Applied Soil Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Applied Soil Ecology

journal homepage: www.elsevier.com/locate/apsoil

Effects of different concentrations of glyphosate (Roundup 360®) on earthworms (*Octodrilus complanatus*, *Lumbricus terrestris* and *Aporrectodea caliginosa*) in vineyards in the North-East of Italy

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ARTICLE INFO

Keyword:
Deep-burrowing earthworms
Anecic earthworms
Glyphosate
Survival rate
Cocoon production
Resistance mechanisms
Vineyards
Octodrilus complanatus
Lumbricus terrestris
Aporrectodea caliginosa

ABSTRACT

The study aims to analyse the effects of the herbicide glyphosate (Roundup 360®) on three key earthworm species. The taxa collected from treated and untreated sites were subsequently bred in uncontaminated soil terraria with serial concentrations of the herbicide. The chosen taxa included two anecic (*Lumbricus terrestris* and *Octodrilus complanatus*) and one endogeic (*Aporrectodea caliginosa*) earthworms. *Lumbricus terrestris* collected from uncontaminated soil (Lt1) and in a conventionally managed vineyard (Lt2). *Aporrectodea caliginosa* and *Octodrilus complanatus* collected from the same uncontaminated site where *L. terrestris* (Lt1) were collected.

Uncontaminated natural grassland soil filling the terraria was characterized for physical-chemical properties. The reproduction rate expressed in earthworms cocoon production was the measure for the effects on worms survival after 21 and 42 days of exposure to 0.59, 2.9, 5.79 g/m^2 of glyphosate, where the dose applied in vineyards in the North-East of Italy goes from 0.72 g/m^2 to 4.32 g/m^2.

Earthworms in untreated terraria were found all alive, while specimens exposed to glyphosate (Roundup 360®) showed a decreasing survival rate and a sharp decline in the number of cocoons. *L. terrestris* (Lt2) showed a survival rate between 64% and 92% after 21 days, and between 44% and 76% after 42 days. *L. terrestris* (Lt1) showed a lower resistance to herbicide (survival rate: 36%–84% after 21 days and 12%–76% after 42 days). *A. caliginosa* was also affected (survival rate: 32%–76% after 21 days and 12%–68% after 42 days). Glyphosate demonstrated severe effects on *Oc. complanatus,* collected on non-contaminated soils, with the lowest values of survival rate (33% after 21 days and 7% after 42 days of exposure). A significant reduction in the cocoons number (about 70%) was observed for *L. terrestris* (Lt1) and *A. caliginosa* after 21 days of exposure, whereas *L. terrestris* (Lt2) showed about 50% of cocoon production.

Results indicate the occurrence of some resistance mechanisms on anecic earthworms in vineyards that have been exposed to glyphosate for at least three decades.

However in spite of the long period of application of glyphosate the impact of this largely applied herbicide is still serious (up to 26% of mortality) especially on the deep-burrowing earthworms species (*Oc. complanatus* and *L. terrestris*).

1. Introduction

Intensification of soil tillage (Edwards and Bohlen, 1996), higher inputs of fertilisers and pesticides, heavy metal soil contamination (Schlegel and Manceau, 2013; Zhang et al., 2009), soil compaction exerted by heavy agricultural machinery are the most important drivers of biodiversity loss in many agroecosystems (Hole et al., 2005; Paoletti, 1999; Paoletti and Pimentel, 2000; Lindenmayer et al., 2012), especially in orchards and vineyards (Paoletti et al., 1995a,b; Altieri et al., 2005). These agricultural practices negatively affect some of different groups of soil fauna (e.g. earthworms, micro-arthropods, nematodes, microorganisms) (Ponge et al., 2013) known for their sensitivity to disturbances associated to agriculture, as soil tillage (Cortet et al., 2002a; Krogh et al., 2007; Lagomarsino et al., 2009), trampling and soil compaction (Cluzeau et al., 1992; Heisler and Kaiser, 1995), fertilizer addition (Cole et al., 2005; Van der Wal et al., 2009), and

