

Thesis of University Doctoral (PhD) dissertation

**Biotic reactions and spread of woolly cupgrass (*Eriochloa villosa*
(Thunb.) Kunth) in the Tiszántúl region**

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1. Research history

Among the factors most likely to threaten biodiversity, the **spread of non-native plant species, i.e. invasive plants**, should be noted. These plants can cause significant nature conservation, forestry, agricultural, human health damage and economic damage (Csiszár, 2012). In domestic conditions, due to the economic change, land-use also changed, which favoured the invasive plants gaining ground. This also contributed to that researchers started dealing with these invasive plants in an increasingly wide range (Mihály - Botta-Dukát, 2004).

One of these invasive species is the Woolly cupgrass (*Eriochloa villosa* (Thunb.) Kunth), which belongs to the *family Poaceae*, as also genus *Eriochloa*. This plant is of East Asian origin, which has already been found in several parts of the world, and which causes significant yield loss in agricultural crops (mainly in row crops) (Virók *et al.*, 2020).

Under Hungarian conditions, following its first detection in 2008, has somewhat fallen out of the researchers' view (Partosfalvi *et al.*, 2008). Therefore no detailed research was carried out in Hungary, despite it is a spreading, invasive plant. Near Debrecen-Szepes, a herbicide susceptibility test was accomplished on Woolly cupgrass, and its results were published (Somogyi *et al.*, 2011). Foreign studies, besides herbicide tests, also investigated physiological parameters of the plant (germination, developmental, reproduction biology, morphological parameters, etc.).

Because of the spread of the Woolly cupgrass in Hungary, propagation dynamics study of the species, would be useful, however, very little data were available on this.

Besides the propagation study, another relatively less researched area is the investigation of the “special” feature of the Woolly cupgrass interference, namely allelopathy, which has a not sufficiently clarified role in the competitiveness of the species. Allelopathy research can be an important pillar in the development of bio-herbicides, which is even more important (with respect to the actual significant withdrawal of herbicidal agents). During my allelopathy research, and the analysis of the results thereof, several factors were observed in mind.

Several pathogens were isolated on Woolly cupgrass, which can be a potential host plant of these microorganisms, used as a natural enemy, but so far complete success has not yet been reached with any of these pathogens (Cummins, 1971, Brunt *et al.*, 1996). During the

collection of domestic samples, we also identified a *Fusarium* fungi species, but because of the polyphagous lifestyle, this fungus cannot be used as a natural enemy either.

Objectives of the research

The subject of the thesis is an invasive weed, the Woolly cupgrass. I investigated its propagation dynamics, spread possibilities, and an important factor of its competitiveness, the allelopathy. On the collected samples, a pathogen (*Fusarium culmorum*) was isolated, which is a natural enemy of the plant, and which can play a role in the weed control.

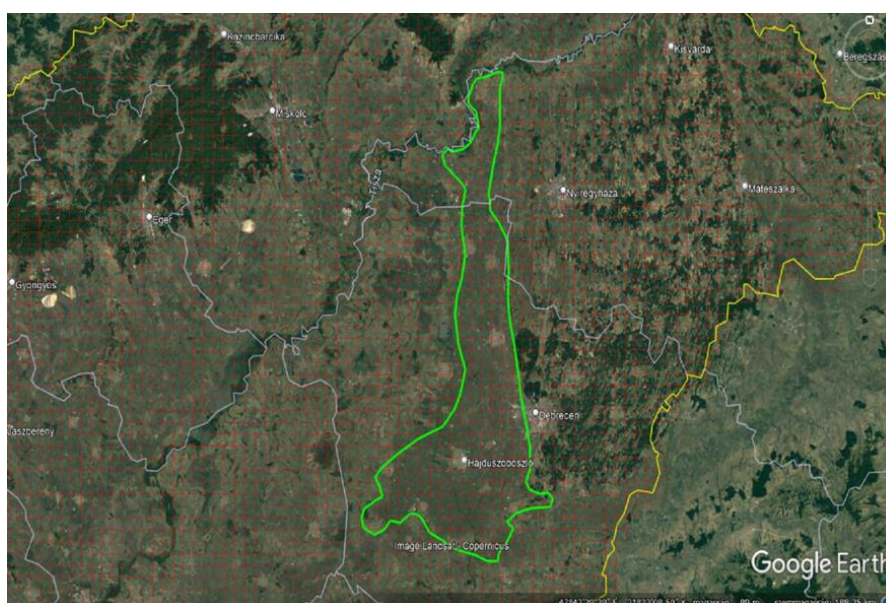
During my PhD work my goals were:

- The propagation dynamics of the Woolly cupgrass and its monitoring in the area of the Hajdúság sub-region. The Hajdúság sub-region (an area of 1161 km²) is where I monitored and investigated the intensity and extent of the spread, concerning the first available data from 2008.
- I investigated one of the most difficult means to grasp, of the competition between weeds, the possibility of allelopathy in the case of Woolly cupgrass. During my investigations I closely monitored several influencing factors of allelopathy.
- I analysed the allelopathic impact of Woolly cupgrass on the morphologic and biologic parameters of a fodder corn hybrid.
- In order to seek after a possible natural enemy against Woolly cupgrass, we prepared microbiological isolations from the plant samples collected during the propagation dynamics investigation.

2. Research methods

2.1 Monitoring and propagation dynamics observation of Woolly cupgrass in the Hajdúság sub-region

One of the objectives of the investigation was to find out the extent of spread in the subregion, of the Woolly cupgrass population, found in the vicinity of Debrecen. This was founded on the data recorded in 2011. The investigation plot was established in the area of the Hajdúság sub-region. This is a 1161 km² area, situated in the Northern-North-eastern part of the Alföld plains. On this plot I fitted a 2,5x2,5 km size UTM (Universal Transverse Mercator) grid system (Varga, 2015), which resulted in 315 UTM grid elements. Within each grid elements, I designated 3 to 5 randomly chosen recording points, where I searched for Woolly cupgrass.



1. Figure: 2,5 x 2,5 km size UTM (Universal Transverse Mercator) grid system on the area of the Hajdúság sub-region (the part marked with a green frame)

Investigation of natural dispersal and dissemination by human means

The spread of seeds is possible through the mediation of animals. The seeds of the investigated weed are large, so the birds can digest them. I also tried this in an experiment, when seeds of Woolly cupgrass (100-100 seeds) were added to the mixture of birdseed (wheat, corn, sunflower seeds) of captive collared doves (*Streptopelia decaocto*), and provided *ad libitum* feeding for the birds. I checked the mixture every seventh day, and collected the bird droppings. The droppings then were cleaned, and the seeds found, were germinated. We repeated this investigation three times.

Another part of the dispersal investigation was to find out, if agricultural harvesting machines can play a role in the spread of Woolly cupgrass. In order to do so, I collected corn stalk/leaves samples in several places, from the siftings of combine harvesters, three times from each area. Until sorting, these samples were kept in paper bags.

2.2 Allelopathic characteristics of Woolly cupgrass

2.2.1 Assessment of the allelopathic characteristics of Woolly cupgrass, and the juglone index

Investigation of the allelopathy of Woolly cupgrass with respect to the different parts of the weed

I carried out this laboratory test in 2016, among laboratory circumstances, when I assessed the effects of extracts, prepared from roots, stem, leaves and panicle of freshly collected Woolly cupgrass. Plant parts were chopped in small pieces, and I used 4 to 8 grams of these for the extracts. I poured 100 ml of distilled water on the samples, and I kept those in darkness for 1 day. The next day I filtered the samples, and these extracts were used for the treatments. The test plants used in this experiment were corn (*Zea mays* L.), sunflower (*Helianthus annuus* L.) and lettuce (*Lactuca sativa* L.). For lettuce I used 11cm diameter Petri dishes, while for corn and sunflower, I used 17cm diameter ones. The extracts were into these dishes, for control I used clean distilled water. The Petri dishes were kept at room temperature (20 ± 1 °C). The experiment was set in three repetitions, and the germination of test plants was measured on the 5th day.

The measured average root, sprout length and total plant length of each treatment was assessed by one-way variance analysis (ANOVA), while normality was assessed by the Kolmogorov-Smirnov and Shapiro-Wilk tests. The standard distribution samples were compared by Tukey HSD test, while non-normally distributed samples were compared by the Kolmogorov-Smirnov Z and the Mann–Whitney U non-parametric tests, at a significance level of $P \geq 5\%$.

