Article citation info:
Kryszak A., Strychalska A., Klarzyńska A., Zielewicz W., Szulc P. 2022. Preliminary Studies on Allelopathy in Tufted Hair Grass (*Deschampsia caespitosa L.*). *Journal of Research and Applications in Agricultural Engineering* 67 (1): 21-28. https://doi.org/10.53502/XTHT9388

Łukasiewicz
Poznań
Institute of
Technology

**Journal of Research and Applications
in Agricultural Engineering**

Journal website: https://tech-rol.eu/

Preliminary Studies on Allelopathy in Tufted Hair Grass (*Deschampsia caespitosa L.*)

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Article info
Received: 5 June 2022
Accepted: 4 August 2022
Published: 30 August 2022

Keywords
allelogens
Deschampsia caespitosa
medows communities
utilization intensity
phytocenoses

In the presented study, *Deschampsia caespitosa* was shown to have allelopathic properties, which can affect the species that co-create of the phytocenosis. In the course of experiments the occurrence of allelopathic compounds was determined in soil samples from phytocenoses differing with respect to proportions of *Deschampsia caespitosa* as well as utilisation intensity. They were tested against a standard (acceptor – ground cucumber). Additionally, the total content of one group of allelopathic compounds (phenolic compounds) in the substrate was determined. Soils of phytocenoses with *Deschampsia caespitosa* exhibited contents of allelopathic compounds which quantities depended on the proportion of this species in the sward as well as the method and intensity of meadow and pasture utilisation. Higher amounts of allelopathic compounds were determined in soils collected from pastures with higher proportions of *D.c.* in the community. Despite small quantities of phenols in the soil, a positive correlation was found between the share of *D.c.* and phenol compounds in soil.

DOI: 10.53502/XTHT9388

1. Introduction

Meadow communities are characterised by considerable floristic variability. Their structure to a significant degree is influenced by habitat conditions and anthropopressure [32]. A certain role is also played by interactions between species related with their biological traits, which may result in some species being outcompeted by others [11]. Under natural conditions vegetation is in a certain equilibrium, while in seminatural habitats it is difficult to assess which of the factors to the greatest degree determines the current floristic composition. Only detailed studies may indicate causes of these changes. One of these may be connected with allelopathic compounds released by higher plants and present in the soil, as it was shown e.g. by Sharma and Raghubanshi [31], Bertin *et al.* [5] or Pruchniewicz and Halarewicz [28]. Allelopathic compounds can affect the growth and development of ecosystems either stimulating or inhibiting [16]. With that said, the key to understanding allelopathic mechanisms is to isolate, identify, and

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enumerate the causative allelochemicals that are present in various plant parts or soils [24].

Meadows and pastures primarily provide fodder for ruminants, but they also perform many significant non-productive functions. It is therefore necessary to monitor their species composition. Both from the point of view of agricultural practice and aspects of nature conservation the appearance of large populations and the expansive spread of such species as e.g. *Deschampsia caespitosa* (*D.c.*) are causes for concern [19]. Modern research provides information primarily on allelopathic interactions between invasive and native species, allowing the former to eliminate the latter more rapidly [34, 36, 25, 33, 4]. Long-term studies [14, 18, 35] concerning the occurrence of *Deschampsia caespitosa* in meadow and pasture communities in river valleys indicate that the broad ecological scale and inappropriate utilisation of the sward contribute to a dynamic spread of this native species with great dynamism. This is confirmed by the occurrence of this species in many meadow habitats, as well as the untended swards in communities with different utilisation types. Its occurrence at a high proportion leads to a considerable depletion of the species composition of the flora. However, the spread of a species may be also connected with the presence of allelopathic compounds in soil. The aim of this study was to verify whether *D.c.* releases allelopathic compounds, as well as whether the share of this species in the sward resulting from the intensity of its utilisation affects the amounts of allelopathic compounds.

2. Material and methods

Studies on the contents of allelopathic compounds were conducted on soil material collected from under swards of meadow phytocenoses (*Alopecuretum pratensis*) (Regel 1925, Steffen 1931) and community with *Deschampsia caespitosa*) and pasture phytocenoses (community *Poa pratensis-Festuca rubra* Fijałk 1962 pro ass., community *Deschampsia caespitosa*), differing in their shares of *D.c.* and the manner of their utilization (Tab. 1). A soil sample from an area with no *D.c.* ground cover, i.e. arable field, was used as the control (Tab. 2).

