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Rye (*Secale cereale* L.) and squarrose clover (*Trifolium squarrosum* L.) cover crops can increase their allelopathic potential for weed control when used mixed as dead mulch

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Highlights

- *Rye and squarrose clover are cover crops with potential allelopathic effects.*
- *Aqueous extracts of residues of rye, squarrose clover, and their mixture reduced and/or slowed weed germination of A. retroflexus and C. canadensis in the in vitro bioassays.*
- *Depending on the concentration of residues, the aqueous extracts had inhibitory effects on radicle and shoot growth of A. retroflexus, C. canadensis, and D. sanguinalis.*
- *The mulch of a mix of rye and squarrose clover under field conditions suppressed weeds better than the single species.*

Abstract

Cover crops are essential tools in agro-ecosystems for reducing the reliance on synthetic inputs and associated environmental risks. Alongside their benefits to soil fertility, cover crops can control weeds by their competitive and allelopathic attributes. Laboratory and field experiments were conducted to assess the allelopathic potential of two cover crop species, rye (*Secale cereale* L.) and squarrose clover (*Trifolium squarrosum* L.), alone or in a mixture, on seed germination and growth of arable weeds. Aqueous extracts of the two cover crops and their mixture were tested in a bioassay on *Conyza canadensis* (L). Cronq., *Amaranthus retroflexus* L. and *Digitaria sanguinalis* (L.) Scop. *In vitro* effects of aqueous extracts varied in a dose-dependent manner, with cover crops and weed species. All three extracts were able to reduce the germination of *A. retroflexus* (–87%) considerably. Inhibitory effects by rye and mixture extracts on radicle growth of all weed species ranged between 51 and 82%. Rye extract was the best at reducing shoot length of *C. canadensis* and *D. sanguinalis* (–39 to 44%), while squarrose clover was more effective on *A. retroflexus* (–79%). Plant extracts also delayed the germination time of weed species with a substantial effect of the mixture on *C. canadensis* seeds. In the field experiment, no significant weed suppression was provided by cover crop residues incorporated as green manure compared to control plots, despite tillage being more effective in reducing weed density than no-till. Still, the mulch of the mixture controlled weed emergence significantly better than single cover crop mulches. The chemical characterization of cover crop residues, both shoots and roots, revealed a notable richness of allelopathic phenolic acids and flavonoids, which may constitute potential natural herbicides through slow decomposition. From the analysis of the aqueous extracts, other non-analysed and/or unidentified water-soluble allelopathic compounds should underlie the phytotoxicity observed *in vitro*, at least for rye. For cover crop mixture, positive interactions among plant materials leading to a better release of allelochemicals and weeding effectiveness are discussed according to chemical profiles and field data. Our study demonstrated the allelopathic activity of the cover crops and their potential to be included in weed management strategies according to cropping system needs. Additional trials are needed to confirm the performance of cover crop residues under field conditions.

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Introduction

Integrated weed management is crucial for preventing significant losses in cash crop yield while preserving the environmental sustainability of agro-ecosystems. Recently, there has been a growing interest in weed management that exploits plant physical

and chemical interactions and focuses on agroecological practices (Petit *et al.*, 2018). These approaches come in response to the increasing concerns over reduced soil fertility (Smith *et al.*, 2011), herbicide-resistant weeds (Baucom *et al.*, 2019), and the negative impacts on humans, animals, food, and the environment arising from excessive mechanical and chemical weed management (Annett *et al.*, 2014; Pelleix *et al.*, 2020). Cover cropping is a standard practice that increases soil organic matter (Freibauer *et al.*, 2004), ensures nutrient recycling (Blanco-Canqui *et al.*, 2012), and reduces soil erosion (Kaspar *et al.*, 2001) while delivering some weed control (Bàrberi and Mazzoncini, 2001). Cover crop-weed interference is attributed primarily to the competition of the living plants with weeds for vital resources such as nutrients, light, water, and space (Bastiaans *et al.*, 2008). However, these plant interactions, often inhibitory, can also be due to a complex physiological phenomenon known as allelopathy observed in some species (Macias *et al.*, 2014; Sturm *et al.*, 2018). Allelopathy involves the discharge of phytochemicals to the soil, primarily phenolics, terpenoids, and alkaloids, from living and decomposing shoots and roots (Macias *et al.*, 2019). Although this phenomenon is not completely understood due to its complexity, it is thought that inhibitory effects are often the result of joint action of different compounds (Cheng and Cheng, 2015). Cover crop species influence the ability of cover crops to control weeds. A mix of cover crops can maximize competitiveness with weeds by exploiting the different traits of each component species, such as biomass production, root system, height, growth habit, growth rate, and allelopathy (Baraibar *et al.*, 2017). Combining species with distinct functional traits-also offers additional services to the agro-ecosystems, such as nitrogen provision with legume species (Gerhards and Schappert, 2020).

In recent years, numerous cover crop extracts and residues have been investigated for their weed suppressive capabilities. Several studies reported a reduction in the germination and growth of weeds following the use of legumes such as *Trifolium pratense* L., *Trifolium alexandrinum* L., *Vicia faba* L. (Price *et al.*, 2008; Fernández-Aparicio *et al.*, 2010; Álvarez-Iglesias *et al.*, 2018), and grasses such as *Sorghum bicolor* (L.) Moench and *Secale cereale* L. (Teasdale *et al.*, 2012; Reiss *et al.*, 2018; Farooq *et al.*, 2020). Rye (*S. cereale*) is one of the most investigated and successful winter cover crops due to its large adaptability, rapid growth and soil coverage, and recognized ability to suppress weeds. This latter is owed to the great aboveground biomass produced and hence the quality of its mulch, as well as to the release of phytotoxic benzoxazinoids and phenolic compounds during and after rye growth (de Bruin *et al.*, 2005).

In soil, multiple factors may interact and impact the release, activity, and persistence of allelochemicals. In addition to edaphic factors such as soil sorption and microbial activity, allelochemicals properties in the field are affected by the quality, the quantity, and the management of residues input. For instance, residues placed upon the soil rather than incorporated may have a slower but more prolonged release of allelochemicals (Kruidhof *et al.*, 2009).

Investigating cover crops' allelopathic potential would allow the selection of species and mixtures to improve weed management. In addition, allelopathic cover crops can be particularly beneficial for standard organic and organic no-till agriculture where means for weed control are limited. In the following study, we aimed to investigate the allelopathic weed suppressive ability of two winter cover crop species: rye and squarrose clover (*Trifolium squarrosum* L.), grown as sole crops and in a mixture. The objectives of our work were: i) to test the effects of aqueous extracts of the selected cover crops on germination potential, germination time, and seedling growth of three common weed species: *Amaranthus retroflexus* L., *Conyza canadensis* (L.) Cronq., and *Digitaria sanguinalis* (L.) Scop., through *in vitro* incubation experiments; ii) to evaluate residues allelopathic activity under different management practices (residues incorporation *vs* mulch) on weed suppression in field conditions; and iii) to determine the chemical composition of cover crops shoots and roots aqueous extracts and their residues.

