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## Some Effects of Biochar on Soil Microorganisms: A review article

### *A bioszén néhány hatása a talaj mikroorganizmusaira: áttekintő cikk*

Evan Dayoub<sup>1\*</sup>, Zoltan Toth<sup>1</sup> and Angela Anda<sup>1</sup>

<sup>1</sup>*Institute of Agronomy, Georgikon Campus, Hungarian University of Agriculture and Life Sciences, Keszthely, 8360-Hungary*

*toth.zoltan@uni-mate.hu*

*Anda.Angela@uni-mate.hu*

*\*Correspondence: dayoubevan@gmail.com*

**Abstract:** Using biochar as a soil amendment is suggested to be a win/win technology for enhancing physical and chemical soil properties, yet little is known about the effects of biochar on soil microorganisms. This review underscores twofold of soil microbiological features studied in short-term experiments. 1) microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and basal soil respiration (BSR). 2)  $\beta$ -glucosidase, dehydrogenase, and urease enzymes activities under different doses and types of biochar and soil. MBC, MBN, BSR  $\beta$ -glucosidase, dehydrogenase, and urease and enzymes activities responded to biochar application depending on biochar dose, type, inorganic fertilizer application, soil type and cultivated plant. MBC, MBN, and BSR increased linearly after gradual amendments of cotton straw biochar while just low doses were effective for raising  $\beta$ -glucosidase, and dehydrogenase activities. Only high doses of wheat and corn straw biochar were effective to increase MBC while linear increments were witnessed under swine manure biochar. Across all biochar types, MBN showed an upward trend with increasing biochar rates hitting the heyday at the highest doses. On the other side, wheat straw and apple branch biochar caused gradual increments in  $\beta$ -glucosidase and urease activity with NPK (nitrogen-phosphorous-potassium) amendment after 72 months.

**Keywords:** *biochar, microbial biomass carbon, microbial biomass nitrogen, basal soil respiration, enzyme activity*

**Összefoglalás:** A bioszén talajjavítóként való felhasználása a talaj fizikai és kémiai tulajdonságainak javítására szolgáló win/win technológia, ugyanakkor keveset tudunk a bioszén talaj mikroorganizmusokra gyakorolt hatásairól. Ez az áttekintés a talaj mikrobiológiai jellemzőinek változásaira hívja fel a figyelmet, melyek a következők: 1) mikrobiális biomassza szén (MBC), mikrobiális biomassza nitrogén (MBN) és bazális talajlégzés (BSR) alakulása. 2) A  $\beta$ -glükózidáz, dehidrogenáz és ureáz enzimek aktivitása különböző dózisokban és eltérő típusú bioszenekben. Az MBC, MBN, BSR  $\beta$ -glükózidáz, dehidrogenáz, ureáz enzimek aktivitása reagált a bioszén kijuttatására a dózistól, a bioszén típustól, a szervesetlen műtrágya kijuttatásától és a termesztett növénytől függően. Az MBC, MBN és BSR lineárisan nőtt a gyapotszalma bioszén kijuttatást követően, még az alacsony dózisok is hatásosak voltak a  $\beta$ -glükózidáz és a dehidrogenáz aktivitás növelésére. Csak nagy dózisú búza és kukorica szalmából származó bioszén hatékonyan növelte az MBC-t, míg a sertéstrágyából nyert bioszén esetében ez az emelkedés lineáris volt. Az MBN az összes bioszén típust tekintve emelkedő tendenciát mutatott, és a bioszén arányának növekedése a legmagasabb dózisok mellett volt a

legnagyobb. A búzaszalma alkalmazása három időszakban (48, 60 és 72 hónap) jelentősen csökkentette az ureáz aktivitást, míg a BSR csak a leghosszabb távú megfigyelésben csökkent nagyobb mértékben. A másik oldalon a búzaszalma bioszén a  $\beta$ -glükózidáz és az ureáz aktivitás fokozatos növekedését okozta NPK (nitrogén-foszfor-kálium) adagolásnál 72 hónappal a kijuttatás után.

**Kulcsszavak:** bioszén, mikrobiális biomasz szén, mikrobiális biomasz nitrogén, bazális talajlégzés, enzimaktivitás.

## 1 Introduction

Biochar is a solid carbonaceous residue made by burning biomass under oxygen-free to oxygen-deficient conditions. Wood chips, crop residues, nut shells, seed mill screenings, algae, animal manure, and sewage sludge are some of the many feedstocks used in biochar production. It is highly resistant to decomposition when applied to soil, and its residence time ranges from tens of years to millennia (Preston and Schmidt, 2006; Verheijen et al., 2010). This reuse of what would otherwise be agricultural waste has become an emerging technology for sustainable soil management to add biomass as an organic amendment (Cernansky, 2015). Its application can improve soil fertility and plant productivity (Jeffery et al., 2014; Lehmann, 2007), as well as improve soil porosity (Omondi et al., 2016). Compared to its effect on soil characteristics and fertility and eutrophication management (Jia et al., 2018), the effects of biochar on the microbial communities of soil have been less thoroughly assessed (Lehmann et al., 2011, 2015).

Biochar may interact with soil microorganisms either directly, by being degraded and utilized, or indirectly, by improving soil properties and habitat conditions (Ameloot et al., 2013) as well as by indirectly i) serving as a refuge habitat, which protects microbes against grazers and predators, ii) improving physical soil properties, e.g., water holding capacity, bulk density, and aeration, and iii) modifying chemical soil properties, e.g. pH, cation exchange capacity (CEC), nutrient retention and sorption of soil organic matter (Lehmann et al., 2011). Overall effects of biochar on soil bacterial diversity and community structure depend on biochar type, pyrolysis temperature, experiment type, precipitation conditions (Wang et al., 2023), soil type, and agricultural management, such as crop type and planting duration (Abujabhah et al., 2016; Dai et al., 2016; Herrmann et al., 2019; Liu et al., 2018; Yu et al., 2018).

This review examines two groups of soil microbiological aspects affected by biochar: First, MBC MBN, BSR. Second,  $\beta$ -glucosidase, dehydrogenase, and urease enzymes activities.

## 2 Biochar effects on Soil Microorganisms

### 2.1 MBC, MBN and BSR

MBC, MBN, BSR, and enzyme activities are commonly determined biochemical properties due to primary regulators of many soil processes, thus considered important indicators of soil quality (Shao et al., 2008).

MBC increased across all soil types except the sandy loam soil with a low OC content (1%). MBC in calcareous Fluvisol and fluvo-aquic soils has markedly increased with (swine-manure and cotton straw) biochar rates increment while wheat and corn straw biochar revealed significant increases in sandy loam and fluvo-aquic soil MBC at the highest rates of amendment generally. On the other hand, a short-term experiment (2 years) showed a significant reduction in MBC after the addition of gradual biochar doses in alkaline sandy loam soil with only 1% of OC growing wheat with no fertilizer. A significant increase of MBC could be seen at 5 t ha<sup>-1</sup> when

mash bean was sown in the same soil with no fertilizer while a different pattern was clear after introducing the fertilizer showing a marked decline only at 5 t ha<sup>-1</sup>.

Generally, MBN showed an upward trend with increasing biochar rates hitting the heyday at the highest amendments across all examined soil types regardless of (sandy loam soil growing wheat under NPK fertilizer) which experienced a significant reduction compared to the control (Azeem et al., 2019). MBC was initially higher but decreased in the second year of biochar amendment (both with and without fertilizer) which may be attributed to the positive priming effect at the start of the experiment and DOC (dissolved organic carbon) significant reduction to 0.45 g kg<sup>-1</sup> after the second year (Azeem et al., 2019). However, some other studies showed a significant increase in MBC under a low biochar application rate (<2%) ( Prayogo et al., 2014; Mingkui and Walelign., 2015). No significant change in MBC was also observed under a low biochar addition ratio (<8%) in temperate soil (Anders et al., 2013). An explanation for MBC changes in response to additions of biochar includes enhanced availability of soil nutrients (i.e P, Ca, and K), adsorption of toxic compounds, and improved soil water and pH status. All these changes have an impact on the activity of soil microorganisms (Lehmann et al., 2011).

BSR showed an upward trend across all the studied soil types except in the sandy loam soil amended with wheat straw biochar. BSR values after the amendment of cotton and corn straw biochar to calcareic Fluvisol and fluvo-aquic soils showed significant increments but regarding the corn straw biochar application there were not any significant differences among the doses. However, sandy loam soil BSR responded after the amendment of sewage sludge biochar with rising at the highest dose only whereas wheat straw biochar negatively affected sandy loam soil BSR at the highest rate only.

Higher respiration rates for soils treated with biochar could have been mediated by an improved soil structure, leading to enhance both aeration and microbial activity (Busscher et al., 2010). The reduction in BSR could be linked to the improved efficiency in carbon use because of the co-location of microorganisms and carbon on biochar surfaces, which reduces the need for enzyme production (Lehmann et al., 2011).

