Atrazine efficiency in an Andisol as affected by clays and nanoclays, Cea et al.

ATRAZINE EFFICIENCY IN AN ANDISOL AS AFFECTED BY CLAYS AND NANOCLAYS IN ETHYLCELLULOSE CONTROLLED RELEASE FORMULATIONS

M. Cea*, P. Cartes, G. Palma and M.L. Mora

Center of Plant-Soil Interaction and Natural Resources Biotechnology, Scientific and Technological Bioresource Nucleus, Universidad de La Frontera, P.O. Box 54-D, Temuco, Chile.

*Corresponding author: mcea@ufro.cl

ABSTRACT

Atrazine, a herbicide used for the control of broadleaf weeds of different crops, was incorporated in ethylcellulose controlled release formulations (CRFs) by using the solvent evaporation technique. Allophanic clays and nanoclays were incorporated as matrix modifying agents. The formulations were characterized by scanning electron microscopy (SEM) and infrared spectroscopy (FTIR), and their behavior was determined in aqueous phase. Dissipation studies and soil columns experiments with CRFs were also carried out, and compared with commercial formulations (CFs). In addition, a short-term bioassay was performed to evaluate the effect of CRFs and CFs on the emergence and growth of field mustard (Brassica campestris L.) under greenhouse conditions. The matrix modifiers (clays and nanoclays) showed little influence on the particle morphology and atrazine encapsulation efficiency. Furthermore, these matrix modifiers had a slight effect on the atrazine release into water, its dissipation and its behavior in soil compared with the formulation without them. All CRFs increased the atrazine herbicidal activity and reduced their loss by leaching in soil. Although some similarities were found in the efficiency to reduce the seedling emergence between CRFs and CFs, a greater death of seedlings was observed in the CRFs, especially when nanoclays were added into the formulation. These facts denote an advantage of CRFs over CFs due to prolonged bio-efficiency, and longer applications intervals will be produced, minimizing the harmful impact on the environment.

Keywords: Atrazine, natural clays and nanoclays, controlled release, soil, herbicidal activity.

INTRODUCTION

Among herbicides, atrazine, a selective pre- and post-emergence herbicide has been extensively used in forestry, grasslands, maize, sugarcane and non-crop areas. Atrazine and its degradation products constitute potential contaminants (Abdelhafid et al., 2000; Müller et al., 2002), and a frequency of water contamination of about 38% with variable concentrations from 0.1 to 27 mg L⁻¹ (Costa et al., 2004) could present.

The undesirable side effects arising from the use of conventional agricultural chemicals like atrazine have been minimized by the introduction of polymer supports constituting controlled release
formulations. The delivery of agrochemicals by controlled release formulations is a promising concept offering advantages for health, nutrition, environmental pollution control, and economic development. In this sense, it has been reported that the microencapsulation of atrazine by different polymers showed excellent controlled release properties (Dailey et al., 1993; Gerst et al., 1998).

Ethylcellulose, a natural, stable, non-toxic and cheap polymer (Moldenhauer and Nairn, 1990) has been commonly used as additive to foods and in various biomedical, pharmaceutical and biotechnological applications because of its low reactivity. Previous reports showed that pesticide-loaded ethylcellulose microspheres had a high herbicidal efficacy (Dailey et al. 1993; Sopeña et al., 2007a).

On the other hand, chemical surface functionality and surface structure of clays and nanoclays play an important role in many technological application fields. Natural and synthetic clays and nanoclays have an attracting interest due to their potential and successful utilization in controlled release systems as modifying agents (Gerstl et al., 1998; Fernández-Pérez et al., 2000, 2004; Dong and Feng, 2005; Hermosín et al., 2006). Clays commonly used in controlled release systems include montmorillonite, bentonite, and synthetic silice particles. Most of them have been modified to improve their efficiency in the active ingredient retention. For example, acidification has improved the adsorption capacity of bentonite used in alginate controlled release formulation of atrazine (Fernández-Pérez et al., 2004), reducing the release rate of the active component.

