Original Paper

Effect of cover crops undersown in maize on the mycotoxin content in maize biomass

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The effect of growing maize with undersown crops on the content of mycotoxins in maize biomass was studied. Small plot experiments were conducted in 2019 on two sites with different soil and climatic conditions: Žabčice and Troubsko. Three treatments of intermediate crops (Italian ryegrass; Fodder vetch and a mixture of both) were undersown into the space between the rows of maize. The maize was harvested at a dry matter content of 35% at the Troubsko experimental site and 43% at the Žabčice experimental site. After the harvest of maize, samples of green biomass (shreddings) were dried at 60 °C and then analyzed for the content of mycotoxins such as deoxynivalenol (DON), aflatoxin (AF, L), and fumonisin (FUM). An average yield of maize shreddings ranged from 16.50 to 21.57 t/ha of dry matter within the individual treatment. The contents of mycotoxins from the sites differed in their statistical significance, and both experimental sites showed the lowest concentrations of AFL in maize shreddings while average concentrations of FUM and DON were always the highest. In most observations, treatments with the undersown crops reached the same values as the control treatment. Only in one treatment (mixture of Italian rye grass and Fodder vetch), an increase in the AFL content (by 0.3 μg/kg) was detected. Based on the performed analyses, it is possible to state that no adverse influence of undersown crops on the occurrence of mycotoxins in maize shreddings was recorded using the chosen methodology of cultivation. Exceeded limit values for the content of mycotoxins in feeds according to 2006/576/were not recorded.

Keywords: mycotoxins, silage, AFL, DON, FUM

1 Introduction

The EU policy of renewable resources has contributed to the increased production of biogas in biogas plants (BPS). Thanks to this, the European Union has become a leader in biogas production, which has not only economic but also environmental and climatic benefits (Scarlat et al., 2018). The most important energy crop for the anaerobic digestion (AD) is maize (*Zea mays* L.), which is the main reason for extending fields with this crop (Britz and Delzeit, 2013).

Vasileiadis et al. (2011) inform that a widespread use of maize led to the development of systems based only on the production of maize, denoted as maize-based cropping systems (MBCS) which can have a potentially adverse effect on the environment. New technologies for the production of maize shreddings and their impact on biogas production as well as a potential effect on the environment were verified for example by Vítěz et al. (2020). In order to achieve high production of methane, it is possible to use a system of growing two or more crops at one place simultaneously (Brooker et al., 2015; Kintl et al., 2020), i.e., to use the principles of mixed culture (intercropping). Brooker et al. (2015) claim that mixed cultures consist mainly of representatives from the

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Poaceae and Fabaceae families. The technology of mixed culture in the combination of maize and white-sweet clover (*Melilotus alba*; WSC) for biogas production was described by Kintl et al. (2020).

The AD depends on microbial communities (MCS) which transform complex organic waste to biogas (. Nevertheless, the choice of substrate mixture for the stable AD is not trivial as it requires experience and technical knowledge about the process (García-Gen et al., 2014). A certain limitation in using legumes can be represented by the higher content of nitrogen in the biomass of leguminous plants, which could hamper biogas production (Pop et al., 2015). On the other hand, Hutňan et al. (2010) claim that the AD process is unstable due to the low N content in the maize silage, and in order to stabilize it, they recommend to add a substrate with a higher N content, which favors just legumes. A significant influence on the overall status of MCS in AD could be that of coumarin present in WSC or in other legumes because its presence leads to AD inhibition (Popp et al., 2015; Kadaňková et al., 2019). The content of coumarin in silage corresponds to the percentage of WSC in silage produced from a mixed culture (Kadaňková et al., 2019) and can be reduced by reducing the amount of WSC shreddings used in the silage. Popp et al. (2015) inform that MCS have to become used to the presence of coumarin in biogas plants during the AD, and thus the technology of mixed culture cannot be used by fits and starts but over a long time only (Kintl et al., 2020; Huňady et al., 2021). It is not only the content of nutrients that is used in evaluating the total production of biomass but also the health condition of plants. One of possible health risks is the occurrence of mycotoxins as the secondary metabolites of various fungi such as *Alternaria*, *Aspergillus*, *Penicillium* and *Fusarium* (Skladanka et al., 2011).

Under certain conditions, dangerous metabolites called mycotoxins arise in the infested plant biomass. These may further spread from the infested plant biomass into subsequent products, e.g. silage. Fungi of the above-mentioned genera produce a range of mycotoxins of which the most known and most dangerous are zearalenon, deoxynivalenol and fumonisins (Driehuis, 2013). Mycotoxins have a great amount of adverse impacts on the health of both animals and humans. The most serious of them are generally the loss of appetite, vomiting, impaired conversion/availability of essential nutrients, carcinogenic, teratogenic, nephrotoxic and hepatotoxic effects (Driehuis, 2013). While the use of maize contaminated with mycotoxins for feeding mammals, poultry or fish can cause manifestations of toxicity, Giorni et al. (2018) assume that using contaminated maize in the production of biogas is a good alternative. There are already several hundreds of mycotoxin species (over 400) described in detail by now, which are produced as secondary metabolites by the above-mentioned microorganisms – fungal pathogens (Driehuis, 2013). The pathogens occur very often on the host plants at different stages of development and are for the most part a natural component of the soil ecosystem. Their complete elimination is therefore not possible for example at growing maize biomass and in the subsequent production of maize silage. Nevertheless, measures are necessary to be taken in order to prevent their excessive spreading (Driehuis, 2013). It is also necessary to check their content in plant products such as silage and use the contaminated products for purposes other than feeding animals (Giorni, 2018).

The primary goal of the research was to assess the potential influence of plant species from the families of Fabaceae and Poaceae sown together with maize on the presence of mycotoxins in shreddings as a basis for further research. The secondary goal was to find out whether the detection of mycotoxins before the harvest can potentially affect the decision on using the maize biomass in biogas plants.

2 Material and methods

2.1 Description of field experiment

In our experiment, we studied the issue of growing maize with undersown crops. Small plot experiments (6 × 3 m) were conducted in 2019 on two sites with different soil and climatic conditions: Žabčice and Troubsko (Figure 1). Three treatments of intermediate crops were undersown into the space between the rows of maize (*Zea mays* L.) including a control treatment without the undersowing in four replicates (Table 1).

Figure 1 Two sites in the Czech Republic (Troubsko and Žabčice) where the small plot experiments took place

In 2018, a preceding crop on the sites of Troubsko and Žabčice was winter wheat (*Triticum aestivum* L.). When it

Table 1 Species and varieties of plants undersown in maize

Treatment	Species	Variety	Sowing rate (kg/ha)
Control	without undersowing	–	–
MC 1	Italian ryegrass (*Lolium multiflorum* Lamk.)**	Svatava	18
MC 2	fodder vetch (*Vicia villosa* Roth)*	Latigo	90
MC 3	fodder vetch (*Vicia villosa* Roth)*	mixture	12.5
	hybrid ryegrass dipl. (*Lolium × hybridum*) **	Soufflet	12.5

* Selgen a. s. (owner of the variety); ** Oseva PRO s. r. o. (owner of the variety); MC – mixed culture

was harvested, stubble tillage and plowing lowed. Prior to the sowing of maize, urea (46% of N) was applied at both sites at a dose of N 120 kg/ha. Before the sowing of maize, the soil on experimental sites was prepared by smoothing harrow. Walterinio (FAO 280; KWS SAAT SE & Co. KgaA) hybrid of maize (*Zea mays* L.) was sown using the Kinze 3500 (Kinze Manufacturing, Williamsburg, IA, USA) seeding machine into a depth of 8 cm at a row spacing of 75 cm in the amount of 80 thousand individuals per hectare. Before emergence, STOMP AQUA herbicide (pendimethalin as an active substance; manufacturer BASF Ltd.) was applied. The undersown crops were seeded in the BBCH 14–15 maize development stages (Table 2). Stands of undersown crops were established using an experimental seeding machine which seeded four inter rows of 0.3 m in width. The distance of undersown plants from each row of maize was 21.5 cm. All three experimental treatments were established in the same way, which is presented in Appendix A illustrating the establishment of one replication of one experimental treatment. All treatments had four replications, and the method of sowing and fertilization was similar on the two sites: Žabčice and Troubsko.