**Table 1 Soil microbial biomass carbon MBC, MBN Soil microbial biomass nitrogen and BSR under different biochar feedstock and rates**

Feedstock type	Pt	Soil type pH	OC g kg <sup>-1</sup>	Plant	Application rate	MBC mg kg <sup>-1</sup>	MBN mg kg <sup>-1</sup>	BSR mg CO <sub>2</sub> eC kg <sup>-1</sup> soil	References
Cotton straw	450	Calcaric Fluvisol 7.8	16.2	Cotton	With NPK				(Liao et al., 2016)
					0	367 <sup>b</sup>	34.1 <sup>b</sup>	15.6 <sup>b</sup>	
					2.25	427 <sup>ab</sup>	33.4 <sup>b</sup>	16.1 <sup>ab</sup>	
					4.5 t ha <sup>-1</sup>	485 <sup>a</sup>	52.5 <sup>a</sup>	17.7 <sup>a</sup>	
Wheat straw	350–550 2- mm	Sandy loam 18 months 5.92	20.1	Rice paddy	No fertilizer				(Chen et al., 2016)
					0	558.0	30.63	32.92	
					20	579.4	39.46	29	
					40 t ha <sup>-1</sup>	620.8	43.51	25.63	
					LSD=				
					58.22				
						LSD=	LSD=		
						11.7	6.06		

Swine-manure	350	Laterite	2.84	Tea	No fertilizer	62.40 <sup>a</sup>	8.34 <sup>a</sup>	NA	(Jiang et al., 2021)
	9 months	5.4			0	65.98 <sup>b</sup>	9.36 <sup>b</sup>	NA	
					0.5	80.63 <sup>c</sup>	10.33 <sup>b</sup>	NA	
					1	85.14 <sup>d</sup>	11.12 <sup>c</sup>	NA	
					2%			NA	
Sewage sludge	600	Sandy loam	8.87	No plant	0	1055 <sup>a</sup>		1631 <sup>a</sup>	(Paz-Ferreiro et al., 2011)
	70 days	6.50			SI4	1292 <sup>a</sup>		1197 <sup>c</sup>	
					SI8	599 <sup>b</sup>		1364 <sup>b</sup>	
					B4	1375 <sup>a</sup>		1382 <sup>b</sup>	
					B8%	1404 <sup>a</sup>		808 <sup>d</sup>	
Sugarcane bagasse	350	Sandy loam	1	Mash bean	No fertilizer			NA	(Azeem et al., 2019)
	2 years	8.5			0	426 <sup>b</sup>	20.2 <sup>b</sup>	NA	
					5	440 <sup>ab</sup>	23.6 <sup>ab</sup>	NA	
					10 t ha <sup>-1</sup>	432 <sup>b</sup>	25.5 <sup>ab</sup>	NA	
					NPK				
					Fertilizer				
0	462 <sup>a</sup>	24.6 <sup>ab</sup>	n						

					5	450 <sup>ab</sup>	23.1 <sup>ab</sup>		
					10 t ha <sup>-1</sup>	460 <sup>a</sup>	26 <sup>a</sup>		
Sugarcane bagasse	350	Sandy loam	1	Wheat	No fertilizer				(Azeem et al., 2019)
	2 years	8.5			0	430 <sup>a</sup>	18.5 <sup>ab</sup>	NA	
					5	401 <sup>b</sup>	21.4 <sup>ab</sup>	NA	
					10	377 <sup>cd</sup>	21.7 <sup>ab</sup>	NA	
					NPK				
					Fertilizer				
					0	444 <sup>a</sup>	26.3 <sup>a</sup>	NA	
					5	373 <sup>d</sup>	19.7 <sup>ab</sup>	NA	
					10 t ha <sup>-1</sup>	394 <sup>bc</sup>	19.7 <sup>b</sup>	NA	
Corn straw	500	fluvo-aquic	9.51	No plant	250 kg N ha <sup>-1</sup>				(Xu et al., 2016)
	<1 mm	8.1		150 days	0	75.12 <sup>b</sup>	8.24 <sup>b</sup>	70.68 <sup>b</sup>	
					2	79.45 <sup>ab</sup>	8.56 <sup>ab</sup>	89.19 <sup>a</sup>	
					4	75.31 <sup>b</sup>	8.59 <sup>ab</sup>	96.86 <sup>a</sup>	
					8%	83.27 <sup>a</sup>	8.86 <sup>a</sup>	94.53 <sup>a</sup>	

Pt: Pyrolysis temperature. (Liao et al., 2016) NPK: s 300 kg N ha<sup>-1</sup> urea, Triple super phosphate (105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium sulfate (60 kg K<sub>2</sub>O ha<sup>-1</sup>).



## 2.2. $\beta$ -glucosidase, Dehydrogenase and Urease Enzyme Activity

Soil enzymes have different roles such as the C-degrading enzymes include  $\alpha$ -glucosidase,  $\beta$ -cellobiosidase, and  $\beta$ -glucosidase (Chen et al., 2016). In addition to the dehydrogenase activity that has been used as a parameter for the evaluation of the degree of recovery of degraded soils (Gil-Sotres et al., 2005). Urease and phosphatase are two important enzymes involved in the nitrogen and phosphorus cycles, respectively (Pascual et al., 1998). Urease is involved in the hydrolysis of urea-type substrates and its origin is basically microbial and its activity is extracellular (Bremner and Mulvaney, 1978). This enzyme may form stable complexes (urease–humus) (Nannipieri et al., 1980).

$\beta$ -glucosidase enzyme activity decreased almost in all studied soil types and carbon contents except in silty clay soil amended with apple branch biochar accompanied by urea.  $\beta$ -glucosidase enzyme activity of cotton straw with NPK and apple branch biochar with urea has been increased importantly starting from the lowest dose but with no important differences among doses for cotton straw biochar. On the other side,  $\beta$ -glucosidase enzyme activity in sandy loam and silty clay soils declined markedly under gradual doses of wheat straw, sewage sludge, and apple branch biochar without urea amendment. Volatile compounds in biochar produced at low temperatures (350-500 °C) stimulate enzymatic activity, including dehydrogenase activity and  $\beta$ -glucosidase activity (Ameloot et al., 2013; Bailey et al., 2011). While reductions in  $\beta$ -glucosidase activity were reported under the amendment of fast-pyrolysis biochar produced from switchgrass (Bailey et al., 2011). Lammirato et al. (2011) also found that biochar addition caused a decrease in the rate of the reaction catalyzed by  $\beta$ -glucosidase.

The dehydrogenase activity decreased under poultry litter and wheat straw amendments to loamy sand soil, as well as in sandy loam soil amended with sugarcane bagasse but without fertilizer. On the other hand, dehydrogenase activity increased in sandy loam soils after the addition of sewage sludge and sugarcane bagasse biochar with no fertilizer. Although, no significant changes in dose variations in biochar additions of wheat straw and sugarcane bagasse growing rice and mash bean were observed, the dehydrogenase activity increased significantly after the addition of wheat straw and sugarcane bagasse growing rice and mash bean (without fertilizer) but among the treatments, the variations were not significant. But for wheat straw biochar amendment with NPK, after 72 months in a loamy sand soil growing winter rye, dehydrogenase activity rose markedly with the increasing dose ones. On the same grounds, its activity grew significantly only after the usage of 8% (the highest rate) of sewage sludge biochar. However, the application of poultry litter biochar in a loamy sand soil growing pasture grass caused a significant drop compared to the control, but not among the biochar rates. The previous results are consistent with Demisie et al. (2014) who revealed that the highest dehydrogenase activity was measured in both oak wood and bamboo biochar pyrolyzed at 600 °C at the lowest rate of 0.5% in a clay loam soil. Similarly, Irfan et al. (2019) indicated this improvement under biochar application rate of 1% C (w/w).

Urease enzyme activity showed a downward trend across all soil types except for loamy sand soil treated with poultry litter biochar. Urease activity decreased significantly in sandy loam soil with increasing biochar rates of sugarcane bagasse biochar without NPK. A similar trend could be seen in silty clay soil in the treatment without urea and apple branch biochar but without significant variations between 1-4% amendment rates. When NPK was introduced to the Sugarcane bagasse biochar for mash bean plant, urease activity lessened significantly at 5 t ha<sup>-1</sup>. But under urea usage and apple branch biochar, it witnessed a significant fluctuation starting with an increment at 2% followed by a drop at 4%.

Urease activity in loamy sand soil for both biochar types (poultry litter and wheat straw biochar after 72 months) increased gradually when biochar doses were used compared to the use of NPK treatment only; while by comparing the three periods for wheat straw biochar use

(48, 60, and 72 months) it has decreased significantly. Woody biochar amendment to silt loam also caused a considerable increase in urease activity at the dose of 22 Mg ha<sup>-1</sup>.

Biochar produced at a pyrolysis temperature of 350–550 °C with a pH of > 10 and C/N ratio of < 50 increased the urease activity to a greater extent than those produced at other pyrolysis conditions (Pokharel et al., 2020). However, the activities of N and P enzymes were related to the application rate and biochar type. The addition of 10 mg kg<sup>-1</sup> biochar stimulated the activities of alkaline phosphatase and urease (Huang et al., 2017). On the other side, the reduction in urease activity could have been attributable to the decline in soil properties due to monoculture cropping of rye and also to the effect of biochar aging (Futa et al., 2020). Gul et al. (2015) detected changes in biochar characteristics due to its aging in soil, in particular on account of its oxidation and the accumulation of H<sup>+</sup> from the soil solution.

Table 2  $\beta$ -glucosidase, dehydrogenase and urease activity under different rates and feedstock of biochar

Feedstock type	Soil type	OC g kg <sup>-1</sup>	Pt	Plant	Application rate	$\beta$ -glucosidase mg p-nitrophenyl kg <sup>-1</sup> soil h <sup>-1</sup> )	Dehydrogenase activity [mg TPF kg <sup>-1</sup> h <sup>-1</sup> ]	Urease activity [mg N-NH <sub>4</sub> <sup>+</sup> kg <sup>-1</sup> h <sup>-1</sup> ]	References	
Poultry litter	Loamy sand	8.87	300	Pasture mix	grass 0	NA	0.74 <sup>ab</sup>	8.61 <sup>a</sup>	(Mierzwa-Hersztek et al., 2016)	
					NPK	NA	0.63 <sup>a</sup>	4.78 <sup>c</sup>		
					PL	NA	0.88 <sup>b</sup>	12.4 <sup>b</sup>		
					2.25+	NA	0.70 <sup>a</sup>	8.38 <sup>a</sup>		
					5 t ha <sup>-1</sup> +	NA	0.72 <sup>a</sup>	11.1 <sup>b</sup>		
Wheat straw	Loamy sand	5.95	650	Winter rye	With NPK				(Futa et al., 2020)	
					72 months	0	NA	1.25 <sup>a</sup>		2.05 <sup>a</sup>
						10	NA	2.80 <sup>b</sup>		2.32 <sup>b</sup>
						20	NA	3.29 <sup>c</sup>		2.98 <sup>c</sup>
			30 t ha <sup>-1</sup>	NA	5.04 <sup>d</sup>	2.51 <sup>d</sup>				

