**Annex A – Mycotoxin producing fungi**

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1. Mycotoxin producing fungi in main crops
   1. *Aspergillus flavus*

*Aspergillus* spp. comprise all validated aflatoxin-producing fungi. Most of the known species belong to the *Aspergillus* section *Flavi*, including *Aspergillus flavus* and its close relative *A. parasiticus*. These two species are the most commonly occurring aflatoxin-producing species in maize. *A. flavus* and *A. parasiticus* are the most similar strains of *A.* section *Flavi*, sharing 96% DNA identity of the aflatoxin gene clusters (Cary, 2006). They can be distinguished from one another by using morphological and physiological characters, but *A. flavus* usually only produces B series aflatoxins (AFs), while *A. parasiticus* can produce both B and G series AFs. However, non-aflatoxigenic strains also naturally occur in both species (Horn et al., 1996). *A. flavus* is the almost exclusive species occurring in maize (Giorni et al., 2007).

* + 1. Infection cycle

Because more is known about the infection cycle of *A. flavus* in maize, general conclusion are drawn from this crop. The life cycle of *A. flavus* can be divided into two major phases: the colonization of plant residues in soil and the infection of crop tissues. Soil serves as the primary habitat of *A. flavus; the fungus is* capable of surviving and overwintering in soil and crop debris. Overwintering structures, namely sclerotia and conidia, serve as the source of new conidia to start the infection cycle on new host plants. They germinate into mycelia when the environmental conditions become suitable. The major factors that influence soil population of these fungi are soil temperature and moisture. Mycelia produce numerous conidiophores and release conidia into the air. Neither *A. flavus* nor *A. parasiticus* has a known sexual stage, and conidia are considered to be the primary inoculum (Payne, 1998). The dispersal of the inoculum is known to be by air movement and by insect vectoring. In the field, arthropods and pathogens are exposed to each other and can mutually influence either directly or indirectly by altering the plant-host susceptibility. Post-harvest arthropods may also interact with pathogens acting as potential feeders on both fungi and grain during storage. Direct interactions include insects or mites acting as vectors of inoculum by carrying fungal spores on the external surface of their bodies (Parry et al., 1995). Indirect interactions have consequences related to the disease process in ways that are mediated by the host plant.

* + 1. Ecology

Temperature (T), relative humidity (RH) and, above all, the water activity (aw) of the substrate are the most important factors influencing *A. flavus* activity (Pitt and Miscamble, 1995). In vitro trials indicate that the growthof *A. flavus* occurs within a wide range of suitable temperatures, with a minimum ranging from 10 to 12.8 °C, a maximum between 43 °C and 48.8 °C and an optimum near 33.8 °C. Proper aw for growth are for instance 0.82 at 25.8 °C, 0.81 at 30.8 °C and 0.80 at 37.8 °C. Usually, the optimum aw for *A. flavus* growth is considered to be in the range from 0.96 to 0.98 at 25 °C, 0.98 at 30 °C and 0.96 at 37 °C (Giorni et al., 2011; Battilani et al., 2016). In the field, *A. flavus* can grow and produce mycotoxins down to 0.73 and 0.85 aw respectively. This corresponds to 8-12 % and 17-19 % moisture content (MC) in maize (Battilani et al., 2013).

AFs production in vitro occurs at water activity values down to 0.85 aw with a potential maximum occurring between 0.95 and 0.99 aw. AFB1 production starts at 0.85 aw and increases rising to 0.98-0.99 aw. In vivo trials demonstrated that AFB1 result to be positively correlated with aw, in agreement with *in vitro* data, when aw ≥ 0.95 and negatively correlated when aw< 0.95. Therefore, this aw value is suggested as a kind of threshold; when it is reached, fungal metabolism changes (Giorni et al., 2016).

The influence of abiotic stresses on *A. flavus* infection is complicated by the co-existing of different fungi species in maize kernels during the growing season. Previous in vitro studies considered the competition between *Fusarium verticillioides* and *A. flavus* (Giorni et al., 2014) and the dominance of one species over the other was demonstrated only in extreme conditions, while mutual antagonism was more commonly observed (Giorni et al., 2016).

