Maximize your grain quality by minimizing mycotoxins.

MYCONTROL

KWS MYCOTOXIN MANAGEMENT

SEEDING
THE FUTURE
SINCE 1856

KWS

Contents

General background

Growing corn means achieving high yields, energetic crops and economic benefit for most farmers. However, pests and diseases cast a cloud over this positive attributes, which corn contributes to an agricultural farm.

Among the many pests and diseases, which can effect corn, mould fungi named Fusarium play an important role. They do not only cause yield losses and quality problems, but also lead to a severe health risk for humans and animals (Musa et al, 2011). This also results in economic losses.

To deal with these issues, detailed knowledge about the fungus, its ways of infections and further effects, is necessary for a healthy crop and therefore a successful agriculture. For this purpose, KWS provides information and services regarding Ear Fusarium and ways of prevention – with competence against Fusarium!

What you should know about Ear Fusarium

During the whole vegetation period, fungi of the species Fusarium can infect the stem, root or ear of a corn plant. First described in 1809, this ubiquitous mould develops falcate, asexual conidia (Lemmens, 2012). Conidia are spores, which are responsible for the asexual mass reproduction of Fusarium. With the help of falling raindrops, the spores are distributed in close proximity. (Please note, that there are sexual types of this and other fungi. This article only refers to the asexual Fusarium spp.). Besides legumes, corn and wheat seem to be more affected than other crops. In both cases, Fusarium can survive during the winter period and infect the new seed, once the environment is favorable for its development and growth.

The main problem regarding Fusarium are the mycotoxins, which contaminate the plants. These are metabolic toxins, produced by Fusarium spp., which cause severe health problems. The fungi produce these mycotoxins to protect themselves. More than 400 of these toxins are known so far. The whole effects of all of them are not yet discovered. By count, approximately 25% of the world-wide crops are contaminated by mycotoxins (Lemmens, 2012).

Not only Fusarium produces mycotoxins. Other mycotoxins produced by fungi are Aspergillus, Penicillin, Alternaria and Claviceps. The following table shows the fungi and their metabolic products.

This corn cob was attacked by corn borer and contaminated by Fusarium.

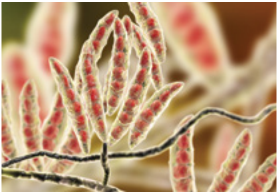
Important fungi and their mycotoxins

The most important fungi and their mycotoxins	
Fungus	**Mycotoxin(s)**
Fusarium	Trichothecenes (Desoxynivalenol, Nivalenol, Diacetoxyscirpenol, T-2 and HAT-2-Toxin), Zearalenon, Moniliformin, Fumonisines, Beauvericin, Fusaric acid
Aspergillus	Aflatoxin B1, G1, M1 Ochratoxin A, Fumi-tremorgens, Fumigaclavines, Fumitoxines, Nivalenol, Gliotoxin, Monacolin
Penicillium	Ochratoxin A, Patulin, Citrinin, PR Toxin, Penicillin acid, Penitrem
Alternaria	Alternariol, Alternariolmethylether, Tenuazonacid
Claviceps	Ergot Alkaloids
Monascus	Monacolin K, Lovastanin, Compactin

Source: Lemmens (2012), modified

One conclusion from this table is that these fungi produce different mycotoxins and some of them produce more than one mycotoxin (Dorn et al, 2009).

Furthermore, the fungi can be differentiated regarding the habitat. Some of the fungi occur on the field before harvest, whereas others start developing under incorrect storage conditions.



Fusarium produce mycotoxins that affect humans and animals
3D illustration

The most important mycotoxins are Fumonisins (FUMO), Desoxynivalenol (DON) and Zearalenon (ZEA). For example, Fusarium graminearum produces DON and ZEA besides Nivalenol. DON and Nivalenol belong to type B Trichothecenes, which is a family of toxic sesquiterpenoids. They are mainly responsible for intoxications of humans and animals. Other mycotoxins like Moniliformin, are also important.

Infection pathways

There are three main options of fungal entry or infection pathways, by which Fusarium spp. enter maize ears:

- by fungal spores landing on the silks, germinating and then the fungal mycelia grow down the silks to infect the kernels and cob (rachis) (Koehler, 1942);
- wounds created by insects or from bird or hail damage offer a point of entry for fungi (Sutton, 1982); and some Fusarium
- spp. are systemic, such as F. verticilliodes, and can enter the ear from infected stalks (Foley 1959, Munkvold et al., 1997b).

