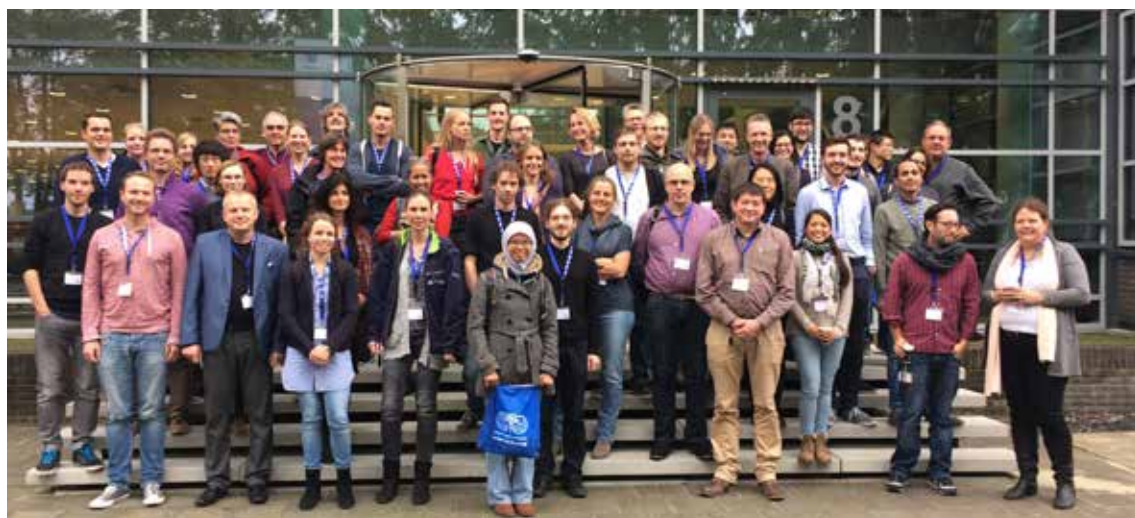


Working group Fusarium

Abstracts of presentations held at the 31st Meeting of the Fusarium working group of the Koninklijke Nederlandse Plantenziektkundige Vereniging, 26 October 2016, CBS-KNAW Fungal Biodiversity Centre

On Wednesday the 26th of October 2016, the KNPV working group on *Fusarium* convened for the 31st time. The meeting was hosted by the CBS-KNAW Fungal Biodiversity Centre in Utrecht and attended by approximately 60 participants. Thirteen presentations were given by the participants on subjects ranging from newly observed *Fusarium* infections observed in different crops to the cellular organization of pathogenicity within the fungal cells and fungi-host interactions. The next meeting of the working group *Fusarium* will be at the same venue on Wednesday the 25th of October 2017.



OPROEP

De Koninklijke Nederlandse Plantenziektkundige Vereniging roept kandidaten op voor de functie van

Voorzitter KNPV

Omdat de laatste zittingstermijn van onze huidige voorzitter Piet Boonekamp dit voorjaar afloopt is de KNPV op zoek naar een nieuwe voorzitter. Vanwege de spilfunctie die een voorzitter heeft binnen onze vereniging is onderstaand functieprofiel opgesteld:

De voorzitter (m/v) is:

- Een netwerker en samenbinder.
- Representatief voor de gehele KNPV, met sterke voeling voor de praktijk.
- In staat buiten de eigen werkomgeving te kijken en te denken.
- Een professionele procesbegeleider
- Een strategische denker met visie op de rol van gewasbescherming in de samenleving
- Een natuurlijke leider met een krachtige en energieke uitstraling

De voorzitter van de KNPV is in staat om bruggen te bouwen en draagt actief bij aan het profiel en de visie van de vereniging. Hij/zij is in staat om scholieren en studenten te interesseren voor de gewasbescherming en initieert activiteiten met een nationale uitstraling. Hij/zij geeft leiding aan het bestuur en de staf van de KNPV en vormt met de secretaris en de penningmeester het dagelijks bestuur. Hij/zij is nauw betrokken bij de organisatie van bijeenkomsten en is het gezicht en de vertegenwoordiger van de vereniging in internationaal verband.

