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The other way around: the utility of a plant invader

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Abstract

Invasive species control management involves a large amount of plant material. The present work evaluated the allelopathic potential of the invasive species *Ulex europaeus* L. (Fabaceae) or 'Gorse' and its possible use as a bioherbicide, taking advantage of the extracted plant material after control measures, particularly needed in invaded areas. Specifically, we investigated the efficacy of dried plant material from *U. europaeus* in the control of the adventitious plants, *Lolium multiflorum* Lam. and *Lolium rigidum* Gaud., using the *Avena sativa* L. crop as a case study. We only used vegetative plant parts because it is essential to avoid the dispersion of *U. europaeus* with its use, especially in invaded areas. A greenhouse pot experiment was conducted, using activated carbon (AC). The target species (*L. multiflorum*, *L. rigidum* and *A. sativa*) were subjected to a mixture of organic substrate with *U. europaeus* mulch applied pre-emergence and a subsequent application of aqueous extracts from the mulch. Emergence, height and biomass of the target species were determined. After 2 months, we also tested a possible legacy effect of the substrate on the germination of the target species. We noticed a negative effect of *U. europaeus* mulch on the emergence of *L. rigidum*, which can be attributable to the allelopathic compounds released from *U. europaeus* mulch because the effect was non-significant in presence of AC. Conversely, no effect on *L. multiflorum* or *A. sativa* was produced by mulch treatments. Nevertheless, the combination of *U. europaeus* mulch and its extracts demonstrated a phytotoxic effect on the biomass of the crop species *A. sativa*, and a fertilizing effect on the weeds *L. multiflorum* and *L. rigidum*, which is why this use is discouraged. With our results we cannot recommend the use of *U. europaeus* as a bioherbicide in oat crops, but this study emphasizes the capability of *U. europaeus* to structure plant communities through the chemic- and bio-properties of its tissues that modifies the soil environment.

Introduction

Synthetic herbicides are known to cause many environmental problems as they accumulate in soil (Bhowmik and Inderjit, 2003; Shah *et al*., 2016). These herbicides are widely and continuously used in agriculture to deal with undesirable weeds, which in turn affects the balance of agricultural fields and neighboring ecosystems, changing soil physico-chemical and biological properties, and increasing the ecotoxicological risk for aquatic and terrestrial non-target organisms, including plants, animals, soil microorganisms and even humans (Pardo-Muras *et al*., 2020*b*; Mehdizadeh *et al*., 2021; Monteiro and Santos, 2022). Besides, several weeds causing economically crop losses, such as *Lolium rigidum*, have evolved resistance to synthetic herbicides (Owen and Zelaya, 2005; Taberner Palau *et al*., 2007). Therefore, the demand for new environmentally safe, low risk alternatives, the so-called bioherbicides, is nowadays increasing. Bioherbicides are biologically based control agents for weeds (Soltys *et al*., 2013) that can be used alone or together with other practices (integrated weed management) to reduce the use of synthetic herbicides (Scavo and Mauromicale, 2020). Their main downside is that the process of development and commercialization takes many years and the costs associated are rather high (Kremer, 2005; Weaver *et al*., 2007; Bailey, 2014).

Some of the characteristics of a good bioherbicide comprise: (1) efficacy on target species, (2) no effect on crop species or other non-target plants growing around the application, (3) limited reproduction if it is a plant-based bioherbicide, (4) low persistence in soil, and therefore the least possible legacy effect on the ground (Bailey, 2014). Plant-based bioherbicides have the following advantages regarding other bioherbicides: (1) many plant phytotoxic compounds are soluble in water, which avoids the use of a surfactant for its application, and (2) the mode of action of allelochemicals is similar to that of synthetic herbicides, but they are safer because of their low persistence and activity in soil (Soltys *et al*., 2013). There is a consistent literature that compiles traditional agronomic uses of plants as bioherbicides (Araniti *et al*., 2012; Pacanoski, 2015; Puig *et al*., 2018; Souza-Alonso *et al*., 2020), but it is necessary to explore the efficacy of each donor plant in each specific context and the best application techniques (e.g., aqueous extracts or mulches; pre-emergence or postemergence) (Soltys *et al.*, 2013; Marble, 2015). Bioherbicides based on the allelopathic compounds naturally produced by plants can be cheap and environmentally friendly alternatives (Albuquerque *et al.*, 2011; Soltys *et al.*, 2013). Particularly, cover crops and mulches are ecofriendly choices for sustainable agriculture that can be used not only for weed control but also as fertilizers to improve crop performance (Campiglia *et al.*, 2010).

Allelopathy can be defined as the ability of plants to inhibit the survival, growth, development and reproduction of other organisms through the release of chemical compounds present in their tissues (Lorenzo and González, 2010; Cheng and Cheng, 2015). Several plant species can use allelopathy as a strategy to compete with other plants for environmental resources, but in some species this strategy is more relevant (Pisula and Meiners, 2010). Additionally, some plant species can be more susceptible to the allelopathic compounds released to the environment by other plants (Medina-Villar *et al.*, 2017). Also, the quantity and composition of allelochemicals greatly vary among plant species, plant organs and specific contexts (Cappuccino and Arnason, 2006; Pisula and Meiners, 2010; Bauer *et al.*, 2012; de las Heras *et al.*, 2020). Allelopathy is a phenomenon with ecological implications, as it influences the species distribution in a community (Hierro and Callaway, 2021). Therefore, studying how species chemically interact with each other by means of allelopathy it is essential to better understand their role structuring plant communities in different ecosystems, including agroecosystems, but also, this knowledge can be applied to map adequate candidates to be used as bioherbicides for a more sustainable agriculture (Albuquerque *et al.*, 2011; Hasan *et al.*, 2021; Lopes *et al.*, 2022).

Exotic invasive plants arise as good candidates to study new bioherbicides because of different reasons. First, many invasive plant species are allelopathic, some of them with high allelopathic potential, such as *Ailanthus altissima* (Mill.) Swingle (Pisula and Meiners, 2010; Chengxu *et al.*, 2011; Kalisz *et al.*, 2021). Second, there are numerous exotic invasive plant species causing serious ecological and socio-economic impacts worldwide, which need to be managed and eliminated for native ecosystem recovery (Pimentel *et al.*, 2005; Pyšek *et al.*, 2012; Seebens *et al.*, 2017). The measures to eliminate these species are extremely difficult and expensive and involve the extraction of a huge amount of biomass, which is frequently burned releasing CO_2 to the atmosphere (Pimentel *et al.*, 2005; Lovell *et al.*, 2006; Villamagna and Murphy, 2010). Therefore, using the removed biomass of invasive species, instead of burning it, provides an environmentally friendly and cost-effective solution for weed management. Some studies verified the effectiveness of using invasive species, such as *Parthenium hysterophorus* L., *Acacia dealbata* Link., *Eucalyptus globulus* Labill. or *Cytisus scoparius* (L.) Link, for weed management in agroecosystems without endangering the culture species (Singh *et al.*, 2005; Marwat *et al.*, 2008; Puig *et al.*, 2013; Souza-Alonso *et al.*, 2020; Pardo-Muras *et al.*, 2020*a*, 2020*b*). The alterations of soil properties produced by using plant material from invasive species should also be considered (legacy effects), as for example, the possible increase in soil nitrogen let by the tissues from Fabaceae plants, able to fix N_2 from the atmosphere (Elgersma *et al.*, 2011; Grove *et al.*, 2012, 2015; Von Holle *et al.*, 2013). Besides, increases in soil nitrate may interfere with allelopathic effects by promoting seed germination (Duermeyer *et al.*, 2018). From an ecological point of view, studying how an exotic invasive plant interacts with other plants (e.g., weeds, crop species) is interesting to better understand the bio-properties of the invasive species and its capability to impact plant diversity and agroecosystems.