Which infection pathway is more important depends on the Fusarium spp. that is predominant and insect pressures in a given geographical location. In some locations, ear rot outbreaks are mainly associated with infection through the silk while in other locations where maize boring insects are a problem and are not controlled by other measures, infection through the kernels is predominant. Insect wounds, especially to the ear, from pests such as Europian corn borer (Ostrinia nubilalis), Southwestern corn borer (Diatraea grandiosella), Western corn rootworm (Diabrotica virgifera virgifera), Corn earworm (Heliocoverpa zeae) and thrips (Frankliniella spp.), increase the levels of Fusarium infection by creating new points of entry for the fungus to enter the plant.

Disease symptoms

The symptoms of Giberella Ear Rot, caused mainly by F. graminearum, are characterized by a pinkish coloured mould (White, 1999). Similar symptoms are found with F. culmorum infections. Infection of the ear occurs by colonizing maize silks and commonly begins as a white mycelium moving down from the ear tip. This mycelium later turns reddish-pink on infected kernels.

In some cases, pinkish fungal growth can be found on the exterior husk leaves, and in severe infections, it is impossible to separate the husks from the kernels as the entire ear becomes a tightly bound mass of fungal and plant tissue that appears 'mummified' (Reid and Sinha, 1998). Once the kernels reach 22–23% moisture, it is difficult for the fungus to further infect (Christensen and Kaufmann, 1969); however cob (rachis) moisture can be 15–25% higher than kernel moisture, so the infection may spread in the cobs and can enter younger kernels via the pedicel.

In some cases, only the cob is infected. The ear may appear to be symptomless but when squeezed by hand, it will feel spongy and the cob will be wet and often pink/red in color. How fast symptoms develop in a given year is highly dependent on the environment that influences not only ear development and subsequent kernel dry down but also fungal growth.

The optimum temperature for Giberella Ear Rot development is 26–28°C, while Fusarium Ear Rot has a broader range extending to higher temperatures (Reid et al., 1999). Giberella Ear Rot also requires a much longer period of precipitation after infection usually around the time of plant (Reid et al., 1996a). Infection through the silks cannot proceed once the silks have dried out (Reid et al., 1992a, Reid and Sinha, 1998). Husk tightness (Koehler, 1959), ear declination and physiological resistance mechanisms all influence the spread of infection. Stalk rot and ear rot are strongly interrelated (Mesterházy, 1983, Mesterházy and Kovács, 1988, Mesterházy et al., 2000) as stalk rot interrupts the water supply to the ears and speeds up development and drying of the ear.

In contrast to Giberella Ear Rot, symptoms of Fusarium Ear Rot from F. verticillioides infection occur mainly on scattered individual kernels or on limited areas of the ear (White, 1999). In some ears, many independent infection sites may develop. Infected kernels develop a cottony growth or may develop white streaks radiating from the top of the kernels where the silks were attached (STARBURST-syndrom). Infected kernels may turn tan or brown.
Fungal growth on the cob may be white, pink or salmon-coloured.
Infection is more frequent on damaged ear tips and on kernels with pericarp injuries due to damage caused by insects.
This type of ear rot can also infect kernels without showing visible symptoms.

Clean (first-grade) grain occasionally has an infection rate of up to 90% without showing symptoms.

Eller et al. (2008a) state that the disease is prevalent in warm, dry conditions, like those common to the southern United States, and F. verticillioides is found in grain or crop residue of virtually all mature maize fields in the United States. Reid et al. (1996a) adds that rainy weather or irrigation at silking thereafter significantly increases disease severity.

A summary of important symptoms

- Husks are covered with a white, salmon-to cinnamon-coloured coating
- The husks are sticky
- Under the husks, the ear is partly or entirely coated with a white to pink, purple or red-coloured mycelium
- The kernels are red to brown-coloured and partly split open
- With further infection, the cob turns brownish-red and starts to rot

Fusarium affected cobs

Which factors are important regarding an Ear Fusarium infection?

Ear Fusarium is a multifactorial disease. Many factors interact and it is difficult to control all these factors. Below, you find the most important factors, which have to be considered.

Annual weather conditions

The weather conditions determine the composition of the fungi population in the environment and the concentration of produced mycotoxins.

Environment

The environment determines the severity of infections based on the soil and local pressure of pests and diseases.

Time of harvest

The longer the crop stays in the field, the higher the risk of a mycotoxin infection.

Soil cultivation

It is crucial to prepare the soil, otherwise infections can be transmitted from plant debris from the previous year.