The physico-chemical properties of natural clays like allophane, which is the most abundant clay of Andisols of Southern Chile, make them attractive to be used in pesticides controlled release formulations. Allophane has a high specific surface area and variable charge, and it has been demonstrated that allophane-organic matter associations play a key role in controlling soil nutrient availability (Mora and Canales, 1995) and contaminant behavior (Cea et al., 2005, 2007).

Atrazine is widely used for broad leaf weed control in maize production in Andisols of Southern Chile. The high organic matter content of Andisols and agronomical practices like the application of high animal manure dosages to improve crop production affect the efficacy and fate of this herbicide (Briceño et al., 2008; Aguilera et al., 2009). These facts have led farmers to increase the atrazine doses commonly applied to ensure an adequate weed control, with the consequent increase of the environmental risk.

The aims of this work were: (i) to study the atrazine behavior as ethylcellulose controlled release formulations in an Andisol and (ii) to investigate the improvement of atrazine efficiency in the soil-plant system by the addition of allophanic clays and nanoclays to the controlled release formulations.

MATERIAL AND METHODS

Materials

Technical atrazine (90% purity) was kindly provided by Agrícola Nacional SACEI (ANASAC), Chile. Commercial formulations of atrazine (Atranex 90WG and Atrazina 500 SC) were also tested. Ethylcellulose (EC), 30-50 mPa, was purchased from Sigma-Aldrich (St. Louis, USA). Polyvinyl alcohol (PVA, 31-50
Atrazine efficiency in an Andisol as affected by clays and nanoclays, Cea et al.

kDa. 87% hydrolysed) was obtained from Sigma-Aldrich (St. Louis, USA). All reagents were of analytical grade. HPLC-grade acetonitrile, methanol and dichloromethane were purchased from J.T. Baker (USA).

Clays and nanoclays were extracted from an Andisol of Temuco Series in Southern Chile. The characterization of clays and nanoclays from this site was previously reported by Calabi et al. (2008).

Soil samples (0 – 20 cm depth) were collected from an Andisol of Freire Series in Southern Chile. The samples were air-dried, sieved through 2 mm and stored in plastic container until their use. The chemical properties of soil were: 15% of organic matter, pH 5.65 and 20 mg kg⁻¹ of P₂O₅. The soil chemical analyses were performed according the methodology described by Sadzawka et al. (2006).

Microsphere Preparation and Characterization

Microspheres were prepared by the solvent evaporation technique according to the methodologies described by Fernandez-Urzuno et al. (2000) and Dong and Feng (2005). Briefly, ethylcellulose (1.2 g) was dissolved in 20 mL of dichloromethane (DCM). Atrazine (0.2 g) was dissolved in this polymer solution at 40°C. The herbicide/polymer solution was emulsified in 200 mL aqueous solution containing 0.15% w/v PVA and various amount of clays (0, 0.04 and 0.06% w/v) and nanoclays (0, 0.0025, 0.005 and 0.01% w/v) under stirring (300 rpm). About two drops of 1-octanol were added to the stirred emulsion to reduce foaming. After 8 h of stirring, the products were filtered, washed with 200 mL of distilled water and air-dried at 30°C.

Characterization by scanning electron microscopy and infrared spectroscopy

The surface morphology of the microspheres was examined by scanning electron microscopy (SEM) (JEOL JSM-6380LV). The microsphere size was measured by image analysis. Infrared spectra of KBr pellets of pure atrazine, pure ethylcellulose, atrazine microspheres and atrazine microspheres containing either clays or nanoclays were obtained using a Brucker Tensor 27 - FTIR spectrometer.

