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Production of Trichothecenes and Other Secondary Metabolites by *Fusarium culmorum* and *Fusarium equiseti* on Common Laboratory Media and a Soil Organic Matter Agar: An Ecological Interpretation

HELLE HESTBJERG,[†,‡] KRISTIAN F. NIELSEN,[§] ULF THRANE,[§] AND SUSANNE ELMHOLT*[,†]

Department of Crop Physiology and Soil Science, Danish Institute of Agricultural Sciences, Research Centre Foulum, DK-8830 Tjele, Denmark, and Mycology Group, BioCentrum-DTU, Technical University of Denmark, Kgs. Lyngby, Denmark

Fusarium culmorum and *F. equiseti* were characterized with regard to production of trichothecenes and other secondary metabolites. Results following growth on laboratory media are interpreted with the aim of increasing the understanding of fungal metabolism in the field environment. While trichothecene production was detected for 94 of 102 *F. culmorum* isolates, only 8 of 57 *F. equiseti* isolates were positive. Profiles of secondary metabolites were compared by following growth on yeast extract sucrose agar (YES), potato sucrose agar (PSA), and an agar medium, prepared from soil organic matter (SOM), which was included to simulate growth conditions in soil. SOM supported the production of chrysogine by *F. culmorum*. The two species utilized the media differently. *F. culmorum* produced zearalenone (ZEA) on YES, whereas some *F. equiseti* isolates produced ZEA on PSA. Other *F. equiseti* isolates produced equisetin. These differences may reflect that *F. culmorum* depends on a pathogenic life style while *F. equiseti* has a more saprotrophic mode of existence.

KEYWORDS: YES; PSA; mycotoxins; deoxynivalenol; nivalenol; zearalenone; equisetin; chrysogine

INTRODUCTION

Fusarium culmorum (W. G. Smith) Sacc. and *F. equiseti* (Corda) Sacc. are soil fungi commonly found in agricultural soil (*1*). Both species are potentially toxigenic toward vertebrates (*2*). Much research has been directed toward the elucidation of the linkage between the morphologically based taxonomy and the production of mycotoxins (*2*−*7*). As many metabolites are produced by an array of *Fusarium* species, studies on profiles of metabolites for characterization of species have appeared more promising than the study of single metabolites. For *F. equiseti*, production of fusarochromanone, equisetin, zearalenone, and types A and B trichothecenes are included in the well-documented profile of metabolites. For *F. culmorum* this profile includes zearalenone, fusarin C, type B trichothecenes, and others (*8*, *9*).

The production of secondary metabolites is mostly studied on nutrient-rich laboratory media (*4*, *10*, *11*). These can be based on e.g. sucrose, yeast extract, potatoes, corn, or rice and often with the amendment of metals (Mg, Zn, Cu) to enhance metabolite production. Different media are known to support the production of different metabolites (*12*−*14*), but the ecological meaning of this has not been discussed. This is due to the fact that studies on production of metabolites have been performed for classification purposes (*7*) or for characterization of isolates (*4*, *11*, *12*, *15*). Often extracts from several media have been combined prior to analysis of metabolites and information on the role of the medium in the expression of fungal metabolism is thereby lost.

It is well-known that many secondary metabolites are produced by some isolates of a given species and not by others (*2*, *11*, *12*, *16*). Lack of detection of a metabolite may be due to its concentration being below the detection level of the analytical method used. Lack of production of a metabolite may relate to the well-known deterioration problems associated with prolonged culturing of *Fusarium* isolates. Altered appearance of cultures may cause misidentification of isolates, or the culture conditions may cause actual loss of ability to produce certain metabolites (*2*, *3*, *8*). The effect of culturing on isolates must be taken into account when comparing isolates with different history. Differences in profiles of metabolites between isolates within a species may also relate to the site of origin (*4*) and thus be due to differences in ecology. Our hypothesis is that climatic and edaphic conditions constitute different selection pressures and cause the evolution of different chemotypes.

* To whom correspondence should be addressed: Fax: +45 8999 1619. E-mail: susanne.elmholt@agrsci.dk.
† Danish Institute of Agricultural Sciences.
‡ Current address: Gl. Tungelundsvej 12, DK-8881 Thorsø, Denmark.
§ Technical University of Denmark.

