Iheringia
Série Botânica
ISSN ON-LINE 2446-8231

Chemical characteristics and phytotoxicity of root exudates from cover crops

Maicon Reginatto[1,*], Lisandro Tomas da Silva Bonome[2], Leonardo Khaoê Giovanetti[3], Henrique von Hertwig Bittencourt[2], Luciano Tormen[2]

[1]Universidade Tecnológica Federal do Paraná, Pato Branco, Paraná, Brasil
[2]Universidade Federal da Fronteira Sul, Laranjeiras do Sul, Paraná, Brasil
[3]Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brasil
*maicon.agroeco@gmail.com

Recebido em 28.III.2020
Aceito em 08.III.2023

DOI 10.21826/2446-82312023v78e2023009

ABSTRACT – This work aimed to evaluate the influence of root exudates from cover crops: *Raphanus sativus*, *Avena strigosa* and *Vicia villosa* on the germination and development of *Zea mays*, *Amaranthus spinosus* and *Ipomoea grandifolia*. The tests performed were gas chromatography with mass spectrometry, germination rate, vigor, root protrusion and dry matter. Aliphatic amino acids (Alanine, Glycine, Isoleucine, Leucine, Proline, Serine, Threonine and Valine) were identified as well as phenylalanine and cinnamic acid. The root exudate of the cover crops tested affects negatively a number of tested parameters. The plants that had the greatest negative effect on the target plants were *Avena strigosa* and *Vicia villosa*. *Raphanus sativus* did not differ from the control group in maize, it inhibited the germination, vigor and growth of the *Amaranthus spinosus* and the *Ipomoea grandifolia.*

Keywords: agroecology, allelopathy, maize, organic agriculture.

RESUMO – Características químicas e fitotoxicidade de exsudatos radiculares de plantas de cobertura. Este trabalho objetivou avaliar a influência dos exsudatos radiculares das plantas de cobertura: *Raphanus sativus*, *Avena strigosa* e *Vicia villosa* na germinação e desenvolvimento do *Zea mays*, *Amaranthus spinosus* e *Ipomoea grandifolia*. Os testes realizados foram: cromatografia gasosa com espectrometria de massa, taxa de germinação, vigor, protrusão radicular e matéria seca. Foram identificados aminoácidos alifáticos (Alanina, Glicina, Isoleucina, Leucina, Prolina, Serina, Treonina e Valina), fenilalanina e ácido cinâmico. O exsudato radicular das plantas de cobertura testadas afeta negativamente uma série de parâmetros testados. As plantas que apresentaram maior efeito negativo sobre as plantas alvo foram a *Avena strigosa* e a *Vicia villosa*. *Raphanus sativus* não diferenciou do grupo controle no milho, inibiu a germinação, vigor e crescimento de *Amaranthus spinosus* e *Ipomoea grandifolia*

Palavras-chave: agroecologia, agricultura orgânica, alelopatia, milho.

INTRODUCTION

The use of cover crops increases the sustainability of agricultural systems by increasing the soil organic matter content and improving its physical, chemical, and biological properties. In the process of developing their root systems, these plants can release soil chemical compounds capable of interfering positively or negatively in germination and crop development in succession and uncultivated plants.

The inhibitory or beneficial effect of one plant on another via the production of chemical compounds that are released into the environment is called allelopathy (Rice 1984). During the plant development period or through degradation of plant tissues, the release of bioactive chemical compounds in the environment, which modify the growth and development of other organisms, may occur (Trezzi *et al*. 2016).

Root exudation is a form of release of substances such as mucilage, amino acids, sugars, phenols, and organic acids. The nature and amount of the released compounds, however, vary between the species and the environmental conditions in which the plants are found (Alves 2009, Oliveros-Bastidas *et al*. 2009). Also, the roots are constituted by a series of organic compounds that can be released from the decomposition of the tissues of the root system and act as allelochemicals. However, there is no direct relationship between the constituent compounds of the root structure and those released by exudation (Wu *et al.* 2001).

