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Phytochemistry 66 (2005) 2474–2480
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Identification and heritability of fumonisin insensitivity in *Zea mays*

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Received 1 March 2005; received in revised form 12 July 2005
Available online 29 September 2005

Abstract

Landraces of maize (*Zea mays* ssp. *mays*) and its wild teosinte relatives (*Zea mays* spp. *parviglumis* and *mexicana*) were surveyed for sensitivity to fumonisin B_1, a phytotoxin produced by the maize pathogen *Gibberella moniliformis*. Only two of 42 *Z. mays* samples were highly insensitive to FB_1 (ED_{50} = ca. 200 μM). The teosintes and 76% of the maize landraces were moderately or highly sensitive to FB_1 ($ED_{50} \leqslant 30$ μM), which indicates that FB_1 sensitivity is likely to be an ancestral trait in *Z. mays*. F_1 generations derived from crosses between FB_1-sensitive maize inbred B73 and insensitive landraces were significantly less sensitive than B73. Thus, our data indicate that FB_1-insensitivity is a relatively rare but heritable trait in maize. We also report the sensitivity of maize to other *Gibberella* toxins – beauvericin, diacetoxyscirpenol, and moniliformin.
Published by Elsevier Ltd.

Keywords: *Zea mays*; Gramineae; Maize; Teosinte; *Gibberella moniliformis*; *Fusarium verticillioides*; Phytotoxicity; Fumonisins; Beauvericin; Diacetoxyscirpenol; Moniliformin

1. Introduction

The wild annual teosintes (*Zea mays* L. spp.) of Mexico are the closest relatives and probable ancestors of domesticated maize (*Zea mays* ssp. *mays* Iltis and Doebley). Despite dramatic differences in their ear and seed morphology, cytological and molecular genetic analyses indicate that maize diverged from the Mexican annual teosinte *Zea mays* ssp. *parviglumis* Iltis and Doebley only ca. 9000 years ago (Doebley, 2004). Although phylogenetic analyses support a single ancient domestication event, landraces of maize in the Americas have evolved a higher level of phenotype and genetic diversity than any other major cereal crop (Lawrence et al., 2004). Landraces of maize and teosinte are an invaluable source of genes for improving commercial maize and for increasing its genetic diversity. The danger of genetic uniformity in the maize crop was first demonstrated by the Southern Corn Leaf Blight epidemic in the United States in 1970, which was caused by the fungus *Cochliobolus heterostrophus*. This epidemic resulted from the nearly universal use of closely related maize hybrids that inadvertently contained a gene for sensitivity to a toxin produced by the fungus (Wolpert et al., 2002).

Commercial maize hybrids in use today are susceptible to seedling blight, stalk rot, and ear rot caused by *Gibberella moniliformis* (asexual stage: *Fusarium verticillioides*). This fungus is not only the most common pathogen of maize, but also is among the most common fungi found colonizing symptomless seeds of maize and teosinte (Desjardins et al., 2000; Munkvold and Desjardins, 1997). The ability of maize-based feeds contaminated by G. *moniliformis* to cause animal diseases like equine leukoencephalomalacia has been known for a hundred years. Fumonisins, the causative agent of equine leukoencephalomalacia, were purified and characterized from *G. moniliformis* in South Africa in 1988 (Marasas, 1996). After their discovery as toxic to animals, fumonisin B_1 (FB_1) (**1**) and the homologues fumonisin

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0031-9422/$ - see front matter. Published by Elsevier Ltd.
doi:10.1016/j.phytochem.2005.08.013

B_2 (FB_2) (**2**) and fumonisin B_3 (FB_3) (**3**) lacking hydroxyl groups at C_{10} and C_5, repectively, were shown to be toxic to plants as well. Fumonisins are a family of amino polyalcohols with structural similarity to the long-chain base backbones of sphingolipids (Fig. 1(**1**–**3**)). Fumonisins inhibit the activity of sphingosine *N*-acetyl transferase and thereby alter sphingolipid biosynthesis, which is thought to be the mechanism of their toxicity to both animals and plants (Abbas et al., 1994; Gilchrist et al., 1992; Marasas, 1996).