* 1. *Aspergillus ochraceus*

*Aspergillus ochraceus* belongs to Aspergillus section Circumdati (also called the *A. ochraceus* group) and it is characterized by densely sporulating ochra colonies with the production of pinkish to purplish brown sclerotia. It represents one of the major sources of ochratoxin A in stored cereals (Pitt and Hocking, 2009) and in coffee (Taniwaki et al., 2003). *A. ochraceus* has been shown to consist of two species (Varga et al., 2000; Frisvad et al., 2004). The second and new species producing large amounts of ochratoxin A consistently has been described as *A. westerdijkiae* (Frisvad et al., 2004).

* + 1. Infection cycle

*A. ochraceus* reproduces by asexually produced spores, known as conidia, which are formed from specialized cells called phialides, where mitosis takes place and from which conidia are generated rapidly and in great amounts (Kirk et al., 2001). The conidia are dispersed in the environment mainly by air but they do not germinate before they reach a suitable substrate.

Spore germination is the first stage in the fungal colonization of substrates and it is mainly determined by specific conditions of aw and temperature. Minimal aw required for spores germination vary on substrates and temperature but no germination is possible with aw <0.75. In particular, minimum aw ranged from 0.77-0.85 on synthetic agar media, while *A. ochareus* growth was found at a minimum aw level of 0.80-0.85 on cereal grains or green coffee beans (Pardo et al., 2006b).

Regarding the temperature effects, *A. ochraceus* growth has been described between 8 and 37°C, with the optimum at 30°C (ICMSF, 1996; Ramos et al., 1998).

Minimum aw level required for spore germination is higher with decreasing temperature level, so at 20-30°C spore germination occur at 0.80 aw while at 10°C the minimum level required is 0.85 aw (Pardo et al., 2005). *A. ochraceus* spore germination is possible under to 10°C, however in order to have 100% spore sporulation aw has to be ≥0.90. At lower aw level, germination stopped at <50% germination spores (Pardo et al., 2006b).

* + 1. Ecology

*A. ochraceus* is common from a wide range of habitats such as soil, agricultural and stored foods (Kozakiewicz, 1989; Frisvad et al., 2004; Morello et al., 2007; Noonim et al., 2008; Gil-Serna et al., 2009; Gil-Serna et al., 2011). *Aspergillus ochraceus* is most commonly found in dried and stored foods, such as smoked and salted dried fish, soya beans, chick peas, nuts, pepper and dried fruit (Milani, 2013). It has been reported infrequently in cereals (Czerwiecki et al., 2002) and green coffee beans (Frank, 1999; Urbano et al., 2001; Taniwaki et al., 2003). Recent records include rice (Pacin et al., 2002), barley (Medina et al., 2006), maize (Sepulveda and Piontelli, 2005; Magnoli et al., 2006; Magnoli et al., 2007) and wheat (Aziz et al., 2006). *A. ochraceus* is the most important mould that produces ochratoxins; however, *A. ochraceus* is generally present at low levels and rarely causes spoilage. Its presence may not be a good indicator of significant ochratoxin contamination (JECFA, 2001).

*A. ochraceus* plays a role even in grapes and wine contamination with OTA; in particular, even if in these substrates main OTA responsible fungi belong to *A.* section *Nigri*, some authors detected higher percentage of OTA positive isolates among *A. ochraceus*, which produced higher amounts of OTA (Da Rocha Rosa et al., 2002; Bellì et al., 2004) thus this species should be regarded as a possible contributor to OTA presence in grape derivates.

* 1. *Fusarium verticillioides*

*Fusarium verticillioides* (teleomorph *Gibberrella monilifomis*) belongs to the Fusarium *Liseola* section, and is the primary cause of pink ear rot in maize. *F. verticillioides* produces asexual (conidia) and abundant sexual (ascospores) spores on crop residues (Battilani et al., 2003).