Investigation of the allelopathy of Woolly cupgrass with respect to different concentrations

The experiment was carried out in 2019, among laboratory circumstances. The roots and sprouts of the plant, were used for the assessment, which were dried and then ground into powder. From the meal I prepared 1%, 5% and 10% aqueous extracts, which were kept in a dark place for 1 day, and then the filtered extracts were used. In this assessment, the test plant was white mustard (*Sinapis alba* L.). Seeds of the test plant (100 pieces in each) were placed in 11cm diameter Petri dishes, and 5ml of the extracts were measured into each Petri dish, respectively. For the control samples, I used clean distilled water. The experiment was carried out at room temperature (20 ± 1 °C), in four repetitions, which were assessed on the fourth day.

The germination differences measured in the treatments, were compared by one-way variance analysis (ANOVA). If the variance of the result was significant ($P < 0,05$), then the comparison of treatments was checked with the Kruskal-Wallis non-parametric test, while pairwise comparison was carried out by the Mann-Whitney U-test.

Determination of the juglone index of Woolly cupgrass

During the assessment, I used the juglone index to quantify the measure of allelopathy (Szabó, 1999). The measurement principle is that significant allelopathic impact can be shown at several species of the family *Juglandaceae* (e.g. walnut-*Juglans regia* L. and black walnut-*Juglans nigra* L.) (Daglish, 1950; Pratavia et al., 1983). Juglone, found in walnuts, has an inhibitory effect on the germination of many plants (Kocacaliskan – Terzi, 2001).

The formula of the juglone index was created as part of the works of Szabó (1999). The essence of the method is that the extract, made from the plant with unknown allelopathic potential, is compared to the treatment carried out with 1 mM juglone. In this assessment, the test plant was white mustard (*Sinapis alba* L.). I investigated its germination percentage, root and sprout length growth treated with extracts of juglone and the plant with allelopathic potential. The ratio of the two will give you the value of the juglone index. If this ratio is larger than one, then the allelopathic effect is stronger than that of juglone (inhibition is more pronounced), if the ratio is smaller than one, then the inhibition is weaker than that of juglone.

If this value is under 0.5, then we can no longer speak of considerable allelopathic effect (Szabó, 1999).

Establishing the juglone index (I_j/x) in the case of the plant extract with unknown allelopathic potential:

$$I_j / x = (H_j + R_j + G_j) / (H_x + R_x + G_x)$$

Where:

H_j: Average sprout length of 3x10 mustard seeds, treated with 1 mM juglone (mm),

R_j: Average root length of 3x10 mustard seeds, treated with 1 mM juglone (mm),

G_j: Average germination capacity of 3x100 mustard seeds, treated with 1 mM juglone (piece),

H_x: Average sprout length of 3x10 mustard seeds, treated with plant extract of unknown allelopathic potential (mm),

R_x: Average root length of 3x10 mustard seeds, treated with plant extract of unknown allelopathic potential (mm),

G_x: Average germination of 3x100 mustard seeds, treated with the plant extract of unknown allelopathic potential (piece) (Csiszár *et al.*, 2012; Csiszár *et al.*, 2013).

The experiment was carried out in 2018, among laboratory circumstances, using fresh plant samples. From these samples, 5 grams was measured into 100 ml of distilled water each, and then soaked for one day. From the test plant (white mustard) I used 100 seeds in each 11cm diameter Petri dish, and repeated this experiment three times. I used 5 ml of each extract, for control I used clean distilled water. Germination was carried out at room temperature (20 ±1 °C) for 4 days, then I measured the number of germinated seeds, and the length of roots and sprouts (these two will provide the total length as well). Besides determining the juglone index, each parameter was also assessed statistically. The measured average root, sprout length and total plant length of each treatment was assessed by one-way variance analysis (ANOVA). With the help of Q-Q diagrams (Q-Q plots) and Levene-test it was verified if the conditions are met. If the difference between results was bigger, pairwise comparison of treatments was analysed using the Bonferroni post-hoc test.

2.2.2 Assessment of the biotic response caused by the allelopathy of Woolly cupgrass, in fodder corn

Introduction of experimental conditions

The experiments were carried out in the greenhouse of DE MÉK (University of Debrecen, Faculty of Agricultural and Food Sciences and Environmental Management), in 2021. The fodder corn hybrid, used in the experiments, was Armagnac (*Zea mays* L. cv. *Armagnac*). In the greenhouse, the typical humidity was 50-60%, the typical temperature was 34/26 °C (day/night). The peat used for growing: 70% brown and 30% medium-mature fog, 1,5 kg/m³ fertiliser KS1 (15-12-29+micro elements), with 14-16-18% MeO additive. The plants were grown in 11cm diameter pots.

In the pots, seeds of the Woolly cupgrass were sowed next to the examined corn plant. For control, only corn was in the pots. After sowing, the pots were watered daily, so that there is no shortage in the plant's water supply. The experiment was set up in 5 repetitions.

During sampling, we examined the morphological (root parameter, stem diameter and plant height) and biochemical (relative chlorophyll content, quantity of photosynthetic pigments, measure of lipid peroxidation, activity of antioxidant enzymes) parameters of the corn plant. Plant sampling was carried out at 4-5 leaf stage of the corn growth, at two times (14 and 21 days after emergence).

Morphological examinations

Stem diameter was measured with a calliper. This value was measured between the second and third nodes of the corn. Height of the plant was measured from soil surface to the tip of the top leaf. Stem diameter and plant height was measured on both the 14th and 21st days after emergence.

Root parameters (root length, root volume, root diameter and root surface) were measure after cleaning the roots, using digitalized images made with an Epson Expression 11,000 XL scanner, and calculated using the WinRHIZO software (Regent Instrument Inc., Québec, QC, Canada).

For the measurement of dry weight content of the corn, the samples were dried in an OHAUS AX223 oven, for three days, at 65 °C.

Methodology of antioxidant enzymes and protein moiety examinations

From the leaves of corn, 0.2 gram samples were used, which were excoriated with liquid nitrogen. Subsequently a buffer solution was added (50 mM KH_2PO_4 buffer (pH 7.0), 1 mM EDTA, 2% PVPP, 0.1% Triton X-100, 1mM sodium ascorbate). The samples thus obtained, were centrifuged at 4 °C. After centrifugation, the supernatant was used for enzyme examinations (Pukacka – Ratajczak, 2005).

The ascorbate peroxidase (APX) examination consisted of 570µl 50 mM KH_2PO_4 buffer (pH 7.0), 200µl H_2O_2 (0.1 mM), 150µl sodium ascorbate (0.5 mM), 50µl EDTA (0.1 mM EDTA), and 30µl enzyme extract. The measurement started by adding the enzyme extract obtained, which was measured against a blank test (in this case measured with phosphate buffer instead of enzyme extract). Decrease of the absorbance was measured with spectrophotometer for 5 minutes (at 20 °C) (Mishra et al., 1993).

For POD (guaiacol-peroxidase) examination 50 µl 0.2 mM H_2O_2 , 100 µl 50 mM guaiacol, 340 µL distilled water, 500 µl 80 mM phosphate buffer (pH 5.5) and 10 µl enzyme was used. During the examination, tetraguaiacol is formed, which was measured with spectrophotometer. The measurement duration was 3 minutes at 30 °C. This was also compared to a blank test, which did not contain any enzymes (Zieslin – Ben-Zaken, 1991).

Protein content was examined according to Bradford (1976).

For SOD (superoxide dismutase) examination 400 mg samples of leaves and 200 mg samples of roots, were used. The samples were crushed with liquid nitrogen, to which 50 mM phosphate buffer (mM EDTA, 1% PVP, 1 mM PMSF in case of pH 7.8) were added (for leaf samples 4 ml, for root samples 2 ml extract), subsequently the samples were centrifuged for 15 minutes (Giannopolities – Ries, 1977; Beyer – Fridovich, 1987).

Quantification of the relative chlorophyll content and quantity of photosynthetic pigments

For the measurement of chlorophyll content, an SPAD-502 Plus device was used. The measurements were made 14 days after emergence, on the fourth leaf of the plant, in five repetitions.