The phytotoxicity of fumonisins to maize, the high frequency of fumonisin production among strains of *G. moniliformis* that are pathogenic to maize, and the high frequency of fumonisin contamination in maize provide circumstantial evidence that fumonisins play a role in pathogenesis on maize (Doehlert et al., 1994; Lamprecht et al., 1994; Munkvold and Desjardins, 1997). In addition, studies with fumonisin-non-producing mutants of *G. moniliformis* showed that the ability to produce FB_1 increases seedling blight, but not ear rot, in maize (Desjardins et al., 1995, 2002). In principal, if production of a toxin increases fungal pathogenicity on maize, then differences in maize sensitivity to the toxin should correlate with differences in susceptibility to the disease. Such rigorous proof of toxin function has been achieved for two fungal pathogens of maize: *Cochliobolus carbonum* and HC-toxin and *C. heterostrophus* and T-toxin, but has not yet been demonstrated for *G. moniliformis* and fumonisin (Wolpert et al., 2002).

Despite the availability of extensive collections of maize landraces, sensitivity of phenotypically and genetically diverse maize landraces to fumonisins and other *Gibberella* toxins has received little attention. This deficiency is due in large part to the difficulty and expense in obtaining these fungal toxins in highly purified form in quantities sufficient for phytotoxicity assays. For this study, we isolated and purified FB_1 (**1**), FB_2 (**2**), FB_3 (**3**), beauvericin (**4**), 4,15-diacetoxyscirpenol (**5**), and moniliformin (**6**) from *Gibberella* and *Fusarium* culture materials. In this paper, we report the results of an initial survey of FB_1-sensitivity of teosinte and maize landrace populations from North and South America in which we found that a high level of insensitivity

