

Ergot sclerotia and ergot alkaloids occurrence in wheat and rye grains produced in Croatia



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Abstract

Ergot alkaloids (EAs) are mycotoxins produced by several species of fungi of the genus *Claviceps*, among which *Claviceps purpurea* is the most widespread in Europe. This species has been found in many economically important cereal grains, such as rye, wheat, triticale, barley, millet and oats. The distribution of EA contamination has a sporadic incidence, with many factors involved in its occurrence, greatly varying between fungal strains, geographic regions, host plants and regional/local weather conditions. Cool, damp weather favours ergot by enhancing the germination of sclerotia. The aim of this study was to investigate the occurrence of ergot sclerotia and EAs in wheat and rye grain samples (n = 64) collected during 2021 from Croatian cereal producers in central and eastern Croatia. In two rye samples, the presence of ergot sclerotia was detected in the amount of 259 mg/kg and 536 mg/kg,

whereas no wheat samples tested positive for ergot sclerotia. A higher contamination with EAs was determined in the rye samples (18% contaminated; max 167.4 µg/kg), while a lower frequency of contamination was determined in wheat, with only one positive sample (1.9%; 68.5 µg/kg). The results indicate low-level EA contamination of wheat and rye cultivated by Croatian producers during the study period. However, despite the low incidence of positive rye samples with EAs, the contents of ergot sclerotia in two samples were higher than permitted by the legislation for foodstuffs. Since the levels of these mycotoxins and ergot sclerotia content can vary depending on a number of factors, further research is required over a longer period of time and under different cereal cultivation and processing conditions.

Key words: ergot alkaloids, ergot sclerotia, ELISA, wheat, rye, croatian fields

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Introduction

Ergot alkaloids (EAs) are mycotoxins produced by several species of fungi of the genus *Claviceps*. Among them, *Claviceps purpurea* is the most widespread in Europe, whereas other species mostly occur in tropical and subtropical areas. This species has been found in more than 400 monocotyledonous plants, grasses and many economically important cereal grains, such as rye, wheat, triticale, barley, millet and oats (Haarmann et al., 2009; EFSA, 2017). All these cereals represent an important part of the daily human diet and are widely consumed by the population, including infants, children, adolescents, and the elderly, and therefore have to be safe (Agriopoulou, 2021). Today over 50 EAs are known, among which ergometrine, ergotamine, ergosine, ergocristine, ergocryptine (mixture of α - and β -isomers), ergocornine and their corresponding -ine epimers, and specifically monitored in European countries (EFSA, 2012).

The entire life cycle of *Claviceps purpurea* is complex and starts when windborne ascospores land on the featherlike stigmas of susceptible wild, forage grasses in spring (Richard, 2007; Miedaner and Geiger, 2015). The fungus gains entry into the host plant from sclerotia that are present in the soil. The infecting fungal elements (ascospores) are ejected forcibly but also are assisted in gaining access to the host plant by the wind and splashes of rain. Ascospores infect the seed heads of plants during the flowering period, and replace the developing fungal tissue with an alkaloid wintering body, recognized as ergot, an ergot body or sclerotia. The sclerotia are brown to purple-black in colour and contain EAs (Krska and Crews, 2008; Mulder et al., 2015). They can be harvested with the grain and if not eliminated before final processing and thus can end up in food or feed produced from contaminated grains (Richard, 2007).

Sclerotia have a negative impact on the quality of grasses and grains due to the presence of different classes of alkaloids, which are considered undesirable substances for human and animal health. However, advances in science and improvements in the application of agronomy and technology measures, such as milling and heat treatment that have proven effective in the reduction of ergot sclerotia, have eradicated the outbreak of major ergotism epidemics in recent decades (EFSA, 2005, 2012). On the other hand, EAs have been industrially produced and used as active pharmaceutical drugs in medical applications, predominantly in obstetrics and in the treatment of migraine headaches (Haarmann et al., 2009; EFSA, 2012). Also, it is known that controls put in place by millers and grain processors include processes such as physical separation and cleaning through visual supervision, and separation using optical sorters to remove discoloured and misshapen grains also removes the sclerotia.

