Moniliformin production in the genus *Fusarium*

Frank Schütt*, Helgard I. Nirenberg and Günther Deml

Federal Biological Research Centre for Agriculture and Forestry, Institute for Microbiology, Königin-Luise-Straße 19, 14195 Berlin, Germany
* corresponding author

Abstract

More than 600 *Fusarium* strains were screened for their ability to produce moniliformin in vitro. They represented 90 species and 15 sections (without section *Pseudomicrocera*). A simple TLC-method (agar plug method) was tested for its applicability to detect moniliformin in fungal cultures. It revealed moniliformin production in 146 strains representing 32 species, all belonging to sections of which the teleomorph is known to be *Gibberella*. The results by and large agree with earlier published data. However, some species did not show any moniliformin production in contrast to other reports. Furthermore, moniliformin production is reported for the first time in two known and in two not yet described species. 58 species were unable to generate the toxin. The results support the importance to include mycotoxin production of fungi as a criterion in a polyphasic taxonomy.

Introduction

Since the discovery and characterization of moniliformin by Cole et al. (1) the production of this mycotoxin has been recorded for at least 24 *Fusarium* species (2, 3, 4). Nevertheless, there are difficulties to attribute a toxin production potential to a certain species because literature data exhibit high discrepancies. A typical example can be seen in *F. moniliforme*, the species that lent its name to moniliformin. Different explanations have been given why in various reports some or even all examined *F. moniliforme* strains produced no moniliformin (13, 4), but neither different culture conditions nor identifications of the strains according to various taxonomic systems explain the contradictory results sufficiently. For the synonymy of species consult monographs (22, 23, 24, 25) and articles (8, 26, 27, 17) on the genus *Fusarium*. Reports of moniliformin production by species such as *F. culmorum, F. graminearum*, and *F. sambucinum* have to be questioned, too (2, 5, 6).

This study intends to give an updated overview on moniliformin production in the genus *Fusarium* and intends to reveal existing contradictions. For this reason previously studied species are reexamined. In addition, isolates of rare species, as well as newly described or yet undescribed species are tested. Furthermore, the applicability of the agar plug method (7) for the detection of moniliformin in *Fusarium* cultures is tested. This simple TLC technique has already been used to screen for other secondary metabolites produced in fungal cultures. If possible, several strains of each species are examined. The results are verified by analyzing culture extracts of randomly chosen moniliformin producers by HPLC.

Materials and Methods

All *Fusarium* isolates were obtained from the culture collection of the Federal Biological Research Centre for Agriculture and Forestry (BBA). Whenever possible, isolates from plants were selected that serve as food for humans. The isolates were cultivated on SNA for 7 days at room temperature in day and night rhythm (8). Before testing for moniliformin production, each strain was checked for its identity and purity.

For TLC determination (agar plug method) 100 g cereal meal (maize and durum wheat, respectively), 20 g agar, and 1 l distilled water were autoclaved for 20 min at 121 °C. 30 g of cereal meal were poured into a petri dish and, as soon as it became solid, inoculated with a plug taken from an SNA culture (diameter 0.5 cm). The isolates grew in an incubator at 25 °C in darkness for 21 days. Analysis was carried out using the method of Filtenborg et al. (7). For extraction, a mixture of acetonitrile / water (9+1) was used. The extracts and a moniliformin standard were spotted onto TLC plates (silica gel 60 F_{254}), which were developed in the solvent system chloroform / methanol / formic acid (55 + 45 + 1). Moniliformin was both detected by absorption in UV light (254 nm) and after derivatization with methylbenzothiazolinonehydrazone (MBTH; 1 % methanol solution). After spraying with MBTH plates were heated for 10 min at 140 °C. Moniliformin appeared as an orange-red spot.

For HPLC determination, 20 g of dry maize or durum wheat meal were autoclaved in 250 ml flasks; then 25 ml of sterile water were added. Each mixture (corn or durum wheat) was inoculated with a plug of SNA culture. The isolates grew in an incubator in darkness for 21 days. Extraction, clean-up, and HPLC determination were performed using the method of Scott and Lawrence (9).

Results and Discussion

The TLC separation of the agar plug extracts of known moniliformin producers gave spots which corresponded to the moniliformin standard. These spots as well as the standard absorbed UV light and formed an orange-red pigment after derivatization. In the HPLC chromatogram the moniliformin standard and the corresponding sample fraction showed the same retention times. No new peak appeared after the addition of moniliformin standard to the samples. Thus, the agar plug method is applicable for the detection of moniliformin.