The quantity of photosynthetic pigment was quantified by adding 5 ml N, N-dimethylformamide to 50 mg of fresh leaves, which then was kept refrigerated for 72 hours. Subsequently the absorbance of the samples was measured with spectrophotometer (Wellburn, 1994; Moran – Porath, 1980).

Quantification of the measure of lipid peroxidation

The measure of lipid peroxidation can be quantified by the concentration of malondialdehyde (MDA). This was obtained by crushing a 100 mg leaf sample with liquid nitrogen, then adding 1 ml 0.25% TBA (thiobarbituric acid) and 10% TCA (trichloroacetic acid). Subsequently the sample was centrifugated for 25 minutes at 4 °C. From the supernatant 0.3 ml was used, by adding 0.7 ml 20% TCA and 0.5% TBA. Then it was heated for half an hour at 95 °C, and finally cooled down on ice bed. After it cooled down, it was centrifugated for 10 minutes at 4 °C, then the absorbance of the sample was measured with spectrophotometer (Heath - Packer, 1968).

For statistical analysis, the IBM SPSS Statistics 25 software was used, where the results by treatment were checked by one-way variance analysis (ANOVA). For normality examination, the Kolmogorov-Smirnov and Shapiro-Wilk tests were used, then the average values were compared with an independent t-test ($P \geq 0.05$). Significant differences were marked with small letters (Kim, 2015).

Possibilities of biologically control against Woolly cupgrass.

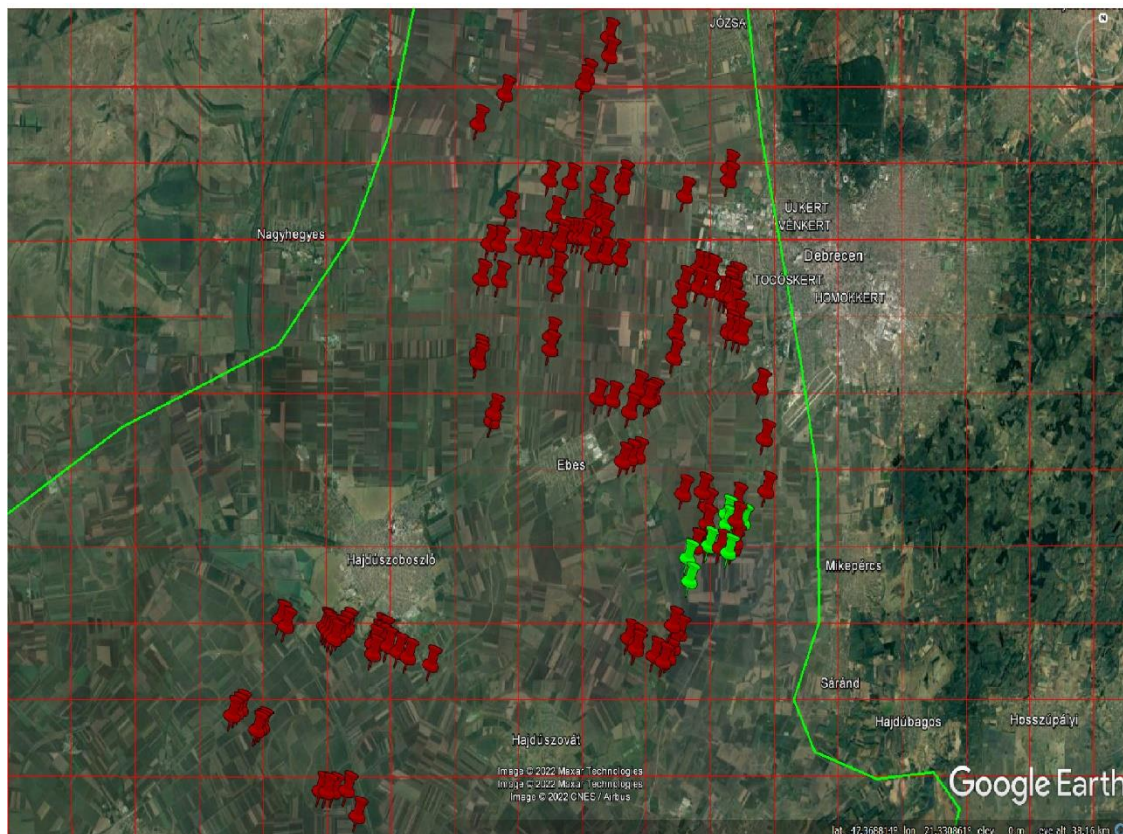
The infected plant samples were collected on November 13, 2015 (GPS coordinates: 47;26;29.20 - 21;34;19.72). After cleaning the samples, their surface was disinfected by 1% chlorogenic solution (Neomagno), which then was rinsed by sterile distilled water. Then the plant parts were placed on moist filter paper (in a 9cm diameter Petri dish), where they spent seven days. The emerged mycelial mat were placed on PDA substrate, using an inoculating loop, and let it grow at 20 °C.

For the morphologic identification of the fungus was carried out by examination of the amphigamous spores (macroconidia) morphology under light microscope, and by the specification of the fungus colony (Leslie - Summerell, 2006).

3. Results

3.1 Propagation dynamics and monitoring of Woolly cupgrass in the area of the Hajdúság sub-region

The propagation dynamics of the Woolly cupgrass was investigated in the area of the Hajdúság sub-region. The investigation was based on the survey conducted by Somogyi and co-workers in 2011. I placed a 2.5 x 2.5 km size UTM grid system (a total of 315 grids) on the monitoring area, within which I designated several recording points. The investigation lasted from 2016 to 2019, and the results obtained, are shown in Figure 2.



2. Figure: Spread of the Woolly cupgrass in the area of the Hajdúság sub-region (📌 published results of Somogyi et al. (2011), 📌 own results)

The results obtained point out, that the presence of Woolly cupgrass can be observed at longer distances from its original, first detection site in 2011. Compared to the data published by Somogyi and co-workers, there were areas 20 kilometres away from the original point of

detection. Thus in a couple of years (5 to 8 years) this weed could spread to such a distance. Therefore, its possibilities of spread, were also investigated.

The Woolly cupgrass has grains, which are 4 to 5mm long and 2 to 3mm wide, thus quite large. The surface of the grain is normally completely bare, sometimes the arista remains on it.

One of the possibilities of natural spread is that by animals. After consuming the seeds, and passing through the digestive system, those still can be viable. The collared doves involved in the experiment, consumed the Woolly cupgrass seeds, but those were not detectable in their droppings. I got the same result after several repetitions.

Based on observations, field-voles also collect the seeds and spiculum of the Woolly cupgrass, but the limited movement of these animals does not justify the spread to larger distances.

The role of people in the spread of seeds, however, can be much greater. There are several publications concerning the spread of weeds by agricultural machines and implements. In my investigation I examined the spread possibilities of combine harvesters. In the corn stalk/leaves residues from the siftings of combines, harvesting on infected areas, I found the seeds of Woolly cupgrass. This points out to that harvesting machines can play an important role in the spread.

3.2 Allelopathic characteristics of Woolly cupgrass

Investigation of the allelopathic effect of Woolly cupgrass with respect to the different parts of the weed

During the investigation it was proven that in the competition the Woolly cupgrass is using the allelopathy. With one of the experiments I wanted to investigate, how much these effects are different within the weed. In this experiment, I inspected the difference between root, sprout and spiculum extracts of the weed, in concentrations of 4% and 8%. The effects were measured on three test plants (sunflower, lettuce, corn).

In the case of sunflower, the 4% solution of the sprout and the root, were different from control, but there was no statistically detectable difference between each other. The 4% solution made from spiculum, did not show any difference from the control samples. In the

case of the 8% extracts, the extracts made from the root and the sprout, were significantly different from the control samples, but the sprout had a stronger inhibition effect, which could also be verified statistically. The solution made from spiculum was not greater than the effect of either the control or the root extract. It was noticeable, that increasing the concentration also increased the anti-sprouting effect. In the case of higher concentrations, the solution made from the sprout extract was the greatest on sunflower.