Table 2 Dates of sowing maize and undersown crops on the two sites

Sowing	Žabčice site	Troubsko site
Maize	10 April 2019	11 April 2019
Undersown crops	31 May 2019	3 June 2019

2.2 Site characteristics

2.2.1 Žabčice

The experiment was established on the Field Experimental Station of Mendel University in Žabčice. The site is situated

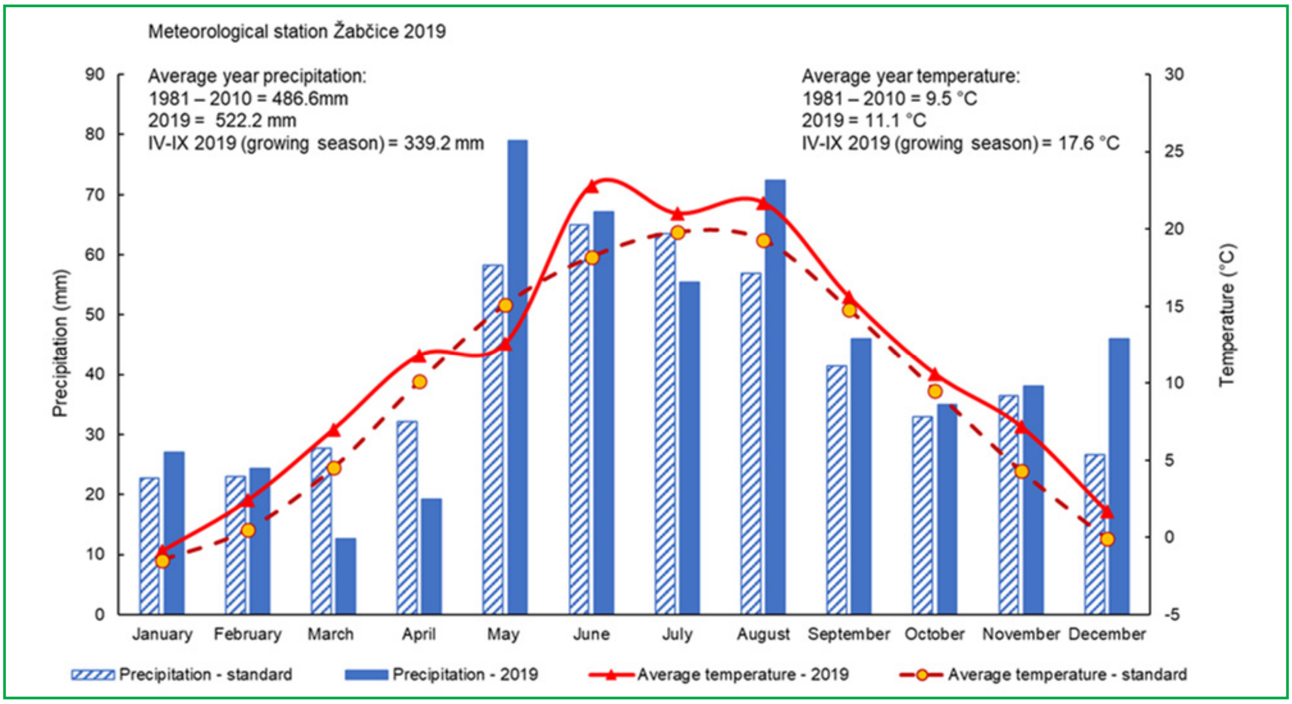

Figure 2 Climate diagram in Žabčice for 2019. Long-term standards for the Žabčice area were calculated on the basis of data from 1981 to 2010
Data provider: Czech Hydrometeorological Institute; http://portal.chmi.cz/historicka-data/

in the south of Moravia, ca. 30 km south of Brno, in the maize production area with a mean annual precipitation amount of 487 mm and a mean annual temperature of 9.5 °C . The region is characterized according to the World Reference Base, by the loamy clay soil of Fluvisol type. In terms of climatic conditions, the growing period of 2019 was slightly above-average compared with the long-term precipitation and temperature means. The total precipitation was higher by 22 mm (107% of the long-term total amount) and the mean air temperature was higher by 1.4 °C (108% of long-term average) (Figure 2).

2.2.2 Troubsko

The experiment was established in ZVT (Agricultural Research Troubsko (South Moravia, ca. 10 km from Brno). As to agro-ecological classification, the experimental area is situated in an agricultural region typical of sugar beet production. In terms of climatic conditions, the region is mildly warm, mildly dry, with an average altitude of 287 m a. s. l., mean annual temperature 8.95 °C and long-term mean annual precipitation amount 525.6 mm (the values correspond to the climatic normal of years 1981–2010). Parent rock is loess and loess loam of the Bohemian Massif, according to the World Reference Base, the soil type is Haplic Luvisol. Climatic conditions were slightly above-average during the growing season of 2019 with the total precipitation amount higher by 3.8 mm (101% of climatic normal in 1981–2010) and the mean air temperature higher by 1.8 °C (119.6% of average in 1981–2010) (Figure 3).

2.3 *Biomass sampling and determination of mycotoxins*

The above ground biomass production of inter-crops was collected by hand at a height of 5 cm above the ground during the maize growth stage BBCH 77–83 (early milk to early wax ripeness) from an area of 0.09 m^2 (0.3 × 0.3 m) from each treatment in 4 replications. The biomass was washed, dried at 60 °C to constant weight, and weighed. The amount of maize biomass produced was determined by the direct sampling of plants from the respective experimental treatments according to the methodology by Loučka et al. (2014). For the purposes of laboratory analyses, the maize biomass was harvested at a stubble height of 10 cm, whole plants were chopped into shreddings (15–20 mm) using Deutz-Fahr MH 650s (Deutz-Fahr, Lauingen, DEU) cutter (Appendix B). It is a mounted one-row cutter with the feeding device, cutting mechanism with 12 knives and a sweeper (Kintl et al., 2020). On the Troubsko and Žabčice sites, the maize biomass was harvested at DM 35% (BBCH 77–83) and DM 45% (BBCH 83–85), respectively. A mixed sample of fresh chopped maize was collected from each repetition of individual treatments. The principle of maize harvest including the collection of individual samples is shown in Appendix B.