No obvious differences could be recognized in moniliformin production between strains grown on maize and durum wheat, respectively. Table 1 shows the species which were able to produce moniliformin. Moniliformin production of *F. redolens*, *F. arthrosporioides*, two new *Fusarium* species and a taxonomically yet uncertain entity is reported for the first time. Since *F. chlamydosporum* is synonymous with *F. fusarioides* and has priority over this species, our findings of moniliformin production in some strains of *F. chlamydosporum* agree with the report of Rabie and coworkers (20). Moniliformin production of *F. acuminatum*, *F. anthophilum*, *F. avenaceum*, *F. beomiforme*, *F. dlaminii*, *F. fujikuroi*, *F. napiforme*, *F. nygamai*, *F. oxysporum*, *F. proliferatum*, *F. subglutinans*, and *F. tricinctum* has been described intensively before for analyzed field samples and in vitro tests (3, 4, 10, 11, 12, 13). The large number of moniliformin producers in section *Liseola* (inclusive *Dlaminia*) (14) can be attributed mostly to a revision of this section rendering many new descriptions and one new combination (15, 16, 17). The large number of tested *F. oxysporum* isolates in section *Elegans* is explained by the large number of different formae speciales and varieties of this species. Moniliformin production of *F. redolens* has probably not been reported before because of its similar morphology with *F. oxysporum*. For the same reason *F. arthrosporioides* might have been overlooked as a moniliformin producer in section *Roseum*: It resembles strongly *F. avenaceum*.

Since *F. subglutinans* and *F. proliferatum* often occur on maize, *F. avenaceum*, *F. tricinctum* and *F. proliferatum* on durum-wheat, *F. fujikuroi* on rice, *F. thapsinum* and *F. proliferatum* on sorghum, *F. denticulatum* on sweet potato and *F. ramigenum* on figs, moniliformin can be expected in these crops. For some of them (maize and durum-wheat) this conclusion was already proven to be correct (28, 29).

Tab 1 – *Fusarium* species producing monili

***Fusarium* species**	**No. of strains tested / positive**
Section *Liseola*	
F. acutatum Nirenberg & O'Donnell	5 / 4
F. anthophilum (A. Braun) Wollenweber	6 / 6
F. begoniae Nirenberg & O'Donnell	4 / 4
F. bulbicola Nirenberg & O'Donnell	4 / 3
F. concentricum Nirenberg & O'Donnell	6 / 2
F. denticulatum Nirenberg & O'Donnell	6 / 6
F. dlaminii Marasas et al.	5 / 4
F. fujikuroi Nirenberg	7 / 6
F. lactis Pirotta & Riboni	6 / 3
F. napiforme Marasas et al.	3 / 1
F. nisikadoi Aoki & Nirenberg	8 / 3
F. nygamai Burgess & Trimboli	9 / 2
F. phyllophilum Nirenberg & O'Donnell	5 / 3
F. proliferatum (Matsushima) Nirenberg	6 / 6
F. pseudoanthophilum Nirenberg et al.	7 / 5
F. pseudocircinatum O'Donnell & Nirenberg	4 / 3
F. pseudonygamai O'Donnell & Nirenberg	3 / 3
F. ramigenum O'Donnell & Nirenberg	3 / 3
F. sacchari (Butler) W. Gams	8 / 5
F. subglutinans (Wollenweber & Reinking) Nelson et al.	6 / 4
F. thapsinum Klittich et al.	8 / 7
Section *Elegans*	
F. beomiforme Nelson et al.	3 / 3
F. oxysporum Schlechtendahl: *Fries*	68 / 18
F. redolens Wollenweber	8 / 7
F. spec. nov. 1[1]	5 / 5
Section *Sporotrichiella*	
F. tricinctum (Corda) Saccardo	6 / 4
F. chlamydosporum Wollenweber & Reinking	12 / 2
Sektion *Roseum*	
F. arthrosporioides Sherbakoff	6 / 6
F. avenaceum (Corda : Fries) Saccardo	11 / 6
F. spec.[2]	4 / 4
Section *Gibbosum*	
F. acuminatum Ellis & Everhart	10 / 6
Section ?	
F. spec. nov. 2[3]	5 / 2

[1] *F.* spec. nov. 1 from peaty soils, resembling *F. oxysporum*
[2] *F.* spec. from elm tree galls, resembling *F. avenaceum*
[3] *F.* spec. nov. 2 from legumes, resembling *F. tumidum*

No moniliformin production was found in strains of *F. graminearum, F. culmorum, F. sambucinum,* and *F. solani* (Table 2). These results agree with numerous investigations (11, 18, 19, 21). Divergent results in other publications are probably due to incorrect identifications of the isolates examined. In this study *F. moniliforme* is not used as a species name since nowadays the correct name of the corn pathogen is *F. verticillioides* (8). This fungus does not produce moniliformin. Studies that ascribe moniliformin production to *F. moniliforme* actually tested not one species but rather an aggregate consisting of more than one species of the section *Liseola* - therefore containing also moniliformin producing strains probably belonging to *F. thapsinum* and *F. fujikuroi.* Most of the other non-moniliformin producing species were examined for the first time. Further studies have to be carried out to understand why in some species only a small number of strains were able to produce moniliformin. For this reason additional morphological, ecological and biomolecular data have to be gathered and compared.