Determination of encapsulation efficiency and herbicide loading

In order to determine both the efficiency of the microencapsulation process and the atrazine content of microspheres, samples in triplicate were dissolved in methanol (1 mg mL⁻¹). Later, the samples were filtered with PTFS membrane (0.45 μm pore size; Millipore) and then analyzed by high performance liquid chromatography (HPLC). Briefly, the samples were injected in a Merck Hitachi L-7110 pump, a Rheodyne 7725 injector with 20 μL loop and a Merck Hitachi L-7400 UV detector. The detection was set up at 220 nm and the column was a reverse phase (Lichrosphere RP18, 5 μm and 250 mm). The mobile phase consisted of acetonitrile: ammonium acetate 0.001 M (40:60 ratio) with a flow rate of 1 mL min⁻¹ at 30°C.

The encapsulation efficiency (EE) was defined as the percentage of herbicide encapsulated by the microspheres in respect to the herbicide used. The herbicide loading (HL) was calculated from the ratio of herbicide encapsulated by microspheres and the final weight of microspheres.
Sorption studies

To determine the effect of clays and nanoclays on the encapsulation process, sorption experiments were conducted as follows: 1 mL of aqueous potassium chloride solutions (0.01 M) containing initial concentrations of atrazine ranging between 2.5 and 20 mg L⁻¹ were prepared. Amounts of 5 mg of clays were mixed with the atrazine solutions, placed in centrifuging tubes and shaken at 25 ± 1ºC. Preliminary experiments were conducted for various time intervals to determine the sorption equilibrium. The time required to reach the equilibrium was 48 h. After shaking, the suspensions were centrifuged and filtrated (membrane of 0.45 μm). The atrazine concentration in the supernatant was determined by HPLC.

Due to the incapacity to separate the nanoclays from the aqueous phase the sorption experiments were conducted using a dialysis technique described by Seol and Lee (2000). Spectral / Por 6 dialysis tubing with a molecular weight cut off of 1000 Da was washed in distilled water. Freeze-dried nanoclays samples were dissolved in potassium chloride solution (0.01 M) at a concentration of 100 mg nanoclays L⁻¹. Aliquots of 10 mL were placed in dialysis tubing, and put in 30 mL test tubes containing 10 mL of (0.01 M) potassium chloride solution ranging in herbicide concentration from 0 to 20 mg L⁻¹. Tubes were shaken at 25 ± 1ºC. Each experiment included three tubes without nanoclays to verify that the atrazine concentrations inside and outside the bag after 48 h were equal.

Atrazine concentration inside and outside dialysis tubing was determined measuring the UV absorbance at 220 nm in a spectrophotometer Spectronic Genesis™ 2PC, using an atrazine calibration curve. The difference between atrazine concentration inside and outside dialysis tubing was assumed to be the atrazine bound to nanoclays. To evaluate the sorption capacities of clays and nanoclays, the experimental data were fitted to the empirical Freundlich-type isotherm (Eq.[1]):

\[ C_s = K_f \times C_e^{1/n} \]  

where \( C_s \) is the atrazine mass sorbed per mass of soil (mg kg⁻¹); \( C_e \) is the concentration of atrazine remaining in solution (mg L⁻¹) after equilibration; \( K_f \) is the Freundlich distribution coefficient (L kg⁻¹); and \( 1/n \) is an exponential empirical parameter that accounts for non-linearity in sorption behavior (Sposito, 1984).

Water release studies

To compare the release behavior of different controlled release formulations (CRFs), 10 mg of microspheres (three replicates) were immersed in water (200 mL). The systems were put in a shaker at 25ºC with stirring at 120 rpm. At different time intervals, aliquots of 100 μL were removed to determine atrazine by HPLC, as described above. The experiment was stopped once, more than 90% of the active ingredient initially present in the formulations was released. The release kinetics of atrazine from microspheres was evaluated by fitting the data to the Ritger and Peppas (1987) generalized model (Eq. [2]):

\[ \frac{M_t}{M_z} = k t^n \]  

where \( M_t \) is the amount of herbicide released from the controlled-release microsphere at time \( t \), \( M_z \) is the total amount of herbicide encapsulated, \( k \) is a constant that incorporates characteristics of the macromolecular network system and the active ingredient, and \( n \) is the diffusional exponent that indicates the release mechanism. The time needed for 50% release of atrazine (\( t_{1/2} \)) was calculated from these constants.
Atrazine dissipation in soil

