Allelopathic interference of wheat crop root exudates on germination, seedling growth and leaf chlorophyll attributes of purslane and annual ryegrass



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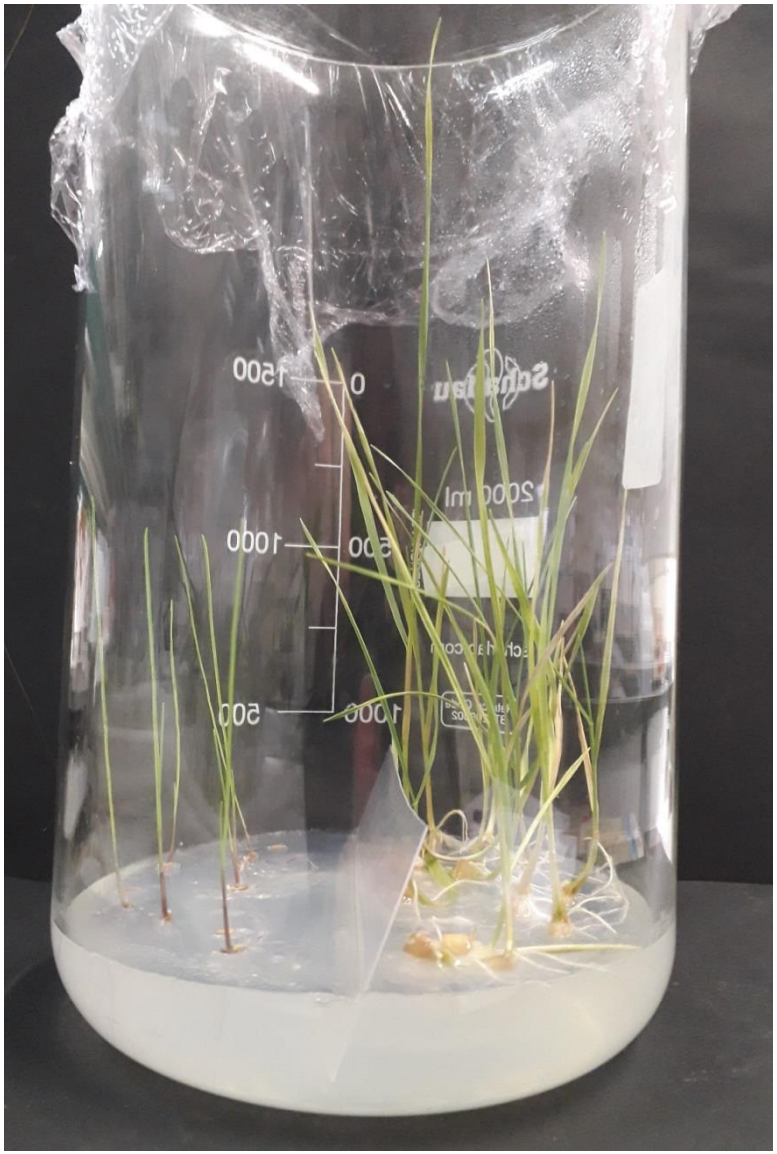
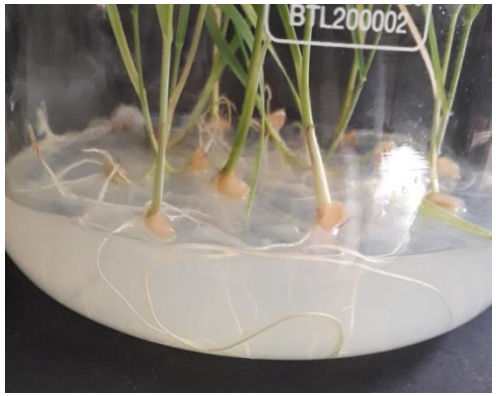
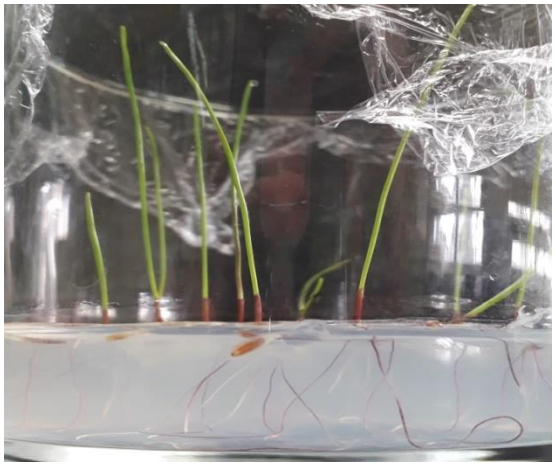


[Equal-compartment-agar Method (Wu et al., 2000)]



Introduction

What is allelopathy?