A N_2-fixing species, able to alter soil properties is *Ulex europaeus* L. (Fabaceae), commonly known as 'gorse' (Bateman and Vitousek, 2018). It is a native species from NW Europe and a serious invader in many world regions, such as Chile, Australia, Sri Lanka, Hawaii or in the west coast of USA (Clements *et al.*, 2001; Bateman and Vitousek, 2018). This species has been intentionally introduced in many countries as a livestock fodder plant or as a living fence, but it has also arrived accidentally by zoochory in animal's fur, being now an invasive species distributed worldwide (Norambuena and Piper, 2000; Clements *et al.*, 2001). This plant species is able to affect soil properties in the invaded ecosystems, e.g., reducing soil pH (Bateman and Vitousek, 2018), and it has been classified as one of the 100 worst invasive species of the world by the International Union for Conservation of Nature (IUCN/SSC, 2000). For instance, in the case of Chile, if no action is taken, the United Nations Development Program (UNDP) has estimated the economic loss associated with the presence of *U. europaeus* in more than 49 millions of dollars in the next two decades (IUCN/SSC, 2000). Considering the ecological and socio-economic impact caused by *U. europaeus* (Galappaththi *et al.*, 2022), the need to eradicate or reduce its density is evident.

To recover management costs, the use of the extracted biomass of *U. europaeus* as biofuel (Viana *et al.*, 2012), fertilizer (Galappaththi *et al.*, 2022), promotor of secondary metabolism (Tighe-Neira *et al.*, 2016) in crops and bioherbicide (Pardo-Muras *et al.*, 2020*a*, 2020*b*) has already been suggested. The ability of its compounds (volatile oxygenated monoterpenes and water-soluble phenolic compounds) to significantly inhibit germination and growth of *Amaranthus retroflexus* L. and *Digitaria sanguinalis* (L.) Scop. has been reported (Pardo-Muras *et al.*, 2018, 2019, 2020*a*, 2020*b*). Besides, one of the most prominent secondary compounds in *U. europaeus*, known as maackiain (Cappuccino and Arnason, 2006), extracted from another Fabaceae species, *Trifolium pratense* L., showed phytotoxic effects on grass species (Liu *et al.*, 2013). The efficacy and potential pre-emergence use of fresh plant material from flowering *U. europaeus* against weeds in maize crops was demonstrated in a previous pot experiment conducted by Pardo-Muras *et al.* (2020*b*). However, different target species, experimental conditions and approaches may greatly change the outputs in allelopathic studies (Haugland and Brandsaeter, 1996; Kobayashi, 2004; Zhang *et al.*, 2021).

Here we suggest the use of different target species and experimental conditions as those used in the studies of Pardo-Muras *et al.* (2018, 2019, 2020*a*, 2020*b*). These conditions raise new and relevant insights to study the allelopathic potential of *U. europaeus* and its possible use as a bioherbicide. For instance, we used dried instead of fresh plant material because it is a useful way to conserve and store the surplus plant material before its use, preventing decomposition and keeping allelopathic potential (Bonanomi *et al.*, 2018; Gatto *et al.*, 2021). We also used activated carbon (AC), as an allelopathy neutralizer, commonly used to discriminate if the effects of plants are driven by allelopathic compounds (Sturm *et al.*, 2018; Lorenzo *et al.*, 2021). Moreover, as the use of *U. europaeus* materials is relevant in invaded areas, because there is already an enormous quantity of undesirable *U. europaeus* plants needed to be removed, the plants used in invaded areas need to be cut before seeding, and even before flowering, to avoid any possibility to spread seeds or add invasive seeds to the crop field, which could increase the invasion problem and affect the crop yield. Therefore, we were interested in assessing whether the vegetative part of *U. europaeus* is allelopathic. Harvesting and transporting big amount of biomass of an invasive plant species from its established habitat to crop fields would inevitably cost farmer extra money. Thus, in our opinion the best way to act, an also the most sustainable, is using the extracted plant material on crops close to geographical areas that are already invaded by *U. europaeus*.

Having into account all these considerations, in this study, we aimed to evaluate the suitability of *U. europaeus* plants as a bioherbicide in oat crops (*Avena sativa* L., commonly named 'oat') as a case study, testing the allelopathic potential of dry plant material (vegetative parts) from *U. europaeus* on the following target plants belonging to the same family (Poaceae) and subfamily (Pooideae): two common weeds of oat crops, *Lolium multiflorum* Lam. and *L. rigidum* Gaud. (commonly referred to as 'ryegrass'), and on the crop species. We also aimed to evaluate the soil legacy effect of *U. europaeus*. We selected oat as the crop species because it is a very common crop in several countries where *U. europaeus* is a troublesome invader that reaches high densities and hinders crop practices (Quiroz *et al*., 2009; Koch *et al*., 2016; ODEPA, 2019). *L. rigidum* and *L. multiflorum* are native from Mediterranean Basin and widespread grain weeds (Romero and Fraga, 1990; Diez de Ulzurrun and Leaden, 2012), coexisting with *U. europaeus* (Moreno-Chacón *et al*., 2018). The allelopathic potential of *U. europaeus* and its soil legacy effect has not been previously evaluated on *A. sativa* and *Lolium* weeds.

Based on previous studies showing the ability of *U. europaeus* to negatively affect different herb species, but not the crop species (Pardo-Muras *et al*., 2020*a*, 2020*b*), we could expect less effectiveness of *U. europaeus* on oat than on *Lolium* weeds, but also bigger seed size of the former supports our expectation (Liebman and Sundberg, 2006). To firmly consider *U. europaeus* as a proper bioherbicide, negative effects need to be demonstrated on the weeds but not on the crop species and the soil legacy effect should be the minimum, reducing possible effects to non-target species. If so, this study will be the starting point to consider the implementation of the *U. europaeus* as a bioherbicide in oat crops. Besides, exploring the biological properties of invasive plant material contributes to understand the ability of *U. europaeus* to affect coexistent herb species by means of allelopathy or by changing other soil properties.