Atrazine dissipation in soil from the commercial formulations (Atrazina 500 SC (CF1) and Atranex 90WG (CF2)) and CRFs was studied using the Andisol previously characterized (see above). For this purpose, triplicate soil samples (150 g) at 60% of water holding capacity (WHC) were placed in plastic pots and mixed with CRFs and commercial formulations at application rate of 1.5 mg atrazine kg\(^{-1}\) of soil. After mixing, the samples were incubated in dark for 18 weeks at 20 ± 1°C. The soil moisture level was maintained by periodic application of distilled water. Samples (10 g) were taken from each pot at 0, 1, 2, 5, 7, 12, 18 weeks after treatment and stored at -20 ºC until their analysis. Atrazine residues were extracted with methanol and analyzed by HPLC. Linear regression equations describing the relationship between atrazine soil concentration \((y = \ln \left(\frac{C}{C_0}\right))\) and time \((x = \text{weeks})\) were used to calculate dissipation half-lives of the formulations as follow (Eq. [3]):

\[
L_n C = L_n C_0 - kt
\]

where \(C\) is the soil atrazine concentration after \(t\) time, \(C_0\) is the initial herbicide concentration in soil, and \(k\) is the dissipation rate constant. The time required to reach an atrazine dissipation of 50% \((t_{1/2})\) was also calculated.

Soil column experiments

To determine atrazine leaching, duplicate undisturbed soil columns were taken with PVC cylinders of 30 cm length and 11 cm internal diameter from the previously characterized Andisol. The bottom of each column was filled with a thin layer of glass wool and covered with a PVC cap provided with a 0.5 cm diameter hole in the center, where the leachates emerged. All columns were saturated with 500 mL of distilled water, and allowed to drain for 24 h. Then, 1.36 mg of active ingredient from each formulation (CF1, CF2, CRFs), equivalent to field application of 1.44 kg atrazine ha\(^{-1}\) were applied to the top of soil columns. Later, the columns were covered with glass wool to avoid damage in soil structure by water. The columns were subjected to watering with distilled water at rate of 50 mL d\(^{-1}\) during 30 days. The watering rate was equivalent to the rainfalls registered in Region de La Araucania of Southern Chile in October 2009. Leachates were collected every day, and atrazine concentration was determined by HPLC after sample concentration.

At the end of the experiment, 10 g (in triplicate) from each column at different depth (0-10 cm, 10-20 cm and 20-30 cm) were extracted with 20 mL of methanol, shaken for 1 h and finally sonicated for 5 min. The suspensions were centrifuged, concentrated (1mL), filtered and analyzed by HPLC in order to determine the residual atrazine amount.

Short time bioassay

To compare the herbicidal activity of atrazine between some selected CRFs (CRF1, CRF2 and CRF5) and commercial formulations (CF1 and CF2), a short time bioassay was carried out using field mustard \((Brassica campestris L.)\). Andisol samples (150 g) at 60% WHC were put in plastic pots. After that, five field mustard seeds were sown in each pot, and placed in a growth chamber at 20 ± 1°C with a photoperiod of 16 h. Field doses of the commercial formulations and its equivalent in active ingredient (1.44 kg atrazine ha\(^{-1}\)) of the CRFs were added to the pots. Three pots were used as replicates for each atrazine formulation, and pots without active ingredient served
as control. During the growth period, the seedlings were watered daily with distilled water. The emerged seedlings of each pot were quantified 7 days after sowing, and the percentage of emerged seedlings was calculated. At the end of the experiment (15 days) the dead seedlings were quantified, and the aerial and roots length of the surviving seedlings was recorded.

Statistical Analysis

For the column experiment, data were analysed using analysis of variance (ANOVA), and comparisons between averaged values from different treatments were made by the least significant difference (LSD) test at 0.05 probability significance level.

