

## Detection of the Mycotoxin F-2 in the Confused Flour Beetle<sup>1</sup> and the Lesser Mealworm<sup>2,3</sup>

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### ABSTRACT

The mycotoxin, F-2, produced by the fungus *Fusarium roseum* var. *graminearum* Snyder & Hansen was ingested and retained by the confused flour beetle, *Tribolium confusum* Jacquelin du Val, and the lesser mealworm, *Alphitobius diaperinus* (Panzer). F-2 persisted in the

insects through metamorphosis and was detected after starvation and death. The amount of F-2 recovered from the insects increased with increasing F-2 concentrations in the insect culture media.

Certain strains of the fungus *Fusarium roseum* var.

*graminearum* Snyder & Hansen can produce toxic metabolites while growing on various crops including grain and cereal products. Some of these metabolites have deleterious effects when ingested by swine, commonly referred to as the estrogenic syndrome. This involves the development of swollen, edematous vulva in females, shrunken testes in young males, enlarged mammary glands in the young of both sexes and possibly abortion in pregnant gilts or sows (Stob

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ume of about 2 ml in a flash evaporator at 35°C and then rinsed into a separatory funnel with equal amounts of petroleum ether (bp 30-60) and acetonitrile. The 2 phases were mixed by shaking, allowed to come to equilibrium and the acetonitrile was saved. The acetonitrile was then concentrated in the flash evaporator.

Several quantities of the acetonitrile insect extract and a known concentration of F-2 standard were spotted on thin-layer chromatoplates. An extract sample size of 200  $\mu$ liter provided an adequate amount for spectrophotometric analysis. The chromatoplates were developed for about 50 min in a 97:3 (v/v) chloroform and absolute ethanol mixture in a developing chamber. After development, the plates were air-dried and the spots were examined with an ultraviolet lamp. Fluorescing spots corresponding to the F-2 standard were outlined with a pencil, and the silica gel within the spots was scraped off with a spatula into 15-ml centrifuge tubes. The silica gel was then suspended in 2 ml absolute ethyl alcohol and centrifuged at 11,000 rpm for 10 min. The supernatant liquid was examined in a DB Beckman spectrophotometer, and the amount of F-2 was determined from a standard curve at 274 nm. When F-2 concentrations were not adequate for spectrophotometric analysis, the sample was estimated by comparison of spot size with F-2 standards.

Because of the large insect sample required for detection of F-2, each value reported is based on a single analysis.

**RESULTS AND DISCUSSIONS.**—No F-2 was detected in the methylene chloride used to rinse the exterior of the insects. Thus the F-2 detected in subsequent experiments was primarily from within the insects.

F-2 was obtained from the confused flour beetles and the lesser mealworms that fed 6 days on rice flour containing 10,000 ppm F-2. The amount of F-2 detected was 8 and 14 ppm in the confused flour beetles and lesser mealworms, respectively.

Table 1 shows the amounts of F-2 recovered from the lesser mealworms after feeding 6 days in F-2 infested rice flour. Insects extracted and analyzed immediately after feeding and those kept at room temperature for 1 week after freezing contained similar concentrations of F-2. Starving the insects apparently reduced the amount of F-2. Subsequent experiments to determine if this loss is via the excretory products.

Fumigation with 80:20 (CCl<sub>4</sub>:CS<sub>2</sub> v/v) apparently did not alter the F-2 within the insect as indicated by the typical absorption spectrum obtained. How-

Table 1.—Amount of F-2 recovered from the adult lesser mealworm receiving different treatments after feeding 6 days in rice flour containing 8000 ppm F-2.

Insect treatment	F-2 recovered from insects (ppm)
Analyzed immediately after feeding <sup>a</sup>	34
" " " " <sup>b</sup>	32
Stored 7 days at room temperature following death <sup>a</sup>	30
Starved 7 days before analysis <sup>a</sup>	20
Untreated control <sup>a</sup>	0

<sup>a</sup> Killed by exposure to -20°C.

<sup>b</sup> Killed by fumigation with 80:20 (CCl<sub>4</sub>:CS<sub>2</sub> v/v).

Table 2.—Quantity of F-2 recovered from the adult lesser mealworm after different feeding periods in rice flour containing F-2 at 8000 ppm.

Length of feeding (days)	F-2 in insects (ppm)
0	0
1	13
4	12
8	18
12	24
17	26
23	24
28	29
33	21

ever, the methylene chloride extracts from fumigated insects did appear clearer than similar extracts of frozen insects. The fumigants may have removed certain pigments interfering with the F-2 fluorescence in the chromatoplates. The amount of interfering pigments also decreased following fumigation with CCl<sub>4</sub> or CS<sub>2</sub> alone as compared with the masked fluorescence of F-2 in the frozen insects. However, the F-2 spots were more pronounced from insects fumigated with 80:20 (CCl<sub>4</sub>:CS<sub>2</sub> v/v) than with either component alone. It appeared that the fumigation did not alter the chemical structure of F-2 since fumigation did not decrease the amount recovered and the UV spectrum obtained was similar to the F-2 standard. Consequently, we continued to use fumigation instead of freezing as a method of killing the insects in subsequent experiments.

