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FUNGI THAT PRODUCE MYCOTOXINS: CONDITIONS AND OCCURRENCE

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Abstract

The occurrence of mycotoxins, in agricultural commodities is a worldwide problem with almost all commodities being potentially susceptible to contamination under the proper conditions. The genera of fungi most implicated are *Aspergillus*, *Penicillium* and *Fusarium*, although the potential for toxin production varies considerably within any given species.

Conditions that affect toxin production include fungal strain variation, genetic susceptibility of the host plant or commodity, moisture content, commodity composition, temperature, aeration, microbial population and stress factors. There is undoubtedly interaction between these factors so that laboratory studies involving limited variables can only, at best, approximate field conditions.

Natural contamination with mycotoxins has been reported for most of the major agricultural commodities in the world including corn, wheat, rice, millet, barley, oats, sorghum, peanuts, beans, copra, some fruits and nuts and various forages; strangely, soybeans do not appear to be involved to any major extent. The major mycotoxins on commodities reported to date include aflatoxin, the trichothecenes, ochratoxin, citrinin, zearalenone, sporidesmins and some tremorgens. However, laboratory studies have shown that the fungi are capable of producing hundreds of toxic chemicals, most of which are not included in routine analyses. In addition, since toxin effects are often insidious and may go undetected, the true dimensions of the mycotoxin problem are unknown.

The presence of mycotoxins in agricultural commodities and ensuing mycotoxicoses represent an extremely complex series of interactions between the causative fungi, the contaminated product, physicochemical environmental factors and the intoxicated host. The various components involved are still being unravelled but, at best, will probably never be fully understood since laboratory studies, where interacting variables are of necessity limited, can only approximate the infinite number of variables manifested in nature, e.g., the chemical composition of the commodity, the complex interactions of the microbial flora, oxygen availability, presence or absence of herbicides and pesticides, insects, irrigation, weather conditions, age, sex, health of the host, host species, presence of multiple toxins and synergism between toxins.

There is even some uncertainty as to which fungal metabolites can be considered to be mycotoxins. For simplicity of discussion, any mold metabolite, regardless of concentration, that results in an undesirable effect in a biological host will be considered to be a mycotoxin. Hence, the range of fungi that can be implicated becomes almost limitless. But again, for simplicity, we will consider only those fungi and substances indicated in mammalian mycotoxicoses.

Table 1. Mycotoxins currently of most concern

Mycotoxin	Producing fungi
Aflatoxins B_1, G_1, M_1	*Aspergillus flavus* *A. parasiticus*
Ochratoxin	*Penicillium viridicatum* *A. ochraceus* series
Citrinin	*P. viridicatum* *P. citrinin*
Patulin	*P. urticae* *P. expansum*
T-2 toxin	*Fusarium tricinctum*
Vomitoxin	*F. graminearum*
Monoacetoxyscirpenol	*F. roseum*
Diacetoxyscirpenol	*F. roseum*
Zearalenone	*F. roseum*
Sporidesmins	*Pithomyces chartarum*
Penitrem A	*P. palitans* *P. crustosum*

Table 1 shows those toxins and their producing fungi currently of most concern or interest to investigators in the field.

Table 2 indicates those substances and fungi that could be of potential concern.

Factors that influence mold growth on a commodity also influence toxin production; the most important of these are moisture, type of substrate and temperature, in that sequence. This may be somewhat of an oversimplification, because developing kernels or seeds in the field possess very high moisture levels at times, yet they are not necessarily susceptible to fungal growth.

Based on moisture requirements, various investigators have divided the fungi into two groups: field molds that require a moisture content of 22–25 % and storage fungi that grow between 13–18 % moisture. This is somewhat of a simplification for there are fungi that overlap the two categories. Table 3 shows moisture requirements for growth on cereals of some of the mycotoxin-producing fungi.

