Reduction of deoxynivalenol, T-2 and HT-2 toxins and associated *Fusarium* species during commercial and laboratory de-hulling of milling oats

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**ABSTRACT**

Oats (*Avena sativa* L.) are well known for their nutritional properties but are susceptible to the growth of different *Fusarium* fungi resulting in mycotoxin contamination of harvested oats. In this study, oat samples from harvest years 2011 to 2017 were preselected for their suitability as milling oats for food purposes with DON contents below 1750 mg/kg. The reduction of DON, T-2 and HT-2 toxins during the commercial de-hulling process was analysed. While the average reduction for the sum of T-2 and HT-2 toxins in large oat kernels was 85\%, the reduction for thin kernels was 66\%. The reduction for DON was about 60\% and did not differ for the two kernel fractions. In laboratory de-hulling experiments, milling oat samples and de-hulled oat kernels with known DON, T-2 and HT-2 toxin content were correlated with the associated DNA amount of *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium langsethiae*. The reduction of the *Fusarium* DNA amount after de-hulling was comparable to the reduction of the associated mycotoxins. Notably, the correlation between *F. langsethiae* DNA amounts and the sum of T-2 and HT-2 toxin contents was $R^2 = 0.85$ in de-hulled oat kernels. In laboratory tests, at least one third of the initial levels of DON and the sum of T-2 and HT-2 toxins could be removed by polishing off the first parts of the outer layers; two thirds remained in the polished oat kernels. These observations indicate that de-hulling alone may not be completely sufficient to remove mycotoxin contamination in oats. These findings are of high importance in the discussion of determining legal maximum levels for DON or the sum of T-2 and HT-2 toxins in intermediate and final products.

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**INTRODUCTION**

Oat products are an important and valuable source of vitamins, minerals, essential fatty acids, carbohydrates and fibres (Hampshire 1998; Butt et al. 2008; Biel et al. 2009; Rasane et al. 2015). The latter contain an increased proportion of $\beta$-glucans (Redaelli et al. 2013), which have an approved cholesterol-lowering effect (European Food Safety Authority 2010, 2011). Furthermore, oat products show great potential for reducing glycaemic response (Zhang et al. 2021) and can contribute to a gluten-free diet if cross contamination during processing can be avoided (Størsrud et al. 2003; Smulders et al. 2018).

Oat grains consist of oat kernels and firmly attached husks, named palea and lemma. Oat grains intended for food purposes are subject to special physical requirements as described by Ganßmann and Vorwerck (1995). Therefore, oats complying with these requirements will be referred to as milling oats in this study. The terms ‘unprocessed oats’ or ‘oats (with husk)’ or ‘unprocessed cereals’ (oats) are used as synonyms corresponding to European legislation (European Commission 2006a, 2013) or newly proposed maximum levels for the sum of T-2 and HT-2 toxins (Verstraete 2022).

Oat kernels consist of the whole kernel with outer layers and germs remaining after removal of attached husks. There are two different processing methods for milling oats. The older method is called ‘dry-shel-ling’; milling oats are first kilned and then
de-hulled. In the later 1960s the second method, the so-called ‘green-shelling’, was introduced. Here, the milling oats are first de-hulled and then kilned (Kent and Evers 1994). The general operations of an oat mill include cleaning and sorting, de-hulling, kilning and steaming, partially cutting and flaking. The order of these processing steps may vary between oat mills. In this study, examinations were carried out according to the ‘green-shelling’ process. The production of oat flakes starts with the cleaning of milling oats. The removal of foreign matter includes impurities (husks, straw, stones, metals, glass, weed seeds, etc.) and oat impurities (foreign cereals, shrivelled, broken and sprouted oats). Typical machines for oat cleaning are aspirators, length and width graders, de-stoners, scourers and magnets.

Milling oats are traded to contain less than 10% of thin oats below 2 mm slotted hole sieve (Ganßmann and Vorwerck 1995). Despite this, oat grains have different sizes. This is due to the structure of oat spikelets which can consist of one, two or even three oat grains, with the first grain being significantly larger and heavier than the others (Doehlert et al. 2002, 2005). Therefore, milling oats are usually graded into at least two different sizes, large and thin, to facilitate de-hulling. This sorting allows fine adjustment of the impact de-huller as larger oat grains require less impact energy than thin ones (Doehlert et al. 2004). During the de-hulling process, the oat grain is thrown with an adjusted speed against an impact ring in the de-huller to separate the husks without damaging the kernels and thus avoiding yield losses. Too much energy leads to higher breakage, too little leads to a lower de-hulling efficiency (Ganßmann and Vorwerck 1995; Doehlert et al. 1999; Doehlert and McMullen 2001). Oat kernels, loose husks and dust are finally separated from each other by means of scouring machines and circulating air aspirators. In later stages, the oat kernels are subjected to two thermal treatments. They are first kilned and then steamed before being processed into oat flakes.

Oats as well as other small-grain cereals such as wheat and barley are susceptible to infections by fungi of the genus *Fusarium* (Placinta et al. 1999; Bottalico and Perrone 2002). In Northern Europe, *Fusarium* diseases of oats are caused by several co-occurring species, e.g. *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium langsethiae* and others (Torp and Langseth 1999; Fredlund et al. 2013; Yli-Mattila et al. 2013; Hietaniemi et al. 2016). The known factors leading to *Fusarium* colonisation of oats and in particular their spikelets include oat variety and its susceptibility (traits such as flowering time and duration, plant height, and anther extrusion), agronomic factors like crop rotation and soil cultivation, and weather conditions during flowering (Šlíková et al. 2010; Parikka et al. 2012; Edwards 2017; Hautsalo et al. 2018; Parikka et al. 2018; Tekle et al. 2018; Kaukoranta et al. 2019; Schönberg et al. 2019). Moreover, Divon et al. (2019) assumed oats may be more susceptible to a *F. langsethiae* infection due to their ability to retain humidity in the spikelets. The longer flowering duration of oats (10–11 days) compared to wheat (4–5 days) might play an additional role (Rajala and Peltonen-Sainio 2011), since flowering is the most sensitive stage of development for severe infections as anthers and pollen provide a conducive environment for *Fusarium* growth in oat grains (Tekle et al. 2012; Divon et al. 2019, 2012b; Xue et al. 2015). The subsequent growth of *F. langsethiae* and *F. graminearum* into the outer layers of the oat kernel has been impressively described by Divon et al. (2019) and Tekle et al. (2012, 2015).

Associated with these infections, *Fusarium* mycotoxins are often produced and accumulated in oats and oat products, which can cause a significant risk to human and animal health when entering the food and feed chain. Mycotoxins most commonly found in oats grown in temperate regions of Northern Europe are the trichothecenes which include deoxynivalenol (DON) as well as T-2 and HT-2 toxins (Edwards 2009; Schwake-Anduschus et al. 2010; Pettersson et al. 2011; Fredlund et al. 2013; Edwards and Jennings 2016; Hietaniemi et al. 2016; Opoku et al. 2018; De Colli et al. 2021; Meyer et al. 2021). DON is a group B trichothecene and mainly produced by *F. graminearum* and *F. culmorum* (Jestoi et al. 2008; Kokkonen et al. 2010; Fredlund et al. 2013; Yli-Mattila et al. 2013).
F. graminearum is the foremost producer of DON compared to F. culmorum (Parikka et al. 2012; Yli-Mattila et al. 2013).