Tab. 1. Species composition of the analyzed meadow communities

Community	**with *Deschampsia caespitosa***	**with *Deschampsia caespitosa***	**with *Deschampsia caespitosa***	**com. *Poa pratensis-Festuca rubra***	***Alopecuretum pratensis***
Utilization	**Meadow, 2 cut**	**Meadow, 1 cut**	**Pasture, occasionally-utilization 1 UL ha^{-1}**	**Pasture utilization 2 UL ha^{-1}**	**Meadow, 3 cut**
Number of species	**13**	**26**	**17**	**19**	**18**
Cover [%]	**98**	**75**	**85**	**85**	**95**
1 *Achillea millefolium*	+	+		+	+
2 *Agropyron repens*		+		+	
3 *Alopecurus pratensis*	2				4
4 *Bellis perennis*			+	+	r
5 *Bromus hordeaceus*					+
6 *Cardamine pratensis*					+
7 *Carex gracilis*	+		+		
8 *Carex hirta*		+	+	+	
9 *Cerastium holosteoides*		+	+		
10 *Cirsium arvense*			r		
11 *Cirsium oleraceum*		r			
12 *Cirsium vulgare*				r	
13 *Daucus carota*					r
14 *Deschampsia caespitosa*	4	3	3	1	+
15 *Eleocharis palustris*			r		
16 *Equisetum arvense*					+
17 *Festuca pratensis*			1		
18 *Festuca rubra*		+	2	3	
19 *Galium mollugo*		+			

Community	with *Deschampsia caespitosa*	with *Deschampsia caespitosa*	with *Deschampsia caespitosa*	com. *Poa pratensis-Festuca rubra*	*Alopecuretum pratensis*
Utilization	**Meadow, 2 cut**	**Meadow, 1 cut**	**Pasture, occasionally-utilization 1 UL ha^{-1}**	**Pasture utilization 2 UL ha^{-1}**	**Meadow, 3 cut**
Number of species	**13**	**26**	**17**	**19**	**18**
Cover [%]	**98**	**75**	**85**	**85**	**95**
20 *Geranium pratense*					+
21 *Glechoma hederacea*	+	+		+	1
22 *Heracleum sphondylium*		r			
23 *Holcus lanatus*		2			
24 *Juncus conglomeratus*		r	+		
25 *Lathyrus pratensis*		+			
26 *Leontodon autumnalis*				+	
27 *Lolium perenne*			+		
28 *Lysimachia nummularia*		r			
29 *Phalaris arundinacea*			+		r
30 *Phleum pratense*			r	+	
31 *Plantago lanceolata*		+			
32 *Plantago major*	r	+			
33 *Poa pratensis*	1	+		2	2
34 *Polygonum persicaria*		+			
35 *Potentilla anserina*	+	1	1	+	1
36 *Potentilla reptans*	r	+	r	1	+
37 *Ranunculus repens*	1	1	+	+	+
38 *Rumex acetosa*		+		+	+
39 *Rumex crispus*	+	+			
40 *Taraxacum officinale*	+			+	+
41 *Trifolium repens*	+	+	+	+	+
42 *Urtica dioica*		r		r	
43 *Veronica chamaedrys*		+			

Tab. 2. Characteristics of research objects

Samples	Share of *D.c.* (%)	Utilisation
1	50	Meadow, 2 cut
2	30	Meadow, 1 cut
3	30	Pasture, occasionally-utilization 1 UL ha^{-1}
4	10	Pasture utilization 2 UL ha^{-1}
5	1	Meadow, 3 cut
6	Control test	Arable land
7	Control test	Distilled water

Soil material for analyses was collected within a radius of approx. 20 cm from tussocks of tufted hair grass. Moreover, in the same plot species of flora were recorded together with the estimated share of tufted hair grass.

The analysed communities were found on permanently or periodically water-logged muck soils.

Allelopathic compounds were extracted from the analysed soil material using cold water [1, 9, 15]. Following extraction the extracts were filtered and the supernatant was tested in bioassays using seeds of pickling cucumber (the sink). In bioassays cucumber seed germinability was assessed as well as the length of formed radicles wetted on filter paper using 4 ml supernatant. The control consisted of seeds of the sink, germinating on blotting paper wetted solely with distilled water (4 ml) (Fig. 1). Bioassay immersed seeds as well as control seeds were germinated in an incubator (temperature 27 °C) for 72 h. The assessment was performed in 5 replications, each comprising a Petri dish with 20 seeds.