Table 2. Mycotoxins of potential concern

Alternariol	*Alternaria alternata* *A. tenuis*
Aspergillic acid	*A. flavus*
Aspertoxin	*A. flavus*
Byssochlamic acid	*Byssochlamys fulva*
Citreoviridin	*P. citreoviride*
Citromycetin	*P. frequentans*
Cyclopiazonic acid	*P. cyclopium* *A. tenuis*
Fumigatoxin	*A. fumigatus*
Fusaranon	*F. nivale*
Islanditoxin	*P. islandicum*
Kojic acid	*A. flavus* *A. glaucus*
Luteoskyrin	*P. islandicum*
Maltoryzine	*A. oryzae*
Nivalenol	*F. nivale*
Penicillic acid	*P. martensii* *P. cyclopium* *P. palitans* *A. ochraceus* group
Psoralens	*Sclerotinia sclerotiorum*
Roridans	*Stachybotrys atra*
Rubratoxin B	*P. rubrum*
Secalonic acid D	*P. oxalicum* *P. aculeatus*
Secalonic acid F	*P. aculeatus*
Sterigmatocystin	*A. versicolor* *A. nidulans*
Verrucarins	*Stachybotrys atra* *Myrothecium roridum*
Viriditoxin	*A. viridi-nutens*

Table 3. Moisture content (MC) requirement for growth of some mycotoxin-producing fungi

MC (%)	Fungi	Toxins produced
10.0-17.0	*Aspergillus ochraceus*	Ochratoxin
15.6-21.0	*Penicillum palitans* *P. oxalicum* *P. viridicatum* *P. cyclopium* *P. citrinum* *A. versicolor*	Tremorgens Secalonic acid Citrinin, ochratoxin Penicillic acid Citrinin Sterigmatocystin
18.0-20.0	*A. flavus* *A. parasiticus*	Aflatoxin Aflatoxin
22-33	*Fusarium roseum* *F. tricinctum* *Alternaria* spp. *F. moniliforme*	Vomitoxin, F-2 T-2 Alternariol, tenuazonic acid (?)

With respect to mycotoxins, the *Fusaria* appear to be the most important of the field fungi; however, recent evidence shows that *Aspergillus flavus* can occur in corn (12, 26) as well as in cottonseed in the field (15). *Alternaria* species also are often present, but their role as important mycotoxin producers has yet to be determined. *Helminthosporium graminearum* can cause major crop destruction in the field, but evidence from our laboratory and others indicates that it is not an important mycotoxin producer (2).

In stored grain at moisture levels below 15 % *Aspergillus glaucus* is dominant, but mycotoxin synthesis has not been attributed to this group. Above 15 % moisture those fungi primarily involved in mycotoxin synthesis begin to develop, particularly the Aspergilli and Penicillia. Above 25 % moisture the Fusaria become of major concern.

The importance of substrate as a governing factor in secondary metabolite synthesis is well known to those versed in fermentation. The cereal grains in general are good substrates for toxin production, whereas those seeds high in protein, e.g., soybeans and peanuts, appear to support production of certain toxins but not of others. Again, one must be careful in generalizing because soybeans, normally a poor substrate for aflatoxin production, can become an adequate medium if the zinc that is tied up by phytic acid is released or supplemented with additional zinc. (6). Unknown factors may also be involved, e.g., although vomitoxin, a trichothecene, is produced by Fusaria on corn in the field and in the laboratory, two additional toxins present in fieldcontaminated samples are not detected in laboratory-inoculated corn (23).

Substrate may also play a role in selecting for or against toxin producing strains of a given species, e.g., there is a higher proportion of toxin-producing strains of *A. flavus* isolated from peanuts and cottonseed than from rice or sorghum. We have found that strains of ochratoxin and citrinin-producing *P. viridicatum* isolated from meat were more unstable than those isolated from grain and rapidly lost toxin-producing ability. It would be interesting to grow cultures isolated from grain on meat to determine if the substrate contributes to genetic instability. A converse experiment might also yield useful information on selective factors.

Molds can grow and produce mycotoxins under a wide temperature range with optima generally between 20 to 30 °C. However, temperatures optimum for toxin production need not correspond to those optimum for growth: *Fusarium tricinctum* grows well at 25 °C but produces T-2 toxin best near freezing temperatures. *Penicillium martensii* produces penicillic acid rapidly at 20–30 °C, but considerably more toxin eventually accumulates between 4 to 10 °C. Toxin destruction or binding to substrate during the production cycle as affected by temperature is a factor that has not been studied in any detail and warrants further investigation.

