

## Spatial dynamics of T-2 and HT-2 toxins in Swedish oats

Thomas Börjesson<sup>1</sup>, Kristin Persson<sup>2</sup> and Anders Lindgren<sup>3</sup>

<sup>1</sup>Agroväst Livsmedel AB, PO Box 64, SE-532 23 Skara, Sweden

<sup>2</sup>Swedish University of Agricultural Sciences, Department of Soil and Environment, SE-532 23 Skara, Sweden

<sup>3</sup>Lantmännen R&D, PO Box 30192, SE-104 25, Stockholm, Sweden

e-mail: thomas.borjesson@agrovast.se

The contents of T-2 and HT-2 toxins (T2+HT2) were analysed in 677 samples of oats delivered to silos in Sweden during the years 2020–2022. The spatial and temporal variation patterns in the prevalence of elevated T2+HT2 contents were explored and the covariation with temperature means or precipitation sums computed for windows of 15 days and T2+HT2 levels in the previous year were tested. There was a statistically significant spatial variation structure (Moran's  $I$ ;  $p < 0.05$ ) in two of the years, and high T2+HT2 content was positively related to high content the previous year ( $p < 0.001$ ). There were positive correlations between T2+HT2 content and warm weather in July and negative correlations with precipitation in May and July in two of the years. This is the first time a pronounced regional variability for T2+HT2 is presented. The results can be valuable when searching for safe regions to produce grains intended for food for infants. In a sampling campaign in 11 fields, it was found that T2+HT2-levels can vary substantially within fields.

**Key words:** cereals, Fusarium toxins, spatial variability, weather variables, baby foods

### Introduction

Oats (*Avena sativa* L.), together with spring wheat (*Triticum aestivum* L.), have been found to be more prone to high levels of deoxynivalenol (DON) and T-2 and HT-2 toxins (hereafter T2+HT2) compared with other cereals in Sweden (Karlsson et al. 2023). The reasons for this may partly be due to oats retaining moisture in their spikelets to a higher degree than other cereals (Divon et al. 2019) and the longer flowering period for oats than for other cereals (Rajala and Peltonen-Sainio 2011, Persson et al. 2017) may also play a role. Since high levels of DON seem to be more pronounced in oats, an extensive monitoring program was launched by grain merchants in Sweden in 2010 (Hartman et al. 2024). The farmer-owned agricultural cooperative Lantmännen (Stockholm, Sweden) has analysed around 6 000 samples of oats for DON each year since 2011. However, T2+HT2 has not been studied in detail yet and the objective of this investigation was to cast more light on the current situation regarding these toxins, while also relating the contents to DON that had been analysed using ELISA technology (either ROSA Lateral Flow Test DONQ2 Quantitative test (Charm Sciences Inc., Lawrence, MA, USA) or RIDASCREEN DON, wellplate technique (Rhone Biopharm, Darmstadt, Germany. Art. No. R5906) as part of Lantmännen's routine control program. All oat samples are run with the LFD device when received at the elevator and samples with higher contents of DON (too low signal for the LFD), are also analysed using the wellplate technique. To be able to also include T2+HT2 in a control program encompassing a similar number of analyses, it would be an advantage to use a similar technique for these toxins, with the combination of speed, cost and acceptable reliability.

*Fusarium langsethiae* have been found to be the main producer of T2+HT2 in Sweden, while DON is primarily produced by *F. graminearum* (Fredlund et al. 2013, Karlsson et al. 2023). *F. langsethiae* is favoured by somewhat dryer and warmer conditions than *F. graminearum*. Faster-growing fungi such as *F. graminearum* may outcompete *F. langsethiae* in wetter conditions (Imathiu et al. 2013). Edwards (2009) noted that high T2+HT2 levels coincided with warm and dry weather in the summer, and there was a significant interaction between year and region, probably reflecting fluctuations in weather between years and regions. He also reported a negative correlation between the contents of DON and T2+HT2 toxins. Likewise, Janavičienė et al. (2022) noted that T2+HT2 levels in Lithuania were higher in 2018, with higher temperatures and humid conditions during flowering compared with preceding years, which saw cooler conditions during this period. Humid conditions 1–2 weeks before mid-anthesis and high temperatures onwards have also been reported by Kaukoranta et al. (2019) to be associated with T2+HT2 accumulation.

Hjelkrem et al. (2018) have also concluded that weather is an important factor for determining variations in T2+HT2 contents in Norwegian oats. They found that the weather variables influencing T2+HT2 levels differed from DON; the period before flowering was more important to determine the T2+HT2 levels than the DON levels.

In a survey in which 164 oat samples from trials in different parts of Sweden between the years 2004–2018 were analysed, it was found that the DON contents were higher in the western parts of Sweden, coinciding with wetter climatic conditions, while higher T2+HT2 levels were found in the eastern parts of the country, with dryer weather conditions (Karlsson et al. 2023). In this study, as well as in earlier studies (Fredlund et al. 2013, Karlsson et al. 2017), *F. graminearum* was shown to be more present in the western part of the country, while *F. langsethiae* was more prevalent in the south and to some extent in the eastern part of the country. Thus, we could expect to find clear differences between regions in Sweden and differences between years depending on weather conditions.