For the short term bioassay, data were subjected to ANOVA, and significantly different means between treatments were separated with the Tukey’s multiple test ($P \leq 0.05$).

RESULTS AND DISCUSSION

Microsphere Characterization

Scanning electron microscopy and infrared spectroscopy

SEM micrographs show the shape and surface morphology of CRFs in presence and absence of clays or nanoclays (Figure 1). In all cases, we observed microparticles with plain, highly irregular and rough surfaces. Nevertheless, it is noteworthy that a high percentage of microparticles showed spherical shapes. We also observed that at increasing clays or nanoclays content, the microparticles formulation lost the spherical form and the roughness and irregularity of the surface increased. The size of CRFs diminished as a consequence of the incorporation of clays or nanoclays into the formulations. However, the microparticle size was not affected by the clays or nanoclays content increment.

The infrared spectra of microparticles were compared with pure atrazine, pure polymer (ethylcellulose), clays and nanoclays spectra (Figure 2 a-c). No differences were observed between the CRFs spectra. We noted the existence of some significant IR bands of atrazine in the spectrum of all CRFs at the same wavenumber: at 3267 cm$^{-1}$ (stretching N-H secondary amine), 1622 cm$^{-1}$ (C=N conjugated cyclic system), 1555 cm$^{-1}$ (N-H bending) and 803 cm$^{-1}$ (stretching C-Cl). The microparticles IR spectra are a sum of pure atrazine and polymer spectra. In this context, El Bahri and Taverdet (2007) found the prevalence IR band characteristics of the active ingredient in their formulations, which also contained ethylcellulose and other polymers as encapsulating agents.

Herbicide loading and encapsulation efficiency

HL values of CRFs ranged from 7.3 to 9.9% (Table 1), and they were very different from the theoretical value (14.28%). This difference has been attributed by diverse researchers to PVA, the emulsifying agent, which increases the solubility of herbicide in the continuous phase, influencing negatively the herbicide loading in microparticles (Sopeña et al., 2007a, b; Fernandez-Urrusuno et al., 2000).

EE values for the different CRFs indicated that in all cases the herbicide
content in the microparticles was lower than the theoretical content (Table 1). Although the range of EE values obtained for this parameter is wide (14 – 37 %), most of them were around 35 %.

The presence of either clays or nanoclays in the aqueous phase seems to have a little influence on EE at low concentration. At higher than 0.005% values for nanoclays and 0.04% for clays, EE value decreased considerably. This phenomenon can be partially explained by the high retention of the active ingredient (atrazine) by clays or nanoclays as it will be discussed later. On the other hand, clays and nanoclays can act as emulsion

Figure 1. SEM images of the controlled release microparticles of (a) CRF1, (b) CRF2, (c) CRF3, (d) CRF4, (e) CRF5 and (f) CRF6.
Figure 2. FTIR spectra of controlled release microparticles of (a) CRF1, polymer and atrazine technical grade; (b) CRF2, CRF3, polymer, clays and atrazine technical grade; (c) CRF4, CRF5, CRF6, polymer, nanoclays and atrazine technical grade.
Atrazine efficiency in an Andisol as affected by clays and nanoclays, Cea et al.