The Commission Regulation (EC) No 1881/2006 set maximum levels for deoxynivalenol for unprocessed oats and cereals intended for human consumption (oat milling products, oat kernels). These are 1750 and 750 µg/kg, respectively (European Commission 2006a). The discussion about lowering maximum levels for DON was reopened with the European Food Safety Authority (EFSA) opinion of Knutsen, Alexander, et al. (2017) having set a new group-TDI of 1 µg/kg bodyweight for DON and its acetylated and modified forms, which are de-acetylated or cleaved in the intestine (Berthiller et al. 2011; Ajandouz et al. 2016). The toxicological effects of DON include vomiting and diarrhoea, changes in the immune system as well as an increased susceptibility to infection and reduced absorption of nutrients (Pestka 2010; Sobrova et al. 2010; Payros et al. 2016). An adjustment of the DON maximum levels for unprocessed oats to include its derivatives is currently not being pursued due to a lack of data to provide an overview on their presence in European oats. In January 2022, new draft regulations on maximum levels of DON were published as part of a targeted stakeholder consultation. In this draft, the maximum level for unprocessed oats is to be maintained at 1750 µg/kg DON, while it is proposed to set the maximum level for cereal milling products at 600 µg/kg DON (Verstraete 2022). While the assumed reduction performance according to Commission Recommendation (2013/165/EU) starting from the maximum level for oats (with husk) to cereal grains for direct human consumption (oat kernels) was 80% for the sum of T-2 and HT-2 toxins, this would increase to 96% according to the new proposal.

Scudamore et al. (2007) showed in a first comprehensive study on milling oats that a large part of the mycotoxins DON and T-2 and HT-2 toxins can be reduced during commercial processing including de-hulling. Other studies mainly focused on the reduction of mycotoxins during laboratory trials (Edwards 2007; Schwake-Anduschus et al. 2010; Ivanova et al. 2017; Tittlemier et al. 2020) or estimated it from available data (Pettersson et al. 2011).

The aim of the present study was to demonstrate the reduction of the Fusarium mycotoxins DON, T-2 and HT-2 toxins during de-hulling at commercial scale according to good manufacturing practice and to find possible limitations of mycotoxin reduction. For this purpose, milling oats, pre-selected for their physical properties and DON content below 1750 µg/kg were investigated for the reduction of Fusarium mycotoxins.

In addition to studies on the commercial scale, de-hulling was to be carried out in the laboratory in order to determine and correlate the Fusarium DNA amounts of F. graminearum and F. culmorum as well as F. langsethiae and the levels of the associated mycotoxins DON and the sum of T-2 and HT-2 toxins before and after laboratory de-hulling. Furthermore, laboratory polishing tests...
were used to investigate the extent to which DON and T-2 and HT-2 toxins are found in the oat kernel. These investigations should provide information on the distribution of *Fusarium* mycotoxins in the oat kernel.

**Materials and methods**

All oat samples in this study were naturally contaminated with DON, T-2 and HT-2 toxins.

**Commercial de-hulling – reduction of DON, T-2 and HT-2 toxins**

All milling oat samples were preselected for their DON content to be below 1750 µg/kg as required by Commission Regulation (EC) No 1881/2006 (European Commission 2006a), their physical characteristics as summarised by Meyer et al. (2021), and their intended use for food purposes from harvest years 2011 to 2017. Twenty six milling oat consignments harvested in Finland, the United Kingdom (UK), Sweden and Ireland were selected for this part of the study, of which nine consignments were analysed for DON and T-2 and HT-2 toxins, 7 only for DON and 10 only for T-2 and HT-2 toxins. The reduction of mycotoxin levels was analysed in 16 production runs for DON and 19 for the sum of T-2 and HT-2 toxin. Sampling took place in accordance with Commission Regulation (EC) No 401/2006 (European Commission 2006b) and was made for milling oats before mill cleaning and after de-hulling and its subsequent cleaning and aspiration steps for large and thin oat kernels. Forty single samples of 100 g each were taken in a timely manner and aggregated for a mixed sample. The processing time from silo discharge to the cleaned, oat kernel took around 1 hour for both kernel sizes. Husk samples were also taken on the same production day, but without a time connection due to the length of the process.

**Laboratory de-hulling – reduction of DON, T-2 and HT-2 toxins and DNA amounts of *Fusarium langsethiae***

Two sets of cleaned milling oat samples were pre-selected for their initial contamination with DON \((n = 20)\) from harvest years 2011 to 2017 and T-2 and HT-2 toxins \((n = 32)\) from harvest years 2011 to 2016. These samples originated from Finland, Germany, Sweden, UK, Poland, Estonia and Ireland. Samples of 200 g were divided into portions of 100 g each using a Retsch sample splitter. Half of each oat sample was de-hulled at a pressure of 6.5 bar for 80 seconds with a compressed air peeler (Streckel und Schrader, Hamburg, Germany). These parameters were set to ensure comparable results for better adjustment of the de-hulling performance during oat milling processing. Subsequently, the fractions of milling oats and oat kernels were separated from each other and analysed separately for DON, T-2 and HT-2 toxins as well as for the related *Fusarium* DNA amounts.

**Laboratory polishing – localisation of DON and T-2 and HT-2 toxins**

Milling oat kernels \((n = 8)\) originating from Finland from the harvest years 2012 and 2013 were selected due to their concurrent contamination with DON, T-2 and HT-2 toxins. Aliquots of 200 g of oat kernels were divided using a Retsch sample splitter; 100 g of de-hulled oat kernels were polished for 22 seconds in a laboratory polishing machine (Streckel & Schrader, Hamburg, Germany). DON, T-2 and HT-2 toxins were analysed in oat kernels before and after polishing.

**Sample preparation**

Milling oats and oat kernels were ground for mycotoxin analysis by an ultra-centrifugal mill ZM 200 using a sieve size of 0.5 mm (Retsch, Haan, Germany). For DNA extraction, milling oats and oat kernels originating from the laboratory-de-hulling trials were ground in a speed rotor mill Pulverisette 14 (Fritsch, Idar-Oberstein, Germany) to 0.2 mm particle size.