De zittingstermijn voor elk KNPV-bestuurslid is in principe drie jaar; een termijn kan eenmaal, en in bijzondere gevallen tweemaal, worden verlengd. Het bestuur van de KNPV roept kandidaten op om te reageren. Indien u interesse hebt in deze functie kunt u dit kenbaar maken aan onze secretaris Frits van der Zweep (secrknpv@gmail.com). Voor verdere informatie kunt u contact opnemen met de huidige voorzitter Piet Boonekamp (tel. 0317480626).

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The impact of flavonoids on trichothecene production by *Fusarium culmorum* and *F. graminearum*

ABSTRACT. Flavonoids are a group of hydroxylated polyphenolic compounds widely distributed in plants. They are involved in the response mechanisms of plants to a wide range of stressful conditions including plant defense against Fusaria. In the present work, the effect of five flavonoids apigenin, luteolin, naringenin, kaempferol and quercetin was evaluated on growth and trichothecene biosynthesis by *F. culmorum* and *F. graminearum*. Plates were supplemented with flavonoid concentrations (400 and 800 µg/g⁻¹) close to the amounts produced by plants in the response of *Fusarium* infection and incubated for 21 days. Our results indicated that flavonoids had a variable effect on fungal growth and mycotoxin production, depending on the strain, the type and concentration of compound assayed. Fungal growth was inhibited by naringenin and quercetin, stimulated by apigenin, and not affected

by luteolin and kaempferol treated plates. With emphasis on quercetin, a decrease in mycotoxin accumulation was revealed in naringenin, luteolin, kaempferol and most apigenin treated plates. Gene expression experiments of Tri genes encoding the trichothecene biosynthesis pathway (Tri4, Tri5 and Tri10) proved that the inhibition of trichothecene production in quercetin, naringenin and luteolin treated plates occurred at the transcriptional level. However, the changes in Tri transcript levels were not evident in all kaempferol and most apigenin treated cultures. We finally attempted to establish link between their effects on fungi and their lipophilic, antioxidant and antiradical properties.

This work was funded by research project: Grant DEC-2013/11/B/NZ9/01788 from the National Science Center, Poland.

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Fusarium research at the HLB

ABSTRACT. Applied research on *Fusarium* spp. typically focuses on potato. This research includes baseline studies and storage trials. Before these studies can be done inoculum of either mycelium or spores needs to be produced. HLB has a fungal collection with multiple strains of multiple plant pathogenic *Fusarium* spp. strains, most of these are isolated from practical samples. The baseline studies are lab scale studies in Petri dishes. These studies are performed as a screening for new treatments against plant pathogens (like *Fusarium* spp.) and the dosages of these treatments.

Following the results of the baseline study a storage trial is typically done. The potato tubers are (lightly) damaged and artificially infected with the desired *Fusarium* spp. Different treatments will be applied usually using a disc atomizer. After a storage period the tubers will be assessed on disease level. Besides the applied research HLB performs diagnostics of practical plant samples. These samples include potato, onion, flower bulbs and grains. In 10% of the samples received and diagnosed at HLB *Fusarium* spp. is the primary cause.

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Fusarium avenaceum causing postharvest decay on apples and pears in the Netherlands

ABSTRACT. Apple (*Malus domestica*) and pear (*Pyrus communis*) are important fruit crops in the Netherlands, with a total production of 353,000 and 349,000 tons in 2014, respectively. In the Netherlands, apples and pears are kept in controlled atmosphere cold storage up to 11 months after harvest. Lesions were observed on pears of cv. Conference in a survey carried out in packing houses in the Netherlands. In general low incidences of 1% to 5% were recorded. Lesions showed brown and watery circular necrosis,

were slightly sunken, often with visible whitish, yellowish or pink mycelia covering the lesions. Symptoms of apple wet core rot were observed on cv. Elstar after 4 to 6 months' storage in different packing houses at controlled atmosphere. Apples exhibited light brown wet rot, initially developing in the core and subsequently spreading into the surrounding cortex, often with a white to rose-red-dish mycelium. Both cultural and morphological characteristics of the isolated pathogens were similar to those described for *Fusarium* spp. The

