Factors Affecting Production of the Mycotoxin F-2 by Fusarium roseum

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ABSTRACT

All 43 isolates of *Fusarium roseum* that were tested, chiefly from corn stored on the cob in cribs on farms in Minnesota, produced some F-2, and seven were rated as high producers. No isolate of any of the other eight species of *Fusarium* tested produced detectable amounts of F-2 [6-(10-hydroxy-6-oxo-trans-1-undecenyl)-β-resorcylic acid lactone]. After preliminary incubation for 2 weeks at 22-25 C, more F-2 was produced with continued incubation at 15 C than at other temp tested. In polished rice as a substrate, more F-2 was produced at a moisture content of 60-65%, and in corn at a moisture content of 45%, w/w basis, before autoclaving, than at other moisture contents tested. A larger amount of F-2 was produced on polished rice than on any other substrate tested, followed by corn, then wheat; very small amounts of F-2 were produced on oats or barley, and none on soybeans or peas. No more than trace amounts of F-2 were produced on any of the liquid media tested. When *F. roseum* and each of a number of other microflora were inoculated onto autoclaved moist grain at the same time, little or no F-2 was produced, and when the competing organisms were inoculated after *F. roseum* had grown for 1 week, F-2 production was lower than in the controls, but still moderately high. Single-ascospore lines of the two mass isolates that were highest in F-2 production differed greatly in the amount of F-2 they produced. Phytopathology 60:1055-1057.

Fusarium roseum (Lk.) Snyd. & Hans., the imperfect stage of *Gibberella zeae* (Schw.) Petch, under suitable circumstances produces a compound variously known as F-2, RAL, FES, or Zearalenone. The compound is of interest because of its physiological activities when administered to or consumed by animals. The compound may be present in corn invaded by *F. roseum*, or in feed made from such corn, and when consumed by swine may produce the estrogenic syndrome. Administered to beef steers and to sheep, the compound may result in increased feed efficiency and increased weight gain. The nature and effects of this compound have been investigated by Stob et al. (3), by Urry et al. (4), and by Mirocha et al. (1, 2) and others. The major factors affecting production of this compound have not, so far as we know, been investigated in detail, and the work here described was undertaken to do this.

MATERIALS AND METHODS.—*Source of mass isolates, and production and extraction of F-2.*—The following isolates were tested for production of F-2; 43 of *F. roseum*, 42 from corn on the cob collected from cribs in Minnesota and South Dakota or from feeds that contained ground corn, and 1 from wheat; 12 of *F. tricinctum* (Cda.) Snyd. & Hans. and 5 of *F. moniliforme* (Sheldon) Snyd. & Hans., also from corn; 2 of *F. nivale* (Fr.) Ces. Snyd. & Hans., 2 of *F. oxysporum* (Sheldon) Snyd. & Hans., and one each of *F. solani* (Mart.) Snyd. & Hans., *F. episphaeria* (Tode) Snyd. & Hans., *F. rigidiusculum* (Br.) Snyd. & Hans., and *F. lateritium* (Nees) Snyd. & Hans. obtained from other workers. For production of F-2, 0.1 g of soil containing the fungus (stock cultures were kept in soil) was added to 100 g of moist autoclaved polished rice in a quart bottle, after which the bottle was shaken to distribute the inoculum and, unless otherwise stated, was incubated for 2 weeks at 22-25 C, followed by 8 weeks at 8-12 C. After incubation, the fungus-invaded grain was removed, dried for 24 hr, ground in a Stein mill, the moisture content adjusted to about 25%, w/w basis, and the F-2 extracted and the amount determined according to the methods described by Mirocha et al. (1).

Effect of substrate, environmental conditions, and mixed cultures upon production of F-2.—The mass isolate selected as being the highest producer of F-2 was grown on various solid and liquid substrates to determine the one or ones most favorable to the production of F-2. The solid substrates tested were rice (*Oryzae sativa* L.) (both rough rice, the grains of which are not divested of the hulls, and polished rice); corn, *Zea mays* L.; wheat, *Triticum aestivum* L.; barley, *Hordeum vulgare* L.; oats, *Avena sativa* L.; soybeans, *Glycine max* (L.) Merr.; and peas, *Pisum sativum* L. Liquid media included Czapek-Dox, Saboraud's, and potato-dextrose broths—both the standard formulations (Difco) and these plus additions of 1% peptone, 1% yeast extract, and 1% corn steep solids, alone and in combination.

To determine the effect of moisture content, temp, and time on production of F-2, the same isolate was grown on either autoclaved moist corn or on autoclaved polished rice, since the highest yields of F-2 were consistently obtained on these substrates. Different moisture contents in the corn or rice can be only approximate, since some water was lost in autoclaving, and, if the fungus grew vigorously during incubation, it produced metabolic water and increased the moisture content of the substrate.