Wheat straw	loamy sand	5.95	650	After 48 months	Average biochar rates for	NA	4.27 <sup>a</sup>	3.76 <sup>a</sup>	(Futa et al., 2020)	
				After 60 months		NA	3.32 <sup>a</sup>	3.24 <sup>b</sup>		
				After 72 months		NA	3.10 <sup>a</sup>	2.47 <sup>c</sup>		
Cotton straw	Calcaric Fluvisol	16.2	450	Cotton	With NPK				(Liao et al., 2016)	
						0	13.5 <sup>b</sup>	NA		NA
						2.25	14.9 <sup>a</sup>	NA		NA
						4.5 t ha <sup>-1</sup>	15.4 <sup>a</sup>	NA		NA
Wheat straw		20.1	350–550	Rice paddy	No fertilizer	54.40	0.91		(Chen et al., 2016)	
						0	50.55	1.72		NA
						20	43.09	2.01		NA
						40 t ha <sup>-1</sup>	LSD=4.56	LSD=0.74		
Sewage sludge	Sandy loam	8.87	600	No plant	0	2.64 <sup>a</sup>	0.11 <sup>a</sup>	NA	(Paz-Ferreiro et al., 2011)	
				High organic matter	SI 4	1.98 <sup>ab</sup>	0.12 <sup>a</sup>	NA		
					SI 8%	0.58 <sup>c</sup>	0.10 <sup>a</sup>	NA		
					B4	1.71 <sup>b</sup>	0.16 <sup>a</sup>	NA		
					B 8%	1.22 <sup>bc</sup>	0.29 <sup>b</sup>	NA		

Sugarcane bagasse	Sandy loam	1	350	Mash bean	No fertilizer				(Azeem et al., 2019)
					0	NA	4.37 <sup>b</sup>	17.83 <sup>b</sup>	
					5	NA	4.91 <sup>a</sup>	17.33 <sup>b</sup>	
	2years	1	350	Mash bean	10 t ha <sup>-1</sup>	NA	5.04 <sup>a</sup>	17.75 <sup>b</sup>	
					NPK fertilizer				
					0	NA	5.33 <sup>a</sup>	19.45 <sup>a</sup>	
					5	NA	5 <sup>a</sup>	17.62 <sup>b</sup>	
					10 t ha <sup>-1</sup>	NA	5.20 <sup>a</sup>	17.70 <sup>a</sup>	
Sugarcane bagasse	Sandy loam	2	350	Wheat	No fertilizer				(Azeem et al., 2019)
					0	NA	4.45 <sup>a</sup>	17.95 <sup>a</sup>	
					5	NA	4.5 <sup>a</sup>	16.5 <sup>c</sup>	
					10 t ha <sup>-1</sup>	NA	4.62 <sup>a</sup>	15.5 <sup>d</sup>	
		1			NPK Fertilizer				
			0	NA	5 <sup>a</sup>	17.20 <sup>b</sup>			
			5	NA	4.70 <sup>a</sup>	17.08 <sup>b</sup>			
			10 t ha <sup>-1</sup>	NA	4.79 <sup>a</sup>	17.37 <sup>b</sup>			

Woody	Silt loam	13	500-600	Corn	NPK	58 <sup>a</sup>	NA	19 <sup>b</sup>	(Bera et al., 2016)	
					3 years	DE	64 <sup>a</sup>	NA		19 <sup>b</sup>
						NPK+ biochar 22 Mg ha <sup>-1</sup>	44 <sup>b</sup>	NA		22 <sup>a</sup>
						DE+biochar	62 <sup>a</sup>	NA		21 <sup>a</sup>
Apple branch	Silt-clay	5	450	108 days	No urea				(Li et al., 2017)	
					0	99.39 <sup>c</sup>	NA	0.194 <sup>b</sup>		
					1	85.19 <sup>b</sup>	NA	0.178 <sup>a</sup>		
					2	83.31 <sup>ab</sup>	NA	0.186 <sup>ab</sup>		
	4%	77.23 <sup>a</sup>	NA	0.179 <sup>a</sup>						
Apple branch	Silt-clay	5	450	108 days	Urea 0.2 g kg <sup>-1</sup>				(Li et al., 2017)	
					0	99.44 <sup>c</sup>	NA	0.218 <sup>cd</sup>		
					1	136.37 <sup>c</sup>	NA	0.221 <sup>cd</sup>		
					2	126.01 <sup>d</sup>	NA	0.224 <sup>d</sup>		
	4%	131.17 <sup>de</sup>	NA	0.209 <sup>c</sup>						

**PL:** Poultry litter 5.00 t DM ha<sup>-1</sup>, **MF:** 100 kg N ha<sup>-1</sup>, 40 kg P ha<sup>-1</sup> and 120 kg K ha<sup>-1</sup>, 336, 50, and 140 kg ha<sup>-1</sup> N, P, and K (Bera et al., 2016), **DE:** Dairy manure effluent 168,000 l ha<sup>-1</sup>. (Liao et al., 2016) NPK: s 300 kg N ha<sup>-1</sup> urea, Triple super phosphate (105 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and potassium sulfate (60 kg K<sub>2</sub>O ha<sup>-1</sup>). (Futa et al., 2020) NPK: 70 kg ha<sup>-1</sup> N (ammonium nitrate), 26 kg ha<sup>-1</sup> P (triple superphosphate), and 66 kg ha<sup>-1</sup> K (muriate of potash, KCl).

### 3 Conclusions

Soil is the most important nutrient and water sources not only for crops, but for soil microflora. The biochar, an organic amendment, a carbon-enriched and porous substance, increases soil water and nutrient retention improving microbial activity. Carbonization of organic materials beyond sequestration of soil carbon, modifies its physical, chemical and biological features. Biochar induced pore structure and water movement changes in the soil improves the life conditions of microbes. It is important to mention that the influence of biochar on soil properties including microbes is highly variable because wide range of soil, biochar and plant variables such as (biochar type, pyrolysis temperature, experimental and environmental conditions, soil type, and agricultural management, etc.).

Although biochar has the ability to improve MBC, MBN, BSR in coarse textured soil except for sandy loam soils with low OC contents. The ability of well-OC content, coarse-textured soils to break down organic matter was diminished. Also, the ability of soil to convert urea into ammonium (the activity of the urea enzyme) has reduced in all soil and biochar types, with the exception of loamy sand soil treated with chicken litter biochar. Among other biochar types cotton and wheat straw biochar seemed to be a promising tool to enhance soil biological activity in coarse to medium textured soils under short term experiments. For example, cotton straw biochar positively affected MBN and BSR, even the lowest doses were enough for promoting  $\beta$ -glucosidase activity. Wheat straw biochar increased  $\beta$ -glucosidase and urease activity while just the lowest rate was positive for dehydrogenase improvement. Another good feedstock for MBC, the sugarcane bagasse has the same behavior as wheat straw regarding its enzyme activities. The critical function that biochar plays in modifying soil enzyme activity, which can also enhance nitrogen mineralization and utilization by activating N assimilation enzymes including glutamine synthetase, nitrate reductase, and glutamate synthase (Khan et al., 2022). Proper biochar application may provide better crop growing conditions, contributing to sustainable agriculture. One of the most important characters in biochar use is that the special technique used for its production makes it suitable for farm-scale conditions. Some of the investigators reported that biochar application may has a positive to neutral and even negative impact on crop growth. This is why its' crucial to understand how the biochar is acting in different soils and crops when it is planned to apply locally.

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## Selections on domestic grape pests

### *Szemelvények a hazai szőlőkártevőkről*

András Hágen<sup>1</sup>, Pál Sümegi<sup>2</sup>

<sup>1</sup>Déli ASZC Máté Berezcki Agricultural and Food Industry Technical School, Vocational Training School, 6500 Baja. Szent Antal u. 96.

<sup>2</sup>University of Szeged, Faculty of Natural Sciences and Informatics. Department of Geology and Paleontology. 6720 Szeged, Egyetem u. 2.

Correspondance: [hagena@freemail.hu](mailto:hagena@freemail.hu)

**Abstract:** Climate fluctuation has turned into climate change in the last 10-20 years, the results of which can be seen in extreme weather events. From a climatic point of view, our country is divided into three sub-zones, of which the first sub-zone is warm-temperate or Mediterranean, the second sub-zone is medium-temperate, while the third sub-zone is cold-temperate. So it is essential to monitor the climate changes of these subzones in the future. Since the transformation of the flora requires a relatively long time, while these pest insects world does not, therefore the change in the climate of our country is already noticeable, which is connected with the completion of globalized networks and the arrival of insects in a new environment. Such new damage recipients in grape culture are the green wandering bug (*Nezera viridula* LINNAEUS 1758), the american grape cicada (*Scaphoideus titanus* BALL 1932), the snake-mining vine moth (*Phyllocnistis vitegenella* CLEMENS 1859), the grape-mining bright moth (*Antispila oinophylla* VAN NIENKERKEN & WAGNER 2012) and the small glow moth (*Holocacista rivillei* STANTON 1855). Two of these are interesting because of global climate change, two are interesting because of globalized trade, and one is interesting partly because of trade and partly because of climate change.

**Keywords:** *climate change, climate zones, Mediterranean insects, invasive insects, biotic reasons, abiotic reasons*

**Összefoglalás:** Az éghajlatingadozás klímaváltozássá alakult az elmúlt 10-20 évben, amelyek eredménye a szélsőséges időjárási eseményeken is meglátszik. Hazánk éghajlati szempontból három alzónára tagolódik, ezek közül az első alzóna a meleg-mérséklet vagy mediterrán, a második alzóna a közepesen-mérséklet, míg a harmadik alzóna a hideg-mérsékelt. Természetesen ezek az alzónák meghatározzák, hogy bizonyos területeken milyen növényt természetünk gazdaságilag optimális befektetéssel, ezért elengedhetetlen, hogy nyomon kövessük ezeknek az alzónáknak az éghajlati változásait a jövőben. Mivel a növényvilág átalakulása viszonylag hosszú időt igényel, a rovarvilágé nem, ezért már észrevehető hazánk klímájának megváltozása, amelyhez kapcsolódik a globalizált hálózatok kiteljesedésével a rovarok új környezetbe kerülése. Ilyen új kártevők a szőlő kultúrában a zöld vándorpoloska (*Nezera viridula* LINNAEUS 1758), az amerikai szőlőkabóca (*Scaphoideus titanus* BALL 1932), kígyóaknás szőlómoly (*Phyllocnistis vitegenella* CLEMENS 1859), a szőlőaknázó fényesmoly (*Antispila oinophylla* VAN NIENKERKEN & WAGNER 2012), és a kis fénymoly (*Holocacista rivillei* STANTON 1855). Ezek közül kettő, amely a globális éghajlatváltozás miatt érdekes, kettő a globalizált kereskedelem miatt, egy pedig részben a kereskedelem,

részben a klímaváltozás miatt érdekes. A továbbiakban nem elképzelhetetlen további délebből, a mediterrán éghajlat irányából érkező rovarok megjelenése.