Although cover crops are more commonly used as green manure to promote physical, chemical, and biological improvements in soil, they can also act as a physical and chemical barrier to plants (Altieri *et al*. 2011). After being released in the environment, the allelochemicals do not distinguish the cultivated species from the uncultivated

ones, and studies are still required to understand the selectivity of the compounds and their performance in the different vegetal species. In one of these studies, the influence of root exudates of several cover crops, among them *Vicia villosa* L. and *Avena strigosa* (Schreb) in the germination percentage of *Glycine max* seeds (Bortolini & Fortes 2005) was verified. In another work with *Sorghum bicolor* the release of allelochemicals was observed due to both the decomposition of green matter and root exudation, reducing the development of *Euphorbia heterophylla* L., *Ipomoea triloba* L. and *Cenchrus echinatus* L. (Gomes *et al.* 2014).

The phytotoxicity of root exudates on other plants is attributed to the diversity of allelochemicals, originating from the secondary metabolism of the plants present in their composition. Among the variety of chemical compounds produced by plants that present phytotoxicity, the most studied are phenolic compounds (Inderjit 1996). These compounds constitute a chemically heterogeneous group with varying molecule sizes and complexity. Thus, the identification of the allelochemicals present in root exudates of green manure plants allows the observation of the causes of the morphological and physiological effects of these compounds on the target plants.

The objective of this work was to evaluate the bioactivity of root exudates in the germination and development of maize (*Zea mays* L. cv. PRE 22D11), *Amaranthus spinosus* L. and *Ipomoea grandifolia* (Dammer) O'Donnell and to identify compounds present in the root exudates in the following cover crops: *Raphanus sativus* L., *Avena strigosa* (Schreb) and *Vicia villosa* L..

MATERIAL AND METHODS

The bioassays were carried out in the laboratories of Plant Physiology, Germination and Plant Growth and Analysis Center of the Federal University of the Fronteira Sul, Campus Laranjeiras do Sul, from March 2017 to February 2018. The experimental design used was completely randomized blocks with four replications, with the following species: *Zea mays* L., *Amaranthus spinosus*. and *Ipomoea grandifolia*, and four treatments: control, *Vicia villosa*, *Avena strigosa* and *Raphanus sativus.*

Seeds of hybrid maize cv. 22D11 by *Sempre Sementes* (donated by Cooperative Oestebio), and of cover crops of *R. sativus*, *A. strigosa* and *V. villosa* were used. The seeds of *A. spinosus* were collected in the municipality of São Miguel do Oeste, SC, Brazil, located at the geographic coordinates (26°40'47.1 "S 53°31'21.5" W), while the *I. grandifolia* seeds were collected in the municipality of Marmeleiro, PR, Brazil (26°13'28.0" S 53°08'09.6" W).

After collection, the seeds passed through the seed blower to remove impurities and damaged seeds and were submitted to moisture measurements by the oven-drying method at 105°C and germination standard, both described in the Rules for Seed Analysis (RAS) (Brasil 2009). The seeds of *I. grandifolia* underwent dormancy by the mechanical scarification method, and the seeds of cover crops were sterilized with sodium hypochlorite (2.5%) for two minutes.

Identification of compounds

Seeds of the cover crops were arranged in germitest paper moistened with distilled water in a volume equivalent to 2.5 times their mass and incubated in a germinating chamber of the Mangelsdorf type (brand Tecnal model TE-405), at 20ºC until reaching a 5 cm ± 0.5 root size.

The seedlings were removed from the germitest paper containing their root exudates. The germitest paper was washed twice with distilled water and compressed to extract all the liquid. The liquid collected was frozen and taken to the lyophilizer (model L101 Liotop), remaining until the material was completely dried (48 hours). To the solid resulting from this process, 5 mL methanol was added for material dissolution.

The identification of phenolic compounds followed a methodology adapted from Dias (2010). The analysis of the extracts was carried out using a gas chromatograph with a mass detector (model GC-2010 plus Shimadzu), and column NST 05 ms, with a 30-meter length, a 0.24 μm stationary phase thickness, and a 0.25 mm column diameter.

The derivatization of the sample was performed by transferring 0.25 mL of the methanolic extract obtained to a vial; the solvent was removed by a constant stream of nitrogen, leaving only a solid fraction. To the resulting solid, 30 μL of pyridine and 70 μL of N,O-Bis (trimethylsilyl) trifluoroacetamide were added. The mixture was subjected to heating at 70°C in a sandbath for 70 minutes. From the resulting solution, 2 μL was manually introduced into the micro-syringe chromatograph. Identification of the compounds was performed by comparing the results with the internal library of the mass spectrometer (NIST08, NIST08s, NIST11, and NIST11s).