In the European Union, there has been legislative limits put on DON since 2006 (EC 2006). Although T2+HT2 toxins are more toxic (tolerable daily intake  $0.02 \mu\text{g kg}^{-1}$  bodyweight (bw) [Arcella et al. 2017]) than DON ( $1 \mu\text{g kg}^{-1}$  bw [Knutson et al. 2017]), legislative limits for T2+HT2 toxins in grains intended for human consumption have not been set until now. These have come into force on 1<sup>st</sup> of July 2024 (EC 2024).

The limit for unprocessed oats is set at  $1\ 250 \mu\text{g kg}^{-1}$  for the sum of T-2 and HT-2 while the limit for baby foods is set at  $10 \mu\text{g kg}^{-1}$ . Bearing in mind that one can count on an average reduction of the T2+HT2 contents of 80% by dehulling (Meyer et al. 2022), the proposed official limit for unprocessed oats is thus irrelevant for sourcing of grains intended for baby foods and a more relevant target for this in farmers' deliveries of this commodity should be  $40 \mu\text{g kg}^{-1}$ .

Thus, it is now of outmost concern for actors in the grain market to put more emphasis on the control of T2+HT2 levels in oats in particular, since levels are expected to be higher than in other cereals. Mapping "safe" regions (i.e., regions with a high probability of producing grains with low toxin contents) is essential for the grain industry. It is also important to keep deliveries with low levels separate from other batches and to further study the influence of weather on the production of T2+HT2 toxins. The aims of this work have been to study how T2+HT2 levels vary between regions and years and how weather variables influence this variability. We have used ELISA technology (RIDASCREEN T2+HT2 Toxin, Rhone Biopharm, Darmstadt, Germany. Art. No. R3805), due to it being cost effective and it is permitting us to gain a clear understanding of the spatial variability for T2+HT2 in all important oat producing regions in Sweden. The use of this technology also paves the way for including this technique as a routine within Lantmännen for testing incoming grain.

## Materials and methods

### Study area and study overview

The study was conducted in southern Sweden (Fig. 1). In Sweden, oats are the third most important cereal after winter wheat (*Triticum aestivum* L.) and spring barley (*Hordeum vulgare* L.). Oat production covers just over 150 thousand hectares, and the average yield is 4.8 tonnes per hectare (Swedish Board of Agriculture 2023). The production is rainfed and about 20% of the area is organic (ibid.).

In the present study, two types of grain samplings were carried out, regional sampling in farmers' deliveries during the years 2020–2022 and sampling within fields at maturity 2021–2023. The regional samples were analysed for both DON and T2+HT2, while the within field samples were analysed for T2+HT2 only. The DON levels were generally low, and no spatial autocorrelation in the occurrence of elevated DON content could be demonstrated in the present study. Therefore, no maps were produced, and no correlation analyses were carried out for this toxin – only descriptive statistics and results from the test of spatial autocorrelation are presented.

### Regional sampling

The oat samples used in this investigation were all collected at the grain intakes of Lantmännen's elevators all over Sweden. They were sampled according to a set procedure agreed upon by the Swedish grain merchants, "Gula Boken" (Svenska Kvarnföreningen and Föreningen Foder & Spannmål 2019). In this procedure, incoming loads of grain are sampled at three spots for each unit, truck and trailer. Thus, when a truck with a trailer arrives, which is the most common method of transporting grain, the delivery is sampled at six spots. At each spot, the whole

depth is sampled, either using a RakoRaf truck sampler (Pfeuffer 2024) or similar, or manually using spears with multiple holes.

Of approximately 6 000 samples collected and analysed for DON each year, 192 samples from the harvest year 2020, 221 from harvest year 2021, and 264 samples from 2022 (a total of 677 samples) were analysed for T2+HT2 toxins. All were the same variety, Galant. The reason for only choosing this variety was that it dominated among the samples collected and it was homogeneously distributed between the different regions. Galant constituted between 70 and 75% of the samples all three years and the rest was distributed on several varieties with less than 500 samples to choose from each year. To choose other varieties as well would thus have resulted in more unreliable results. For all samples, coordinates (projected coordinate system RT90 2.5 gon V; EPSG: 3021) for farm centres were available. To study regional differences, the samples chosen for T2+HT2 analyses were not taken randomly but collected from delimited regions in Sweden in the most important oat producing counties; these were chosen to cover different types of landscapes and agrometeorological conditions (Fig. 1).