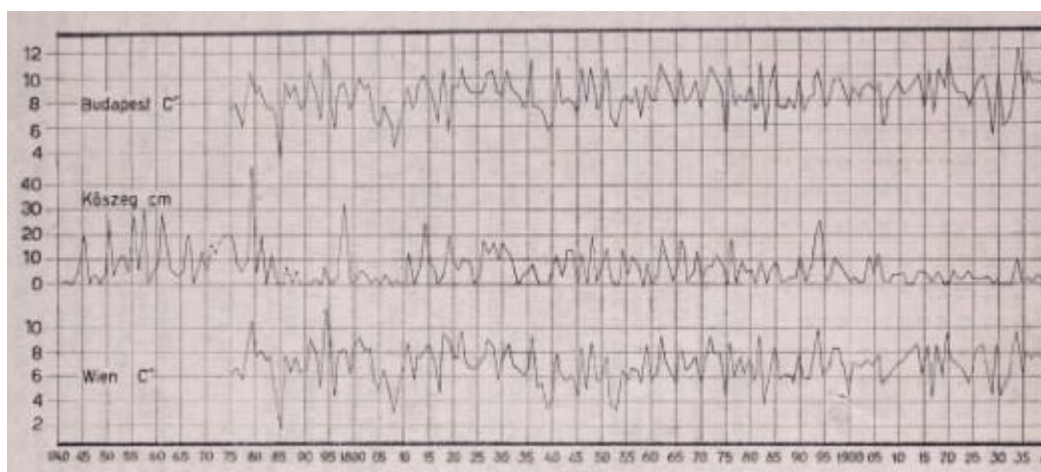
**Kulcsszavak:** klímaváltozás, klímazónák, mediterrán rovarok, inváziós rovarok, boiotikus ok, abiotikus ok.

## 1 Introduction

According to the fossilized seeds, the ancient varieties of the grape genus appeared in the warm-temperate climate zone in the Pliocene period. During the Ice Age, their production area shrank. In the Holocene after the ice age, different grape species appeared in the refugia, which began to spread within the northern flora kingdom (holarctic) due to the heat. The forest grape (*Vitis silvertis* GMELIN 1805) achieved its greatest success in the territory of today's Iran, Armenia and Azerbaijan 4-5,000 years ago. The wine-producing grape (*Vitis vinifera* LINNAEUS 1753) developed from this 2-3,000 years ago.

In the area of the Carpathian basin, viticulture appeared as early as the Roman period. After the fall of the Roman Empire and during the period of migration, the grape almost died out, and then flourished again in the Middle Ages. Grapes were mostly grown in areas suitable for it, in designated majorities, as well as in church areas. Harvests were held simultaneously. The date was determined by the council, each grape variety based on its clusters.

Berkes (1942) established that climate fluctuations can best be traced in the phenomena of plant physiology (phenology) of grapes. In the Kőszeg museum, you can find the "Book of Grape Harvest", which has been reporting on the phenological phase of the noble plant and the length of the grape shoots since 1740. According to Berkes' research, strong temperature fluctuations have occurred in the last 150-200 years. Based on the samples from Kőszeg, Budapest, Vienna and Berlin, the average annual temperature fell everywhere from 1790 to 1860, and has risen since then (Figure 1). The minimum temperature falls between 1850-60. So there is a strong correlation between shoot length and temperature. This shows that the shoot lengths in Kőszeg were the largest between 1740 and 1780, so the spring temperature was basically warmer.



**Figure 1** Length of vine shoots in Budapest, Kőszeg and Vienna from 1740 to 1940 (Berkes 1942)

Benedeffy (1972) compared the phenological data of grapes from three locations. The archives in Sopron contained data from 1576 until 1763 with few interruptions. In Kőszeg from 1649 to 1820 in the "Book of Grape Harvest". He also examined the samples from Kecskemét, which



were recorded from 1698 until 1830. He also examined the data from Szombathely, one part of which covers from 1668 to 1704, and the second part from 1797 to 1848. The ripening times of the grapes show a regular periodicity, the duration of which is approx. 36 years. From Benedeffy's investigations, a 55-year period and a slightly curving 210-220-year period can be detected in the air temperature conditions.

The last decades have proven that our climate is warming, but what does the future hold for us, to what extent will it warm, and how will the precipitation map of our country change, as well as what effect this will have on the basically little precipitation (depending on the location, one vine consumes 4-14 liters of water) for demanding viticulture. We are looking for an answer to this in the following.

## **2 Materials and Methods**

It is necessary to examine the climate system from two fundamental points of view. According to one, it is necessary to examine how the system works, while according to the other, it is necessary to examine how it affects the environment and the human activity carried out in it (Varga-Haszonits 2003).

If we want to find out about the future development of the climate, there are two possibilities (Easterling et al. 1992):

1. We assume that the climate does not change. According to this, the climatic elements will fluctuate between the many-year average.
2. We assume that it changes, then its change can be analyzed and detected by statistical tests. It will be similar to past analogies, and may be modeled with general circulation models.

The scenario of the evolution of the climate is the assumption based on analogy and the estimation of the global climate models can be proposed (Varga-Haszonits 2003).

In this study, we basically list the climatological scenarios, from which the conclusion can be drawn that our climate will warm and the amount of precipitation will become fluctuating and extreme.

We examine five grape pests, whether they are invasive species or have arrived in their "new" environment due to global climate change.

This study shows how global climate change (warming, erratic rainfall intensity) creates a new environment in Hungarian farmlands.

## **3 Changes in climate zones**

The fact of global climate change has become accepted nowadays. Climate change has and will have serious medical meteorological effects in the future (Bartholy et al. 2003, Bartholy et al. 2007). The changes affect not only the animal kingdom, but also the flora, albeit at a slower pace than the insects (Skendžić et al. 2021).

Hungary belongs to the temperate climate zone, where three sub-zones are distinguished:

The first is the warm-temperate or Mediterranean, the second is the mid-temperate and the third is the cold-temperate subzone. The variability within the belts is determined by the amount and distribution of precipitation. Borhidi (1961) was the first to show these climate zones on a map. Dobor et al. (2014) presented four periods in their analysis:

1. 1901-1950: The basic state;
2. 1970-2009: The onset of climate change;

3. 2011-2050: Acceleration of climate change;
4. 2051-2100: Peak climate change.

According to the analyses, those belonging to group 3 show an increase of around 2 °C. For group 4, the 2 °C limit is well exceeded (Table 1).

**Table 1** The evolution of our country's climate in the future (Borhidi 2019)

Annual mean temperature averages (°C)					
	1901-1950	1970-2009	2011-2050	2051-2100	Difference
<i>National</i>	9,8	10,5	11,4	13,1	3,3
<i>NE-Hun.</i>	9,2	9,9	10,7	12,7	3,5
<i>Great Basin</i>	11,2	11,9	12,9	14,9	3,7
<i>S-Hun.</i>	10,1	11,0	11,8	13,5	3,4

In addition to the temperature, a decrease in the amount of precipitation can also be forecast by an average of 58-60 mm per year (Borhidi 2019). This means a full month of precipitation loss per year, especially in the summer months. Borhidi (2019) drew an interesting conclusion between the climatic water balance, that is, the evaporative water loss and the climatic water income from precipitation. As a result, the climatic zone map of our country will change. So, due to the decrease in precipitation, the previously known montane or submontane beech in Kőszeg will be replaced by the end of the century by the hornbeam-oak currently populating South Transdanubia, that is vegetation that requires less precipitation.

Based on this, it can be concluded that the current grape culture and grape varieties are changing at the national level, including at the beginning of the Alps.

### 3.1 Pests in viticulture

We encounter many pests in grapes. Many species of insects can wreak havoc on crops. Listed below, we encounter such pests as the grape root bug (*Viteus vitifoliae* FITCH 1855), the vine beetle (*Otiorhynchus ligustici* LINNAEUS 1758), the leaf mite (*Calepitrimerus vitis* NALEPA 1905), the cotton bug (*Eriophyes vitis* HILGARDIA 1955), the spider mite, the plum borer (*Byctiscus betulae* LINNAEUS 1758) and many others.

In addition to the insects that have been found so far, there are other pests in our country.

1. green wandering bug (*Nezera viridula* LINNAEUS 1758);
2. american vine cicada (*Scaphoideus titanus* BALL 1932);
3. snake-mined vine moth (*Phyllocnistis vitegenella* CLEMENS 1859);
4. grape mining moth (*Antispila oinophylla* VAN NIENKERKEN & WAGNER 2012);
5. small bright moth (*Holocacista rivillei* STANTON 1855).

The first two were basically moved from their "birthplace" to a foreign environment due to globalization. In its "new" location, it is a true invasive species that has adapted to the local climate and lives without natural enemies.

The green wandering bug lives in large numbers in tropical and subtropical areas, and was first observed in Hungary in 2002 (Vétek et al. 2014, Kóbor 2017). The American cicada is native to North America and came to Europe in 1958 with American root cuttings imported due to the phylloxera blight (Szolárdi et al. 2014).

The snake-mined vine moth is an intermediate pest, because it basically originates from North America, but was first detected in Italy in 1994, and then spread from there to neighboring Slovenia (2004), the southern part of Switzerland (2009) and Hungary (2014). This species can also be called an invasive species and a Mediterranean species (Pobleccki 2018).

The grape-mining moth came to our country from Italy. Its larvae damage the leaves of the grapes, which reduces the assimilation surface, and thus the sugar and acid content of the grapes deteriorates (Pastorális 2012).

The other insect coming from Italy is the small bright moth. Its damage is similar to that of the grape-mining moth.

So, the presented species spread in our country due to global trade and climate change.

#### **4 Results**

The history of the pest species we have examined can be traced very well from scientific records and farmers' comments. Two types, the green wandering bug (*Nezara viridula* LINNAEUS 1758) and the American vine cicada (*Scaphoideus titanus* BALL 1932), are cosmopolitan species, so they can survive in other environments where the conditions are right. In other words, invasive species.

The snake-mined vine moth (*Phyllocnistis vitegenella* CLEMENS 1859) is considered an eccentric, as it lives in the Mediterranean regions of America, but it did not come directly to our country, it came from northern Italy. So it is both an invasive species and a pest from the Mediterranean area, but not in the literal sense.