Material and methods

We evaluated in a greenhouse pot experiment two possible techniques to apply *U. europaeus* as a bioherbicide in crop fields: (a) direct use of dried *U. europaeus* material (hereby named as 'mulch') preemergence added and (b) preemergence mulch combined with a subsequent postemergence application of aqueous extracts from the mulch. We also analyzed the legacy effect (2 months period), let by *U. europaeus* (mulch + extracts) in the soils, on the germination of the target species.

Plant material

The donor species whose phytotoxic potential was tested is *U. europaeus*, a shrubby legume species with entomophilous pollination, from humid acidic habitats (Rapoport *et al*., 2009). Branches of *U. europaeus* were collected from at least 20 randomly selected individuals in non-herbivorized native populations located in NW Spain, specifically in Orense (N 42°18′21″; O −8°7′7″) and Pontevedra (N 42°11′53.6″; O −8°39′53″) in late Spring 2018 and 2019. In Orense, the mean annual temperature is 13.1°C and total annual precipitation is 1224 mm whereas in Pontevedra it is 14.9°C and 1303 mm, respectively (average data of the years 1982–2012; www.climatedata.org). The collected branches were oven dried (60°C, >48 h).

The target species (i.e., the ones on which it is desired to test the bioherbicide effect of *U. europaeus*) are three therophyte grasses (Poaceae family): the crop species *A. sativa* ('oat') and the weeds: *L. rigidum* and *L. multiflorum* (Romero and Fraga, 1990; Michitte *et al*., 2007). The fact that the three target species are grasses will be worthwhile to better assess the selective capacity of the allelopathy effect of *U. europaeus* on different species of the same family. This will also eliminate possible different responses due to taxonomic peculiarities of the species.

A. sativa is cultivated in most temperate regions of the world (http://faostat.fao.org). *L. rigidum* is a very problematic weed, considered among the ten species with the highest resistance to herbicides including glyphosate (Lemerle *et al*., 2001; Soltys *et al*., 2013). Multiple biotypes of *L. multiflorum* are also resistant to different herbicides of the families of ALS and ACCasa inhibitors and to glyphosate (Espinoza *et al*., 2009; Diez de Ulzurrun and Leaden, 2012). Therefore, it seems an urgent need to find new effective herbicides to face weed resistance. If they are ecofriendly alternatives, we would reduce negative impacts in crops and ecosystems.

Seeds from *L. rigidum* and *L. multiflorum* were purchased in Semillas Silvestres (www.semillassilvestres.com) and those from *A. sativa* in Fitoagrícola (www.fitoagricola.net). As commercial seeds, their viability is ensured. They were all disinfected before use with consecutive 1-min baths of sodium hypochlorite (50%) and ethanol (69%) to reduce fungus proliferation during the germination bioassays. Finally, they were rinsed with plenty of deionized water. The allelopathic effect of *U. europaeus* was tested at different development stages of the target plants (germination, seedling emergence and plant growth).

Mulch and extract preparation

Mulch for the experiments was prepared using dried branches, thorny twigs and phyllodes from *U. europaeus*, that were cut in fractions (c.a. 2 cm) to simulate the crushing or chopping process traditionally carried out by an electric fodder cutter after *U. europaeus* remotion for agricultural application (Jamil *et al*., 2009; Atlan *et al*., 2015; Khan *et al*., 2016). Also, as a spiny shrub, *U. europaeus* would be difficult to farmers to handle if it is not cut. This fraction size of the mulch was used in similar agricultural studies (Jamil *et al*., 2009; Puig *et al*., 2013; Stagnari *et al*., 2014; Souza-Alonso *et al*., 2020). Flowers and fruits were discarded to prepare the mulch, as the focus in this study was on the vegetative part. As explained before, for field studies in invaded areas, *U. europaeus* plants need to be collected out of their fructification and even floriation period to avoid reproduction of the mulch in the cultivar.

Aqueous extract was prepared with *U. europaeus* mulch at a concentration of 10% (10 g of *U. europaeus* mulch per 100 ml of deionized water), keeping the mixture or 'tea' stirring for 24 h at 90 rpm. Similar concentrations have been used in previous phytotoxicity studies (e.g., Singh and Sangeeta, 1991; Cheema and Khaliq, 2000; Jamil *et al*., 2009; Soltys *et al*., 2013). After stirring time, with the help of a suction pump, the resulting liquid was filtered in a MILLIPORE Express® PLUS container with a 0.22 μm filter. The extract was preserved at 4°C until use the day posterior to the preparation.

Effect of U. europaeus *mulch on target species*

Thermoformed pots (11 × 11 × 12 cm; base with aeration) were filled in as follows: (1) only with commercial substrate, (2) with commercial substrate and AC—20 ml per liter of substrate (Callaway, 2003)—with a homogeneous mix of commercial substrate and mulch from *U. europaeus*—4 g of mulch per kg of substrate (Singh *et al.*, 2005)— and (4) with a homogeneous mix of AC-supplemented substrate and mulch from *U. europaeus*. The commercial substrate was a blend of commercial growth media (50% fine blond peat and 50% black peat; Projar Professional Seed Pro 5050; www.projar.es) supplemented with 10% (v/v) vermiculite to avoid desiccation. Therefore, this fully factorial design includes, for each target species, two factors with two levels each: (1) *U. europaeus* mulch (presence or absence; hereafter called as 'Ulex') and (2) 'AC' (presence or absence). A total of 120 pots (10 × 3 target species × 2 AC × 2 Ulex) were placed in a greenhouse at Real Jardín Botánico Alfonso XII (Complutense University of Madrid, Spain). Twenty disinfected seeds of each target species (*A. sativa*, *L. rigidum* y *L. multiflorum*) were sown (c.a. 2 cm deep) in each pot. Several studies used AC as a substance to reveal allelopathic effects because this substance can adsorb organic compounds released by plant species (Tian *et al.*, 2007; Yuan *et al.*, 2013; Del Fabbro and Prati, 2014).

Pots were weekly watered enough to maintain optimal humidity conditions for plant growth, and they were frequently relocated in the greenhouse to homogenously distribute possible micro-environment effects or border effects among pots. Seedling emergence was daily monitored. For each target species and pot, the emergence percentage and emergence speed were then calculated. After 1 month since seed sown, plant height was measured and averaged per pot, and after 48 days since seed sown, we kept four plants per pot, the biggest ones, which ranged from 12 to 16 cm. The rest of the plants were harvested, oven-dried (48 h at 60°C) and weighted. To obtain the aerial biomass per plant, the weight was divided by the number of plants harvested in each pot. For each variable the number of replicates per treatment was 10 (10 pots).