**Analysis of mycotoxins**

All samples were analysed for the *Fusarium* mycotoxins DON, T-2 and HT-2 toxins by the Gesellschaft für Bioanalytik Hamburg (GBA) in

1166  J. C. MEYER ET AL.
Germany. The method used for analysing T-2 and HT-2 is described in detail by Pettersson et al. (2011) and has been validated for the analysis of T-2 and HT-2 toxins in oat grains, for all fractions used in this study. The linear range was 5–400 ng/ml. The limit of quantification (LOQ) was 5.0 µg/kg for both toxins; the limit of detection (LOD) was 0.5 µg/kg. Recoveries of T-2 toxin were in the range of 81–105% while they were 80–95% for HT-2 toxin. The relative repeatability standard deviation (RSDr) determined in oat grains was 8.9% for T-2 toxin and 5.2% for HT-2. DON analysis was performed according to DIN EN 15891: 2010-12 (Foodstuffs–Determination of deoxynivalenol in cereals, cereal products and cereal based foods for infants and young children - HPLC method with immunoaffinity column clean-up and UV detection) (DIN EN 15891 2010) and has been validated for the analysis of DON in oat grains. The linear range was 30–200 ng/ml. The LOQ was 50 µg/kg and the LOD was 12 µg/kg. The recovery of DON was in the range of 89–107%. The RSDr determined was 14.2%. The laboratory’s methods are accredited according to ISO 17025 and the laboratory has participated successfully in various proficiency tests on DON as well as T-2 and HT-2 toxins. Suppliers of proficiency tests were either BIPEA or FAPAS. Samples with a concentration below the limit of quantification (LOQ) value were assigned a concentration equal to half of the LOQ in the calculations.

**Determination of Fusarium DNA amount by quantitative PCR (qPCR)**

All samples of the laboratory de-hulling study were analysed for the DNA amounts of *Fusarium* DNA of *F. graminearum* (Fg), *F. culmorum* (Fc) and *F. langsethiae* (Fl) by the Institute of Phytopathology, Division of Plant Diseases and Crop Protection of the Kiel University, Germany.

The *Fusarium* isolates of *F. graminearum* (DSM-1095), *F. culmorum* (DSM-1094) and *F. langsethiae* (DSM-8051) used for positive controls and standard curves for the determination of DNA amounts by quantitative PCR (qPCR) were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and Aarhus University (Faculty of Agricultural Sciences, Department of Integrated Pest Management, Aarhus, Denmark). The isolates were grown on potato dextrose agar (PDA) (Carl Roth, Karlsruhe, Germany) for 2 weeks at 20 °C under 12 h light and 12 h darkness.

DNA from ground milling oats and de-hulled oat kernels was extracted from 100 mg ground material using the NucleoSpin®Plant II extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer’s instructions. The mycelium of each *Fusarium* isolate was scraped off the PDA and ground in liquid nitrogen. DNA was extracted from homogenized mycelium using the DNeasy extraction kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The purity and concentration of DNA from the fungal isolates used for standard curves and from the extracted DNA from grain samples was determined using a NanoDrop™ OneC (Thermo Scientific, Waltham, MA, USA). DNA concentrations of samples were adjusted to 20 ng/µL. DNA samples were stored at −20 °C (Birr et al. 2020).

Quantitative PCR was performed on DNA isolated from milling oats and de-hulled oat kernels with species-specific primers designed by Nicolaisen et al. (2009) for *F. graminearum*, *F. culmorum* and *F. langsethiae* as well as for plant DNA by using a qTOWER 2.2 (Analytik Jena, Jena, Germany) according to Birr et al. (2020). All samples were run in triplicate 20 µL reactions containing 10 µL SsoAdvanced™ SYBR® Green Super-mix (Bio-Rad Laboratories, Hercules, CA, USA), 2 µL total genomic DNA (20 ng/µL), 6 µL water (HPLC grade) and 1 µL of each primer (10 pmol/µL). The qPCR conditions for each *Fusarium* primer assay were 2 min at 50 °C, 95 °C for 10 min, 40 cycles of 95 °C for 15 s and 62 °C for 1 min followed by dissociation analysis from 60 to 95 °C, whereby for the plant assay annealing and extension were performed at 60 °C. All samples were analysed using the three *Fusarium* primer assays together with the plant assay. A five-fold dilution series (0, 0.05, 0.5, 5 and 50 ng/µL) with pure fungal and oat DNA using the aforementioned *Fusarium* isolates and healthy oat kernels for plant DNA was run to generate a standard curve for each *Fusarium* and the plant
assay. The results of each investigated sample from each species-specific and plant assay were evaluated by studying the dissociation curve and cycle threshold (Ct) value. The amount of *Fusarium* DNA was calculated from the Ct values using the standard curve, and these values were normalized with the estimated amount of plant DNA based on the plant assay. The DNA amount of each species was calculated as pg fungal DNA per ng plant DNA. Standards were included in every plate to account for differences in qPCR efficiencies between runs.

**Data analysis**

Paired t-tests were used to compare statistically the reduction rates of DON and the sum of T-2 and HT-2 toxins before and after de-hulling on commercial and laboratory scale as well as the difference between large and small oat kernels during commercial de-hulling. In addition, the paired t-test was also used for statistical comparison of the DON levels and the sum of T-2 and HT-2 toxins before and after laboratory polishing. The same test was used to compare the reduction performance of the total DNA amounts of both *F. graminearum* and *F. culmorum* or *F. langsethiae* before and after laboratory de-hulling. Correlation analyses were performed to determine the relationships between individual variables, such as DON or the sum of T-2 and HT-2 toxins and the corresponding DNA amounts of *Fusarium* species before and after laboratory de-hulling. All confidence levels were specified as either \( p < 0.05 \) or \( p < 0.01 \). These and all other calculations were performed using Microsoft® Excel 365.

**Results and discussion**

The reduction of DON, T-2 and HT-2 toxins was investigated in milling oat samples harvested in different countries in 2011–2017. Milling oats were de-hulled under commercial and laboratory conditions, and the mycotoxin levels of the resulting fractions (oat kernels and husks) were compared with the levels of the starting material. In addition, the fractions obtained by laboratory de-hulling were analysed for their *Fusarium* DNA amounts and correlated with the associated mycotoxin levels. Finally, the distribution of mycotoxins within oat kernels was determined by polishing under laboratory conditions.

**Commercial de-hulling**

**Reduction of DON contents**

A total of 16 production runs were examined for the reduction of DON (Table 1). The milling oats to be examined had on average the following

| Sample no | Origin | Harvest year | DON [μg/kg] | Reduction [%] |
|-----------|--------|--------------|-------------|--------------|
|           |        |              | Milling oats | Large oat kernels | Thin oat kernels | Husks | Large oat kernels | Thin oat kernels |
| 1         | Finland | 2011         | 122         | 54            | 69            | 212 | 56            | 44            |
| 2         | Finland | 2011         | 1175        | 546           | 611           | 1529 | 54            | 48            |
| 3         | Finland | 2011         | 796         | 271           | 347           | 2473 | 66            | 56            |
| 4         | Finland | 2012         | 579         | 153           | 202           | 1354 | 74            | 65            |
| 5         | Finland | 2012         | 1168        | 280           | 381           | 2493 | 76            | 67            |
| 6         | Finland | 2012         | 1059        | 291           | 273           | 2850 | 73            | 74            |
| 7         | Finland | 2012         | 227         | 218           | 267           | na   | 4             | 9             |
| 8         | Finland | 2012         | 1194        | 318           | 477           | na   | 73            | 60            |
| 9         | Finland | 2012         | 942         | 297           | 368           | 2399 | 68            | 61            |
| 10        | Finland | 2012        | 606         | 145           | 285           | 1177 | 76            | 53            |
| 11        | Finland | 2012        | 236         | 98            | 63            | 873  | 58            | 73            |
| 12        | Finland | 2012        | 1357        | 360           | 467           | 2393 | 73            | 66            |
| 13        | Finland | 2013        | 435         | 358           | 442           | 1090 | 18            | –2            |
| 14        | Finland | 2014        | 755         | 153           | 189           | 2034 | 80            | 75            |
| 15        | Finland | 2016        | 1384        | 364           | 128           | 3508 | 74            | 91            |
| 16        | Finland | 2017        | 189         | 75            | 58            | 440  | 60            | 69            |
| Mean ± SD |        |              | 764 ± 436   | 249 ± 131     | 285 ± 166     | 1773 ± 965 | 61 ± 21       | 57 ± 24       |
| Min       |        |              | 122         | 54            | 33            | 212  | 4             | –2            |
| Max       |        |              | 1384        | 546           | 611           | 3508 | 80            | 91            |