To determine the effect of mixed cultures on production of F-2, autoclaved moist corn was (i) inoculated with *F. roseum* and with each of several other microflora at the same time, then incubated for 2 weeks at 22-25 C, followed by 8 weeks at 8-12 C, or (ii)

inoculated with *F. roseum,* incubated for a week at 22-25 C, then inoculated with each of the other organisms and kept for an additional week at 22-25 C before transfer to 8-12 C for additional incubation.

Production of F-2 by single ascospore lines.—The isolate that produced the greatest amount of F-2 commonly formed mature perithecia in culture. A suspension of ascospores was poured onto the surface of clear agar in petri dishes, the excess water allowed to evaporate and, after about 24 hr, isolated germinating ascospores were transferred to sterile soil in perfume bottles. For production of F-2 these lines were grown on autoclaved polished rice having 45% moisture, w/w basis, before autoclaving, for 2 weeks at 22-25 C and 8 weeks at 8-12 C.

Results and Discussion.—All of the 43 isolates of *F. roseum* tested produced some F-2, the amount ranging from 90 to 1,900 ppm of the dry wt of the substrate. Seven isolates of *F. roseum* produced more than 1,000 ppm of F-2, and were rated as high producers. These came from three locations in Minnesota and one in South Dakota. Isolate No. 1, from corn from Mapleton, Minnesota, was the highest producer (1,900 ppm), and, according to Duncan's multiple range test, it exceeded the next highest producer, 1,420 ppm by isolate No. 2 from corn from Yankton, South Dakota, by an amount significant at the 1% level. No isolate of any other species of *Fusarium* tested produced a detectable amount of F-2, but the small number of isolates tested precludes any generalization as to the likelihood of occurrence of isolates that might produce F-2.

The largest amount of F-2 was produced on parboiled polished rice, followed by corn, then wheat (Table 1). Much lower amounts of F-2 were produced on rough rice and on barley, and still less on oats. No F-2 was produced on either soybeans or peas, although the fungus grew at least moderately well on them. No more than trace amounts of F-2 were found in any of the liquid media.

The amounts of F-2 produced by isolates No. 1 and 3 of *F. roseum* after incubation for 2 weeks at 22-25 C, followed by 8 weeks at various temp, are shown in Fig. 1-A. Both isolates produced considerably more F-2 at 15 C than at any of the other temp tested. Several isolates were grown at a constant temp of 24-27 C, and produced only small amounts of F-2 or none at all.

Table 1. Amount of F-2 mycotoxin produced by *Fusarium roseum* isolate No. 1 on different solid substrates after incubation for 2 weeks at 24-27 C followed by 8 weeks at 12 C

Substrate	μg F-2/g dry wt of substrate[a]
Parboiled polished rice	3,087
Corn	2,585
Wheat	2,485
Rough rice	1,053
Oats	363
Barley	993
Soybeans	0
Peas	0

[a] Average of 3 tests.

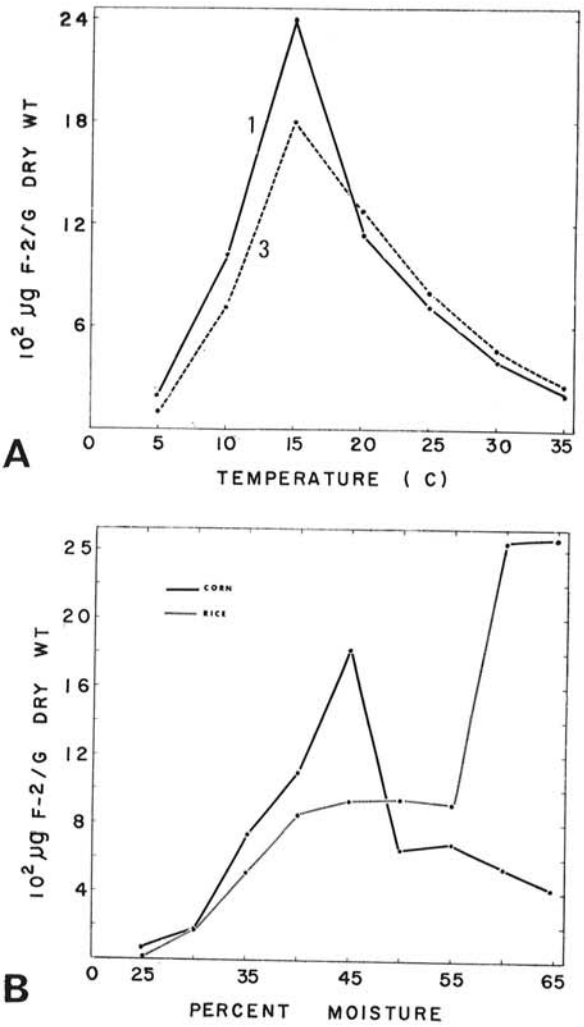

Fig. 1. A) Amount of F-2 mycotoxin produced by isolate No. 1 and isolate No. 3 of *Fusarium roseum* on corn, at different temp. **B)** Amount of F-2 mycotoxin produced by isolate No. 1 growing on corn and rice seed with different moisture contents, incubated 2 weeks at 22-25 C and 8 weeks at 12 C.