* + 1. Infection cycle

Conidia and spores are air- and/or spash- dispersed and enter the plant through natural opening or wounds. This pathway is the main infection route for in planta inoculum. In addition, spora-carrying arthropods can favor the dispersal of inoculum and enhanced the infection (Parry et al., 1995). The infection can also occur systemically from mycelium growth after plant penetration, or from seed transmission (Marin et al., 1996; Munkvold and Carlton, 1997). The fungus survives for longer than one year on crop debris in the soil, which are considered the main source of infection (Cotten and Munkvold, 1998). The production of thickened hyphae apparently prolongs the pathogen survival on buried residues (Munkvold and Desjardins, 1997). Vegetative hyphal fusion (VHF) helps fungi exchange nutrients and signals within and between hyphae, which could be critical for their success in surviving hostile environments or the infection processes. Guo et al. 2015 have functionally characterized the FvSO gene in *F. verticillioides* and demonstrated that it is essential for VHF, normal vegetative growth and sporulation, as well as fumonisin production (Guo et al., 2015). Only few transcription factors (TFs) have been characterized in this corn pathogen (Malapi-Wight et al., 2013).

* + 1. Ecology

Several factors may influence the development of *F. verticillioides*, primarily temperature (T) and activity water (aw) which are directly influenced by meteorological conditions. Besides T and aw, several biotic factors can change during the maize growing season, including relative humidity (RH), amount and timing of rainfall (R) and wind (W) patterns, affecting both fungal growth and fumonisin production. The optimal conditions for the development of *F. verticillioides* include warm temperatures and moderate rainfall. Moderately warm temperatures and high rainfall during maturation are more conductive for infections by *F. graminearum* (Leslie and Logrieco, 2014).

Previous studies showed that *F. verticillioides* growth occurs within a wide range of temperatures, with the optimum ranging from 22.5 to 27.5°C and a minimum water availability of 0.87 (Medina et al., 2013). The optimum T and aw reported for inducing fumonisin production ranged from 20 to 25°C and 0.95–0.99 aw, while no production is observed at ≤0.93 aw and 10°C.

The influence of climatic factors on *Fusarium* diseases is complicated by the fact that *Fusarium* fungi can cause disease individually or in complex infections (Doohan et al., 2003). There are numerous reports showing how species differentially respond to various environmental variations. It has been demonstrated that *F. verticillioides* dominates over almost all the other fungal species commonly present in maize, especially in conditions of high aw. At lower aw levels (0.98 and 0.95), however, mutual antagonism has been more commonly observed (Giorni et al., 2014).

The effect of environmental conditions on growth and toxin production in *F. temperatum* has been recently studied in-depth in two strains isolated from Andean maize harvested in Argentina. *F. temperatum* strains reached a maximum growth rate at values higher than 22°C and the lowest growth rates at 15°C and 0.95 aw. *F. temperatum* produced the maximum levels of fumonisin B1 (FB1) in conditions of low water activity and temperature, 0.98 aw and 15°C (1000 μg g−1) (Fumero et al., 2016).

It is known that fungi also respond to environmental stresses by accumulating compatible solutes. Boudreau et al., (2013) observed dramatic temperature-dependent changing in the inventory of compatible solutes in *F. verticillioides*, especially trehalose (Boudreau et al., 2013).

A summary of the ecological needs of *F. verticillioides, F.graminearum and* *F. temperatum* is given in (**Table A.1**).

**Table A.1:** Summary of ecological needs of *F. verticillioides, F.graminearum and F. temperatum* reported by different authors