Combined effect of mulch and extract from U. europaeus *on target species*

After 48 days since the seed sown, with the help of an aerosol vaporizer, we added to the pots with the remaining four plants the following: (1) extract from *U. europaeus* (10%) in the pots with the presence of *U. europaeus* mulch and (2) deionized water in the pots without the mulch. We applied 2 ml per day and pot (in two consecutive days), keeping the extract cold (4°C) from one day to the next (Tighe-Neira *et al.*, 2016). After 28 days since the vaporization of the extract, i.e., 76 days from the seed sown, plants were harvested, separating the above- and belowground biomass. Roots were washed to remove the residual substrate. Plant aboveground and belowground biomass were dried in the oven at 60°C for 48 h and weighed. We divided the plant weight by the number of remained plants (1–4) in each pot (number of replicates per treatment = 10). Most of the four remained plants survived, except for three plants of *L. multiflorum* in one pot and one plant of *L. rigidum* in other pot. Therefore, we did not consider pertinent analyzing differences in seedling survival among treatments.

Soil legacy effects

The substrates where the species grew were collected after plant harvest and kept in a freezer at −20°C until its use. Treatments to test mulch and extract legacy effect left on substrate were: (1) substrate, (2) substrate + AC, (3) substrate + Ulex (mulch and extract) and (4) substrate + Ulex (mulch and extract) + AC. In Petri dishes (Ø 10 cm) we added 40 ml of substrate of each treatment substrate (5 replicates), which was composed by an equal mixture of two randomly selected replicates of the same treatment. On the surface of each substrate, 20 seeds of each target species were placed, always placing each target species in the soil where it had grown. A total of 20 Petri dishes by target species: 5 × 2 Ulex (with or without mulch + extract) × 2 AC treatments (with and without). The substrate was moistened with 4 ml of deionized water. Petri dishes were kept in a germination chamber, in the dark, at 20°C. The number of germinated seeds was recorded each day until no germination of any seed was observed after 3 days. During this time, dishes were moistened with deionized water as required. For each species, the germination percentage (%G) and germination speed (GE) were then calculated.

Variable's calculation and statistical analysis

Final percentage of emerged and germinated plants (final number of emerged or germinated seedling × 100/total number of sown seeds) were calculated. Emergence speed (ES) and germination speed (GS) were as ES or $GS = [N1 + N2/2 + N3/3 + \ldots + Nn/n] \times 100$, where N1, N2, N3, Nn, are the proportions of emerged seedlings or germinated seeds at 1, 2, 3, …, *n* days (Wardle *et al.*, 1991). ES and GS range from 0 (if no seedlings emerge or germinate, respectively, at the end of the study period) to 100 (if all seedlings emerge or germinate, respectively, on the first day). To check variables normality and homoscedasticity, Kolmogorov–Smirnov and Levene tests were done and when required, transformation of the variables (Arcsen $\sqrt{\text{variable}/100}$) was done. For each target species (*L. multiflorum*, *L. rigidum* and *A sativa*), a two-way ANOVA was used to assess differences in emergence (%E), germination (%G), ES, GS, height and biomass among treatments: AC (presence–absence) and Ulex (presence–absence) and the interactions among them. After ANOVA, Least Significance Difference (LSD) post hoc test was used for multiple comparisons between factor levels. A permutational multivariate analysis of variance (PERMANOVA) using distance matrices was implemented to assess the influence of Ulex and CA accounting for all the variables: emergence, ES, height and biomass of the target species. All statistical analyses were performed using StatSoft Statistica software except for PERMANOVA that was performed in R software 3.4.3 (R Core Team, 2022) using 'adonis2' function (vegan package).

Results

Effect of U. europaeus *mulch on target species*

Both Ulex and AC significantly affected biomass of *L. rigidum* (Table 1). The interaction between *U. europaeus* mulch and AC

Table 1. Summary results of the two-way ANOVA assessing the effects of mulch from *U. europaeus* (Ulex), activated carbon (AC) and their interactions on the percentage of emergence (%E), emergence speed (ES) and height of each target species (*Avena sativa*, *Lolium multiflorum* and *Lolium rigidum*)

		Target species					
		A. sativa		*L. multiflorum*		*L. rigidum*	
	Factor	*F*	*P*	*F*	*P*	*F*	*P*
%E	Ulex	0.03	0.857	2.04	0.162	0.89	0.352
	CA	1.61	0.213	0.37	0.544	2.97	0.094
	Ulex × CA	0.30	0.590	3.37	0.075	9.66	**
ES	Ulex	0.06	0.812	1.55	0.221	3.18	0.083
	CA	0.63	0.433	0.27	0.606	0.70	0.408
	Ulex × CA	0.26	0.615	1.76	0.193	5.17	*
Height (cm)	Ulex	3.66	0.064	1.15	0.292	8.98	**
	CA	0.69	0.412	2.31	0.138	3.99	0.053
	Ulex × CA	0.33	0.569	16.09	***	0.57	0.456
Aboveground biomass (g)	Ulex	0.94	0.339	19.37	***	28.87	***
	CA	5.44	*	5.44	*	26.33	***
	Ulex × CA	2.94	0.095	10.76	**	0.13	0.719

Significance level (*P*): *** <0.001; ** <0.01; * <0.05.

(Ulex × AC) significantly affected the percentage of emergence (%E) and the emergence speed (ES) of *L. rigidum* and the height and aboveground biomass of *L. multiflorum* (Table 1). *U. europaeus* mulch decreased the %E, ES and height of *L. rigidum*, but the effect was absent in the presence of AC (Fig. 1), indicating that these effects were likely caused by allelopathic compounds possibly released by *U. europaeus* mulch. In the case of *L. multiflorum*, *U. europaeus* mulch also decreased height, but differences were only significant in the presence of AC (Fig. 1). *U. europaeus* mulch increased while AC decreased *L. rigidum* biomass (Fig. 1). In the absence of *U. europaeus* mulch and AC, *A. sativa* and *L. multiflorum* developed less biomass (Fig. 1).

Combined effect of mulch and extract from U. europaeus *on target species*

The combined effect of *U. europaeus* mulch and extract (Ulex) significantly affected belowground and total biomass of *L. rigidum* and *L. multiflorum* (Table 2). Specifically, under the combined effect of *U. europaeus* mulch and extract, weed species (*L. rigidum* and *L. multiflorum*) reached more biomass, while *A. sativa* reached lower biomass (Fig. 2). The effect on *A. sativa* disappeared in the presence of AC (Fig. 2), indicating that allelopathic compounds of *U. europaeus* could be implicated. The interaction term Ulex × AC significantly affected *A. sativa* biomass (Table 2).

General effect of U. europaeus *and AC*

Accounting for all dependent variables, only in *L. rigidum* we found a significant effect produced by the interaction Ulex × CA (Table 3), indicating that *L. rigidum* was the most sensitive species to the treatments, but the direction of the treatment effects changed depending on the variable (Figs. 1 and 2).

