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Taxonomic Study of Fusaria of the Sporotrichiella Section Used in Recent Toxicological Work

A. Z. JOFFE* AND J. PALTI

Laboratory of Mycology and Mycotoxicology, Department of Botany, The Hebrew University of Jerusalem, Jerusalem, and Agricultural Research Organization, Bet Dagan, Israel

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Eight isolates of *Fusarium* of the Sporotrichiella section were critically studied as to their taxonomic position. Most of these isolates had been used for comprehensive toxicological work on mycotoxin effects on animals, chiefly in the United States and Japan. Isolates NRRL 3299 and NRRL 3287, supplied from the United States, were determined to be *Fusarium poae*. Isolates NRRL 3249, NRRL 5908, 2061-C, and YN-13 from the United States and isolate T-2 from South Africa belonged to *Fusarium sporotrichioides*. Isolate NRRL 3509 belonged to *F. sporotrichioides* var. *tricinctum*. The relevance of recent toxicological work with these isolates on animals to earlier work on alimentary toxic aleukia is discussed in light of the above identifications.

The taxonomy of *Fusarium* species of the Sporotrichiella section may once have appeared a subject of purely academic interest, but this no longer holds true. Ever since species of the section have been proved to cause serious disorders in humans who died after consuming overwintered grain (7, 9-12) and various diseases in animals (1, 2, 6, 17, 19, 22, 23, 25-27) the true identity of these species is of acute importance. Establishment of this identity, which is the aim of this paper, will enable us to relate, at least from a taxonomic angle, a large body of toxicological work carried out in recent years (mainly in the United States and Japan) to the earlier work carried out principally in the Soviet Union.

The taxonomic problem derives from the following situation. The great majority of taxonomists, ever since Wollenweber and Reinking (24), have recognized several distinct species in the Sporotrichiella section (3, 5, 14, 18). Moreover, Joffe (7, 9, 11-13) and Seemueller (20) have proved that these species differ in their toxicological properties as well, with what has generally been called *Fusarium tricinctum* producing lower numbers of isolates with strongly toxic properties and *Fusarium poae* and *Fusarium sporotrichioides* producing such isolates in large numbers.

However, in the United States Snyder and Hansen (21), for reasons never adequately explained, decided to bunch all the species in the section together under *F. tricinctum*. Since most of the toxicological work in recent years has been carried out by researchers not particularly interested in the taxonomic identity of the isolates they worked with, Snyder and Hansen's nomenclature was adopted uncritically. The result was a breakdown in communication, since no one was quite sure how these isolates related to those with which toxicological work had been carried out ever since the 1940s in the Soviet Union (7-12), England (19), and Israel (15, 16).

MATERIALS AND METHODS

Sources of isolates. For the purpose of this study, the isolates with which toxicological work was performed were kindly supplied by the colleagues and institutions listed in Table 1.

Culturing and identification. The spore suspensions obtained from lyophilized tubes, pure cultures, or isolates preserved in soil were added to plates containing potato dextrose agar (PDA), adjusted to pH 5.6 by the addition of lactic acid. The plates were incubated at 24 C for 3 to 5 days and then kept for further study at 5 C. After growth at 24 C and transfer from petri dishes to test tubes, monospore cultures were made on PDA from all cultures. For this purpose spore suspensions were made with 10 ml of sterile water and diluted to no more than one spore per drop. These spores were transferred by sterile pipette to petri dishes with PDA for incubation at 24 C. After another 1 to 2 days, by which time the slow-growing conidia should have appeared, they were examined under stereoscopic microscope. Subsequently, very small squares of the agar with a single germinating spore were transferred to PDA slants, incubated at 24 C for 5 to 6 days, and then kept at 5 C. The resulting colonies were identified on the basis of the cultural and morphological characteristics and behavior in culture.

Growth rates of *Fusarium* strains were determined

TABLE 1. *Isolates used*

Isolate no. and designation	Form	Supplied by
F. tricinctum NRRL 3249	Lyophilized	C. W. Hesseltine[a]
F. poae NRRL 3287	Lyophilized	C. W. Hesseltine
F. tricinctum NRRL 3299	Lyophilized	C. W. Hesseltine
F. tricinctum NRRL 3509	Lyophilized	C. W. Hesseltine
F. tricinctum NRRL 5908	PDA culture	H. R. Burmeister
F. tricinctum T-2	PDA culture	W. F. O. Marasas[b]
F. tricinctum 2061-C	Preserved in soil	C. J. Mirocha[c]
F. tricinctum YN-13	Preserved in soil	C. J. Mirocha

[a] Northern Regional Research Laboratory, U.S. Department of Agriculture, Peoria, Ill.
[b] Plant Protection Research Institute, Pretoria, South Africa.
[c] Department of Plant Pathology, St. Paul, Minn.

on PDA in petri dishes incubated at 24 C. The growth rate was expressed as the mean diameter (in centimeters) attained by six to eight monoconidial cultures in 5 days.