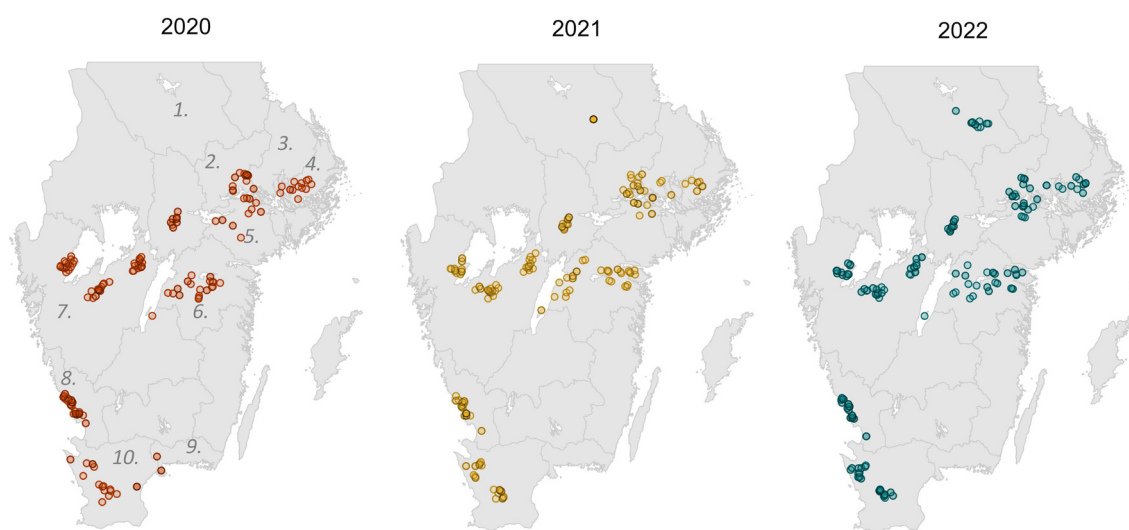


Fig. 1. Sampling locations in southern Sweden (slightly jittered for better visibility). Numbers denote counties: 1 = Dalarna, 2 = Västmanland, 3 = Uppsala, 4 = Stockholm, 5 = Södermanland, 6 = Östergötland, 7 = Västra Götaland, 8 = Halland, 9 = Blekinge, and 10 = Skåne

### Sampling within fields

In order to quantify variations in T2+HT2 content within fields, 3–12 samples per field were taken from eleven fields in Halland and Södermanland in 2021, 2022 and 2023. Those regions were chosen because T2+HT2 levels were comparatively high in the samples taken in 2020. The samples were taken using the Minibatt+ handheld harvester (Goudé, Le Catelet, France) according to a specific protocol: At each sampling point, nine subsamples were taken, one at a central point, and two in each of four perpendicular directions. The first sample in each direction was taken about 1.5 m from the central point and the others were taken 3 m from the central point. Thus, each sampling area covered about 36 m<sup>2</sup>. The coordinates were collected at the central point using the app My GPS coordinates (Android Apps & Tools), and the precision was usually 6 m or better.

### Sample storage and preparations

The water content of the samples varied between 10% and 20%, and the samples were stored below 8 °C until analyses, which were performed after a storage period of up to 3 years. According to studies by Mylona and Magan (2011), these conditions should not result in growth of *Fusarium langsethiae* and T2+HT2 production. At 20% moisture content ( $A_w$  0.89) no *F. langsethiae* growth or T2+HT2 accumulation was observed at 15 °C. Although the experiment was only conducted during a short period (10 days) the authors claim that it can be used to set limits for safe storage of oats for longer periods. Moreover, no physical changes or mould growth has been observed and samples from 2020 were reanalysed after a storage period of approximately two years and no significant change in the T2+HT2 levels were observed irrespective of initial water content (data not shown).

Sample mass varied between 300 g and 500 g. Portions to grind were taken out using a 3 l riffle sample divider with 10 slots (Pfeuffer GmbH, Kitzingen, Germany). Samples were then ground using a knife mill (GM200, Retsch GmbH, Haan, Germany) operated at 10 000 rpm for 30 seconds per sample.

First, 5 g portions were taken out, milled and analysed, based on the instruction that 5 g of a milled sample be weighed for analysis. However, when analysing duplicates (see results in the results section), we were not satisfied with the repeatability of the results, and 50 g of each sample was instead milled and thoroughly mixed whereafter 5 g was taken out for analysis. This is also in accordance with the routine procedure used for analysing DON in the laboratory.

Since the analyses of the 50 g milling portions were analysed up to 2 years after the 5 g portions and a reduction of T2+HT2 contents has been reported for flour under moderate water activity ( $A_w$  0.8, Janavičienė et al. 2022), we also studied whether there was a correlation between water content and the difference between the first and the second analyses, but no such trend could be discerned (data not shown).

### Laboratory analyses

Samples were analysed using the ELISA kit RIDASCREEN T2+HT2 Toxin (Rhone Biopharm, Darmstadt, Germany. Art. No. R3805). Analyses were performed according to kit producer instructions using a centrifuge (Sigma 6 – 16S) operated at 3 000 rpm. Absorbance values were recorded at 450 nm with a DS2 ELISA System (Dynex Technologies, Chantilly, VA, USA). The recovery rate for spiked oat samples is reported to be 69%, and the cross-reactivity to T2 toxin is 72%. The reference substance used is HT2 and therefore 100%.

A wheat control sample, TR-MT100, from Trilogy, Washington, USA, with a specified mean T2+HT2 toxin level of  $79 \mu\text{g kg}^{-1}$  was used. The laboratory standard deviation was  $13 \mu\text{g kg}^{-1}$  and the RSD 24% ( $n=17$ ).

Results were obtained using the software DS-Matrix and curve fitting option Cubic Splines. The detection limit for oats, according to the manufacturer, for the procedure corresponds to  $16 \mu\text{g toxin kg}^{-1}$ , and the limit of quantification is reported to be  $24 \mu\text{g toxin kg}^{-1}$ . With every run, a control sample with a known content of T2+HT2 was included. Samples with T2+HT2 contents below the limit of detection were set to half the limit of detection (LOD;  $8 \mu\text{g toxin kg}^{-1}$ ).