The influence of length of incubation time on production of F-2 by isolate No. 1 of *F. roseum* growing on autoclaved moist corn at 12 and at 25 C (preceded by 2 weeks at 24-27 C) is illustrated in Fig. 2. With incubation at 25 C, the greatest amount of F-2 was produced at 8 weeks, and with incubation at 15 C the greatest amount was produced at 10 weeks. The influence of moisture content of the substrate on production of F-2 by isolate No. 1 of *F. roseum* growing on corn and on rice after incubation for 2 weeks at 22-25 C and 8 weeks at 12 C is shown in Fig. 1-B. The fungus grew very sparsely in the samples with 25% moisture before autoclaving (probably 20-22% after autoclaving), but grew vigorously in all of the samples

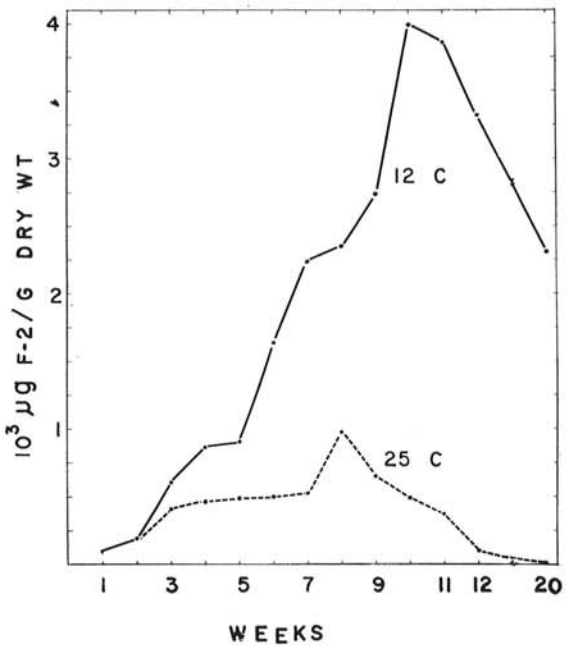

Fig. 2. Amount of F-2 mycotoxin produced by isolate No. 1 growing on corn seed at 12 and 25 C, after incubation for various lengths of time.

with preautoclaving moisture contents of 35% and higher.

Table 2 summarizes the amounts of F-2 produced when various microflora were introduced onto the substrate at the same time as *F. roseum,* and also 1 week after inoculation with *F. roseum,* by which time *F. roseum* was well established, with nearly all kernels in the substrate surrounded by a layer of mycelium. Very few of the mixed cultures in which the competing organisms were inoculated at the same time as *F. roseum* produced much F-2, but in most of those in which the competing organisms were inoculated after *F. roseum* had become established in the substrate, appreciable amounts of F-2 were produced; in several cases from about 50 to 75% of the amount produced in the controls, with *F. roseum* alone. In corn stored on the cob in cribs we often have found *F. roseum* in what appeared to be pure culture on portions of the cobs, but more frequently it was associated with fungi

TABLE 2. Quantity of F-2 mycotoxin produced by *Fusarium roseum* on corn seed in the presence of other microorganisms

Species added	µg F-2/g dry wt	
	Seeded same time *Fusarium roseum*	Seeded 1 week after *F. roseum*
Aspergillus flavus	50	875
A. ruber	81	1,800
A. ochraceous	112	1,660
A. niger	64	2,600
Penicillium sp.	0	988
Chaetomium globosum	101	2,303
Bacteria (unidentified)	0	
Control (*F. roseum* only)	2,800	3,216

such as were tested here in mixed cultures. Once *F. roseum* becomes established in such corn, evidently F-2 can be produced even in the presence of competing organisms.

Production of F-2 by 90 single-ascospore lines of isolate No. 1 of *F. roseum* grown on moist autoclaved corn ranged from 70-1,623 ppm, and of 30 single-ascospore lines of isolate No. 2 from 140-4,130 ppm. The two sets of single-ascospore lines were not tested at the same time, and so the higher production of many of the 30 lines of isolate No. 2 may have been a function of environment rather than of inherent characters. However, there seems to be no doubt that single-ascospore lines differ in amount of F-2 produced under a given set of conditions. Selection of high-yielding lines offers one means of increasing production of F-2 in the laboratory.

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