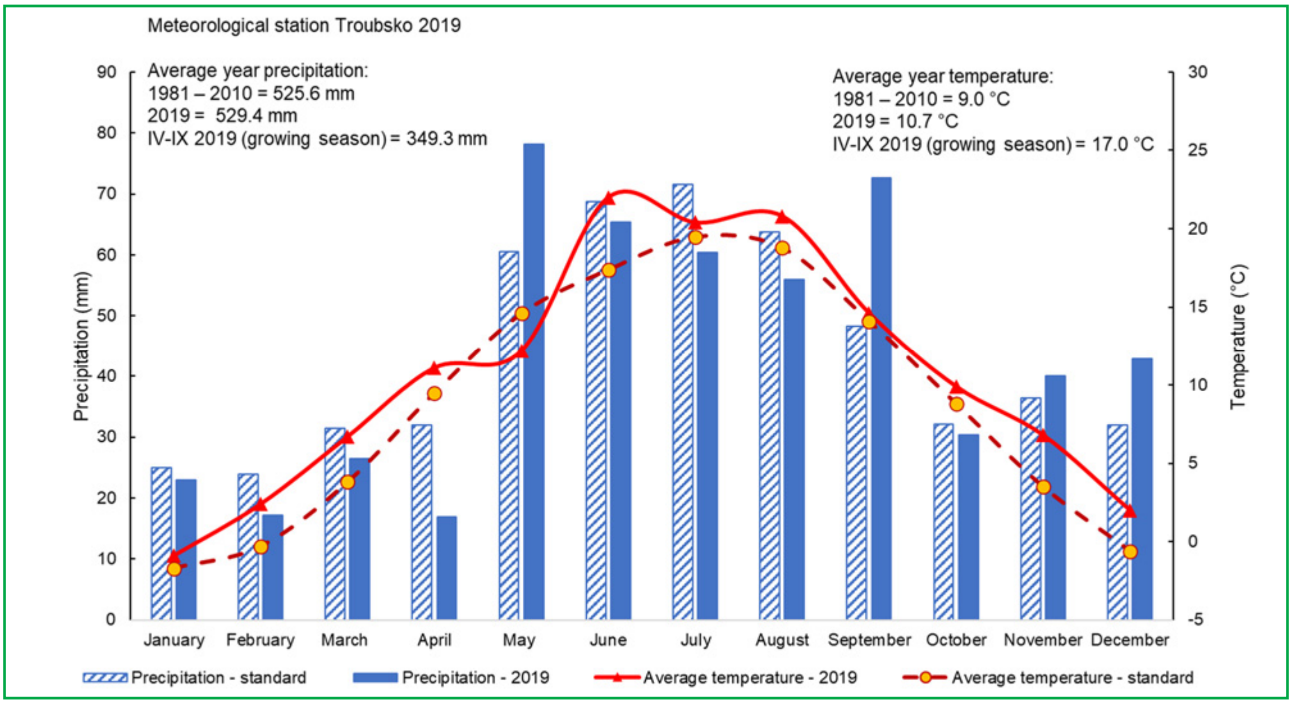

Figure 3 Climatic diagram in Troubsko for 2019. Long-term standards for the Troubsko area were calculated on the basis of data from 1981 to 2010
Data provider: Czech Hydrometeorological Institute; http://portal.chmi.cz/historicka-data/

Samples of green biomass (shreddings) were dried at 60 °C, ground to a particle size <1 mm, and then analysed for the content of mycotoxins such as deoxynivalenol (DON), aflatoxin (AFL) and fumonisin (FUM) using the enzyme-linked arian-sorbent assay (ELISA) according to Skládanka et al. (2011). ELISA is a competitive, direct enzyme test for the quantitative analysis of plant biomass for the content of mycotoxins. Concentrations of individual toxins were expressed in micrograms per kg of plant biomass (shreddings).

2.4 Statistical processing of data

The measured values were subjected to statistical assessment at a significance level of $P < 0.05$. At first, the exploratory data analysis (EDA) was performed to determine the symmetric division of the data. Then, the one-factor ANOVA analysis was made, which was followed by the post-hoc Tukey´s HSD test. In addition, the measured data were subjected to the correlation and personal component analysis (PCA). All analyses and subsequent graphical data processing were implemented in the R program.

3 Results and discussion

3.1 Žabčice

The average yield of maize shreddings (Table 3) on the site ranged from 16.50 to 19.59 t/ha in dry matter (DM) and from 37.59 to 45.95 t/ha in green matter (GM) with the average dry matter being 43.46%. Although the measured values showed a relatively wide range, no significant differences were found between the individual experimental treatments, which holds for the yield of both DM and GM.

In addition to the yield of maize shreddings, we also monitored the yield of individual inter-crops on the Žabčice site (Table 4). The yields of inter-crops were more variable than the yield of maize biomass. The highest yields were recorded in *Vicia villosa* in treatments MC 3 (0.29 t of DM and 0.81 t of GM per hectare) and *Lolium multiflorum* (0.26 of DM and 0.69 t of GM per hectare) as compared with MC 2. We also analyzed the relation between growing inter-crops and maize biomass yield in treatments MC 1–MC 3 using the factor analysis (Table 5); measured values are illustrated by the correlation matrix. No dependence was found between the growing of the above-mentioned inter-crops and the yield of maize.

As mentioned above, the dependence between maize yield and inter-crops was analyzed to reveal a possible influence of growing inter-crops on the yield of the main crop. No negative or positive influence was recorded (Table 5) when the yields of inter-crops and maize (GM and DM) were analyzed. The correlation matrix showed (as expected) dependence between the yields of GM and DM in the respective crops.

However, we failed to demonstrate that those higher yields of inter-crops would mean lower yields of maize. PCA results are presented in Appendix C and Appendix D. The measured data show again that there was no dependence between the yields of inter-crops and maize. Further on, two main factors were identified (Dim1 43% and Dim2 38%) covering more than 80% of the variability of measured values, whose action on

Table 3 Yield of maize shreddings

Treatments	Yield of dry matter		Yield of green matter	
	t/ha	±SD	t/ha	±SD
Control	18.90	3.87a	43.05	6.43a
MC 1	17.81	1.15a	40.56	2.9 a
MC 2	19.59	7.35a	45.95	15.57a
MC 3	16.50	1.69a	37.59	3.66a

MC 1 – *Lolium multiflorum*; MC 2 – *Vicia villosa*; MC 3 – *Vicia villosa* and *Lolium × hybridum*. Different lowercase letters indicate a significant difference between the respective treatments ($P < 0.05$; post-hoc Tukey´s HSD test)

Table 4 Yield of inter-crops

Treatments	Yield of dry matter		Yield of green matter	
	t/ha	±SD	t/ha	±SD
MC 1	0.26a	0.022	0.69a	0.054
MC 2	0.16b	0.003	0.46b	0.015
MC 3	0.29a	0.025	0.81a	0.081

MC 1 – *Lolium multiflorum*; MC 2 – *Vicia villosa*; MC 3 – *Vicia villosa* and *Lolium × hybridum*. Different lowercase letters indicate a significant difference between the respective treatments ($P < 0.05$; post-hoc Tukey´s HSD test)

Table 5 The relationship between the yield of green and dry matter of plant biomass of inter-crops and maize

Treatments	Yield of intercrops (t/ha) – green matter	Yield of inter crops (t/ha) – dry matter	Yield of maize (t/ha) – green matter	Yield of maize (t/ha) – dry matter
Yield of intercrops (t/ha) – green matter	1.00	**0.98**	-0.04	-0.09
Yield of inter crops (t/ha) – dry matter	**0.98**	1.00	-0.06	-0.10
Yield of maize (t/ha) – green matter	-0.04	-0.06	1.00	**0.99**
Yield of maize (t/ha) – dry matter	-0.09	-0.10	**0.99**	1.00

values of pearson correlation coefficient (*r*) are shown. Bold values indicate significant dependence between the individual variables at a significance level of $P < 0.05$

the respective variables was balanced. However, the factors did not act identically on the concentration of mycotoxins in the biomass of maize and on the yields of maize and inter-crops. This is also confirmed by the biplot graph (Appendix D) which indicates that the influence of individual grown inter-crops on the yield of maize was minimal, and was also variable as to the content of mycotoxins in the maize silage. Dim1 for example had no influence on the yield of maize but strongly correlated with the yield of inter-crops. On the other hand, Dim2 exhibited a strong correlation with the yield of maize and strongly adversely correlated with AFL and weakly with DON. In contrast, Dim1 correlated with the content of FUM. This shows that the content of mycotoxins in the maize silage was influenced on the Žabčice site by more factors, not only by inter-crop species.