### Spatial data analyses

As has been discussed above, the contents of T2+HT2 should not exceed  $40 \mu\text{g kg}^{-1}$  to ensure its safe use as a raw material for baby food. Although a reading of  $40 \mu\text{g kg}^{-1}$  may in practice be higher, considering the method's limitations in expected low recovery rate and cross-reactivity, we chose this because a lower limit would approach the limit of quantification. To assess whether there was a statistically significant spatial variation structure in exceedances of the  $40 \mu\text{g kg}^{-1}$  threshold, the T2+HT2 content was converted to a binary variable with a value of 1 for samples exceeding the threshold and a value of 0 for samples with a content equal to or below  $40 \mu\text{g kg}^{-1}$ . The reason for the conversion to a binary variable was that the data was zero-inflated with a large fraction of observations under the LOD. Then Moran's I was calculated for each year using the inverse Euclidian distances between sample locations as weights.

After testing, the prevalence of threshold exceedances was mapped. A hexagonal grid was created. For all grid cells with at least five samples within a distance of less than 20 km from a central point in all three years, the percentage of these samples below  $40 \mu\text{g kg}^{-1}$  was calculated. There were 53 hexagonal cells with enough data for this mapping exercise. Spatial analyses were conducted using the software R (R Core Team 2023) and ArcGIS (ESRI, Redlands, CA, USA)

### Weather data

Weather data was provided by the Swedish Hydrological and Meteorological Institute (SMHI 2024, Norrköping, Sweden) and extracted using the SLU VPE/Fältforsk – JSON service. For each, daily mean values of air temperature ( $^{\circ}\text{C}$ ), and precipitation (mm) were taken from the  $2.5 \times 2.5$  km MESAN grid (Häggmark et al. 2000). Air temperature means and precipitation sums were then computed for moving windows of 15 days with a time step of one day. This was done for all time-windows with a centre day from April 1 till August 31 and resulted in a total of 306 weather variables.

## Statistics

Bivariate and multivariate correlation analyses were conducted for the 53 grid cells. This was done year by year ( $n = 53$ ), and across years ( $n = 159$ ). Pearson correlation coefficients ( $r$ ) were calculated between the fraction of samples with T2+HT2 content over  $40 \mu\text{g kg}^{-1}$  and each of the weather variables. In addition, bivariate correlations were tested between T2+HT2 content in one year and T2+HT2 content in the same grid cell the year before, and between the T2+HT2 contents in 2022 and in 2020. Multivariate Partial Least Square (PLS) models were constructed using the software Aspen Unscrambler Version 12.2 (Aspen Technology Inc., Bedford, MA, USA), and  $r$  values were determined. When constructing the models, it was decided not to include more variables than necessary. All variables were included to start, and then the number of variables was reduced as long as no or only a very limited reduction in model performance was observed. To reduce the number of variables, variables with lower variable importance were removed step by step. Variable importance was based on weighed sums of absolute regression coefficients.

## Results

### Comparison of milling weights

An experiment with 3 replicate grindings of 5 g and 50 g portions of 5 samples showed that the larger sample size resulted in much more reliable results (Table 1). Standard deviation (SD) and Relative standard deviations ( $\text{RSD} = 100 \times \text{SD} / \text{mean}$ ) were calculated.

Table 1. Comparisons of means, standard deviations (SD) and relative standard deviation (RSD) when either 5 g or 50 g of five oat samples were ground and mixed before taking out 5 g for analysis.

Sample number	Ground amount (g)	Mean ( $\mu\text{g kg}^{-1}$ )	SD ( $\mu\text{g kg}^{-1}$ )	RSD *
28	50	126.8	20.8	16.4
28	5	85.2	20.8	24.4
41	50	107.1	10.1	9.5
41	5	41.6	28.5	68.3
145	50	362.0	120.7	33.4
145	5	172.9	64.8	37.5
178	50	51.8	11.6	22.5
178	5	21.2	3.4	15.8
187	50	77.4	21.5	27.8
187	5	74.5	63.4	85.2

\*Mean RSD when 50 g portions were ground is  $22 \mu\text{g kg}^{-1}$  and for 5 g portions it is  $46 \mu\text{g kg}^{-1}$ .

### Descriptive statistics

Descriptive statistics for the regional sampling in grain deliveries is presented in Table 2. The DON levels were relatively low during 2020–2022, and more than half of the samples had levels below the level of quantification, which is  $100 \mu\text{g kg}^{-1}$ . Less than 10% of the samples had DON contents over  $200 \mu\text{g kg}^{-1}$ , which is the threshold used to classify “elevated content” in the present study. The levels of T2+HT2 were somewhat higher in 2021 and 2022 than in 2020. In 2020, 12% of the samples had higher contents than  $40 \mu\text{g kg}^{-1}$ , which is the presently used threshold for high values, and around 40% of the samples had T2+HT2 contents over this threshold in 2021 and 2022.