The physiological effects of EAs have been recognised through the consumption of contaminated cereals, flour and bread. Contaminated feed with toxic levels of EAs has been found to affect the reproductive cycles of pigs, poultry and cattle, causing terminated pregnancies (Coufal-Majewski et al., 2016). They also have been shown to impact digestive systems, resulting in under-performance in weight-gain of farm animals for meat production (Klotz, 2015). When consuming small amounts of contaminated cereals, indigestion occurs, whereas higher amounts cause ergotism, a disease that is manifested in hallucinations, pain and strong vasoconstrictions, which eventually lead to dry gangrene and limb death. In the worst case, kidney and heart failure can

occur, causing death. Cases of domestic animal poisoning have been reported in cattle, horses, sheep, pigs and poultry (Nicholson, 2012).

Recently it has been shown that EAs are more common in foods and feed containing rye and wheat (Agriopoulou, 2021). Taking into account this evidence, the toxicity of EAs, the lack of research on ergot sclerotia and EA occurrence in Croatia, and the newly prescribed maximum levels (MLs) recently defined by the European legislation (Regulation (EU) 2021/1399), the aim of this study was to investigate the occurrence of ergot sclerotia and EA contents in wheat and rye grain samples taken from Croatian cereal producers during the 2021 harvest season.

Materials and Methods

Sampling and sample preparation

During 2021, a total of 64 samples of wheat ($n=53$) and rye ($n=11$) were collected from cereal producers located in central and eastern Croatia. Sampling and sample preparation were performed in accordance with Commission Regulation (EC) No 401/2006, which lays down the methods of sampling of the official control of the levels of mycotoxins in foodstuffs, and Commission Regulation (EC) No 152/2009, which lays down the methods of sampling and analyses for the official control of feed. The samples were stored in a cool and dry place. After the determination of ergot sclerotia content, samples were used for further ELISA analysis of EAs. Prepared test portions were ground into a fine powder with a particle size of 1.0 mm using an analytical mill (Cylotec 1093, Tecator, Sweden), and then stored at 4°C until EA analyse.

Visual method for the determination of ergot sclerotia

Ergot sclerotia were determined according to IAG method-A4 "Method

for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff" (IAG, 2008). Qualitative determination was performed macroscopically on ergot sclerotia fragments. Quantification was performed by selecting and weighing the ergot sclerotia fragments per kilogram of sample. Ergot sclerotia were identified based on their characteristic features. The identification was facilitated by comparison with reference material (from previous proficiency testing materials) and the existing descriptions. Ergot sclerotia are elongated with a length of up to several centimetres, dark violet to black in colour. The shape is similar to cereal kernels. They only consist of fungal hyphae. The material identified as ergot was selected and weighed. The amount of ergot fragments in mg/kg of the sample was calculated using the following formula:

$$C = \frac{BC \times 1000}{E}$$

where:

C = amount of ergot sclerotia in mg/kg [ppm] of the sample

BC = selected fragments of ergot sclerotia in the sample [mg]

E = total weight of the sample [g]

ELISA performance

Extraction procedure and ELISA test were performed according to the manufacturer's instructions with the Ergot Alkaloids ELISA kit (Randox, Crumlin, United Kingdom) which determines the total content of ten EAs. For each 2.5 g sample of milled cereals, 25 mL extraction solution from the ELISA kit was added, then shaken vigorously on the vortex for 1 min and rolled "head over head" for 15 mins. The content was then centrifuged at 1500 rpm for 2 mins and the extracted samples were diluted with a diluted washing buffer at 1:4 (200 µL + 800 µL) for wheat samples and at 1:9 (100 µL + 900 µL) for rye samples. Samples

were mixed thoroughly and prepared for application on an ELISA microtitre plate. In the plate wells, pipetting of standards, samples, quality control and conjugation, and all incubation and washing steps were performed according to manufacturer's instructions. Any colour reaction was stopped by the addition of 100 μ L stop solution per well. After the last step in which the colour changed from blue to yellow, the optical density was measured at 450 nm within 10 mins. The ELISA assay was performed using a Chemwell 2910 automated analyser (Awareness Technology, Inc., USA).