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Table 1. Origin of *F. culmorum* and *F. equiseti* Isolates

field	location	elevation *m*	*F. culmorum* no. of isolates	*F. equiseti* no. of isolates
N9	Ås, Østfold, Norway	95	44	11
N10	Rygge, Norway	40	7	0
DK1	Kalø, Denmark	80	4	0
DK2	Høng, Denmark	10	4	3
DK3	Østermarie, Denmark	100	35	11
D8	Güterfelde, Germany	81	4	24
D6	Osterseeon, Germany	527	2	0
A4	Elixhausen, Austria	434	0	1
A5	Ansfelden, Austria	263	2	7

This study was undertaken to investigate the intraspecies variation within *F. culmorum* and *F. equiseti* from northern and central Europe, with regard to production of trichothecenes and other selected metabolites and relate the metabolite profiles to the ecology of the fungi. Isolation and culturing conditions were the same for all isolates. The procedures were chosen to be as lenient as possible, according to the present knowledge, to preserve the wild type state of the isolates.

MATERIALS AND METHODS

Fungal Isolates. Isolates of *F. culmorum* and *F. equiseti* were derived from organic soil particles and identified as described in Hestbjerg et al. (*17*). Identification was based on morphological and physiological criteria according to Nirenberg (*18*) and Domsch et al. (*1*) and was confirmed by the general metabolite profile according to Thrane (*8, 9*). A total of 39 of the isolates are kept at the IBT Culture Collection at BioCentrum-DTU, Kgs. Lyngby, Denmark (**Tables 3** and **4**). The isolates originated from wheat field soils in Norway, Denmark, Germany, and Austria. In each field, soil was sampled in June−July 1995 from nine points being disposed in a 3 × 3 grid with 10 m distance between grid points (total area 400 m^2). Approximately 225 g of soil was sampled at each point (depth of 0−10 cm), and the soil from each field was combined to composite samples of approximately 2 kg.

Information on the origin of the isolates is given in **Table 1**. Soil particles from soil cultures were transferred to 9 cm Petri dishes containing "Spezieller Närhstoffarmer Agar" (SNA; *19*) and incubated for 7 days at 20 °C and a 12 h near-UV-light/12 h dark regime. The isolates were then transferred to fresh SNA plates and incubated in the same way for 14 days. From these plates, 4 mm agar plugs, cut approximately 2 cm from the margin of the colony, were transferred to the center of plates containing the appropriate media.

Media. Yeast extract sucrose agar (YES) contained Difco yeast extract, 2%, sucrose, 15%, agar, 2%, and trace elements according to Frisvad and Filtenborg (*20*). Potato sucrose agar (PSA) was prepared from potato extract, 50%, sucrose, 2%, and agar, 1.5% (*21*). The total N content in YES and PSA was determined according to AOAC (*22*), and the total C content was calculated on the basis of the sucrose content and information from Difco with regard to the yeast extract (the contribution from the potato extract was considered negligible). Soil organic matter agar (SOM) consisted of soil organic matter, 2%, and agar, 2%, in soil extract. Soil organic matter was extracted from a soil collected in a wheat field immediately after harvest. The soil was washed through a set of sieves (4 and 1 mm), and floating organic matter was collected from the 1 mm sieve, dried, and ground for 15 min in a ball mill. The organic matter was analyzed for C and N content, determined according to Leco (*23*) and Hansen (*24*), respectively. Soil extract was prepared by autoclaving 500 g of soil from a wheat field after harvest in 1 L of tap water for 1 h (121 °C) and repeatedly filtering through coffee filters to enable final filtering through a Whatman no. 1 filter. For each medium, one batch was prepared for the whole experiment.

Incubation and Extraction. Each isolate was inoculated onto one plate (9 cm diameter) of each medium and incubated for 14 days at 25 °C in the dark. The plug extraction method by Smedsgaard (*25*) was used, slightly modified: 10 6-mm agar plugs, cut along a diameter of the fungal culture, were extracted with 1 mL of extraction solvent (methanol−dichloromethane−ethyl acetate (1:2:3, v/v/v) containing 1% formic acid). The samples were redissolved in pure methanol, and the extracts were kept at −80 °C prior to analysis.