Table 1. Conditions used during the preparation procedures of formulations, their herbicide loading (HL) and encapsulation efficiency (EE).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>PVA (%)</th>
<th>Clay (%)</th>
<th>Nanoclay (%)</th>
<th>HL (%)</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF1</td>
<td>0.15</td>
<td>0</td>
<td>0</td>
<td>8.25 ± 1.48</td>
<td>35.78 ± 3.09</td>
</tr>
<tr>
<td>CRF2</td>
<td>0.15</td>
<td>0.04</td>
<td>0</td>
<td>9.15 ± 1.63</td>
<td>37.04 ± 2.23</td>
</tr>
<tr>
<td>CRF3</td>
<td>0.15</td>
<td>0.06</td>
<td>0</td>
<td>8.13 ± 0.20</td>
<td>13.83 ± 0.91</td>
</tr>
<tr>
<td>CRF4</td>
<td>0.15</td>
<td>0</td>
<td>0.0025</td>
<td>8.05 ± 0.78</td>
<td>31.48 ± 2.10</td>
</tr>
<tr>
<td>CRF5</td>
<td>0.15</td>
<td>0</td>
<td>0.005</td>
<td>7.30 ± 0.71</td>
<td>34.53 ± 4.29</td>
</tr>
<tr>
<td>CRF6</td>
<td>0.15</td>
<td>0</td>
<td>0.01</td>
<td>9.90 ± 0.57</td>
<td>19.26 ± 2.62</td>
</tr>
</tbody>
</table>

stabilizers (Dong and Feng, 2005). By analogy, if high concentrations of emulsifiers like PVA can decrease the encapsulation efficiency (Fernandez-Urrusuno et al., 2000), similar effects of inorganic matrix (e.g. clays or nanoclays) are expected.

Sorption studies

The sorption isotherms were adequately described by the Freundlich equation (Eq. [1]), with $R^2$ values of 0.98 and 0.95 for clays and nanoclays, respectively. The $K_f$ values were 2,500 mg kg$^{-1}$ for clays and 3,981 mg kg$^{-1}$ for nanoclays indicating the high capacity of both of them to sorb atrazine.

The $1/n$ values obtained (0.73 for clays and 0.78 for nanoclays) showed that atrazine has a strong affinity with the sorbent (Fernández–Perez et al., 2004).

Water release studies

From the six CRFs, three of them were selected to carry out a water release experiment due to their higher EE. Figure 3 shows the cumulative atrazine release from the selected controlled release formulations (CRF1, CRF2 and CRF5) into water.

![Figure 3. Atrazine released (%) into water from controlled release formulations.](image)

70
It appears that the atrazine release behavior is not markedly affected by the presence of either clays or nanoclays. The values of \( k \) and \( n \) derived from Equation [2] are shown in Table 2.

**Table 2.** Constant from fitting the empirical equation \( M_t/M_\infty = k t^n \) to release data of atrazine in water.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>( k )</th>
<th>( n )</th>
<th>( R^2 )</th>
<th>( t_{1/2} ) (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF1</td>
<td>0.13</td>
<td>0.42</td>
<td>0.92</td>
<td>24.7</td>
</tr>
<tr>
<td>CRF2</td>
<td>0.18</td>
<td>0.38</td>
<td>0.94</td>
<td>14.8</td>
</tr>
<tr>
<td>CRF5</td>
<td>0.12</td>
<td>0.47</td>
<td>0.88</td>
<td>20.8</td>
</tr>
</tbody>
</table>

We observed an increase in the rate \( (k) \) of atrazine release in the following order: CRF5 < CRF1 < CRF2. The \( n \) values ranged from 0.38 (CRF2) to 0.47 (CRF5). Values of \( n \) close to 0.43 are indicative of a Fickian diffusion mechanism in spherical monolithic matrices (Ritger and Peppas, 1987). According to Flores-Cespedes et al. (2007) the differences of \( n \) values are a consequence of: (i) the higher complexity of systems containing matrix modifier (e.g. clays), and (ii) the capacity of these agents to interact with the active ingredient. Thus, higher sorption capacity of modifying agent could result in a slower release of active ingredient.

In fact, in our work, the CRF containing nanoclays (CRF5) showed a slight deviation from \( n = 0.43 \), and therefore a slower release of atrazine which is supported by our sorption experiments. In contrast, the presence of clays in the formulation (CRF2) slightly increased the rate of active ingredient release. This result is in agreement with SEM images presented in Figure 1, since in CRF2 the roughness and irregularity of surface was higher compared with those of CRF1 and CRF5. In addition, CRF2 exhibited a greater formation of cracks and pores at the surface than the other ones. Such differences in the surface properties could explain the higher rate of atrazine release in CRF2 in respect to CRF1 and CRF5.

