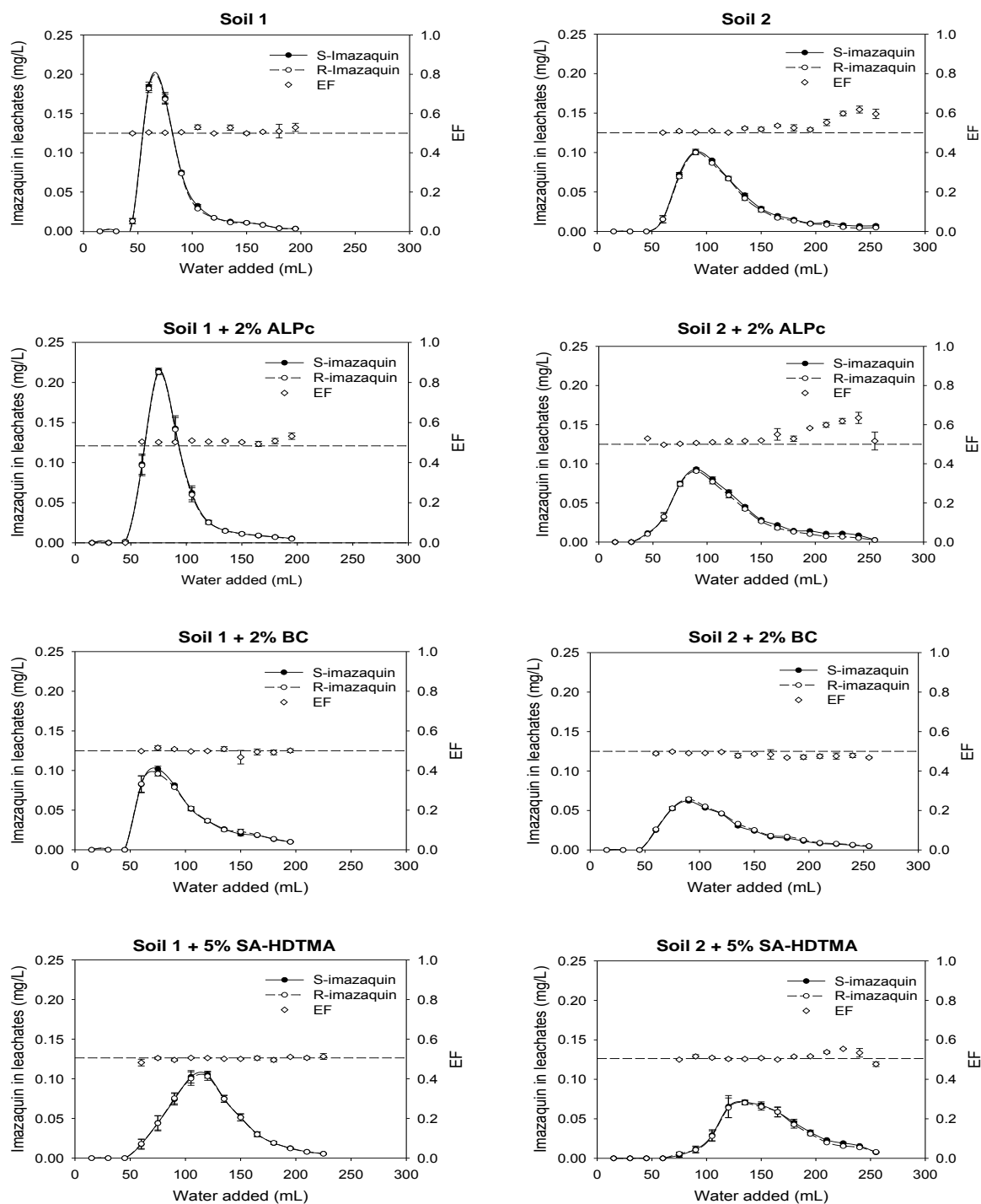
Fig. 5. Breakthrough curves (BTCs) of *S*- and *R*-imazaquin in unamended and amended soil

columns after application of the herbicide as racemic mixture. Enantiomer fractions (EF) in the

leachates are indicated in the relative BTCs. Error bars correspond to standard errors of

triplicate measurements.

Supplementary data for

Behavior of the enantiomers of the herbicide imazaquin in agricultural soils under

different application regimes

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Number of tables: 3

Number of figures: 4

Table S1. Physicochemical properties of the organic residues.