Soil legacy effect

No soil legacy effects were observed in *L. rigidum* and *L. multiflorum* (Table 4 and Fig. 3). The interaction effect of 2-month conditioned substrates by AC and mulch and extracts from *U. europaeus* (Ulex) significantly affected the germination percentage (%G) of *A. sativa*, but any of the factors significantly affected germination speed (GS) of the target species (Table 4). Only the presence of CA in the absence of Ulex had a negative legacy effect on *A. sativa* (Fig. 3).

Discussion

Effects of U. europaeus

The present study provides useful information on the effect of *U. europaeus* mulch and extract on the target species; this is a starting point for testing the feasibility of using *U. europaeus* as a bioherbicide in sustainable agriculture. Beginning with *U. europaeus* mulch, we found that it exerted a negative effect on the emergence and height of the weed *L. rigidum*, but not on the performance of the other target species. Thus, our previous expectations were not completely fulfilled because we expected that the germination of both weeds would be more affected than that of the crop species due to the bigger size of the later (Liebman and Sundberg, 2006) and due to previous studies reporting greater phytotoxic effects of *U. europaeus* amendments and extracts on weed species than on maize (Pardo-Muras *et al*., 2020*a*, 2020*b*). The negative effect on both emergence and height of *L. rigidum* was only detected in the absence of AC, indicating that it can be attributable to the presence of phytotoxic compounds likely released by *U. europaeus* mulch in the soil. From an ecological perspective, our results indicate that *U. europaeus* is able to allelopathically hinder the germination and establishment of *L. rigidum*, which can be advantageous for *U. europaeus* to reduce competitors for its own seeds in the field.

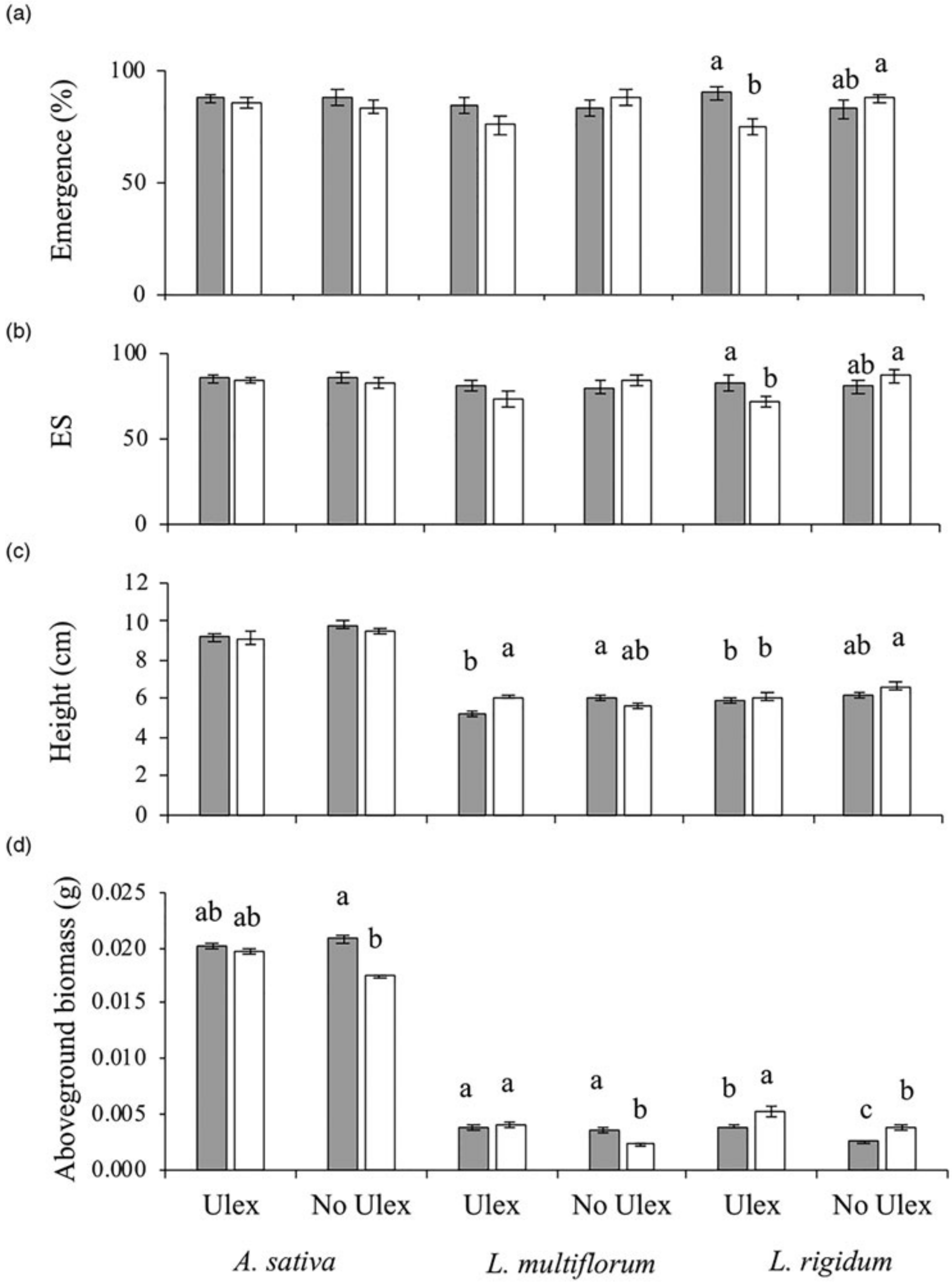

Fig. 1. Mean values (±SE, $N = 10$) of (a) the percentage of emergence, (b) emergence speed (ES) and (c) height and (d) aboveground biomass of the target species (*Avena sativa*, *Lolium multiflorum* y *Lolium rigidum*) grown in the presence or absence of *U. europaeus* mulch (Ulex and No Ulex, respectively) and with or without activated carbon (AC) (grey and white bars, respectively). Different letters stand for statistically significant differences among treatments (Ulex × AC) at $P < 0.05$ (LSD test).

Although significant, the effects on *L. rigidum* were rather small. Besides, the height of the weed species *L. multiflorum* significantly decreased in the presence of *U. europaeus* mulch, but the effect was also small and only detected in the presence of AC. This may indicate that some compounds released by *U. europaeus* hinder the growth in height of *L. multiflorum* in conditions of elevated carbon. Following the results of mulch effects, we could say that *U. europaeus* mulch can be used as a pre-emergence bioherbicide in oat crops, being safe for *A. sativa* and showing a small but significant effect on *L. rigidum*. However, we cannot certainly report this because the mulch also increased the aboveground biomass of weeds in a greater or lesser extent. This result unadvised the use of *U. europaeus* mulch to control *Lolium* weeds. Future investigations would be to focus on how to increase the effectiveness of *U. europaeus* mulch on *Lolium* weeds, for instance by increasing the quantity added or by adding the mulch only on the topsoil, creating a physical barrier for weed germination (Facelli and Pickett, 1991).