Materials and methods

Plant material and extractions

Rye (*S. cereale* L. var. Dukato), squarrose clover (*T. squarrosum* L. var. OK), and their mixture were grown in plots (6×10 m), set up according to a split-plot design with three blocks, on an experimental field at the Centre for Agri-environmental research 'Enrico Avanzi' of the University of Pisa, Pisa, Italy (43°40' N Lat; 10°19' E Long; 1 m above mean sea level and 0% slope). The site is characterized by a typical Mediterranean climate with an average annual rainfall of 907 mm occurring primarily in autumn and spring and a mean annual temperature of 15.5°C. The soil is classified as a Typic Xerofluvent according to the USDA taxonomy and had a sandy clay loam texture with a pH of 7.9, an EC of 45.2 µS cm^{-1}, an organic matter content (Walkley-Black method) of 1.3%, a total N (Kjeldahl method) of 0.8 mg g^{-1} of soil, and available P (Olsen method) of 4.2 µg g^{-1} of soil in the top 30 cm. The two species were broadcast sown on tilled soil on 26th October 2017 at a seeding rate of 180 kg ha^{-1} for rye, 50 kg ha^{-1} for squarrose clover, and their half rates for the mixture. Cover crops were grown as winter rainfed crops without supplemental irrigation nor fertilization. Whole plants (shoots and their corresponding roots) were collected from the different replications in the field by digging them out manually from 30 cm depth over a total area of 1.5 m^2 for each cover crop. Roots were washed and cleaned from soil residues, and plant samples were then kept to dry in a greenhouse in the shade. At sampling time, squarrose clover was almost at the full flowering stage (BBCH 65), and rye was at the early milk stage (BBCH 73). The phenological stages followed the BBCH scale of Meier (2001). In order to prepare the stock extract (Puig *et al*., 2018), plant samples were chopped to 1 cm^2-sized pieces, and a total of 66.7 g of dry weight from each cover crop was soaked in 1 L of distilled water and then kept in the dark at ambient temperature for 24 h, being shaken gently regularly. Thereafter, extracts were filtered by a vacuum pump through a Büchner funnel using Whatman filter paper no. 2, then through a cellulose membrane of 0.45-µm pore size. Filtrates were kept at –20°C until the start of the bioassay.

In vitro dose-response bioassays: weed seeds germination and growth

Based on Puig *et al*. (2018), dilutions of 10, 25, and 50% were prepared from the stock extract to obtain the equivalent concentrations of 6.67, 16.67, 33.35, and 66.7 g of dry plant material per 1 L of distilled water (g DW L^{-1}). Aliquots of 600 µL from every concentration were then added to each 3.48 cm diameter well of a six-well plate containing Whatman no. 1 filter papers. The extracts were compared to a control consisting of distilled water as the one used for dilutions. Fifteen seeds of *D. sanguinalis* or *C. canadensis* or twenty seeds of *A. retroflexus* were placed in each well. The plates were then sealed with parafilm and incubated in a germination chamber. Weed seeds were collected from the plants growing in the same experimental field as the cover crops. *In vitro* treat-

ments were replicated six times and were distributed randomly in the germination chamber. *C. canadensis* was incubated at 20°C for a 12 h photoperiod, whereas *A. retroflexus* and *D. sanguinalis* were kept in darkness at 30°C and 27°C, respectively. Germinated seeds (rupture of the seed coat and radicle emergence at ≥1 mm length) were counted regularly, starting 24 h after the incubation until maximum germination. The total percentage of germinated seeds (Gt) was calculated from the cumulative germination data. Germination indices were calculated from data registered daily (Álvarez-Iglesias *et al.*, 2014):

Mean germination time:

$$MGT = \frac{\sum(N_n * n)}{\sum N_n} \quad (1)$$

with N_n the number of seeds germinated at n hours, and n the number of hours from the start of the germination test;

Coefficient of the rate of germination:

$$CRG = \frac{\sum N_n}{\sum(N_n * T_n)} * 100 \quad (2)$$

with N_n the number of germinated seeds in time Tn (hours);

Speed of germination:

$$S = \frac{\sum(N_n - N(n-1))}{n} \quad (3)$$

with N_n the number of seeds germinated in n day;

Speed of accumulated germination:

$$AS = \frac{\sum N_n}{n} \quad (4)$$

with N_n the cumulative number of seeds germinated in n day.

For weed growth bioassays, ten seeds of each weed species already germinated with radicle length between 1 and 3 mm were kept in incubation for 48 hours. After that, the radicle and shoot lengths of each seedling were measured.

Field experiment

The experiment was conducted during 2017-2018 at the Centre for Agri-environmental research 'Enrico Avanzi' of the University of Pisa, Pisa, Italy. Site characteristics are the same as described earlier. The experiment was laid out in a split-plot design of three blocks with tillage as the main plot and cover crops (rye, squarrose clover, and the mixture) and control (no cover crop) as subplots. Subplots had an area of 60 m^2 each (6×10 m). Starting on 30th May 2018, cover crops in half of the plots were rolled and flamed to obtain dead mulches, whereas, in the remaining half, they were incorporated with a rotary cultivator. Termination methods are detailed in Abou Chehade *et al.* (2019). Cover crop dry biomass was 6.1 (Standard deviation SD=1.8) t ha^{-1} for squarrose clover, 8.9 (SD=1.2) t ha^{-1} for rye, and 8.2 (SD=1.7) t ha^{-1} for the mixture. Tomato plants (*Solanum lycopersicum* L. cv. Elba F1) were then transplanted in a single row at a density of 2.2 plants m^{-2} on 6th June 2018. Total and individual weed species density from each treatment were measured over 3 random areas (50×50 cm) at 15-20 cm proximity of tomato plants after 42 and 62 days of cover crop termination.