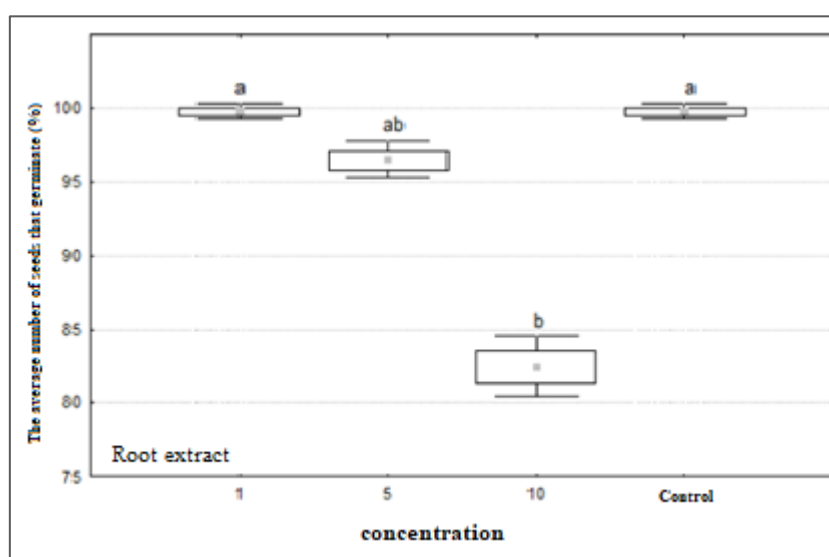
The 4% and 8% plant extracts were also used in the case of lettuce. All of the 4% extracts made from different parts of the Woolly cupgrass, differed significantly from the control sample, and inhibited the test plant. However, the inhibition effect of the extract made from spiculum was not as strong as that of the root and sprout extracts. The extracts of sprout and root were not significantly different from each other. The effect of the 8% solution was similar to that of the 4% extracts. Thus the extract made from all three plant parts showed a significantly different effect. The germination inhibiting effect of the extract made from spiculum was not as strong as that of the solutions made from roots and sprouts, and the latter two did not differ from each other, statistically. Lettuce, as a sensitive test plant, shows well, that Woolly cupgrass does have an allelopathic effect, and the germination inhibiting effect of the extracts made from different parts of the plant is also different, as it was the case with the sunflower test plant.

The third test plant was fodder corn, which I used to analyse the effect on germination, of the 4% and 8% extracts made from different parts of Woolly cupgrass. The result of the examinations showed that there is some difference between the germination inhibiting effect of the extracts prepared from the different parts and with different concentration but this could not be significantly verified. Presumably corn tolerated the allelopathic compounds produced by the Woolly cupgrass, better.

Overall, the germination inhibiting effect of the different parts of Woolly cupgrass, was successfully demonstrated. Lettuce, as a more sensitive test plant, reacted much better. The difference in the inhibitory effect between the extracts prepared from different parts of the weed plant, was demonstrated. In conclusion it can be said that the extract made from the spiculum, has the weakest inhibiting effect. The extracts prepared from roots and sprouts, were the strongest, in some cases the sprout extract had the most powerful germination inhibiting effect. By increasing the concentration, the inhibiting effect also increased.

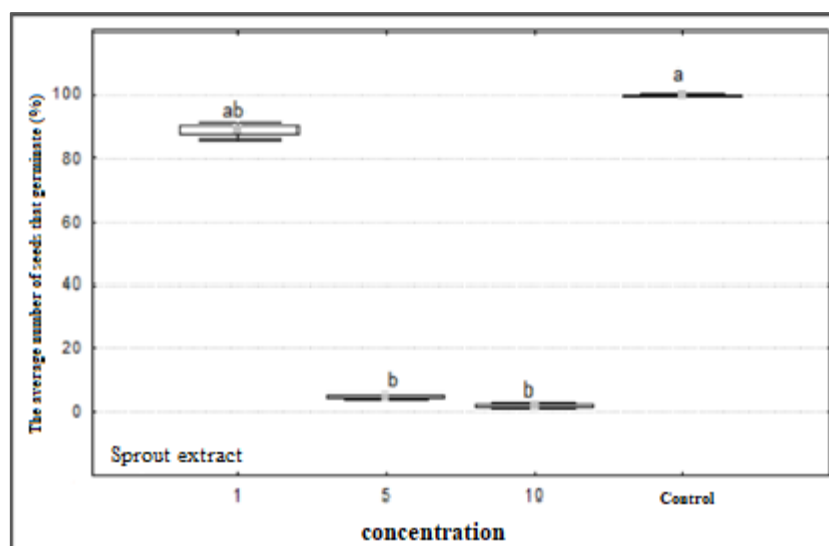
Investigation of the allelopathy of Woolly cupgrass with respect to different concentrations, on white mustard

The examination showed that the root extract of Woolly cupgrass has a significant inhibiting effect on the germination of the test plant. The 10% root extract of the examined plant significantly excelled both the control and the 1% root extract solutions. There was no significant difference between the 1% solution and the control sample. For the 5% extract, significant difference could not be detected with control sample and 1% extract (Figure 3.).



3. Figure: Root extracts' effect on the germination percentage of white mustard. Small letters indicate significant differences (Mann-Whitney U-test: $p < 0.05$)

The extracts prepared from sprouts of the Woolly cupgrass, have significantly different effects on germination of the white mustard test plant. The inhibiting effect of the 5% and 10% solutions, excelled the results of the control samples. The 1% solution, prepared from the sprout of Woolly cupgrass, did not significantly differ from the effect of the control sample and the 5% and 10% extracts (Figure 4.).

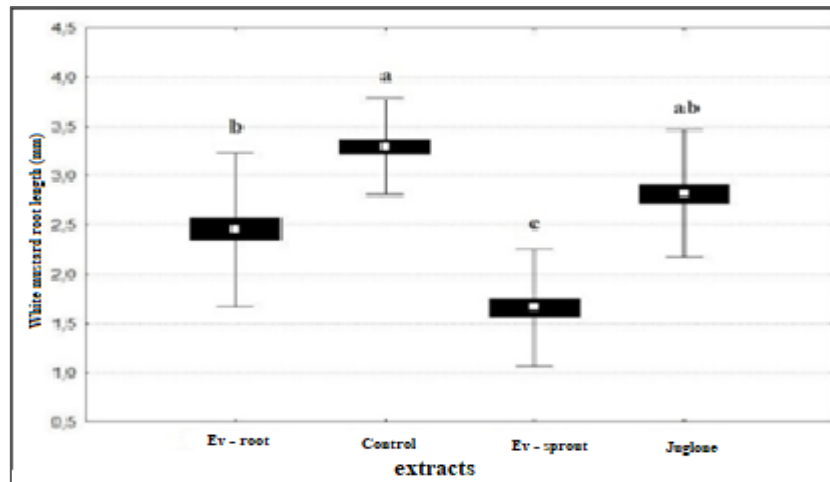


4. Figure: Sprout extracts' effect on the germination percentage of white mustard. Small letters indicate significant differences (Mann-Whitney U-test: $p < 0.05$)

Overall, it can be demonstrated, that extracts prepared from the root and sprout of Woolly cupgrass, show statistically verifiable inhibiting effect on the germination of the white mustard test plant, compared to the control samples. Increasing the concentration further enhanced the effect, in case of the 10% extract the difference was significant. In the case of extracts prepared from the sprouts of the examined plant, the effect was greater on the white mustard.

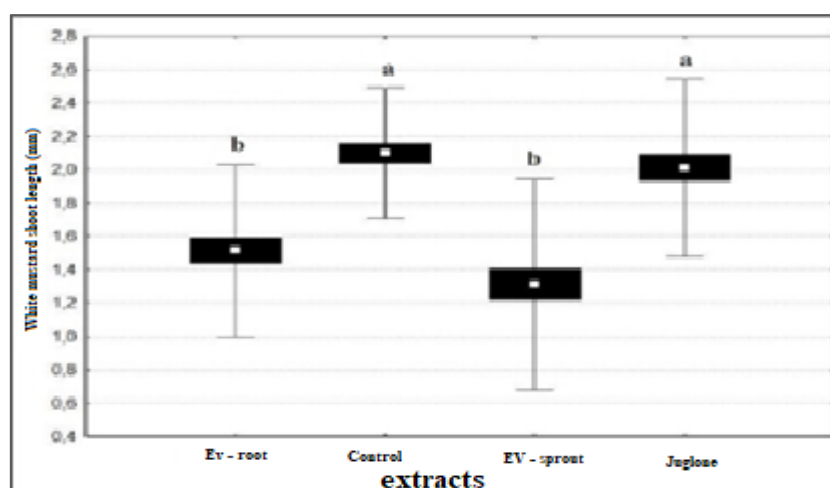
Results of the germination test to determine the juglone index

The root length of the white mustard, measured on the 4th day after treatment, was significantly shorter than that of the control plants, which clearly showed that aqueous solution of the root and sprout extract of Woolly cupgrass, inhibited the development of test plants. The extent of this inhibiting effect was significant for both solutions. The effect of the 1mM juglone solution did not differ from that of the control sample and the root extract of Woolly cupgrass. The strongest inhibiting effect resulted from the extract prepared from the sprout, which differed significantly from all three treatments (*Figure 5*).