na: not analysed; SD: standard deviation.
physical parameters: proportion of oat kernels 74%, husks 26%, and thin oats (<2 mm slotted hole sieve) 8%. The mean content of DON was 764 ± 436 μg/kg in milling oats ranging from 122 to 1384 μg/kg. In large oat kernels a mean DON contamination of 249 ± 131 μg/kg was found, whereas in thin oat kernels it was 285 ± 166 μg/kg. While the reductions of milling oats were

Figure 1. Commercial de-hulling – (a) DON contents (µg/kg) in milling oats and the corresponding percentage reduction (%) for large and thin oat kernels after commercial de-hulling. The auxiliary line at 66% represents the assumed reduction performance resulting from the proposals to change the maximum levels of DON in unprocessed oats and cereal milling products (Verstraete 2022). (b) DON contents (µg/kg) in milling oats and the resulting DON contents (µg/kg) for large and thin oat kernels after commercial de-hulling. The auxiliary line at 600 µg/kg represents the proposed maximum level of DON in cereal milling products (Verstraete 2022).
significant for both large and small oat kernels ($p < 0.01$), no significant difference ($p = 0.07$) was found between large and small oat kernels. Nevertheless, a strong relationship of DON concentrations was found between large and thin kernels after de-hulling ($R^2 = 0.69; p < 0.01$). The reduction by de-hulling varied in a wide range. For large kernels, DON was reduced by 61% after de-hulling and varied between 4% and 80%; for thin kernels it was similar with 57%, ranging from /C02% to 91% (Figure 1(a)). Ninety per cent of the 16 production runs showed a reduction of DON between 14% and 77% for the large kernels and 7% and 87% for the thin kernels. The combined reduction of large and thin oat kernels was 61%. In conclusion, the reduction of DON contents was similar for large and thin kernels from the same milling oats.

Brodal et al. (2020) reported that sieving may reduce DON concentrations in large oats with husks to a maximum of 24% at initial levels between 100 and 309 mg/kg using a 2.2 mm slotted hole sieve. In comparison to our study, it must be noted that the mean percentage of thin oats in the study of Brodal et al. (2020) was 21%, compared to 8% of this study.

All de-hulled oat kernels complied with the maximum level of DON for intermediate products of 750 mg/kg (European Commission 2006a). As shown in Figure 1(b), only one production run with an initial contamination of 1175 mg/kg DON originating from Finland would not meet the newly proposed limit of 600 µg/kg (Verstraete 2022).

Hietaniemi et al. (2008) reported that commercial sorting and de-hulling reduced DON levels in unsorted oat samples by 75–91%. The difference to our investigations can be explained by the circumstance that we examined only pre-cleaned milling oats. Scudamore et al. (2007) showed a reduction for DON of 88% in four commercial milling trials down to oat flakes (three from Finland and one from Sweden). On average these samples contained 628 mg/kg DON and showed a range between 253 and 1230 mg/kg. We observed that the assumed DON reduction of 66% for large oat kernels was not achieved in 9 out of 16 production runs with a mean reduction of 74%. Here, the mean initial DON content was 1005 µg/kg and varied between 579 and 1384 µg/kg; the median was 1059 µg/kg. In contrast, 7 of 16 production runs achieved a mean reduction of 45%. For these samples the mean content was 454 µg/kg and varied between 122 and 1175 µg/kg; the median was 236 µg/kg (Table 1, Figure 1(a,b)). This observation supports the hypothesis of

| Sample no | Origin        | Harvest year | Sum of T-2 and HT-2 toxins [mg/kg] | Reduction [%] |
|-----------|---------------|--------------|-----------------------------------|---------------|
|           |               |              | Milling oats | Large oat kernels | Thin oat kernels | Husks | Large oat kernels | Thin oat kernels |
| 1         | Finland       | 2011         | 63          | 25               | 54             | 103   | 59               | 15             |
| 2         | Finland       | 2011         | 173         | 46               | 82             | 389   | 73               | 52             |
| 3         | Finland       | 2011         | 220         | 58               | 113            | 675   | 73               | 49             |
| 4         | Finland       | 2012         | 252         | 16               | 69             | 428   | 94               | 73             |
| 5         | Finland       | 2012         | 146         | 26               | 56             | na    | 82               | 62             |
| 6         | Finland       | 2012         | 224         | 26               | 61             | 292   | 88               | 73             |
| 7         | UK            | 2014         | 338         | 108              | 135            | 1292  | 68               | 60             |
| 8         | UK            | 2014         | 733         | 92               | 169            | 1370  | 87               | 77             |
| 9         | UK            | 2014         | 485         | 69               | 106            | 897   | 86               | 78             |
| 10        | Finland       | 2014         | 314         | 22               | 74             | 611   | 93               | 76             |
| 11        | Finland       | 2014         | 355         | 38               | 70             | 1298  | 89               | 80             |
| 12        | Finland       | 2014         | 318         | 30               | 67             | 898   | 91               | 79             |
| 13        | Ireland       | 2014         | 532         | 26               | 209            | 2270  | 95               | 61             |
| 14        | UK            | 2014         | 219         | 48               | 131            | 535   | 78               | 40             |
| 15        | UK            | 2014         | 1856        | 50               | 125            | 4436  | 97               | 93             |
| 16        | Sweden        | 2015         | 537         | 56               | 195            | 954   | 90               | 64             |
| 17        | Ireland       | 2015         | 641         | 30               | 139            | 1530  | 95               | 78             |
| 18        | Finland       | 2016         | 227         | 25               | 16             | 741   | 89               | 93             |
| 19        | Finland       | 2017         | 165         | 39               | 98             | 368   | 76               | 41             |
| Mean ± SD |               |              | 409 ± 393   | 44 ± 25          | 104 ± 51       | 1056 ± 999 | 85 ± 10 | 66 ± 20           |
| Min       |               |              | 63          | 16               | 16             | 103   | 60               | 15             |
| Max       |               |              | 1856        | 108              | 209            | 4436  | 97               | 93             |

SD: standard deviation.
Figure 2. Commercial de-hulling – (a) Sum of T-2 and HT-2 toxins contents (µg/kg) in milling oats and the corresponding percentage reduction (%) in large and thin oat kernels after commercial de-hulling. The auxiliary line at 96% represents the assumed reduction performance according to Verstraete (2022) resulting from the proposals to introduce maximum levels for the sum of T-2 and HT-2 toxins in oats (with husk) and cereals placed on the market for the final consumer (oats) b) Sum of T-2 and HT-2 toxin contents (µg/kg) in milling oats and the resulting sum of T-2 and HT-2 toxin contents (µg/kg) for large and thin oat kernels after commercial de-hulling. The auxiliary line at 50 µg/kg represents the proposed maximum level of the sum of T-2 and HT-2 toxins in oats as cereal grains placed on the market for the final consumer (oats) (Verstraete 2022).
Scudamore et al. (2007) that the reduction in heavily contaminated unprocessed oats was higher compared to less contaminated consignments.