Chemical characterization of cover crop aqueous extracts and residues

The aqueous extracts of each concentration were firstly characterized for their pH (CRISON micropH 2001), electrical conductivity (EC) (CRISON CDTM-523), and osmolarity (Gonotec OSMOMAT 030 cryoscopic osmometer). The aqueous extracts, predominantly shoots, and roots aqueous extracts (extracted as described in the earlier section) at the highest concentration were also evaluated for phenolic acids and flavonoids composition after the procedure detailed in Souto *et al.* (2001). For plant residues, phenolic characterization, 6.67 g of plant material were soaked in 100 mL of water and ethanol (50:50) and shaken for 24 h. The extracts obtained followed the same extraction procedure as the aqueous extracts. The analysis was performed using an HPLC (Shimadzu chromatograph) equipped with a UV-DIODE ARRAY detector to identify flavonoids and phenolic acids. Identification was achieved using a reverse-phase Waters Nova-Pak C-18 (4.6×250 mm) column with a 4 µm particle size. The extracts were analysed using two mobile phases for flavonoids: (A) methanol:phosphoric acid 999:1 and (B) water:phosphoric acid 999:1. HPLC grade solvents were used. Linear gradients starting with 20% (A) and ending with 100% (A) were used over the first 50 min with an additional 5 min at 100% (A). The flow rate of the mobile phase was 1 mL min^{-1}, and the eluate was analysed at 250-400 nm (Hussain *et al.*, 2011). For phenolic acids, linear gradient elution was carried out at a flow rate of 1.5 mL min^{-1}. Solvent A was 0.5% acetic acid in pure water, and solvent B, acetonitrile with 0.5% acetic acid. A gradient from 0% to 20% B over 45 min, followed by 15 min re-equilibration with A was used. Flavonoids and phenolic acids were identified and quantified by comparing retention times, wavelength detection, and peak areas to those of standard compounds. Derivatives were quantified using peak areas of the correspondent aglycones.

Statistical analysis

Data for total germination and radicle and shoot lengths were expressed as a percentage with respect to control and were fitted to non-linear regression models using the *drc* package (Ritz *et al.*, 2015) of RStudio statistical software. A lack-of-fit test was performed on each model to ensure it provides an adequate description of the relation between extract concentrations and variables in evaluation. Whenever a lack of fit was reported, as in the case of shoot length inhibition for *D. sanguinalis* and *C. canadensis*, analyses of variance were performed. The following are the non-linear regressions adopted depending on the variable assessed:

Log-logistic model:

$$Y = c + \frac{(d-c)}{1+exp\{b[log(x)-log(e)]\}} \quad (5)$$

Exponential decay model:

$$Y = c + (d-c)\left[exp\left(-\frac{x}{b}\right)\right] \quad (6)$$

Weibull model:

$$Y = c + (d-c)exp\{-exp[b(log(x) - log\,(e))]\} \quad (7)$$

where *Y* is the response (seed germination, and radicle and shoot lengths), *d* is the upper limit, *c* is the lower limit, *b* is the slope (at the inflection point in case of log-logistic and Weibull functions), *x* is the extract concentration, and *e* is the concentration generating a half response between the upper and the lower limits (the inflection point or ED_{50}).

Concentrations of aqueous extracts resulting in 10% (ED_{10}) and 50% (ED_{50}) inhibition from the control were calculated from the fitted equations for seed germination and radicle and shoot lengths. The regression parameters and effective doses were compared using the approximate t-tests of compParm and EDcomp functions, respectively (package *drc*).

Germination indices, shoot length of *D. sanguinalis* and *C. canadensis*, and field weed density were analysed with generalized mixed-effects models. In the case of germination indices and shoot length, extract type and concentration were the fixed factors, and replication was considered random. For field weed density, cover crop, tillage, and time were fixed factors, and random factors included block and replication, considering the split-plot design and hence the nested structure of the errors. Comparison between lsmeans was performed using Tukey's test at $P \leq 0.05$. Data were reported as estimate/lsmean±standard error.

Results

Effects of cover crops extracts on weed seed germination and seedling growth: in vitro experiment

Cover crops aqueous extracts affected the germination rate of the selected weed species (Figure 1). Total germination of *A. retroflexus* seeds declined with the three extracts progressively as their concentrations increased, reaching between 9 and 20% at the highest concentration of the extracts (Table 1). ED_{10} and ED_{50} showed clover extract more effective at low concentrations, although statistical differences were seen only with ED_{50}, needing 5.5 g compared to around 10 g DW L^{-1} for rye and mixture extracts (Table 1). All extracts alike reduced germination of *C. canadensis* seeds slightly (11.8 % on average), as shown in Figure 1. On the contrary, *D. sanguinalis* germination was not affected by the three cover crop extracts (data not shown). In addition, cover crop aqueous extracts could delay weed seed germination, as seen with the calculated germination indices in Table 2. The three extracts at all concentrations succeeded in delaying seed germination of *A. retroflexus*, as evaluated by S and AS. The clover seemed to be the most effective among the extracts (AS: 7.80±0.75 *vs*. 9.11±0.80 and 11.05±0.91 respectively, averaged over concentrations, for rye and mixture, and S: 3.35±0.31 *vs* 4.00±0.34 and 4.93±0.39 respectively, averaged over concentrations, for rye and mixture). The reduction of the speed of accumulated germination/germination varied between 43 and 85% averaged over the cover crops extracts passing from 6.67 to 66.7 g DW L^{-1} of concentration. CRG confirmed the potential of squarrose clover extract at the highest concentration to slow down germination of *A. retroflexus*. The mean germination time varied between 24 and 74 h for *A. retroflexus* but without statistically significant differences among the extracts and their concentrations. Despite the very slight reduction in the germination of *C. canadensis* seeds, a significant delay in germination onset compared to control for the three extracts was also observed, as all four indices show. However, clover extracts seemed active only at the highest concentration. The mixture was the most effective extract and was able at 66.7 g DW L^{-1} concentration to increase germination time by 68%. Cover crop aqueous extracts had no effects on the speed and germination time of *D. sanguinalis* represented by the four germination indices.

Cover crop aqueous extracts also had an inhibitory effect on seedling growth in a dose-dependent manner (Figure 1). For *A. retroflexus*, radicle length could be reduced by a maximum of 51 to 65% with respect to control in the presence of the aqueous extracts, without evident differences between rye, squarrose clover, and the mixture (Table 1). Shoot growth of *A. retroflexus*

Table 1. Estimated parameters and absolute effective doses of the log-logistic equation (Eq. 5) describing total seed germination of *A. retroflexus* and radicle length of *C. canadensis* and *D. sanguinalis*, the exponential decay equation (Eq. 6) describing seed germination of *C. canadensis* and radicle length of A. retroflexus and the Weibull equation (Eq.7) describing shoot length of *A. retroflexus*, in response to extracts concentration.