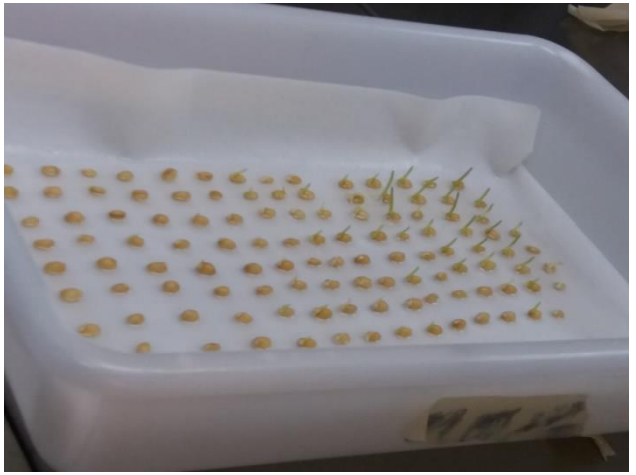
Molisch (1937) and Rice (1984) define allelopathy as:

"...biochemical interactions between all types of plants including micro-organisms....which also includes *inhibitory* and *stimulatory* reciprocal biochemical interactions"

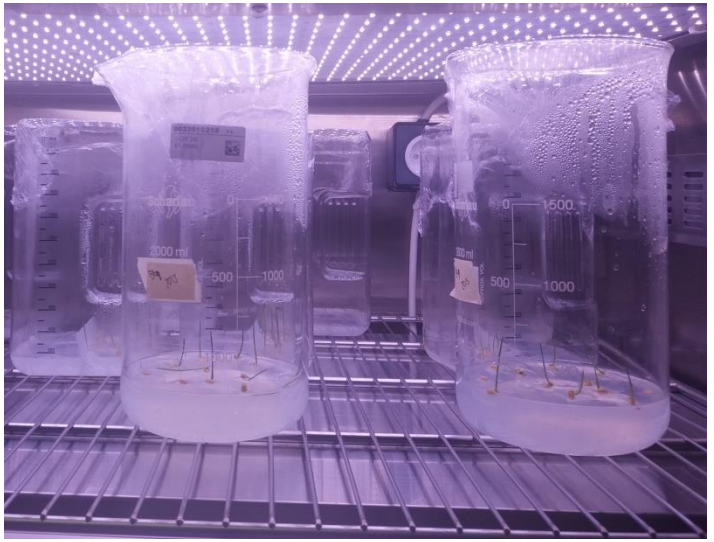
"…studies any process primarily involving secondary metabolites produced by plants, algae, bacteria, and fungi that influence the growth and development of biological and agricultural systems, including positive and negative effects " (IAS 1996)

- This is distinct from resource competition which involves the removal or reduction of an environmental factor required by another plant which occupies the same habitat (Rice 1984)

- Investigations were carried out under the project "**Increasing the efficiency and competitiveness of organic crop breeding (ECOBREED)"** during 2019 - 2020.



- The experiments were conducted under the growth chamber condition was related to accomplish the relevant TASK 2.3 of the ECOBREED.



WP2: Wheat
TASK 2.3 Allelopathic activity screening (months 13-48)

Strategic Objectives: To evaluate allelopathic potential of 40 varieties (breeding lines, commercial varieties, approved cultivars, selected accessions) from different eco-geographic regions of Europe against selected monocot and dicot weed species, *in vitro*.

UVigo Mission

Our specific objectives were to;

1) **To Identify and quantify secondary metabolites [(Benzoxazinoids and Polyphenols] profiling by LC-MS/MS from leaves, roots and rhizospheres of various wheat cultivars**

2) **elucidate the allelopathic effects of different wheat varieties on germination, seedling growth, biomass, chlorophyll pigments and carotenoids of target weed species (*Lolium rigidum* (Gaud), *Portulaca oleracea*).**

3) **characterize varietal differences of dose response wheat allelopathy (planting density) on annual ryegrass and common purslane.**

Objectives

- Identification of hydroxamic acids and polyphenols (Phenolics and flavonoids) present in shoots, roots and agar growth medium of wheat varieties and their quantification through LC-MS analysis

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- Allelopathic growth chamber trial – Equal-compartment-agar Method (Wu et al., 2000)]. Allelopathic effect of four wheat varieties on **germination**, **seedling growth**, **root length** and **chlorophyll pigments** on two important weed species ryegrass (*Lolium rigidum* (Gaud), and common purslane (*Portulaca oleracea*).