Adult lesser mealworms contained 13 ppm of F-2 after feeding 1 day in rice flour containing F-2 at 8000 ppm (Table 2). The amount of F-2 increased to 24 ppm after 12 days and remained relatively constant until the 33rd day.

About 3 ppm of F-2 was recovered from the insects feeding 2 weeks on the rice flour containing 100 ppm of F-2 (Table 3). The amounts recovered increased with increasing concentrations of F-2. Also there was generally more F-2 in the insects after the 2nd week of feeding than after the 3rd week. These data agree with previous results that about 2 weeks of feeding is sufficient for detecting maximum amounts of F-2 in the insects.

Newly emerged confused flour beetle adults from larvae fed on F-2-contaminated rice flour contained F-2 at a concentration of 6 ppm indicating that the F-2 is retained by the insects through metamorphosis.

The analyses of feed suspected to have caused illnesses of domestic animals revealed F-2 concentrat-

Table 3.—Quantity of F-2 recovered from the adult lesser mealworm after feeding 2 and 3 weeks on rice flour containing various concentrations of F-2.

F-2 concentration in flour (ppm)	F-2 in insects (ppm)	
	2nd week	3rd week
0	0	0
100	3 <sup>a</sup>	4 <sup>a</sup>
500	9	8
1,000	12	11
5,000	20	19
10,000	29	16

<sup>a</sup> Estimated by thin-layer chromatography.

tions of 10 to 200 ppm.<sup>6</sup> The amounts of F-2 recovered from insects reported in this paper are within this range. It should be noted, however, that the total amount of F-2 available from the flour would be considerably greater than that from the insects. Still, the insects represent a mobile source of this contaminant.

The possible destruction or inactivation of the toxin ingested by the insects was not fully determined. UV spectrophotometric analyses showed no shift in the absorption maxima of the F-2 extracted.

In summary, F-2 is ingested and harbored by the confused flour beetle and the lesser mealworm, retained through metamorphosis, and detected despite starvation and death. Consequently these data indicate that both species of insects may be potential carriers of F-2 to foods and feeds.

<sup>6</sup> Unpublished results. C. J. Mirocha. Department of Plant Pathology, University of Minnesota, St. Paul 55101.

#### REFERENCES CITED

- Abdel-Rahman, H. A., C. M. Christensen, and A. C. Hodson. 1969. The relationship between *Plodia interpunctella* (Hb.) (Lepidoptera, Phycitidae) and stored-grain fungi. *J. Stored Prod. Res.* 4 (4): 331-7.
- Agrawal, N. S., C. M. Christensen, and A. C. Hodson. 1957. Grain storage fungi associated with the granary weevil. *J. Econ. Entomol.* 50 (5): 659-63.
- Agrawal, N. S., A. C. Hodson, and C. M. Christensen. 1958. Development of granary weevils and fungi in columns of wheat. *Ibid.* 51 (5): 701-2.
- Christensen, C. M., G. H. Nelson, and C. J. Mirocha. 1965. Effect on the white rat uterus of a toxic substance isolated from *Fusarium*. *Appl. Microbiol.* 13 (5): 653-9.
- Cotton, R. T. 1956. *Pests of stored grain and grain products.* Burgess Publishing Co., Minneapolis, Minn. 59 p.
- Harding, W. C. Jr., and T. L. Bissell. 1958. Lesser mealworms in a brooder house. *J. Econ. Entomol.* 51 (1): 112.
- Mirocha, C. J., C. M. Christensen, and G. H. Nelson. 1967. Estrogenic metabolite produced by *Fusarium graminearum* in stored corn. *Appl. Microbiol.* 15 (3): 497-503.
1968. Toxic metabolites produced by fungi implicated in mycotoxicoses. *Biotech. Bioeng.* 10: 469-82.
- Misra, C. P., C. M. Christensen, and A. C. Hodson. 1961. The Angoumois grain moth, *Sitotroga cerealella*, and storage fungi. *J. Econ. Entomol.* 54 (5): 1032-3.
- Sinha, R. N. 1966. Development and mortality of *Tribolium castaneum* and *T. confusum* on seed-borne fungi. *Ann. Entomol. Soc. Amer.* 59: 192-201.
- Stob, M., R. S. Baldwin, J. Tuite, F. N. Andrews, and K. G. Gillette. 1962. Isolation of an anabolic uterotrophic compound from corn infected with *Gibberella zeae*. *Nature* 196: 1318.
- Urry, W., H. L. Wehrmeister, E. B. Hodge, and P. H. Hidy. 1966. The structure of zearalenone. *Tetrahedron Letters* no. 27, p. 3109-14.
- Van Wyk, J. H., A. C. Hodson, and C. M. Christensen. 1959. Microflora associated with the confused flour beetle, *T. confusum*. *Ann. Entomol. Soc. Amer.* 52: 452-63.