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http://dx.doi.org/10.1016/j.apsoil.2017.07.028
Received 12 January 2017; Received in revised form 13 July 2017; Accepted 18 July 2017
0929-1393/

Please cite this article as: Stellin, F., Applied Soil Ecology (2017), http://dx.doi.org/10.1016/j.apsoil.2017.07.028

pesticide treatment (Frampton, 1997; Rebecchi et al., 2000; Cortet et al., 2002b). The use of pesticides (insecticides, fungicides and herbicides) in the agroecosystems represents one of the most controversial agricultural activities because of its impact on soil fauna (Paoletti and Bressan, 1996). Herbicides have pronounced negative effects on nematode soil abundance (Sánchez-Moreno et al., 2014), on interactions between earthworms and symbiotic mycorrhizal fungi (Zaller et al., 2014) on dehydrogenase activity of bacterial, fungal and actinomycetes soil communities (Sebiomo et al., 2011). Recent studies highlighted how the magnitude of micro-arthropods responses (phytophagous and predatory mites species) to weed management practices (glyphosate applications) may be linked to changing in habitat, such as loss of vegetation cover and elimination of food sources (Belden and Lydy, 2000).

In many agroecosystems and natural environments, earthworms are probably the most important group of soil biota in terms of soil formation and maintenance of soil structure and fertility (Coleman and Wall, 2015). As ecosystem engineers, especially the deep-burrowing species (anecic ones) (Lavelle et al., 1997) they have fundamental roles in soil functioning because of their burrowing activities, as well as their ingestion of soil and production of castings (Latif et al., 2009; Gavinelli et al., 2017). The importance of earthworms includes their influence on soil aeration, water infiltration and mixing of horizons that improve the soil structure. Also they represent an important source of food for birds and moles (Lavelle et al., 2006). The beneficial effects of earthworms was described for the first time by Darwin (1881) which summarized his study on earthworms after 40 years of experimental work and observations (Edwards, 2004). Since then, a vast literature has confirmed the great importance of earthworms as biological agents in soil formation (Paoletti et al., 1995a,b, 1998) and suitable bioindicators of soil pollution (Paoletti, 1999; Paoletti and Sommaggio,1996; Rodriguez-Castellanos and Sanchez-Hernandez, 2007) in terrestrial ecosystems providing an early warning of deterioration in soil quality.

Pesticides (Lüscher et al., 2014; Mosleh et al., 2003), especially fungicides (Paoletti et al., 1998) and herbicides (Pizl, 1988), have negative effects on earthworms at all organization levels (Pelosi et al., 2014) causing their death (Slimak, 1997), depressing their growth (Dalby et al., 1995; Springett and Gray, 1992; Correia and Moreira, 2010), affecting their reproduction (Yasmin and D'Souza, 2010) and their behaviour (Buch et al.,2013; Kaneda et al., 2009). Also the fatty acids content can be affected (Hossam et al., 2012). Moreover, herbicides via earthworms can achieve higher levels of the trophic chain (Jarmuł-Pietraszczyk and Jastrzebska, 2012).

Glyphosate (GLY) (N-phosphonomethyl-glycine) base product is the leading non-selective aminophosphonate-type herbicide for the annual and perennial weeds control both in agriculture and in non-agricultural landscapes (Piola et al., 2013). It has been registered as a broad spectrum herbicide in the U.S. since 1974 and in the early twenty-first century, glyphosate became the largest-selling single crop protection chemical product on the market (Woodburn, 2000). Glyphosate is generally regarded as an environmentally-friendly herbicide due to its rapid biodegradation, strong adsorption to soil minerals, especially Fe and Al oxides (Norgaard et al., 2014) and fast microbial degradation (Barja and Santos, 2005; Giesy et al., 2000; Vereecken, 2005). Some studies demonstrated that the presence of glyphosate could significantly reduce the acute toxicity of Cu to earthworms (Zhou et al., 2012, 2013).