All chemicals and solvents used during the extraction process were of analytical grade. Quality control of the implemented ELISA method was performed using the reference QC material of rye flour T22180QC (Fapas, York, England) with an assigned value in total 10 EAs, ranging from 266–572 μ g/kg, and a mean value of 419 μ g/kg. On this QC material, trueness was determined by analysing six replicates of this material, and repeatability was determined using the same steps on two other occasions in the same analytical conditions and also in six replicates per analysis.

Calculation and statistical analysis of EAs

The total concentration of EAs (μ g/kg) was calculated from a six-point calibration curve using a mathematical interpolation. The results of EA sample concentrations were multiplied by a dilution factor of 50 for wheat and 100 for rye. The obtained values were further corrected for the mean value of recovery determined by the kit manufacturer's instructions through validation at three levels of fortification (50, 75 and 150 μ g/kg for wheat and 125, 250 and 500 μ g/kg for rye).

Statistical analysis was performed using the Statistica Software Ver. 10.0 (StatSoft Inc. 1984 to 2011, USA), with

the statistical significance level set at 95% ($P=0.05$).

Results and Discussion

As data on the ergot sclerotia and EAs presence in different cereals are very scarce for Croatia, this study investigated their occurrence in wheat and rye, as these cereals are most commonly contaminated with EAs in other countries (Agriopoulou, 2021). The European Commission recommends the monitoring of EAs in food and feed (2012/154/EU) for Member States, requiring them to gather reliable data on the year-to-year variations of these mycotoxins in cereals and cereal products intended for human consumption, and animal consumption. It is recommended that at least the following six main EAs and corresponding epimers (12 in total) be tested: ergocristine, ergocristinine, ergotamine, ergotamine, ergocryptine, ergocryptinine, ergometrine, ergometrinin, ergozine, ergozinine, ergocornine, and ergocorninine. According to the same recommendation, Member States should also determine, where possible, the content of ergot sclerotia in a sample, to improve the knowledge of the relationship between the presence of ergot sclerotia and the concentrations of individual EAs. Further, a new Regulation (EU) No. 2021/1399 entered into effect in January 2022, amending Regulation (EC) No. 1881/2006, gives the first separate specifications of MLs of ergot sclerotia and EAs.

In this study, the total EA content was monitored in unprocessed wheat and rye produced in 2021 by Croatian producers, so the investigated samples were not cleaned in an industrial ergot sclerotia removal process. The ELISA method used for EAs determination was validated by the ELISA kit manufacturer, and prior to the determination of EA

Table 1. Results of validation and quality control of the implemented ELISA method for the determination of ergot alkaloid (EA) concentrations

Parameter	Wheat	Rye
LOD ($\mu\text{g}/\text{kg}$)	50	125
LOQ ($\mu\text{g}/\text{kg}$)	70	140
Recovery (%)	75	99
Trueness* ($\mu\text{g}/\text{kg}$)	-	319.22 \pm 52.42
Repeatability* ($\mu\text{g}/\text{kg}$)	-	313.30 \pm 37.21

* Results obtained on rye flour QC material FAPAS T22180QC (assigned value: total EA range 266–572 $\mu\text{g}/\text{kg}$; mean 419 $\mu\text{g}/\text{kg}$) are given as mean value \pm SD

Table 2. Determined ergot sclerotia and ergot alkaloids (EAs) in wheat and rye samples taken from Croatian producers