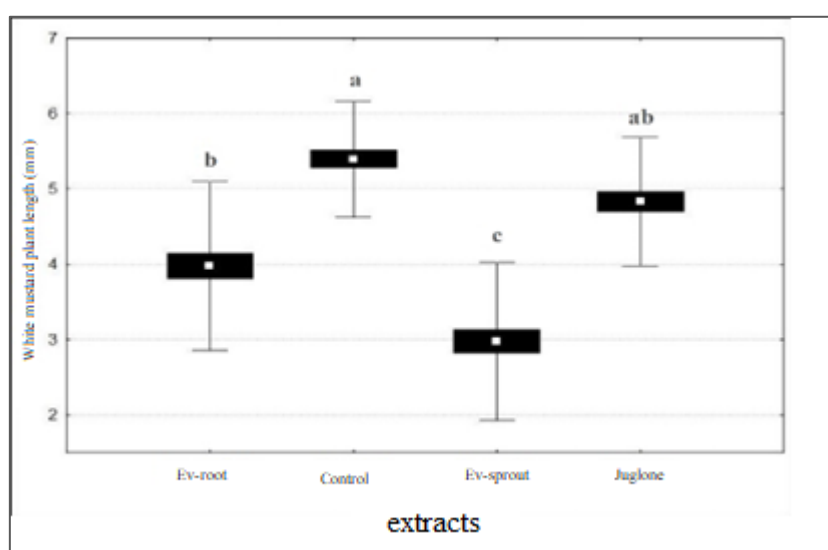
5. Figure: Effect of the root and sprout extract of Woolly cupgrass (Ev-root, Ev-sprout), and juglone (1mM) solution, on the root growth of the white mustard test plant. Different small letters indicate significant differences, based on Bonferroni test: ($p < 0.05$).

The sprout length results, measured on the 4th day, also showed differences between the treatments. The most powerful inhibiting effect on the development of white mustard, was caused by the sprout and root extract of Woolly cupgrass. On the sprout development of white mustard, it was also the sprout extract which caused the most powerful inhibition. The 1mM juglone solution did also have an effect on the sprout development of white mustard, but the results of samples did not differ significantly from the control samples. However, the extract prepared from the root and sprout of Woolly cupgrass, did indeed significantly inhibit the sprout development of white mustard (6. Figure).



6. Figure: Effect of the root and sprout extract of Woolly cupgrass (Ev-root, Ev-sprout), and juglone (1mM) solution, on the sprout growth of the white mustard. Different small letters indicate significant differences, based on Bonferroni test: ($p < 0.05$)

The total length of the plant, was calculated by adding the root length and sprout length. As per the results of the experiment, the root and sprout extract of Woolly cupgrass did significantly, that is, in a statistically verifiable way, inhibit the development of white mustard, compared to the control samples. The 1mM juglone solution did have an inhibiting effect on the whole plant, but it did not significantly differ from the control sample. The most powerful inhibiting effect on the development of white mustard, was caused by the sprout extract of Woolly cupgrass, but the inhibiting effect of the root extract, was also considerable (Figure 7.).



7. Figure: Effect of the root and sprout extract of Woolly cupgrass (Ev-root, Ev-sprout), and juglone (1mM) solution, on the growth of white mustard. Different small letters indicate significant differences, based on Bonferroni test: ($p < 0.05$)

The results obtained point out again, that Woolly cupgrass does have an allelopathic effect. Considering the different organs of Woolly cupgrass it is shown that the concentration of allelopathic compounds is different within the plant. The extract made from the sprout proved to have the most powerful inhibiting effect.

Assessment of the juglone index of Woolly cupgrass

Entering the data measured in the case of **extract prepared from the sprout of Woolly cupgrass**, in the calculation formula of the juglone index (Ij), we got the following result:

$$Ij = \frac{(2,011 + 2,747 + 97,600)}{(1,311 + 1,660 + 92,000)} = \mathbf{1,078}$$

The value obtained is greater than one, which shows that the inhibiting effect is stronger, than that of the 1mM juglone solution, used for reference. We got the same result in the previous measurements as well.

Entering the data measured in the case of **extract prepared from the root of Woolly cupgrass**, in the formula of the juglone index (Ij), we got the following result:

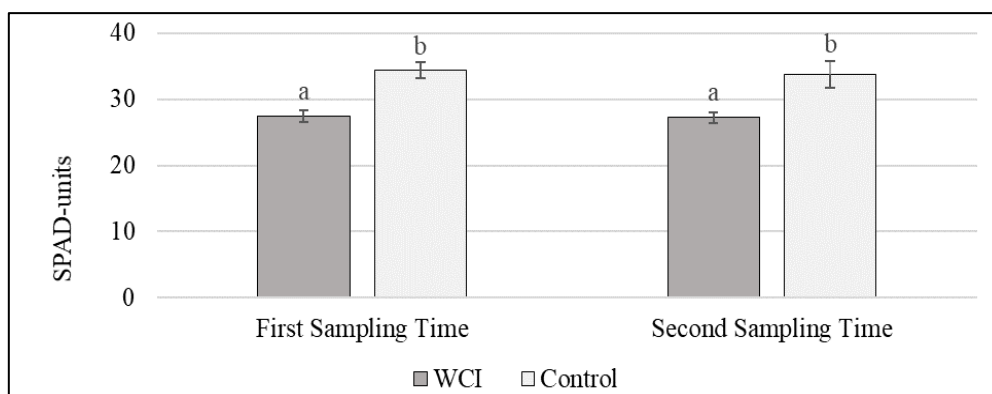
$$Ij = \frac{(2,011 + 2,747 + 97,600)}{(1,516 + 2,637 + 98,000)} = \mathbf{1,002}$$

The quotient value obtained is almost one, which shows that the inhibiting effect of the aqueous solution prepared from the root of Woolly cupgrass, is nearly the same as the effect of the 1mM juglone solution.

Investigation of the physiological response caused by the presence of Woolly cupgrass, on Armagnac corn hybrid

Effect of Woolly cupgrass on the relative chlorophyll content of corn and on photosynthetic pigments

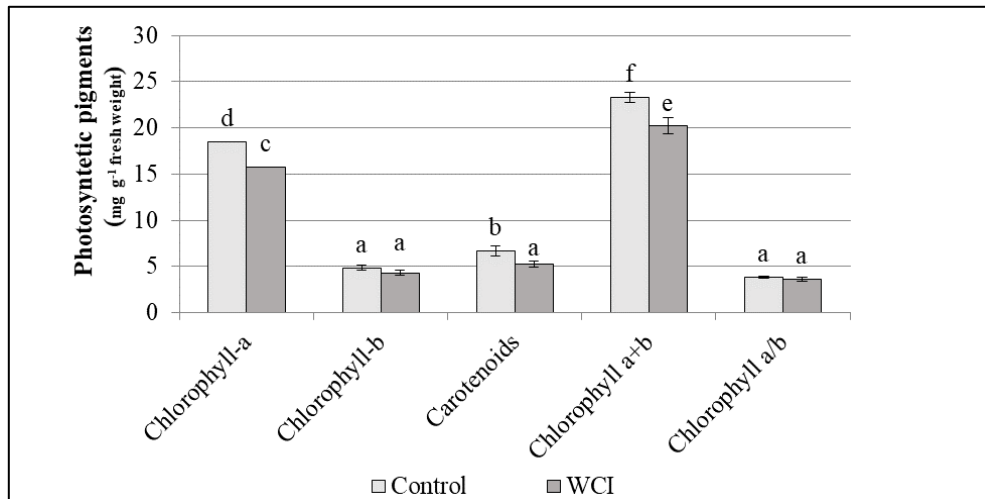
In the experiment it was demonstrated, that because of the effect of Woolly cupgrass, the quantity of photosynthetic pigments decreased in corn. The relative chlorophyll content measured at both times, showed a significant decrease, compared to the values measured in control plants (*Figure 8*).