**Reduction of the sum of T-2 and HT-2 toxin contents**

For the investigation on reduction of T-2 and HT-2 toxins by de-hulling, 19 milling oat consignments were selected according to their DON content to be below 1750 µg/kg (Table 2). The proportion of oats kernels, husks and thin oats (<2 mm slotted hole sieve) in these samples was on average 74%, 26% and 7%, respectively. For these milling oats a mean value of 409 ± 393 µg/kg was found for the sum of T-2 and HT-2 toxins, ranging from 63 to 1856 µg/kg (Table 2). After de-hulling large oat kernels contained on average 44 ± 25 µg/kg for the sum of T-2 and HT-2 toxins, ranging between 16 and 108 µg/kg. Thin kernels contained on average 104 ± 51 µg/kg for the sum of T-2 and HT-2 toxins, ranging from 16 to 209 µg/kg. The reduction rates from milling oats were significant (p < 0.01) for both large and small oat kernels, as there was a significant difference (p < 0.01) between large and small oat kernels. The sum of T-2 and HT-2 toxin between both fractions did not correlate (p = 0.11).

A similar observation was made by Brodal et al. in 2020; thin oats with husk exhibited a higher T-2 and HT-2 toxins content. Investigating samples from two different harvest years they showed a reduction of up to 56% and a weight reduction of up to 21% by removal of thin oats with husk using a sieve size of 2.2 mm. This is interesting as Imathiu et al. (2013) and Martin et al. (2018) reported that *F. langsethiae* infections showed no visible symptoms during field trials and additionally no changes in oat yield (Imathiu et al. 2013) or thousand kernel weight (Martin et al. 2018). The difference in T-2 and HT-2 toxins reduction hints at differences in the growth of *Fusarium langsethiae* during oat flowering. Rajala and Peltonen-Sainio (2011) reported that within a spikelet, the first flower is pollinated on average one day before the second flower. Divon et al. (2012b, 2019) and Tekle et al. (2012) showed that during flowering *Fusarium* growth into the oat grain including the oat kernel is facilitated by anthers and pollen as they provide a conducive environment. Furthermore, Tekle et al. (2012) reported that fungal hyphae were observed spreading from the primary to secondary and tertiary flower and that the latter was infected a few days after inoculation. Therefore, we assume that the second and third flowers, with an almost comparable pollen count and a larger surface due to a lower kernel weight, meet a comparable number of *F. langsethiae* hyphae which grow into the kernels to produce higher T-2 and HT-2 toxin contents.

The reduction of the sum of T-2 and HT-2 toxins after de-hulling was sufficient for all oat kernels, except one Irish sample, to meet the indicative level of 200 µg/kg as laid down in the Commission Recommendation 2013/165/EU (European Commission 2013) (Table 2, Figure 2(b)). However, based on the recently proposed level of 50 µg/kg for the sum of T-2 and HT-2 toxins for cereals placed on the market for the final consumer (oats) (Verstraete 2022), five of the large oat kernel samples received from these 19 production runs would not have complied with this level. Interestingly, the initial levels of five of these milling oat samples were below the newly proposed maximum level of 1250 µg/kg for unprocessed cereals (oats) at levels between 220 and 723 µg/kg (Table 2). We obtained a different picture for thin oat kernels as 18 production runs were above the proposed maximum level of 50 µg/kg and only one of these unprocessed cereals (oats) contained more than 1250 µg/kg. The reduction between large and thin oat kernels from the same milling oat consignments differed markedly. This was in contrast to the reduction of DON. While the reduction for the sum of T-2 and HT-2 toxins for large kernels was 85% on average (ranging between 60 and 97%), it was 65% (in the range of 15% and 93%) for thin kernels. These differences are presented in Table 2. Ninety percent of the 19 production runs showed a reduction by de-hulling between 67 and 96% for the large kernels and between 38% and 93% for thin kernels, respectively. The combined reduction of larger and thin oat kernels was 83%. Only one of 19 production runs for large oat kernels with an initial content of 1856 µg/kg for the sum of T-2 and HT-2...
toxins achieved a reduction of 97%. Six other production runs showed a reduction of at least 93% (Figure 2(a)). Here, the initial contents were between 252 and 641 mg/kg for the sum of T-2 and HT-2 toxins. The remaining 12 production runs achieved a reduction performance of 60% on average; this varied between 15% and 93%. The initial levels of these production runs were between 63 and 723 mg/kg for the sum of T-2 and HT-2 toxins (Table 2, Figure 2(a)).

Scudamore et al. (2007) reported a higher average reduction of up to 95% compared to our study. The maximum reduction of 99% was achieved for milling oats with an initial contamination of 2467 and 3528 μg/kg for the sum of T-2 and HT-2 toxins, respectively. The higher reduction rate could be explained by testing of mainly UK milling oats, as these are supplied to UK oat mills without prior cleaning (Croucher 2020; Gowlett 2020). In comparison, Swedish and Finnish oats are dried and aspirated (cleaned) directly after harvesting before storage (Heeschen and Säddler 2010; Lähdetie 2020; Pekkala 2020; Pettersson 2020). This prior cleaning could account for the slightly lower estimate of Pettersson et al. (2011) for the reduction of 82% for T-2 toxin and 88% for HT-2 toxin from milling oats to rolled oats.

In summary, five production runs with initial levels for the sum of T-2 and HT-2 toxins between 220 and 723 mg/kg would have corresponded to the newly discussed maximum level of 1250 mg/kg, resulted for both large and thin oat kernels in not complying with the newly discussed maximum level of 50 μg/kg (Verstraete 2022). The reduction of these five production runs were between 68 and 90% for large kernels and between 49% and 78% for thin kernels.