	Wheat (n=53)	Rye (n=11)
Ergot sclerotia		
No (%) of positives	0 (0)	2 (18)
Mean of sclerotia content (mg/kg)	-	398
Max (mg/kg)	-	536
Min (mg/kg)	-	259
EAs		
No (%) of positives*	1 (1.9)	2 (18)
Mean of positives* ($\mu\text{g}/\text{kg}$)	68.5 \pm 0.0	121.8 \pm 64.5
Max ($\mu\text{g}/\text{kg}$)	68.5	167.4
Min ($\mu\text{g}/\text{kg}$)	<LOD	76.2

*Samples with EA concentrations over the limit of detection (LOD) of the implemented ELISA method

concentrations in the samples in this study, trueness and repeatability were analysed/checked in the laboratory with CRM material. The results of validation and quality control of the implemented ELISA method for determination of EA concentrations are shown in Table 1. Based on the all validation and quality control results, the implemented ELISA method was recognised as suitable for the efficient quantitative determination of EA concentrations in the sampled cereals.

The results of the obtained ergot sclerotia particle content and total EA concentrations in wheat and rye samples

obtained in this study are shown in Table 2. Two rye samples exceeded the ML for the content of sclerotia (259 mg/kg and 536 mg/kg) for foodstuffs (200 mg/kg; Regulation (EC) 1881/2006), but were below the ML defined for feedstuffs (1000 mg/kg; Directive 2002/32/EC). No ergot sclerotia were found in any of the wheat samples.

EA contamination was detected in two rye samples from which the ergot sclerotia were isolated (Figure 1). EA presence was determined in total in a low percentage of the analysed cereals (mean 4.7%), with a determined low number of all positives

(three samples in total, i.e., two rye and one wheat) and a mean EA concentration of $104.0 \pm 55.0 \mu\text{g}/\text{kg}$. Overall, the EA levels in this survey were low, with higher contamination determined in rye samples (18% contaminated, mean EAs $121.8 \pm 64.5 \mu\text{g}/\text{kg}$; max EAs $167.4 \mu\text{g}/\text{kg}$), while a lower contamination with only one positive sample (1.9%) was determined in wheat ($68.5 \mu\text{g}/\text{kg}$). The results indicate low-level contamination of wheat and rye cultivated by Croatian producers during the study period. All the obtained EA concentrations were in accordance with the MLs stipulated in Regulation (EU) No. 2021/1399, of $150 \mu\text{g}/\text{kg}$ for wheat and $500 \mu\text{g}/\text{kg}$ for rye as unprocessed cereals.

The obtained results of ergot sclerotia in this study are lower than those reported by Mulder et al. (2012) in which 75% of rye samples contained ergot sclerotia (mean $449 \text{ mg}/\text{kg}$) and 25% of wheat samples (average $198 \text{ mg}/\text{kg}$). Babič et al. (2020) observed that although ergot sclerotia fragments were present, EAs were not detected (20 samples) and *vice versa* (54 samples). Contrary to this study and the study by Babič et al. (2020), a strong linear relationship between the concentration of EAs and the presence of

ergot sclerotia was reported by Tittlemier et al. (2015) in Canadian wheat and other cereals and by Orlando et al. (2017) in French cereals.

In earlier European studies of EAs in cereals, as summarized by the European Food Safety Authority (EFSA, 2017), the profile and concentration of individual EAs varies considerably in different grains and batches of grain. The occurrence of EAs was investigated on 4,528 food samples collected between 2011 and 2016, with almost 70% sampled between 2014 and 2015 in 15 different European countries, with more than 50% coming from the Netherlands and around 28% from Germany. Among the EAs, ergotamine, ergocristine and ergosine were quantified in about 15% of samples, while ergometrinine and ergocorninine were only reported in just 6% and 8% of samples, respectively. Overall, in the food samples, only 11% of the analytical results were quantified. This general result is in accordance with the results of this study, although some samples of the large European study had significantly EA concentrations than reported here. In the same report by EFSA, the study on a total of 654 feed samples collected in five European countries during the same



Figure 1. Ergot sclerotia fragments A) in rye sample; B) separated ergot sclerotia fragments

period showed that in more than half of these samples, not a single EA was found above the limit of quantification (LOQ) of the implemented analytical method ($n = 352$).

