Fusarium spp. Causing Dry Rot of Seed Potato Tubers in Michigan and Their Sensitivity to Fungicides

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Abstract

Gachango, E., Hanson, L. E., Rojas, A., Hao, J. J., and Kirk, W. W. 2012. *Fusarium* spp. causing dry rot of seed potato tubers in Michigan and their sensitivity to fungicides. Plant Dis. 96:1767-1774.

A survey of seed potato tubers in Michigan seed production storage facilities was carried out during 2009 and 2010. *Fusarium* spp. associated with tuber dry rot symptoms were identified to species and tested for sensitivity to difenoconazole, fludioxonil, and thiabendazole. Symptomatic tubers ($n = 370$) were collected from a total of 51 seed lots, from which 228 isolates of *Fusarium* were recovered and identified to 11 species. *Fusarium oxysporum* was the most commonly isolated species (30.3%), followed by *F. equiseti* (19.3%). *F. sambucinum* and *F. avenaceum* were third most prevalent (each at 13.6%). Less prevalent species (each at 4 to 10%) included *F. cerealis*, *F. solani*, and *F. acuminatum*; and species present at $\leq 3\%$ included *F. sporotrichioides*, *F. torulosum*, *F. tricinctum*, and *F. graminearum*. Representative

isolates of all species were pathogenic when inoculated onto seed tubers ('Dark Red Norland'). Isolates of *F. sambucinum* were the most virulent. All 228 isolates of *Fusarium* were sensitive to difenoconazole (effective fungicide concentration that caused 50% inhibition of mycelial growth [EC_{50}] < 5 mg/liter). Insensitivity to fludioxonil ($EC_{50} > 100$ mg/liter) was detected only for *F. sambucinum* and *F. oxysporum* isolates at 8.9 and 20.4%, respectively. All isolates were sensitive to thiabendazole ($EC_{50} < 5$ mg/liter), except for those of *F. sambucinum* ($EC_{50} > 100$ mg/liter). Therefore, knowledge of what *Fusarium* spp. are present in seed potato storage facilities in Michigan may be important if using fludioxonil or thiabendazole for seed piece treatment but not when using difenoconazole.

Fusarium dry rot of potato (*Solanum tuberosum* L.) can be a devastating postharvest disease worldwide and is caused by several species of *Fusarium* (5,42). Dry rot affects both tubers in storage and seed tuber pieces in the field (7,48). Losses associated with dry rot have been estimated to range from 6 to 25%, and occasionally losses as great as 60% have been reported during long-term storage (12,42).

Infection of potato tubers by dry rot pathogens occurs through wounds inflicted during harvesting, grading, cutting, and handling of tubers (39,42). Thirteen species of *Fusarium* have been implicated in fungal dry rots of potato worldwide (8,20); of which eight species have been reported in the northern United States (19). The most prevalent species infecting potato are *F. sambucinum* Fuckel; (syn. *F. sulphureum* Schltdl., tel. *Gibberella pulicaris*), *F. solani* var. *caeruleum* (Lib. ex Sacc.) C. Booth (syn. *F. caeruleum*, tel. *Nectria haematocca*), and *F. oxysporum* Schltdl. (19). Other species reported in the northern United States include *F. avenaceum* (Fr.) Sacc., *F. culmorum* (W.G. Sm.) Sacc., *F. acuminatum* Ellis & Everh., *F. equiseti* (Corda) Sacc., and *F. crookwellense* L.W. Burgess, P.E. Nelson & Toussoun (*F. cerealis*) (19,34). Most of these species were also recovered in a study in the Pacific region of the United States, with *F. sambucinum* being the most prevalent (34). Recently, *F. graminearum* was reported to be the most prevalent *Fusarium* sp. causing potato dry rot in North Dakota (2,13).