8. Figure: Effect of Woolly cupgrass on the relative chlorophyll content of the Armagnac corn leaves. Different small letters indicate significant differences: ($p < 0.05$)

Since relative chlorophyll content is a relative value, the quantity of each photosynthetic pigment is to be determined.

In the quantitative exercise of photosynthetic pigments of corn, the neighbourhood of Woolly cupgrass had an unfavourable effect on chlorophyll-A, on carotenoids and on chlorophyll-A+B, which were significantly different. There was no statistically verifiable difference in the quantity of chlorophyll-B (*Figure 9*).



9. Figure: Effect of Woolly cupgrass (WCI) on some photosynthetic pigments of Armagnac corn leaves (chlorophyll-A, chlorophyll-B and carotenoids). Small letters indicate significant differences: ($p < 0.05$)

Effect of Woolly cupgrass neighbourhood on root parameters, growth and dry weight of the Armagnac corn.

Effect of Woolly cupgrass on the root parameters of the Armagnac corn.

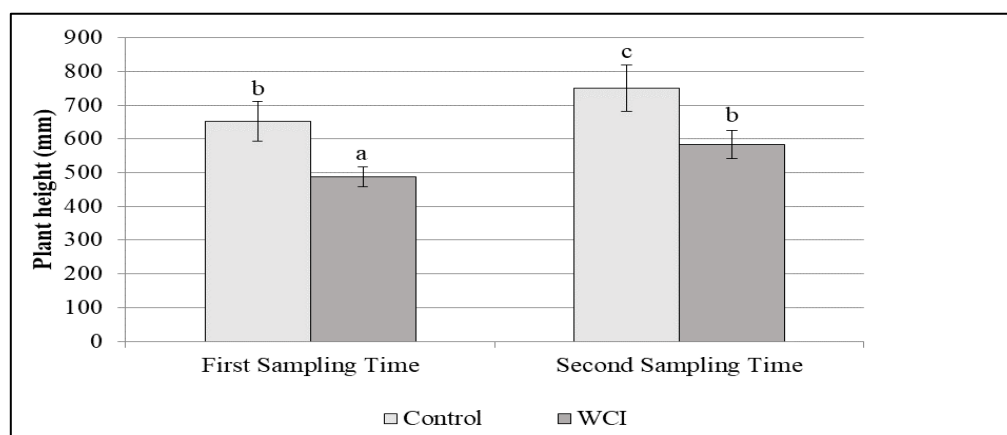
Woolly cupgrass had a negative effect on the root parameters of the corn plant, e.g. On total root length, root volume, root surface and root diameter. Except the latter one, all parameters showed a significant difference (*Table 1.*).

1. Table: Effect of Woolly cupgrass on root parameters of the Armagnac corn on day 21 (n=5)

Root parameters	Treatments	
	Control	Plant grown together with Woolly cupgrass
Total root length (cm)	757.17 ± 41.22 a	478.49 ± 79.62 b
Adventitious root length (cm)	1.54 ± 0.070 a	0.76 ± 0.11 b
Diameter (mm)	0.49 ± 0.011 a	0.46 ± 0.03 a
Surface (cm²)	117.58 ± 12.05 a	67.24 ± 13.39 b
< 0.2 mm	382.67 ± 30.58 a	283.89 ± 55.53 b
0.2 - 0.4 mm	118.95 ± 20.75 a	53.77 ± 8.15 b
0.4 - 0.6 mm	27.91 ± 4.85 a	11.21 ± 2.61 b
0.6 - 0.8 mm	61.81 ± 1.19 a	42.19 ± 3.45 b
0.8 - 1 mm	84.18 ± 6.18 a	41.10 ± 5.10 b
> 1.0 mm	94.79 ± 7.48 a	46.23 ± 10.14 b

Effect of Woolly cupgrass on the growth of the Armagnac corn

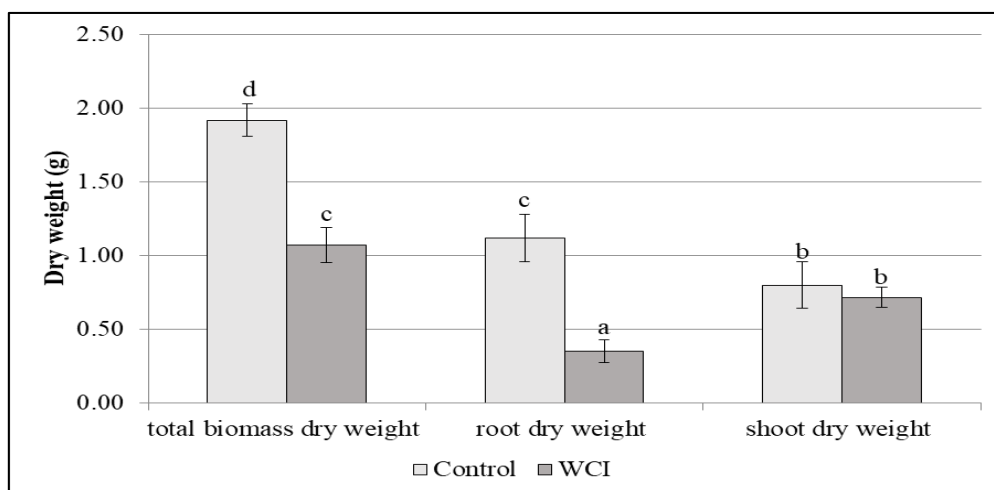
Plant height of corn grown together with Woolly cupgrass, was smaller at both measurement times (14th and 21st day), compared to the control plants. This difference was also significant in both cases (*Figure 10.*).



10. Figure: Effect of Woolly cupgrass (WCI) on the plant height of the Armagnac corn. Different small letters indicate significant differences: (p<0.05)

Effect of Woolly cupgrass on the dry weight of the Armagnac corn

Dry biomass weight of corn grown together with Woolly cupgrass, was smaller, compared to the weight of control plants. This growth inhibition was statistically verified for the dry weight of the root, and the total biomass (root+sprout). The weight of the stem or sprout was not significantly different, compared to the control samples (*Figure 11.*).

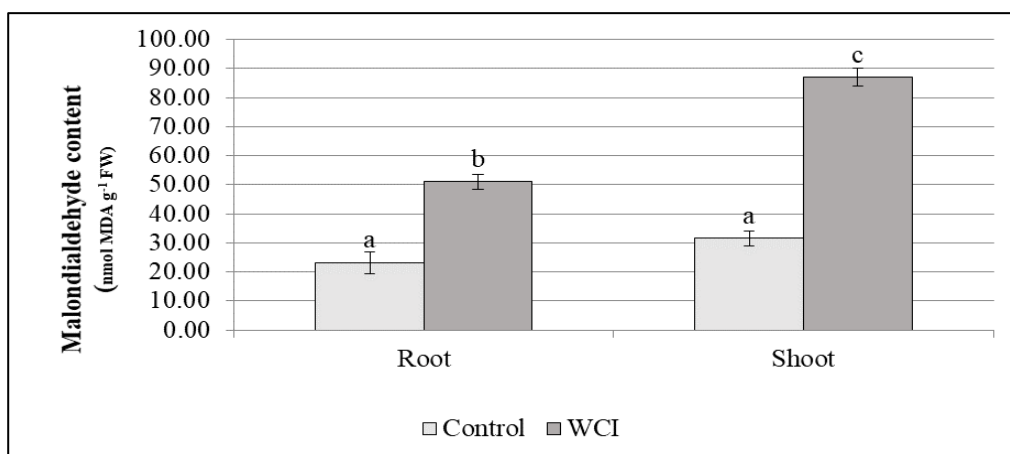


11. Figure: Effect of Woolly cupgrass (WCI) on the dry weight of the Armagnac corn. Different small letters indicate significant differences: ($p < 0.05$)

Effect of Woolly cupgrass on the MDA content, on the APX, SOD and POD activity, and the protein content of the Armagnac corn hybrid

