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Glyphosate fate in soils when arriving in plant residues

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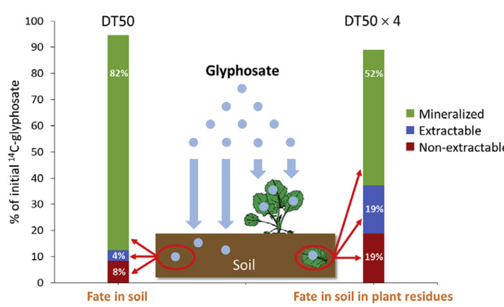
HIGHLIGHTS

- Better insight into the fate of pesticide returned to soils via plant residues is needed.
- Incorporation into oilseed rape residues hampered the mineralization of glyphosate in soils.
- Incorporation into plant residues increased the amounts of glyphosate and of its metabolite in soils.
- The trapping of pesticides into plant materials increases their persistence in soils.

GRAPHICAL ABSTRACT



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ABSTRACT

A significant fraction of pesticides sprayed on crops may be returned to soils via plant residues, but its fate has been little documented. The objective of this work was to study the fate of glyphosate associated to plants residues. Oilseed rape was used as model plant using two lines: a glyphosate-tolerant (GT) line and a non-GT one, considered as a crucifer weed. The effects of different fragmentation degrees and placements in soil of plant residues were tested. A control was set up by spraying glyphosate directly on the soil. The mineralization of glyphosate in soil was slower when incorporated into plant residues, and the amounts of extractable and non-extractable glyphosate residues increased. Glyphosate availability for mineralization increased when the size of plant residues decreased, and as the distribution of plant residues in soil was more homogeneous. After 80 days of soil incubation, extractable ^{14}C-residues mostly involved one metabolite of glyphosate (AMPA) but up to 2.6% of initial ^{14}C was still extracted from undecayed leaves as glyphosate. Thus, the trapping of herbicides in plant materials provided a protection against degradation, and crops residues returns may increase the persistence of glyphosate in soils. This pattern appeared more pronounced for GT crops, which accumulated more non-degraded glyphosate in their tissues.

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1. Introduction

A fraction of pesticides applied to crops (especially for foliar pesticides) is intercepted and absorbed by the leaves of weeds and/or crops. Plant material containing pesticides is returned to the soil during the plant cycle (via leaf senescence) or after harvest (as crop residues). The amounts of pesticide thus returned to soil with plant can be significant (Doublet et al., 2009; Von Wiren-Lehr et al., 1997), in particular with soil conservation practices because they lead to a continuous coverage of the soil surface by plant residues (Beare et al., 1993; Guérif et al., 2001). Although these practices have been known for a long time, their use was strongly increased with the introduction of glyphosate-tolerant (GT) crops, which are

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among the most cultivated genetically modified crops in the world (Cerdeira and Duke, 2006; Jacobsen et al., 2013; Locke et al., 2008). Glyphosate, a broad spectrum post-emergence herbicide, was already applied for weed control in conservation tillage systems (Dorn et al., 2013). Its use in GT crops will lead to additional inputs to soils through plant residues, especially since glyphosate is hardly degraded by most GT plants (Cerdeira and Duke, 2006; Dill, 2005; Locke et al., 2008; Nandula et al., 1999; Nandula et al., 2007). Once glyphosate has been absorbed by plants, its release into the environment depends on the decay rate of dead plant material, since glyphosate will remain sequestered in plant tissue until the plant dies and starts decomposing (Locke et al., 2008).

While the fate of glyphosate in soils is well-known (e.g. Cerdeira and Duke, 2006; Helander et al., 2012; Mamy et al., 2005), the soil fate of glyphosate absorbed in plant material (and that of other pesticides in general) has been little investigated in the literature. A soil incubation of glyphosate associated with soybean cells showed the fate of this pesticide to differ from a direct application on bare soil: glyphosate mineralization involved a lag phase and the fraction of non-extractable residues (NER) increased (Von Wiren-Lehr et al., 1997). Doublet et al. (2009) showed that the soil fate of glyphosate in aerial parts of oilseed rape and/or maize was different from that of glyphosate being directly applied to soils. Soil mineralization of glyphosate in crops decreased, and amounts of ^{14}C-extractable residues, mainly composed by the metabolite aminomethylphosphonic acid (AMPA), and NER increased. The fate of glyphosate was influenced by the type of plant compartment in which the herbicide was absorbed, because of differences in herbicide bioavailability and plant compartments biodegradability. However these two pieces of work do not consider the effects of the agricultural practices on the fate of plant residues and consequently on the fate of the associated glyphosate.