identity representative isolates from different apple and pear lots were confirmed by means of multi-locus gene sequencing. Eventually, confirming the identity of these isolates as *Fusarium avenaceum*. Subsequently, Koch's postulates were performed and fulfilled on apple and pear fruit. *F. avenaceum* is a wound pathogen that has been isolated from apple fruit in Croatia and in the United States. Only few reports describe wet core symptoms associated with *F. avenaceum* on apple

fruit. These are the first reports of wet core rot on apple fruit, and storage decay on pear fruit caused by *F. avenaceum* in the Netherlands. The disease represents an economical problem for apple growers. As wet core of apple is undetectable until the fruit is cut or consumed, it affects consumer confidence. *F. avenaceum* infections may constitute a safety issue due to the potential production of mycotoxins such as moniliformin.

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A new race of *Fusarium oxysporum* f.sp. *lactucae* in lettuce

ABSTRACT. In 2014 a new disease emerged in greenhouse grown lettuce in the Netherlands. Plants wilted and eventually died. In first instance growers supposed that the problem was caused by Symphyllids, root centipedes. Diagnosis performed at the National Reference Centre revealed that the causal agent is *Fusarium oxysporum*. *Fusarium oxysporum* f.sp. *lactucae* race 1 was

recently discovered in Italy and Portugal. In Japan, two more races are known. Collaboration with researchers from the University of Torino (Italy) resulted in the description of a new race of *Fusarium oxysporum* f.sp. *lactucae*, race 4.

This result was published in 'Plant Pathology' in September 2016

Fusarium oxysporum f.sp. *cupense* in Indonesia: diversity and pathogenicity

ABSTRACT. *Fusarium* wilt on banana is caused by *Fusarium oxysporum* f.sp. *cupense* (Foc) and is one of the major constraints in banana cultivation worldwide. Indonesia is part of the center of origin and diversity of bananas in Southeast Asia, and 30 different wild species and more than 500 cultivated banana varieties have been identified in this region. It is hypothesized that Foc co-evolved with banana and that the diversity of the host plants drive the evolution of this pathogen resulting in a huge diversity. We have been collecting Foc throughout Indonesia at 31 different location in Java, Sumatra, Kalimantan, Papua, Sulawesi, and Flores. This resulted in a comprehensive collection of ~200 Foc isolates from over 40 different local banana cultivars. We used genotyping by sequencing, viz. Diversity Array Technology (DArT), to assess Foc genetic diversity. A total of 34,885 DArT markers are polymorphic, with an average genotyping call rate of 98% and

scoring reproducibility of 100%. Cluster analyses of the DArT markers divided the Foc isolates in two major clades, and the clustering is irrespective of the geographical and host origin of the isolates. Additionally, we included 24 reference isolates for each vegetative compatibility group (VCGs), which represent thus far the known global Foc diversity. Our results indicate that the Indonesian Foc isolates displayed a high genotypic diversity, containing isolates from all known VCGs. Moreover, these analyses also revealed additional, novel genotypes. Phenotyping of a subset of these isolates for pathogenicity on Cavendish variety "Grand Naine" showed high variability of aggressiveness. Until now only VCG01213, commonly known as Tropical Race 4 (TR4), is truly pathogenic on the Cavendish subgroup. Our data demonstrate that also other, new Foc genotypes cause disease in Cavendish with even higher aggressivity levels than the reference II5 Foc isolate.

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Virulence factors of Panama Disease pathogen

ABSTRACT. Panama Disease is a wilt disease in banana caused by the fungus *Fusarium oxysporum* f.sp. *cubense* (Foc). To be able to infect the host, generally fungus employ themselves with certain weapons, such as enzyme, toxins, or secreted proteins. There is no knowledge in particular what are the virulence factors in the fungus *Fusarium oxysporum* f.sp. *cubense* – banana interaction. What has been known, however, is that in the *Fusarium lycopersici*-tomato pathosystem, one particular secreted protein, i.e. Six (Secreted in Xylem) protein families are functioning as virulence or avirulence functions. In tomato, at least three out of 14 known *SIX* genes, for example *SIX1*, *SIX3*, and *SIX4* are able to trigger defense mechanism by recognition of the corresponding resistance genes *I-3*, *I-2*, and *I-1*, and therefore it was called as *AVR3*, *AVR2*, and *AVR1*, respectively. Molecules produced by these genes are considered as an effectors, which means small molecules that binds to a protein and regulates its biological activity. In the *Fusarium* – banana pathosystem, these genes have never been explored. We tried to mine the genome, based on the whole genome sequence of several strains from the fungal species representing race diversity. Foc is grouped