Cereal grains, peanuts, cottonseed and some forages appear to be the most important food and feed substances that may be contaminated with mycotoxins. However, probably no edible substance can be regarded as absolutely safe from possible mycotoxin contamination. Mycotoxin production can occur in the field or during harvest, processing, storage and shipment of commodities during the feeding period on the farm or from inadequate storage at home after a cooking process. The extent of the problem is difficult to define, for the occurrence of any given toxin can vary from year to year depending to a considerable extent on weather conditions and biological and technological factors. There appears to be some uninformed sentiment, perhaps indicative of the cynicism of the times, that the problem has been magnified beyond reality. I believe sufficient data is now available to refute this. The natural occurrence of mycotoxins in cereals and in food and feeds has recently been reviewed by Hesseltine (7) and by Stoloff (19).

Most of the literature prior to the 1960's is concerned with incidents involving animals which consumed moldy feeds with ensuing obvious and dramatic results; however, human involvement had also been noted: Yellow rice disease in Japan involved primarily humans; stachybotryotoxicosis and scabby grain in Russia intoxicated humans and animals; moldy corn toxicosis and moldy feed toxicosis (hemorrhagic syndrome) in the United States affected cattle, swine and poultry; facial eczema, a photosensitization disease, affected ruminants in New Zealand; hemorrhagic sweet clover poisoning of cattle in the United States was associated with conversion of coumarin to dicoumarol by fungi.

In the 1960's mycotoxin research received major impetus with the discovery of the aflatoxins, a family of closely related compounds indicated in toxicoses of poultry, swine, cattle and trout. Research subsequently expanded to include a large number of other toxins, particularly those produced by species in the genera *Aspergillus*, *Penicillium* and *Fusarium*. The major toxins attracting interest, in addition to the aflatoxins, include the 12, 13-epoxytrichothecenes (a family of 37 naturally occurring substances), zearalenone, ochratoxin, citrinin, patulin, penicillic acid, the sporidesmins and the tremorgenic mycotoxins.

Of these toxins, aflatoxin has commanded the lion's

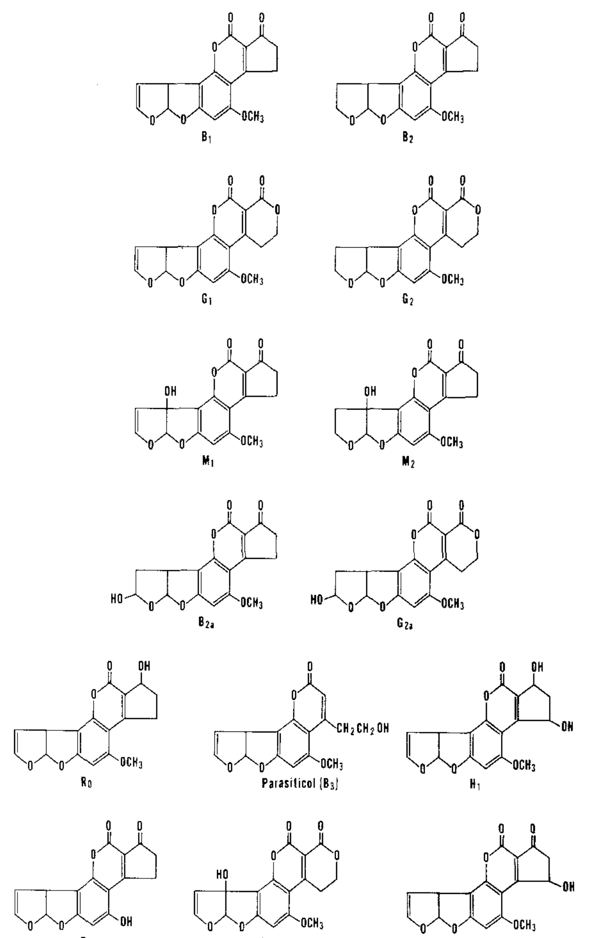


Fig. 1. Structures of the naturally occurring aflatoxins.