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Topic** | | **Range considered** | **T min** | **T opt** | **T max** | **Reference (s)** |
| **TEMPERATURE (T in °C)** | | | | | |  |
| Fungal growth | | | | | | |
| *F. verticillioides* |  |  |  | 20 -25 |  | (Medina et al., 2013) |
| *F. verticillioides* |  | 15-35 |  | 25 |  | (Fanelli et al., 2013) |
| *F. temperatum* |  | 15-30 |  |  |  | (Fumero et al., 2016) |
| **Mycotoxin production** | | | | | | |
|  |  |  |  |  |  |  |
| *F. verticillioides* | Fumonisin A, B and C |  |  | 25 |  | (Lazzaro et al., 2012) |
| *F. verticillioides* | FB1 and FB2 |  |  | 20-30 |  | (Medina et al.,2013) |
| *F. verticillioides* | FB1, FB2 and FB3 | 15-35 |  | 30 |  | (Fanelli et al.,2013) |
| *F. graminearum* | DON |  |  |  |  | (Jingping et al., 2016) |
| *F. temperatum* | MON | 15-30 | 15 |  | 30 | (Fumero et al., 2016) |
| *F. temperatum* | BEA | 15-30 | 15 |  | 30 | (Fumero et al., 2016) |
| *F. temperatum* | Fusaproliferin | 15-30 | 15 |  | 30 | (Fumero et al., 2016) |
| *F. temperatum* | FB1 | 15-30 |  | 15 |  | (Fumero et al., 2016) |
|  |  |  |  |  |  |  |
| **Gene expression** | | | | | | |
| *F. verticillioides* | FUM genes | 20-35 |  |  |  | (Medina et al.,2013) |
| *F. verticillioides* | FUM1 and FUM21 | 15-35 |  | 15 |  | (Fanelli et al.,2013) |
|  |  |  |  |  |  |  |
| **Topic** |  | **Range considered** | **aw min** | **aw opt** | **aw max** | **Reference (s)** |
| **WATER ACTIVITY (aw)** | | | | | | |
| Fungal growth | | | | | | |
| *F. verticillioides* |  |  |  | 0.995 |  | (Medina et al.,2013) |
| *F. verticillioides* |  | 0.93-0.999 |  | 0.998-0.99 |  | (Fanelli et al.,2013) |
| *F. graminearum* |  |  |  | 0.900-0.995 |  | (Jingping et al.,2016) |
| *F. temperatum* |  | 0.95-0.995 |  |  |  | (Fumero et al., 2016) |
| **Mycotoxin production** | | | | | | |
| *F. verticillioides* | Fumonisin A, B and C | 0.955-0.990 |  | 0.990 |  | (Lazzaro et al.,2012) |
| *F. verticillioides* | FB1 and FB2 |  |  | 0.98-0.995 |  | (Medina et al.,2013) |
| *F. verticillioides* | FB1, FB2 and FB3 | 0.93-0.999 |  | 0.99 |  | (Fanelli et al.,2013) |
| *F. graminearum* | DON |  |  | 0.950-0.995 |  | (Jingping et al.,2016) |
| *F. temperatum* | MON | 0.95-0.995 |  | 0.95 |  |  |
| *F. temperatum* | BEA | 0.95-0.995 |  | 0.98 |  |  |
| *F. temperatum* | Fusaproliferin | 0.95-0.995 |  | 0.95 |  |  |
| *F. temperatum* | FB1 | 0.95-0.995 |  | - | 0.98 |  |
| **Gene expression** | | | | | | |
| F. verticillioides | FUM genes | 0.93-0.995 |  |  |  | (Medina et al.,2013) |
| F. verticillioides | FUM1 and FUM21 | 0.93-0.999 |  | 0.99 |  | (Fanelli et al.,2013) |
| **Topic** |  | **Range considered** | **min** | **opt** | **max** | **Reference (s)** |
| **INCUBATION TIME (g)** | | | | | | |
| F. verticillioides | Fumonisin A, B and C | 21-45 |  | 30 |  | (Lazzaro et al.,2012) |

–: no information available;

1. Table footnote a
2. Table footnote b
   1. *Fusarium graminearum*

*Fusarium graminearum* (teleomorph Gibberella zeae Schweinitz) belongs to the Fusarium *Discolor* section, and is the primary cause *Gibberella* ear rot or *red* ear rot in maize (Logrieco et al., 2012).

* + 1. Infection cycle

*F. graminearum* overwinters on crop residues. Both sexual and asexual spores are produced in *F. graminearum*, and ascospores are the primary inocula during the period of flowering (Trail et al., 2002; Leslie and Summerell, 2006). Sexual spores are produced and discharged from the perithecia, the most suitable overwintering structures (Sutton, 1982; Trail et al., 2005). Ascospores are forcibly discharged into the air through flask-shaped perithecia, while conidia are produced from the sporodochia on infected crops and are responsible for secondary infection (Trail et al., 2005). While ascospore dispersal is known to be by air movement, conidia are moved via rain-splash dispersal (Paul et al., 2004). Conidia are considered to serve predominantly in short-distance dispersal (Shaner, 2003).

Since conidia are responsible for disease propagation, and conidiation is a unique characteristic of fungal species, it has been hypothesized that conidiogenesis-related genes might be novel targets for disease control (Trail, 2009). To date, many genes related to various biological and biochemical functions have been known to be important for both conidia and ascospores development in *Fusarium* spp. A recent study on the molecular mechanisms underlying conidiogenesis in *F. graminearum* shows that overexpression of abaA gene resulted in pleiotropic defects such as impaired sexual and asexual development, retarded conidium germination, as well as reduced trichothecene production (Son et al., 2013).