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- Comparative phytotoxic germination bioassays of wheat varieties

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- Specific goals include elucidation of the allelopathic potential of selected wheat varieties and their possible use to promote the plant-based herbicides and in the integrated weed management strategies.

Materials and methods

- Allelopathic effect of the selected four wheat varieties **('Glosa', 'Ursita', 'Ludwig' and PI554101)**

- Target weed species:
  (1) ***Lolium rigidum***
  (2) ***Portulaca oleracea***

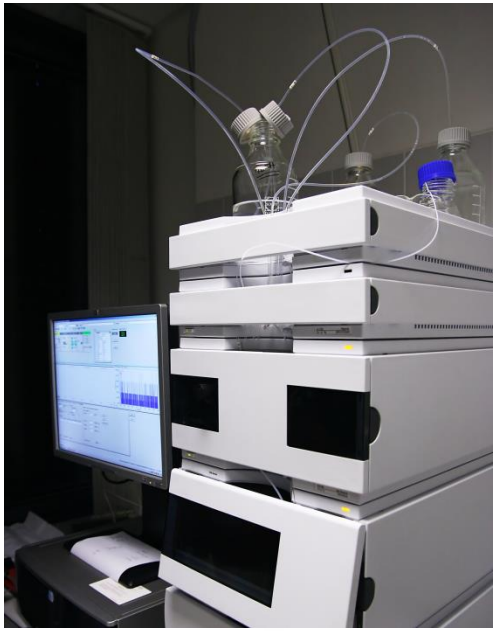
- **Equal-compartment agar method** (Wu et al., 2000).

- **Growth chamber:**
  Day/ night temperatures: 25/15 ºC
  Relative air humidity: 60±5 %
  Photoperiod : Light/dark period 13/11 h

Materials and methods

Biometric parameters:

- ❑ Germination
- ❑ Radicle length
- ❑ Total plant weight
- ❑ Leaf chlorophyll pigments



Photosynthetic pigments content:

HPLC-photo diode array detector (HPLC-PDA) Transferred to Eppendorf tubes (2 mL).
Lyophilization in dark for 24 h & at −40°C. Pigments extraction was done at 4°C by adding 200 µL of methanol (95% purity). Sample homogenization, the Eppendorf tubes were Centrifugation at 14000 *g* for 10 min in a Mini Spin Centrifuge. All the extraction steps were performed in dark and cold conditions. A volume of 40 µL supernatant was collected from each tube and transferred to small vials for HPLC pigment identification and separation (Zapata et al. 2000).

RESULTS

Table 1. Germination bioassays of *Lolium rigidum* grown alone (control) or grown together with different wheat varieties (**Glosa, Ursita and Ludwig**).

Name	% germination	% reduction	Total Weight	% reduction	Root Length	% reduction
Control	78 ±18.7		0.079 ±0.043		2.39 ±1.06	
Glosa	50 ±14.1	35.9	0.030 ±0.006	64.41	0.89 ±0.31	62.76
Ursita	32.5 ±9.6	58.33	0.011 ±0.004	86.44	0.66 ±0.28	72.38
Ludwig	55 ±12.9	29.49	0.060 ±0.008	40.68	1.46 ±0.81	38.91

Each value represents the mean (±S.D.) of three replicates.

- *Lolium rigidum* reduced its germination when growing in combination with all wheat varieties, particularly with Ursita and Glosa.
- Roots length and total weight of *L. rigidum* seedlings decreased.

Table 2. Germination bioassays of *Portulaca oleracea* grown alone (control) or grown together with different wheat varieties (**Glosa, Ursita, Ludwig and Bt1 2017**).

Name	% germination	% reduction	Total Weight	% reduction
Control	91.33 ±7.57		0.037 ± 0.012	
+ PI554101	100 ±0	(+) 8.67	0.03 ±0.007	18.92
+ Glosa	75 ±12.9	17.88	0.026 ±0.009	29.73
+ Ursita	87.5 ±12.58	4.19	0.031 ±0.006	16.22
+ Ludwig	87.5 ±12.58	4.19	0.035 ±0.009	5.41

Each value represents the mean (±S.D.) of three replicates.