Effect of Woolly cupgrass on the MDA content of the different parts of the Armagnac corn

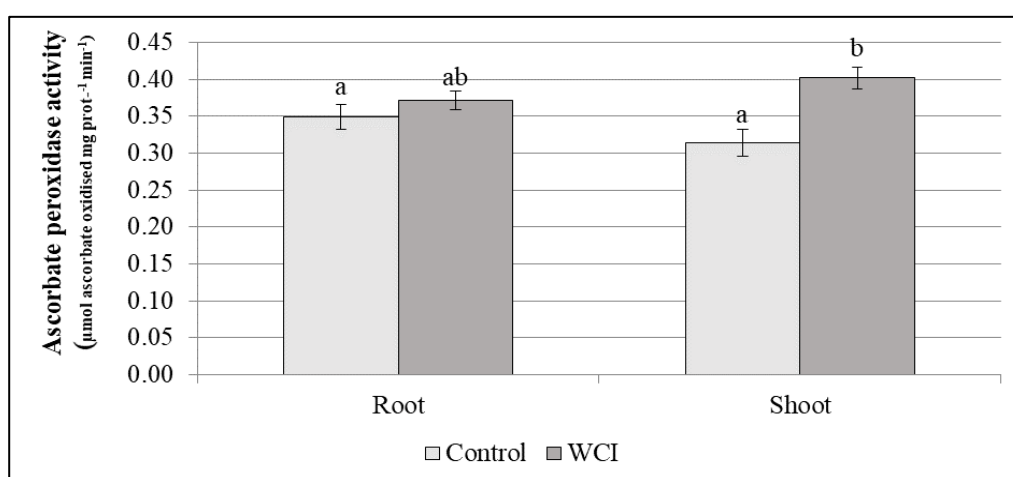
The responses caused by Woolly cupgrass was also investigated, concerning the biochemical parameters of corn. One of these factors is the quantity of malondialdehyde (MDA), which was examined in both the sprout and the root. There was no significant difference between the malondialdehyde content of the sprout and root of the control plant, however in the root of the corn grown together with Woolly cupgrass (23% of difference) and sprout (71% of difference), the MDA content significantly increased (*Figure 12.*).



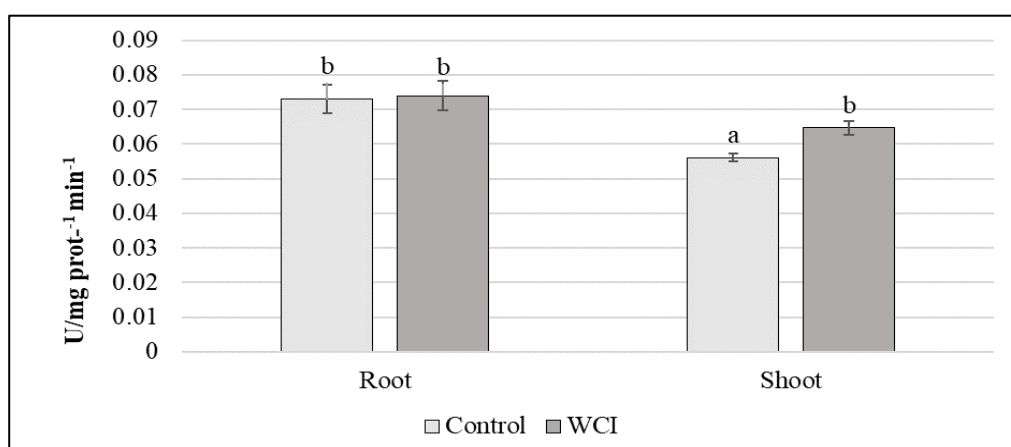
12. Figure: Effect of Woolly cupgrass (WCI) on the MDA content of the different parts of the Armagnac corn. Different small letters indicate significant differences: ($p < 0.05$)

Effect of Woolly cupgrass on the APX and APX activity of the different parts of the Armagnac corn

During the experiment the ascorbate peroxidase (APX) and superoxide dismutase (SOD) activity of the plant, was assessed. Compared to the control plant, in the corn grown together with Woolly cupgrass, the level of activity increased. However, significant difference was only demonstrated in the sprout, resulting in higher levels of APX (28% of difference) and SOD (16% of difference). In the root there was no significant difference demonstrated, compared to the control plants (*Figure 13. – 14.*).

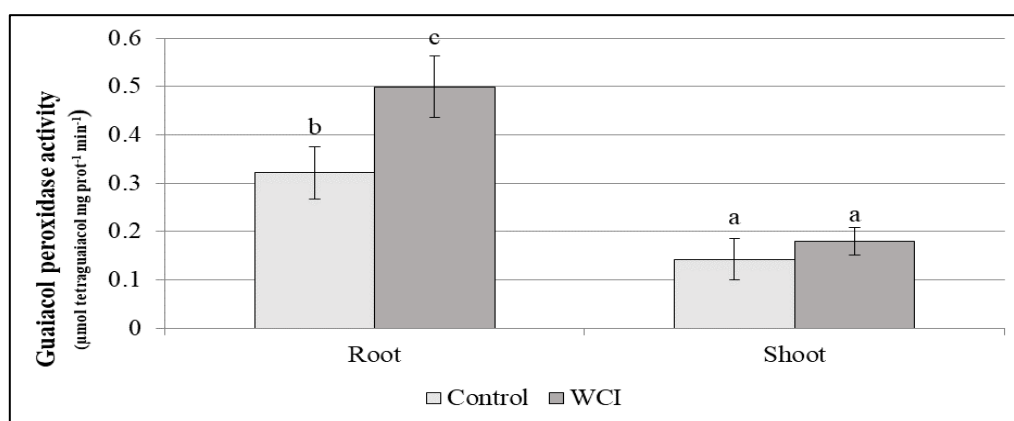


13. Figure: Effect of Woolly cupgrass (WCI) on the APX activity of the different parts of the Armagnac corn. Different small letters indicate significant differences: ($p < 0.05$)



14. Figure: Effect of Woolly cupgrass (WCI) on the SOD activity of the different parts of the Armagnac corn. Different small letters indicate significant differences: ($p < 0.05$).

Among biochemical responses, POD (peroxidase) activity was also examined. The results show, that the POD activity of corn grown together with Woolly cupgrass, increased (both in the sprout and root), compared to the control plants. POD activity was more intense in the root, which also deviated significantly (*Figure 15*).

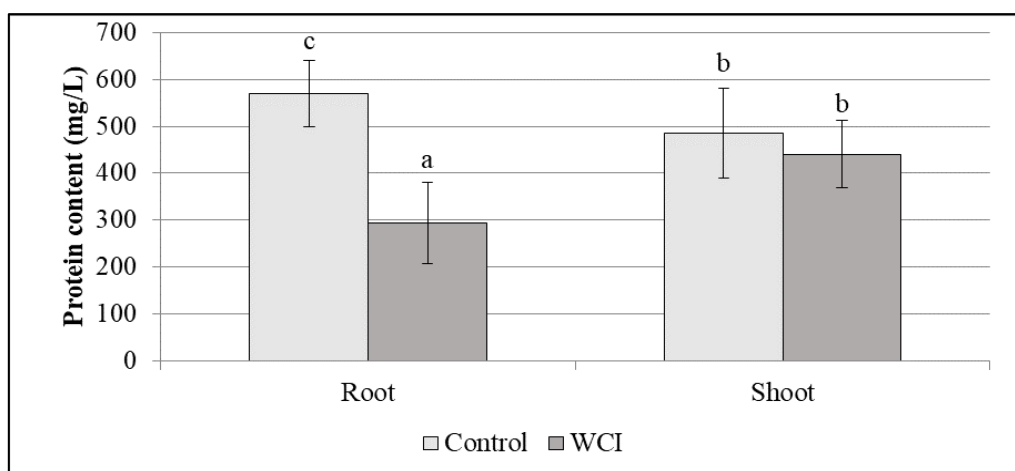


15. Figure: Effect of Woolly cupgrass (WCI) on the POD activity of the different parts of the Armagnac corn. Different small letters indicate significant differences: ($p < 0.05$).