Previous studies found that *U. europaeus* amendments affected weed species (Pardo-Muras *et al.*, 2020*b*). However, the effects greatly varied among target species and dependent variables measured (emergence, biomass, height). Similar to our results, in the presence of *U. europaeus* amendments, the biomass of the weed *Digitaria sanguinalis* (Poaceae) increased, as well as other Monocotyledon species (Pardo-Muras *et al.*, 2020*b*). Contrary, *U. europaeus* amendments decreased the emergence and height of the weed *Amaranthus retroflexus* (Amaranthaceae). Several studies also reported different responses of plant species to allelopathic and fertilizer effects of plant residues (Sturm *et al.*, 2018; Little *et al.*, 2021). In the case of *U. europaeus* aqueous extracts, the effects also varied among target species, negatively affecting *A. retroflexus* but not *D. sanguinalis* or *Zea mays*

Table 2. Summary results of the two-way ANOVA assessing the combined effects of mulch and extract from *U. europaeus* (Ulex), activated carbon (AC) and their interactions on the above- and belowground and total biomass of the target species (*Avena sativa*, *Lolium multiflorum* and *Lolium rigidum*)

		Target species					
		A. sativa		*L. multiflorum*		*L. rigidum*	
Variable	Factor	*F*	*P*	*F*	*P*	*F*	*P*
Aboveground biomass (g)	Ulex	2.6	0.116	0.0	0.953	1.3	0.262
	CA	0.2	0.694	0.7	0.396	0.1	0.773
	Ulex × CA	4.4	*	1.0	0.314	0.0	0.877
Belowground biomass (g)	Ulex	2.7	0.108	23.6	***	15.0	***
	CA	0.0	0.963	0.1	0.770	0.4	0.517
	Ulex × CA	4.8	*	2.5	0.122	0.2	0.626
Total biomass (g)	Ulex	2.7	0.109	14.0	***	8.1	**
	CA	0.1	0.739	0.3	0.576	0.0	0.872
	Ulex × CA	4.6	*	2.6	0.115	0.0	0.872

Significance level: *** <0.001; ** <0.01; * <0.05.

L. (Pardo-Muras *et al.*, 2020*a*). Similarly, in this study, the effect of *U. europaeus* aqueous extract, added post-emergence, was negative for the biomass of *A. sativa* but positive for *Lolium* species. The sensitiveness of *A. sativa* to the water-soluble allelopathic compounds of *U. europaeus* can explain this result, as the negative effect was reduced in the presence of AC. Therefore, the postemergence use of *U. europaeus* aqueous in oat crops is disadvised but could contemplate its use in areas where *A. sativa* is an undesired species, mainly in wheat crops (Rapoport *et al.*, 2009).

The positive effects of *U. europaeus* mulch and aqueous extract on *Lolium* biomass could be due to nitrogen and other nutrients released during mulch decomposition or extracted in the water. Among Fabaceous plants, *U. europaeus* has been identified to produce a voluminous amount of fixed nitrogen through its ability of rapid symbiotic nitrogen fixation in nodules. Common gorse has an annual rate of 100–200 kg ha^{-1} nitrogen accumulation during the rapid dry-matter accumulation period (Galappaththi *et al.*, 2022). In fact, *U. europaeus* has traditionally been used as a natural agricultural fertilizer due to its nutritive effect (Atlan *et al.*, 2015). Soil nitrogen not only favor plant growth, but also plant germination (Duermeyer *et al.*, 2018), which can explain the small negative effects or the absence of them that we found on the germination of the target species exposed to *U. europaeus* mulch. The increase in other soil nutrients, such as phosphorus, after addition of *U. europaeus* amendments (Pardo-Muras *et al.*, 2020*b*), may also explain the positive effect we found in this study. Therefore, allelopathic effects of *U. europaeus* may be counteracted by positive fertilizer effects.

From an ecological point of view, our results indicate that in areas out of the native range of *U. europaeus*, the phenomenon known as invasional meltdown can occur, where an exotic invasive species (in this case *U. europaeus*) facilitates the establishment of other exotic species (in this case, *Lolium* species) (Simberloff and Von Holle, 1999). This phenomenon is common in Fabaceae species (e.g., Von Holle *et al.*, 2006) and there are, in fact, invaded areas (e.g., Chile, Australia, Brazil) where *U. europaeus* coexist with *L. rigidum*, *L. multiflorum* and other *Lolium* species (Gilfedder and Kirkpatrick, 1996; Koch *et al.*, 2016; Moreno-Chacón *et al.*, 2018). However, this possibility needs to be experimentally tested.

The species *L. rigidum* was the most sensitive species to *U. europaeus* treatments, but its response was ontogenetically dependent, negative at germination stage and positive during seedling growth. Besides, the species-specific effects produced by *U. europaeus* highlights the ability of this species to structure plant communities through changes in the soil environment. However, here we have just studied the effect of *U. europaeus* through its plant residues. Testing other components, such as plant root exudates, should be considered for a more realistic understanding of *U. europaeus* allelopathy in future studies.

Side effect of AC

Regarding the isolated effect of AC (i.e., in the absence of *U. europaeus* mulch), it varied among target plant species. We found positive effects on the biomass of *A. sativa* and *L. multiflorum* but negative effects on the biomass of *L. rigidum*. The fertilizing capacity of AC itself, increasing plant biomass has been previously reported (Gómez-Aparicio and Canham, 2008; Lau *et al.*, 2008). Additionally, AC may display other side effects, such as the reduction of mycorrhiza infection or the stimulation of microbial community (Weißhuhn and Prati, 2009). Also, a clear negative effect of AC on plant biomass and survival was also reported in a previous study (Yuan *et al.*, 2021). Although AC was proven to be valid in allelopathic studies by blocking allelopathic effects (e.g., Sturm *et al.*, 2018; Kheirabadi *et al.*, 2020; Lorenzo *et al.*, 2021), experimental designs as the one used in this study must allow the identification of possible isolated and interactive effects of AC on target plants. As AC may have species-specific effects on plant performance through changes in chemical and biological properties of soils, it is essential to account for these effects in allelopathy studies.