In other studies, the occurrence of EAs have also been reported. Malysheva et al. (2014) reported high rates of EA contamination in rye- and wheat-containing foods in northern Europe. Rye-containing foods were found to have 1121 $\mu\text{g}/\text{kg}$ and wheat-containing foods were found to have 591 $\mu\text{g}/\text{kg}$ EAs, respectively. In a Swiss study, 16 *Claviceps* metabolites were detected in 253 barley samples, originating from the fields throughout Switzerland, showing a small percentage of positive samples (3–17%) (Drakopoulos et al., 2021). Their presence was also detected in Italy (Debegnach et al., 2019), Slovenia (Babič et al., 2020), Poland (Bryła et al., 2018), France (Orlando et al., 2017), Netherlands (Mulder et al., 2015), United Kingdom (Crews et al., 2009), and other countries worldwide (Blaney et al., 2009; Menzies et al., 2017; Xue et al., 2017; Wyka et al., 2020). In western Canada, EAs were detected in 49 of 67 naturally contaminated cool-season barley grains, with mean concentrations ranging from 121 to 555 $\mu\text{g}/\text{kg}$ (Shi et al., 2019).

The period of rainfall and mainly the period of flowering are the two critical periods in which the fungus parasitises the seed heads of living plants (Guo et al., 2016). It was concluded that EAs could be detected in grains even when ergot bodies were removed by a hand-cleaning procedure (Shi et al., 2019). The sclerotia are harvested together with the cereals or grass and can thus lead to contamination of cereal-based food and feed products with EAs (Storm et al., 2008). Moreover, during the milling of cereals, sclerotia are not easily separated from the healthy grains, but instead they are fragmented and can be transferred to the finished product such as flour (Pitt and Miller, 2017).

Cleaning protocols become even more unreliable when fungal sclerotia are produced in dry climates, given the similar size to grains, making them difficult to remove (Krska and Crews, 2008). It was found that the cleaning and processing of cereals can remove up to 82% of sclerotia (Agriopoulou, 2021), meaning they are not completely eliminated and the problem of EA contamination of cereals can still be detected at high levels (EFSA, 2005). For example, an EA concentration of 7255 mg/kg was reported in rye flour from Germany (Krska and Crews, 2008). Agriopoulou (2021) reported that it is known that ergot sclerotia can be removed from cereals by grain cleaning machines based on photocells, though this is not standard in all countries and milling companies and the integrated system of a management's approach that includes all the individual control strategies is important to mitigate EAs in cereals and cereal-based foods.

All the mentioned studies show that the EA contamination has a sporadic incidence. It is clear therefore that many factors are involved in EA occurrence, and that their concentrations vary greatly between fungal strains, geographic regions, host plants and regional/local weather, all of which show that cool, damp weather favours ergot by promoting the germination of sclerotia.

Conclusions

During the study period, ergot sclerotia and the detected EA contents indicated a low-level of contamination of wheat and rye cultivated by Croatian producers. Since EA levels can vary depending on a number of factors, further research of these mycotoxins and ergot sclerotia content is required over a longer period of time and under different cereal cultivation and processing conditions. Additional research should focus on the impact of geographic regions, host plants

and regional/local weather, as well as the technological processing procedures (grinding, heat treatment, etc.) on EA concentrations in the final product. These are the most important factors involved in EA occurrence in cereals and by-products. The ultimate goal is to ensure the health safety of food and feed in terms of the occurrence of these mycotoxins and their toxic impacts on human and animal health.