- Germination of *Portulaca oleracea* was reduced when growing in association with all wheat varieties particularly with Ursita and Glosa.
- Total weight of *P. oleracea* seedlings decreased.

Table 3. Photosynthetic pigment composition and content in the leaves of two weed species (*Lolium rigidum* and *Portulaca oleracea*) when grown alone or in association with different wheat varieties for 10 days. Data were expressed as percentage of Chl *a*. Each value represents the mean (± S.E.) of three replicates. Mean values followed by the same letter within a column are not significantly different according to Tukey'test ($p \leq 0.05$).

Plant species/variety	Chlorophyll a	Chlorophyll b	β-Carotene	Lutein	Neoxanthin	Peridinin	Violaxanthin
Lolium rigidium alone	100	137.71a	25.57a	142.62a	26.74a	6.54a	6.59a
Lolium+Ursita (10:10)	100	52.64b	11.2b	52.93b	11.93b	1.85b	3.38b
Lolium+Ursita (10:15)	100	48.76c	10.87b	51.56b	10.95b	1.69b	3.4b
Portulaca oleracea alone	100	31.82c	7.02c	116.22a	7.55b	2.2a	18.69a
Portulaca+PI554101 (10:10)	100	15.19d	25a	31.51d	8.63a	0.68c	13.92b
Portulaca+Ursita (10:10)	100	57.18a	11.71b	55.79b	9.07a	1.6b	5.03d
Portulaca+Ursita (10:15)	100	44.65b	6.74c	48.52c	7.95b	1.12b	6.84c

- ✓ Chlorophyll *a*, chlorophyll *b*, *β*-carotene, lutein, neoxanthin, peridinin, and violaxanthin.
- ✓ In *L. rigidum* leaves, *Chl* showed a significant decline after growing with 'Ursita'.
- ✓ Chlorophyll *b* (64.59%), *β*-carotene (57.48), lutein (63.84%), neoxanthin (59%), peridinin (74.16%), and violaxanthin (48.41%) after growing with 'Ursita' at plant density of 10:15.