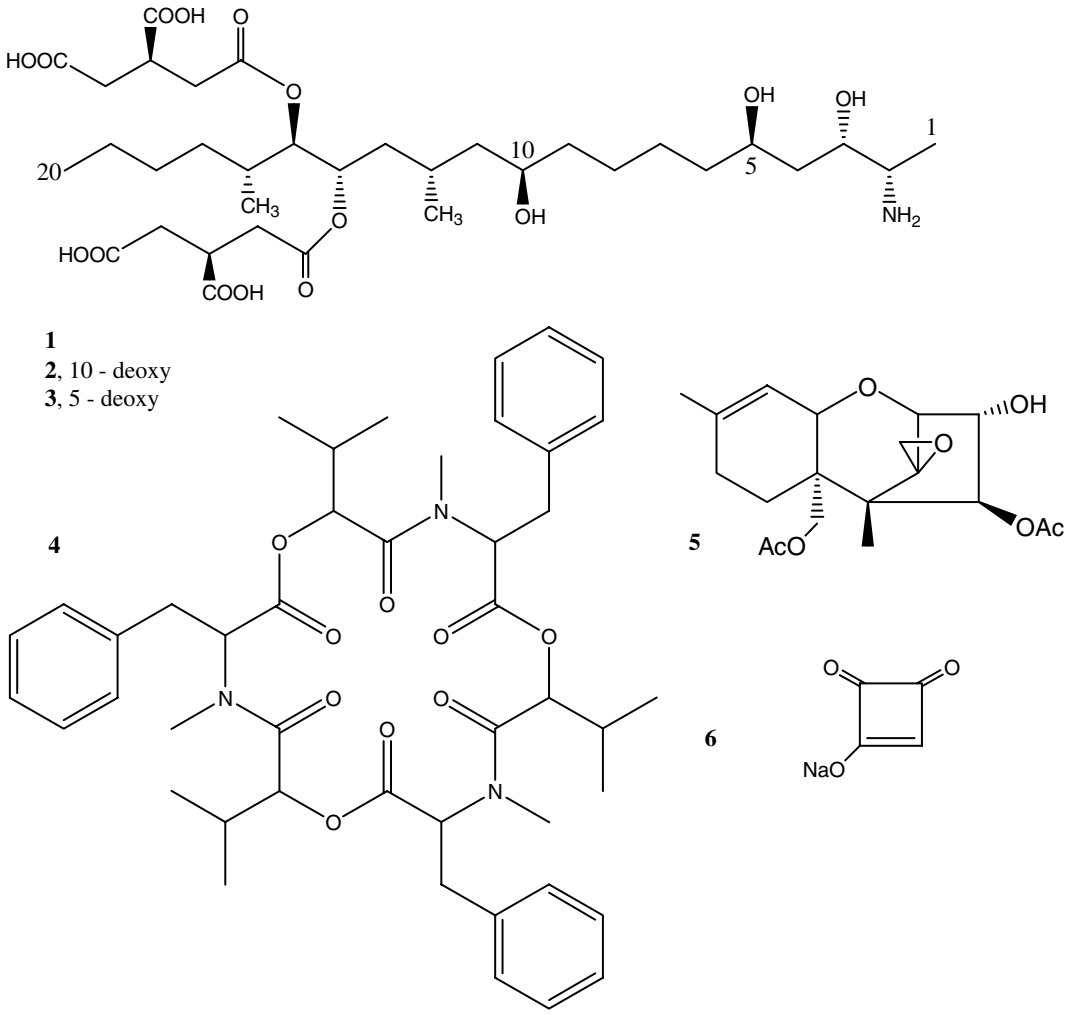

Fig. 1. Structure of fumonisin B_1 (**1**); fumonisin B_2 (**2**) and fumonisin B_3 (**3**) are missing hydroxyl groups at carbon positions 10 and 5, respectively. Structures of beauvericin (**4**), 4,15-diacetoxyscirpenol (**5**), and moniliformin (**6**).

to FB_1 (**1**) is a relatively rare but heritable trait in maize. We also report sensitivity of maize to beauvericin (**4**), 4,15-diacetoxyscirpenol (**5**), FB_2 (**2**), FB_3 (**3**), and moniliformin (**6**) (Fig. 1).

2. Results and discussion

2.1. Sensitivity of maize and teosinte to fumonisin B_1

Zea mays samples for testing were selected to include the historically important US Corn Belt Dent inbred B73, two species of teosinte from Mexico, and 39 maize landraces from North and South America (Table 1). The open-pollinated landrace populations were chosen to represent different segments of the landrace gene pool, including Northern Flint and Flour, Andean Highland, Tropical Highland, and Tropical Lowland. The sensitivity of seeds to FB_1 (**1**) was expressed as an ED_{50}, the concentration at which mean seedling growth (combined root length and shoot length) was inhibited by 50% compared to controls without FB_1 (**1**). Seed samples were scored as highly sensitive ($ED_{50} \leqslant 15$ μM), moderately sensitive (20–30 μM), moderately insensitive (35–50 μM), or highly insensitive (>100 μM). For all 42 *Z. mays* seed samples tested, seedling root growth and shoot growth were inhibited by FB_1 (**1**), but ED_{50} varied 40-fold among samples. For all samples, root growth was more inhibited than shoot growth, as has been reported previously for maize seedlings (Lamprecht et al., 1994). At 1 and 10 μM FB_1 (**1**), seedlings showed no obvious signs of disease other than stunting and occasional root tip browning. At 100 μM FB_1 (**1**), stunting and browning were more frequent and more extensive, and seed germination often was inhibited. Seeds that remained ungerminated in the presence of FB_1 (**1**) were able to germinate and grow after transfer to agar medium or soil without FB_1 (**1**), indicating that FB_1 (**1**) inhibition was not irreversible.

Overall, 76% of the *Z. mays* samples tested were moderately or highly sensitive to FB_1 (**1**) ($ED_{50} \leqslant 30$ μM). Both species of Mexican annual teosinte (*Zea mays* ssp. *mexicana* (Schrader) Iltis and Doebley and *Z. mays* ssp. *parviglumis*) were highly sensitive ($ED_{50} = 10$–15 μM), indicating that FB_1-sensitivity is likely to be an ancestral trait in *Z. mays*. Furthermore, all 12 populations of Mexican maize landraces tested (including a New Mexican population of the Chapalote race) were moderately or highly sensitive to FB_1 (**1**), with a mean $ED_{50} = 18 \pm 7$ μM (mean ± SD). The FB_1-sensitive landraces from Mexico included Tropical Lowland and Tropical Highland populations, and included primitive popcorns, sweetcorn, and dents with cylindrical ears (Wellhausen et al., 1952). A population of the Tuson race, a Cuban cylindrical dent, also was highly sensitive to FB_1 (Hatheway, 1957).

Historical records and analyses of DNA microsatellite loci indicate that Mexican Tropical Lowland landraces were major sources for the Southern Dents, which were introduced into the United States after European colonization (Labate et al., 2003; Liu et al., 2003). Southern Dents were hybridized with Northern Flints during the 1800s to produce the highly productive US Corn Belt Dents. Among Corn Belt Dents, the Iowa Stiff Stalk Synthetic and the Lancaster Surecrop heterotic group (which includes inbreds Mo 17 and C103) have historically contributed heavily to the pedigrees of commercial hybrids. Both inbred B73 (derived from Iowa Stiff Stalk Synthetic) and landrace Lancaster Surecrop were highly sensitive to FB_1 (**1**) ($ED_{50} = 10$–15 μM), indicating that FB_1 (**1**) sensitivity may be widespread among Corn Belt Dents derived from this pedigree.