Amendment	% C	% N	Soluble OC (%)[a]	pH[a]
ALP	44	1.25	7.6	5.3
ALPc	28	2.3	0.9	8.3
BC	30	1.8	0.4	9.4

[a] Value measured in 1 g ALP/ALPc/BC : 20 mL $CaCl_2$ (0.01 M).

Table S2. Sorption percentage (% Sorbed) and distribution coefficient (K_d) for *S-*, *R-* and *(S+R)-*imazaquin on unamended soils and on soils amended with ALP, ALPc, BC and SA-HDTMA measured at a single initial racemic-imazaquin concentration of 1 mg/L.

		% Sorbed	K_d (L/kg)	pH[a]
Soil 1	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.4
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 1 + 2% ALP	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	7.9
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 1 + 2% ALPc	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.3
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 1 + 2% BC	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.4
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 1 + 2% SA-HDTMA	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.2
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 1 + 5% SA-HDTMA	*S*-imazaquin	3.6 ± 0.4[b]	0.3 ± 0.0	
	R-imazaquin	3.9 ± 0.7	0.4 ± 0.1	8.3
	(S+R)-imazaquin	3.8 ± 0.4	0.3 ± 0.0	
Soil 2	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.1
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 2 + 2% ALP	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	7.7
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 2 + 2% ALPc	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.0
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 2 + 2% BC	*S*-imazaquin	< 1%	< 0.1	
	R-imazaquin	< 1%	< 0.1	8.2
	(S+R)-imazaquin	< 1%	< 0.1	
Soil 2 + 2% SA-HDTMA	*S*-imazaquin	1.5 ± 0.2	0.1 ± 0.0	
	R-imazaquin	2.1 ± 0.4	0.2 ± 0.0	8.1
	(S+R)-imazaquin	1.8 ± 0.3	0.1 ± 0.0	
Soil 2 + 5% SA-HDTMA	*S*-imazaquin	10.3 ± 0.4	0.9 ± 0.0	
	R-imazaquin	9.6 ± 0.8	0.8 ± 0.1	8.1
	(S+R)-imazaquin	10.0 ± 0.6	0.8 ± 0.1	

[a] pH values of the equilibrated suspensions.

[b] Value ± standard error.

Table S3. Freundlich coefficients and thermodynamic index of irreversibility for *S*-, *R*- and

(*S+R*)-imazaquin sorption-desorption isotherms on soils amended with SA-HDTMA.

|		K_f	N_f	R^2	TII
Soil 1 + 5% SA-HDTMA	*S*-imazaquin	0.26 (0.21-0.32)[a]	1.37 ± 0.22[b]	0.952	0.70
	R-imazaquin	0.29 (0.25-0.34)	1.23 ± 0.15	0.970	0.65
	(S+R)-imazaquin	0.22 (0.19-0.26)	1.31 ± 0.49	0.961	0.68
Soil 2 + 5% SA-HDTMA	*S*-imazaquin	0.97 (0.90-1.05)	0.96 ± 0.08	0.985	0.25
	R-imazaquin	0.96 (0.91-1.01)	1.07 ± 0.05	0.995	0.34
	(S+R)-imazaquin	0.95 (0.90-1.00)	1.02 ± 0.06	0.993	0.30