Toxicity tests on rabbit skin were performed and evaluated by methods outlined elsewhere (7, 11, 12, 15).

RESULTS

Characteristics of the section Sporotrichiella and its species. The basis for classification in the section Sporotrichiella Wr. emend. Joffe has recently been defined by Joffe (14) by the following: (i) shape of microconidia, whether lemon or pear shaped, globose, ellipsoid, or elongate, dispersed in aerial mycelium, or formed in false heads; (ii) relative frequency of micro- and macroconidia.

Macroconidia were sparse, small, oblong, narrowly fusoid to falcate, and pedicellate formed in aerial mycelium or in sporodochia. Chlamydospores were intercalary, terminal, in chains or knots, and occasionally had plectenchymatous sclerotia. Perithecial states were absent.

Isolates identified as F. poae (Peck) Wr. A detailed study of isolate NRRL 3299, originally isolated from toxic corn (1), yielded the following data.

(i) Cultural characteristics. Aerial mycelium was friable, felted, and assumed a powdery appearance as microconidia form; it was white, slightly rose, yellowish, or brown. The growth rate was 7.7 cm during a 5-day period.

(ii) Spores. Microconidia scattered in the mycelium, appeared white in mass, and were abundant, round to oval, or spherical with a basal papilla, ellipsoid or sometimes elongate, and rarely pear shaped. Macroconidia were sparse, falcate, and slightly curved and formed only in aerial mycelium, mostly 3-septate. Intercalary, hyaline, and mostly smooth chlamydospores appeared rarely, singly or in small chains and rarely in knots (Fig. 1). The measurements (in micrometers) of 50 conidia were in the following ranges: 0-septate, 5.6 to 10.4 by 3.5 to 7.6; 1-septate, 8.8 to 14.5 by 5.2 to 7.4; 3-septate, 20.5 to 33.0 by 4.0 to 5.6.

(iii) Toxicity. In rabbit skin tests isolate NRRL 3299 provoked extremely strong toxic reactions, i.e., severe leucocytic reaction and extensive necrosis.

The above characteristics match those of *F. poae* (Peck) Wr. Very similar characteristics, including extremely strong toxicity to rabbit skin, were determined for isolate NRRL 3287 from an unknown source, which had been sent to us correctly designated as *F. poae* (Hesseltine, personal communication); however, in the literature it had previously been referred to as *F. tricinctum*.

Isolates identified as F. sporotrichioides Sherb. A detailed study of isolate NRRL 3249, originally isolated from tall fescue (*Festuca arundinacea* Schreb.) in the United States (6, 22, 27), yielded the following results.

(i) Cultural characteristics. Aerial mycelium was whitish at first, later assuming various shades of yellow, rose, carmine-red, or light brown. The stroma and coloring of substrate on PDA were carmine-red, purple, or violet. The growth rate was 4 cm in 5 days.

(ii) Spores. Microconidia were hyaline, smooth walled, and highly heterogeneous and formed as lateral branches in the aerial mycelium; they were almost oval, pear shaped or spindle shaped to elliptical, oblong or slightly falcate, and scattered in the mycelium singly or in false heads. In a mass they appeared white or cream colored. Macroconidia developed from branched conidiophores formed in the aerial mycelium or in sporodochia; spindle-shaped, falcate, or curved, apedicellate or with a pedicellate basal cell, and tapering slightly at each end, with a small apical cell. The macroconidia were typically 3- and 5-septate and formed in amounts more or less equal to that of microco-