Table 4. Natural occurrence of aflatoxins in foods and feeds

Commodity or product		
Corn	Pecans	Noodles
Wheat	Walnuts	Bread
Rice	Filberts	Wheat flour
Millet	Beans	Wheat grits
Sorghum	Figs	Wheat meal
Barley	Capsicum pepper	Rye meal
Oats	Wine	Rice cake
Copra	Milk	Peanut cake
Palm kernels	Dried milk	Peanut meal
Peanuts	Evaporated milk	Coconut oil meal
Cottonseed	Cheeses	Cottonseed meal
Pistachio	Dried fish	Mixed feeds
Brazil nuts	Garlic	Soy sauce
Almonds	Spaghetti	

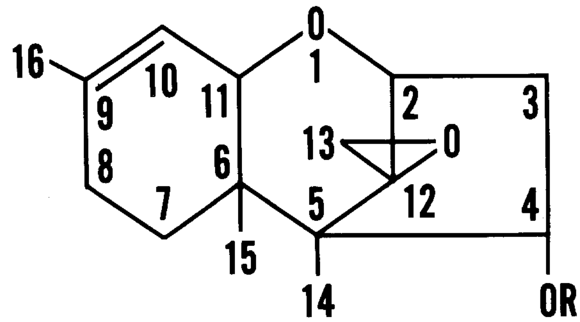


Fig. 2. Basic ring structures of trichothecene and 12, 13-epoxytrichothecenes.

share of attention for it constitutes a worldwide problem. Of the 13 naturally occurring aflatoxins (Fig. 1), aflatoxins B_1, G_1, and M_1 are the most important with respect to contamination of foods and feeds. Only *A. flavus* and *A. parasiticus* have been definitively shown to produce the aflatoxins although numerous production claims have been made for other fungi. Representatives of almost all of the major agricultural commodities, with the possible exceptions of soybeans, have been found to be naturally contaminated. The incidence and level of aflatoxin in commodities varies with numerous factors, many ill-defined, including country, time of year, agricultural practices, crop types and varieties, weather, storage and shipping conditions, hence, cannot be readily summarized. In general, commodities grown in warm and humid areas where high levels of insect damage and poor farming and storage practices prevail appear to be the most susceptible. Table 4 shows those commodities reported to contain aflatoxin as a result of natural contamination.

Previously thought to be primarily a storage contamination problem, the comprehensive studies of Lillehoj and his colleagues have revealed that aflatoxin contamination of corn can start in the field prior to harvest (12, 26); similar findings have been reported by Marsh et al. for cottonseed (13). Some selected data for the incidence of aflatoxin in corn is shown in Table 5. For additional quantitative data, the excellent reviews of Hesseltine (7) and Stoloff (19) should be consulted.

Other toxins of considerable import with respect to natural occurrence and toxicity to man and animals are those possessing the 12, 13-epoxytrichothecene ring structure (Fig. 2). These compounds affect the blood-producing centers and cause severe hemorrhaging, vesicant effects, vomiting and, in the case of swine, refusal of the feed. A number of fungal genera and species are capable of producing the trichothecenes, but from the viewpoint of food and feed contamination various *Fusaria* and *Stachybotrys atra* are the most important. Commodities affected include corn, millet, wheat, oats, possibly other cereal grains and hay. Unfortunately, lack of adequate analytical methods has prevented extensive surveys for these toxins.

Until comparatively recently, considerable confusion was associated with *Fusarium*- and *Stachybotrys*-contaminated commodities because the mycotoxicoses and associated toxins could not be correlated. In stachybotryotoxicosis, a mycotoxicosis of horses, calves, sheep and swine caused by ingestion of feed contaminated with *S. atra*, Eppley & Bailey (5) isolated five 12, 13-epoxytrichothecenes that are probably responsible for the disease. In alimentary toxic aleukia, a severe hemorrhagic disease of humans resulting from consumption of overwintered cereals molded with *F. poae* and *F. sporotriciodes*, the situation was particularly confused because of Russian reports implicating four steroid-type substances in the disease. These substances could not be isolated by other investigators, but Israel researchers have now definitively shown the trichothecenes to be the causative agents. Even more confusing has been the phenomena of feed refusal

Table 5. Aflatoxin occurrence in corn in some countries

Country	Year	Number of samples	Per cent frequency of aflatoxin	Toxin average or range ppb
USA	1964,65,67	1594	2.5*	9
USA	1968-69	293**	2.7	18
USA	1969-70	60***	35.0	66
USA	1973	297****	51.5	3-500
USA	1973-74	2866	8.2	5 and higher
Thailand	1972	62	35	265
Uganda	1971	48	40	133
Philippines	1969	98	51	110
Dominican Rep.	1974	24	83	229

* Most contaminated samples were in the lowest grade
** Corn samples were taken from export channels
*** Samples were from the Southern U.S.
**** Samples were from a specific area of Southeastern U.S.