Effect of Woolly cupgrass on the protein content of the Armagnac corn

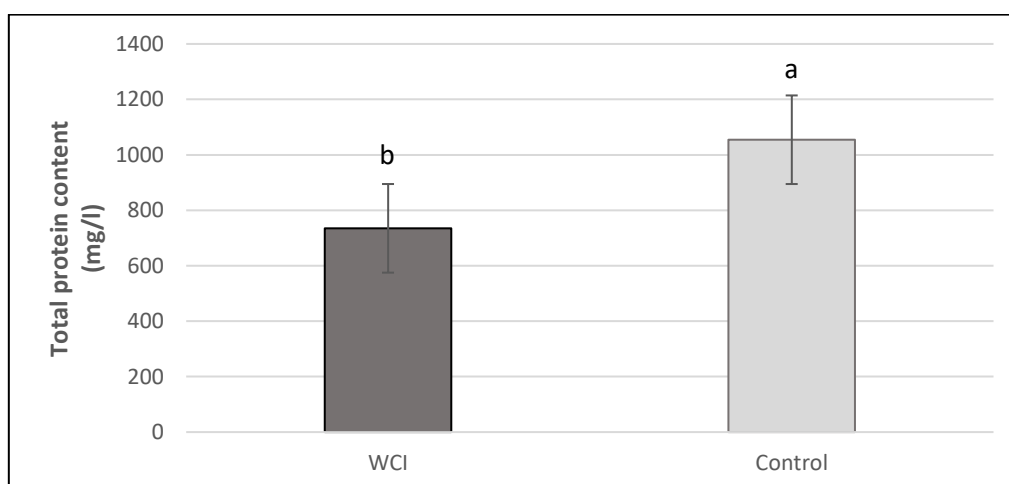
During the investigation, the protein content of the corn plants was also examined. The results obtained point out that the plants grown together with Woolly cupgrass have a decreased

protein content, compared to the control plants. Decrease was more intense in the root, which also deviated significantly from control. In the control plant, protein content was higher in the root, than in the sprout (*Figure 16.*).



16. Figure: Effect of Woolly cupgrass (WCI) on the protein content of the Armagnac corn (measured on day 21)

The total protein content was also examined, where the results obtained, were similar to the results of the sprout and root assessment. The total protein content of the corn grown together with Woolly cupgrass was lower, which significantly differ from the control plants (*Figure 17.*).



17. Figure: Effect of Woolly cupgrass (WCI) on the total protein content of the Armagnac corn (measured on day 21)

3.3 Possibilities of biologically control against Woolly cupgrass

Growing the cleaned bloom of fungi on PDA substrate the morphology of fungi, and morphologic features of the macroconidia of conidic stroma, clearly indicate the species *Fusarium culmorum* (Wm.G. Sm.) Sacc. The samples of Woolly cupgrass collected, were taken at the end of the growing season. Other than the mycelial mat on the roots of the weed, no other disease symptoms were detected, which makes it likely, that the fungi *Fusarium culmorum*, as a saprophytic pathogen, was present in the rhizosphere of the weed.

4. New and innovative results of the dissertation

1. I conducted a field investigation in the Hajdúság sub-region, regarding the dispersal of Woolly cupgrass. For geostatistical evaluation, I fitted an UTM grid system on the sub-region. Since 2016, I investigated 315 UTM grids (2.5 x 2.5 km size), detected the intense dispersal of the weed species, after the (locally initial) detection in 2011. **I identified, that newly found populations are more than 20km away from the original site.**
2. In order to find out the reasons for intense dispersal, I conducted propagation dynamics monitoring, and feeding studies were also set up. Based on their results, it is likely that the reason of the rapid dispersal is **primarily the random spreading by cultivating and harvesting equipment.** Wind and animals have a secondary importance in dispersal. However, this fact emphasizes the necessity of informing and explaining to agricultural producers of the areas involved.
3. I investigated the allelopathic effect of water-based (4% and 8%) extracts, prepared from different parts of Woolly cupgrass. I found that the most powerful inhibition comes from the sprout extracts. The sprout and root extracts (at both concentrations) took a powerful inhibiting effect on the germination of **sunflower achenes**. In the case of **lettuce**, even the extracts prepared from the spiculum showed a significant germination inhibiting effect. At the same time, in the case of **corn seeds**, I was unable to verify statistically, the germination inhibiting effect of the examined solutions (root, sprout, spiculum).
4. In order to exactly characterise the allelopathic effect of Woolly cupgrass, I determined the juglone index of each aqueous extract (5% and 10%). During experiments, I compared the effect of 1mM solution of clean juglone, with that of the aqueous extracts, prepared from root and sprout, on white mustard test plant. The juglone index of the 10% root extract was 1.002, while the juglone index result of the 10% sprout extract was 1.078. **In both cases it was verified, that the above extracts have a stronger allelopathic effect, than walnut leaves.**

5. The allelopathic effect was clearly shown in the experiments of jointly growing Woolly cupgrass with corn. The **measurements made on corn test plants** verified, that 22% decrease of the plant height, and 31% decrease of the total biomass dry matter was measurable on the 21st day after hatching. The investigation of root development showed that all parameters (except root diameter) were significantly smaller than on control plants. All three experiments showed significant differences, compared to the control (no weeds) plants. It can therefore be concluded that **the development inhibiting effect of Woolly cupgrass is observable immediately after hatching.**
6. The combination of the plants resulted in significant biochemical variations in the test plant. The **decrease of the relative chlorophyll content** showed a value of 18.2%. By analysing the photosynthetic pigments i determined, that this is **mainly caused by significant decrease of chlorophyll-A (-15%) and carotenoids (-20%)**. It can be demonstrated, that **the quantity of proteins also decreased significantly in the roots of young maize plants**, when Woolly cupgrass was present.
7. Further biochemical tests verified the **significant increase of MDA (malondialdehyde) level, which characterises lipid peroxidation, in the root (+23%) and sprout (+71%) of the weedy crop.**
8. As far as the formation of antioxidant enzymes, I determined that **both APX (ascorbate peroxidase +28%) and SOD (superoxide dismutase +16%) increased significantly in the sprouts of the weedy corn.** At the same time the POD (guaiacol-peroxidase) enzyme level increased measurably in the roots, as a result of Woolly cupgrass being present.
9. During the field investigation, i succeeded (the first time in Hungary) to verify the presence of *Fusarium culmorum* in the rhizosphere of Woolly cupgrass. Between the pathogen and the host plant, there is a probable endophytic relation, which has to be confirmed by further investigation.

5. Practical application of the results

- Clarification of the potential ways of dispersal of the examined weed species, can substantiate the accurate transfer of knowledge to the agricultural producers. In my experience this new, potentially very dangerous, invasive weed species is practically unknown for agricultural producers. In order to prevent its further dispersal, and effective suppression, intense education, and the close cooperation between practice and research shall be established.
- Exploitation of allelopathy is increasingly a key issue in bioherbicide development. It is verified, that Woolly cupgrass is producing various allelochemicals. Some of these get to the soil, and get in touch with the roots (seeds) of other plants, while others probably take effect in gaseous state as well. The strongest inhibiting effect was shown in the extracts prepared from sprouts and roots of the weed. In both cases a stronger inhibiting effect was verified, than that of the walnut leaves, during the examination of crop seeds.
- The physiological response of young maize plants was established, in the case of close proximity to Woolly cupgrass. Growing these plants together showed significant differences in the quantity of photosynthetic pigments and some enzymes, in the particular corn hybrid. Such tests can provide sufficient indications to the research of resilience of hybrids, and can substantiate the breeding research aimed at minimizing early stress responses, and such selection of the hybrids in production.

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Publication list



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List of publications related to the dissertation

Hungarian book chapters (1)

1. Nagy, A., **Szilágyi, A.**: A fenyércirok (*Sorghum halepense* (L.) Pers.) lehetséges herbicidrezisztens biotípusainak levélfelületen becsült antociánosodás mértékének vizsgálata.
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18. Radványi, C., **Szilágyi, A.**, Szőke, L.: Az ázsiai gyapjűfű (*Eriochloa villosa* /Thunb./ Kunth) allelopatikus hatása a kukorica főbb morfológiai paramétereire.
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20. **Szilágyi, A.**, Radócz, L.: Egy új inváziós gyomnövény: az ázsiai gyapjűfű (*Eriochloa villosa* (THUNB.) KUNTH) helyzete Magyarországon.
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Total IF of journals (all publications): 15,412

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The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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