Variety

Some varieties demonstrate a higher genetic tolerance.

Mechanical damage

Damaged plants (e.g. by insects) are weaker and therefore less resistant to fungal attacks.

Crop rotation

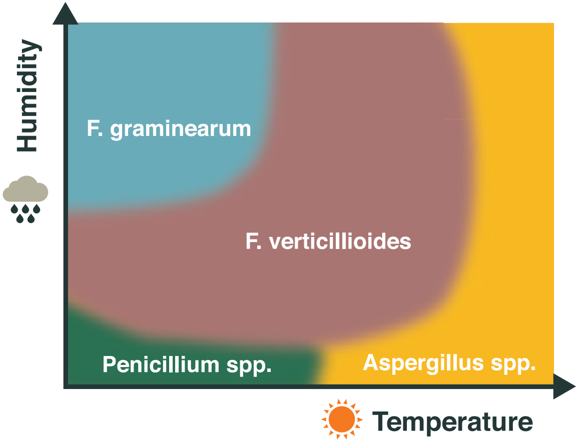
The previous crop can have a negative effect on corn regarding Ear Fusarium. There are crops, which are more susceptible than others.

More in detail

One important factor, which cannot be controlled, is **the weather**. The atmospheric condition in a certain year is one main aspect regarding the development and growth of Fusarium and the production of their mycotoxins.

The weather conditions determine the composition of fungi species in the environment and the type and concentration of produced mycotoxins. Varying climate conditions result in a shift of the range of species. Rainy and warm weeks in late summer or autumn and additionally a high humidity support the infection with Ear Fusarium (Musa et al, 2011).

However, there are different Fusarium spp. with diverse comfort zones. F. graminearum is e.g. dominant in a humid and cool environment, whereas F. verticilloides prefers hot and dry conditions.



This graph shows the interaction between fungi and environment and the importance of humidity and temperature regarding the appearance of individual fungal species.

The environment also determines the severity of infection. This includes the soil, hillslope and local pressure of pests and diseases.

Mechanical damage is another key factor. If the plant is already harmed by natural injury (e.g. caused by hail, insects or birds, early frost), the risk of an infection with Fusarium is higher. The natural protection of the plant is weakened, which makes it easier for the mould to infect it. In all cases, the rainwater infiltrates the ear, due to the harmed and partly opened husks, and the fungus is washed into the ear (Lemmens, 2012).

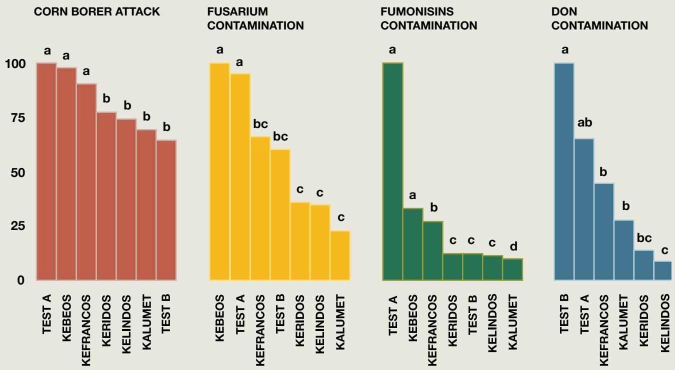
Mechanical damage caused by the corn borer is a crucial evidence for a more intense control of this insect. When the plant is already infected with other pests or diseases, the immune system of the plant is weakened, which benefits to the infection with Ear Fusarium. In addition, the damage by the corn borer serves as an entry for the moulds.

The time of harvest affects the type and concentration of the mycotoxins and is therefore a variable of significant importance (Lew et al, 2001). The later the harvest date, the higher the contamination with mycotoxins. Earlier varieties show a lower content of mycotoxins and ensure a safe maturation. Especially the DON-content tends to increase, the later the harvest time (Musa et al, 2011). The production of ZEA starts later than the one of DON, therefore the DON-content and concentration is always higher than the ZEA-content (Lew et al, 2001).
Please note, that mycotoxins, which are produced before harvest, remain in the harvested product (silage, grain corn, CCM).

Corn varieties show different tolerances to Fusarium fungal attacks based on their genetics. This has been proven in scientific researches, for example by Prof. Causin, Dip. TESAF, University of Padova. Therefore, it is important to choose the right variety adapted to regional environmental conditions.