into four different races, i.e. Race 1 which infect cultivar Gross Michel, Race 2 which infect cultivar Bluggoe, Race 4 which able to infect almost all cultivars, divided into Sub-Tropical Race 4 (ST4) and Tropical Race 4 (TR4). Employing comparative genomics methods we search for the homology of *SIX* genes in *Fusarium oxysporum* f.sp. *cubense*, as well as other possible putative effectors. Based on the MIMP presence and the homology to *SIX* genes, we found around 23 putative effectors, 9 of them are *SIX* homologs, predicted in the genome. Following that we checked the presence and absence of the predicted genes by PCR to our 50 Foc isolates collection. Phylogenetic tree which was generated based on EF-1 alpha and from the PCR detection showed that *SIX1*, is clustered in one race group in particular TR4. To be precise, the *SIX1* homologs, *SIX1b* and *SIX1c* are present only in TR4 isolates. We then test the function of these genes employing gene knock out and complementation. Result indicates that *SIX1a* has a virulence function in Foc. The *six1a* delta mutant showed lower disease index compare to the wild type, and the complemented strains restore its virulence. Further work is still on going to check the gene functions.

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Novel intracellular and apoplastic *Fusarium oxysporum* effectors suppress MAMP-triggered defense responses

ABSTRACT. Fungi are widespread colonizers of plants, and some species can also cause devastating diseases. Colonization success largely depends on the ability to manipulate the host plant, often achieved via effectors, secreted proteins that act either inside plant cells or in the apoplast. *Fusarium oxysporum* (Fo) is a soil inhabiting fungus that can infect many plant species via the roots. Its effectors were previously characterized as small, secreted proteins that accumulate in the xylem vessels during infection. For several Fo effectors a contribution to disease development has been demonstrated, but the underlying virulence mechanisms remain unknown. For in depth analysis we selected 12 candidate effectors from

a Fo strain that infects *Brassicaceae* plants including oilseed rape and Arabidopsis. Using a recently developed uptake assay we discovered that the majority of these candidate effectors enter living plant cells during root colonization, suggesting that they manipulate intracellular host processes. We also tested whether Fo effectors could suppress generic plant defense responses induced by microbe-associated molecular patterns (MAMPs), e.g. bacterial flagellin. Remarkably, three newly discovered Fo effectors inhibited such a defense response in the non-host plant *Nicotiana benthamiana*, indicating that they target conserved plant defense mechanisms.

Like Fokkens, Peter van Dam & Martijn Rep

University of Amsterdam,
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Host-specific regions in the pangenome of *F. oxysporum* reveal evolutionary trajectories of host-switches

ABSTRACT. The *Fusarium oxysporum* species complex (FOSC) consists of both pathogenic and non-pathogenic isolates that occur in very large populations. Pathogenic isolates enter the vascular system of host plants through the root and cause wilting or root rot disease symptoms. Individual isolates are host-specific and can therefore be grouped into formae speciales, but collectively the species complex has a very wide host range. Host-specificity is polyphyletic and for tomato-infecting isolates it has been demonstrated that virulence towards a specific host can be transferred through the exchange of chromosomes.