* + 1. Ecology

The temporal pattern of *F. graminearum* inoculum production is conditioned by several ecological factors such as temperature, moisture conditions and nutritional status of the residues supporting fungal development as well as intrinsic variability among strains of the fungus. Perithecia is produced at temperatures between 5 and 30°C (the optimum is 21.7°C) but matured only in a narrow range of temperatures (at 20 and 25°C). Low moisture conditions inhibit or slow maturation and ascospore production. Perithecium production and maturation increased with relative humidity (Son et al., 2013; Manstretta and Rossi, 2015, 2016). Further details on the ecological needs of *F.graminearum* are given in (**Tables A.1**).

* 1. *Penicillium verrucosum*

*P. verrucosum* (teleomorph: not known) belongs to the section Fasciculata (Houbraken et al., 2011) and is characterized by globose, rough walled conidia and terverticillate conidiophores with appressed elements born from surface or subsurface hyphae. A very little understanding of its life history and ecology outside storage environments is available (Lillehoj and Elling, 1983; Wicklow, 1995) while a lot of studies have been dedicated over the years to assess its mycotoxin production ability.

Due to its similarity with *P. nordicum*, *P. verrucosum* is hardly discriminated from the former one when growing on the same culture medium: the reverse color of the colony on YES medium, which is reddish-terracotta brown for *P. verrucosum*, may be helpful in this sense; moreover, they can also be distinguished by the production of citrinin (CIT), which is only produced by some strains of *P. verrucosum*, while both of them produce ochratoxin A (OTA), as reported by (Frisvad and Samson, 2004).

* + 1. Infection cycle

For *P. verrucosum*,airborne dissemination of conidia and mycelium fragments represents the main strategy to colonize an environment, and airborne spore load is one of the most important parameters affecting seed grains in warehouses (Sawane and Saoji, 2005) although spora-carrying arthropods should also be considered (Greif and Currah, 2007).

* + 1. Ecology

The genus *Penicillium* contains opportunistic polyphagous fungi, generally saprophytes and living on a wide range of organic substances, food and feed matrixes such as fruits, bread, meat and cereals, leading to their decay and eventually mycotoxin contamination. Most of them are nutritionally undemanding and able to grow in presence of a sprinkling of mineral salts and almost any form of organic carbon (the most complex ones are excluded); moreover, Penicillia are able to grow under a wide range of physico-chemical environmental conditions of *aw*, temperature, pH and redox potential (Pitt and Hocking, 2009).

In particular, *P. verrucosum* is commonly distributed in cold-temperate regions (Norway, Denmark, Sweden, United Kingdom, Germany, Poland, Netherlands, Belgium, Switzerland, France, Portugal, Italy, Canada, USA, Russia) and is mostly related to stored cereal grains (such as barley, oats, rye and wheat) while its closely related species *P. nordicum*, is more common on proteinaceous food matrixes such as cured-meat products and cheese; despite this, *P. verrucosum* was also reported on cheese in the past (Elmholt, 2003; Frisvad and Samson, 2004).

According to the scientific literature, *Penicillium verrucosum* can grow at temperatures ranging from 0 to 31°C, with the optimum belonging to the range 20-25°C (Northolt et al., 1979; Cairns‐Fuller et al., 2005); the minimum *aw* required for germination is ca. 0.80, but a minimum of 0.85 *aw* is required for growth (Pardo et al., 2006a), while an optimum value of 0.98 on grain-based media was reported by Cairns-Fuller et al. (2005). Those data were partially confirmed *in vitro* by (Camardo Leggieri et al., 2017), which found that fungal growth on CYA (Czapek Yeast Agar) (Frisvad and Samson, 2004) added with NaCl when proper occurred from 5 to 30°C (optimum: 20°C) and that 0.87-0.99 *aw* was a range suitable for growth too, with optimum at 0.99. Moreover, it was also reported in the past that *P. verrucosum* grew over the pH range 2.1-10.0 at least (Wheeler et al., 1991).