Table 2. Descriptive statistics for the regional sampling in farmers' deliveries. For DON, the limit of detection (LOD) is  $100 \mu\text{g kg}^{-1}$  and for the T-2 and HT-2 toxins (T2+HT2), it is  $16 \mu\text{g kg}^{-1}$ . Threshold values,  $200 \mu\text{g kg}^{-1}$  for DON and  $40 \mu\text{g kg}^{-1}$  for T2+HT2, were set for the present study only and are not anchored in legislative regulations. *Below LOD* is the percentage of all samples with concentrations below the LODs and *Below threshold* is the percentage of all samples with concentrations below the thresholds. n = number of samples, --- = below LOD.

Year	n	Min ( $\mu\text{g kg}^{-1}$ )	Median ( $\mu\text{g kg}^{-1}$ )	Max ( $\mu\text{g kg}^{-1}$ )	Below LOD (% of samples)	Below threshold (% of samples)
DON						
2020	192	---	---	921	72	93
2021	221	---	---	1 160	71	91
2022	264	---	---	5 100	83	96
T2+HT2						
2020	192	---	---	127	61	88
2021	221	---	27	330	38	58
2022	264	---	30	342	37	61

Descriptive statistics of T2+HT2 content in the within-field samples are presented in Figure 2. Fields 8 and 9 are located in Södermanland, while the rest of the fields are located in Halland (counties are marked in Figure 1). The magnitudes of within-field variation varied from 6 to  $302 \mu\text{g kg}^{-1}$ . In fields with greater within-field variation, it was usually not a single sample that was high, but rather a relatively uniform distribution of concentrations from low to high. The fields 1, 5 and 11 are adjacent fields.

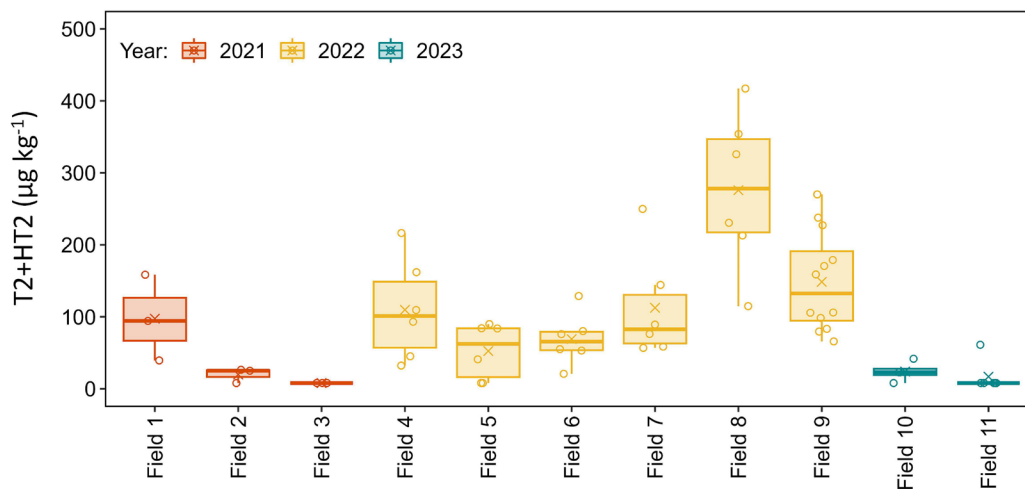


Fig. 2. Descriptive statistics for of T-2 and HT-2 toxins (T2+HT2) content in the within-field samples. Crosses = means, midlines = medians, boxes = interquartile ranges (IQR, i.e. the difference between the third and the first quartile of the data), whiskers = highest and lowest observation within  $1.5 \times$  IQR from midline. Observations outside whiskers are considered outliers. Points show individual observations (slightly jittered in x-direction for clarity). Colours indicate sampling year (2021–2023).

### Spatial variation at regional scale

For the years 2021 and 2022, the geographical dependence in the exceedance of  $40 \mu\text{g kg}^{-1}$  T2+HT2 was statistically significant (Table 3). Positive Moran's I-values mean that the exceedances were more clustered than would normally be expected if randomly distributed, i.e., there was a positive spatial autocorrelation. The fractions of samples exceeding  $40 \mu\text{g kg}^{-1}$  within a 20 km neighbourhood is mapped in Figure 3. The pattern is more obvious during the years 2021 and 2022 compared with 2020, and in particular the county Västergötland showed lower levels than other regions.

Table 3. Global Moran's I for the exceedance of  $200 \mu\text{g kg}^{-1}$  deoxynivalenol (DON) or  $40 \mu\text{g kg}^{-1}$  T2+HT2. Levels of statistical significance: N.s. =  $p > 0.05$ , \*\*\* =  $p < 0.001$ .