Northern Flint and Flour landraces were the dominate genotypes of maize in the northern United States and Canada prior to European colonization, but at the present time these landraces survive mainly in germplasm collections and among Native American groups. Furthermore, although Northern Flints played a historical role in the development of Corn Belt Dents, DNA microsatellite analysis indicates that the contribution of Northern Flint germplasm to Corn Belt Dent inbreds is only about 25% (Liu et al., 2003). Seventy-one percent of 14 maize landraces from the United States and Canada were moderately or highly sensitive to FB_1 (**1**), with a mean $ED_{50} = 15 \pm 6$ μM. Highly sensitive landraces included a Northern Flint from the Mohawk tribe of New York; a representative of the Southeastern Flint-Flour racial complex from the Quapaw tribe of Oklahoma; blue flour corns from the desert Southwest; and Country Gentlemen, a sweet corn derived from a Northern Flint background. Three landraces were moderately insensitive ($ED_{50} = 35$ μM): Kokoma, a blue flour corn from the Hopi tribe of Arizona, and the Northern Flints Smutnose and Gaspe Flint, which are genetically closely related to each other and to flints widely grown in North and Eastern Europe (Rebourg et al., 2003). Tama Flint, a blue seeded landrace from the Mesquakie tribe of Iowa, was the most FB_1 (**1**)-insensitive maize found in the study, with an $ED_{50} =$ ca. 200 μM. In fact, growth of Tama Flint seedlings was inhibited by only 58% at 300 μM, the highest concentration of FB_1 (**1**) tested. DNA microsatellite analysis indicates that Tama Flint is closely related to a cluster of the Northern Flint-Flour race that contains the FB_1-sensitive landraces Mohawk Roundnose and Sac-Fox Blue (Santicruz-Varela et al., 2004; Ugalde, 1997).

In addition to the North American landraces, the survey included 10 maize landraces from the Andean highland region of South America – Chile, Colombia, and Peru. In contrast to the North American landraces, only one Andean landrace, a popcorn from highland Colombia, was highly sensitive to FB_1 (**1**). A group of five landraces were moderately insensitive, with a mean $ED_{50} = 40 \pm 6$ μM. In addition, Magdalena 469, a landrace of the Guirua race from Colombia, was highly insensitive to FB_1 (**1**), with an $ED_{50} =$ ca. 200 μM. The Guirua race has been collected only at elevations of 1850–1870 m in the Department of Magdalena and has a semiflint endosperm and predomi-

Table 1
Populations of *Zea mays* screened for seedling sensitivity to fumonisin B_1 (**1**)