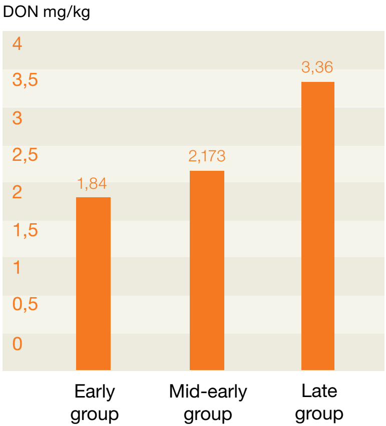
Parameters revealed in 6 locations in Italy over two years

(y-axis: normalized values, %)



The values are normalized on the highest value (highest value = 100%). The statistic analysis has been done separately for each parameter, different letters represent values statistically different from each other (P<0,05)

Trial results from Germany:



DON Content mg/kg DM of maturity groups
Frankendorf

Source: Ifl Bayern (2007)

The previous crop can have a negative effect on corn regarding Ear Fusarium. There are crops, which are more susceptible than others, for example grain corn:

High risk of contamination

- Grain corn
- Silage corn
- Grass
- Winter wheat
- Sugarbeet
- Other cereals
- Potatoes
- Rapeseed

Low risk of contamination

What kind of damage do mycotoxins cause?

Mycotoxins lead to severe health problems for humans and animals. Especially, when used directly without testing, physiological stress and risks remain undetected (Musa et al, 2011). Intoxications with mycotoxins often remain undiscovered, since a decrease in performance and problems with fertility are unspecific problems (Lemmens, 2012). This is especially important, when the intoxication becomes chronic.
Therefore, a diligent control of the harvested corn is crucial. The main problem here is the local contamination with mycotoxins in the product.

Ruminants are more tolerant towards infections due to their stomach system. With the help of endogenous microorganisms, they are able to transfer the toxic form of e.g. DON or ZEA into a non-toxic form. Therefore pigs, because they have only one stomach, are more susceptible than cows.
This is especially problematic for CCM or grain corn fed pigs.

DON inhibits the protein biosynthesis. Resulting, secondary effects are haemolysis, failure of proliferation and chlorosis, necrosis and wilt. Furthermore, DON leads to vomiting, diarrhea, loss of weight because of a reduced feed intake and a general weakening of the immune system. This can then result in further diseases.

ZEA has an oestrogenic effect and is carcinogenic. This is expressed in a swelling of the teats and the vulva. It also has a very negative effect on the fertility, due to a delayed mating season, false pregnancies and ovary abnormities.

The following table gives an overview about the several symptoms caused by different mycotoxins:

Mycotoxin	Effect
Deoxynivalenol	Gastro intestinal toxin, reduced feed intake, vomiting and diarrhea, growth delay, immunosuppression, destroys red blood cells
Zearalenon	Estrogen analog, reduced fertility
Fumonisin	Carcinogenic, harms grey brain substance, harms liver and kidneys, can lead to lung edema
T2-Toxin	Gastro intestinal toxin, causes shortage or missing of white blood cells, harms bone marrow, skin necrosis, bleedings
Nivalenol	Skin irritating, immunosuppressive
Beauvericin	Destroys cells
Enniatine	Destroys cells
Moniliformin	Dyspnea, muscle weekness (especially heart muscle), loss of weight
Aflatoxin	Highly carcinogenic

Source: Dorn et al (2009)

What can you do to fight Ear Fusarium? Prevention!

The best and most effective methods to fight Fusarium and their mycotoxins include all actions regarding prevention. This means that every step during a vegetation period, e.g. soil preparation, harvest, storage etc., is involved and should be remembered. The implementation of prevention strategies is of essential importance for the successful and sustainable fight against mycotoxins (Lemmens, 2012). These 6 prevention strategies are important for you:

Field hygienics / soil preparation: The plant debris after harvest have to be incorporated into the soil. Ploughing is the best method for an efficient incorporation. In the deeper soil layers, the plant debris rot and do not longer provide organic material for the fungus. Non-turning cultivation supports the development of fungi. Ploughing is also a crucial point regarding the control of the corn borer. When you fight against the corn borer, you decrease the risk of getting an Ear Fusarium infection.

Time of harvest: The earlier the date of harvest, the lower the contamination.

Crop rotation / variety: Crop rotation has a huge effect on infection with pests. A susceptible crop increases the risk for the following crop to become re-infected. Especially grain corn leaves a lot of plant debris on the field during harvest, which enhances the risk of fusarium (see: soil preparation). As the harvest date is of essential importance, choose early varieties, to ensure a safe maturation.

