Soil Biology & Biochemistry 95 (2016) 30–39

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Effects of the allelochemical coumarin on plants and soil microbial community

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ARTICLE INFO

Article history:
Received 20 April 2015
Received in revised form
25 November 2015
Accepted 28 November 2015
Available online 22 December 2015

Keywords:
Coumarin
Weed control
Wild oat
Wheat
Microbial biomass and activity
Bacterial and fungal genetic diversity

ABSTRACT

Coumarins are a large family of plant secondary metabolites with allelopathic properties. Coumarin activity against weeds has been demonstrated in *in-vitro* conditions, but the effects of coumarin on weeds and other organisms in natural conditions have not been yet investigated. A necessary step before field study is represented by microcosm experiments where natural conditions can be partially simulated by using undisturbed soil cores and the effect of coumarin can be verified before application in field. This study evaluates the effects of coumarin (0, 100, 200, 300 mg kg^{-1} dry soil) on a weed (wild oat), a crop (wheat), and microbial community in *soil* and *soil + plants* microcosms. For each treatment and experimental system, we determined plant growth (length and mass of shoot and root), soil microbial biomass and activity, and bacterial and fungal genetic diversity. The results indicate that coumarin is a promising natural herbicide with potential application in sustainable agriculture, and point to doses in the range 100–150 mg kg^{-1} dry soil for optimal harm-to-benefit compromise. Comparison with data from the literature reveals that responses to coumarin by plants and microorganisms in natural systems may significantly diverge from behaviours observed *in vitro*, probably reflecting multifaceted interactions of biotic and abiotic components.

1. Introduction

The systematic use of synthetic herbicides in agriculture may induce resistance in weeds (Pergo et al., 2008; Chuah et al., 2013) and damages to non-target plants (Boutin et al., 2012), animals and microorganisms (Aktar et al., 2009; García-Orenes et al., 2010; Chen et al., 2014), with a consequent loss of biodiversity (Boutin et al., 2012). Herbicides may also cause adverse effects on human health (Pimentel, 2005), therefore, European Community (Directive 2009/128/EC; Regulation 1107/2009) recommended their sustainable use, as for other pesticides, by encouraging the development and application of alternative approaches or techniques to reduce dependency on its use. Growing concern over the ecological impact of synthetic herbicides has stimulated the research for alternative methods of weed control based on natural products (Pergo et al., 2008).

Plants are a rich source of compounds with biological activity, mainly secondary metabolites (Pergo et al., 2008). These molecules perform a diversity of functions including plant protection from fungi and bacteria (Macías et al., 2007), deterrence against herbivores, inhibition of competing plant species (Bourgaud et al., 2001; Bhadoria, 2011). The last effect, known as allelopathy, is now regarded as a major ecological mechanism structuring plant communities (Mallik, 2008).

Among secondary metabolites known for their allelopathic properties, particularly widespread are coumarins, a group of lactone derivatives of cinnamic acid present in numerous plant species and encompassing at least 1300 molecules classified as coumarins, hydroxycoumarins, pyranocoumarins and furanocoumarins (Hoult and Payá, 1996). Among these, particularly abundant and active is coumarin (2H-chromen-2-one) that also represents the core structure of molecules of pharmaceutical importance for man (as novobiocin, coumaromycin, chartesium) and its numerous derivatives have been used as anticoagulant, antibacterial, antifungal, antioxidant, antitumour, anti-HIV, anti-hypertension, anti-inflammatory, anti-arrhythmia, anti-

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http://dx.doi.org/10.1016/j.soilbio.2015.11.028
0038-0717/

osteoporosis and for the treatment of lymphoedema (Katsori and Hadjipavlou-Litina, 2014).

Coumarin is present in plants of the Fabaceae, Apiaceae, Rutaceae, Asteraceae, Poaceae and Lamiaceae families (Esposito et al., 2008; Haig et al., 2009; Yamamoto, 2009; Razavi, 2011; Tesio et al., 2011). This compound inhibits the growth and development of many plant species, its main target being the root system (Lupini et al., 2014). *In-vitro* experiments have demonstrated reduced seed germination and/or reduced plant growth in response to coumarin treatment for several weeds including *Bidens pilosa* (Pergo et al., 2008), *Lolium rigidum* (Haig et al., 2009), *Avena fatua* (Goodwin and Taves, 1950), *Eleusine indica* (Chuah et al., 2013). Coumarins also display anti-microbial activity *in vitro* (Hooper et al., 1982; Razavi et al., 2010). Very little is known about the effects of soil-applied coumarins on microorganisms (Luo et al., 2007). This is a cause of concern when considering the possible use of coumarins for weed control in agriculture, as soil microbial community is an essential component of terrestrial ecosystems. Largely unexplored also are possible effects of coumarins and other biologically active natural compounds on crops. Before field application of coumarins or other allelopathic compounds, it is necessary to verify, at a lower scale (i.e., microcosm), if they are effective on weeds in soil environment too and to exclude that they can negatively affect no target organisms.

The present study aimed to evaluate coumarin effects on plants and soil microbial community in microcosm systems. The selected plants were wheat (*Triticum durum* Desf.), a crop of high economic relevance, and wild oat (*A. fatua* L.), a weed frequently associated with wheat in the field.