However, many common glyphosate-containing products (Roundup360®) are made with a polyethoxylated tallow amine surfactant that is more toxic than glyphosate itself and the combination of the two is yet more toxic (Monsanto, 1996). These chemicals are acutely adverse to animals (IBR, 1991a,b; Martin, 1982; Morowati, 2000); Monsanto (1988a,b) demonstrated that Roundup increases the frequency of recessive lethal mutations in fruit flies. Acute toxicity tests on laboratory rats showed that the absorbed glyphosate dose had distributed throughout the body seven days after administration (Monsanto, 1988a,b). In humans, the main effects involve cardiac depression, accumulation of excess fluid in the lungs, eye and skin irritation, gastrointestinal pain and vomiting (Cox, 1995a). Moreover, residues of the commonly-used herbicide glyphosate can be detected long after glyphosate treatments have been made (Cox, 1995b; Landry et al., 2005). However, some earthworms species (*Aporrectodea caliginosa* and *Allolobophora chlorotica*) showed the ability to acclimate to residual glyphosate contamination in agricultural soils through accelerated activation of detoxification and antioxidant enzymes (Bon et al., 2006; Givaudan et al., 2014; Omar et al., 2012).

The current study aimed at testing the effects of a commercial formulation of glyphosate (Roundup 360 Power, Monsanto Europe N.V. Belgium), on the growth and survival rate of three key earthworms species (two being large burrowing species: *Lumbricus terrestris* and *Octodrilus complanatus* and one*, A. caliginosa* an endogeic) collected in either treated or untreated sites and subsequently reared within terraria supplemented with increasing dosages of glyphosate.

Concentration-dependent weight loss has been reported for glyphosate intoxication in *Eisenia andrei* (Piola et al., 2013) and *Eisenia foetida* (Yasmin and D'Souza, 2007). On *E. foetida* a reduced locomotor activity was also observed (Verrell and Van Buskirk, 2004). Negative impact of glyphosate on reproductive ability and on growth inhibition and in nervous system has been reported in *Dendrobaena veneta* and the production of deformed cocoons under laboratory conditions was observed (Jarmuł-Pietraszczyk and Jastrzebska, 2012). *Lumbricus terrestris* featured a significant decrease in sperm numbers (Sherwan, 2013).

2. Material and methods

2.1. Species analyzed

Lumbricus terrestris is a deep-burrowing, anecic species native in Europe; an earthworm that recently has invaded some areas of Canada, parts of US and South America, New Zeland, Australia. It is pinkish to reddish-brown in colour and typically reaches 10–25 cm in length. It is found in deciduous natural forests, planted forests, range/natural grasslands, riparian zones, ruderal/disturbed, scrub/shrub lands, urban areas, wetlands and it is often present also in agricultural fields, although it is negatively affected by fungicides, herbicides, tillage operations and lack of leaf litter. It can inhabit all soil types except coarse sands, bare rock and acidic peat (*Sphagnum*), but it is not frost-tolerant and hibernates into deep soil layers during the winter (Addison, 2009; Tiunov et al., 2006; Wironen and Moore, 2006). By rapidly consuming leaf litter, *L. terrestris* affects biochemicals cycles and plant communities through its interaction with seeds. It may also displace native earthworm species.

Oc. complanatus is a large deep-burrowing species and it is present on hills, mountains, woodlot remnants and in rural landscapes. Although large specimens also disappear upon shifting to agricultural intensification, they generally recolonize fields abandoned to fallow (Paoletti, 1999). It lives into deep soil galleries but occasionally feeds on surface litter when soil humidity is high enough, e.g., in the North-Eastern Italy (Paoletti, 1985).

A. caliginosa is the most common endogeic species in European, North American and New Zealand fields (Paoletti and Pimentel, 2000).

2.2. Experimental design

The experiment consisted of two trials. Every trial had the aim to analyze two groups of taxa. Adults earthworms thoroughly identified by morpho-anatomical characters using the LOMBRI software (Paoletti and Gradenigo, 1996) were collected in farms under different agricultural management and geographical areas:

Lumbricus terrestris (Lt1 group), and *Aporrectodea caliginosa* were collected from non-treated natural grassland close to the experimental farm at Legnaro (45°20′42.87″N; 11°57′20.26″E);