Gas Chromatography−Mass Spectrometry. Following growth on YES, all isolates were analyzed for production of trichothecenes by gas chromatography−mass spectrometry (GC−MS) as described in details by Nielsen and Thrane (*16*). Briefly, the trichothecenes were derivatized to their pentafluoropropionyl esters and analyzed twice, first using GC-negative ion chemical ionization (NICI)-MS and second using GC-electron impact ionization (EI^+)−tandem mass spectrometry. Injection of 1.0 μL was performed by a Finnigan A200S autosampler, splitless (45 s, split 1:40) with hot needle, 2 s in a 4 mm i.d. Focusliner with glass wool (SGE, Ringwood, Australia) at 280 °C on a 0.25 mm, 0.10 μm, and 30 m HP-5 Trace column (Hewlett-Packard, Avondale, PA). The syringe (10 μL) was washed with 10 μL of acetone 10 times before and 15 times after injection. GC program: 80 °C for 1 min, 40 °C/min to 160 °C, then 4 °C/min to 205 °C, then 8 °C/min to 240 °C, and then 40 °C/min to 300 °C holding 3 min. Helium of a purity of 99.999% (local supplier) was used as carrier gas at a constant linear gas velocity of 40 cm/s. The transfer line temperature was 275 °C. Details on MS and MS−MS settings have been given elsewhere (*16*).

The following standards were available: acetyl T-2 toxin (AT-2); T-2 toxin (T-2); HT-2 toxin (HT-2); T-2 triol (T-2TR); T-2 tetraol (T-2TE); scirpentriol; 15-monoacetoxyscirpenol (15-MAS); neosolaniol (NEO); 15-acetyldeoxynivalenol (15-ADON); 3-acetyldeoxynivalenol (3-ADON); diacetoxyscirpenol (DAS); nivalenol (NIV); fusarenon-X (F-X); deoxynivalenol (DON). They were all obtained from Sigma (St. Louis, MO). Detection limits for the 1 μL injected samples using the most abundant ion in the MS-MS spectrum were for AT-2 100 pg, for the PFP derivatives of NIV, DON, and F-X about 5−15 pg, for TRI about 2 pg, and for 3ADON, 15MAS, DAS, NEO, HT-2, and T-2 about 20−70 pg.

High-Performance Liquid Chromatography. A few isolates of each species were selected from each location for determination of the production of selected metabolites on YES, PSA, and SOM. High-performance liquid chromatography (HPLC) with diode array detection was used (*25*). Briefly, the separation was done on a 100 mm × 4 mm ID HP Hypersil BDS C_{18} column with a 4 × 4 mm Hypersil RP 18 guard column (all from Hewlett-Packard, Waldbronn, Germany) at 40 °C. The gradient solvent system consisted of water and acetonitrile, starting at 85% water and 15% acetonitrile going to 100% acetoniltrile in 40 min, maintained at 100% acetonitrile for 3 min before returning to 85% water and 15% acetonitrile in 7 min. Both solvents were added 50 μL/L of trifluoracetic acid. All chemicals were of HPLC grade. The extracts were thawed and used without further treatment. Metabolites were detected at 225 nm and characterized by their UV spectrum (200−600 nm). Results were interpreted qualitatively, that is the presence or absence of metabolites. The HPLC chromatograms were compared to standards for fusarochromanone (FCHR) (C. Mirocha, University of Minnesota, St. Paul, MN), equisetin (EQ) (H. R. Burmeister, Northern Regional Research Center, Peoria, IL), zearalenone (ZEA) (Sigma, St. Louis, MO), fusarin C (F-C) (L. F. Bjeldanes, University of California, Berkeley, CA), and chrysogine (CHRY) (J. Bergman, Stockholm University, Stockholm, Sweden). Quantitative analyses and determination of detection limits were not possible due to the unknown purity of standards.