On both research sites, the content of mycotoxins was assessed in maize shreddings prepared for the process of ensiling. The assessment was focused on the concentrations of AFL (Figure 4 a), DON (Figure 4 b) and FUM (Figure 4 c). The lowest concentrations were recorded in the AFL mycotoxin (Figure 4 a); its average content across the treatments was 1.15 μg/kg. The lowest AFL content was recorded in the treatment with the undersown *Lolium multiflorum*; the other treatments exhibited similar AFL concentrations, i.e., about 1.20 μg/kg. Thus, the measured AFL concentrations were very even. Differences between the individual treatments were minimal and therefore statistically non-significant.

Another determined mycotoxin was DON whose mean concentration in the maize shreddings from individual treatments was highly variable. While the lowest values were hitting the boundary of 200 μg/kg, the highest value surmounted 1,000 μg/kg. This concentration of DON was recorded in the control treatment with no undersown plants. The lowest DON values (≤200 μg/kg) were recorded in treatments with the undersown plants (*Lolium multiflorum*; *Vicia villosa*; *Vicia villosa* and *Lolium* × *hybridum*). The measured values indicated minimum DON concentration differences in maize shreddings gained from the stand with the undersown plants and a difference when compared with the control treatment. It should be pointed out, however, that the differences were not significant. The last monitored mycotoxin was FUM (Figure 4 c). Its concentration in maize shreddings fluctuated similarly as in the above two mycotoxins (Figure 4 a and b). Compared with them, the average FUM content in maize shreddings was the second highest after DON with its values ranging from

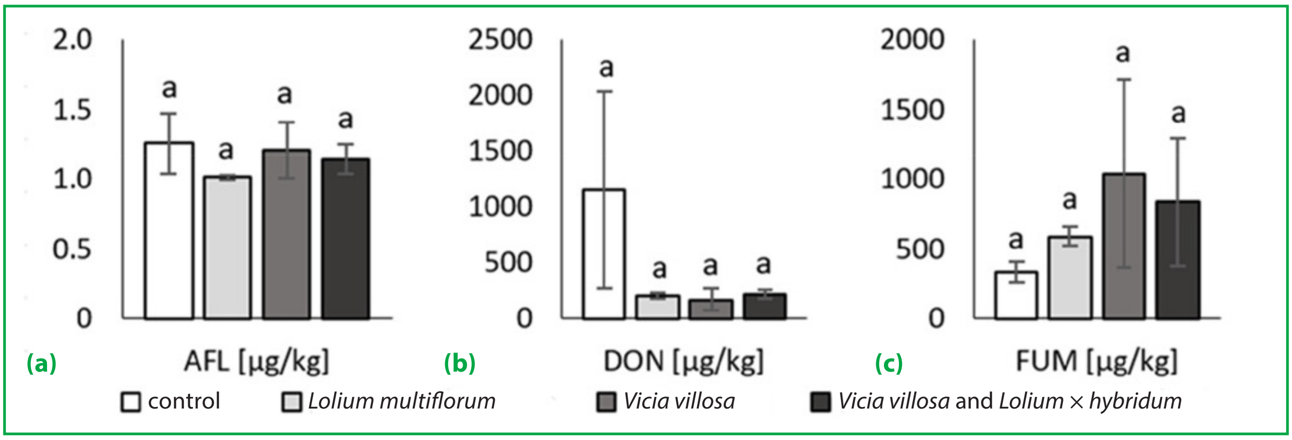

Figure 4 Contents of AFL (a), DON (b), and FUM (c) in the maize silage of individual experimental treatments. Different lowercase letters indicate a significant difference between respective treatments
$P < 0.05$; post-hoc Tukey´s HSD test

300 to 1,000 µg/kg. Once again, in spite of relatively high differences between the individual treatments, no significant differences were recorded. The lowest value was determined in the control treatment (337 µg/kg) and the highest value was recorded in the treatmentwith the undersown *Vicia villosa* (1,041 µg/kg). Similarly, as in the DON mycotoxin, the values measured in the individual treatments exhibited an increased variability and hence a lower significance in terms of statistical exploration.

3.2 Troubsko

On the experimental site of Troubsko, the average yield of both dry and green mass was determined after the harvest of maize shreddings similarly as on the Žabčice site (Table 6). Average values of the yield of maize shreddings fluctuated from 16.42 to 21.57 t/ha in the dry mass and from 45.60 to 57.60 t/ha in the GM with an average DM content of 36.43%. The measured values indicate that the highest yield (t/ha) of DM and GM mass was recorded in the control treatment and MC 2 treatment. The other treatments exhibited a pronounced drop in the yield of maize biomass. Differences between these treatments were non-significant though (Table 6).

In addition to the yield of maize shreddings, also the yield of respective inter-crops was monitored on the Troubsko site (Table 7). Compared with the Žabčice site, the yield of individual inter-crops was more variable and different, which is demonstrated also by more statistically significant differences. As compared with the other treatments, the highest yield (*P* <0.05) was achieved in MC 2 where the inter-crop (*Vicia villosa*) reached a DM yield of 0.76 t/ha and a GM yield of 3.72 t/ha. The yield of other crops was then significantly lower in the following order: MC 2 >MC 3 (0.59 t of DM per ha) >MC 1 (0.38 t of DM per ha). Thus, a difference between the measured values was greater than 30%.

Similarly, as on the Žabčice site, the relation was analysed between the yield of maize biomass and the yield of inter-crop biomass (GM and DM). The correlation matrix (Table 8) illustrates similarly as in the case of Žabčice site (Table 5) that no positive or negative relation was found between the yield of inter-crop and yield of maize. The expected dependence between the yield of GM and DM was demonstrated again, which was greater than *r* >0.9. PCA results (Appendix C and D) summarize the absence of the influence of growing inter-crops on the yield of maize as the main crop. Further on, two main factors were identified, similarly as on the Žabčice site, which explain a significant part of the variability of measured values (>70%). The first factor (Dim1 – 52%) positively correlated with the content of AFL and with the yield of inter-crops, but negatively correlated with the concentration of DON. The second factor (Dim2 – 22%) negatively correlated with the content of mycotoxin DON, and positively correlated with the yield of maize and the content of FUM. Based on the PCA results, it is not possible to determine unambiguously whether the measured values of mycotoxin contents were affected by the growing of inter-crops; the influence of other factors (probably meteorological conditions) is apparent. Nevertheless, no effect of grown inter-crops on the yield of maize was observed.