2. Materials and methods

2.1. Coumarin and soil

The coumarin tested was 2H-chromen-2-one, the most abundant coumarin extracted from *Melilotus neapolitana*, a highly competitive annual legume of the Mediterranean maquis (Esposito et al., 2008). Synthetic 2H-chromen-2-one was purchased from Sigma–Aldrich. This compound is referred to as coumarin hereafter.

Soil cores for microcosms were collected at Maddaloni (Southern Italy, 41°02′37″N; 14°24′45″E) in an olive grove, abandoned since at least 15 years, with a high herbaceous cover. The lack of agrochemical inputs and soil disturbance during the last 15 years was considered suitable situation to have appropriate soil baseline conditions to evidence the effect of a new stressor added to the soil. The soil was identified as a Molli-Vitric Andosol (di Gennaro, 2002) with a sandy loam texture (Marzaioli et al., 2010), pH of 7.7, water holding capacity 70.4%, bulk density 0.7 g cm^{-3}, electrical conductivity 0.4 dS m^{-1}, organic carbon content 71.3 g kg^{-1}.

2.2. Preliminary assays

Since studies available in literature concerning the effect of coumarin on plants or microorganisms were generally done *in-vitro* conditions, it was necessary to carry out preliminary assays in order to define the appropriate range of effective coumarin concentrations and incubation time to be applied in a more complex system, as the soil, where the effect of coumarin could be different than *in-vitro* conditions because of interactions of coumarin with soil components. Undisturbed soil cores were collected at 0–10 cm depth and transferred to 40 pots (13.5 cm in diameter, 10 cm in height) for plant assay and 32 pots for soil assay. For both series of assays, coumarin was applied in aqueous solution at three concentrations (50, 100, 150 mg kg^{-1} dry soil, plus control), with five (plant assay) and four (soil assay) replicates for each treatment. The experiments were carried out at environmental temperature (about 20 °C) and 60% of field capacity. For plant assay, 10 seeds of wild oat or 10 seeds of wheat were placed in each pot before coumarin addition and the percentage of seedling emergence from soil was determined 7 days later. Mass and length of shoot and root were measured 20 days after seedling emergence. For soil assay, soil samples were collected 14 and 28 days after coumarin addition and were analysed for extractable organic carbon, microbial biomass, fungal mycelium, total microbial activity (evaluated as soil respiration) and some specific activities (N mineralization, nitrification and N-acetyl-β-D-glucosaminidase activity), using the methods described in the following section.

The preliminary assays showed no effect of coumarin on seedling emergence of either plant species, a negative effect on oat only at the highest concentration tested (150 mg kg^{-1}) and a positive significant effect on wheat (Table 1). Moreover, no persistent effect of coumarin was observed on soil parameters through the whole

Table 1
Mean values (±standard deviation) of seedling emergence (expressed as number of seedlings emerging from soil in % of seeds add to pot), shoot and root length (cm) and mass (mg) of oat and wheat grown in soil treated with different coumarin amounts (0, 50, 100, 150 mg kg^{-1} dry soil) obtained in preliminary assay on plants. Different superscript letters indicate significant differences ($P < 0.05$) among treatments. The parameter values showing significant differences among treatments are indicated in bold.

Plant and variable	Coumarin concentration (mg kg^{-1} dry soil)			
	0	50	100	150
Oat				
Seedling emergence	50.0 (±7.1)	46.0 (±16.7)	38.0 (±13.0)	42.0 (±27.7)
Oat				
Shoot length	**16.3 (±4.0)**^{a}	**14.6 (±4.7)**^{ab}	**13.3 (±5.3)**^{ab}	**11.6 (±5.1)**^{b}
Root length	5.8 (±1.7)	5.2 (±2.1)	6.6 (±3.2)	4.7 (±1.8)
Oat				
Shoot mass	**57.2 (±14.6)**^{a}	**44.3 (±21.9)**^{ab}	**47.2 (±29.8)**^{ab}	**36.9 (±20.9)**^{b}
Root mass	**3.6 (±2.3)**^{a}	**2.3 (±1.6)**^{ab}	**2.8 (±1.7)**^{ab}	**2.0 (±1.0)**^{b}
Wheat				
Seedling emergence	72.0 (±8.4)	82.0 (±14.8)	80.0 (±12.2)	92.0 (±8.4)
Wheat				
Shoot length	**12.8 (±3.2)**^{a}	**14.2 (±3.8)**^{a}	**17.3 (±3.2)**^{b}	**17.0 (±3.6)**^{b}
Root length	**6.7 (±1.9)**^{a}	**9.0 (±3.6)**^{bd}	**11.3 (±3.8)**^{ce}	**10.0 (±3.3)**^{de}
Wheat				
Shoot mass	**76.8 (±36.0)**^{a}	**93.1 (±36.1)**^{ac}	**116.3 (±44.0)**^{b}	**108.2 (±35.1)**^{bc}
Root mass	7.3 (±4.6)	7.3 (±2.9)	7.0 (±3.6)	6.5 (±2.6)