The grape mining moth (*Antispila oinophylla* VAN NIENKERKEN & WAGNER 2012) and the small bright moth (*Holocacista rivillei* STANTON 1855) species came to our country from Mediterranean areas due to global climate change.

#### **5 Discussion**

The phenological phases of species belonging to the grape genus show a strong correlation with climate change. This can be traced in the research of Berkes and Benedeffy, detected in the growth of vines.

The current state is supported by climate maps (Borhidi 2019). In terms of climate change in the future, the rate of change can be divided into four time units. As for the future, we have to pay attention to the last 2 stages. Based on these, in the 3rd stage (2011-2050), the temperature increase below 2°C will be kept narrowly, but in the period after 2050 we will exceed 2°C.

And the amount of precipitation will change drastically (Rakonczai 2006). An entire month's amount of precipitation will be missing, and as a result, the vegetation zone of our country will probably also be rearranged. In terms of grapes, the grape varieties typical of a given area will no longer be relevant in the future, so other varieties that manage with little rainfall and are economically profitable will spread.

Basically, the migration of animal species is a natural process to find a suitable habitat for them. The reasons for migration can be biotic and abiotic. The biotic causes are a decrease in the amount of food, disease, overgrowth of the species (gradation), or possibly the appearance of a competing insect population. Abiotic causes include changes in climate, temperature or soil.

Of the five species we have presented, two are species that came from the Mediterranean area for biotic reasons (invasion species), and two for abiotic reasons.

Among the pest insects in the grape culture of our country, in addition to the usual species, invasive species and species arriving here from the Mediterranean areas will be observed (Venette - Hutchinson 2021, Reineke - Thiéry 2016). Of the five species we have presented, two are invasive species, and two are species introduced from the Mediterranean area. The snake-mined vine moth is partly an invasive species, because it was brought from North America to the northern part of Italy, so it entered our country from a relatively Mediterranean area.

If the scenario of Dobor et al. (2014) really comes true (and it probably will), the grape culture of our country will fundamentally change in terms of varieties and new pests.

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## Differences between ex-situ and in situ germination and seedling establishment in the case of the marsh gladiolus (*Gladiolus palustris*)

### *Különbségek ex-situ és in situ csírázásban és a csíranövények növekedésében a mocsári kardvirág (Gladiolus palustris) esetében*

Vivien Lábadi<sup>1,2\*</sup>, Bálint Pacsai<sup>1,2</sup> and Judit Bódis<sup>2</sup>

<sup>1</sup>Hungarian University of Agriculture and Life Sciences, Festetics György Doctoral School; labadi.vivien@uni-mate.hu, pacasai.balint@uni-mate.hu

<sup>2</sup>Hungarian University of Agriculture and Life Sciences, Institute for Wildlife Management and Nature Conservation, bodis.judit.zsuzsanna@uni-mate.hu

\*Correspondence: labadi.vivien@uni-mate.hu

**Abstract:** The marsh gladiolus (*Gladiolus palustris* Gaud.) is a species of community interest in the European Union, and it's strictly protected by law in Hungary. Survival of its populations is threatened by a number of factors, so thorough knowledge on the biology of the species is required for its conservation and long-time survival. To interpret the data collected in local populations, it is essential to examine the dynamics between age-stage categories and study germination rates of seeds in the populations. For this purpose, ex-situ and in-situ germination biology studies were set up to monitor the development of individuals starting from germination and establish a suitable age-stage categorization system for demographic purposes. During the autumn of 2023, seeds were sown in an open forest and in a meadow (in-situ), and seeds from these sites were sown in containers (ex-situ) at the same time. Germination rates were considerably higher in the ex-situ sowing than in the in-situ experiment for seeds of both origins. Moreover, there were significant differences between the average height (leaf length) of the seedlings. Under in-situ conditions average height was significantly larger in the open forest than in the meadows. There were significant differences between average height of seedlings in the in-situ and ex-situ experiments only during the first measurement, which differences became less pronounced later on.

**Keywords:** endangered plants; recruitment; population biology; age-state; *Gladiolus palustris*

**Összefoglalás:** A mocsári kardvirág (*Gladiolus palustris* Gaud.) az Európai Unióban veszélyeztetett, a hazai flóra fokozottan védett faja. Az állományok fennmaradását több tényező is veszélyezteti, így a faj biológiájának minél mélyebb ismerete rendkívül fontos a faj védelme és fennmaradása szempontjából. A hazai állományokban gyűjtött adatok értelmezéséhez elengedhetetlen a korállapot-kategóriák közötti átmenetek vizsgálata és a faj állományában a magok csírázóképeségének megállapítása. Ennek érdekében ex-situ és in-situ csírázásbiológiai vizsgálatokat állítottunk be, hogy az egyedek fejlődését a csírázástól kezdve nyomon követhessük, illetve felállítsunk egy alkalmas rendszert az egyedeknek korállapot-kategóriákba való besorolásához. 2023 őszén két termőhelyen, egy nyílt erdőben és egy kiszáradó lápréten végeztünk in situ vetést, valamint az ezekről a termőhelyekről származó magokat ládákból is (ex situ) elvetettük. A csírázási arány az ex-situ kísérletben jelentősen magasabb volt, mint az

in-situ vetésben mindkét termőhelyről származó magok esetében, továbbá a csíranövények magasságában (levélhosszában) is szignifikáns különbségeket tapasztaltunk. In situ körülmények között a magoncok átlagos magassága szignifikánsan nagyobb volt az erdőben, mint a réten. Az in situ és ex situ kísérletekben a magoncok átlagos magassága között csak fejlődésük kezdeti szakaszában figyeltünk meg szignifikáns különbséget, mely a későbbiekben mérséklődött.

**Kulcsszavak:** veszélyeztetett növényfaj; szaporodás; populációbiológia; korállapot-kategória; *Gladiolus palustris*

## 1 Introduction

Marsh gladiolus or sword-lily (*Gladiolus palustris* Gaud.) is a 20–100 cm tall, perennial bulbous species, with 4–5 sword-shaped leaves. It has 3–6 bisexual rosy violet or magenta flowers that bloom in June–July in Hungary. *G. palustris* is native in Europe, its distribution ranges from eastern France to the southern Balkans (Vidéki & Máté, 2006). It has a declining population trend all across Europe and considered to be threatened in many countries (Bilz et al. 2011), and as a result, *G. palustris* became legally protected in the European Union (listed in Annexes II and IV of the Habitat Directive 92/43/EEC). It has been strictly protected by law in Hungary since 1982 (1/1982. (III. 15.) Annex 1 to the OKTH Regulation), conservation value of the species is HUF 250,000. According to Social Behaviour Types described by Borhidi (1995, it is categorized as a rare specialist.

While original habitats of this species may have been clearings and margins of open forests with varying water regime, today its largest populations live in meadows of anthropogenic origin. *G. palustris* prefers habitats alternately wet and dry, therefore in Hungary it occurs mostly on transitions between wet meadows and steppes in lowlands and hilly regions. In the Transdanubian region it has only two populations; one situated in an open birch forest and one in a *Molinia* meadow.

In the case of perennial herbaceous plants, such as *G. palustris*, it is difficult to determine the exact age of individuals, therefore age-state categorization is a more suitable approach for studying demographic characteristics of wild populations than the usage of actual age in years (Rabotnov, 1969; Harper, 1977; Silvertown et al., 1993; Vakhrameeva et al. 2008).

The potential 'age groups' or 'age-states' categories what were described by Rabotnov (1969) have been modified later in several ways by different authors, one of the better known being Gatsuk et al. (1980), who grouped age-states into ontogenetic periods.

In the case of *G. palustris*, the suitable age-state categorization has not been established yet, which makes it very difficult to interpret field data collected in order to support the protection of the species.

We started in-situ germination studies with the species in 2019 when we had sown seeds into plots, which we managed in different ways. When we set up our demography studies in 2023, has become necessary for the analysis and interpretation of our field data to define the characteristics of every age-state category applicable for *G. palustris*. In order to understand the life cycle of the species in detail, we carried out more detailed in-situ and ex-situ germination biology studies as well. Since in-situ germination experiments are carried out in the original habitats, these setups are suitable for population biology studies and age-state calibrations, but are difficult to manage and have uncertain outcomes, therefore we also started ex-situ germination experiments at the same time. In ex-situ experiments it is much easier to manipulate conditions and as we can almost freely choose location for the setups, it could also enable constant monitoring as well.



In-situ and ex-situ sowing was the first part of our population biology studies. Our goal was to study whether there were differences between germination of seeds and seedlings establishment originated from different environments (from an open forest and from a hay meadow). Seeds originated from each site were sown in-situ and in containers (ex-situ) at the same time. Our short-term goal was to examine germination characteristics and our long-term goal was to follow the life history of the individuals developing from the seed sown.

We wanted to find answers to the following questions:

- Question 1: Are there differences in germination percentages between ex-situ and in-situ conditions?
- Question 2: Are there differences in germination percentages between seeds sown in forest and meadow habitats?
- Question 3: Are there differences in seedling heights between ex-situ and in-situ conditions?
- Question 4: Are there differences in seedling heights based on in-situ habitat conditions?
- Question 5.: Did seedlings that germinated earlier (so they had more time to grow) produce larger leaves?

## 2 Materials and Methods

As *G. palustris* is a strictly protected species in Hungary, therefore studying biology of the species required an official permission. This permission has been granted (File No: PE-KTFO/2595-13/2023.) prior the setup of our experiments.

### 2.1 Study areas and experimental garden of endangered species

Both in-situ sowing sites are located in the Balaton-felvidéki National Park. One woodland population was selected in Nyirádi Sár-álló, at the edge of an open birch forest with *Molinia caerulea* undergrowth, near the northern edge of a *Molinia* meadow, and one meadow population in the Péntzes-rét meadow near Raposka, where seeds were collected from a *Molinia* meadow habitat (*Succiso-Molinetum caeruleae*) (Figure 1).



**Figure 1** In-situ sowing sites at Nyirád (left), and at Raposka (right)

In the experimental garden of Balaton-felvidéki National Park in Pécsely, the species has been grown in ex-situ conditions for several years (seeds originally sourced from Nyirád). The propagules produced here obliged by law to be returned to the original habitat. Therefore, we did not collect seeds in Nyirád (where the population is relatively small anyways), instead we

used seeds and one-year old corms came from the experimental garden and we had to adapt the experimental setup to the amounts of propagules available.