Table 6 Yield of maize shreddings

Treatments	Yield of dry matter		Yield of green mass	
	t/ha	±SD	t/ha	±SD
Control	20.42	1.26a	55.20	3.39a
MC 1	16.42	2.42a	45.60	6.73a
MC 2	21.57	6.01a	57.60	16.04a
MC 3	17.55	2.39a	49.80	6.77a

MC 1 – *Lolium multiflorum*; MC 2 – *Vicia villosa*; MC 3 – *Vicia villosa* and *Lolium × hybridum*. Different lowercase letters indicate a significant difference between respective treatments (*P* <0.05; post-hoc Tukey´s HSD test)

Table 7 Yield of inter-crops

Treatments	Yield of dry matter		Yield of green matter	
	t/ha	±SD	t/ha	±SD
MC 1	0.38c	0.029	1.33c	0.168
MC 2	0.76a	0.041	3.72a	0.127
MC 3	0.59b	0.043	1.91b	0.120

MC 1 – *Lolium multiflorum*; MC 2 – *Vicia villosa*; MC 3 – *Vicia villosa* and *Lolium × hybridum*. Different lowercase letters indicate a significant difference between the respective treatments (*P* <0.05; post-hoc Tukey´s HSD test)

Table 8 Relationship between the yields of green and dry matter of plant biomass of inter-crops and maize

Treatment	Yield of intercrops (t/ha) – green matter	Yield of inter crops (t/ha) – dry matter	Yield of maize (t/ha) – green matter	Yield of maize (t/ha) – dry matter
Yield of intercrops (t/ha) – green matter	1.00	**0.99**	-0.45	-0.28
Yield of inter crops (t/ha) – dry matter	**0.99**	1.00	-0.53	-0.37
Yield of maize (t/ha) – green matter	-0.45	-0.53	1.00	**0.91**
Yield of maize (t/ha) – dry matter	-0.28	-0.37	**0.91**	1.00

values of pearson correlation coefficient (r) are shown. Bold values indicate significant dependence between the individual variables at a significance level of $P < 0.05$

Furthermore, contents of mycotoxins AFL, DON and FUM (Figs 5 a–c) were assessed in maize shreddings prepared from the plant matter grown with/without the selected undersown plants. The contents of respective mycotoxins in the maize silage considerably fluctuated. The highest values were recorded in FUM (on average over 250 µg/kg) and the lowest content was measured in AFL, the concentration of which did not exceed 1 µg/kg in the shreddings from individual treatments. Vbn Thus, the measured values of AFL can be considered as the absolutely lowest ones in respect of determined mycotoxins (DON and FUM). On the other hand, the content of this mycotoxin in the maize shreddings showed significant differences ($P < 0.05$). The control treatment contained demonstrably the lowest AFL concentration as compared with the treatmentin which maize was grown with two undersown crops, i.e. *Vicia villosa*, and *Lolium* × *hybridum*. In contrast, the remaining two treatments with the undersown either only *Lolium multiflorum* or *Vicia villosa* contained less AFL at a level comparable with the control treatment. The average content of DON in the maize silage ranged from 105 to 270 µg/kg. In spite of a wide range of values measured between the respective treatments, no influence of undersown crop on the occurrence of DON in the maize silage was observed.

Further on, the concentration of FUM in the maize silage was assessed. Compared with AFL and DON, this mycotoxin exhibited the highest occurrence value. Its lowest concentration (<250 µg/kg) was recorded in treatments with the undersown *Lolium multiflorum* and with the combination of *Vicia villosa* and *Lolium* × *hybridum*. The demonstrably highest presence of FUM was found out in the treatment with *Vicia villosa*. The control treatment did not show significant differences as compared with the other treatments.

3.3 *Plant biomass production*

The values of yield measured on both sites (Table 3 and Table 6) do not indicate an influence of growing maize together with the other undersown crops on its yield. No demonstrable influence of undersown crops on the yield of the main crop was found on any of the experimental sites. The measured values show (as expected) there is a dependence between the yields of green and dry matter in the respective crops. However, we failed to demonstrate that the higher yields of inter-crops would mean the reduced yield of maize (Tables 5 and 8). This finding was not surprising as there are scientific studies (Mohammadi 2010; Youngerman et al., 2018) pointing out potential benefits of this system of growing maize.

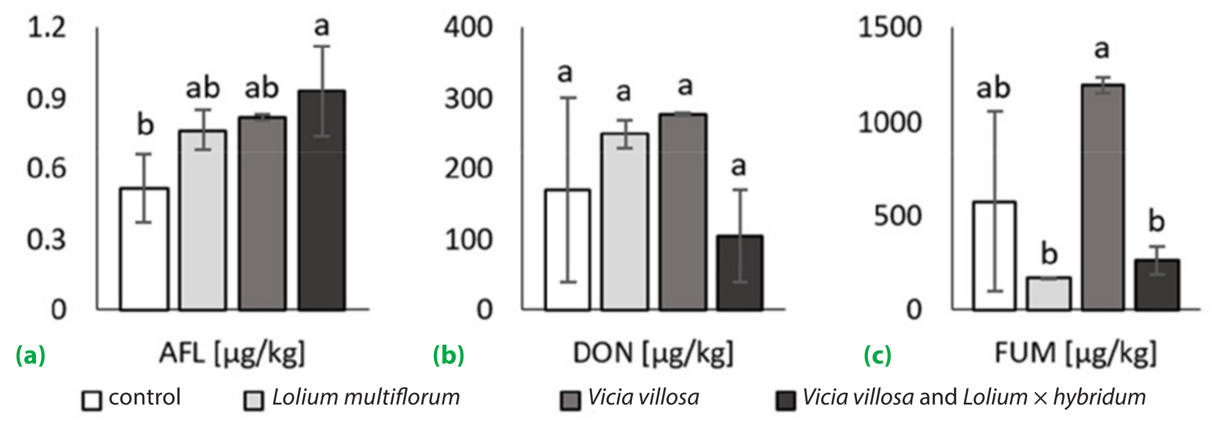

Figure 5 Contents of AFL (a), DON (b), FUM (c) in the maize silage of individual experimental treatments. Different lowercase letters indicate a significant difference between respective treatment
$P < 0.05$; post-hoc Tukey´s HSD test

As mentioned above, no influence of undersown crops was found on the yield of maize biomass, and the same result was reported by Youngerman et al. (2018). These authors found out that the system of undersowing is useful, particularly for enterprises applying environment-friendly management because it helps eliminate weeds. On the other hand, it can have a mildly negative influence on the yield of maize, which can be eliminated by reducing the amount of undersown plants. Using inter-crops as undersown crops can contribute to reduce the negative influence of growing maize on the soil environment and to enhancing soil fertility (Song et al., 2007). There is a risk however that the inter-crops may adversely affect the growth of the main crop. This adverse effect can be eliminated by selecting plant species whose growth is slower than that of the main crop (Xu et al., 2020) or by modifying their sowing rate. The lower density of the crop on the plot and the reduced sowing rates then result in reduced competitive pressure onto the main crop (Xu et al., 2020). In our experiment, the inter-crops were sown in strips and represented 22% from the area of one treatment. This is why they probably did not represent more serious competition for the main crop due to their lower density on the plot, and no negative correlation was found between the yield of inter-crops and the yield of maize (main crop).

The possibility of eliminating weeds in maize using undersown crops was also confirmed by Mohammadi (2010) who performed experiments directly with *Vicia villosa*. Moreover, Mohammadi (2010) claims that an optimal sowing rate should range around 50 kg/ha, as the quality of grown maize (number of seeds per hectare as well as total biomass) improved with such a sowing rate. In our experiment, the sowing rate was 90 kg/ha in the MC 2 treatment where it was grown as pure culture and 12.5 kg/ha in the MC 3 treatment where it was grown in combination with another crop; thus, it will be necessary to work also with lower sowing rates (if maize is grown as monoculture) in the follow up research. In addition to the positive influence on the yield of maize, a way opens to reduce weeds with using both undersown plants. However, a potential risk can be a higher density of the stand, which may result in an increased stand moisture content and hence to conditions favorable for the development of fungal infections. Another possible benefit is the use of legumes as undersown crops which can increase the content of nitrogen (through its biological fixation) in the soil, and thus to enhance the overall N balance in growing maize (Youngerman et al., 2018).