Considering mycotoxin production, Camardo Leggieri at al. (2017) showed that ecological needs were more stringent compared to those required for growth: in fact, *in vitro* OTA production started at 15°C while fungal growth was observed from 5°C (optimum: 25°C); regarding *aw*, *P. verrucosum* only produced OTA at 0.99 within 14 days, while longer incubation times were required for producing this toxin at lower *aw* values according to what reported by (Schmidt-Heydt et al., 2012); moreover, Cairns-Fuller et al. (2005) specifically reported that only one isolates over three produced OTA at 15°C and 0.85 *aw*, after 56 days and that both fungal growth and OTA production were significantly inhibited by a concentration of 50% v/v CO2 in the atmosphere at 0.90-0.995 *aw* and 25°C.

The complete OTA production gene pathway in *P. verrucosum* was firstly described by (Geisen et al., 2006).

A summary of the ecological needs of *P. verrucosum* is given in (**Table A.2**).

**Table A.2:** summary of ecological needs of *P. verrucosum* reported by different author

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Topic** | | **Range considered** | **T min** | **T opt** | **T max** | **Reference (s)** |
| **TEMPERATURE (T in °C)** | | | | | |  |
| **Fungal growth** | | | | | | |
| *P. verrucosum* |  | 0 - 40°C | 5°C | 20°C | 30°C | Camardo Leggieri et al. (2017)\* |
|  |  | 10 - 30°C | 10°C | 20°C | - | (Pardo et al., 2006a)x |
|  |  | 10 - 25°C | 10°C | 25°C | 25°C | Cairns-Fuller et al. (2005)+ |
| **Mycotoxin production** | | | | | | |
| *P. verrucosum* |  | 20°C | - | - | - | Camardo Leggieri et al. (2017)\* |
|  |  | 10 - 25°C | 15°C | 25°C | 25°C | Cairns-Fuller et al. (2005)+ |
| **Topic** |  | **Range considered** | ***aw* min** | ***aw* opt** | ***aw* max** | **Reference (s)** |
| **WATER ACTIVITY (aw)** | | | | | | |
| **Fungal growth** | | | | | | |
| *P. verrucosum* |  | 0.78 - 0.99 | 0.87 | 0.99 | 0.99 | Camardo Leggieri et al. (2017)\* |
|  |  | 0.75 - 0.99 | 0.85 | 0.95 | - | (Pardo et al., 2006a)x |
|  |  | 0.75 - 0.995 | 0.80 | 0.98 | 0.995 | Cairns-Fuller et al. (2005)+ |
| **Mycotoxin production** | | | | | | |
| *P. verrucosum* |  | 0.78 - 0.99 | 0.99 | 0.99 | 0.99 | Camardo Leggieri et al. (2017)\* |
|  |  | 0.75 - 0.995 | 0.85 | 0.95 | 0.995 | Cairns-Fuller et al. (2005)+ |
| **GENE EXPRESSION** | | | | | | |
| *P. verrucosum* |  | *Otapks* |  |  |  | Geisen et al. (2006) |
|  |  | *Otanps* |  |  |  | Geisen et al. (2006) |
|  |  | *Otachl* |  |  |  | Geisen et al. (2006) |
|  |  | *Otatra* |  |  |  | Geisen et al. (2006) |

–: no information available.

\* In Camardo Leggieri et al. (2017) fungal growth under various T regimes at 14 days of incubation was considered. Moreover, OTA production was studied at various *aw* regimes at 20°C (because that T regime was similar to those adopted in the ripening chambers) and 14 days of incubation were considered also in this case; therefore, no optimum T regime for OTA production was established. Medium used: CYA.

X In Pardo et al. (2006) optimal fungal growth did not show significant difference between 20 and 30°C; thus, the minimum values of T and *aw* suitable for fungal growth at the least favourable conditions were reported. Medium used: BMEA (Barley Malt Extract Agar).

+ In Cairns-Fuller et al. (2005) fungal growth and OTA production on artificial culture medium (milled wheat medium) were considered to have a more comparable set of results; incubation time was up to 56 days with scheduled measurement at 7, 14, 21, 28, 42 and 56 days.

**List of abbreviations**

|  |  |
| --- | --- |
| AFB1 | Aflatoxin B1 |
| aw | Activity water |
| MC | moisture content |
| OTA | Ochratoxin A |
| RH | Relative humidity |
| T | Temperature |
| W | Wind |

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