## 2.2 In-situ sowing

### Preliminary study

*G. palustris* corms were planted and seeds were sown at two sites in a *Molinia* meadow (not in the forest) at Nyirád in 2019. The distance between the original habitat and the two sites were 460 (Site 1) and 900 m (Site 2) respectively. The sites were receiving four different types of grassland managements as a part of an experiment: control (without treatment), mowed (cutting and removing the hay once in a year in July), topped (similarly as in ‘mowing’, but without collecting the hay) and burned (burned every 3 years in winter and topped in the other years). We set up sowing plots in every treatment type at both sites, so we had 2×4 sowing plots. The propagules were sown in 1 m × 1 m plots, each plot consisted of 5 rows. The distance between rows was 25 cm, the distance between seeds in a row was 2 cm.

We planted 22 corms in a mown area and 25 corms in a control area, sown 2×100 and 2×125 seeds in Site 1 and 4×125 seeds in Site 2. The 2 cm distance between seeds proved to be not enough; it was difficult to identify individuals over the years.

In all years after sowing (except 2022) we monitored the presence of individuals and measured their heights.

### Sowing experiment

The sowing experiment was set up in the summer of 2023. One in-situ sowing area was designated on clearings in the open birch forest of Nyirádi Sár-álló. It was important that the species should be present in the surrounding habitat but absent in the close vicinity of the sowing plots.

Seeds collected from the Pécsely experimental garden were sown at four sites, 110 seeds overall in each plot (05.10.2023). The beginnings of the rows were marked with individually numbered nails and the end with unlabelled nails to simplify later retrieval. A distance of 20 cm was left between rows and 5 cm between seeds.

The other in-situ sowing area was set up near Raposka in a *Molinia* meadow. Seeds used for this setup were collected from the same meadow on the 17<sup>th</sup> of August in 2023. Seeds were sown not far from a *G. palustris* population, but the species was not present in the close vicinity of the plots, similarly as in Nyirád. 110 seeds were sown in each plot, in the same arrangement as in Nyirád, but with 6 replicates (16.10.2023).

## 2.3 Ex-situ sowing

Seeds were also sown in ex-situ conditions, in containers (24–25.10.2023), in total 150 seeds from the *Molinia* meadow near Raposka and 150 seeds from the Pécsely experimental garden. The containers were filled with soil from the original habitats (Raposka and Nyirád) and seeds were sown at a maximum depth of 0.5 cm and at a maximum distance of 3 cm between them. Throughout the study, the containers were kept in the open air, without controlling temperature and precipitation, but emerging weeds in the containers were removed.

## 2.4 Germination and seedling establishment

During the spring of 2024, we started following the germination and seedling establishment (Figure 2). In-situ sowings were examined twice (17.04.2024 and 23.05.2024), in both cases we checked the presence or absence of the seedlings and measured their height. Germination in

the containers was checked on a weekly basis and the height of the seedlings was also measured on two occasions (13–14.04.2024 and 18.05.2024).



*Figure 2* Individuals germinated in spring 2024. Left: individual from in-situ sowing, right: from ex-situ sowing

## 2.4 Data analysis

Procuring the experimental data was done with Microsoft Office Excel 2016 software, and analyses were done with R (version 4.3.1.). Normality of the datasets tested with Shapiro-tests, homogeneity of replicates tested with ANOVA, comparing the seedlings height were done with pairwise T-tests and connection between time of germination and final seedling length was examined with Pearson-correlation.

## 3 Results and Discussion

### 3.1 Preliminary study

In the preliminary study (Figure 3) corms were planted in 2 replicates, but data were collected successfully only from the mowed area, as the corms planted in the control area were destroyed by moles in 2020 and no plants have been found there since.





**Figure 3** Established plants arised from the preliminary study at the Nyirádi Sár-álló in 2023 (left) and in 2024 (right).

During the 4 years of the preliminary study, we recorded 50% of the planted corms emerging at least once, while the rate of recoveries from sown seeds of the same condition was between 11 and 14,4%. Even in terms of all managed plots varied between 10.4 and 21.6% (Table 1). Therefore, corms had a noticeably higher survival rate than seedlings over the years.

Seedlings were found in almost all plots in the first year, most of which had disappeared later in the majority of the plots, especially at Site 2 (Table 1). As numerous seeds could germinate in the first year but could not survive later, this may indicate that the species is highly habitat dependent: plants established better in Site 1 which is closer to the original habitat. Since from the control plot at Site 1 had all seedlings disappeared, we think that the litter (which accumulated in large amounts only in the control plots) also inhibited their survival. In actively treated areas we found developing plants 4 years after sowing, suggesting that seedlings prefer mown, topped and burnt areas instead of the undisturbed vegetation of control areas.

**Table 1 Percentage of individuals recovered during the preliminary study. 100% means total number of seeds/corms sown at the site.**

	Site	No. of seeds/corms sowed/planted	Germination rate (%)				
			2020	2021	2023	2024	total
Mowing Corms	1	22	31.82	0.00	22.73	18.18	50.00
Control Corms	1	25	0.00	0.00	0.00	0.00	0.00
Mowing Seeds	1	100	6.00	2.00	1.00	4.00	11.00
	2	125	14.40	0.00	0.00	0.00	14.40
Control Seeds	1	100	2.00	0.00	0.00	0.00	2.00
	2	125	12.80	6.40	0.00	1.60	17.60
Topped Seeds	1	125	14.40	5.60	2.40	4.80	21.60
	2	125	10.40	0.00	0.00	0.00	10.40
Burned Seeds	1	125	11.20	3.20	6.40	1.60	14.40
	2	125	13.60	0.00	0.80	0.00	13.60

### 3.2 Germination

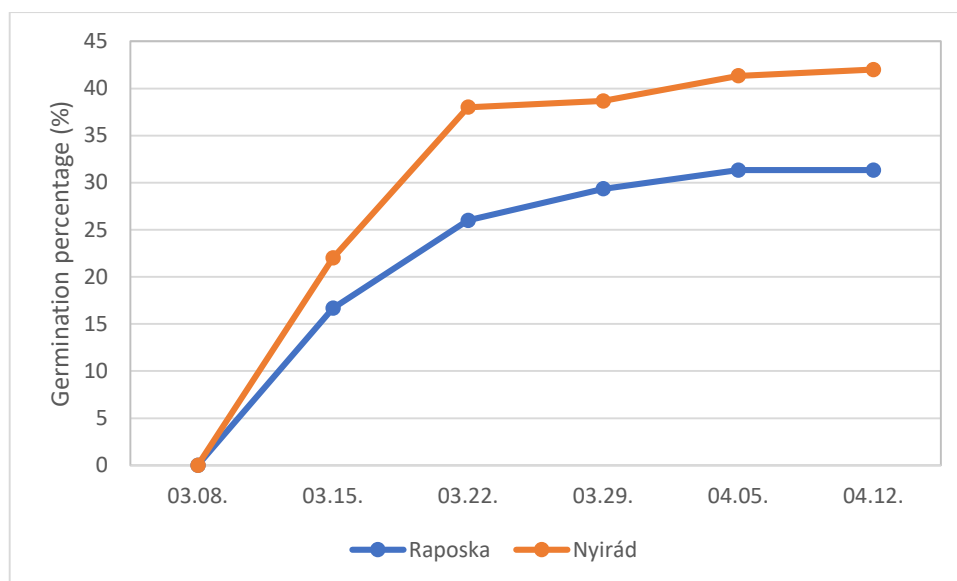
At the in-situ site at Nyirád no seedlings were found during the first survey (17<sup>th</sup> April). During the second measurement (23<sup>rd</sup> May) 7 seedlings were found, resulting in a total of 1.59% germination. At Raposka we surveyed 102 seedlings during the first measurement. On the second occasion 22 new seedlings appeared, but 31 previously recorded individuals were not found, thus we surveyed 93 seedlings during the second survey altogether. A total of 124 seedlings were found at Raposka, which results in a 18.79% overall germination (Table 2), however, it cannot be ruled out that despite a careful search for seedlings, some still might be overlooked at both sites due to close resemblance between *Gladiolus* seedlings and young blades of several grass species.

**Table 2 Number of individuals germinated in the first and second measurement and germination percentage.**

area	seeds sown	no. of seedlings					germination percentage (%)
		1 <sup>st</sup> survey	2 <sup>nd</sup> survey			total	
		total	new	missing	total		
Raposka	6x110	102	22	31	93	124	18.79
Nyirád	4x110	0	7	-	7	7	1.59

In the ex-situ experiment 31.33% of seeds from Raposka and 42.00% of seeds from Nyirád germinated in the first year. In both containers the first seedlings emerged during the week before 15<sup>th</sup> March and this initial period was also the peak in terms of germination rate during the experiment (Figure 4). After this initial peak the number of seedlings germinated per week decreased gradually until 12<sup>th</sup> April.

Our results show that in the case of *G. palustris* germination percentages are tend to be higher in meadows than in forest habitats, and germination percentages are higher in ex-situ than in in-situ conditions.



**Figure 4** Germination percentages of *G. palustris* seeds of different origin over the spring of 2024 in the ex-situ experiment.

### 3.3 Seedling height (leaf length)

At Nyirád, seedlings emerged only in two from four plots. There was no significant difference in average seedling height between these two replicates. At Raposka there were no significant differences in the average height of the 6 replicates during the first survey. During the second survey, there was significant difference ( $p=0.006$ ) only between the plot with the lowest (3<sup>rd</sup> rep) and the highest mean (4<sup>th</sup> rep), which we found still acceptable as one cohort with slight in-between differences and we used all 6 replicates combined for the comparisons with the data collected at Nyirád (Table 3).

**Table 3** Number of seedlings, average height and the corresponding standard deviation, minimum and maximum values during the two in-sit surveys.

Study area	Code	count1	mean1 (mm)	sd1	min1 (mm)	max1 (mm)	count2	mean2 (mm)	sd2	min2 (mm)	max2 (mm)
Rap	1	23	33.70	10.80	9	58	21	59.70	14.50	29	93
Rap	2	8	31.20	5.65	24	41	6	57	19.20	31	81
Rap	3	17	28.50	10.30	9	44	19	52.40	12.70	25	69
Rap	4	18	30.80	6.90	18	42	18	68.10	10.30	49	88
Rap	5	20	27.70	6.00	18	40	17	62.60	13.90	35	78
Rap	6	16	32.60	9.01	18	48	12	62.30	11.40	40	78
Nyir	3	0	-	-	-	-	3	67.30	5.03	62	72
Nyir	4	0	-	-	-	-	4	76.50	13.70	58	91

In the in-situ experiment at Nyirád no seedlings were found for the first time (17<sup>th</sup> of April, 5 months after sowing). At the same time at Raposka average height of seedlings was  $30.8 \pm 8.7$

mm. During the second survey (23<sup>rd</sup> of May, 6 months after sowing) average height of seedlings at Raposka was 60.5±13.9 mm and 72.6±11.2 mm at Nyirád.