RESULTS

The N contents in YES, PSA, and SOM were 2.2, 0.1, and 0.37 g L^{-1}, respectively. The carbon:nitrogen ratios were calculated to be 33 in YES, 84 in PSA, and 18 in SOM. All GC-MS chromatograms were inspected for peaks corresponding to the above listed trichothecene standards. HPLC chromatograms were compared to standards for FCHR, EQ, ZEA, F-C, and CHRY. Derivatives of these metabolites (metabolites with a similar UV spectrum but a different retention time index) and a few unknown metabolites with distinct UV spectra were also selected for the data analysis.

Table 2. Production of Trichothecenes by *F. culmorum* and *F. equiseti* on YES Agar, Where Figures Indicate the Number of Isolates with the Different Profiles

profile of trichothecene production	N9	N10	DK1	DK2	DK3	D8	D6	A4	A5
F. culmorum									
DON, 3-ADON	42	7	2	4	19	4	2		2
DON	1				2				
NIV, FX			2		7				
no trichothecenes	1				7				
F. equiseti									
NIV						1			
FX					1				
DAS						1			
NIV, FX, DAS				1	1	1			
NIV, FX, DAS, SCR				1					
NIV, FX, DAS, MAS, SCR					1				
no trichothecenes	11			1	8	21		1	7

Production of Trichothecenes. Results on the production of trichothecenes are given in **Table 2**. Of 57 isolates of *F. equiseti*, only 8 produced trichothecenes in detectable amounts. The profiles of these 8 producers were very different, and the isolates originated from three different locations. Of 102 isolates of *F. culmorum*, trichothecene production was detected for 94. Most isolates of *F. culmorum* produced DON and 3-ADON. From two Danish locations, 9 isolates produced NIV and F-X.

Secondary Metabolite Profiles from HPLC Analyses. The production of selected metabolites by 15 isolates of *F. equiseti*, grown on YES and PSA, is shown in **Table 3**. *F. equiseti* did not produce any of the selected metabolites on SOM. Three isolates (IBT 40221, 40218, and 40269) were characterized by their production of ZEA on PSA and the lack of EQ on the same medium. These isolates also produced metabolites with the same RI as FCHR derivatives (P797 and P805) but with different UV spectra. Results from the HPLC analyses of 24 isolates of *F. culmorum* grown on YES, PSA and SOM are shown in **Table 4**. Most isolates of *F. culmorum* produced CHRY on all three media. On YES, most isolates also produced ZEA and a range of derivatives of F-C. Metabolite Y679 was produced by all four isolates from field N9 and only by these isolates. Metabolite Y845, having a UV spectrum similar to that of CHRY, was produced by two isolates from soil D8 and only by these isolates.

DISCUSSION

For both *F. culmorum* and *F. equiseti* a considerable intraspecies variation in the production of secondary metabolites was found. It is speculated that this as well as the interspecies differences relates to differences in the ecology of the fungi.

All the isolates originated from organic soil particles. During isolation and culturing they were treated identically. Nutrient-rich media and multiple transfers were avoided. Thus, cultural practices are considered to have caused no differences in the metabolism of the isolates. The task to classify *Fusarium* species according to their production of mycotoxins has been a very difficult one due to misidentifications of fungal isolates (*2*, *3*, *8*). In this study identification was based on a combination of conidial morphology, physiological traits such as growth rate and colony color, and metabolite profiles. For the trichothecenes, detection methods such as thin-layer chromatography (TLC) or high-performance liquid chromatography (HPLC) with UV detection have caused the report of many false positives in the literature. While useful for the detection of many metabolites, the UV spectra for trichothecenes are not specific. GC with electron capture detection or methods based on mass spectrometry are reliable for the detection of these compounds (*16*, *26*−*29*). In each field, fungi were isolated from composite soil samples of a 400 m^2 area. It cannot be excluded that some isolates belong to the same individual, as some fungi are known to invade quite large areas of soil. We isolated only one strain from each particle and applied a technique that washes off conidia from the soil particles. This decreases the risk of recovering several isolates of a heavily sporulating individual, which may have been transferred to large areas by e.g. moisture flow or tillage. Five fields revealed more than one chemotype (**Table 2**) indicating the coexistence of several individuals. For example both DON/3-ADON and NIV/F-X producing isolates of *F. culmorum* were found in DK3. Three different chemotypes of *F. equiseti* were also found in DK3.