Table 6. Reported mycotoxicoses probably involving trichothecenes

Fungus	Source	Host	Symptoms	Country
Fusarium roseum, *F. poae*, *F. nivales*, others	Wheat, oats, barley, corn	Man, horses, sheep, pigs, dogs	Vomiting, diarrhea	Japan, Korea
F. moniliforme, *F. tricinctum* *F. poae*	Oats, hay, feeds	Horses, sheep, cows, chickens, pigs	Anorexia, growth loss, respiratory, gastric	Sweden
F. tricinctum	Corn	Cows	Haemorrhage, death	USA
F. roseum	Corn, mixed feed	Pigs, dogs	Feed refusal, vomiting	USA
F. poae	Cereals	Man	Haemorrhage, death	Russia
Stachybotrys atra	Hay	Horses, man	Haemorrhage, death, skin vessicant	Russia

or vomiting in swine fed corn contaminated with *F. graminearum*. A variety of trichothecenes were implicated, but Vesonder et al. (22, 23) have indicated that one trichothecene, vomitoxin, was probably responsible for both phenomena. Table 6 gives examples of mycotoxicoses probably attributable to the trichothecenes.

In addition to the trichothecenes, *F. graminearum*, *F. tricintum*, *F. oxysporium*, *F. culmorum* and *F. moniliforme* can produce an estrogenic metabolite zearalenone (F-2) (Fig. 3) that effects swine. Corn and barley appear to be the major crops contaminated in the United States as well as in various European countries (14). Considerable research remains to be done on factors affecting production of zearalenone in the field although low temperatures are known to favor accumulation of this toxin.

Ochratoxin A (Fig. 4), a nephrotoxin, is produced by various members of the *A. ochraceous* group as well as by *P. viridicatum*. This latter organism appears to be responsible for the nephroses noted in Danish pigs eating contaminated barley (8). Ochratoxin has been found in wheat,

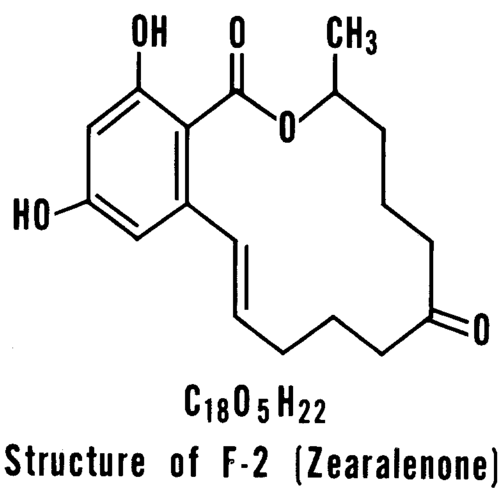


Fig. 3. Structure of F-2 (zearalenone).

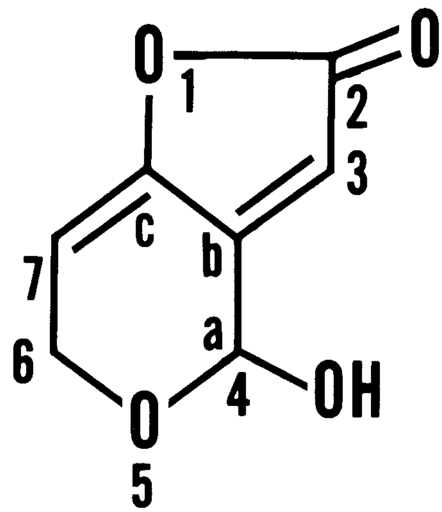


Fig. 4. Formulae of ochratoxin A and citrinin.