DON and the sum of T-2 and HT-2 toxin contents in husks

After de-hulling, highest contents of DON, T2 and HT2 toxins were found in the husk fraction (Tables 1 and 2). In 16 commercial production runs, oat husks showed a mean DON contamination of 1773 μg/kg and in 19 production runs mean for the sum of T-2 and HT-2 toxin concentrations of 1056 μg/kg. The maximum DON concentration was 3508 μg/kg in husks of a Finnish milling oat sample with an initial

| Sample No | Origin | Harvest year | Total DNA amount of Fg/Fc (µg) | DON [µg/kg] | Total DNA amount of Fg/Fc (µg) | DON [µg/kg] | Reduction after de-hulling |
|-----------|--------|--------------|-------------------------------|-------------|-------------------------------|-------------|---------------------------|
|           |        |              |                               |             |                               |             | Fg/Fc [%] |
|           |        |              |                               |             |                               |             | DON [%]    |
| 1         | Finland | 2013        | 2.95                          | 1163        | 0.92                          | 315         | 69          | 73          |
| 2         | Finland | 2014        | 6.82                          | 1130        | 1.35                          | 545         | 80          | 52          |
| 3         | Finland | 2016        | 2.85                          | 988         | 0.53                          | 290         | 82          | 71          |
| 4         | Finland | 2017        | 5.98                          | 1792        | 1.86                          | 885         | 69          | 51          |
| 5         | Finland | 2012        | 1.85                          | 873         | 0.54                          | 256         | 71          | 71          |
| 6         | Germany | 2012        | 0.42                          | 116         | 0.70                          | 25          | 80          | 52          |
| 7         | Finland | 2012        | 4.00                          | 876         | 1.61                          | 316         | 48          | 64          |
| 8         | Finland | 2012        | 2.47                          | 572         | 0.65                          | 98          | 74          | 83          |
| 9         | Finland | 2012        | 3.46                          | 667         | 1.13                          | 310         | 67          | 54          |
| 10        | Finland | 2012        | 1.59                          | 484         | 0.58                          | 79          | 63          | 84          |
| 11        | Finland | 2012        | 2.36                          | 232         | 1.01                          | 140         | 57          | 40          |
| 12        | Sweden  | 2011        | 1.37                          | 531         | 0.49                          | 52          | 64          | 90          |
| 13        | Finland | 2012        | 4.86                          | 1589        | 0.37                          | 435         | 92          | 73          |
| 14        | Finland | 2013        | 3.66                          | 430         | 0.35                          | 132         | 90          | 69          |
| 15        | Sweden  | 2013        | 0.87                          | 425         | 0.40                          | 78          | 54          | 82          |
| 16        | Finland | 2013        | 1.99                          | 456         | 0.83                          | 156         | 58          | 66          |
| 17        | Finland | 2013        | 1.55                          | 462         | 0.59                          | 25          | 62          | 95          |
| 18        | Finland | 2013        | 1.96                          | 202         | 1.15                          | 25          | 41          | 88          |
| 19        | Finland | 2013        | 2.02                          | 349         | 0.03                          | 84          | 98          | 76          |
| 20        | Finland | 2013        | 1.48                          | 582         | 0.43                          | 51          | 71          | 91          |
| Mean ± SD |        |              | 2.73 ± 1.66                    | 696 ± 449   | 0.78 ± 0.46                    | 215 ± 216   | 62 ± 15      | 72 ± 34     |
| Min       |        |              | 0.42                          | 116         | 0.03                          | 25          | –69          | 40          |
| Max       |        |              | 6.82                          | 1792        | 1.86                          | 885         | 98          | 95          |

(*) pg fungal DNA/ng of plant DNA; SD: standard deviation.
content of 1348 μg/kg and for the sum of T-2 and HT-2 toxins it was 4436 μg/kg in husks in a sample from the United Kingdom with an initial content of 1856 μg/kg. The oat husks thus amounted to almost 2.5 times the initial mycotoxin contents in milling oats. This is in line with observations of Ivanova et al. (2017), Pettersson et al. (2011), Šliková et al. (2010) and Scudamore et al. (2007). The higher mycotoxin contents in husks can be explained by moulds being able to colonise the inside of husks more easily due to weaker cells and thinner walls (Schmidt 1981).

**Laboratory de-hulling**

For a better understanding of the effectiveness of commercial de-hulling, laboratory tests were carried out. Therefore, oat samples selected for their initial DON, T-2 and HT-2 toxins content, were subjected to a pre-cleaning and showed a husk content of 23–31%. These samples were divided...
and either analysed for DON, T-2 and HT-2 toxins and the related DNA amounts of *F. graminearum*, *F. culmorum* and *F. langsethiae* or subjected to laboratory de-hulling. Oat kernels were manually removed before the subsequent analysis. Due to the smaller sample size it was not possible to differentiate between large and thin kernels. Therefore, the reduction rates achieved during these experiments refer to the total amount of oat kernels obtained in contrast to the mill trials described above.

**Reduction of DON and DNA amounts of *F. graminearum* and *F. culmorum***

In total, 20 milling oat samples were pre-selected for their DON content (Table 3). This was on average 696 ± 449 µg/kg and ranged from 116 to 1792 µg/kg between samples. Total DNA amounts of *F. graminearum* and *F. culmorum* ranged between 0.42 and 6.82 pg fungal DNA/ng of plant DNA, with an average of 2.73 ± 1.66 pg fungal DNA/ng of plant DNA. In our study, *F. graminearum* was the dominant DON producing species and showed a 25-fold higher DNA amount compared to *F. culmorum* (Supplementary Data 2). The DON content of the oat kernels was on average 215 ± 216 µg/kg and ranged between 25 and 885 µg/kg. The total DNA amounts of *F. graminearum* and *F. culmorum* in the oat kernels was 0.78 ± 0.46 pg fungal DNA/ng of plant DNA in the range between 0.03 and 1.86 pg fungal DNA/ng of plant DNA. The reduction of the DON content as well as the total DNA amount of *F. graminearum* and *F. culmorum* were