For *F. culmorum*, 92% of the isolates (94 of 102) produced trichothecenes while, for *F. equiseti*, only 14% (8 of 57) were positive. This is in accordance with the findings of others (*12*, *15*, *30*). It is assumed that all isolates possess the genes involved in trichothecene production. Under this assumption, possible explanations for the lack of detected trichothecene production by an isolate may be (i) production in amounts below the detection level of the applied method, (ii) mutations in the

Table 3. Metabolites Produced by *F. Equiseti* on YES and PSA Agar[a]

		YES					PSA					
isolate IBT no.	field	CHRY	EQ	775	781	FCHR	CHRY	EQ	797	805	FCHR	ZEA
40221	DK2			+	+	9			+	+		7
40219	DK2		+			5	+	+				
40218	DK2	+	+	+	+	8	+		+	+		7
40269	DK3	+	+	+	+	8	+			+		5
40270	DK3	+		+	+	6	+	+				
40256	DK3		+			3		+			1	
40271	A4	+	+	+	+	7	+	+			2	
40257	A5	+	+	+	+	7	+	+				
40243	A5	+	+	+		7		+			1	
40272	D8	+	+	+	+	6	+	+				
40273	D8	+	+	+	+	7	+	+				
40274	D8	+	+	+	+	7		+			1	
40255	N9	+	+	+	+	9	+	+			1	
40275	N9	+	+			5	+	+				
40217	N9	+	+	+	+	9	+	+			1	

[a] See text for explanation of metabolites. + indicates production of a given metabolite. Where several derivatives of the same metabolite are produced, the number of derivatives is given.

Table 4. Metabolites Produced by *F. culmorum* on YES, PSA, and SOM[a]

isolate		YES						PSA			SOM:
IBT no.	field	CHRY	ZEA	679	746	845	FC	CHRY	ZEA	845	CHRY
40258	DK1		+				6	+			+
40259	DK1	+						+			+
40260	DK1	+	+		+		4	+			+
40261	DK2	+	+		+		3	+			
40253	DK2	+	+				2	+	+		
40220	DK2	+	+				2				
40262	DK3	+	+		+		1	+			+
40263	DK3	+	+		+		8				+
40264	DK3	+	+		+		3	+			+
40265	DK3						3				
40254	A5	+					5	+			
9480	A5	+	+		+		2	+			
9551	D6	+	+		+		3	+			+
9479	D6	+			+		7	+			+
40252	D8	+	+			+	4	+			
40251	D8	+	+			+	1	+			+
40266	D8	+	+				4	+			+
40267	N9		+	+	+		2	+			+
40268	N9		+	+	+		2	+			+
40244	N9	+	+	+	+		7	+			+
40225	N9		+	+	+		3	+			+
40232	N10	+	+		+		3	+			+
40224	N10	+	+		+		3	+			+
40238	N10	+	+		+		2	+			+

[a] See text for explanation of metabolite abbreviations. + indicates production of a given metabolite. Where several derivatives of the same metabolite are produced, the number of derivatives is given.

biosynthetic pathway, and (iii) down regulation of the biosynthetic pathway by unknown mechanisms. Irrespective of the mechanism involved, the obtained results can be related to the ecology of the fungi as a larger need for *F. culmorum* to produce trichothecenes than for *F. equiseti*. The production of trichothecenes by fusaria has been related to plant pathogenesis (*31*−*33*). These compounds are believed to play a role in the aggressiveness of an isolate. It was recently shown in a competition assay that DON production is affected by competing fungi (*34*). The production of DON by *F. graminearum* was reduced in the presence of various isolates of *Trichoderma harzianum* and *Fusarium* species that normally co-occur with *F. graminearum*. For example all isolates of *F. subglutinans* caused reductions in DON production. The fact that competing organisms are able to partly suppress the production of trichothecenes indicates that these metabolites may be important in the ecology of the fungi. *F. culmorum* is regarded as a serious plant pathogen, especially on cereals, whereas *F. equiseti* generally is considered a weak pathogen (*1*, *35*). If the existence of *F. culmorum* to a larger degree depends on the ability to infect plants and the trichothecenes increase this ability, the selection pressure would result in a more consistent production of trichothecenes by *F. culmorum* than by *F. equiseti*.