During the first measurement (14<sup>th</sup> of April) of ex situ containers, when the seedlings were about 3 weeks old, their average height was 48.1±22.2 mm from Nyirád and 45.5±21.0 mm from Raposka. During the second measurement (18<sup>th</sup> of May), when the seedlings were about 8 weeks old, the average height of seedlings from Nyirád was 70.8±20.6 mm and 64.1±21.8 mm from Raposka (Figure 5).

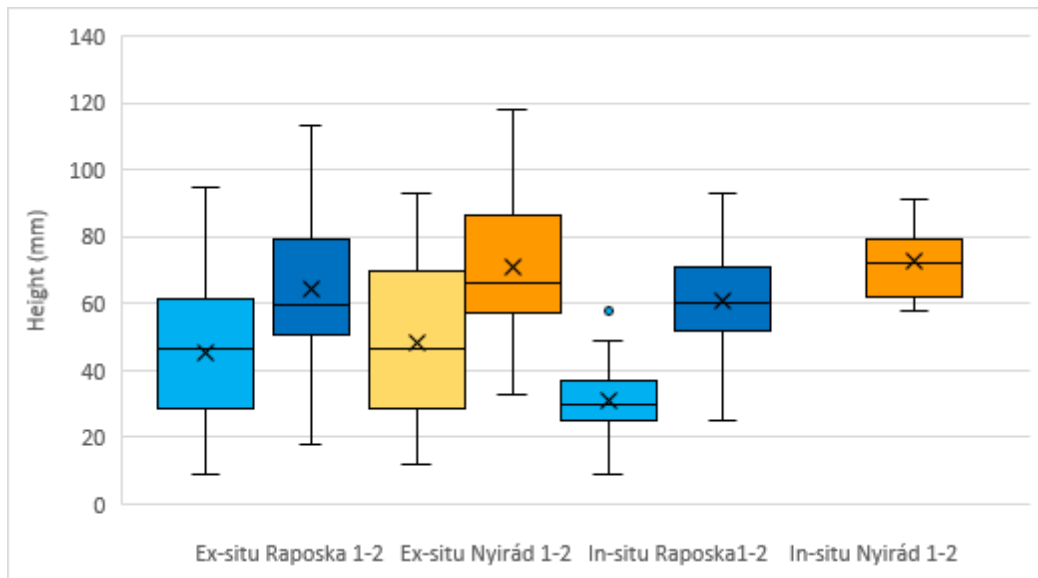


Figure 5 Average height of seedlings in both ex-situ and in-situ sowings at the time of the two measurements.

In the ex-situ experiments there was no significant difference between average height of seedlings from Nyirád and Raposka in either during the first and second height measurement. In the in-situ experiments average height of seedlings at Nyirád was significantly higher than seedlings at Raposka (Table 4). These results show that the average height of seedlings in the open forest is higher than in meadow populations.

The average height of ex-situ seedlings originated from Raposka was significantly higher than the in-situ seedlings in Raposka during the first measurement, but we found no such difference on the second measuring. Also, no statistically justified difference was found between average heights of in-situ and ex-situ seedlings of Nyirád (Table 4).

Table 4 Significance levels of differences between sites and in-situ/ex-situ experiments at the time of the two surveys. \* means significant difference.

		Time of measurement	p-value
Between sites / origin of seeds	ex situ	1	0.586
		2	0.189
	in situ	1	-
		2	0.029*
Between in situ and ex situ	Nyirád	1	n/a
		2	0.746
	Raposka	1	<0.001*
		2	0.379

We also investigated whether there is a correlation between the number of days elapsed between germination and the second measurement and the height of individuals measured for the second time. We found almost no correlation ( $R=0.007$ ,  $p=0.95$ ) between these two factors, thus, seedlings that had germinated earlier and had more time for development did not produce larger leaves.

#### 4 Conclusions

To interpret the data collected in local populations, it is essential to examine the dynamics between age-stage categories and study germination rates of seeds in the populations. The seedling ‘age-state’ category is a critical phase as several factors alone can cause high mortality. The examination of seedlings is complicated by the fact that seedlings are often present aboveground for a short time and are difficult to detect, especially in a dense vegetation. To monitor development of individuals starting from germination, ex-situ and in-situ germination studies were set up. During the autumn of 2023 seeds were sown in an open forest and in a meadow (in-situ), and seeds collected from these sites were sown in containers (ex-situ) at the same time.

Germination rates were considerably higher in the ex-situ sowing than in the in-situ experiment for seeds of both origins. Although the germination rate was low in open forest habitats, seeds originated from the same population germinated well in ex-situ conditions. There were significant differences between the average height (leaf length) of the seedlings. Under in-situ conditions average height was significantly larger in the open forest than in the meadows. There were significant differences between average heights of seedlings in the in-situ and ex-situ experiments only during the first measurement, which difference disappeared with the subsequent growth of the plants.

Successful germination and survival of recruitment are generally very important factors in long-time survival of a species. Nevertheless, we have very few information about the germination biology of *Gladiolus* species.

Several germination experiments were done previously with the genus *Gladiolus*, but these experiments mostly carried out in laboratory conditions.

Ramzan (2010) examined the effect of priming with potassium nitrate ( $KNO_3$ ) on seed germination percentage and germination time on seeds of *G. alatus*. He achieved the highest germination success was with distilled water.

Amișculesei (2022) studied the effect of length of stratification and different temperature regimes on germination percentages of three *Gladiolus* species (*G. imbricatus*, *G. × byzantinus* and *G. tristis*) in a germination chamber. In the case of *G. × byzantinus*, stratification had a much stronger influence on germination than temperature conditions. On the contrary, temperature had a major influence on germination time and percentage of *G. tristis*. In the case of *G. imbricatus*, the duration of stratification has a marked impact on seed germination.

Fernández (2005) investigated the effect of temperature and light conditions on germination of *G. illyricus*, *G. italicus* and *G. tristis*. In the case of *G. illyricus* and *G. tristis* seeds, the highest germination percentages were obtained at 13–15°C. The germination percentage of *G. italicus* was almost zero in all treatments, probably as a result of seed dormancy.

Dutt et al. (2000) studied the effect of soaking in gibberellic acid ( $GA_3$ ) solution on the germination of 10 different *Gladiolus* hybrids. All concentrations of  $GA_3$  significantly increased germination percentages and reduced the number of days required for germination compared to water.

Our knowledge on the germination of *G. palustris* is rather incomplete. Brunzel (2010) found that the germination percentages of *G. palustris* in pot cultures kept outdoors were lower than



in the laboratory (which was over 90%). His findings confirm our results as he demonstrated in the outdoor sowing experiments that significantly higher number of seedlings were recorded in plots where the vegetation and litter were removed prior. This indicates the potential importance of small-scale vegetation-free areas in the germination success of *G. palustris*.

After a one-year study we have a better understanding of the germination and seedling establishment of *G. palustris*, however, there are still many questions that we will only be able to answer during the following years. For example, there was a high disappearance among the in-situ seedlings, for which we do not yet know whether they went into dormant phase early or perished.

Our long-term goal for the next years is to establish a suitable age-stage categorization system for demographic purposes. We hope to have answers to our additional questions in the following years to get closer to providing detailed information on the conservation methods of a species of Community importance.

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## Effect of precipitation on wheat (*Triticum aestivum*), maize (*Zea mays*), and sunflower (*Helianthus annuus*) yields in the district of Szentes

### *A csapadék hatása a búza (Triticum aestivum), a kukorica (Zea mays) és a napraforgó (Helianthus annuus) termésére a Szentesi járásban*

Dorottya Szám<sup>1,2\*</sup>, Gábor Keve<sup>1</sup>, Dániel Koch<sup>1,2</sup> and Zsolt Hetesi<sup>3,4</sup>

<sup>1</sup>Faculty of Water Sciences, Department of Regional Water Management

Ludovika University of Public Service, Baja, Hungary

<sup>2</sup>National Laboratory for Water Science and Water Security, Faculty of Water Sciences,

Ludovika University of Public Service, Baja, Hungary

<sup>3</sup>Faculty of Water Sciences, Department of Water and Environmental Security

Ludovika University of Public Service, Baja, Hungary

<sup>4</sup>Faculty of Natural Sciences, Institute of Mathematics and Informatics

University of Pécs, Pécs, Hungary

\*Correspondence: [szam.dorottya@uni-nke.hu](mailto:szam.dorottya@uni-nke.hu)

**Abstract:** Hungary's three most important arable crops are wheat, maize, and sunflower. These crops have the largest area under cultivation. In our study, we compared the average yields of a 200-ha farm on the outskirts of Szentes city with variations in rainfall conditions. We have shown that the amount of rainfall during the growing season affected the yield averages of wheat and maize and their prices on the world market. Yields of drought-sensitive maize, which prefers humid conditions, were most dependent on rainfall. However, rainfall also proved to be a significant factor in wheat yield averages. Sunflower yield averages did not correlate with rainfall, which may be because water demand is highly dependent on the phenological stage. Therefore, a good rainfall distribution over time is also important for this crop. Changes in rainfall patterns due to climate change do not favor any crop. This is because climate change is reflected in a linear trend in annual rainfall amounts and greater rainfall variability over time and space.