Variable	Weed species	Extract	Regression parameters			ED_{10}	ED_{50}
			b	*c*	*e*	g DW L^{-1}	
Seed germination	*A. retroflexus*	Rye	1.4±0.4^{b}	10.1±7.3^{a}	8.2±1.3^{a}	1.9±0.7^{a}	9.7±1.8^{ab}
		S. clover	1.4±0.6^{b}	8.8±6.1^{a}	4.8±0.8^{b}	1.1±0.8^{a}	5.5±0.8^{b}
		Mixture	3.6±0.8^{a}	20.3±2.9^{a}	9.1±0.7^{a}	5.3±0.5^{a}	10.5±1.1^{a}
	C. canadensis	Rye	13.9±6.0^{a}	87.5±1.8^{a}	-	22.5±9.7^{a}	n.d.
		S. clover	8.8±4.1^{a}	89.3±1.4^{a}	-	23.8±11.2^{a}	n.d.
		Mixture	10.1±4.6^{a}	88.6±1.5^{a}	-	21.2±9.8^{a}	n.d.
Shoot length	*A. retroflexus*	Rye	4.1±1.6^{a}	36.1±3.9^{a}	12.6±2.1^{a}	8.2±2.0^{a}	14.0±2.3^{a}
		S. clover	1.3±0.4^{a}	21.3±4.0^{a}	9.0±1.4^{a}	2.0±0.9^{ab}	9.1±1.4^{a}
		Mixture	0.7±0.3^{a}	28.4±7.4^{a}	8.6±2.9^{a}	0.7±0.8^{b}	11.0±4.3^{a}
Radicle length	*A. retroflexus*	Rye	32.0±10.0^{a}	35.1±9.8^{a}	-	5.3±1.7^{a}	47.0±14.7^{a}
		S. clover	13.5±3.7^{a}	45.9±4.5^{a}	-	2.8±0.7^{a}	35.1±9.5^{a}
		Mixture	13.5±3.5^{a}	48.6±4.3^{a}	-	2.9±0.8^{a}	48.4±12.6^{a}
	C. canadensis	Rye	1.0±0.2^{b}	18.1±6.9^{b}	8.8±1.6^{a}	1.3±0.4^{a}	13.6±3.6^{ab}
		S. clover	2.7±0.4^{a}	43.3±1.9^{a}	10.0±0.7^{a}	5.6±0.5^{a}	20.9±3.0^{a}
		Mixture	1.2±0.3^{b}	24.3±5.5^{b}	7.0±0.9^{a}	1.4±0.5^{a}	12.4±3.0^{b}
	D. sanguinalis	Rye	-	-	-	-	-
		S. clover	-	-	-	-	-
		Mixture	5.0±2.1	18.1±6.3	19.8±2.2	13.3±1.5	21.7±3.1

b, slope of the curve; *c*, lower limit of the curve or the maximum response (% of control); *e*, inflection point or dose of plant material (g DW L^{-1}) causing a half response between the upper and lower limits of the curve; ED_{10} and ED_{50}, effective doses resulting in 10 and 50% inhibition respectively; n.d., not defined. ^{a,b}For each weed species, means with different letters within a column are statistically different at $P \leq 0.05$.

was also inhibited (64 to 79%) (Figure 1). The mixture had a low ED_{10} as compared to rye (Table 1). ED_{50} was similar among the extracts ranging between 9.1 and 14 g DW L^{-1}.

Contrary to the low germination inhibition, cover crop aqueous extracts had a noticeable effect on the radicle length of *C. canadensis.* Maximum inhibition of radicle length ranging between 76 and 82% as a percentage of control was observed for rye and mixture extracts at 66.7 g DW L^{-1} compared to 57% reduction by squarrose clover extract (Figure 1). Estimated ED_{50} confirmed the higher potency of the mixture over squarrose clover extracts (Table 1). Shoot length of *C. canadensis* could not be fit to a non-linear response (Table 3). However, a considerable reduction (44%) of *C. canadensis* shoot growth was obtained with rye at a full concentration, as shown in Table 3. In *D. sanguinalis,* radicle inhibition followed a significant log-logistic dose-response curve only in response to the mixture, reaching an 82% decline (Figure 1 and Table1). The response of *D. sanguinalis* shoot growth to extracts concentration could not be described by a non-linear response. However, the analysis of variance confirmed a reduction by 39% of hypocotyl length only with rye extract at the highest concentration (Table 3).

Effects of cover crop residues management on weed abundance under field conditions

The statistical analysis showed a significant interaction between cover crops and tillage, and tillage and time of sampling for total and broadleaf weed density (Table 4). Averaged over the two sampling times (42 and 62 days after cover crop termination), total weed density was the least in conventional tillage (CT) treatments regardless of the presence of a cover crop. Despite being less effective than CT, cover crop mulches in no-till (NT) systems reduced weed density significantly compared to control, with the mixture having the highest weed suppressive potential. Weed density in the two tillage regimes increased with time irrespective of cover crop type and presence. Cover crops in no-till reduced broadleaf weed density significantly, reaching a 74% decline with mixture mulch. Yet, cover crop mulches significantly increased grass population density, namely where squarrose clover was present.

Chemical characterization of cover crop aqueous extracts and residues

Values for pH, electrical conductivity (EC), and osmolarity at each plant material concentration in the aqueous extracts are reported in Table 5. The pH ranged between 4.50 and 7.31, differing between the extracts and decreasing with increasing concentration. Except the clover at the lowest concentration, the three extracts were slightly acid, with the mixture having the highest acidity. EC values varied from 0.19 to 2.69 dS m^{-1} and osmolarity from 0.006 to 0.106 Osmol kg^{-1} as the concentration of cover crop in the extract goes from 6.67 to 66.7 g DW L^{-1}. For both characteristics, clover extract registered the highest values.

Phenolic profiles characterizing each extract are reported in Table 6. The HPLC analysis showed the presence of the flavonoid prunetin and its derivative in rye aqueous extract. On the contrary, squarrose clover aqueous extract had the phenolic acids ρ-hydrox-

Table 2. Germination indices of weed species as affected by the different concentrations of rye, squarrose clover, and their mixture in the aqueous extracts.