	Name[a]	Origin	Description	Accession no.	ED_{50}[b]
1	Tama Flint[cd]	Iowa, USA	Northern Flint-Flour race, flint, blue seed	PI217411	ca. 200
2	Magdalena 469[cd]	Colombia	Guirua seed, semiflint, blue and yellow seed	PI445007	ca. 200
3	CHZM 10 021	Chile	Araucano race, flint, yellow seed	PI485703	50
4	Narino 369	Colombia	Pira Naranja race, pop, yellow and orange seed	PI445082	45
5	Lambayeque 15	Peru	Mochero race, flour, yellow and red seed	PI485322	40
6	Smutnose[cd]	Michigan, USA	Northern Flint-Flour race, flint, yellow and orange seed	PI222490	35
7	Gaspe Flint	Quebec, Canada	Northern Flint race, flint, yellow seed	PI401757	35
8	Kokoma[cd]	Arizona, USA	Southwestern 12-row racial complex, flour, blue seed	PI213733	35
9	Tacna 1	Peru	Coruca race, Flour to semident, yellow seed	Ames 8563	35
10	Boyaca 406	Colombia	Pira race, pop, white and red seed	PI444125	35
11	San Martin 91	Peru	Piricinco race, flour, brownish seed with bluish cast	PI571941	30
12	Durango 102	Mexico	Tabloncillo race, semiflint to semident, white seed	PI484870	30
13	Chihuahua 129[d]	Mexico	Cristalino de Chihuahua race, flint, yellow seed	PI484404	30
14	Sinaloa 2[d]	Mexico	Chapalote race, pop, brown seed	NSL 283388	25
15	Choco 356	Colombia	Chococeno race, flour, white seed	PI 444741	25
16	Mesita Pueblo	New Mexico, USA	Southwestern 12-row racial complex, floury, blue seed	PI 218146	25
17	Sonora 124	Mexico	Dulcillo de Noreste race, sweet, white seed	PI515464	25
18	Chihuahua 138	Mexico	Apachito race, semiflint, white and red seed	PI484413	25
19	CHZM 13 040	Chilie	Choclero race, flour to semident, yellow seed	PI586727	20
20	Mandan Clay Red	North Dakato, USA	Northern Flint-Flour race, flour, red seed	PI213807	20
21	Sac-Fox Blue	Iowa USA	Northern Flint-Flour race, flint, yellow and blue seed	PI213768	20
22	Michoacan 66[d]	Mexico	Zamorano Amarillo race, semident, yellow seed	Ames 19589	20
23	Jalisco 133	Mexico	Complejo Serrano de Jalisco race, semident, white and orange seed	PI 628446	15
24	Quapaw Red	Oklahoma USA	Southeastern Flint-Flour racial complex, flint, red seed	PI 213757	15
25	Chapalote	New Mexico USA	Chapalote race, semiflint, brown seed	PI 420245	15
26	Oaxaca 26	Mexico	Negrito race, semiflint, blue seed	PI 515390	15
27	Jalisco 142	Mexico	Palomero de Jalisco race, pop to flint to semiflint, white and orange seed	Ames 19536	15
28	Tesuque Pueblo	New Mexico USA	Southwestern Semident racial complex, flint to flour to semident, white seed	PI 218137	15
29	Moencopi Pueblo	Arizona USA	Southwestern Semident racial complex, flour to semident, blue seed	PI 218175	15
30	Huhni	Arizona USA	Pima Papago race, flour, white seed	PI 420251	15
31	*Z. mays* ssp. *mexicana*	Mexico	Teosinte	PI 566683	15
32	Cundinamarca 327	Colombia	Pira race, pop, white seed	PI444512	10
33	Chihuahua 160[d]	Mexico	Gordo race, flint to flour, white seed	PI484433	10
34	B73[d]	Iowa USA	Com Belt dent inbred, dent, yellow seed		10
35	Olotillo Blanco	Mexico	Olotillo race, dent, white seed	PI438943	10
36	*Z. mays* ssp. *parviglumis*	Mexico	Teosinte	PI384061	10
37	Mohawk Roundnose	New York USA	Northern Flint-Flour race, flint, white seed	PI483087	10
38	Zuni Blue	North Dakota USA	Landrace with characteristics between Northern Flint-Flour race and Southwestern racial complexes, flour, blue seed	PI213799	10
39	Oaxaca 570[d]	Mexico	Mixteco race, dent, blue seed	Ames 19654	10
40	Lancaster Surecrop	Pennsylvania USA	Corn Belt Dent race, dent, yellow seed	PI 213697	10
41	Cuba 67	Cuba	Tuson race, semident to dent, yellow seed	NSL 283507	10
42	Country Gentleman[d]	Wisconsin USA	Northern Flint-Flour Sweetcorn subrace, sweet, white seed	Ames 22639	5

[a] Seed samples of maize inbred B73 were obtained from Michael Muhitch, USDA, Peoria, IL, USA. Seed samples of 38 open-pollinated landrace populations of maize and of two subspecies of annual teosinte were obtained from USDA, North Central Regional Plant Introduction Station, Ames, Iowa, USA. Descriptions of seed samples are from (Gonzalez-Ugalde, 1997; Goodman and Brown, 1988; Hatheway, 1957; Lawrence et al., 2004; Roberts et al., 1957; USDA, ARS, GRIN [Online Database], Wellhausen et al., 1952). Teosinte samples were pools of seed samples of the same subspecies and geographic region. PI 56683 was a pool of Z *mays* ssp. *mexicana* "Acece" from Mexico, Mexico, including seeds from PI 56683, PI 56684, and PI 56685. PI 384061 was a pool of Z. *mays* ssp. *parviglumis* from Guerrero, Mexico, including seeds from PI 384061, PI 384062, PI 384063, and PI 384064.

[b] ED_{50} indicates μM FB_1 (**1**) at which growth of the longest root and shoot of each seedling was reduced by 50% as described in Section 3. The last unit of each ED_{50} value was rounded off to the nearest unit step (5). All populations were tested once at 0, 1, 10, and 100 μM FB_1; eleven populations (marked [d]) were retested at 0, 1, 10, and 100 μM; four populations (marked [c]) were tested further at 0, 10, 100, 200 and 300 uM. Thus, up to three values of seedling length for each value of fumonisin concentration were used for linear regression analysis. Germination $\geqslant$70% for all samples without FB_1. Correlation coefficient $\geqslant$0.80 for all samples.

nately a deep blue aleurone (Roberts et al., 1957). Growth of Magdalena 469 seedlings was inhibited by 85% at 300 μM FB_1 (**1**), indicating that this landrace is likely not as insensitive as Tama Flint.