**Keywords:** *Triticum aestivum*, *Zea mays*, *Helianthus annuus*, rainfall extremities, drought, Great Hungarian Plain

**Összefoglalás:** Magyarország három legjelentősebb szántóföldi kultúrája a búza, a kukorica és a napraforgó. Vetésterülete ezen kultúráknak a legnagyobb. Tanulmányunkban egy Szentes település határában fekvő kétszáz hektáros gazdaság átlagos terméshozamait vetettük össze a település csapadékviszonyainak változásával. Kimutattuk, hogy a vegetációs perióduson belüli csapadék mennyisége hatással volt a búza és a kukorica termésátlagaira és ezen keresztül a világpiacon árai alakulására is. Leginkább a humid körülményeket kedvelő, aszályjelző kukorica terméshozama függött a csapadékösszegetől. Jelentős tényezőnek bizonyult azonban a búza termésátlagában is a csapadékösszeg. A napraforgó termésátlagai nem mutattak

összefüggést a csapadék mennyiségével, melynek oka abban kereshető, hogy a vízigény erősen függ a fenológiai fázistól. Emiatt ezen kultúra esetében a csapadék megfelelő időbeli eloszlása is lényeges. Az éghajlatváltozás okozta csapadékviszonyok megváltozása egyik növénynek sem kedvez. Azért mert az éghajlatváltozás nem csak az éves csapadékösszeg lineáris trendjében mutatkozik meg, hanem a csapadék időbeli és térbeli nagyobb változékonyságában is.

**Kulcsszavak:** *Triticum aestivum*, *Zea mays*, *Helianthus annuus*, extrém csapadék, aszály, Magyar Alföld

## 1 Introduction

Of particular relevance among the impacts of climate change on agriculture is the increase in the frequency of extreme precipitation events and droughts (Stott, 2016). Such changes in precipitation patterns also affect crop yields in the Carpathian Basin (Jánosi et al., 2023; Hetesi et al., 2023). This is particularly true in cultivated areas where natural precipitation is the only source of water taken up by plants (Varga-Haszonits and Varga, 2005). Sufficient and timely natural precipitation is necessary for plant growth and development and achieving optimal crop yields. Depending on their phenological stage, knowledge of the water requirements of plants is also the key to the time-differentiated planning of artificial water supply.

The critical period for common wheat (*Triticum aestivum* L.) is the development of its generative organs and achieving yield potential (Nyiri, 1993). If wheat does not receive sufficient water, it will limit its further development, reduce the amount of genetic yield available, and reduce the quality of the crop (Alaei et al., 2010; Xuemei et al., 2010; Nouri et al., 2011; Ragheid et al., 2011).

For maize, the early growing season is critical. Water loss due to evaporation in rows that have not yet closed is high (Lacolla et al., 2023). Early growth, in addition to full reproduction, requires significant amounts of water. Without irrigation, this period must coincide with a natural rainfall peak. In Hungary, irrigation of maize is essential to achieve optimal yields, as the crop's water requirements are almost always more significant than the precipitation of the growing season (Tamás et al., 2022).

It is also true for maize and wheat that insufficient rainfall leads to drought stress. However, excessive precipitation and the high humidity that often accompanies it are not beneficial either, as they promote the growth and reproduction of many hydrophilic plant pathogenic fungi (Pál-Fám and Rudolf, 2014) and weed infestation (Varga, 2002; Márton et al., 2013).

In the case of sunflowers, the most important role in achieving optimal yields is played by balanced rainfall conditions. Sunflowers require the most water until plate formation. However, from plate formation to stalk maturation, high rainfall is particularly harmful because it encourages (mainly fungal) infections.

Our study aimed to investigate the precipitation conditions in the Szentes district and how the annual and growing season precipitation amounts affect the yields of wheat, maize, and sunflowers on a farm. In the broader region, the largest area (209 706 ha) is under wheat. This is followed by maize (221 541 ha) and sunflower, which has a significant area (KSH, 2020; KSH, 2022). This order is also valid for all regions of Hungary, which is why these three crops were chosen.

## 2 Materials and Methods

Our study was conducted in the Szentes District in the Southern Great Plain region (*Figure 1*). Szentes is the third most populous settlement in the Csongrád-Csanád county, on the River

Tisza's left bank. The Lower Tisza Water Management Directorate (ATIVIZIG, 2024) measures daily rainfall at the *Felsőveker* hydrometeorological station in the municipality. As is standard, the rainfall totals are automatically read at exactly 07:00 every day with an accuracy of tenths of a millimeter.

We investigated whether annual wheat, maize, and sunflower yields from 2006 to 2019 and rainfall totals show a linear relationship. Rainfall totals were determined by summing daily rainfall data separately for the growing season and separately for the whole calendar year. Yields were obtained from a large farm based in Szentes city, which did not use artificial irrigation water supplementation during the study period. Therefore, natural precipitation was the primary source of water for the crops.

Finally, annual yields were compared with average market selling prices for wheat and maize. We examined the extent to which yields show a negative correlation with sales prices and whether successive years of low yields could result in a rolling effect on prices.

### 3 Results and Discussion

Figure 1 shows how sensitive the yield of different crops is to rainfall. A medium-strong correlation is observed for maize, considered a drought indicator crop, and a medium-strong correlation for wheat. As a control, the yields of the sunflower fields occupying the third largest area of the farm were also examined. Our original hypothesis was supported by the fact that sunflower yields did not show any correlation with rainfall.

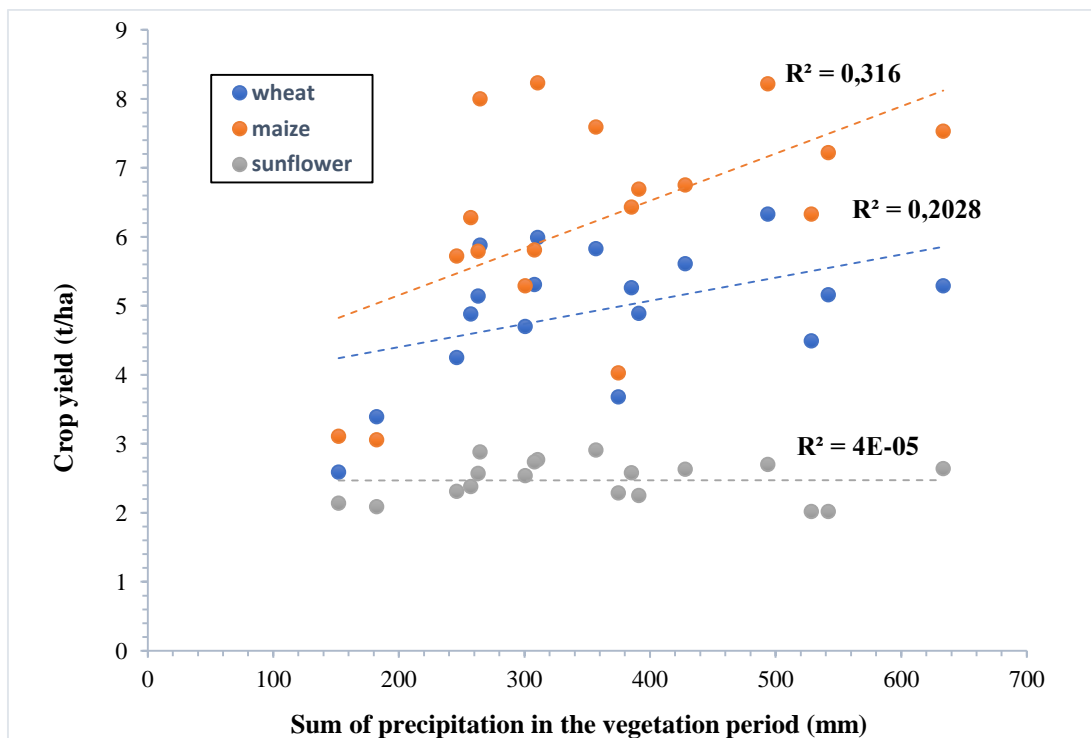


Figure 1 Yields (t/ha) of maize, wheat, and sunflower on a farm near Szentes and the sum of precipitation in the vegetation period data in Szentes between 1978 and 2020 (Source of data: ATIVIZIG, 2024; Szentes farm, 2023).

The lowest yields shown in *Figure 2* belong to 2003 and 2007, when the growing season rainfall did not even reach 200 mm. The year with the highest rainfall was 2014, when 633.6 mm of rainfall was recorded during the growing season. Even so, this is not the year with the highest average yields, which indicates the impact of the high rainfall not being positive (+78% compared to the growing season average). The highest yield averages typically occurred in years close to the multi-year rainfall average (2016, 2018, 2020).



**Figure 2** Trends in wheat and maize crop yields and average buying-in prices 2003–2020.  
(Source of data: KSH, 2024; Szentes farm, 2023)

A similar positive correlation was found between maize yield ( $=0.21$ ) and wheat yield and annual precipitation between 2006 and 2019 in a study in Poland. Still, Poland's climate is rainier than Hungary's (Wójcik-Gront and Gozdowski, 2023).

Several factors can explain the lack of a strong correlation, the most important of which is plants' phenological, phase-dependent water demand, which varies during the growing season. The water requirements of plants are more or less constant and are a well-known varietal characteristic. Like many abiotic environmental factors, rainfall is optimal for crops within a range. Too much rainfall can lead to anaerobic soil conditions and soil erosion, as the soil has a finite capacity to absorb water. It can also increase the growth and spread of weeds, pests, and pathogens that require a wet and humid environment. Among pathogens, fungal diseases such as rust (*Puccinia sorghi*, *Puccinia striiformis*, *Puccinia triticina*) and fusarium (*Fusarium* sp.) are particularly susceptible to humid conditions. Aphids (Aphidoidea) and leaf beetles (*Oulema* sp.) are also important among pests.

It is also worth comparing wheat and maize crop yields with buying-in prices (*Figure 2*). It can be seen that the two driest years (2003 and 2012) had the lowest yields, which drove up buying-in prices. In the years with better yields, prices fell.

An even more accurate relationship between crop yields and buying-in prices would be obtained by inflation-adjusted prices. However, the drought year 2012 is still striking, with only 182.3 mm of rainfall in the vegetation period and prices at a record high (wheat: 60 425 HUF/t, maize: 56 697 HUF/t). The previous year, also dry, was also low, with 246.1 mm of rainfall. Thus, the high buying-in price in 2012 probably reflects the rolling effect of the drought; just as the fall in prices was delayed, the rise in yields appeared with a lag. Limited storage time also plays a role in this.

## 4 Conclusions

The dependence of wheat and maize yields on the amount of precipitation is shown using the example of the average yields of a large farm from 2003 to 2020. The drought-sensitive nature of maize, which prefers humid conditions, was shown by the dependence of the yield average on higher precipitation. Wheat yield averages were found to be less dependent on rainfall than maize, which is consistent with the lower water requirement of the crop. This result confirms the role of intra- and inter-annual rainfall forecasting in crop protection.

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