Year	Moran's I	Significance level
Exceedance of $200 \mu\text{g kg}^{-1}$ DON		
2020	0.00	N.s
2021	-0.02	N.s
2022	-0.01	N.s
Exceedance of $40 \mu\text{g kg}^{-1}$ T2+HT2		
2020	0.00	N.s
2021	0.15	***
2022	0.07	***

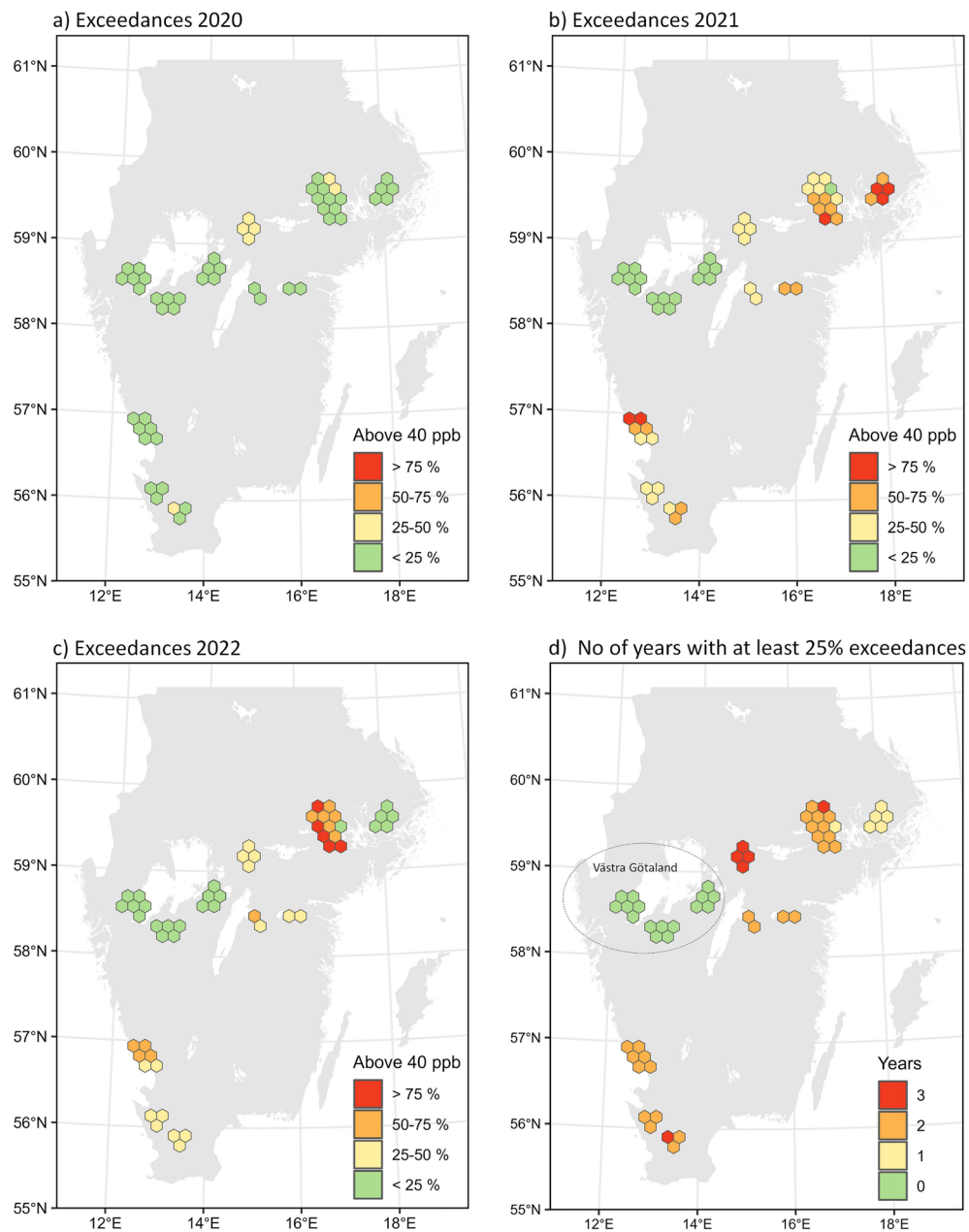


Fig. 3. Percent of samples with T2+HT2 contents  $> 40 \mu\text{g kg}^{-1}$  in different regions in southern Sweden in 2020 (a), 2021 (b) and 2022 (c). Map d shows the number of years (2020–2022) during which at least 25% of samples exceeded  $40 \mu\text{g kg}^{-1}$ .

## Correlations and multivariate statistics

Many of the weather variables were correlated ( $p < 0.05$ ) with the risk of obtaining a T2+HT2 content above  $40 \mu\text{g kg}^{-1}$  (Fig. 4). Air temperatures showed positive correlations for most time-windows in July, both for individual years and when all three years were analysed together. The temperatures in July were quite moderate for all years, but with quite pronounced differences between the years,  $15.3 \text{ }^\circ\text{C}$  in 2020,  $19.5 \text{ }^\circ\text{C}$  in 2021 and  $17.1 \text{ }^\circ\text{C}$  in 2022 (mean temperatures for the 53 grid cells). At approximate beginning of stem elongation (DC 31) there were statistically significant negative correlations between air temperature and the risk of obtaining a high T2+HT2 content in two individual years and for all years together. The mean precipitation in July was 101 mm in 2020, 62 mm in 2021 and 52 mm in 2022. Precipitation showed negative correlations with the prevalence of high T2+HT2 content for many time-windows throughout the season, but when the three years were combined there was no statistically significant correlation. In the years 2021 and 2022 there were positive correlations between the amounts of rain towards the end of the growing season and the prevalence of high T2+HT2 content. It should also be noted that mid-flowering (DC stage 65; Zadoks et al. 1974) occurred at approximately the same time during the years 2020–2022, at the end of June 2020 and a few days later, in the beginning of July 2021 and 2022 according to gradings performed by Swedish Board of Agriculture.