References

- AGRIOPOULOU, S. (2021): Ergot alkaloids mycotoxins in cereals and cereal-derived food products: characteristics, toxicity, prevalence, and control strategies. *Agronomy* 11, 931. 10.3390/agronomy11050931
- BABIĆ, J., G. TAVČAR-KALCHER, F. A. CELAR, K. KOS, M. ČERVEK and B. JAKOVAC-STRAJN (2020): Ergot and ergot alkaloids in cereal grains intended for animal feeding collected in Slovenia: Occurrence, pattern and correlations. *Toxins* 12, 730. 10.3390/toxins12110730
- BLANEY, B. J., J. B. MOLLOY and I. J. BROCK (2009): Alkaloids in Australian rye ergot (*Claviceps purpurea*) sclerotia: Implications for food and stockfeed regulations. *Anim. Product. Sci.* 49, 975-982. 10.1071/AN09030
- BRYŁA, M., E. KSINIOWICZ-WOŹNIAK, G. PODOLSKA, A. WAŚKIEWICZ, K. SZYMCZYK and R. JĘDRZEJCZAK (2018): Occurrence of ergot and its alkaloids in winter rye harvested in Poland. *World Mycotoxin J.* 11, 635-646. 10.3920/WMJ2018.2322
- COMMISSION RECOMMENDATION 2012/154/EU on the monitoring of the presence of ergot alkaloids in feed and food. *Off. J. Eur. Union* 2012, L77, 20-21.
- COMMISSION REGULATION (EC) No 152/2009 laying down the methods of sampling and analysis for the official control of feed. *Off. J. Eur. Union* 2009, L 54, 1-130.
- COMMISSION REGULATION (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. *Off. J. Eur. Union* 2006, L364, 5-24.
- COMMISSION REGULATION (EU) 2021/1399 of 24 August 2021 amending Regulation (EC) No 1881/2006 as regards maximum levels of ergot sclerotia and ergot alkaloids in certain foodstuffs. *Off. J. Eur. Union* 2021, L 301, 1-5.
- COUFAL-MAJEWSKI, S., K. STANFORD, T. MCALLISTER, B. BLAKLEY, J. MCKINNON, A. V. CHAVES and Y. WANG (2016): Impacts of cereal ergot in food animal production. *Front. Vet. Sci.* 3:15. 10.3389/fvets.2016.00015
- CREWS, C., W. A. C. ANDERSON, G. REES and R. KRŠKA (2009): Ergot alkaloids in some rye-based UK cereal products. *Food Addit. Contam. Part B* 2, 79-85. 10.1080/02652030903042509
- DEBEGNACH, F., S. PATRIARCA, C. BRERA, E. GREGORI, E. SONEGO, G. MORACCI and B. DE SANTIS (2019): Ergot alkaloids in wheat and rye derived products in Italy. *Foods* 8, 150. 10.3390/foods8050150
- DRAKOPOULOS, D., M. SULYOK, R. KRŠKA, A. F. LOGRIECO and S. VOGELGSANG (2021): Raised concerns about the safety of barley grains and straw: A Swiss survey reveals a high diversity of mycotoxins and other fungal metabolites. *Food Control* 125, 107919. 10.1016/j.foodcont.2021.107919
- EFSA (European Food Safety Authority), D. ARCELLA, J. Á. GÓMEZ RUIZ, M. L. INNOCENTI and R. ROLDÁN (2017): Scientific report on human and animal dietary exposure to ergot alkaloids. *EFSA J.* 2017, 15, 4902, 53. 10.2903/j.efsa.2017.4902
- EFSA PANEL ON CONTAMINANTS IN THE FOOD CHAIN (CONTAM) (2012): Scientific opinion on ergot alkaloids in food and feed. *EFSA J.* 2012, 10, 2798. 10.2903/j.efsa.2012.2798
- EUROPEAN FOOD SAFETY AGENCY (EFSA) (2005): Opinion of the scientific panel on contaminants in food chain on a request from the Commission related to ergot as undesirable substance in animal feed. *EFSA J.* 2005, 225, 1-27. 10.2903/j.efsa.2005.225
- EUROPEAN PARLIAMENT COUNCIL DIRECTIVE 2002/32/EC on undesirable substances in animal feed. *Off. J. Eur. Union* 2002, L 140, 10-21.
- GUO, Q., B. SHAO, Z. DU and J. ZHANG (2016): Simultaneous determination of 25 ergot alkaloids in cereal samples by ultraperformance liquid chromatography-tandem mass spectrometry. *J. Agric. Food Chem.* 64, 7033-7039. 10.1021/acs.jafc.6b02484
- HAARMANN, T., Y. ROLKE, S. GIESBERT and P. TUDZYNSKI (2009): Ergot: From witchcraft to biotechnology. *Mol. Plant Pathol.* 10, 563-577. 10.1111/j.1364-3703.2009.00548.x
- IAG (2008): Method for the Determination of Ergot (*Claviceps purpurea* Tul.) in Animal Feedingstuff. IAG-Method A4. Method. https://www.iag-micro.org/files/iag-a4_ergot.pdf.
- KLOTZ, J. L. (2015): Activities and effects of ergot alkaloids on livestock physiology and production. *Toxins* 7, 2801-2821. 10.3390/toxins7082801
- KRŠKA, R. and C. CREWS (2008): Significance, chemistry and determination of ergot alkaloids: a review. *Food Addit. Contam. Part A* 25, 722-731. 10.1080/02652030701765756
- MALYSHEVA, S. V., D. A. LARIONOVA, D. J. DI MAVUNGA and S. DE SAEGER (2014): Pattern and distribution of ergot alkaloids in cereals and cereal products from European countries. *World Mycotoxin J.* 7, 217-230. 10.3920/WMJ2013.1642
- MENZIES, J. G., H. W. KLEIN-GEBBINCK, A. GORDON and D. M. O'SULLIVAN (2017): Evaluation of *Claviceps purpurea* isolates on wheat reveals complex virulence and host susceptibility relationships. *Can. J. Plant Pathol.* 39, 307-317. 10.1080/07060661.2017.1355334
- MIEDANER, T. and H. H. GEIGER (2015): Biology, genetics, and management of ergot (*Claviceps* spp.) in rye, sorghum, and pearl millet. *Toxins* 7, 659-678. 10.3390/toxins7030659
- MULDER, P. P. J., L. W. D. VAN RAAMSDONK, H. J. VAN EGMOND, J. VOOGT, M. W. VAN BRAKEL, G. M. VAN DER HORST and J. DE JONG (2012): Dutch survey ergot alkaloids and sclerotia in animal. *RIKILT report* 2012-005, 54.
- MULDER, P. P., D. P. PEREBOOM-DE FAUW, R. L. HOOGENBOOM, J. DE STOPPELAAR and M. DE NIJS (2015): Tropane and ergot alkaloids in grain-based products for infants and young children in