Soil legacy effects

No negative soil legacy effects left by *U. europaeus* mulch and extracts were detected in this study, indicating the environmental

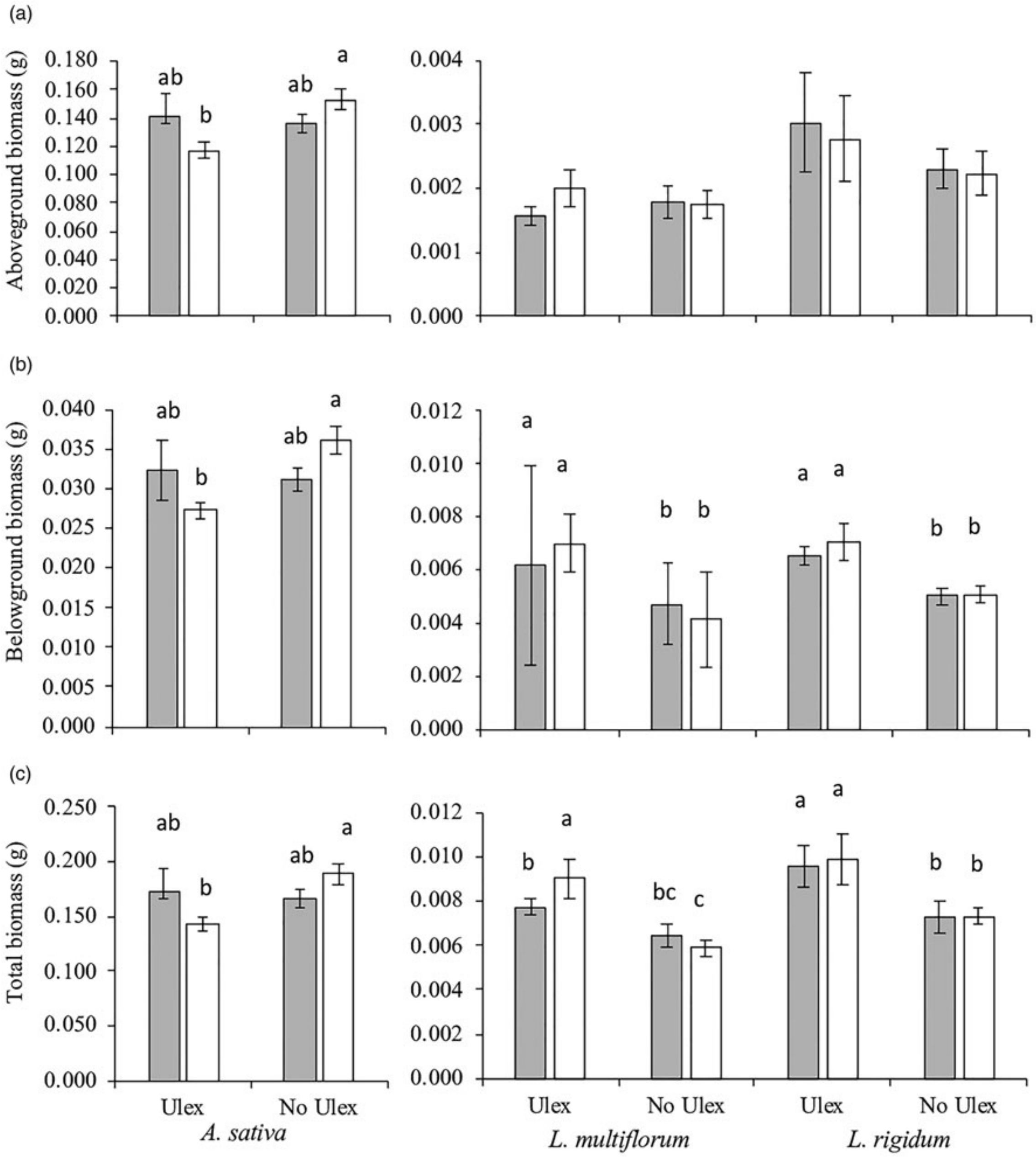

Fig. 2. Mean values (±SE, $N = 10$) of (a) aboveground biomass, (b) belowground biomass (ES) and (c) total biomass of the target species (*Avena sativa*, *Lolium multiflorum* y *Lolium rigidum*) grown in the presence or absence of *U. europaeus* mulch and extract (Ulex and No Ulex, respectively) and with or without activated carbon (AC) (grey and white bars, respectively). Different letters stand for statistically significant differences among treatments (Ulex × AC) at $P < 0.05$ (LSD test).

safety of their use. Therefore, the possibility of allelopathic compounds having unwanted effects on the local flora is limited. However, further research is needed to understand soil legacy effects of *U. europaeus*, not only related to soil allelopathy, but also regarding to soil chemical and biological modifications commonly produced by Fabaceae species (Grove *et al.*, 2012, 2015; Von Holle *et al.*, 2013). The absence of soil legacy effects can be due to the degradation or inactivation of allelochemical compounds of *U. europaeus* in soil, or because the negative effect of allelochemicals was counteracted by positive effect of soil nitrogen on germination (Kobayashi, 2004; Duermeyer *et al.*, 2018), but this need to be further investigated.

The use of *U. europaeus* mulch could be considered adequate as a fertilizer to prepare the soil 2 months before sown because it can add nutrients to the soil and the allelopathic effects may not persist after 2 months, but research is needed to increase its effectiveness as a bioherbicide on the emergence of weeds. For instance, *U. europaeus* mulch can be used in combination with other conventional agricultural practices or to reduce the quantity of herbicide in crops. Good farming practices demand a sensible use of herbicides to avoid over-dependence on a single control measure; herbicide rotation and integration with other measures are recommended to augment system stability (Fernández-Quintanilla *et al.*, 2007). Our way to test the allelopathic potential of *U. europaeus* was intended to be easy, cheap, natural and sustainable, in order to seek for a practical methodology that farmers can effortlessly apply, avoiding the use of chemicals and expensive and complex pretreatments, but we are aware that other methods can be more

Table 3. Summary results of the permutational multivariate analysis of variance using distance matrices, assessing the general effect of *U. europaeus* (Ulex), activated carbon (AC) and their interactions accounting for all dependent variables: percentage of emergence (% E), emergence speed (ES), height, above- and belowground and total biomass of the target species of each target species (*Avena sativa*, *Lolium multiflorum* and *Lolium rigidum*) on the (*Avena sativa*, *Lolium multiflorum* and *Lolium rigidum*)

Target species						
	A. sativa		*L. multiflorum*		*L. rigidum*	
Factor	*F*	*P*	*F*	*P*	*F*	*P*
Ulex	0.36	0.574	1.81	0.191	1.21	0.140
AC	0.90	0.314	0.58	0.445	1.09	0.216
Ulex × AC	0.20	0.717	3.42	0.066	2.18	**0.001****

Significance level: *** <0.001; ** <0.01; * <0.05.