For *F. culmorum*, the production of type B trichothecenes, ZEA, and F-C (**Tables 2** and **4**) is in accordance with the findings by Thrane (*9*). With regard to the trichothecenes, we found the production of DON and 3-ADON to be a very common chemotype in our European isolates of *F. culmorum* (**Table 2**). This result is in accordance with Bottalico et al. (*12*), Miller et al. (*4*), and Nielsen and Thrane (*16*). The common chemotype for American isolates is production of DON and 15-ADON (*5*, *36*) while none of our isolates produced 15-ADON supporting the hypothesis that a biogeographic pattern exists for *F. culmorum*. Both 3-ADON and 15-ADON are phytotoxic (*37*−*39*), and they possibly play the same role in the ecology of the fungi.

Nine isolates of *F. culmorum* produced NIV and F-X. For some of these isolates, trace amounts of DON were detected (not shown). This was confirmed when a quantitative determination of DON was performed for isolates inoculated onto barley seedlings (*33*). The distinction of two main chemotypes within *F. culmorum* is in agreement with recent results by Nielsen and Thrane (*16*), as well as results from France (*40*) and Germany (*30*, *41*).

The production of type B trichothecenes by *F. equiseti* seems rather controversial. Up to 1989, such reports had not been verified (*2*, *8*). For some of our isolates combinations of type A and type B trichothecenes were detected (**Table 2**). Seven of eight trichothecene producing isolates produced one or two type B trichothecenes (NIV, F-X). In addition a few isolates showed a low level of DON; however, this needs to be verified by additional analyses. Production of DON by *F. equiseti* has been reported by Abramson et al. (*5*) and McLachlan et al. (*42*). Abramson et al. (*5*) further reported the production of 15-ADON and F-X by this species. Their isolates are identified according to Nelson et al. (*43*). *F. equiseti* thereby includes *F. scirpi* var. *compactum* and *F. scirpi* var. *filiferum*, which is not the case in our study. The DON producing isolate studied by McLachlan et al. (*42*) was obtained from L. Burgess from Australia and as such is comparable with our isolates (*44*). In summary, the trichothecene production by *F. equiseti* seems variable but some isolates are able to produce both type A and B trichothecenes. The diversity of profiles found among the few isolates that produced trichothecenes, may indicate a low selection pressure for this trait in *F. equiseti*.

Two distinct profiles of secondary metabolites were found for *F. equiseti* on PSA. Three isolates produced ZEA on PSA while the others produced EQ (**Table 3**). Our hypothesis is that differences in environmental conditions prior to isolation have influenced the mechanisms regulating the metabolic pathways. ZEA is biosynthesized from eight acetate units via the acetate−malonate pathway (*45*). The biosynthetic pathway leading to EQ, however, still needs to be elucidated, and it is therefore not known whether the biosynthesis of EQ and ZEA are linked. The three ZEA-producing isolates cannot be distinguished from the others with regard to conidial morphology, growth rate, pigmentation, sporulation, and production of fusarochromanone (*46*). Though all from Denmark, they originate from two distinctly different locations, one being on the island of Bornholm in the Baltic Sea south of Sweden and the other on Zealand. Thus, there is no clear relation to a geographical area. It is concluded that the differentiation with regard to utilization of PSA relates to differences in the physiology that are regulated by unknown mechanisms. ZEA is known for its estrogenic effects (*47*). The compound has also been found to exhibit phytotoxic effects (*37*, *48*−*49*) and possesses antibiotic properties as well (*50*, *51*). EQ is known as an antibiotic (*52*). Thus, there are differences in the biological effect of these compounds. Competition with different microbial populations and/or interactions with different plants or animals in the soil environment may constitute a history that has affected the metabolic regulation mechanisms. The results reveal interesting differences between the two species with regard to the utilization of YES and PSA. The following is an attempt to interpret the fungal physiology by comparing these common laboratory media to natural habitats. *F. culmorum* primarily produced ZEA on YES (**Table 4**), whereas *F. equiseti* produced this compound on PSA (**Table 3**). Further, *F. equiseti* produced 5−7 derivatives of ZEA. This difference is hypothesized to reflect a difference in the life style of these fungi in the field. YES and PSA can be