Under conventional tillage practices, crop residues are partially or totally incorporated into the soil, while in no-tillage management systems, the soil is not plowed and crop residues accumulate on the soil surface as a mulch layer (Beare et al., 1993; Guérif et al., 2001). Tillage directly affects plant residues fragmentation and distribution, and indirectly affects the environmental conditions which drive residues decomposition, such as moisture content and temperature (Angers and Recous, 1997; Guérif et al., 2001). Ground residue materials are more susceptible to microbial attack than intact plant parts due to a better soil-residue contact; however, fine particles are also more likely to be protected against decomposition through physical protection by clay and other particles (Angers and Recous, 1997; Beare et al., 1993; Iqbal et al., 2014; Sørensen et al., 1996). In addition, the placement of crop residues in soil dramatically affects the dynamics of residue decomposition, the latter being faster for buried than surface residues (Coppens et al., 2006; Douglas et al., 1980; Guérif et al., 2001). Soil water content, temperature and nutrient proximity are among the most important variables that are affected by plant residues placement (Beare et al., 1993). To the best of our knowledge, the effect of crop residues form and of their placement on the soil fate of pesticide contained in plants is not known.

In this context, the objective of this work was to study the fate in soil of glyphosate in glyphosate-tolerant and non-tolerant oilseed rape, as a function of the size of plant residues and their placement in soils. Experiments involved a preliminary step of ^{14}C-glyphosate absorption on leaves, followed by laboratory incubations of soil samples combined with leaves using different placement and fragmentation procedures. Oilseed rape was selected because it is one of the most cultivated GT crops in the world (Jacobsen et al., 2013) and also because it may be considered as a model for crucifer weeds.

2. Materials and methods

2.1. Herbicide

[Methyl-^{14}C]glyphosate (N-(phosphonomethyl)glycine) was purchased from Sigma Chemicals (81 MBq mmol^{-1}, 99.2% purity). Water solution of labeled glyphosate was prepared at 372 mg L^{-1} (containing 166 MBq L^{-1}) in order to add 18 μg of glyphosate (0.08 MBq) on each treated leaf (Grangeot et al., 2006; Lutman et al., 2008; Stanton et al., 2010; see section 2.4. Incubation procedure).

2.2. Soil

Soil samples were taken from the top layer (0–10 cm) of a French experimental site (Dijon, Burgundy), immediately placed in a cooler and taken to the laboratory where they were passed through a 3 mm sieve, removing visible organic residues by hand (Angers and Recous, 1997), and stored at 4 °C for 8 days before use. The soil is a clay-loam calcareous Cambisol (IUSS, 2015) with (% of dry soil): 37.7 of clay, 29.6 of silt, 15.2 of sand, 16.7 of $CaCO_3$, 1.63 of organic carbon, and pH in water of 8.2.

2.3. Plant material

Glyphosate-tolerant (Roundup Ready®) and non-tolerant varieties of oilseed rape (*Brassica napus* L.) were sampled in French agricultural experimental sites, both at the 4-leaf development stage. In this work, non-GT oilseed rape is considered as a representative of crucifer weeds, which commonly occur in oilseed rape fields (Légère, 2005). Direct comparison between GT and non-GT oilseed rape crops is not possible as the two oilseed rapes are not issued from the same genetic line.

Twenty-four hours following harvest of oilseed rape, the youngest leaves were cut off and treated in laboratory with ten 5 μL droplets of ^{14}C-glyphosate solution using a 25 μL micro syringe (Hamilton Co, Alltech) (Chamel et al., 1991). Preliminary results showed that there was no difference in glyphosate absorption by leaves between a whole oilseed rape plant and an isolated oilseed rape leaf (Mamy, 2004). The amounts of herbicide applied (18 μg/leaf) corresponded to a glyphosate treatment of 540 g ha^{-1} in an application volume of 150 L ha^{-1} consistent with the dose of glyphosate usually used for weed control in GT oilseed rape (Grangeot et al., 2006; Lutman et al., 2008; Stanton et al., 2010). The treated leaves were selected for the incubation experiments as they contain the highest amounts of glyphosate among all plants parts, and therefore represent the main contribution of glyphosate input to soil through crop residues (Doublet et al., 2009; Nandula et al., 1999).

Eight days after glyphosate application, one half of the treated leaves were washed with 10 mL of ultrapure water (Millipore) (Doublet et al., 2009; Nandula et al., 1999). The amounts of absorbed herbicide in washed leaves were estimated by the difference between radioactivity contained in wash solution, determined by liquid scintillation counting (see section 2.5. Chemical analysis), and the applied radioactivity. The washed leaves allowed incubation of the amount of herbicide that was absorbed in the leaves, while the non-washed leaves represented the maximum amount of herbicide that can be returned to the soil through leaves.

Treated leaves were prepared to study different conditions of plant materials when they reach the soil (Table 1). The effect of the size of plant residues was studied comparing entire leaves, leaves fragments of 3-mm size obtained after fractionation with scalpel, or crushed leaves finely ground in a glass mortar. To study the effect of plant residues placement in soil, leaves will be put on the soil

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