3.4 Content of mycotoxins in maize silage

The quality of maize grain and silage is monitored every year not only in the Czech Republic but also in other EU countries for infestation by fungi from the genus of *Fusarium* and *Aspergillus* and their subsequent contamination by mycotoxins. The reason is a negative influence of the presence of mycotoxins both on the health of animals and their persistence and possible transfer into milk used for human food (Driehuis and Elferink, 2000). In our experiment, maize shreddings were studied from two sites where maize was grown by the original and innovative technology. The reason for using the innovative technology (with new undersown crops) was an effort to reduce the impact of growing maize on arable land (Kabelka et al., 2021), namely the risk of water erosion. Apart from that, the method of growing maize affects also other parameters such as yield and quality of maize shreddings, i.e., also the content of mycotoxins in them (Drakopoulos et al., 2021).

The results of mycotoxin contents from the sites differed in their statistical significance. On the other hand, both experimental sites showed the lowest concentration of AFL in maize shreddings while average concentrations of FUM and DON were always the highest. According to Driehuis and Elferink (2000) and González-Pereyra et al. (2008), AFL occurs most frequently in the biomass for preparing silage but also in silages themselves, which is then often accompanied by DON, FUM and other mycotoxins (e.g., ochratoxin A and zearalenone). Although the concentration of AFLB1 form is often relatively low, the substance is highly toxic (Focker et al., 2021). If ingested, AFLB1 is metabolized in the body of a dairy cow and excreted in milk as AFLM1 of lower toxicity; however, it still represents a considerable risk of contamination for subsequent products. This is why the AFL values measured in our experiment have to be taken seriously in spite of the fact that the increased AFL concentration was found only in one treatment with undersown crops on one site as compared with the control treatment. Limit values are for example stipulated in European Commission Regulation no. 650/2010/EC with a max. Tolerable value of 5 µg AFLB1/kg for maize and rice which are to be sorted out or given another physical treatment prior to being used for human consumption as food ingredients. It follows from these values that the AFL concentrations established in our experiment cannot be considered dangerous.

According to Gallo et al. (2015), ensiled fodder crops can contain a mixture of mycotoxins originating from the preharvest contamination by *Fusarium* and *Aspergillus*. Cavallarin et al. (2011) inform that the content of AFL can increase when the silage is exposed to air during ensiling or during feeding. There are more authors (Bahrami et al.,

2016) who claim that depending on climatic conditions, AFLB1 in maize silage ranges from 0.68 to 4.57 µg/kg unless a non-standard situation would occur, which would create optimum conditions for the growth of aflatoxigenic molds. Such conditions are increased air humidity and temperature (Bahrami et al., 2016).

González-Pereyra et al. (2008) state that the average contents of DON and FUM in maize shreddings range around 150 or 600 µg/kg, respectively, with this value multiplied at least twice after the process of ensiling. This corroborates the transport of mycotoxins from biomass used in the production of silage further into the feed and hence into farm animal bodies. In our experiment, DON and FUM mycotoxins represented the most serious problem. Although the growing of maize together with undersown crops was not demonstrated to have an influence on their occurrence, their increased combinations were recorded on both experimental sites. European Commission Recommendation no. 2006/576/EC states that tolerable contents of DON and FUM in feed materials are 12 mg/kg (12,000 µg/kg) and 60 mg/kg (60 000 µg/kg), respectively. If we compare these values with the values measured in our research, it is obvious that the (recommended) limit values for the contents of these mycotoxins in biomass for preparing maize silage were not exceeded on any of the experimental sites. Similar conclusions were arrived at also by Drakopoulos et al. (2021) who studied the influence of inter-crops on the content of mycotoxins in winter wheat after maize as a preceding crop. They found out that the growing of inter-crops had the same influence on the reduction of mycotoxins in the plant biomass as for example ploughing (treatment without inter-crops), probably due to increased diversity of plant residues in the soil. This could explain why only minimal differences were recorded between the control treatment and the treatments with inter-crops (MC 1 – MC 3). It should also be pointed out that in our experiment, the inter-crops were grown in rows (strips) and represented only a small part of the seeded area as compared with the experiment conducted by Drakopoulos et al. (2021), but also with other experiments with inter-crops or cover crops (Šišić et al., 2018). This could have led to the lower potential positive effect on the reduced occurrence of mycotoxins in the soil and hence in the plant biomass.

The occurrence of mycotoxins in maize biomass and in subsequent silage is affected by a great number of factors. It was found out that most cereals (wheat, barley and maize) are prone to infections caused by the *Fusarium* and *Aspergillus fungi* (Šišić et al., 2018; Giorni et al., 2018; Ogunade et al., 2018). In the case that the assessment is focused on green biomass, the most significant factors are climatic and meteorological conditions (Leggieri et al., 2019). Some studies (Drakopoulos et al., 2021; Šišić et al., 2018) confirm the potential of inter-crops to reduce the occurrence of mycotoxins in the main crop. The potential however depends on how suitable the individual species of plants used as inter-crops are as alternative hosts for the genera of fungi producing mycotoxins (Šišić et al., 2018). This is why the choice of inter-crops with respect to their phytopathological risk exhibits an important property for reducing the occurrence of mycotoxins in the biomass of grown main crops (Skládanka et al., 2011; Šišić et al., 2018). Thus, it is possible to make a preliminary statement based on the recorded concentrations of mycotoxins in the conducted experiment that the selected plant species (*Lolium multiflorum*, *Vicia villosa* and *Vicia villosa* + *Lolium* × *hybridum*) exhibited good resistance to the occurrence of DON but lower resistance to AFL and FUM (on both sites). Another important aspect affecting the occurrence of mycotoxin pathogens are abiotic factors (Liu et al., 2016).

Warm and moist weather contributes to the development of fungal infections too (Liu et al., 2016). Another influence is that of post-harvest residues (biotic factors), i.e., the preceding crop species and the type of tillage, which directly influence soil aeration and soil oxidation processes (Liu et al., 2016). Our two experimental sites had the same preceding crop (winter wheat), which can explain the relatively low concentrations of mycotoxins (Drakopoulos et al., 2021). Winter wheat is generally considered of low risk as its residual biomass decays faster after harvest than for example the biomass of maize. Thus, no environment is created for the survival of fungi (Tillmann et al., 2017). If a stored fodder such as silage is assessed, then the quality of storage and ensiling process is very important, which has a crucial influence on the presence of mycotoxins in silage (Ogunade et al., 2018). The potential influence of precipitation and temperature on the experimental sites was not significant, the reason being similar total precipitation amounts and similar mean temperature in the period of the field experiment. In combination with the preceding crop of winter wheat, this can be one of factors why a more significant development of fungal infections and hence emergence of mycotoxins did not occur.

4 Conclusions

The research work deals with the influence of growing maize undersown with selected crops on the content of mycotoxins in maize shreddings for the preparation of silage. The attention was focused particularly on aflatoxins, fumosines and deoxynivalenol. A great part of the concentration of mycotoxins is produced already during the vegetation period and this is why the contents of mycotoxins in the primary biomass further used for the production of feeds have to be watched. Therefore, the concentrations of above mycotoxins will be studied in detail as the experiment continues in the following years. The authors are aware of the fact that results published here are the first ones from the multi-year experiment, and this is why they should be interpreted with caution.