Table 4. Soil legacy effects

Target species							
		A. sativa		*L. multiflorum*		*L. rigidum*	
Variable	Factor	*F*	*P*	*F*	*P*	*F*	*P*
%G	Ulex	0.470	0.505	0.045	0.835	0.001	0.977
	AC	1.510	0.237	0.423	0.525	1.134	0.303
	Ulex × AC	9.000	***	0.423	0.525	0.614	0.445
GS	Ulex	0.859	0.368	2.508	0.133	0.111	0.743
	AC	0.163	0.692	0.014	0.906	0.678	0.422
	Ulex × AC	0.001	0.973	0.197	0.663	0.211	0.652

Summary results of the two-way ANOVA assessing the soil legacy effects (2-months) left by mulch and extract from *U. europaeus* (Ulex), activated carbon (AC) and their interactions on the percentage of germination (%G) and germination speed (GS) of the target species (*Avena sativa*, *Lolium multiflorum* and *Lolium rigidum*)
Significance level: *** <0.001; ** <0.01; * <0.05.

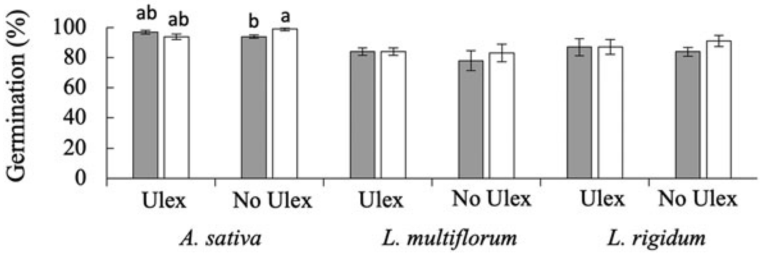

Fig. 3. Soil legacy effects. Mean values (±SE, $N = 5$) of germination percentage of the target species (*Avena sativa*, *Lolium multiflorum* y *Lolium rigidum*) submitted to substrates conditioned during 2 months by the following treatments: presence or absence of *U. europaeus* mulch and extract (Ulex and No Ulex, respectively) and presence or absence of activated carbon (AC) (grey and white, respectively). Different letters stand for statistically significant differences at $P < 0.05$ (LSD test).

effective to extract allelochemicals (see Pardo-Muras *et al.*, 2018, 2019, 2020*a*).

Future research

The effect of *U. europaeus* mulch in the emergence and height of the weed *L. rigidum* and in other weed species need to be further investigated in more realistic conditions in the field. If we intend to use extracted plant material locally and suddenly from the invasive *U. europaeus*, there will not be a need to dry the biomass. In that case, using fresh instead of dried *U. europaeus* can be more effective (Pardo-Muras *et al.*, 2020*b*). For our study, we preserved the branches by oven drying them at 60°C before use, similar to previous studies (Singh and Sangeeta, 1991; Singh and Thapar, 2003; Gnanavel and Kathiresan, 2007; Saeed *et al.*, 2011; Khan *et al.*, 2016). It is known that many allelopathic compounds can be degraded with the heat but there are also many that are thermostable (Gil *et al.*, 2022). For instance, the allelopathic potential of *Pinus koraniensis* Siebold & Zucc. even increased when dried at 90°C (Gil *et al.*, 2022) and allelopathic properties of *Mentha pulgium* L. were not altered when dried at 50°C (Ahmed *et al.*, 2018). Even autoclave temperatures did not reduce the toxicity of *A. altissima* leaves (Heisey, 1990).

Given the variety of biotic and abiotic factors affecting the allelopathic interactions in natural soils, testing our experimental results in farmland would be advisable (Callaway, 2003). Similar studies found drastic differences between *in vitro* and *in vivo* experiments. For example, under experimental laboratory conditions, the germination of the weed *Phalaris minor* Retz. was reduced by 100%, while under natural field experimental conditions only 16%, when applying mulch from the leguminous *Sesbania aculeata* (Willd.) Pers. (Om *et al.*, 2002).

Another aspect to be considered is if *U. europaeus* plants from the invaded range of distribution could have the same phytotoxic effectiveness on weed species as plants from the native range. In this study we tested the allelopathic effects of *U. europaeus* plants from the native range of distribution, but the effects of plants from the invaded range (where there is a need to eliminate the species) may differ. The comparison of the production of allelopathic compounds in plant species between native and invaded ranges has been scarcely examined and the data are not conclusive. For example, as noted by Lankau *et al.* (2009), the production of phytotoxic agents (glucosinolates) in *Alliaria petiolata* (M. Bieb.) Cavara & Grande was reduced throughout the invasion of chronosequence. Conversely, the species *Solidago canadensis* L. produced a greater amount of allelopathic compounds in the invaded than in the native range of distribution (Abhilasha *et al.*, 2008). In the case of *Ageratina adenophora* (Spreng.) R. M. King & H. Rob., the concentration of some volatile compounds increased while others decreased in the invaded range (Inderjit *et al.*, 2011). In the case of *U. europaeus*, Hornoy *et al.* (2012) did not find differences in defensive chemicals (quinolizidine alkaloids) between native and invaded regions. Further research is needed to evaluate whether *U. europaeus* individuals from invaded regions are more allelopathic than those from native populations.

Despite our study failed to find the effectiveness of *U. europaeus* as bioherbicide in oat crops, this invasive plant has promising potential for further studies to focus on the development of a bioherbicide, because allelopathy can change depending on different contexts (soil, climate, target species, etc.) and experimental designs (Haugland and Brandsaeter, 1996; Kobayashi, 2004; Medina-Villar *et al.*, 2017; Zhang *et al.*, 2021). Fertilizer is another possible use of *U. europaeus*. Regarding to management, invasive species are a controversial topic. There are polarized opinions whether plant eradications are feasible, the extent to which stakeholders should influence management decisions, and whether utilization of invasive species is an effective control approach. Innovative ideas based on rigorous scientific research should help improve consensus on how to approach invasive species management (Shackleton *et al.*, 2022).

Conclusions

Our study showed that *U. europaeus* cannot be used as a bioherbicide in oat crops, at least using the methodology we applied, because it favored the growth of the weeds *L. rigidum* and *L. multiflorum* and hindered the growth of crop species. However, some allelopathic effects of *U. europaeus* mulch on *L. rigidum* open the way for further investigations on the bio-properties of this invasive species and on how to increase its effectiveness as a bioherbicide. Our results also emphasized the use of *U. europaeus* as a fertilizer on crops. The use of *U. europaeus* in crops can be considered safe for germination of plants, as no soil legacy effect was detected. Given the intense degree of *U. europaeus* invasion, the need to remove this species and the great presence of oat crops in different areas of the world, we greatly recommend the utilization of *U. europaeus* residues in the invaded areas in a way that compensate the cost of control and elimination practices of this undesired species. For the moment, the use of *U. europaeus* residues as a bioherbicide on different crops needs further research. Our results also indicated the ability of *U. europaeus* to structure plant communities, being able to allelopathically hinder the germination and height of *L. rigidum* and facilitate the growth of both *Lolium* weeds.

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Conflict of interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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