Even among the rather small selection of maize landraces in this study, ED_{50} for seedling sensitivity to FB_1 (**1**) varied 40-fold, from a low of 5 μM for Country Gentleman to ca. 200 μM for Tama Flint. The 40-fold range of FB_1-sensitivity of maize landraces is similar to the 50-fold range (0.4–20 μM) of FB_1-sensitivity for leaf necrosis of tomato (*Lycopersicon esculentum* Mill) genotypes that differ at the *Asc* locus (Gilchrist et al., 1992). The mechanism for FB_1 (**1**) insensitivity

in maize is unknown. In tomato, however, the dominant *Asc* gene for FB_1 (**1**)-insensitivity has been cloned; toxin sensitivity is a recessive trait that evolved by gene mutation (Brandwagt et al., 2000). Genes with sequence similarity to *Asc* have been found in maize, and are logical candidates for FB_1 (**1**)-insensitivity genes (Lawrence et al., 2004).

For the *Cochliobolus* toxins, HC-toxin and T-toxin, toxin insensitivity is a dominant, ancestral trait in maize and its wild progenitor *Z. mays* ssp. *parviglumis*; in addition, toxin sensitivity is a rare, recessive trait that evolved by gene mutations in certain maize inbred lines (Wolpert et al., 2002; Zhang et al., 2002). For FB_1 (**1**), in contrast, it appears that toxin sensitivity is an ancestral trait in *Zea mays* ssp. *parviglumis* and *Zea mays* ssp. *mexicana*, as well as the most common phenotype among North American landraces of maize and two historically important Corn Belt Dents, inbred B73 and Lancaster Surecrop. This pattern is unexpected because the fumonisin-producing species *G. moniliformis* and *G. intermedia* (asexual stage: *F. proliferatum*) are major fungal pathogens associated with maize in North America, thus conditions for selection for fumonisin-insensitivity in maize should exist (Munkvold and Desjardins, 1997).

2.2. Sensitivity of maize to other Gibberella toxins

The relative sensitivity of inbred B73 to FB_1 (**1**) and the less-oxygenated homologues FB_2 (**2**) and FB_3 (**3**) that also are produced by *G. moniliformis* was compared in three replicate tests at concentrations of 1, 10, and 100 μM. All three homologues inhibited seedling growth, as has been reported previously for maize seedlings (Lamprecht et al., 1994). In the previous study, FB_1 (**1**) and FB_2 (**2**) were similar in toxicity, whereas FB_3 (**3**) was less toxic. In our study, in contrast, ED_{50}s were 6, 80, and 65 μM for FB_1 (**1**), FB_2 (**2**), and FB_3 (**3**), respectively. The reasons for the difference in relative sensitivity to FB_2 (**2**) in the two studies are not known.

Maize inbred B73 and half of the landraces were tested for sensitivity to 100 μM moniliformin (**6**), a hydroxycyclobutenedione produced by several *Gibberella* species pathogenic to maize (Bryden et al., 2001). Landraces tested were numbers 1, 2, 3, 4, 6, 13, 16, 17, 19, 20, 22, 23, 24, 32, 33, 35, 37, 39, and 41 as listed in Table 1. Seedling growth of two landraces, Oaxaca 570 and Mohawk Roundnose, was inhibited by nearly one-half, but inbred B73 and the remaining 17 landraces were insensitive. Maize inbred B73 was used to test synergistic interactions between moniliformin (**6**) and FB_1 (**1**); 100 μM moniliformin (**6**) had no effect on phytotoxicity of 10 μM FB_1 (**1**). Moniliformin (**6**) was previously reported to be toxic to maize callus culture ($ED_{50} = 100$ μM) or when 200 μg were placed into the leaf whorl of one-week old maize seedlings (Van Asch et al., 1992; Cole et al., 1973). For comparison, each maize seedling in our assay was exposed to a maximum of 20 μg moniliformin (**6**).