- the Netherlands in 2011-2014. Food Addit. Contam. Part B 8, 284-290. 10.1080/19393210.2015.1089947
27. NICHOLSON, S. S. (2012): Ergot. In: Ramesh, C. G.: Veterinary Toxicology, Basic and Clinical Principles (Second Edition). Academic Press (1200-1204). 10.1016/B978-0-12-385926-6.00103-4
28. ORLANDO, B., C. MAUMENÉ and F. PIRAUX (2017): Ergot and ergot alkaloids in French cereals: Occurrence, pattern and agronomic practices for managing the risk. World Mycotoxin J. 10, 327-337. 10.3920/WMJ2017.2183
29. PITT, J. I. and J. D. MILLER (2017): A concise history of mycotoxin research. J. Agric. Food Chem. 65, 7021-7033. 10.1021/acs.jafc.6b04494
30. RICHARD, J. L. (2007): Some major mycotoxins and their mycotoxicoses - An overview. Int. J. Food Microbiol. 119, 3-10. 10.1016/j.ijfoodmicro.2007.07.019
31. SHI, H., W. SCHWAB, N. LIU and P. YU (2019): Major ergot alkaloids in naturally contaminated cool-season barley grain grown under a cold climate condition in western Canada, explored with near-infrared (NIR) and fourier transform mid-infrared (ATR-FT/MIR) spectroscopy. Food Control 102, 221-230. 10.1016/j.foodcont.2019.03.025
32. STORM, I. D., P. H. RASMUSSEN, B. W. STROBEL and H. C. B. HANSEN (2008): Ergot alkaloids in rye flour determined by solid-phase cation-exchange and high-pressure liquid chromatography with fluorescence detection. Food Addit. Contam. 25, 338-346. 10.1080/02652030701551792
33. TITTEMIER, S. A., D. DRUL, M. ROSCOE and T. MCKENDRY (2015): Occurrence of ergot and ergot alkaloids in western Canadian wheat and other cereals. J. Agric. Food Chem. 63, 6644-6650. 10.1021/acs.jafc.5b02977
34. WYKA, S. A., S. J. MONDO, M. LIU, V. NALAM and K. D. BRODEKS (2020): A large accessory genome, high recombination rates, and selection of secondary metabolite genes help maintain global distribution and broad host range of the fungal plant pathogen *Claviceps purpurea*. BioRxiv. 10.1101/2020.05.20.106880v1.
35. XUE, A. G., Y. CHEN and Y. AI-REWASHDY (2017): Diseases of spring wheat in central and eastern Ontario in 2017. Can. Plant Dis. Surv. 98, 148-149.