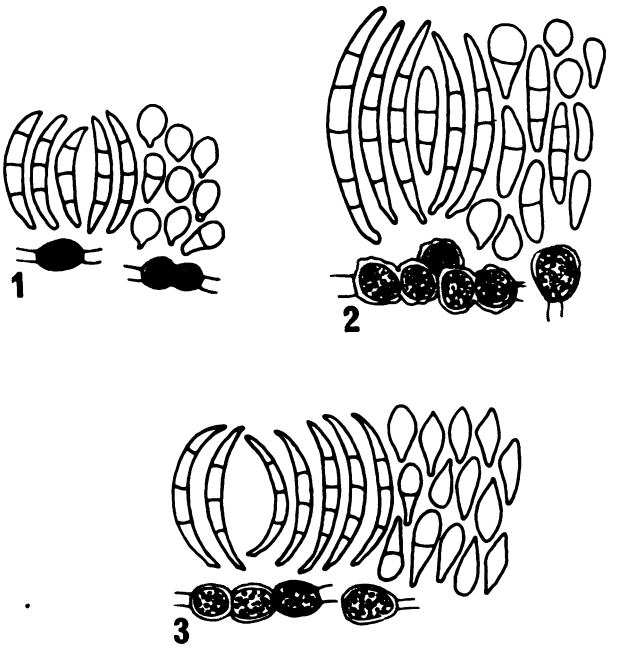

FIG. 1. *Micro- and macroconidia and chlamydospores of isolate NRRL 3299, identified as F. poae.* ×700.

FIG. 2. *Micro- and macroconidia and chlamydospores of isolate NRRL 3249, identified as F. sporotrichioides.* ×700.

FIG. 3. *Micro- and macroconidia and chlamydospores of isolate NRRL 3509, identified as F. sporotrichioides var. tricinctum.* ×700.

nidia in the aerial mycelium. Chlamydospores were intercalary, formed singly, in chains, or in knots, and were hyaline and later light brown (Fig. 2). The measurements (in micrometers) of 50 conidia were in the following ranges: 0-septate, 5.9 to 12 by 3.0 to 5.0; 1-septate, 10.2 to 18 by 3.8 to 5.5; 3-septate, 26 to 34 by 3.5 to 4.6; 4-septate, 31 to 37 by 3.8 to 4.8; 5-septate, 36 to 44 by 3.9 to 4.5.

(iii) Toxicity. In rabbit skin tests, isolate NRRL 3249 provoked strongly toxic reactions, i.e., oedema and leucocytic reaction with some necrosis.

The above characteristics match those postulated for *F. sporotrichioides* Sherb. Similar characteristics (without toxicity tests) were determined for three further isolates: the T-2 isolate suppplied from South Africa, originally isolated from *Zea mays* in France (Marasas, personal communication), which also provoked a strong reaction on rabbit skin; isolate NRRL 5908, originating from fescue hay (1); and isolate 2061-C, originating from corn cobs in the United States, both examined morphologically only. These four isolates can therefore be positively identified as *F. sporotrichioides* Sherb.

A fifth isolate, designated YN-13, exhibited cultural characteristics similar to the above and formed abundant microconidia corresponding to those described here. However, only occasional macroconidia were formed by this isolate. Nevertheless, isolate YN-13 may be assigned to *F. sporotrichioides*.

Isolates identified as F. sporotrichioides var. tricinctum (Corda) Raillo (syn. F. trincinctum (Corda) Sacc.). A detailed study was made of isolate NRRL 3509, the source of which is unknown to us.

(i) Cultural characteristics. Aerial mycelium was sparse to abundant and colored intensive carmine-rose to purple-red, occasionally whitish or ochre colored. Stroma were carmine or purple. The growth rate was 2.9 cm in 5 days.

(ii) Spores. Microconidia were 3- to 5-septate and scattered in the aerial mycelium; they were lemon-shaped, oval to ellipsoid, or pear shaped and somewhat more abundant than macroconidia. Macroconidia, less abundant, formed usually in sporodochia, but also formed in the aerial mycelium; they were falcate and more strongly curved than those of *F. sporotrichioides*, with a well-marked foot cell. Chlamydospores were in hyphae, intercalary, singly or in chains, brownish, and smooth-walled; they were occasionally found in macroconidia (Fig. 3). Measurements (in micrometers) of 50 conidia

of strain NRRL 3509 were in the following ranges: 0-septate, 7.0 to 10.5 by 2.5 to 6.2; 1-septate, 9.0 to 17.5 by 2.3 to 3.9; 3-septate, 21 to 36 by 3.2 to 3.8.

(iii) Toxicity. In rabbit skin tests, isolate NRRL 3509 provoked a moderately toxic reaction, i.e., moderately strong oedema, with some leucocytic reaction but no necrosis.

According to all these characteristics this isolate belongs to what Wollenweber and Reinking (24) and Gerlach (3) termed *F. tricinctum* (Corda) Sacc. and what, for morphological reasons explained elsewhere (14), we consider as the subspecies *F. sporotrichioides* var. *tricinctum*.