Tab 2 – *Fusarium* species not producing moniliformin

F. anguioides Sherbakoff (5)[1]
F. annulatum Bugnicourt (1)
F. aquaeductuum (Radlkofer & Rabenhorst) Lagerheim
F. argillaceum Fries (3)
F. babinda Summerell et al. (7)
F. bactridioides Wollenweber (1)
F. brachygibbosum Padwick (5)
F. brevicatenulatum Nirenberg & O'Donnell (2)
F. buharicum Jaczewski (2)
F. buxicola Saccardo (1)
F. camptoceras Wollenweber & Reinking (5)
F. caudatum Wollenweber (4)
F. cavispermum Corda (3)
F. cerealis Cooke (6)
F. ciliatum Link (2)
F. circinatum Nirenberg & O'Donnell (4)
F. coccophilum (Desmazieres) Wollenweber & Reinking (1)
F. coeruleum (Libert) Saccardo (3)
F. compactum (Wollenweber) Gordon (3)
F. concolor Reinking (4)
F. culmorum (W. G. Smith) Saccardo (5)
F. decemcellulare Brick (3)
F. dimerum Penzig (4)
F. diversisporum Sherbakoff (6)
F. equiseti (Corda) Saccardo (7)
F. filiferum (Preuss) Wollenweber (5)
F. flocciferum Corda (7)
F. globosum Rheeder et al.(6)
F. graminearum Schwabe (8)
F. guttiforme Nirenberg & O'Donnell (6)
F. heterosporum Nees : Fries (5)
F. incarnatum (Roberge) Saccardo (4)
F. inflexum R. Schneider (3)
F. larvarum Fuckel (2)
F. lateritium Nees : Fries (20)
F. longipes Wollenweber & Reinking (3)
F. lunulosporum Gerlach (1)
F. melanochlorum (Caspari) Saccardo (3)
F. merismoides Corda (7)
F. poae (Peck) Wollenweber (6)
F. reticulatum Montagne (1)
F. robustum Gerlach (1)
F. sambucinum Fuckel (12)
F. scirpi Lambotte & Fautrey (8)
F. semitectum Berkeley & Ravenel (2)
F. solani (Martius) Saccardo. (34)
F. sphaeriae Fuckel (2)
F. splendens Matuo & Kobayashi (3)
F. sporotrichioides Sherbakoff (7)
F. staphyleae Samuels & Rogerson (3)
F. stilboides Wollenweber (6)
F. striatum Sherbakoff (6)
F. sublunatum Reinking (4)
F. succisae (Schröter) Saccardo (2)
F. torulosum (Berkeley & Curtis) Nirenberg (8)
F. tumidum Sherbakoff (6)
F. venenatum Nirenberg (3)
F. verticillioides (Saccardo) Nirenberg (6)

[1] number of tested strains in brackets

To answer the question if the cultural condition of the isolates influenced the ability to produce moniliformin a X^2 independence test was carried out with the strains of the species in section *Liseola*. No significant correlation could be found between morphological appearance of the strains and their moniliformin production in culture.

In many cases the good correlation between species classification and moniliformin production demonstrated that an up-to-date polyphasic taxonomy which encompasses besides morphological and biomolecular also physiological and ecological data provides a solid basis in the determination of *Fusarium* species.

References

1 Cole RJ, Kirksey JW, Cutler HG, Doupnik BL, Peckham JC (1973) Toxin from *F. moniliforme*: effects on plants and animals. Science 179: 1324-1326

2 Scott PM, Abbas HK, Mirocha CJ, Lawrence GA, Weber D (1987) Formation of moniliformin by *Fusarium sporotrichioides* and *Fusarium culmorum*. Appl Environ Microbiol 53: 196-197

3 Chelkowski J, Zawadzki M, Zaikowski P, Logrieco A, Bottalico A, (1990) Moniliformin production by *Fusarium* species. Mycotoxin Res 6: 41-45

4 Marasas WFO, Thiel PG, Sydenham EW, Rabie CJ, Lübben A, Nelson PE (1991) Toxicity and moniliformin production by four recently described species of *Fusarium* and two uncertain taxa. Mycopathologia 113: 191-197