Based on the performed analyses and measured values, it is possible to state that no adverse influence of undersown crops on the occurrence of mycotoxins in maize shreddings was recorded using the chosen methodology of cultivation. The fact that above-limit concentrations of the respective mycotoxins were not recorded in any of the experimental treatments does not mean that the mycotoxins should not be monitored in growing maize together with other undersown crops. Therefore, we recommend that mycological and toxicological analyses of plant biomass grown for the production of feeds become a part of the whole feed production process as a preventive measure. Elimination of mycotoxins is very difficult due to the different polarity of their molecules, which affects their physical and chemical inactivation (for example water solubility). It is therefore necessary to prevent the primary (from the source plant biomass – shreddings) as well as the secondary (during storage) silage contamination. A negative influence of growing inter-crops on the yield of the main crop has not been observed so far.

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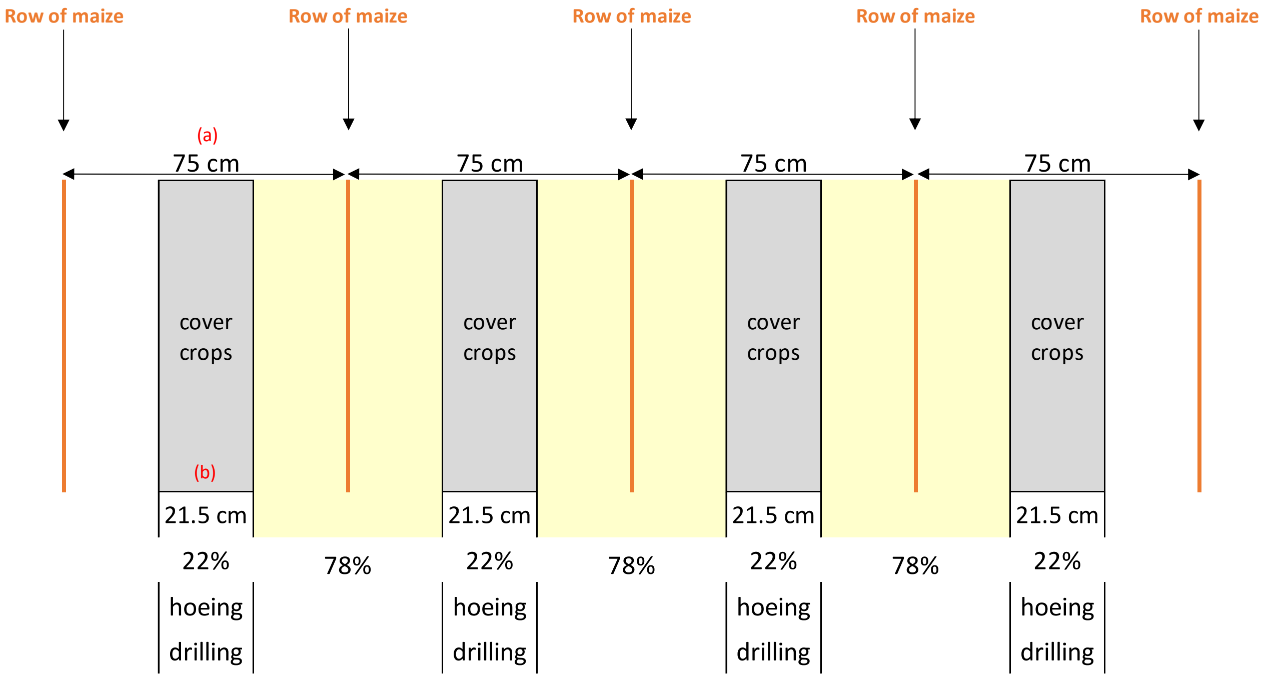
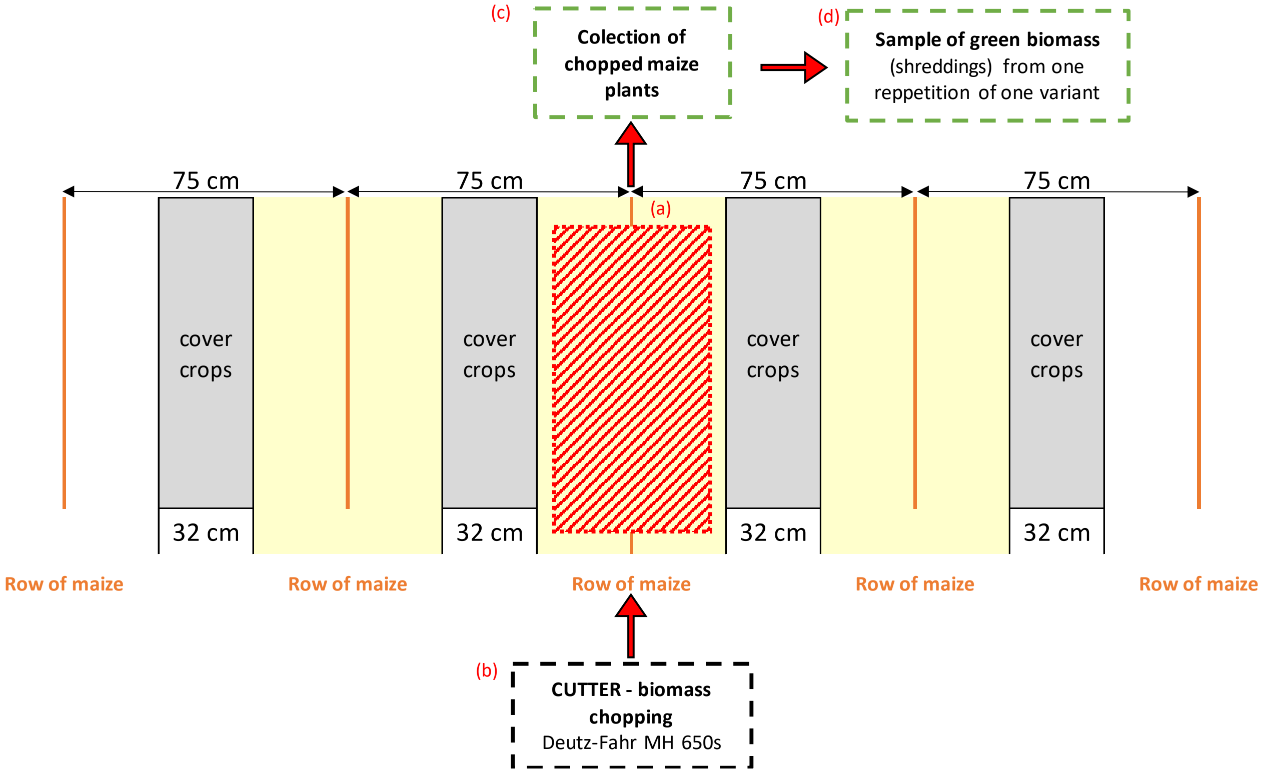