Extract	Concentration (g DW L^{-1})	*A. retroflexus* AS	S	CRG	MGT	*C. canadensis* AS	S	CRG	MGT	*D. sanguinalis* AS	S	CRG	MGT
Rye	6.67	10.60 ±1.71	4.74 ±0.74	1.19 ±0.04^{cde}	59.32 ±10.17^{abc}	12.60 ±0.52^{bcd}	5.31 ±0.16^{bcd}	1.09 ±0.01^{bc}	68.76 ±1.98^{cd}	7.03 ±0.68	3.58 ±0.31	1.30 ±0.01	62.13 ±2.30
	16.68	4.83 ±0.78	2.23 ±0.35	1.13 ±0.04^{de}	69.14 ±11.85^{ab}	10.26 ±0.42^{efg}	4.48 ±0.13^{ef}	1.04 ±0.01^{de}	75.25 ±2.17^{bc}	7.21 ±0.70	3.67 ±0.32	1.31 ±0.01	61.97 ±2.30
	33.35	5.94 ±0.96	2.65 ±0.41	1.32 ±0.04^{ab}	40.00 ±6.86^{c}	9.48 ±0.39^{fgh}	419 ±0.13^{ef}	1.03 ±0.01^{ef}	76.73 ±2.21^{bc}	8.17 ±0.79	4.05 ±0.36	1.33 ±0.01	58.97 ±2.18
	66.70	3.32 ±0.54	1.48 ±0.23	1.35 ±0.05^{ab}	37.00 ±6.34^{cd}	9.36 ±0.38^{fgh}	4.14 ±0.12^{ef}	1.03 ±0.01^{ef}	76.77 ±2.21^{bc}	6.33 ±0.61	3.36 ±0.29	1.28 ±0.01	67.05 ±2.48
S. clover	6.67	8.60 ±1.39	3.63 ±0.57	1.23 ±0.04^{bcd}	47.62 ±8.16^{abc}	15.17 ±0.62^{ab}	6.10 ±0.18^{ab}	1.14 ±0.01^{a}	58.21 ±1.68^{e}	7.33 ±0.71	3.62 ±0.32	1.33 ±0.01	58.64 ±2.17
	16.68	4.57 ±0.74	2.03 ±0.32	1.28 ±0.04^{abc}	46.67 ±7.99^{abc}	13.96 ±0.57^{abc}	5.71 ±0.17^{abc}	1.13 ±0.01^{a}	62.06 ±1.79^{de}	8.44 ±0.82	4.35 ±0.38	1.30 ±0.01	63.39 ±2.35
	33.35	3.59 ±0.58	1.57 ±0.24	1.28 ±0.04^{abc}	42.00 ±7.20^{bc}	13.31 ±0.55^{abcd}	5.43 ±0.16^{bc}	1.12 ±0.01^{ab}	61.99 ±1.78^{de}	8.6 ±0.83	4.32 ±0.38	1.32 ±0.01	60.95 ±2.26
	66.70	1.38 ±0.22	0.64 ±0.09	1.09 ±0.04^{e}	74.00 ±12.68^{a}	11.08 ±0.45^{def}	4.68 ±0.14^{de}	1.06 ±0.01^{cde}	69.97 ±2.02^{cd}	6.89 ±0.67	3.57 ±0.31	1.28 ±0.01	63.59 ±2.35
Mixture	6.67	15.41 ±2.48	6.88 ±1.08	1.23 ±0.04^{bcd}	56.77 ±9.73^{abc}	12.34 ±0.51^{cde}	5.20 ±0.16^{cd}	1.08 ±0.01^{bcd}	68.84 ±1.98^{cd}	8.86 ±0.86	4.37 ±0.38	1.33 ±0.01	57.87 ±2.14
	16.68	7.59 ±1.22	3.35 ±0.52	1.32 ±0.04^{ab}	36.67 ±6.28^{cd}	8.70 ±0.36^{gh}	4.05 ±0.12^{ef}	1.01 ±0.01^{f}	82.75 ±2.38^{b}	7.19 ±0.70	3.64 ±0.32	1.31 ±0.01	61.14 ±2.26
	33.35	6.85 ±1.10	3.50 ±0.55	1.39 ±0.05^{a}	24.00 ±4.11^{d}	8.26 ±0.34^{h}	3.88 ±0.12^{f}	1.00 ±0.01^{f}	83.52 ±2.41^{b}	6.08 ±0.59	3.18 ±0.28	1.29 ±0.01	65.47 ±2.42
	66.70	4.55 ±0.73	2.02 ±0.31	1.33 ±0.05^{ab}	36.67 ±6.28^{cd}	5.59 ±0.23^{i}	3.24 ±0.10^{g}	0.93 ±0.01^{hg}	99.14 ±2.86^{a}	5.07 ±0.49	2.72 ±0.24	1.26 ±0.01	67.77 ±2.51
Control	n.a.	20.86 ±3.36	8.91 ±1.39	1.24 ±0.04^{bcd}	47.26 ±8.10^{abc}	16.02 ±0.66^{a}	6.48 ±0.20^{a}	1.14 ±0.01^{a}	59.03 ±1.70^{e}	7.72 ±0.75	3.90 ±0.34	1.31 ±0.01	61.47 ±2.28

AS, speed of accumulated germination; S, speed of germination; CRG, coefficient of the rate of germination, MGT, mean germination time. n.a., not applicable. $^{a-i}$Means with different letters within a column are statistically different at P≤0.05.

ybenzaldehyde, vanillic acid, caffeic acid, and ρ-coumaric acid, in addition to the flavonoid prunetin, luteolin, and their derivative. The mixture was the richest in water-soluble phenolic acids in terms of the number of compounds detected (protocatechuic acid, ρ-hydroxybenzoic acid, ρ-hydroxybenzaldehyde, vanillic acid,

Table 3. Length (% of control) of *C. canadensis* and *D. sanguinalis* shoots as affected by cover crop aqueous extracts.

Extract	Concentration (g DW L^{-1})	Shoot length (%) *C. canadensis*	*D. sanguinalis*
Rye	6.67	85.4±3.4^{ab}	116.1±8.0^{a}
	16.68	84.7±3.4^{ab}	107.0±8.0^{ab}
	33.35	86.6±3.4^{ab}	117.2±8.0^{a}
	66.70	56.4±2.4^{c}	61.1±8.0^{d}
S. clover	6.67	82.7±3.3^{b}	118.1±8.0^{a}
	16.68	87.7±3.5^{ab}	98.8±8.0^{abcd}
	33.35	88.8±3.5^{ab}	68.9±8.0^{cd}
	66.70	87.0±3.4^{ab}	86.1±8.0^{abcd}
Mixture	6.67	79.4±3.2^{b}	106.8±8.0^{ab}
	16.68	82.6±3.3^{b}	119.8±8.0^{a}
	33.35	82.3±3.3^{b}	93.8±8.0^{abcd}
	66.70	86.6±3.4^{ab}	70.8±8.0^{bcd}
Control	n.a.	99.9±3.9^{a}	100.0±8.0^{abc}

$^{a-d}$Means with different letters within a column are statistically different at P≤0.05. n.a., not applicable.

Table 4. Field weed density (plants m^{-2}) in an organic tomato system as affected by the interaction of tillage with cover crop and time after cover crop termination. Data are back-transformed.

Factor		Density (plants m^{-2}) Grasses	Broadleaves	Total
Tillage	CT	6.1±0.7^{b}	6.0±1.2^{b}	12.4±1.5^{b}
	NT	10.9±1.3^{a}	24.6±4.9^{a}	39.6±4.7^{a}
Cover crop	Rye	7.2±0.9^{b}	13.0±2.0^{b}	21.3±2.0^{bc}
	S. clover	10.6±1.3^{a}	11.8±1.8^{b}	23.0±2.1^{b}
	Mixture	10.1±1.2^{a}	8.0±1.2^{c}	18.5±1.7^{c}
	Control	5.7±0.7^{b}	17.7±2.7^{a}	26.7±2.5^{a}
Time	42 DAT	7.0±0.8^{b}	10.4 ±1.6^{b}	25.5±2.3^{a}
	62 DAT	9.6±1.1^{a}	14.1±1.6^{a}	19.3±1.7^{b}
Tillage × Cover crop	CT Rye	5.2±1.0^{bc}	5.8±1.3^{d}	11.3±1.5^{d}
	CT S. clover	7.1±1.3^{bc}	6.2±1.4^{d}	13.7±1.8^{d}
	CT Mixture	5.9±1.1^{bc}	5.5±0.8^{d}	11.4±1.6^{d}
	CT Control	6.6±1.2^{bc}	6.8±1.5^{d}	13.6±1.8^{d}
	NT Rye	10.3±1.7^{b}	29.3±5.9^{b}	40.3±4.9^{b}
	NT S. clover	15.9±2.6^{a}	22.2±4.5^{c}	38.8±4.7^{b}
	NT Mixture	17.3±2.8^{a}	12.1±2.5^{d}	30.0±3.7^{c}
	NT Control	5.1±0.9^{c}	46.2±9.3^{a}	52.2±6.3^{a}
Tillage × Time	CT 42 DAT	5.2±0.7^{a}	4.4±0.9^{c}	9.8±1.3^{d}
	CT 62 DAT	7.2±0.9^{a}	8.1±1.7^{b}	15.7±1.9^{c}
	NT 42 DAT	9.4±1.1^{a}	24.7±4.9^{a}	37.8±4.5^{b}
	NT 62 DAT	12.7±1.5^{a}	24.5±4.9^{a}	41.4±4.9^{a}