Seedling growth of maize inbred B73 and the FB_1-insensitive landrace Tama Flint was compared at 1, 10, 100, and 200 μM concentrations of 4,15-diacetoxyscirpenol (**5**), a 12,13-epoxytrichothecene produced by *Gibberella*/*Fusarium* species pathogenic to maize. Seedling growth was inhibited by diacetoxyscirpenol (**5**), with $ED_{50} = 8$ μM for inbred B73 and 20 μM for Tama Flint. Like other trichothecenes, diacetoxyscirpenol (**5**) is toxic to a wide range of plants, although its toxicity to maize seedlings specifically does not appear to have been reported previously (Brian et al., 1961). Maize inbred B73 also was tested for sensitivity to 100 μM beauvericin (**4**), a cyclic depsipeptide produced by several *Gibberella*/*Fusarium* species pathogenic to maize (Bryden et al., 2001). Beauvericin (**4**) had no effect on seedling growth and, furthermore, 100 μM beauvericin (**4**) had no synergistic effect, either alone or in combination with 100 μM moniliformin (**6**), on phytotoxicity of 10 μM FB_1 (**1**). To our knowledge, phytotoxicity of beauvericin (**4**) to maize seedlings has not previously been investigated.

2.3. Heritability of fumonisin insensitivity

Heritability of FB_1-insensitivity in maize was tested by crossing the highly sensitive inbred B73 as the maternal parent with the highly insensitive landrace Tama Flint and the moderately insensitive landraces Smutnose and Kokoma. Crosses with the highly insensitive landrace Magdalena 469 were unsuccessful due to its late maturity under field conditions in Illinois. The aleurone is colorless in inbred B73 and Smutnose, and blue in Tama Flint and Kokoma. Thus, blue aleurone color of F_1 generation seeds was a useful marker for successful outcrossing with Tama Flint and Kokoma paternal parents; only ears that contained 100% blue seeds were selected for analysis. Data on sensitivity to 100 μM FB_1 (**1**) were collected on an individual ear basis for four crosses: three ears of inbred B73 × B73, five ears of B73 × Tama Flint, three ears of B73 × Smutnose, and three ears of B73 × Kokoma. Data for individual ears of each cross were similar and thus were combined for statistical analysis and presentation. Sib-crosses of Kokoma, Smutnose, and Tama Flint were lost to animal predation in the field; therefore FB_1-sensitivity data from the original samples of these three landraces were used for comparisons between parents and F_1 generations.

Frequencies of FB_1-sensitivity classes of seeds of the three FB_1-insensitive parents and their F_1 generation progeny were significantly different from parent B73 by chi-square analysis ($\chi^2 = 121$–144, $p < 0.001$) (Table 2). For example, in the presence of 100 μM FB_1 (**1**), seed germination was 86–95% for FB_1-insensitive landraces Tama Flint, Smutnose, and Kokoma; and 56–70% for F_1 generation progeny. In contrast, in the presence of FB_1 (**1**), seed germination was only 14% for the FB_1-sensitive inbred B73. The frequency distribution of fumonisin sensitivity classes for the landrace parents and the F_1 generations showed continuous variation that was skewed toward, but significantly different from, the sensitive parent.

Our results indicate that fumonisin insensitivity is likely to be a heritable trait in maize. Due in large part to the heterogeneity of the fumonisin-insensitive parents, all of

Table 2
Sensitivity of maize parental-line and F_1-generation seedlings to 100 μM fumonisin B_1 (**1**)

Maize	% seeds germinated[a]	% seeds in each sensitivity class (% control seedling median growth)[b]				Total no. seeds
		<26%	26–50%	51–75%	>75%	
B73 × B73 (parent)	14	6	6	2	0	251
Tama flint (parent)	90	22	25	31	12	49
B73 × Tama flint (Fi)	56	29	24	3	0	376
Smutnose (parent)	86	26	32	28	0	50
B73 × Smutnose (Fi)	70	40	25	5	0	176
Kokoma (parent)	95	26	53	12	4	44
B73 × Kokoma (Fi)	66	34	28	4	0	244

[a] Seeds were scored as germinated if the combined root and shoot growth was >10 mm after 5 days incubation. Scores were adjusted for poor growth that was not due to FB_1 treatment by subtracting the percentage (%) of ungerminated control seeds, which was B73 × B73 (16), Tama Flint (19), B73 × Tama Flint (20), Smutnose (14), B73 × Smutnose (40), Kokoma (27), and B73 × Kokoma (19).

[b] Median growth of germinated control seedlings (in cm) was B73 × B73 (8.5), Tama Flint (8.0), B73 × Tama Flint (14), Smutnose (12), B73 × Smutnose (14), Kokoma (10), and B73 × Kokoma (16).

which are open-pollinated landraces, conclusions cannot yet be drawn about the numbers or types of genes for fumonisin insensitivity. This limitation is now being addressed by combining fumonisin selection with sib-crosses of the landrace parents and backcrosses to the B73 inbred parent. This approach should yield less heterogeneous, fumonisin-insensitive lines that will be useful for rigorously assessing the genetic and biochemical basis of fumonisin insensitivity in maize and for determining whether fumonisin insensitivity increases the resistance of maize to *G. moniliformis*.