The data were submitted to hypothesis tests by analysis of variance ($p < 0.05$), and when significant, the means of the treatments were compared by the Tukey test ($p < 0.05$) using the statistical program Sisvar 5.6 (Ferreira 2014).

Bioassays with root exudates

To obtain the root exudates of the cover crops, seeds of each species were arranged between germitest paper moistened with distilled water in a volume equivalent to 2.5 times their mass. After sowing, the seeds were incubated in a germination chamber of the Mangelsdorf type (brand Tecnal model TE-405) at 20 ºC until the beginning of the root protrusion.

Four replicates of two hundred seedlings with radicles of approximately 1 mm were transferred to another germitest paper, where they remained for 13 (*R. sativus* and *A. strigosa*) and 15 days (*V. villosa*) in a germination chamber at 20ºC for the development of the seedlings. After this period the seedlings were discarded and the paper, containing exudates of the seedlings, was reused for the following tests: germination, germination speed

index (GSI), seedling growth and dry mass of shoot and root system. For the control, germitest paper soaked in distilled water was used.

The germination and GSI variables were obtained in the same bioassay using 50, 25 and 25 seeds in each replicate for maize, *A. spinosus* and *I. grandifolia*, respectively. The maize was incubated in a Mangesldorf germination chamber and the weeds in a BOD type germination chamber. Each species remained in the germinator for the time recommended by the Rules for Seed Analysis, and later the number of normal and abnormal seedlings and dead and dormant seeds was recorded (Brasil 2009).

The calculation of the GSI was performed by the sum of the number of normal seedlings each day, divided by the number of days after the seedling formation, using the formula proposed by Maguire as reference (1962): GSI = (G1 / N1) + (G2 / N2) + (G3 / N3) + ... + (Gn / Nn). Where: GSI: Germination Speed Index; G1, G2, Gn: number of normal seedlings recorded at first count, at second count, and at last count; N1, N2, Nn: number of days of sowing to the first, second and last count. After the number of days recommended by RAS for each species, the growth of seedlings was evaluated, which was measured with a digital caliper.

RESULTS AND DISCUSSION

Identification of compounds

One phenolic compound, cinnamic acid, was identified in the radicular exudate of *Raphanus sativus*, but was not identified in the other treatments (Tab. 1). Cinnamic acid is a phytotoxic compound, affecting several physiological processes in the plant, such as selective membrane permeability, root ion absorption, protein synthesis and oxidative phosphorylation (Putnam 1987, Yu & Matsui 1997, Einhellig 2004).

The molecules mostly found in the root exudates of the cover crops were aliphatic amino acids (alanine, glycine, isoleucine, leucine, proline, serine, threonine and valine) and an aromatic, phenylalanine (Tab. 1), in agreement with Oliveros-Bastidas *et al*. (2009).

Certain amino acids may inhibit germination and plant growth. Amino acid biosynthesis is an energetically demanding process for plants. Thus, surplus amino acids in the cell can cause inhibition of enzymes responsible for the biosynthesis of other amino acids by feedback, affecting plant growth (Bright *et al.* 1978). Under laboratory conditions, the exogenous application of amino acids inhibited the germination and formation of *Orobanche ramosa* tubers. This is a parasitic species of several crops of economic interest (Vurro *et al.* 2006).

The amino acids alanine, cysteine, histidine, methionine, isoleucine, tryptophan, phenylalanine, andlysine, were effective in reducing the growth of the *Orobanche minor* parasite species (Fernández-Aparicio *et al.* 2017). While proline and glycine were the most effective in inhibiting the growth of *P. ramosa* (Vurro *et al.* 2006), the reduction in plant growth in response to the presence of amino acids is dependent on the plant species, the stage of development of the plant, the concentration, and the amino acid (Fernández-Aparicio *et al.* 2017). Thus, the aliphatic amino acids found in the root exudates of the cover crops *R. sativus*, *A. strigosa* and *V. sativa* might have been responsible for the reduction in germination and growth of maize seedlings and *A. spinosus* and *I. grandifolia*.

Also, some non-protein amino acids have allelopathic potential on plants. Others, in turn, are precursors of a series of phenolic compounds such as coumarins, cinnamic acids and flavonoids (Li *et al.* 2010).