Fig. 1. A comparison of sprouting cucumber seeds under control conditions (K) and the dish with supernatant from soil from under *D.c.* (test 4)

Moreover, total contents of phenolic compounds constituting the group of allelopathic compounds were determined both in fresh soil and soil dried at 105 °C. The content of phenolics in fresh soil was assessed in two soil samples collected from the vicinity of grass tussock (approx. 20 cm) in the sward of communities differing in the shares of *D.c.* and the control, i.e. soil samples from an arable field (Tab. 1). Phenolic compounds were extracted from fresh soil using 80% ethanol, the extract was placed in an incubator (temperature 100 °C) and heated until boiling. Next it was cooled for 24 h in a refrigerator and centrifuged. The supernatant was supplemented with 0.2 ml Folina reagent and 2 ml 10% Na_2CO_3. After 30 min absorbance was measured colorimetrically at a wavelength of 660 nm in a *Spekolem 11* apparatus. Contents of phenolic compounds in soil dry matter were assessed upon drying at 105 °C by referring the values to the standard calibration curve [12, 27].

The samples were analysed employing the CANOCO 4.5 software [6]. Data were elaborated with the assistance of the PCA (principal component analysis) analysis. The PCA method is applied to numerical data [20]. It gives a first approximation of the regularities underlying a phenomenon because it is based on correlation [20].

It allowed us to determine major variability directions of the examined samples in accordance with the occurrence of phenolic compounds in soil.

3. Results and discussion

3.1. Allelopathic compounds in the soil, and germination of the test plant

Biological assays showed that allelopathic compounds were found in soil extracts obtained from the vicinity of tussocks of tufted hair grass. Regardless of the proportion of the share of the species and the manner of sward utilisation, the presence of allelopathic compounds was confirmed by germinating seeds of the test plant (Fig. 2).

The number of germinating seeds and the length of radicles of the bioassay plant in extracts of soil samples collected from the sward with the same share of *D.c.* utilised as pasture was lower than from the sward used for hay production (Fig. 3). A particularly strong inhibitory effect on the growth of the test plant radicles was observed for the supernatant of soil coming from phytocenoses of pastures with a low, 10% share of tufted hair grass. In turn, the highest germination rate was recorded for seeds of the test plants immersed in the extract from soil samples collected from phytocenoses with the highest share of *D.c.* (50%) used for hay production, but the length of their radicles was smallest (Fig. 3). It may be assumed that the difference in seed germinability is connected with the concentration of allelopathic compounds in soil. Their greater content in soils collected from pastures may be connected with soil sickness, caused by their long-term sodding by vegetation with a practically constant floristic composition [21, 17]. This contributes to adverse changes in the microbial structure and chemical composition of soil [3]. The literature mainly discusses the inhibitory effect of allelogens produced by some species on others [7, 2, 8]. On the other hand, however, allelogens from some plants can also have a stimulating effect on others [23] which is relatively well understood in horticultural plants. Meadow communities are built by a great number of taxa, so the interactions between individual taxa are multidirectional and it is difficult to clearly interpret the results obtained at this stage of the experiment.