| Sample No | Origin   | Harvest year | DNA amount of Fl (µg) | Sum of T-2 and HT-2 toxins (µg/kg) | DNA amount of Fl (µg) | Sum of T-2 and HT-2 toxins (µg/kg) | Reduction after de-hulling |
|-----------|----------|--------------|-----------------------|-----------------------------------|-----------------------|-----------------------------------|-----------------------------|
| 1         | Finland  | 2011         | 1.57                  | 125                               | 0.29                  | 17                                | 82                         |
| 2         | Finland  | 2011         | 0.88                  | 140                               | 0.19                  | 20                                | 79                         |
| 3         | Finland  | 2012         | 0.97                  | 233                               | 0.23                  | 29                                | 77                         |
| 4         | Finland  | 2012         | 0.62                  | 140                               | 0.37                  | 20                                | 44                         |
| 5         | Finland  | 2012         | 0.88                  | 243                               | 0.13                  | 67                                | 85                         |
| 6         | Finland  | 2012         | 1.40                  | 337                               | 0.28                  | 41                                | 80                         |
| 7         | Poland   | 2012         | 0.52                  | 38.5                              | 0.02                  | 11                                | 95                         |
| 8         | Finland  | 2012         | 2.57                  | 879                               | 0.66                  | 128                               | 75                         |
| 9         | Finland  | 2013         | 1.79                  | 719                               | 0.24                  | 39                                | 87                         |
| 10        | Estonia  | 2013         | 4.99                  | 377                               | 0.69                  | 89                                | 86                         |
| 11        | Sweden   | 2013         | 10.09                 | 578                               | 1.99                  | 156                               | 80                         |
| 12        | Finland  | 2012         | 1.00                  | 295                               | 0.26                  | 12                                | 73                         |
| 13        | UK       | 2012         | 5.28                  | 743                               | 1.18                  | 92                                | 78                         |
| 14        | Germany  | 2012         | 1.36                  | 98                                | 0.18                  | 10                                | 86                         |
| 15        | Finland  | 2012         | 2.15                  | 277                               | 0.21                  | 50                                | 90                         |
| 16        | Finland  | 2012         | 1.31                  | 147                               | 0.31                  | 26                                | 76                         |
| 17        | Finland  | 2012         | 1.69                  | 477                               | 0.08                  | 24                                | 95                         |
| 18        | Finland  | 2012         | 1.86                  | 231                               | 0.26                  | 26                                | 85                         |
| 19        | Finland  | 2012         | 2.06                  | 266                               | 0.29                  | 26                                | 86                         |
| 20        | Finland  | 2012         | 1.39                  | 179                               | 0.16                  | 26                                | 88                         |
| 21        | Finland  | 2013         | 1.47                  | 113                               | 0.19                  | 20                                | 87                         |
| 22        | Sweden   | 2013         | 0.32                  | 11                                | 0.11                  | 8                                 | 63                         |
| 23        | Finland  | 2013         | 1.41                  | 132                               | 0.23                  | 36                                | 84                         |
| 24        | Finland  | 2013         | 4.44                  | 373                               | 0.37                  | 34                                | 91                         |
| 25        | Finland  | 2013         | 2.09                  | 166                               | 0.80                  | 25                                | 63                         |
| 26        | UK       | 2013         | 4.27                  | 508                               | 0.94                  | 59                                | 77                         |
| 27        | Finland  | 2013         | 2.12                  | 247                               | 0.46                  | 46                                | 78                         |
| 28        | Finland  | 2013         | 2.50                  | 308                               | 0.56                  | 27                                | 77                         |
| 29        | UK       | 2014         | 13.40                 | 3429                              | 2.92                  | 307                               | 78                         |
| 30        | UK       | 2013         | 7.31                  | 1585                              | 1.82                  | 230                               | 75                         |
| 31        | Ireland  | 2016        | 2.20                  | 421                               | 0.41                  | 13                                | 82                         |
| 32        | Finland  | 2016        | 0.75                  | 103                               | 0.15                  | 19                                | 80                         |

Mean ± SD 2.71 ± 2.87 435 ± 627 0.53 ± 0.63 54 ± 66 80 ± 10.0 84 ± 12.6
Min 0.32 11 0.02 8 44 25
Max 13.40 3429 2.92 307 95 97

(*) pg fungal DNA/ng of plant DNA.
(**) These samples originated from the same milling oat consignments, but were taken on successive production days; SD: standard deviation.
significant \((p < 0.01)\). The laboratory reduction of 72\% – with a range from 40\% to 95\% – was slightly higher compared to the combined reduction of 62\% in the oat mill. The reduction of total DNA amounts of \textit{F. graminearum} and \textit{F. culmorum} in de-hulled kernels was comparable to the DON reduction.

DON contents and total fungal DNA amounts of \textit{F. graminearum} and \textit{F. culmorum} correlated significantly before and after laboratory de-hulling, which is indicated by the coefficient of determination of \(R^2 = 0.81\) \((p < 0.01)\) for DON contents and \(R^2 = 0.34\) \((p < 0.01)\) for the total DNA amounts of \textit{F. graminearum} and \textit{F. culmorum} (Figure 3(a,b)). The coefficient of determination between DON concentrations and total DNA amounts of \textit{F. graminearum} and \textit{F. culmorum} in milling oat samples was \(R^2 = 0.59\) \((p < 0.01)\) and decreased to \(R^2 = 0.42\) \((p < 0.05)\) after de-hulling (Figure 3(c,d)). Fredlund et al. (2013), Haikka et al. (2020) and Yli-Mattila et al. (2008) reported a comparable correlation between DNA amounts of \textit{F. graminearum} and DON contents in oat grains.

In comparison to the reduction of DON of 72\% during laboratory de-hulling described in our work, Tittlemier et al. (2020) reported an average reduction of 87\% whereas Ivanova et al. (2017) determined a reduction of 94\%. While oats grains in the first study had a varying cleaning status, the percentage of husk content was approximately 26\% (Tittlemier 2021). In the second study oat grain samples were cleaned after de-hulling and the content of removed husks was 31 up to 43\%. A higher husk content could also explain a higher reduction as a later point of time for infection may lead to an accumulation of DON in husks (Tekle et al. 2013). This observation is supported by Orina et al. (2017) who noticed that an increase of the husk fraction is correlated with a significant DON contamination. We assume that the oat kernel itself becomes more resistant and fewer nutrients are available for further \textit{Fusarium} growth and that these therefore primarily infest the husks. Yan et al. (2010) recorded reduction between 86\% and 55\% in two different years and assumed that the lower reduction rate depended on lower initial DON concentrations. Unfortunately cleaning status and husk content were not described. In our opinion, differences in the reduction are based on a different cleaning status of the oat samples examined on the one hand and higher husk content on the other hand.

### Reduction of the sum of T-2 and HT-2 toxins and DNA amounts of \textit{Fusarium langsethiae}

A total of 32 cleaned oat samples were selected for their T-2 and HT-2 toxin contents for the laboratory de-hulling experiments (Table 4). The initial mean for the sum of T-2 and HT-2 toxin contamination of these samples was 435 ± 627 \(\mu g/kg\) and varied between 11 and 3429 \(\mu g/kg\). Both toxins exhibited levels above the LOQ in all samples.
samples. A mean DNA amount of *F. langsethiae* of 2.71 ± 2.87 pg fungal DNA/ng of plant DNA was found, ranging from 0.32 to 13.40 pg fungal DNA/ng of plant DNA. The mean content of the sum of T-2 and HT-2 toxin of the oat kernels was 54 ± 66 mg/kg and ranged between 8 and 307 mg/kg. The DNA amount of *F. langsethiae* in the oat kernels was 0.53 ± 0.63 pg fungal DNA/ng of plant DNA in the range between 0.02 and 2.92 pg fungal DNA/ng of plant DNA. The reduction of the sum of T-2 and HT-2 toxins as well as the DNA amounts of *F. langsethiae* (*p* < 0.01) were significant. The concentrations for the sum of T-2 and HT-2 toxins before and after laboratory de-hulling showed a strong correlation (*R*^2^ = 0.83; *p* < 0.01) as shown in Figure 4(a). Moreover, an even distribution of DNA amounts of *F. langsethiae* between milling oats and oat kernels is documented by the coefficient of determination of *R*^2^ = 0.92 (*p* < 0.01) (Figure 4(b)). The correlation between the sum of T-2 and HT-2 toxins and *F. langsethiae* DNA amounts in milling oats is presented in Figure 4(c) (*R*^2^ = 0.70; *p* < 0.01) and is comparable to results of Fredlund et al. (2013) and Edwards et al. (2012). It even rose to *R*^2^ = 0.85 (*p* < 0.01) in the oat kernels (Figure 4(d)) and indicates that *F. langsethiae* is a major producer of T-2 and HT-2
toxins. This shows that *F. langsethiae* is not only found in the husks but also penetrates the oat kernels, as shown by Divon et al. (2019). Expressed as a percentage, the laboratory reduction was 84% (25–97%) on average and thus comparable to the combined mill reduction for large and thin oat kernels of 83%. At the same time, the amount of *F. langsethiae* DNA was reduced by 80%. Even higher reductions of up to 95% were reported in laboratory trials (Edwards 2007; Schwake-Anduschus et al. 2010; Ivanova et al. 2017).