However, one should not disregard that the aliphatic amino acids found in the methanolic extract of root exudates originated from the seed germination process, which may have leached several organic compounds as a function of root protrusion. Further studies should be carried out to clarify these hypotheses.

Table 1. Compounds identified from the chromatographic analysis with mass spectrometry of the root exudates of the cover crops seedlings *Raphanus sativus*, *Avena strigosa* and *Vicia villosa*.

Category	Compounds	Cover crops		
		Raphanus sativus	*Avena strigosa*	*Vicia villosa*
Aliphatic Amino Acids	Alanine	*	*	-
	Glycine	-	*	-
	Isoleucine	*	-	*
	Leucine	*	-	*
	Proline	*	*	-
	Serina	-	*	*
	Threonine	*	*	*
	Valine	*	*	*
Aromatic Amino Acids	Phenylalanine	-	*	*
Phenolic compounds	Cinnamic acid	*	-	-

*Presence of the compounds in the samples analyzed.

- No compounds in the samples analyzed.

On the other hand, phenylalanine, an amino acid found in the root exudate of *A. strigosa* seedlings and *V. villosa*, belongs to the group of aromatic amino acids, which is formed from the shikimic acid route (Taiz & Zeiger 2017). The most abundant class of phenolic compounds in plants is derived from phenylalanine, by the elimination of a molecule of ammonia, forming cinnamic acid. The latter, besides having a high allelopathic effect in plants, is a precursor of several compounds with allelopathic potential such as caffeic acid, coumarins and other simple phenylpropanoids (Blum 2004, Taiz & Zeiger 2017).

Bioassays with root exudates

The root exudates of *A. strigosa* and *V. villosa* reduced the germination, GSI, length and dry mass of root and shoot of the maize in relation to the control. On the other hand, the exudates of the *R. sativus* did not differ from the control for any of the evaluated parameters (Tab. 2).

Maize germination was reduced by 22 and 28% when sown on paper containing root exudates of *V. villosa* and *A. strigosa* respectively (Tab. 2). In this study, the presence of allelopathic activity in the root exudates was observed in the presence of allelopathic growth factors (Pires & Oliveira 2011), as well as the amino acids which, depending on the type and concentration, promote germination and plant growth inhibition (Vurro *et al.* 2006, Fernández-Aparicio *et al.* 2013).

In addition to germination, the root exudates of *V. villosa* and *A. strigosa* contributed to a lower rate of germination of the seeds and restricted the growth and dry matter accumulation of maize seedlings. *Zea mays* seedlings showed abnormality mainly in the root system, such as necrosis and oxidation when sown in papers containing root exudates of *V. villosa*.

These seed vigor parameters determine its potential for rapid and uniform emergence and the development of normal seedlings under a wide range of environmental conditions (AOSA 2009). Seeds that germinate more slowly can give rise to seedlings with reduced size and, which as a result, may be more susceptible to stress and predation, having less chance in competition for resources such as water, light and mineral nutrients (Jefferson & Pennachio 2003).

As regards *A. spinosus*, root exudates of *A. strigosa* and *V. villosa* also affected germination, GSI and seedling initial growth (Tab. 3), a similar result to that observed for maize (Tab 2). The *R. sativus* did not influence the evaluated parameters, except for the seedling growth, which was reduced in comparison to the control. The cover crops with the highest allelopathic potential were *A. strigosa*, followed by *V. villosa* and *R. sativus*; however, the latter only interfered with the *A. spinosus* growth (Tabs. 2 and 3). According to Lesuffleur *et al.* (2007) cultivated species can release some amino acids by root exudation, which depending on the concentration can function as herbicides, impairing the germination and growth of weeds.

Reduction of crop production due to the presence of invasive plants is one of the agricultural activity major problems (Oerke 2006). A practice adopted for the suppression of invasive plants is the use of the dead cover of cover crops, as it promotes a physical barrier to the emergence of invasive plants. However, this study results show that the suppression of invasive plants can also occur due to the release of inhibitory compounds by root exudation.

Table 2. Effect of root exudation of cover crop seedlings on germination and vigor of maize seedlings (*Zea mays*) cv. PRE 22D11.