[a] Values in parentheses correspond to the range in the value of the Freundlich coefficients.
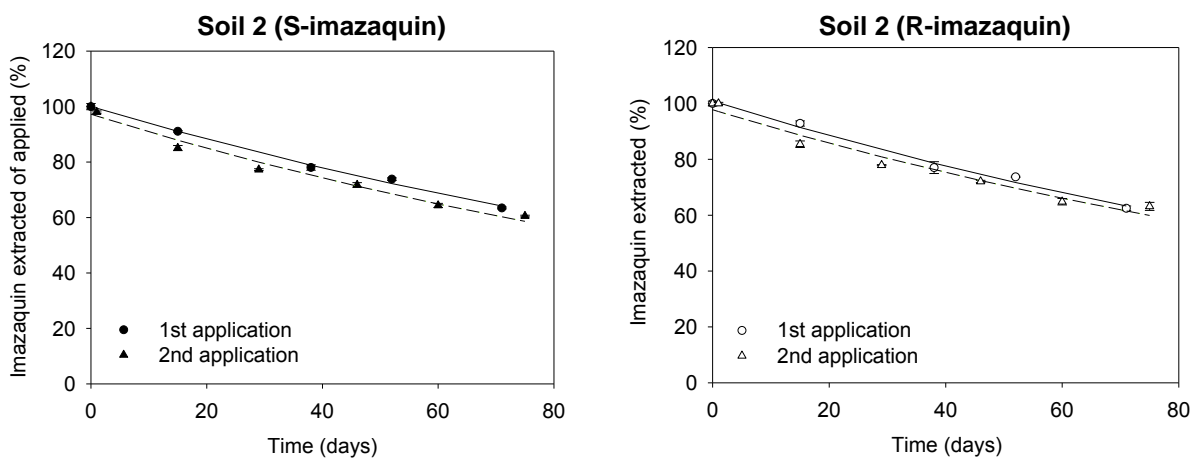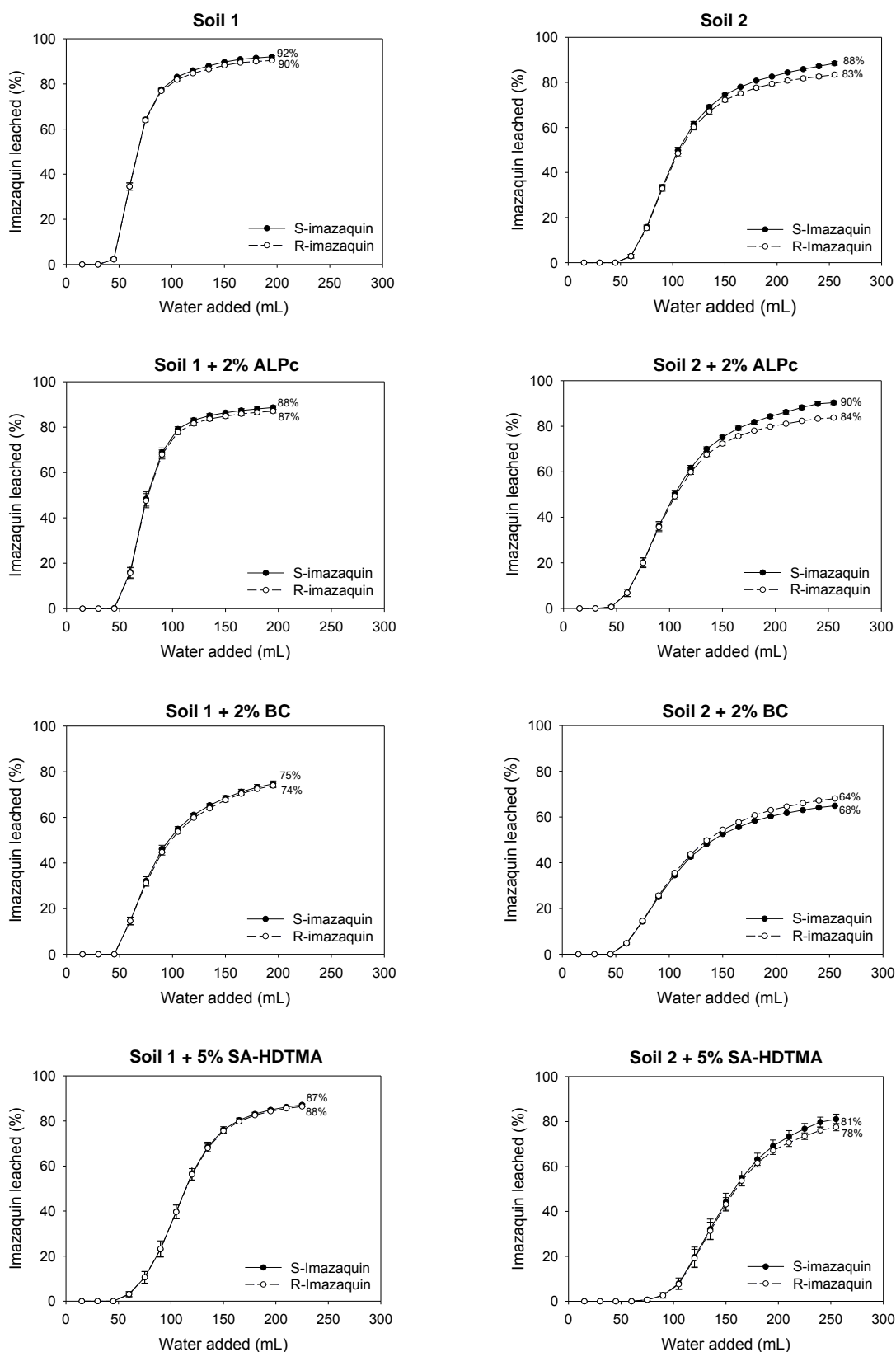
[b] Value ± standard error.
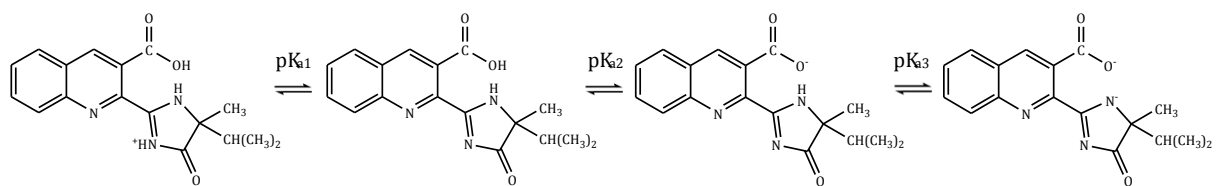
F**igure S1.** Ionization of imazaquin.
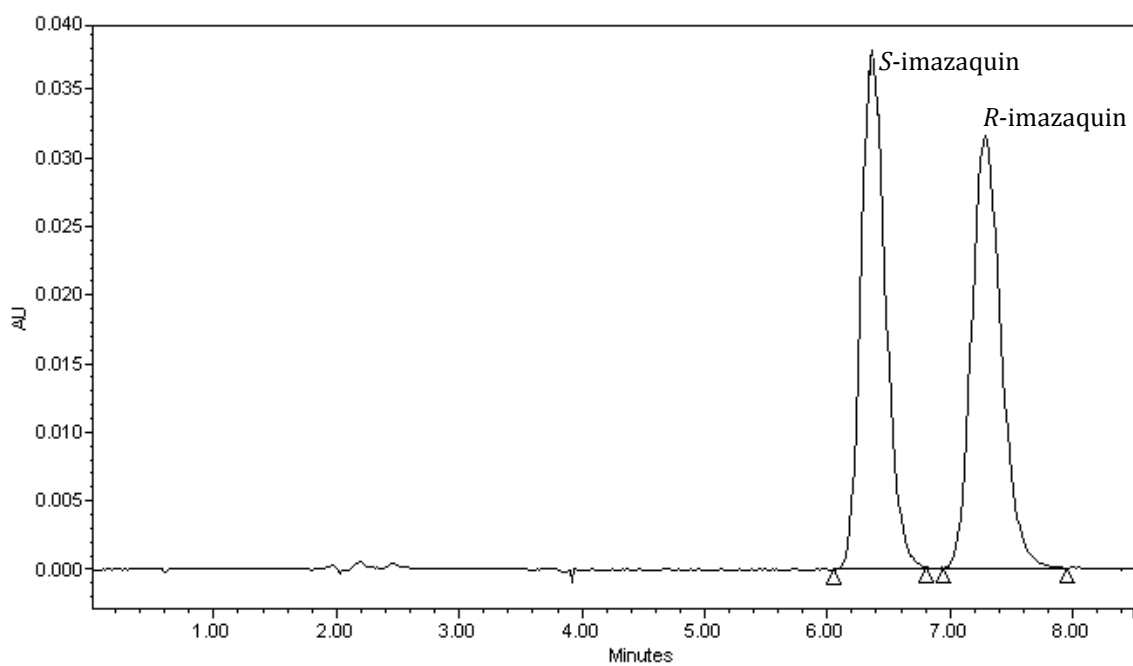
Figure S2. Chromatogram obtained by chiral HPLC for an aqueous solution of rac-imazaquin (6 mg/L). Peak assignation was performed according to the results of a bioassay conducted with *Brassica oleracea botrytis* that indicated higher herbicidal activity of the second eluted enantiomer (data not shown).

Figure S3. *S*- and *R*-imazaquin dissipation curves upon two successive applications of *rac*-imazaquin to soil 2. Symbols represent experimental data, whereas lines are the fittings to single first-order dissipation kinetics. Error bars correspond to standard errors of triplicate measurements.

Figure S4. Cumulative breakthrough curves (BTCs) of *S*- and *R*-imazaquin in unamended and ALPc-, BC- and SA-HDTMA-amended soil columns. Error bars correspond to standard errors of triplicate measurements.