Consistently positive correlations were obtained between the risks of having a T2+HT2 content above  $40 \mu\text{g kg}^{-1}$  and the risk index for the previous year. The  $r$  value was 0.51 between 2020 and 2021, and 0.52 between 2021 and 2022. The  $r$  values were statistically significant in a two-tailed test ( $p < 0.001$ ).

When combining variables using a multivariate method, it was found that the correlations between predicted and observed risks for obtaining values above  $40 \mu\text{g kg}^{-1}$  were stronger than the correlations between observed risk and individual weather variables. When using only weather variables to predict the risk of obtaining values above  $40 \mu\text{g kg}^{-1}$  for all years computed together, the best model ( $r = 0.87$ ,  $p < 0.001$ ) was based on 15 precipitation variables only (Table 4). A model based on only temperature data was also developed. In this case, the best model was almost as good ( $r = 0.84$ ,  $p < 0.001$ ) and 8 variables were sufficient to include (Table 4). It was obvious that the precipitation and temperature variables were more or less equally good as predictors and to include both types of variables in the models did not improve them. To include the risk index the previous year did also not improve the models.

Table 4. Variables used for the best Multivariate Partial Least Square (PLS)-models developed. The centre dates for the moving 15-day precipitation and temperature variables, respectively, are given.

Month	Moving window centre date	
	Precipitation	Temperature
April	2, 25	1, 14
May	8, 13, 20, 23, 25	3
June	9, 24	11, 27
July	8, 12, 23	
August	11, 19, 31	3, 23, 31

The variables' contribution in the PLS-models was well in line with the correlation coefficients for the individual variables (Fig. 4). The precipitation variables in June and August were largely positively related with higher risk for T2+HT2, while they were largely negatively related in June and July. The PLS-coefficients indicated that high risk for T2+HT2 accumulation was associated with low temperatures in April but later during the growing season they were primarily correlated with high temperatures.