Treatment	Germination (%)	GSI	Growth (mm)		Dry matter (g)	
			S.	R. S.	S.	R. S.
Control	98.00 ± 0.82 A	9.58 ± 0.16 AB	91.72 ± 3.0 AB	165.31 ± 10.8 A	0.515 ± 0.118 A	0.576 ± 0.067 A
Raphanus sativus	94.00 ± 2.45 A	10.02 ± 0.65 A	99.82 ± 7.6 A	170.12 ± 12.92 A	0.456 ± 0.047 A	0.525 ± 0.019 A
Avena strigosa	70.50 ± 3.03 B	6.24 ± 0.42 C	78.52 ± 3.16 BC	142.13 ± 10.51 A	0.176 ± 0.014 B	0.338 ± 0.024 B
Vicia villosa	76.00 ± 4.83 B	7.73 ± 0.71 BC	65.21 ± 1.32 C	63.35 ± 9.12 B	0.178 ± 0.042 B	0.292 ± 0.021 B
C.V. (%)	7.56	12.73	10.57	16.16	13.91	14.48

Means followed by the same capital letter in the column did not differ from each other in the Tukey test at 5% probability. GSI: Germination Rate Index. S.: Shoot. R. S.: Root system.

Table 3. Germination, germination speed index and growth of *Amaranthus spinosus* seedlings under the effect of root exudation of cover crops seedlings.

Treatment	Germination (%)	GSI	Growth (mm)
Control	76.00 ± 5.66 A	4.29 ± 0.36 A	21.24 ± 2.33 A
Raphanus sativus	70.75 ± 8.87 AB	3.27 ± 0.52 AB	14.29 ± 2.7 B
Avena strigosa	43.00 ± 3.00 C	1.48 ± 0.14 C	14.34 ± 1.91 B
Vicia villosa	61.25 ± 8.39 B	2.83 ± 0.40 BC	15.86 ± 2.46 B
C.V. (%)	10.74	17.66	8.15

Means followed by the same capital letter in the column did not differ from each other in the Tukey test at 5% probability.

When the *I. grandifolia* was observed, the root exudates of the cover crops did not impair the root protrusion. However, they reduced the root protrusion speed rate and the invasive growth (Tab. 4). The decrease in radicular protrusion speed index and the growth of the *I. grandifolia* can reduce its competition potential for resources such as water, light and mineral nutrients, reducing its infestation capacity. According to Fernandez-Aparicio *et al.* (2017) the amino acid, the concentration, the plant species, and the stage of development of the host species influence the sensitivity of the plants to the amino acids. Those authors reported the inhibitory effect of the amino acids serine, threonine, and valine on root growth of *O. minor*, but these did not affect germination.

Table 4. Root protrusion and initial development of *Ipomoea grandiflora* seedlings under root exudation of cover crops seedlings.

Treatment	Protrusion (%)	RPRI	Growth (mm)
Control	80.00 ± 2.71 A	21.90 ± 0.96 A	51.14 ± 3.79 A
Raphanus sativus	91.50 ± 1.26 A	14.90 ± 0.24 B	30.98 ± 3.18 B
Avena strigosa	85.00 ± 4.8 A	13.67 ± 0.54 B	25.25 ± 2.31 B
Vicia villosa	87.50 ± 1.75 A	13.50 ± 0.60 B	29.43 ± 3.89 B
C.V. (%)	7.06	8.00	16.30

Means followed by the same capital letter in the column did not differ from each other in the Tukey test at 5% probability. RPRI: Radicular Protrusion Rate Index.

The delay in the growth of the *I. grandifolia* seedlings indicates that the adequate choice of cover crops may be an alternative for reducing the use of synthetic herbicides. It is possible to reduce the infestation of weeds by the release of allelopathic compounds by root exudation and/or the straw physical effect, preventing the survival of the germinated seeds on the soil surface (Gomes Jr. & Christoffoleti 2008).

This research results indicate that the choice of cover crops should be made taking into account the culture implanted in succession and the weeds present in the growing area since these can reduce the potential of infestation of invasive plants due to the release of allelochemicals in the soil by the roots. Allelochemicals are common in plants and proven to be toxic to plants, thus can be used as natural herbicides (Pires & Oliveira 2011, Fernández-Aparicio *et al.* 2017, Einhellig 1986).

Thus, although the use of cover crops is a very common practice adopted by small and medium-sized farmers due to the multiple benefits of crop succession, especially regarding soil properties (Bruno *et al.* 2017), the results observed in this study suggest some caution in the choice of a coverage plan.