### Dissipation experiment in soil

In this experiment we used the three before selected CRFs and two commercial formulations (CF1 and CF2). The kinetic studies of atrazine dissipation in soil from different formulations are shown in Figure 4. We observed that in soil treated with CF1 and CF2, more than 75% of the initially applied atrazine was dissipated in 1 week. In contrast, the equivalent level of dissipation for CRFs was reached at 12 weeks, independent from the presence of clays or nanoclays. In general, CRFs protected against herbicide dissipation better than CFs.

Atrazine soil degradation from the different formulations fitted closely to the first-order kinetics evaluated here (Eq [3]), with regression coefficients ranging from 0.92 to 0.98 (Table 3). Half-life values \( (t_{1/2}) \) are also reported in Table 3. \( t_{1/2} \) were 1.29 and 1.26 for CF1 and CF2, respectively. These values are comparable with those obtained by Aguilera et al. (2009) in an Andisol with similar chemical characteristics and with previous history of atrazine application, like our specific soil. Comparatively, the \( t_{1/2} \) values for CRFs were at least 2.79-fold higher than commercial formulations (CF1 and CF2). CRF2 formulation showed a singular behavior. As it can be observed in Table 3, atrazine from this formulation suffered more dissipation than the other CRFs. This behavior could be attributed to the microparticle composition (prepared in presence of clays) and surface irregularity as it was shown in Figure 1. In this way, the presence of pores and cracks in the microparticles surface could make them ore susceptible to environmental damage than CRF1 and CRF5.
Atrazine efficiency in an Andisol as affected by clays and nanoclays, Cea et al.

Figure 4. Atrazine dissipation in soil from ethylcellulose microparticles (CRF1, CRF2 and CRF5) and commercial formulations (CF1 and CF2)

Table 3. Half-Life values ($t_{1/2}$ in weeks) in soil dissipation experiment.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$k$</th>
<th>$R^2$</th>
<th>$t_{1/2}$ (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CF1</td>
<td>0.54</td>
<td>0.97</td>
<td>1.29</td>
</tr>
<tr>
<td>CF2</td>
<td>0.55</td>
<td>0.97</td>
<td>1.26</td>
</tr>
<tr>
<td>CRF1</td>
<td>0.14</td>
<td>0.98</td>
<td>4.87</td>
</tr>
<tr>
<td>CRF2</td>
<td>0.19</td>
<td>0.97</td>
<td>3.61</td>
</tr>
<tr>
<td>CRF5</td>
<td>0.16</td>
<td>0.97</td>
<td>4.26</td>
</tr>
</tbody>
</table>

From our results, it can be inferred that CRFs represent an advantage in comparison with CFs. The lower dissipation given by the encapsulation matrix could result in a greater stabilization of the active ingredient against environmental degradation. Furthermore, according to Tsuji (2001) the active ingredient stabilization produces longer application intervals which results in labor saving.

Soil columns experiment

The behavior of CFs and CRFs in undisturbed soil was determined by measuring the atrazine content in leachates and soil in a column experiment. At the end of the experiment (30 d), atrazine was not detected in the leachates when the CRFs were applied, and only a small amount (0.01% for CF1 and 0.04% for CF2) of active ingredients, referred to the total herbicide applied, was found by effect of CFs application. The quantity of retained atrazine at the different soil depths is presented in Figure 5. At 0-10 cm depth, the retained atrazine was at least 3.8-fold higher in CRFs compared with CFs. We did not observe significant differences in the column distribution pattern of atrazine between...
CFs and CRFs, and most of the retained active ingredient was accumulated in the first 10 cm depth. Previous reports have also shown the greatest retention of pesticides in the upper soil layer (Fernández-Pérez et al., 2001; Sopeña et al., 2007a, b). The total atrazine recovery (soil plus leachates) at 30 d was no more than 3.25% for CFs and 15.49% for CRFs. The difference to 100% of the total atrazine applied was attributed to degradation processes.