3. Experimental

3.1. Fungal metabolites

FB_1 (**1**), FB_2 (**2**), and FB_3 (**3**) were isolated from culture extracts of *G. moniliformis* strain M-3125 and purified by preparative HPLC as previously reported (Nelson et al., 1993; Poling and Plattner, 1999). Beauvericin (**4**) was obtained from culture extracts of *Gibberella intermedia* and purified by preparative HPLC as previously reported (Poling and Plattner, 1999. Moniliformin (**6**) was isolated from culture extracts of *Gibberella subglutinans* (asexual stage: *F. subglutinans*) and purified by ion-exchange chromatography as previously reported (Thiel et al., 1982). 4,15-Diacetoxyscirpenol (**5**) was obtained from culture extracts of a mutant derived from *F. sporotrichioides* strain NRRL 3299 and purified by preparative HPLC and crystallization as previously reported (Hohn et al., 1993). All fungal metabolites were at least 98% pure; structures were verified by LC-MS and NMR analyses.

To standardize fungal metabolite concentrations during the study, master stock solutions of beauvericin, fumonisins, and moniliformin were prepared in water at 10× their final concentrations, filter-sterilized, and frozen at −20 °C. The master stock solution of diacetoxyscirpenol (**5**) was prepared at 100× in MeOH to yield a level of 1% v/v MeOH in the final assay; this level of MeOH had no effect on maize seeds, but controls with and without MeOH were included. Master stock solutions were thawed and added to autoclaved 0.6% w/v Bacto agar in water (Becton, Dickinson Co. Sparks, MD), and immediately dispensed in 2 ml aliquots into sterile plastic test tubes (16 × 90 mm).

Phytotoxicity assays of the maize populations were conducted using 20 seeds per treatment with FB_1 (**1**), FB_2 (**2**), and FB_3 (**3**) at 0, 1, 10, and 100 μM; additional tests were conducted with FB_1 (**1**) at 0, 10, 100, 200 and 300 μM. Diacetoxyscirpenol (**5**) was tested at 0, 1, 10, 100 and 200 μM, and beauvericin (**4**) and moniliformin (**6**) were each tested at 100 μM; all of these assays also used 20 seeds per treatment. Inheritance of fumonisin insensitivity in individual ears from maize crosses was investigated by comparing seedling growth with and without 100 μM FB_1 (**1**) using 100 seeds per treatment per ear.

3.2. Plant materials

Plant materials used for the survey of FB_1 (**1**) sensitivity are described in Table 1. Teosinte fruitcases were split and the seeds were removed. Seeds of teosinte and maize were surface-disinfested by placing them in 0.5% sodium hypochlorite for 1 min, and were rinsed twice in sterile water. Each surface disinfested seed was place in a test tube containing agar with the fungal metabolite(s) to be tested. The test tubes were loosely capped and incubated in the dark for 5 days at 25 ± 1 °C, after which time the lengths of the longest root and shoot of each seedling were measured.

In May 2004, seeds of the FB_1-sensitive inbred B73 and FB_1-insensitive landraces Kokoma, Magdalena, Smutnose, and Tama Flint were sown in a field plot at the National Center for Agricultural Utilization Research, Peoria, IL. To obtain the F_1 generation, pollen from one plant of B73, Smutnose, or Tama Flint was applied to the silks of one ear of the sensitive inbred B73. Developing ears of B73 were protected with glassine bags before and paper bags after pollination to exclude unwanted pollen. Landrace Magdalena matured too late in the season to allow crosses with inbred B73. Approximately 40 ears of each of the successful crosses were harvested and shelled in September. Seeds from each ear were stored in an individual bag and were tested within

5 months of harvest for sensitivity to 100 μM FB_1 as described above.

3.3. Statistical methods

For the initial survey, logarithmic transformation of the fungal metabolite concentration (X) was used to fit data to a straight line ($Y = a + b[\log X]$) and allow linear regression analysis. Metabolite sensitivity was expressed as an effective dose (ED_{50}), the concentration of metabolite at which combined root and shoot growth was equal to 50% of the value of the Y-intercept. For the analysis of heritability, a chi-square (5×2) test was used to compare the frequencies of seeds in each of the five FB_1-sensitivity classes to analyze differences between the B73 parental line and the other three parental lines and three F_1-generations for a total of 6 χ^2 tests.

Acknowledgements

We thank N. Deppe, D. Engel, S. Folmar, and T. Wilson for technical assistance; and D. Palmquist for statistical analysis.

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