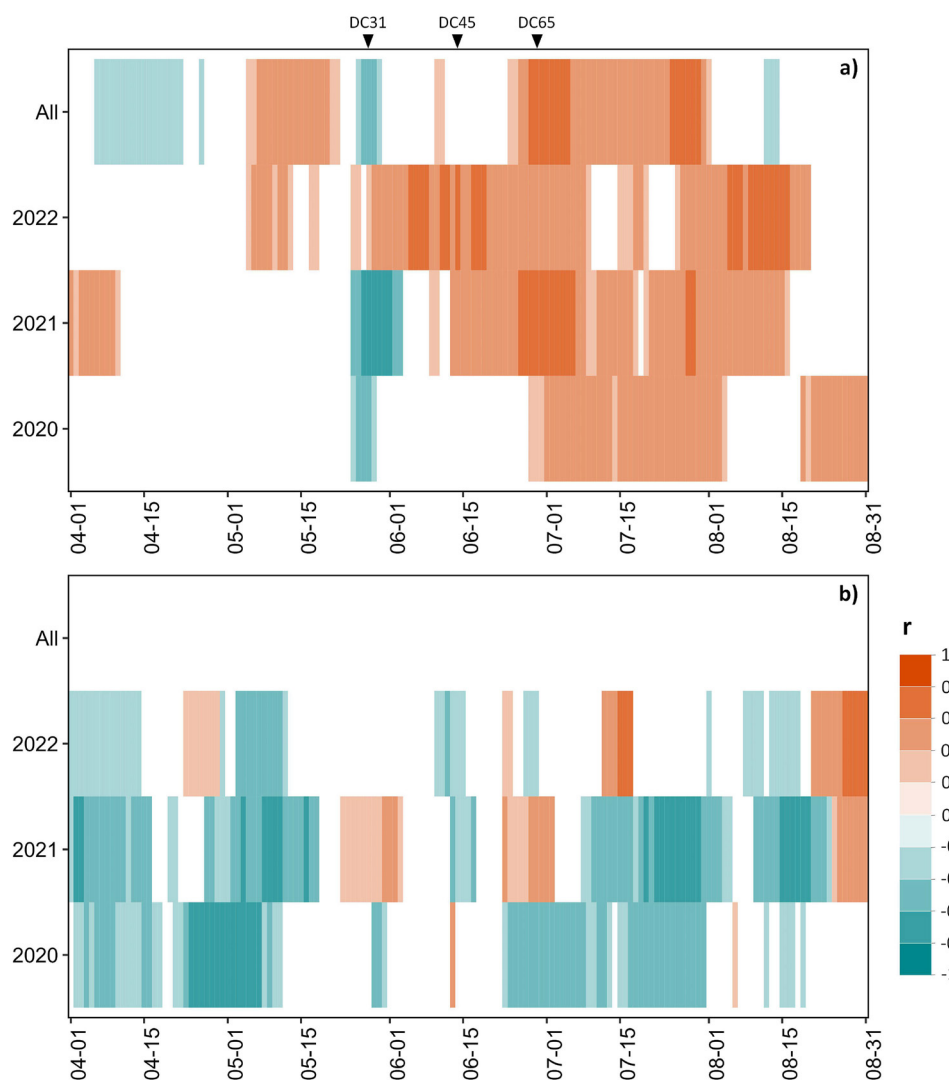


Fig. 4. Pearson correlation coefficients ( $r$ ) between the risk of exceedance of  $40 \mu\text{g kg}^{-1}$  T2+HT2 and a) mean air temperature and b) precipitation sum. Mean air temperatures and precipitation sums are computed for windows of 15 days moved with a one-day step. The dates on the x axis are the centre dates (month-day) of the time-windows. Correlation coefficients, which were not statistically significant at  $p \leq 0.05$  in a two-tailed test, are not shown. Approximate developmental stages (Zadoks et al. 1974) for oats in Sweden are indicated (DC 31 = beginning stem elongation, DC 45 = booting and DC 65 = flowering).

## Discussion

The geographical pattern for T2+HT2 in this investigation agrees well with patterns that have been reported before (Karlsson et al. 2023). This investigation suggests that geographical pattern may actually be more pronounced than what has been seen for DON. The variations between years seem to be more pronounced for DON compared with geographical differences (non-published data on the DON-distribution between years). Likewise, Karlsson et al. (2023) have reported a statistically significant higher content of T2+HT2 in the eastern parts of the oats producing region in Sweden compared with the western and the south-eastern parts of the country, but no statistically significant regional differences for DON were observed.

The present results are in line with numerous previous results, which show that warmer and drier conditions during the summer are correlated with increased levels of T2+HT2 toxins, e.g., Hjelkrem et al. (2018) and Edwards et al. (2009). Xu et al. (2014) also reported a positive relationship between T2+HT2 content at harvest and dry conditions from late May onwards (including the heading/flowering period) in oats. This finding is also in agreement with results presented by Bernhoft et al. (2012), who reported a positive relationship between *F. langsethiae* and the mean July temperature (typically corresponding to the flowering time of oats in Norway). The fact that we note a

negative correlation between T2+HT2 accumulation and rain in July (during flowering) is also in accordance with earlier findings and can probably be explained through the competition from faster growing *Fusarium* species in these circumstances (Imathiu et al. 2013). We observed a correlation with lower temperatures at stem elongation and accumulation of T2+HT2. Kaukoranta et al. (2019) also report that lower temperatures and more precipitation before anthesis can promote T2+HT2 accumulation, although they found that this stage occurs later, 1–2 weeks before mid-anthesis. That the toxin content in previous years is a relatively good predictor has not been studied in detail previously but may be a secondary effect of the fact that the fungus seems to be quite stationary.

As this is an observational study, it is not possible to draw firm conclusions on reasons for the distributions. The spatial variation patterns with rare exceedances in Västra Götaland and common exceedances in the eastern and southern part of south Sweden may be due to geographic variation in weather conditions but may as well be explained by other region-specific conditions or by infection levels in earlier years. Nevertheless, even if exact causes cannot be established, it is useful to discover in which regions and under which weather conditions high T2+HT2 levels are more common. This constitutes an important piece of information when sourcing oat grains of baby food quality.

Future studies should focus on the stability of the geographical patterns that were discovered in the present study and on the extent to which they can be explained by weather variables. The eastern parts of the country, such as Södermanland (Fig. 1), is known for having a drier climate than the western part of the country such as Västra Götaland and Halland. For the period 1991–2020, the average temperatures in July were slightly higher in the counties of Västmanland (17.3 °C) and Södermanland (17.5 °C) than in Halland (17.0 °C) and Västra Götaland (17.0 °C), whereas the mean precipitation in July was lower in Västmanland (75.7 mm) and Södermanland (68.2 mm) compared with 99.3 mm in Halland and 80.1 mm in Västra Götaland (SMHI 2004). To find higher levels of T2+HT2 in the eastern part of the country is thus not surprising but high levels of T2+HT2 in Halland is not what would be expected based on long-term weather trends. In our study, we actually had relatively dry and warm conditions in Halland, but on average, that is not the case. A specific region in Halland had in one particular year (2010) high precipitation in July but also a quite high T2+HT2 content in harvested oats and high levels of *F. langsethiae*-DNA (Karlsson et al. 2023). This data suggest that infection rates are an important factor in determining T2+HT2 production.

In some fields, a considerable within-field variation in T2+HT2 content was observed. In an earlier study, pronounced within-field variation in DON content seemed to be related to soil characteristics (Söderström et al. 2015). Whether also within-field variation in T2+HT2 content is related to soil properties remains to be tested.

## Conclusions

Based on 677 oat grain samples taken in southern Sweden over three years, we found that 40 µg kg<sup>-1</sup> T2+HT2 in delivered grains was commonly exceeded and that there was a regional spatial variation pattern in exceedances that was mostly consistent between years. High levels of T2+HT2 were statistically correlated with T2+HT2 content at the same location in previous years (indicating a stationarity) and warm and dry weather during flowering and maturing of the crop. Based on a separate sampling in 11 fields, we conclude that within-field variation in T2+HT2 content can be notable. We recommend continued monitoring of the T2+HT2 content in oats at intake to build a larger knowledge base, which can be used for targeted sourcing of oats intended for baby food.

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