CT, conventional tillage; NT, no-till; DAT, days after termination. $^{a-d}$Means with different letters, within a column and for each factor separately are statistically different at P≤0.05.

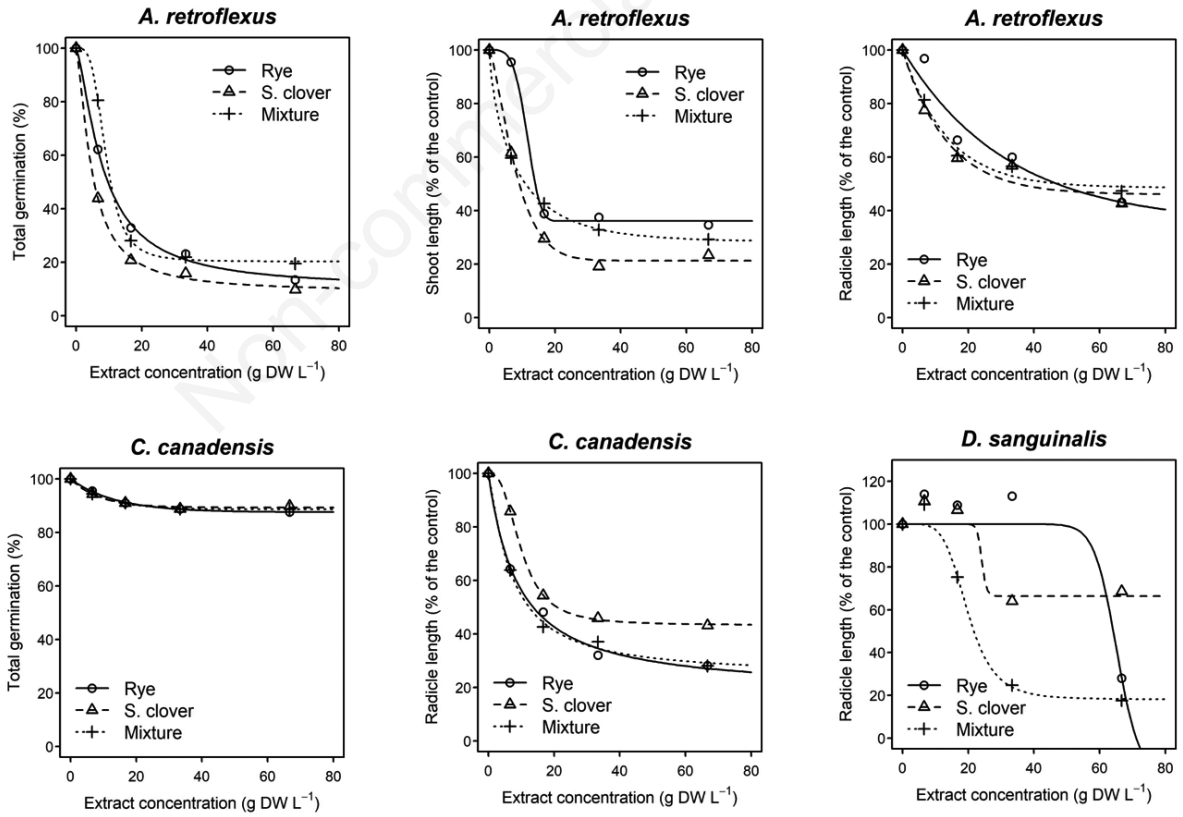

Figure 1. Dose-response curves representing the effects of the aqueous extracts of rye, squarrose clover, and their mixture on seed germination, radicle, and shoot length of three summer weed species. Total seed germination follows a log-logistic (Eq. 5) and an exponential decay (Eq. 6) response in *A. retroflexus* and *C. canadensis,* respectively. Shoot length of *A. retroflexus* follows a Weibull response (Eq. 7) . Radicle length follows an exponential decay in *A. retroflexus* and a log-logistic response in *C. canadensis* and *D. sanguinalis*.

syringic acid, caffeic acid, and ferulic acid). Roots aqueous extracts were also characterized for the presence of phenolic compounds. Multiple phenolic acids were found in squarrose clover roots extracts. Clover roots aqueous extracts contained protocatechuic acid, ρ-hydroxybenzoic acid, and ferulic acid, as well as the phenolic compounds found in the prevalently shoot extracts. The roots of the mixture, predominantly rye roots, combined the flavonoids of both pure rye and clover root aqueous extracts. Cover crop plant materials were also characterized for their phenolic composition using water and ethanol (50:50) as extractants to verify the presence of potential phytotoxic compounds that were not released during the aqueous extraction process (Table 6). The two cover crops and their mixture yielded higher total content of phenolic acids than those occurring in the aqueous extracts. The composition of rye shoots showed the presence of, in order of concentrations, ρ-coumaric acid, luteolin derivative, ferulic acid, vanillic acid, vanillin, and hydroxybenzoic acid as major components. In roots, around half of the phenolics composition was composed of ρ-coumaric acid without ferulic acid and luteolin derivative. Squarrose clover residues had large amounts of luteolin derivative, ellagic acid, and vanillin besides those extracted with water. Mixture phenolics concentrations were luteolin derivative > ρ-coumaric acid and a derivative > vanillin > ferulic acid. Interestingly, ρ-coumaric acid was higher in the mixture than in the single species residues. Despite combining compounds of single squarrose clover and rye, roots of the mixture additionally yielded apigenin and prunetin.

Discussion

Effects of cover crops extracts on weed seed germination and seedling growth: in vitro experiment

Characterization of the extracts showed most of them had values that do not surpass the thresholds considered phytotoxic for

Table 5. Basic characterization of the aqueous extracts of rye, squarrose clover, and their mixture at different concentrations.