Pojavnost ergot-sklerocija i ergot-alkaloida u pšenici i raži hrvatskih proizvođača

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Ergot-alkaloidi (EA) su mikotoksini koje proizvodi nekoliko vrsta gljivica iz roda *Claviceps*, među kojima je i *Claviceps purpurea* najraširenija gljivica u Europi. Ova vrsta je pronađena u ekonomski važnim žitaricama, kao što su: raž, pšenica, tritikal, ječam, proso i zob. Distribucija kontaminacije EA ima sporadičnu pojavu s mnogo čimbenika koji su uključeni u njihovu pojavu, uvelike varirajući između sojeva gljivica, geografskih regija, biljaka domaćina i regionalnog vremena, što ukazuje da hladno i vlažno vrijeme pogoduje ergotu tako što potiče klijanje sklerocija. Cilj je ovog istraživanja bio istražiti pojavu ergot-sklerocija i EA u uzorcima pšenice i raži ($n=64$) uzorkovanih tijekom 2021. godine od hrvatskih proizvođača žitarica u središnjoj i istočnoj Hrvatskoj. U dva uzorka raži utvrđena je prisutnost ergot-sklerocija u količini od 259 mg/kg i 536 mg/kg. Niti u jednom uzorku

pšenice nije pronađen ergot-sklerocij. Veća kontaminacija s EA utvrđena je u uzorcima raži (18 % kontaminirano, max 167,4 $\mu\text{g}/\text{kg}$), dok je manja učestalost kontaminacije, sa samo jednim pozitivnim uzorkom (1,9 %), utvrđena u pšenici (68,5 $\mu\text{g}/\text{kg}$). Rezultati su ukazali na nisku razinu kontaminacije s EA pšenice i raži koje su uzgojili hrvatski proizvođači tijekom cijelog istraživanog razdoblja. Međutim, iako je utvrđen nizak postotak pozitivno testiranih uzoraka raži na EA, sadržaj ergot-sklerocija u dva uzorka bio je veći nego što je to za hranu zakonodavstvom dopušteno. Budući da razine tih mikotoksina i ergot-sklerocija mogu varirati ovisno o brojnim čimbenicima, potrebno je njihovo daljnje istraživanje tijekom dužeg vremenskog razdoblja i pod različitim uvjetima uzgoja i obrade žitarica.

Gljučne riječi: ergot-alkaloidi, ergot-sklerocij, ELISA, pšenica, raž, hrvatska polja