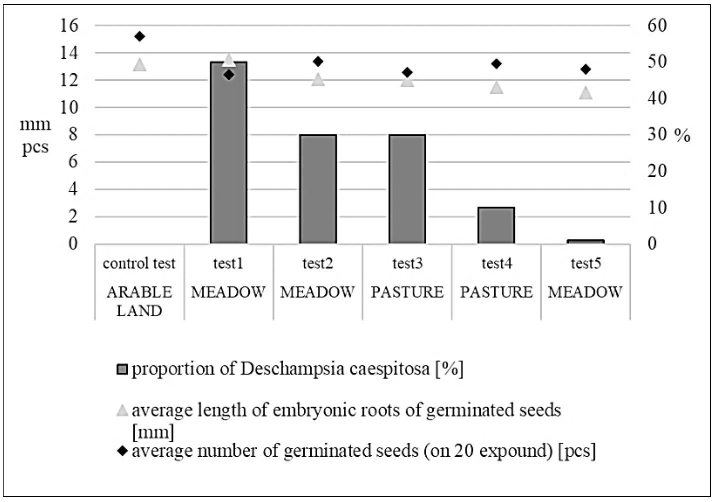

Fig. 2. Number of sprouting seeds – a test of allelopathic compounds in soil

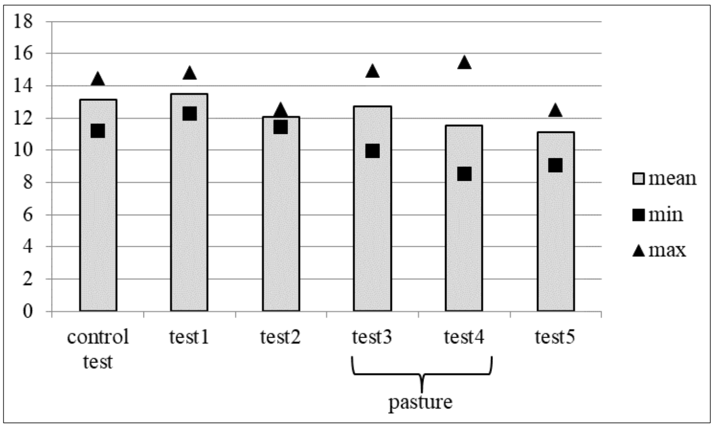

Fig. 3. Length of cucumber root – a test of allelopathic compounds in soil

3.2. Determination of total phenolic contents in soil samples

Allelopathic substances are accumulated in the aboveground parts of plants, on soil surface as well as roots and the rhizosphere [21, 5, 29]. Most of them are secondary metabolites [30], of which phenolic acids belong to the group of stronger acting ones [29]. They frequently inhibit plant growth and cause root deformations [37, 10].

Results indicate that the phenolic content in tested soils is slight (Tab. 3). However, their presence in soils can be connected with the occurrence of D.c., as evidenced by the reference to the standard, i.e. arable field with a 0% share of D.c. (Fig. 4).

In multi-species meadow and pasture communities, however, this may be due to the presence of other plants with allelopathic properties. So, for example, in the root secretions of couch grass (Agropyron repens) there are also many allelogens including phenolic acids [13].

The content of phenolic compounds in soil samples collected from meadows was greater than in those from pastures.

Tab. 3. Phenol content, and seed germination of the test plant

	MEADOW 30% D.c.	PASTURE 10% D.c.	ARABLE LAND 0% D.c.
average length of embryonic roots of germinated seeds [mm]	12.06	11.52	13.13
average number of germinated seeds (on 20 expound) [pcs]	13.4	13.2	15.2
phenolic content [μg/100g]	0.086	0.063	0,038

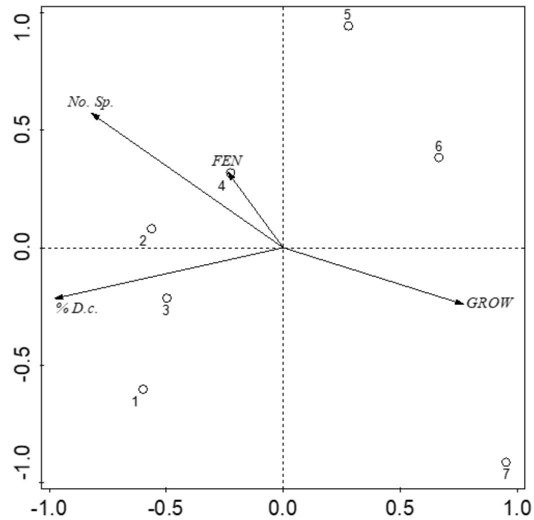

Fig. 4. Occurrence of phenolic compounds in soil (mg /g d.m.) at various shares of D.c. and the richness of species in the sward *(No. Sp.* – number of species; *FEN* – phenolic contents in soil samples, % *D.c.* – share of *D.c.* in percetage; *GROW* – mean length of cucumber roots; 1, 2, 3, 4, 5, 6, 7 – samples, explanation in Tab. 1)

3.3. Allelopathic compou.nds and richness of species

Results of preliminary analyses indicate that the occurrence and the potential spread of *D.c.* may be connected e.g. with the allelopathic compounds released by this species. However, phenolic compounds influenced an increase in the number of species (Fig. 4). This dependence may suggest that other allelopathic compounds may contribute to a reduction of species richness in the sward. This process may result in the decline of the species richness in the sward with a share of this species. Many authors have already showed interspecies interactions between plants in communities, including those referring to the presence of allelopathic compounds, affecting grassy ecosystems [22, 26, 38]. To date the focus has been first of all on the importance of habitat conditions (mainly moisture content) as well as type of utilisation on the share of *D.c.* in the sward, while the potential effect of the allelopathic compounds has not been investigated in depth [18, 35].

PCA analysis (Fig. 4) shows that the number of species in the community is correlated with the phenolic content of the soil. A higher proportion of phenols was found in samples with a higher proportion of D.c. A higher proportion of D.c., however, limits the number of other species.

4. Conclusions

1. Soils of meadow phytocenoses with a share of *Deschampsia caespitosa* exhibit varied contents in allelopathic compounds.
2. Phenolic content in tested soils is slight, however, their presence can connected with the occurrence of D.c., as evidenced by the reference to the standard, i.e. arable field with a 0% share of D.c.
3. A higher number of species in the phytocenoses was recorded with a lower allelopathic potential of the supernatant, but a greater share of phenolic compounds.

Acknowledgements

We would like to thank Professor Barbara Politycka for her help and guidance through the methodological meanderings associated with the study of allelopathy in *Deschampsia caespitosa*.

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