We used comparative genomics on 89 FOSC isolates that belong to different formae speciales to identify host-specific genomic regions and to infer evolutionary trajectories of host-switches. Importantly, 18 isolates were sequenced using SMRT technology, allowing us to achieve near complete assemblies. In these assemblies, approximately one third is sparsely present in other isolates and could thus be considered 'accessory'. We find evidence for both clade-specific contigs as well as host-specific contigs. Virulence on tomato is

strongly associated with large fractions of chromosome 14 and 15 of the reference genome, which is congruent with previous reports that exchange of chromosome 14 can lead to gain of pathogenicity on tomato. For cucumber-infecting isolates we find two different trajectories towards this host: one that involved exchange of (parts of) chromosomes and either in two distinct events, or in one event followed by loss of (parts of) a chromosome in the ancestor of the watermelon-infecting sister clade. The other group of cucumber-infecting isolates is inferred to originate from a melon-infecting ancestor, where either the jump to cucumber was accomplished by gain of very small regions or possibly through loss of avirulence genes. Similarly, for melon-infecting strains we find that two out of three clades share genomic regions with phylogenetically distinct isolates that cause root rot in cucurbits, whereas the other clade has a large clade-specific chromosome and shares a large region with a cucumber-infecting strain. Interestingly, we find that all cucurbit-infecting isolates possess a specific genomic region that is absent in other isolates and is likely to contain genes that are important for infection of this group of hosts.

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Mitochondrial evolution in *Fusarium*

ABSTRACT. The advances made in sequencing technologies and in software development for handling these sequencing reads, allow us to sequence and assemble the mitochondrial genome sequences of large numbers of strains. We assembled and analyzed the mitochondrial genome of more than 60 members of the *Fusarium oxysporum* species complex (FOSC) and *Fusarium fujikuroi* species complex (FFSC) and more than 20 members of the *Fusarium graminearum* species complex (FGSC): The mitochondria revealed drastic differences in genome sizes between the three species complexes, especially due to the absence or presence of intronic sequences in the hardly variable mitochondrial core genes. The comparison also revealed a large ORF (LV-uORF) with unknown function had been described before to be under selection. However, our sequence

variability analysis revealed that the LV-uORF is not under selection, since its DNA sequence variability is equal to that of intergenic regions of the mitochondrial genome. Comparisons of intron presence/absence has shown that in *Fusarium graminearum sensu stricto* the majority of the introns are fixed and conserved, but some introns are variable even within a single phylogenetic species. The variable introns showed little or no correlation with the phylogenetic tree based on the intergenic regions of the mitochondrial genome. In contrast, within FOSC the intron pattern is fixed for the different lineages, which correspond with phylogenetic trees based on other markers. The results also revealed mitochondrial recombination within FOSC. These results show that within the genus *Fusarium* the trends in the evolution of mitochondrial genomes are different for the different species complexes.

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The International Centre for Fusarium Research (ICFR) and the need for a Fusarium monograph

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ABSTRACT. A new infrastructure is urgently needed to encourage and promote international and national research collaborations on the genus *Fusarium*, which includes numerous important pathogens and mycotoxin producers. The International Centre for *Fusarium* Research (ICFR) aims to provide a stable platform for collaborations between *Fusarium* research laboratories and to develop new standard approaches for *Fusarium* research. The ICFR brings together key research institutes working on the genus *Fusarium* and fusarium-like sister genera. As a virtual research centre with its members spread over many research centers and universities worldwide, communication and collaboration on a global scale will be strongly encouraged. The ICFR is governed by a Board of Directors, a revolving Chair and a Secretary-General. The Board of Directors consists

of several prominent international *Fusarium* researchers, which will meet at least once a year, functioning as a scientific advisory committee to the Chair and Secretary-General. The main vision of the ICFR is to provide a stable platform for *Fusarium* research, which will be achieved by providing a comprehensive monograph of the genus *Fusarium*. Therefore, the ICFR is actively involved with the NSF project “ARTS: A phylogenetic revisionary monograph of the genus *Fusarium*” coordinated by Prof. dr. D. M. Geiser. This project aims to produce an open-access electronic and printed monograph for the genus *Fusarium*, which will integrate DNA sequences and other metadata (i.e. ecology and mycotoxicology). For more information on the ICFR, please visit www.fusarium.org or contact Dr. L. Lombard to become involved.