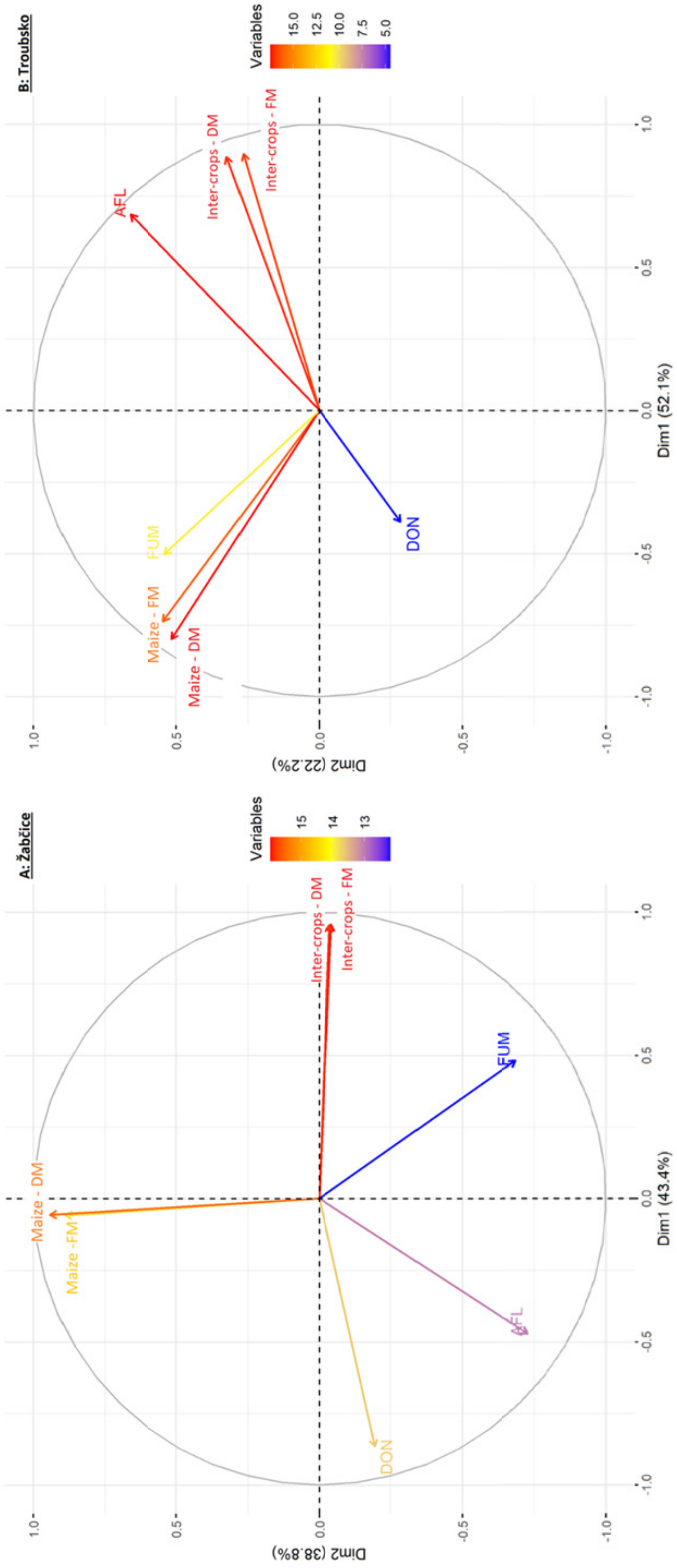
Appendix A

The scheme illustrates establishment of one experimental treatment. The inter-crops were undersown between the maize rows with the spacing of individual maize rows being (a) 75 cm. The undersown crops were seeded into the center of space between two maize rows (b), the strip of undersown plants was 21.5 cm wide and represented 22% of interspace area (b)



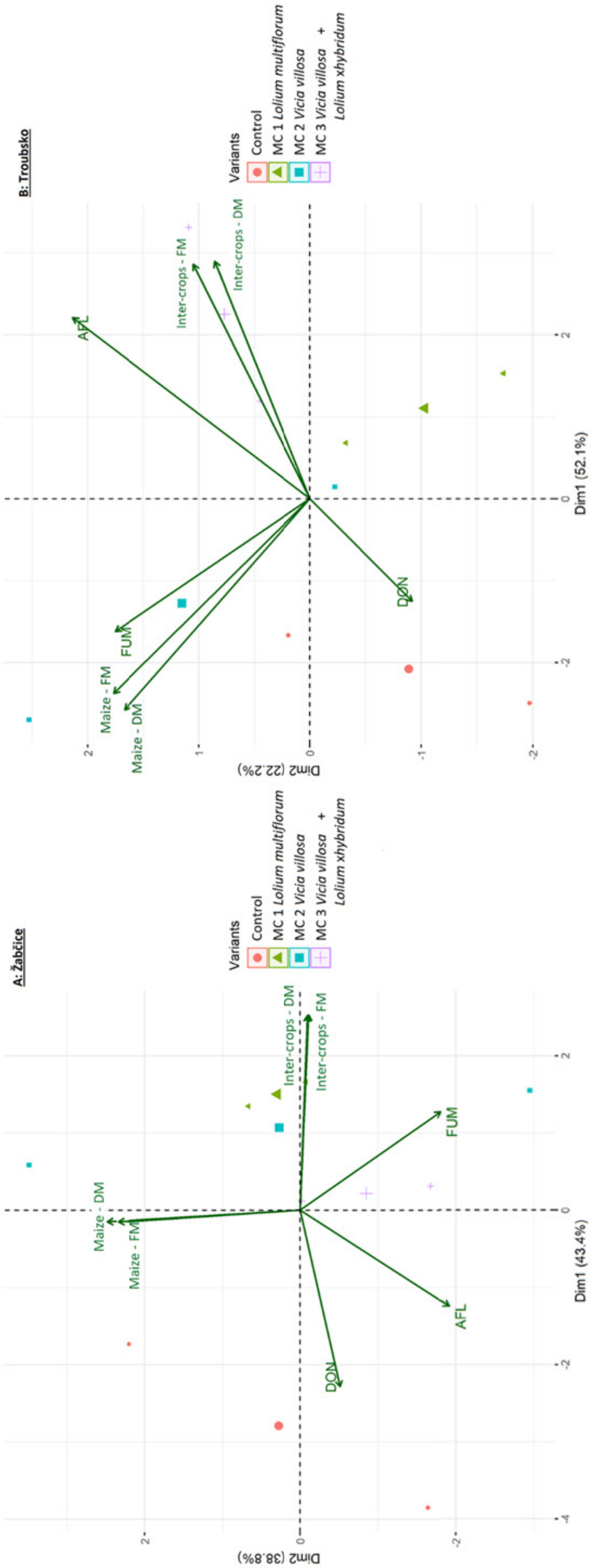
Appendix B

The scheme illustrates harvest of one repetition of individual experimental treatment. The maize stand was harvested at all times only in the middle part (a) of the respective repetition of each treatment. The part is highlighted with red hatching on the scheme. First, the selected plot of maize (a) was harvested using the Deutz-Fahr MH 650s cutter (b). The prepared maize shreddings were mixed (c). The homogenized matter was sampled (d) for laboratory analyses



Appendix C

PCA plot of variables on the correlation circle for two experimental sites A: Žabčice and B: Troubsko. Maize – DM = yield of maize dry matter; Maize – FM = yield of maize fresh matter; Inter-crops – DM = yield of all inter-crops dry matter; Inter-crops – FM = yield of inter-crops fresh matter



Appendix D

PCA biplot graph – plot of individual data for two experimental sites A: Žabčice and B: Troubsko. Maize – DM = yield of maize dry matter; Maize – FM = yield of maize fresh matter; Inter-crops – DM = yield of all inter-crops dry matter; Inter-crops – FM = yield of inter-crops fresh matt