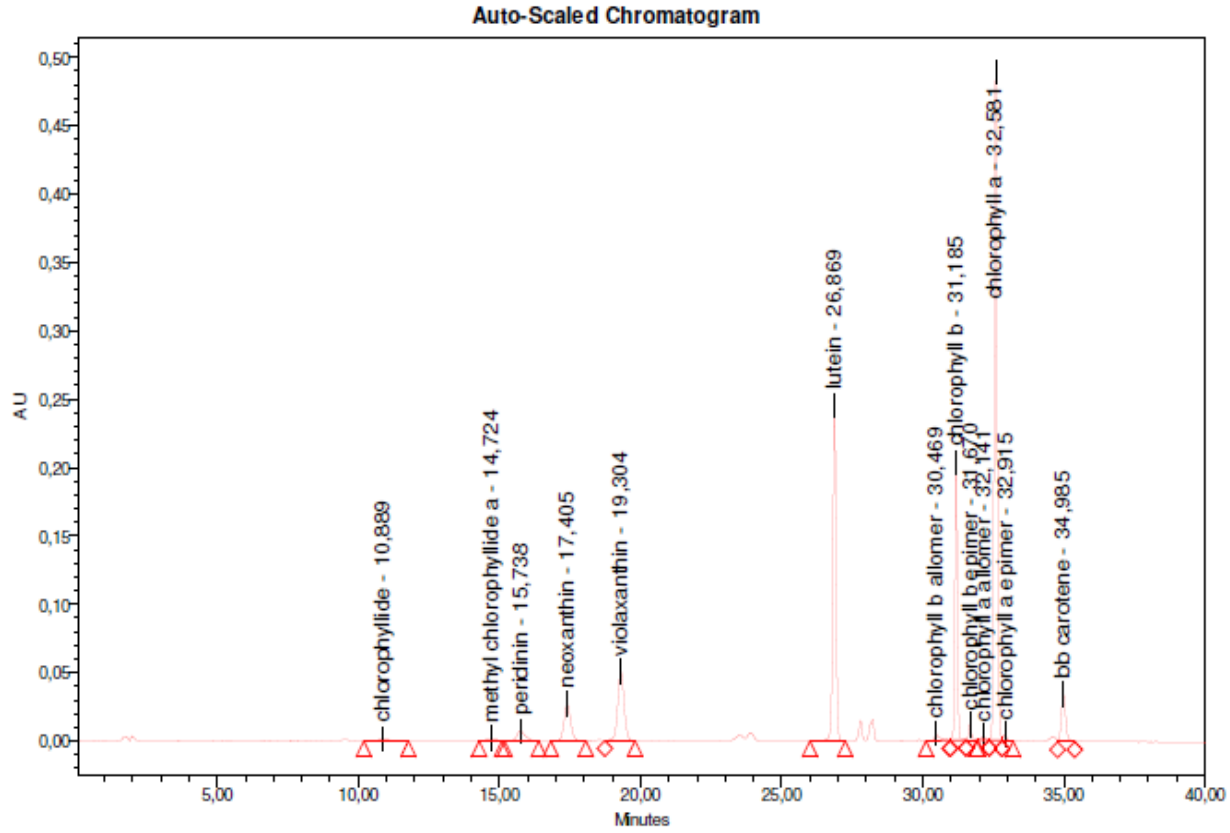


Figure 1. HPLC profile of chlorophyll pigments and carotenoids from leaf tissue extract of *Lolium rigidum*.

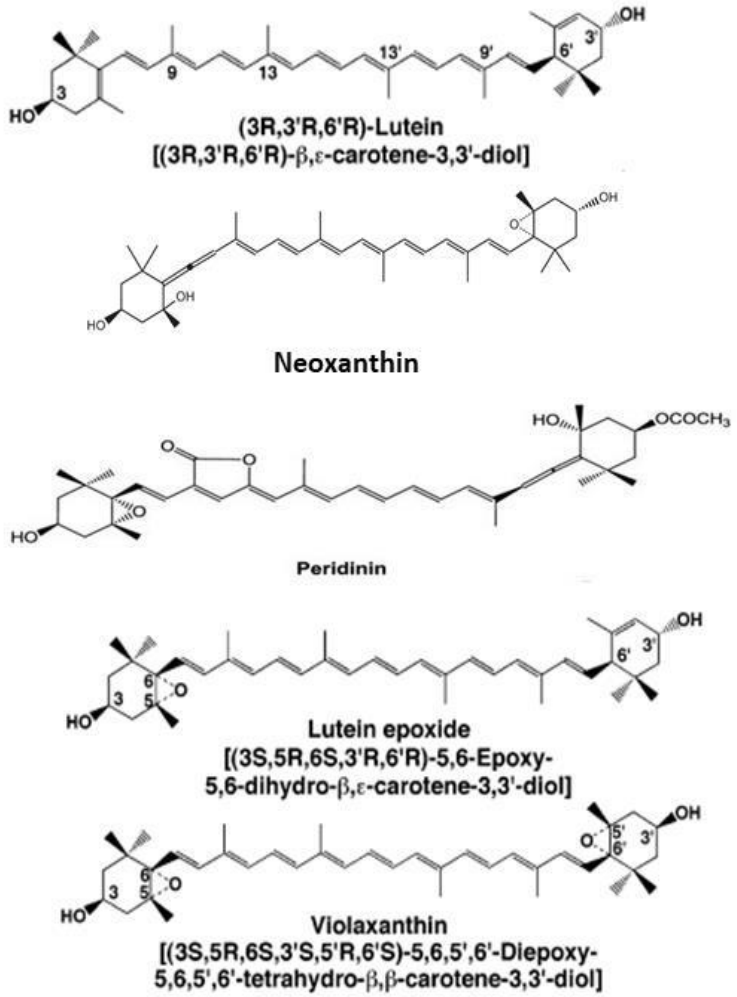


Figure 2. Chemical structure of the major chlorophyll pigments and carotenoids identified and quantified from *Lolium rigidum* and *Portulaca oleracea*.

General conclusion

As far as there is no contact between both species (wheat and the growing weeds), we concluded that the suppressive effect of wheat on weed germination and growth is via exuded compounds to the medium. Therefore, it seems clear that the ability of a variety of wheat to control weed presence could be by affecting multiple processes as germination, growth and photosynthetic efficiency of treated weeds.

Future Tasks

- **Assessment of the allelopathic potential of wheat and buckwheat cultivars** on germination, seedling growth, biomass and chlorophyll pigments and carotenoids of target weed species (*Lolium rigidum* (Gaud), *Portulaca oleracea*)

- **Quantification of the Benzoxazinoids (BXZs) and Polyphenols** (Phenolic acids and flavonoids) in roots/leaves and rhizospheres of various wheat/buckwheat cultivars

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