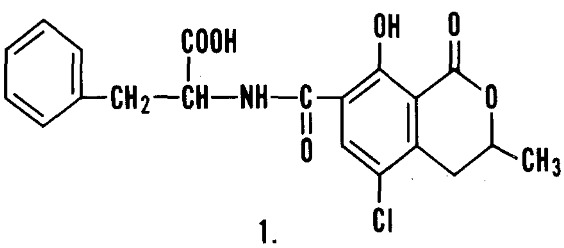
barley, oats, rye and mixed feeds in Canada (17); in corn and barley in the United States (18) and more recently in grains in Sweden (9) and Japan (20). *P. viridicatum* can also produce citrinin (Fig. 4), another nephrotoxin, so that cereals contaminated with ochratoxin may also contain citrinin. This raises the possibility of mycotoxin synergism, but this topic is outside the scope of this paper and has recently been reviewed by Lillehoj & Ciegler (1976) (11).

Tremorgens

Although more than 10 tremorgenic mycotoxins have been described in the literature only one of them, penitrem A, appears to have been definitely involved in mycotoxicoses. Penitrem A production is confined among the Penicillia to several species in the subsection Fasiculata, section Asymetrica, and includes *P. cyclopium*, *P. palitans*, *P. crustosum*, and *P. puberulum*. Wilson et al. (25) isolated *P. cyclopium* as the principal contaminant of feedstuffs causing disease outbreaks among sheep and horses in Tennessee. We isolated *P. palitans* from corn probably responsible for the deaths of dairy cows in southern Illinois. Some of the species that produce penitrem A are common contaminants of grains or specific feedstuffs. *P. crustosum*, a good tremorgen producer, is a contaminant of various refrigerated foods, grains and cereal products and causes a soft brown rot in apples. Whether or not tremorgen is produced during the apple rotting process is not known. The above-mentioned species have been isolated from moldy commercial feedstuffs, but no surveys have been conducted for the occurrence of this class of mycotoxins in agricultural commodities.

Patulin

This toxin (Fig. 5), once believed to have therapeutic activity against the common cold, has been implicated as a potential mycotoxin. *P. urticae (P. patulum)* was isolated from dry malt feed believed to have caused the deaths of over 100 cows in Japan (21). More recently it has been found in commercial apple juice in the United States, Canada and Europe (16, 24). Patulin contamination originates from apples contaminated with *P. expansum* rather than from subsequent contamination of the cider per se. A number of fungi can synthesize this mycotoxin (Table 7).



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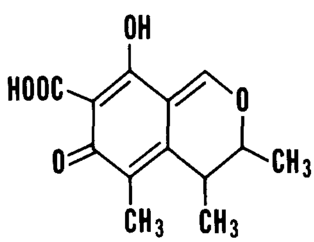

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Fig. 5. Structure of patulin.

Table 7. Molds producing Patulin

P. urticae (P. patulum)	*A. terreus*
P. expansum	*P. melinii*
P. granulatum	*P. cyclopium*
P. lanosum	*P. lapidosium*
P. claviforme	*P. equinum*
A. clavatus	*Byssochlamys nivea*
A. giganteus	

Slaframine

Second cutting red clover hay infected with *Rhizoctonia leguminicola* has been responsible for reduced milk yields and body weight in cows in the Midwest. The responsible mycotoxin, slaframine or slobber factor, elicits a dramatic effect of excess salivation (1). This mycotoxin problem was solved by the simple expedient of not growing red clover. A variety of other mycotoxicoses has been associated with contaminated forages. In New Zealand the sporidesmins produced by *Pithomyces chartarum* on grasses results in liver damage in sheep and cattle and a secondary effect of facial eczema (10).

Tremors and paralysis have been noted in large numbers of cattle grazing on Bermuda grass in some of our southern states, particularly in Louisiana. *Clavicep* species capable of producing alkaloids have been isolated from toxic batches of hay but have not been definitively implicated in the disease per se (3).

Another intriguing disease associated with hay is fescue foot, a lameness in cattle resulting from feeding on toxic tall fescue *(Festuca arundinacea)*. Although the disease has all the manifestations of a mycotoxicosis, neither the organism nor the potentially responsible toxin have been isolated to date (4).

In summary, it is apparent from this brief review that numerous fungi are capable of growing under a wide variety of conditions, on a broad array of substrates, with concomitant production of mycotoxins. The primary factors involved include the type of substrate, moisture level, aerobic conditions, temperature, plant variety and fungal strain.

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