Differences in reduction performance can be explained by varying preliminary conditions such as cleaning level (Brodal et al. 2020), husk content or applied laboratory parameters. Edwards (2007) examined uncleaned oat samples with husk content between 23 and 43%, but noticed, contrary to our assumption, no influence of the husk contents (Edwards 2021). Schwake-Anduschus et al. (2010) cleaned the investigated unprocessed oat samples with a husk content of 24 up to 33% (Schwake-Anduschus 2021), but the de-hulling time was doubled compared to ours and thus abrasion of outer kernel layers (as explained later) might have occurred. Ivanova et al. (2017) cleaned the oat kernels after de-hulling and described unusually high husk contents for milling oats of 31 up to 43%. Only two of the received 32 oat kernel samples showed a sum of T-2 and HT-2 toxins that was above the indicative level of 200 µg/kg (European Commission 2013). However, the initial concentration of these milling oat samples exceed the proposed maximum level for unprocessed cereals (oats) of 1250 µg/kg T-2 and HT-2 toxins (Verstraete 2022) with 1585 and 3429 µg/kg, respectively. Additionally, six oat kernel samples resulting from milling oats complying with the newly proposed maximum level of 1250 µg/kg for the sum of T-2 and HT-2 toxins showed contents of 59–156 µg/kg. Thus, these six oat kernels would no longer comply with the maximum level of 50 µg/kg currently under discussion.

Even though the results are based on a limited number of samples examined, qPCR results showed a dominant influence of *F. langsethiae* on T-2 and HT-2 toxin contents of both milling oats and oat kernels. This assumption is supported by Divon’s (2021) observations in preliminary in vitro experiments on the growth rate of various *Fusarium* species on amylopectin. Results showed that *F. langsethiae* grows more easily on this medium compared to *F. graminearum*.

The situation seems to be different for a contamination with DON. The lower correlations between DON contents and total DNA amounts of *F. graminearum* and *F. culmorum* in both milling oats and de-hulled oat kernels suggest that diffusion and/or transport processes of the highly soluble DON (Karlovsky et al. 2016) via the vascular system of the plant may occur in oats as described by Schwake-Anduschus et al. (2015) for wheat, based on observations of Kang and Buchenauer (1999).

**Laboratory polishing – localisation of DON and T-2 and HT-2 toxins**

It has been shown by Tekle et al. (2012) and Divon et al. (2012a, 2019) that *Fusarium* hyphae may penetrate through the natural openings of the oat flower and infect the internal surfaces of the husks (palea and lemma) and the oat kernel itself. This observation has also been made in the past for wheat grains (Seitz and Bechtel 1985; Jackowiak et al. 2005). Particularly, outer layers of small grains contain higher mycotoxin levels as shown for DON in oats (Ivanova et al. 2017), for T-2 and HT-2 toxins and DON in durum wheat (Visconti et al. 2004; Ríos et al. 2009; Pascale et al. 2011)) and for DON in wheat (Tibola et al. 2016). Schwake-Anduschus et al. (2015) observed a more even distribution of DON and its glycosylated derivative in wheat in the different milling fractions.

In our polishing tests (*n*= 8) using a laboratory polishing machine, 3.2% of the outer layers of the oat kernels were removed (Table 5). While the unpolished oat kernels contained on average 430 ± 184 µg/kg DON in the range of 191–694 µg/kg, in the polished oat kernels remained 305 ± 166 µg/kg (97 to 609 µg/kg). Thus, the DON content could be reduced by 32% on average. The correlation for DON before and after polishing was $R^2 = 0.90$ ($p < 0.01$). From the initial concentration of 56 ± 11 µg/kg for the sum of T-2 and HT-2 toxins (in the range of 39–67 µg/kg) in
the unpolished oat kernels, 33 ± 13 µg/kg (17–54 µg/kg) remained. This corresponds to a mean reduction of 42%. The correlation for the sum of T-2 and HT-2 toxins $R^2 = 0.57$ ($p < 0.01$). In other words, the removal of the outer 3% of an oat grain contained about one third of the mycotoxins, while two thirds were found in the remaining 97%. The reduction by polishing was significant for DON as well as for the sum of T-2 and HT-2 toxins ($p < 0.01$). Ivanova et al. (2017) reported that the outer 10% of an oat kernel contained about 60% of the initial DON content. In addition, they reported that the DON content decreased gradually towards the inner parts of the kernel. The observations of Ivanova et al. (2017) and those of our polishing experiments as well as the findings concerning the colonisation of the oat kernels by Fusarium of Tekle et al. (2012) and Divon et al. (2019) showed that Fusarium toxins can also be found in oat kernels and that de-hulling alone is not sufficient to remove mycotoxin contamination in unprocessed oats.

**Conclusions**

*Fusarium graminearum* is the dominant DON producing species whereas *Fusarium langsethiae* is the major producer of T-2 and HT-2 toxins in milling oats grown in Northern Europe. Commercial de-hulling is able to significantly reduce DON and T-2 and HT-2 toxins (60% and 80%, respectively). Further, the degree of reduction depends on the initial mycotoxin level for DON and the sum of T-2 and HT-2 toxins in milling oats. Remarkably, thin oat kernels contained higher levels of the sum of T-2 and HT-2 toxins than larger oat kernels. Therefore, sieving of oats grains may lead by a separation of large kernels with lower and thin oat kernels with higher levels. In particular, we observed a significant correlation between the sum of T-2 and HT-2 toxins and the DNA amount of *F. langsethiae* in milling oats before and in oat kernels after de-hulling. This observation confirms previous studies that *F. langsethiae* also infects the developing oat kernel during flowering. We were able to show using laboratory polishing that at least one third of the contents of DON and the sum of T-2 and HT-2 toxins are located in the outer marginal layers of the oat kernel; two thirds remained in the rest of the oat kernel.

However, determination of initial contamination of milling oats with T-2 and HT-2 toxins may not provide sufficient information on the expected levels of the corresponding oat kernels. Milling oats that would meet the recently proposed maximum level of 1250 µg/kg of the sum of T-2 and HT-2 toxins will not necessarily result in oat kernels that meet the recently proposed maximum levels of 50 µg/kg of the sum of T-2 and HT-2 toxins by de-hulling only.

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