Extract	Concentration (g DW L^{-1})	pH	EC (dS m^{-1})	Osmolarity (Osmol kg^{-1})
Rye	6.67	5.22	0.19	0.009
	16.68	5.19	0.46	0.012
	33.35	5.12	0.82	0.032
	66.70	5.10	1.52	0.070
S. clover	6.67	7.31	0.34	0.006
	16.68	6.84	0.75	0.022
	33.35	6.66	1.44	0.049
	66.70	6.60	2.69	0.106
Mixture	6.67	4.67	0.24	0.009
	16.68	4.58	0.51	0.014
	33.35	4.55	1.01	0.041
	66.70	4.50	1.67	0.081
Control (distilled water)	n.a.	5.87	0.01	0.000

n.a., not applicable.

Table 6. Phenolic profiles of aqueous extracts (concentrations expressed in μg mL^{-1}) and plant materials (concentrations expressed in μg g^{-1}) identified by HPLC-DAD in rye, squarrose clover, and their mixture.

	Aqueous extract						Dry material					
	Shoots			Roots			Shoots			Roots		
Compound	Rye	S. clover	Mixture	Rye	S. clover	Mixture	Rye	S. clover	Mixture	Rye	S. clover	Mixture
Phenolic acids												
Protocatechuic acid			0.791		1.148			3.096			0.875	
ρ-hydroxybenzoic acid			0.943		1.038		3.523		4.308	0.880		3.969
ρ-hydroxybenzaldehyde		0.054	0.086				2.255	3.599	2.761	2.214		2.391
Vanillic acid		0.172	1.165		0.937		10.544	19.210	5.608	3.678	5.888	4.819
Syringic acid			0.658				2.230			0.714		
Caffeic acid		2.592	3.690		2.084			7.936			7.620	0.746
Vanillin							7.880	12.622	9.574	4.863		5.937
ρ-coumaric acid		0.526			0.168		18.848	11.765	19.200	14.392	4.815	18.777
Ferulic acid			0.355		0.428		18.164		6.498		3.309	2.900
ρ-coumaric acid derivative									8.134			
Total identified		3.344	7.688		5.803		63.444	58.228	56.083	26.741	22.507	39.539
Flavonoids												
Prunetin derivative	0.024	0.429	0.173	0.054	0.854	0.088						
Luteolin derivative 1		0.505										
Luteolin derivative 2								70.702	42.378			
Ellagic acid								33.642				
Luteolin derivative 3							18.602					
Apigenin												18.765
Prunetin	0.059	0.524	0.164		0.120	0.066						28.616
Total identified	0.083	1.458	0.337	0.054	0.974	0.154	18.602	104.344	42.378			47.381

pH, electrical conductivity, and osmolarity (toxicity: pH<5, EC>2 dS m^{-1} and osmolarity >0.07 osmol kg^{-1}) (Macias *et al.*, 2000; Dhima *et al*., 2009; Lawley *et al*., 2012). Exceptions included mixture extracts that had a pH ranging between 4.5 and 4.7. In this case, we do not dismiss a contribution of acidity to the inhibitory effects observed. Similarly, the role of the osmotic pressure and/or salinity in the phytotoxicity observed with the cover crop extracts at full concentration is not excluded. Yet, significant seed germination and seedling growth inhibition were found even when concentrations of extracts had values for both characteristics lower than the limit of phytotoxicity. We assume, therefore, that the presence of active phytotoxins in the aqueous extracts most likely contributed to the inhibitory effects obtained from our *in vitro* tests.

In vitro tests performed herein revealed the success of aqueous rye extracts, even at low doses, in suppressing weed germination and root and shoot lengths of the three weed species. Rye extracts and residues have been widely investigated and were reported to reduce the establishment and growth of many problematic arable weeds, including barnyardgrass (*Echinochloa crus-galli* (L.) Beauv.), common lambsquarters (*Chenopodium album* L.), green foxtail (*Setaria viridis* (L.) P. Beauv.) and redroot pigweed (*Amaranthus retroflexus* L.) (Dhima *et al*., 2006; Tabaglio *et al*., 2008; Teasdale *et al*., 2012). Clovers have been researched for their allelopathic potential, but, to our knowledge, no previous reports exist on the specific potential of squarrose clover. Our results were in agreement with those obtained by Sturm *et al*. (2016) using aqueous extracts of subterranean clover (*Trifolium subterraneum* L.) on lambsquarters, chickweed *(Stellaria media* (L.) Vill), and chamomile (*Matricaria chamomilla* L.*)*. Seedling growth of redroot pigweed*,* wild mustard (*Sinapis arvensis* L.), and other weeds were also inhibited by aqueous extracts from red clover (*Trifolium pratense* L.), Persian clover (*Trifolium resupinatum* L.), and berseem clover (*Trifolium alexandrium* L.) (Ohno and Doolan, 2001, Maighany *et al*., 2007; Fernández-Aparicio *et al*., 2010). Here, the effects of the mixture extract were similar to the other extracts in most cases. An additional aspect that influences weed establishment and their negative interaction with crops is seed germination time. The extracts caused a delay in seed germination, mainly for *C. canadensis,* which confirmed that allelochemicals reduce germination and have the potential to delay weeds germination, benefiting the cash crop whenever verified in the field (Scavo *et al.*, 2018).

Effects of cover crop residues management on weed abundance under field conditions

In field conditions, the effects of cover crop type on weed density depended on residue management. Plots under conventional tillage maintained low rates of weed infestation, but no significant effects of cover crop type on weed emergence were detected. However, although bearing higher weed densities, cover crop mulches in no-till plots provided significant control of broadleaf weeds. This is in accordance with Kruidhof *et al*. (2009), who reported that residues placement might alter weed suppression response of cover crops, with mulches having higher effects. The effect correlates with the results of our *in vitro* tests so that weed control could be due partly to allelochemicals present in plant tissues of rye and squarrose clover, leached or released with degradation by soil microbiota. Cover crop residues in no-till were flamed after rolling for termination, which might have reduced their allelopathic properties demonstrated *in vitro*. However, their effects were still significant in no-till plots. Of course, weed suppression in no-till can also be significantly attributed to the physical impedance of the mulch laid upon the soil and the alteration of the weed environment (Teasdale, 1996). Cover crop residues reduce light penetration that is essential for the germination of most weeds. Light permeability and weed emergence are inversely proportional to the number of residues left upon the soil (Webster *et al*., 2016). Indeed, the palmer amaranth (*A. palmeri* S. Wats.) population declined by half in concomitance with a similar reduction in light at the soil surface, as high biomass of rye (6.2 t ha^{-1}) was produced (Webster *et al*., 2016).