CONCLUSIONS

The results suggest that the amino acids released by the root exudates of *Avena strigosa, Vicia villosa* and *Raphanus sativus* may reduce the germination and growth of maize, *Amaranthus spinosus* and *Ipomoea grandifolia*.

Root exudates of *R. sativus* did not show interference in germination and maize seedlings growth. However, it was detrimental to the growth of the *A. spinosus* and *I. grandifolia*

Avena strigosa root exudation promoted greater interference in germination and growth of maize seedlings and weeds, followed by the root exudate of *V. villosa* and *R. sativus* respectively.

The use of these plants in areas with the presence of target weeds can be an alternative to help reduce the incidence of these plants in crop areas, in addition to helping to improve the soil physical, chemical, and biological conditions.

ACKNOWLEDGMENT

This study was partly funded by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) -Finance Code 001

REFERENCES

Altieri, M. A., Lana, M. A., Bittencourt, H. V. H., Kieling, A. S., Comin, J. J. & Lovato, P. E. 2011. Enhancing Crop Productivity via Weed Suppression in Organic No-Till Cropping Systems in Santa Catarina, Brazil. Journal of Sustainable Agriculture 35: 855-869. 10.1080/10440046.2011.588998

Alves, T. F. D. 2009. Efeito dos exsudatos radiculares na mineralização de resíduos orgânicos aplicados ao solo. Dissertação 78 f., Instituto Superior de Agronomia, Universidade Técnica de Lisboa, Lisboa.

Association of Official Seed Analysts – AOSA. 2009. Seed vigor testing handbook. East Lansing: AOSA, 334 p. (Contribution, 32).

Blum, U. 2004. Fate of phenolic Allelochemicals in soils – the Role of soil and Rhizosphere Microorganisms. *In* Allelopathy chemistry and mode of action of allelochemicals (Macías. F. A., Galindo, J. C.G., Molinillo, J. M.G. &Cutler, H. G.). CRC Press LLC, Boca Raton, p. 57-76.

Bortolini, M. F. & Fortes, A. M. T. 2005. Efeitos alelopáticos sobre a germinação de sementes de soja (Glycine max L. Merrill). Semina. Ciências Agrárias. 26(1): 5-10.

Brasil, Ministério da Agricultura e Pecuária. 2009. Regras para Análise de Sementes. Ministério da Agricultura, Pecuária e Abastecimento. Secretaria de Defesa Agropecuária, - Brasília: Mapa/ACS. 398 p.

Bright, S., Wood, E. A. & Miflin, B. J. 1978. The effect of aspartate derived amino acids (lysine, threonine, methionine) on the growth of excise embryos of wheat and barley. Planta 139(2): 113–117. 10.1007/bf00387135

Bruno, C. L. S., Costa, L. H. M., Montalli, M. H., Carvalho, J. P. & Silva, R. A. 2017. Plantas de cobertura no Sistema Plantio Direto. Revista Conexão Eletrônica 14(1):611-619.

Dias, F. S. 2010. Determinação de compostos fenólicos em vinhos e caracterização de vinhos elaborados na região do Vale do São Francisco Pernambuco. Tese 121 f., Universidade Federal da Bahia, Bahia.

Einhellig, F. A. 1986. Mechanisms and modes of action of allelochemicals. In The Science of Allelopathy (Putnam, A.R.& Tang, C.S. eds.). John Wiley & Sons, New York, p. 171-188.

Einhellig, F. A. 2004. Mode of Allelochemical Action of Phenolic Compounds. *In* Allelopathy chemistry and mode of action of allelochemicals (Macías. F. A., Galindo, J. C.G., Molinillo, J. M.G., & Cutler, H. G.) CRC Press LLC, Boca Raton, p. 217-238.

Fernández-Aparicio, M., Bernard, A., Falchetto, L., Marget, P., Chauvel, B., Steinberg, C., Morris, C. E., Gibot-Leclerc, S., Boari, A., Vurro, M., Bohan, D. A., Sands, D. C. & Reboud, X. 2017. Investigation of amino acids as herbicides for control of *Orobanche minor* parasitism in red clover. Frontiers in Plant Science. 8(842):1-12. 10.3389/fpls.2017.00842

Fernández-Aparicio, M., Cimmino, A., Evidente, A. & Rubiales, D. 2013. Inhibition of Orobanche crenata seed germination and radicle growth by allelochemicals identified in cereals. Journal of Agricultural and Food Chemistry 61(41): 9797–9803. 10.1021/jf403738p.