Fertilisation: Too much nitrogen can delay the maturation. This results in a later harvest date. But also lack of nitrogen can be a negative factor. This is due to a consequential lack of development of the plant that can increase the stress dynamics and therefore reduce natural defences allowing the fungal penetration.

Harvest / storage: Correct cleaning, drying and storing of the harvested crop is important. The ensiling process must be professional and correct (exclusion of air, no re-heating, no wet material…). Beware of the moisture! A storage moisture under 13% leads to no further development of mould. Beginning from 17% moisture, the risk of the development of harmful mould increases significantly. The storage itself must be clean and without stock parasites. A control of these parasites is important and should not be disregarded.

Feeding hygienics: contaminated material has to be thrown away generously. The troughs have to be clean and free of old material. Shovels and forks as well as wheelbarrows etc. have to be free of dirt.

If your harvested crop is contaminated with mycotoxins, there are some methods, which can help to avoid a contamination and an acute intoxication. One method is the physical rejecting of broken grains and cob-tops. Chemical remedies are especially helpful regarding an intoxication with aflatoxin. Absorbers can inhibit the uptake of mycotoxins. Lucerne fiber, for example, is used in case of an intoxication with ZEA. Yeast and activated carbon are further mycotoxin binding agents.

Breeding and testing

Early quality check of young plants.

KWS is working on many breeding programs for different types and maturities of corn and they are located all over the world. Each breeding program is based in the country or the environment in which the developed hybrids will be cultivated.

This guarantees that during the work of selection of future hybrids we can already select in a very early stage those plants, which show higher tolerances to diseases or environmental particularities of the region.

Moreover, we have more time to discover the behaviour of the plants in their final environment and are not limited to just the last years of trials before registration to get a clear picture about each variety.

This local breeding approach proves especially important when it comes to fungal diseases such as Fusarium ear rot, because the appearance of Fusarium depends highly on the environmental conditions.

To select hybrids which are more tolerant to Fusarium ear rot, KWS researchers combine quantitative and qualitative breeding approaches with molecular markers and inoculation methods taking full advantage of natural genetic variation.

Many plant properties are controlled by an entire set of genes, of which each one only makes a small contribution. This is called a “quantitative” trait and resistance to Fusarium ear rot is mainly of quantitative nature. In order to be able to predict such complex traits for further breeding, all active DNA regions must be identified and their influence on different traits assessed. This is possible by linking extensive genetic marker information (DNA fingerprints) for entire plant populations with data measured in the field (intensive phenotyping – for Fusarium ear rot under artificial inoculation -example given below).

Biostatistical models are used to calculate the proportional contributions made to development of a trait and so ultimately the plant's potential for further breeding. These models can be applied to predict the resistance of thousands of so far untested variety candidates. The selected fraction goes into intensive field testing across many locations. With genomic prediction, the breeding cycle and the time until improved varieties reach the farmers field is shortened. With the "qualitative" approach the breeders try to identify which major genes are responsible for an elevated resistance against Fusarium ear rot and how to detect them in early stages of corn breeding to choose the parent lines which are more resistant. Such major genes are rare in our adapted European breeding materials. Therefore, our breeders always try to increase the genetic resources of our breeding gene pool with new resistant material to ensure a higher variability to choose from.

PRIMA – Unlocking the resistance of tropical maize resources for maize production in Europe (Research project in collaboration with Universities Hohenheim, Göttingen and Hamburg; funded by the BLE (Federal office for Agriculture and Food in Germany)). Tropical maize is grown under high pressure of pests and diseases and has therefore a naturally higher level of resistance. KWS has breeding programs all over the world, including tropical countries like Brazil. So far, the use of tropical breeding materials in Europe was always restricted by the lack of adaptation (late maturity) to our environments. The "PRIMA" project now aims at improving the resistance of maize against ear rot caused by Fusarium and the leaf disease "Northern Corn Leaf Blight" by developing new methods for the targeted use of tropical genetic resources. The project is a collaborative approach across disciplines, combining the expertise of three university partners and the breeding materials as well as field-testing expertise from KWS in Brazil and Europe. Phytopathological analysis of the species and race spectra of the fungi as well as modern molecular breeding approaches will be used for a targeted introgression of natural resistance from tropical into European breeding materials. Experimental hybrids with improved resistance will be tested in the KWS trial network all over Europe from Germany to Italy and in South America (Brazil, Argentina) and will be made available to the farmers within a few years after project completion.