DISCUSSION

In many recent studies of the toxic effects of fungi of the Sporotrichiella section on animals, reference has been made to the toxicosis caused in humans by the fungi in the Soviet Union, and termed alimentary toxic aleukia (ATA). Attempts have been made to relate the symptoms observed on poultry (25, 26), cattle (27), and various other animals (23) to published descriptions of ATA. Such comparisons are, in our view, premature at the present stage of knowledge. The properties of isolates made from overwintered grain in the Soviet Union, which caused ATA in humans ingesting such cereals, cannot properly be related to the properties of isolates causing toxic symptoms in animals in other countries, until and unless isolates have been grown and tested under very similar conditions. The limited aim of the present study was to elucidate the true taxonomic identity of isolates used recently in toxicological studies on animals. This is to be considered as only the first step in determining relationships between these isolates and those found to cause ATA.

In the following sections, symptoms produced in man and animals by fusaria of the Sporotrichiella section have been grouped according to what have now been found to be their proper species.

F. poae. In studies of ATA in the Soviet Union (7, 8, 11, 12), *F. poae* has been outstanding in its toxicity; out of 68 isolates from overwintered grain, 46 were strongly toxic, two were mildly toxic, and only two were nontoxic. American isolates of this species can now be considered responsible for toxic symptoms or death in the following cases. Injection with isolate NRRL 3299 induced abscesses and hemorrhages, lung congestion, and other symptoms in cattle (6). Ueno et al. (23) worked with isolate NRRL 3299, which they termed *F. tricinctum*, and with isolate NRRL 3287. Both isolates caused damage to the thymus and to lymph nodes, extremely severe damage to the small intestine, and eventual death in mice. Oral lesions and neural disturbances followed ingestion of isolate NRRL 3299 by chickens (25, 26).

F. sporotrichioides. In investigations of ATA (7, 8, 11, 12) this species proved only very slightly less toxic than *F. poae*; out of 65 isolates from overwintered grain, 44 were strongly toxic and four were nontoxic. The toxic action of American isolates of this species can now be related to the symptoms reported in the following cases. Gangrene and arched back in cattle were caused by an injection of isolate NRRL 3249 (6). Pathological changes in the tail, internal hemorrhages, and death ensued when isolate NRRL 3249 was fed to cattle (22). Ueno et al. (23) conducted their investigations using mice and rats with isolate NRRL 3249, which they referred to as *F. tricinctum*, and with NRRL 3510, an isolate grown in our laboratory in Israel but originating from overwintered grain in the Soviet Union. The isolates were injected intraperitoneally into mice and differed considerably in their effects: NRRL 3510 caused severe damage to the thymus, spleen, small intestine, and bone marrow, leading to death, whereas NRRL 3249 caused only limited damage to the above organs and did not cause death. Marasas et al. (17) induced stunted growth of rats and inflammation of their nose and mouth by feeding them the T-2 strain of this species and induced skin necrosis by topical application. On mice, butenolide from NRRL 3249 had a mean lethal dose of 43.60 mg/kg (body weight) by intraperitoneal injection and an oral toxicity of 275 mg/kg (body weight) (27). Toxicosis of turkey poults, with necrotic lesions in the mouth and eventual death, resulted from feeding on isolate 2061-C (2). Temporary damage to the intestinal tract of trout was induced by feeding on the R-2 strain (17).

F. sporotrichioides var. tricinctum. In the ATA studies carried out in the Soviet Union (8, 12), 27 isolates of this variety were found to comprise only two toxic isolates and one mildly toxic isolate, whereas 24 were nontoxic. We are not aware of published studies of the toxicity to animals of isolate NRRL 3509, the only one among the isolates studied here that belonged to this variety in the section Sporotrichiella.

Significance of the taxonomic identity of toxigenic fungi. The taxonomic investigation reported here was aimed at complementing research into the composition of toxins formed by various species of *Fusarium*. Contrary to the opinion expressed by some toxicologists, it is

not sufficient to classify isolates solely by the nature of the toxins they produce. Since the conditions under which toxins are formed vary markedly according to species and variety (7, 8, 10–12, 15), the quantities of toxic material produced under various sets of conditions differ widely. This is of obvious significance wherever practical aspects of the danger of toxigenesis have to be considered. It is therefore most desirable that in all future toxicological work the fungal isolate involved should undergo careful identification.

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