Here, weed suppression was significantly remarkable for the mixture mulch, which reduced dicots weed density by three quarters. Webster *et al.* (2013) showed how the addition of rye to legumes boosted the overall cover crop biomass and enhanced weed suppression. In our case, the mixture yielded similarly to rye monoculture, rye making up 70% of the mixture biomass, suggesting a possible higher discharge of allelochemicals when both cover crops are mixed. Squarrose clover in the mixture might have sped up the mineralization of cover crop biomass by reducing C/N and hence the release of allelochemicals. Grass weeds are less sensitive to allelochemicals with different uptake and translocation mechanisms than dicots (Norswothy, 2003; Tabaglio *et al*., 2013). In our experiment, the grass population was denser where cover crops were present as mulch. This increase was considerably greater under squarrose clover and the mixture. Stimulatory effects of cover crop mulches can be due to the alteration of the soil environment and, in the case of squarrose clover, due to nitrogen availability.

Chemical composition of cover crop aqueous extracts and residues

Phenolic compounds are the major allelochemicals present usually in aqueous extracts (Rice, 1984), to which many of the suppressive effects on several target species were attributed (Reigosa *et al*., 2007; Puig *et al*., 2018). These substances may negatively affect the hormonal balance, enzyme activities, membrane permeability and mineral uptake, stomatal function, photosynthetic rate, respiration, and biosynthesis of certain compounds in plants (Marchiosi *et al*., 2020).

Despite the similarity in the inhibitory response between the three aqueous extracts, their phenolic profiles were different. In aqueous rye extracts, phenolics analysis via HPLC-DAD detected only shallow peaks of prunetin and its derivative. Prunetin was one of the isoflavones identified in red clover (*Trifolium pratense* L.) that correlated to germination rate decrease and radicle length inhibition of wild mustard (Lou *et al*., 2016). Nonetheless, the absence of other flavonoids and phenolic acids, despite the observed *in vitro* phytotoxicity of the aqueous extract, points to the presence of other water-soluble inhibitors that have not been measured in our study. Secondary metabolites such as benzoxazinoids could have contributed to the allelopathic activity (Reberg-Horton *et al*., 2004). Phytotoxic activity of rye, in many cases, has been ascribed to the benzoxazinones 2,4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one (DIBOA) in shoots and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA) in roots alongside the benzoxazolinones, as their degradation products, BOA and MBOA (Reberg-Horton *et al*., 2004; Copaja *et al*., 2006; Schulz *et al*., 2013). These compounds are well known for their potent phytotoxic effects, which are superior to those of phenolic acids (Jia *et al*., 2006). The content of these compounds varies with rye cultivars and the plant's developmental stage, with a substantial decrease in the advanced stages (Reberg-Horton *et al*., 2005). Benzoxazinoids could have played a role in weed suppression by rye, mainly at the seedling stage in field plots, during

which the release of these compounds through root exudation preferably occurs (Reberg-Horton *et al*., 2005; Schulz *et al*., 2013). Noteworthy, we found many phenolic acids in mixture aqueous extract in contradiction to rye extract, which is its main component. This effect may be due to its acid pH that helped extract such compounds in water (Mota *et al*., 2008), which were abundant in rye plant material extracted in ethanol and water (50:50). Then, following our field data, it is possible that the mixture cover crop facilitated the release of phenolic compounds to soil water from rye residues, thus achieving better weed control in no-till plots. Allelochemicals in mixtures can interact positively, which increases their bioavailability, persistence and, phytotoxicity in the soil matrix (Tharayil *et al.*, 2008). Although, as said above, we do not discard that other compounds in the three extracts released during aqueous extraction and not evaluated herein may have helped induce the phytotoxic effects on weed germination and seedlings growth observed *in vitro*.

Phenolics composition of plant material obtained from ethanolic extraction of rye corresponds to those reported previously in the literature, although with some differences related to the occurrence/absence of other compounds and their concentrations. Otte *et al*. (2020) reported the presence of the same phenolic acids in rye tissues with a difference related to the significant presence of sinapic acid. The same authors found that 4-hydroxybenzoic acid, coumaric acid, syringic acid, and vanillic acid were higher in roots than rye shoots. In accordance with Otte *et al*. (2020) and Carlsen *et al*. (2009), ferulic acid was higher in shoots than roots, although in our case, despite its high quantity, it did not constitute the major phenolic acid. The lower concentrations found in rye tissues in our study may be explained by the advanced growth stage of the plants at the time of termination. Simple phenolic compounds are higher in young rye plants (Schulz *et al*., 2013). Clover species were identified to contain flavonoids and isoflavonoids and lower amounts of phenolic acids in different parts of the plant (Oleszek and Stochmal, 2002; Oleszek *et al*., 2007). Kolodziejczyk-Czepas *et al*. (2017) showed flavonoids representing around 44.5% of squarrose clover tissues, confirming our findings. Luteolin and ellagic acid flavonoids in squarrose clover shoots were reported previously for their phytotoxicity (Beninger and Hall, 2005; Qin *et al*., 2006). Despite all compounds reported having an allelopathic activity (Marchiosi *et al*., 2020), their concentrations in extracts did not surpass the phytotoxicity limits considered 100 ppm for coumaric, vanillic, and ferulic acids and 300 ppm for caffeic acid in crops and weeds (Chou and Patrick 1976; Olofsdotter *et al*. 2002). However, the joint action of very low concentrations of different compounds can lead to complex synergies among them, thus making the natural cocktail phytotoxic enough to exert conspicuous weed control (Pardo-Muras *et al*., 2020). Residues of the two cover crops and their mixture are rich sources of phenolics as well. In the field, roots are also an important contributor of phenolic compounds and other allelochemicals, especially in no-till where they remain in contact with the upper layer of the soil. Here, roots of the mixture yielded a higher quantity of phenolic compounds than rye and squarrose clover grown in pure stands. Roots of rye plants accompanying legumes like hairy vetch (*Vicia villosa* Roth.) or berseem clover (*Trifolium alexandrinum* L.) were found with increased benzoxazinoids concentrations compared to rye monocultures (Hazrati *et al*., 2020; Rakoczy-Trojanowska *et al*., 2020). This effect, alongside phenolic compounds found mainly in roots, may explain the superior reduction of field weed density by the mixture mulch.

Conclusions

Our study investigated the allelopathic potential of three cover crops (rye, squarrose clover, and their mixture) on suppressing three common arable weeds. The aqueous extracts of the three cover crops inhibited *in vitro* weed germination and growth. In the field, the mulch consisting of a mix of rye and squarrose clover under no-till management demonstrated a higher potential for weed suppression over its single counterparts. Such effectiveness could be partially due to an increased release of diverse allelochemicals from plant tissues to water when the cover crops are mixed. The results are promising for creating mixtures of cover crops and their inclusion in weed management strategies. Even so, the diversification of allelopathic cover crop species and management can be essential to maintain a diverse weed flora in the long run and to ensure sustainable weed management. More studies are needed however to reveal the allelochemicals responsible for weed inhibition in these pure and mixture cover crops and to confirm their activity under field conditions on weeds and notably on cash crop growth.

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