When a new potential hybrid has passed the severe early selection process, it will be tested in many trials all over the country to verify its performance in different environments and planting conditions. After this intensive selection process, it will enter the trials for commercial registration and by the time the hybrid becomes commercial our breeders, technicians, and sales teams at KWS know all characteristics of the new hybrid. Combining traditional and modern breeding methods helps KWS breeders to take full advantage of natural genetic variation for resistance and the breeding programs are succeeding in faster and more efficient variety development for the farmer.

Inoculation

In one of the breeding programs of KWS which is located in the KWS breeding station in Monselice, Italy, one part of the activities is focused on the selection of corn plants, which demonstrate a high tolerance to Fusarium toxins. This is done with many trials and selection procedures, by eliminating the most susceptible plants from the pool of plants we test. However, it is difficult to do these trials and selections each year because the environmental conditions are not always in favour of the development of the different fungi we want to select for.

Therefore, the breeders and technicians of KWS use a technology called "artificial inoculation" to assure the equal infection of the corn cobs with the toxic fungus (e.g. Fusarium verticillioides and Fusarium graminearum). With this method it can be guaranteed, that the level of infection is high enough to get a good variation, which enables KWS to select for the most tolerant varieties.
To be sure, that appropriate Fungal species are used for artificial inoculation, which are similar to the natural appearance in the region, the spore suspension contains only spores of one species, which was detected in the region as the main occurring species. This procedure is performed in each breeding program of KWS to identify the main species that naturally occurs.

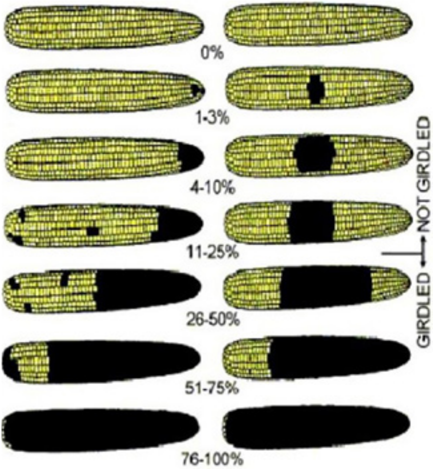
Different techniques exist to apply the fungus to the plant, which simulate the different natural channels how the fungus attacks the plant:

1. Silk channel inoculation consists of the suspension of spores injected into the centre of the silk channel through the use of a syringe. This procedure is performed approximately 4-6 days after silk releases, so with silks still vital and in a receptive condition. This case simulates the fungal entry through the silk channel, as it happens in the case of Fusarium graminearum and Aspergillus Flavus.

2. The kernel inoculation procedure instead consists of suspending spores through the use of a special "fork" directly wounding the caryopsis. In this case we simulate the damage caused by European corn borer, natural vector of the genus Fusarium verticillioides.

3. The silk inoculation aspersion is instead used to convey inoculation of the non-toxic strain of Aspergillus Flavus. This occurs through the aspiration by using a vaporizer of a solution containing mycotoxins directly on the silks. This occurs at a phenological stage where the silks are dehisced or semi-dehisced; and therefore unable to neutralise the entrance and consequently the development of the mycelium.

At the moment of the harvest, each cob will be evaluated for percentage of infected kernels. This is done for cobs, which have been inoculated and for cobs which didn't receive this treatment (control). The evaluation is done based on the percentage of kernels on each cob which present mould, using the scheme in fig3, reaching from 1 (no mould visible) up to 7 (over 75% of kernels are covered by mould).



Not all Fusarium species do show the same infection scheme, therefore the percentage must be sometimes calculated over ears, which show several spots of infection and not a connected area of infection. Usually 2-3 infected kernels = 1%. This selection work is performed every year and on several experimental fields scattered around Italian corn area. Thanks to this important continuous tests and verifications the KWS varieties show a good tolerance against the different types of Fusarium ear rot present in our regions and have therefore healthier grain, compared to varieties from other corn breeders present in the market. Italian farmers therefore have the possibility to choose corn hybrids which guarantee good productions, not only from a quantitative but also from a qualitative point of view.

Of course, also the farmer has to do his part to prevent the development of the fungus and the related mycotoxins, by limiting the stress factors for the plants. To prevent Fusarium toxins it is important to select the right moment for sowing, early sowing is recommended, assure a balanced fertilization, apply a treatment against the European Corn borer and to harvest early with 26-27% of grain humidity. Of course the choice of the hybrid plays a big role and choosing hybrids of KWS is for sure a good decision to prevent Fusarium toxins.

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KWS SAAT SE
Grimsehlstr. 31
37574 Einbeck
Germany
www.kws.com