As annual grasses and small seeded broadleaf weeds germinate in the upper 10 cm of soil (Sopeña et al., 2007a) our CRFs represent an excellent alternative to CFs, since after 30 d of application they retained significantly greater amount of active ingredient (9.81% of herbicide applied) in the soil surface (0 – 10 cm) in comparison with CFs (1.60%).

**Short-term bioassay**

In the short-term bioassay, the emergence percentage of field mustard was evaluated 7 days after atrazine application as shown in Table 4. All formulations (CFs and CRFs) inhibited the seedling emergence as compared with the control treatment. In average, the CRFs caused greater inhibition of emergence than CFs. Likewise, after 15 days of atrazine addition, the percentage of dead seedlings was at least 3.54 fold higher for the CRFs compared with CF2, and the highest lethal effect (67%) was observed when CRF5 was applied. These facts demonstrated that although CFs and CRFs caused an immediate herbicide effect after the atrazine application, in CRFs this effect was maintained within the time. In fact, CRFs caused a further later damage in emerged seedlings. In terms of seedling growth, no differences were observed in the aerial length between CFs and CRFs, but CRFs significantly decreased the root length (Figure 6). Thus, the later negative effect of CRFs on root growth increased the lethal effect of atrazine in respect to CFs.

The overall result of atrazine application was an increase of the lethal effect of CRFs over CFs ranging from 13% to 64% (Table 4). This is in agreement with the results of dissipation (Figure 4) and column (Figure 5) experiments.

The aerial and root length of seedlings grown in pots treated with CRFs containing either clays (CRF2) or nanoclays (CRF5) were not statistical different from those of CRF1 (ethylcellulose atrazine formulation). This fact was as it was expected, as similar behavior of CRFs was found in soil columns and dissipation experiments, irrespective of the presence of clays or nanoclays.

Other reports have shown a low herbicidal activity of controlled release formulations on soybean (Sopeña et al., 2007a) and oat (Sopeña et al., 2007b) in the short-term due to the slow discharge of the active ingredient to the soil. Nevertheless, Akelah et al. (2008) found an effective herbicidal activity from their atrazine controlled release formulations over herbs in maize crop, which agrees with the results of our experiment.

Thus, the use of CRFs could provide an advantage over CFs due to a more prolonged bio-efficiency seems to occur as a consequence of the slow release of active ingredient to the soil.

**CONCLUSIONS**

Matrix modifiers, such as clays and nanoclays added to controlled release atrazine formulations (CRFs), influenced slightly the particle morphology,
Figure 5. Distribution (micrograms) of atrazine at different depths in the soil columns after leaching the five tested formulations.

Figure 6. Effect of atrazine formulations on aerial and root length of surviving seedlings after 15 days of application.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dead seedlings (%)</th>
<th>Emergence of seed (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>67</td>
</tr>
<tr>
<td>CF1</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>CF2</td>
<td>11</td>
<td>53</td>
</tr>
<tr>
<td>CRF1</td>
<td>44</td>
<td>27</td>
</tr>
<tr>
<td>CRF2</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>CRF5</td>
<td>67</td>
<td>40</td>
</tr>
</tbody>
</table>

In all CRFs the atrazine herbicidal activity in soil was increased, and the loss by leaching through the soil profile was reduced. Even though similarities were observed in the efficiency of CRFs and CFs to diminish the seedling emergence, a higher death of seedlings was observed in the CRFs, especially when nanoclays were added into the formulation. These facts indicate an advantage of CRFs over CFs due to prolonged bio-efficiency and longer applications intervals will be produced, minimizing the harmful impact on the environment.

ACKNOWLEDGMENTS. We wish to thank FONDECYT N° 3080039 for its financial support.

REFERENCES


Atrazine efficiency in an Andisol as affected by clays and nanoclays, Cea et al.