seen as representing different field habitats. YES is a more nutrient-rich medium than PSA, containing 7.5-fold more sucrose, containing above 20-fold more nitrogen, and having a carbon-to-nitrogen ratio 2.5 times lower than PSA. In the field environment, living plants offer a nutrient-rich habitat for pathogenic fungi. There is a continuous flow of sucrose to the root tips (*53*). The C:N ratio for young wheat roots depends on the amount of fertilizer applied to the crop and lies in the range 28−50 (*54*). The value of 35, which was calculated for YES, lies within this range. Senescent and dead plant materials are poorer in available nutrients than living plants. The lower content of nitrogen and the high C:N ratio of straw (*55*) resembles the values found for PSA. The production of ZEA on YES by *F. culmorum* may thus express that this species uses ZEA in its pathogenic mode of existence. As a phytotoxin, ZEA possibly acts synergistically with DON (*37*), and *F. culmorum* may use this effect in its pathogenesis. Further research is required to compare the pathogenicity of isolates with and without the ability to produce ZEA. The production of ZEA on PSA by *F. equiseti* may likewise express the use of this compound under saprotrophic conditions. The rich production of ZEA and derivatives of ZEA on PSA may indicate that *F. equiseti* depends more on the antibiotic activity of ZEA when decomposing dead organic matter often in competition with a range of other organisms. It is capable of utilizing cellulose (*1*, *56*) and therefore also part of the carbon in, for example, straw. The high C:N ratio of PSA contradicts the low C:N ratio of SOM. However, both substrates are low in nitrogen content and it has been argued that the nutrient levels are more important than the ratio in regulating decomposition of organic matter by fungi (*57*).

This study attempts to interpret fungal physiology as expressed on common laboratory media like YES and PSA. A further step is the investigation of metabolite production on natural media. SOM provides a means of studying the growth and metabolism of saprotrophic fungi, when supplied with resources that are available in their natural soil habitat. Media based on extracts from natural substrates or living cells cannot be completely standardized. Their nutrient contents will depend on the quality of the added resources, in the case of SOM on the composition of the organic matter from the particular soil used. This aspect has also been touched upon for the natural ingredients of PSA (potato extract; *21*) and YES (yeast extract; *58*). To overcome this problem within a study it is essential to use the same batch for the whole experiment as done in the present experiment. The contents of C and N in all substrates were measured to ease comparison with other studies. Other components than C and N (e.g. micronutrients) can, however, also affect the production of secondary metabolites (*58*).

SOM is easy to handle in the laboratory, and if one bears its limitations in mind, it can give hints to the ecological significance of secondary metabolites in the soil. In this study the only known metabolite produced on SOM was CHRY, which was produced by most isolates of *F. culmorum* (**Table 4**). No investigations on the biological activity of CHRY have been carried out, but its appearance on SOM indicates that it plays a role in the saprotrophic mode of existence of *F. culmorum*. It could be argued that when only one known metabolite is produced on SOM its usefulness is questionable. From a chemotaxonomical viewpoint this is correct. From an ecological viewpoint, however, we find it thought provoking that most metabolites produced on the very energy- and nutrient-rich laboratory media are not produced in detectable amounts when the fungus nourishes on soil organic matter. An explanation could be that there is no need for a large production of all metabolites in natural habitats. There is rather a need for targeted production at microhabitat level and only of specific metabolites in quantities sufficient for a specific purpose. The biological activity of metabolites may take place at a molecular level whereas detection by analytical equipment requires much higher amounts. A massive production (overproduction?) of metabolites as seen during growth on rich laboratory substrates would be a waste of resources (energy) in natural habitats. From an analytical point of view improvements on the extraction procedure are necessary to exceed the detection limit for metabolites produced in only very small amounts. The introduction of other extraction solvents, e.g. water, would be relevant from an ecological point of view and might reveal the production of other secondary metabolites on this medium. At present, we have very little knowledge on the metabolism of *Fusarium* in the soil habitat. Future research needs to address the questions: Which metabolites are produced in natural habitats, and how are their production affected by interactions with the external environment (substrate, living plants, insects, and microorganisms)? The inclusion of media based on natural substrates might be a good indicator of metabolites that are relevant for further studies.

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