5 Cole RJ, Cox RH (1981) Handbook of toxic fungal metabolites: S. 894-897. Academic Press Inc., New York

6 Abbas HK, Mirocha CJ, Gunther R (1991) Production of zearalenone, nivalenol, moniliformin, and wortmannin from toxigenic cultures of *Fusarium* obtained from pasture soil samples collected in New Zealand. Mycotoxin Res 7: 53-60

7 Filtenborg O, Frisvad JC, Svendsen JA (1983) Simple screening method for molds producing mycotoxins in pure cultures. Appl Environ Microbiol 45: 581-585

8 Nirenberg HI (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Mitt Biol Bundesanst Land-Forstwirtsch, Berlin-Dahlem 169

9 Scott PM, Lawrence GA (1987) Liquid chromatographic determination and stability of the *Fusarium* mycotoxin moniliformin in cereal grains. J Assoc Off Anal Chem 70: 850-853

10 Kriek NPJ, Marasas WFO, Steyn PS, van Rensburg SJ, Steyn M (1977) Toxicity of a moniliformin-producing strain of *Fusarium moniliforme var. subglutinans* isolated from maize. Food Cosmet Toxicol 15: 579-587

11 Marasas WFO, Leistner L, Hofmann G, Eckhardt C (1979) Occurence of toxigenic strains of *Fusarium* in maize and barley in Germany. Europ J Appl Microbiol Biotechnol 7: 289-305

12 Rabie CJ, Marasas WFO, Thiel PG, Lübben A, Vleggaar R (1982) Moniliformin production and toxicity of different *Fusarium* species from southern Africa. Appl Environ Microbiol 43: 517-521

13 Marasas WFO, Thiel PG, Rabie CJ (1986) Moniliformin production in *Fusarium* section *Liseola*. Mycologia 78: 242-247

14 Schütt F, Nirenberg HI, Deml G (1998) Moniliformin production within the *Liseola-Dlaminia* complex. Accepted for publication by Cer Res Communic

15 Nirenberg HI and Aoki T (1997) *Fusarium nisikadoi*, a new species from Japan. Mycoscience 38: 329-333

16 Nirenberg HI, O'Donnell K, Kroschel J, Adrianaivo AP, Frank JM, Mubatanhema W (1998) Two new species of *Fusarium: Fusarium brevicatenulatum* from the noxious weed *Striga asiatica* in Madagascar and *Fusarium pseudoanthophilum* from Zea *mays* in Zimbabwe. Mycologia 90: 459-464
17 Nirenberg HI and O'Donnell K (1998) New *Fusarium* species and combinations within the *Gibberella fujikuroi* species complex. Mycologia 90: 434-458
18 Logrieco A, Bottalico A, Ricci V (1990) Occurence of *Fusarium* species and their mycotoxins in cereal grains from some Mediterranean countries. Phytopath Medit 29: 81-89
19 Hussein H, Baxter M, Andrew IG, Franich RA (1991) Mycotoxin production by *Fusarium* species isolated from New Zealand maize fields. Mycopathologia 113: 35-40
20 Rabie CJ, Luebben A, Louw AI, Rathbone EB, Steyn PS, Vleggaar R (1978) Moniliformin, a mycotoxin from *Fusarium fusarioides*. J Agric Fd Chem 26: 375-379
21 Bottalico A, Visconti A, Solfrizzo M (1982) Production of moniliformin by *Fusarium* species in Italy. Phytopath Medit 21: 105-106
22 Booth C (1971) The genus *Fusarium*. CMI, Kew
23 Gerlach W and Nirenberg HI (1982) The genus *Fusarium* – a pictoral atlas. Mitt Biol Bundesanst Land- Forstwirtsch, Berlin-Dahlem 209: 1-406
24 Nelson PE, Toussoun TA, Marasas WFO (1983) *Fusarium* species. An illustrated manual for identification. Pa. St. Univ. Press
25 Burgess LW, Lidell CM, Summerell BA (1988) Laboratory Manual for *Fusarium* Research. Univ. of Sydney
26 Nirenberg HI (1989) Identification of fusaria occuring in Europe on cereals and potatoes. In: Chelkowski J (Ed) *Fusarium*: Mycotoxins, Taxonomy and Pathogenicity. Elsvier Science Publishers BV, Amsterdam: 179-193
27 Nirenberg HI (1990) Recent advances in the taxonomy of *Fusarium*. Studies in Mycology 32: 91-101
28 Sharman M, Gilbert J, Chelkowski J (1991) A survey of the occurence of the mycotoxin moniliformin in cereal samples from sources worldwide. Fd Add Contam 8: 459-466
29 Adler A, Lew H, Brodacz W, Edinger W, Oberforster M (1995) Occurence of moniliformin, desoxynivalenol, and zearalenone in durum wheat (*Triticum durum* Desf.). Mycotoxin Res 11: 9-15

Manuscript received June 30, 1998; accepted July 15, 1998.