Ferreira, D. F. 2014. Sisvar: a Guide for its Bootstrap procedures in multiple comparisons. Ciência e Agrotecnologia. 38(2): 109-112. 10.1590/S1413-70542014000200001

Gomes Jr., F.G. & Christoffoleti, P.J. 2008. Biologia e manejo de plantas daninhas em áreas de plantio direto. Planta daninha. 26(4): 789-798. 10.1590/S0100-83582008000400010

Gomes, D. S., Bevilaqua, N. C., Silva, F. B. & Monquero, P. A. 2014. Supressão de plantas espontâneas pelo uso de cobertura vegetal de crotalária e sorgo. Revista Brasileira de Agroecologia 9(2): 206-213.

Inderjit. 1996. Plant phenolics in allelopathy. The Botanical Review. 62(2): 186–202. 10.1007/BF02857921

Jefferson, L. V. & Pennacchio, M. 2003. Allelopathic effects of foliage extracts from four Chenopodiaceae species on seed germination. Journal of Arid Environments 55(2): 275–285. 10.1016/S0140-1963(03)00028-4

Lesuffleur, F., Paynel, F., Bataillé, M., Le Deunff, E. & Cliquet, J. B. 2007. Root amino acid exudation: measurement of high efflux rates of glycine and serine from six different plant species Plant Soil 294(1-2): 235-246. 10.1007/s11104-007-9249-x

Li, Z. H., Wang, Q., Ruan, X., Pan, C. & Jiang, D.A. 2010. Phenolics and Plant Allelopathy. Molecules 15(12): 8933-8952. 10.3390/molecules15128933

Maguire, J. D. 1962. Speed of germination-aid selection and avaluation for seedling emergence and vigor. Crop Science 2: 176-177. 10.2135/cropsci1962.0011183X000200020033x

Oerke, E. C. 2006. Crop losses to pests. Journal of Agricicultural Science 144(1): 31-43. 10.1017/S0021859605005708

Olivera-Bastidas, A. J., Macias, F. A., Fernandez, C. C., Marin, D. & Molinillo, J. M. G. 2009. Exudados de la raiz y su relevância actual en las interaciones alelopáticas. Química Nova 32(1): 198-213. 10.1590/S0100-40422009000100035

Pires, N. M. & Oliveira, V. R. 2011. Alelopatia. *In* Biologia e manejo de plantas daninhas (Oliveira Júnior, R. S.; Constantin, J. & Inque, M. H.). Omnipax, Curitiba, 348 p.

Putnam, A. R. 1987. Weed allelopathy. *In* Weed Physiology (Duke, S.O. ed.) CRC Press, Boca Raton, p. 131-155.

Rice, E.L. 1984. Allelopathy. 2.ed. Academic, New York. 422 p.

Taiz, L. & Zeiger, E. 2017. Fisiologia e Desenvolvimento Vegetal. 6. ed. Artmed, Porto Alegre, 888 p.

Trezzi, M.M., Vidal, R.A., Balbinot-Junior, A.A., Bittencourt, H.V.H. & Souza-Filho, A.P.S. 2016. Allelopathy: driving mechanisms governing its activity in agriculture. Journal of Plant Interactions 11(1): 53-60. 10.1080/17429145.2016.1159342

Vurro, M., Boari, A., Pilgeram, A. L. & Sands, D. C. 2006. Exogenous amino acids inhibit seed germination and tubercle formation by *Orobanche ramosa* (broomrape): potential application for management of parasitic weeds. Biological Control 36(2): 258–265. 10.1016/j.biocontrol.2005.09.017

Wu, H., Haig, T., Pratley, J., Lemerle, D. & An, M. 2001. Allelochemicals in wheat (*Triticum aestivum* L.): Production and exudation of 2,4-dihydroxy-7-methoxy-1,4- benzoxazin-3-one. Journal of Chemical Ecology 27(8): 1691–1700. 10.1023/a:1010422727899.

Yu, J. Q. & Matsui, Y. 1997. Effects of root exudates of cucumber (*Cucumis sativus*) and allelochemicals on ion uptake by cucumber seedlings. Journal of Chemical Ecology 23: 817-827.