Pierre Hellin &
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New insight on the molecular mechanisms conferring resistance to DMI fungicides in *Fusarium culmorum*

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ABSTRACT. *Fusarium culmorum* is an important mycotoxigenic plant pathogen causing diseases on a variety of crops and weeds. In Belgium and in the neighboring countries, this species has been shown to be the predominant species involved in Fusarium head blight on wheat in 2011. In an assay to characterise this species sensitivity towards triazoles, significant differences were found between strains from a mostly European collection, with up to 10-fold differences in EC_{50} . An *F. culmorum* strain resistant to triazoles was obtained by cultivating the sensitive UK99 strain in tebuconazole-amended media. The obtained resistant strain showed similar levels of fitness (e.g. growth and pathogenicity) than its parental strain and was more resistant to all members of the demethylation inhibitor (DMI) fungicides but not

to other fungicide families tested. Taking advantage of the recently published genome of UK99, a RNA-Seq analysis was performed to compare the response of the resistant strain to that of its parental strain. Numerous genes were found to be differentially expressed between the two strains and some of them could be the key to decipher the triazole resistance mechanisms in *F. culmorum*. Among them, a transporter protein was overexpressed in both strains when subjected to tebuconazole but at a significantly higher level in the resistant strain than in its parental strain. The potential role of this transporter in triazole efflux was confirmed by comparing the expression of this gene in *F. culmorum* strains with distinct triazole sensitivity.

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Crops are a main driver for species diversity and the toxigenic potential of *Fusarium* isolates in maize ears in China

ABSTRACT. Increasing demands for corn and the relatively low-care cultivation of the crop have resulted in an enormous expansion of the acreage of maize in China in recent years. However, *Fusarium* ear rot (FER) forms an important constraint to maize production in China. Members of both the *Fusarium fujikuroi* species complex

(FFSC) and the *Fusarium graminearum* species complex prove to be the causal agents of FER in the main Chinese maize producing areas. *Fusarium verticillioides* producing fumonisin producing was the most prevalent species, followed by fumonisin-producing *Fusarium proliferatum* and 15-acetyldeoxynivalenol producing

F. graminearum. In the Northern regions in China, *Fusarium temperatum* and *Fusarium boothii* were identified for the first time extending their known habitats to colder environments. Mating type analysis of the different heterothallic FFSC species, showed that both types co-occur in each sampling site suggestive of the possibility of common sexual recombination. Virulence tests with *F. boothii* (from maize) and *F. graminearum* from maize or wheat showed adaptation to the host. In addition, *F. graminearum* seems to outcompete *F. boothii* in wheat-maize rotations.

Based on our findings and previous studies, we

conclude that wheat/maize rotation selects for *F. graminearum*, while a wheat/rice rotation selects for *F. asiaticum*. In contrast, *F. boothii* is selected when maize is cultivated without rotation. A higher occurrence of *F. temperatum* is observed on maize in colder climatological regions in China, while *Fusarium meridionale* seems restricted to mountain areas. Each of these species has their characteristic mycotoxin profile and deoxynivalenol and fumonisin are the potential threats to maize production in Northern China.

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A decision support system to control mycotoxin contamination in maize silages

ABSTRACT. Mycotoxins are toxic secondary metabolites produced by a variety of fungal species, such as *Fusarium*, *Penicillium* or *Aspergillus*, among others. Contamination of feed with mycotoxins can cause severe health problems in dairy cattle. Especially high yielding dairy cows with a high feed uptake and rapid ruminal flow are susceptible to gastroenteritis, reduced reproduction and reduced milk production, as a result of mycotoxin contamination.

Maize silage is one of the main components of dairy feed in the region of Flanders, Belgium, and is therefore one of the main sources for mycotoxin uptake in dairy cows. This research aims towards providing dairy farmers in Flanders with a user-friendly prediction model, able to foresee mycotoxin contamination based on weather, cultivation, harvest and silage conditions.

This model will be constructed based on analyses

of maize silages across Flanders, and on own research focusing on methods to prevent mycotoxin contamination. 100 maize silages will be selected based upon geographical spread, cultivation technique and silage conditions. These silages will be sampled once during harvest and 2-3 times during feeding every year for four years, and analyzed for mycotoxin and fungal contamination. Own research will be divided into five separate work packages, with the following topics: biofumigation of the soil using green crop manures, treatment of crop residues with antagonistic microbial populations, impact of harvest date and dry matter content on mycotoxin contamination, microbial detoxification in the silage, and toxicity of mycotoxins in dairy cattle. These results will aid towards constructing and validating the prediction model.

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