In Michigan, potato seed tubers are produced in the Upper and Lower Peninsulas (29). The limited seed tuber generation program in Michigan was adopted in 2002 and utilizes the tissue culture system (47). With tissue culture, plants are produced under aseptic conditions, tested for various bacterial and viral diseases, and

transplanted into greenhouses to produce the nuclear class of seed or what is commonly known as mini-tubers. This involves meristem, tissue culture seed stock development, after which mini-tubers or tissue culture plantlets are planted into a field or fields as the initial source of certified seed potato lots. The seed lots are then multiplied and increased to reach a sufficient quantity for commercial use for up to six generations (Field Year 1 to 6 Class Seed), after which the seed lot is no longer eligible for recertification. The seed lots entered for Michigan certification are tested in the winter in south Florida. On passing this test, the seed lots are evaluated by Michigan State Department of Agriculture seed inspectors prior to shipping seed tubers from the Michigan seed farms. The seed lots that pass this stage achieve "blue tag" certification (U.S. Standards for Seed Potato Grades Regulation number 628) from the Michigan Seed Potato Association (MPSA; Gaylord, MI) and the Michigan Department of Agriculture (MPSA Director E. Dorman, Plant Pathology Laboratory, East Lansing) (29).

Seed and commercial potato growers receive seed as whole tubers to grow for recertification as seed or for commercial production. The tubers are cut into pieces and treated with a seed treatment such as fludioxonil or thiabendazole prior to planting (48). Cutting seed tubers potentially exposes the seed pieces to contamination or infection by pathogens such as *Fusarium* spp., which may lead to dry rot development in the field or in storage once tubers are wounded during harvesting. For most (>50%) potato seed lots in Michigan, some level of dry rot has been reported (22). *F. sambucinum* was the predominant species affecting seed potato tubers selected from a single storage in Michigan in 2006, causing seed piece decay after planting (25) and rotting sprouts of the progeny tubers (49). Since the report of Lacy and Hammerschmidt in 1993 (25), there has been no systematic assessment of *Fusarium* spp. responsible for dry rot on seed potato tuber stocks in Michigan. There are also no commercially grown potato cultivars resistant to dry rot in North America, although the level of resistance varies among some cultivars and breeding lines (26). Understanding the *Fusarium* spp. composition in outbreaks of potato dry rot is of importance in designing both chemical and cultural management (crop rotation) schemes for potato dry rot because different crops

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Accepted for publication 23 June 2012.

may harbor different *Fusarium* spp. that can vary in pathogenicity and sensitivity toward fungicides.

Fusarium dry rot on seed potato can be controlled in two phases during the potato growth cycle: (i) control of seed piece decay prior to planting, which is primarily achieved by seed treatment; and (ii) postharvest control of dry rot on both stored commercial tubers and seed tubers (33). Fludioxonil (Maxim Seed Potato Protectant; Syngenta Crop Protection), a phenylpyrrole, is among the few fungicides registered for potato seed treatment against *Fusarium* dry rot in the United States (48). Studies have shown that fludioxonil is able to reduce seed piece decay as well as the incidence of diseased sprouts (48) that develop into infected plants. Recently, fludioxonil-resistant strains of *Fusarium* spp. were reported in Canada and the United States, including strains of *F. sambucinum*, *F. oxysporum*, and *F. coeruleum* (16,38). However, sensitivity to fludioxonil in other *Fusarium* spp. that cause potato seed piece decay is not known.

Postharvest management of potato dry rot has been achieved primarily by reducing tuber bruising, providing conditions for rapid wound healing (41), and applying thiabendazole, a benzimidazole fungicide (TBZ or Mertect 340F, Syngenta Crop Protection), as tubers are placed into storage (20,34). Isolates of *F. sambucinum* resistant to TBZ were found first in Europe (20) and subsequently in the United States (10,11). Fungicide resistance has reduced the efficacy of TBZ in controlling *F. sambucinum* (34) but may be useful where TBZ-sensitive isolates still occur (10). Resistance to TBZ has also been reported in other *Fusarium* spp. isolated from dry rot potato tubers, including *F. oxysporum*, *F. solani*, *F. culmorum* (19,34), *F. avenaceum*, *F. equiseti*, *F. sporotrichioides* (34), and *F. acuminatum* (19).

To counteract the reduced effectiveness of TBZ (34) and, potentially, of fludioxonil associated with resistant strains of pathogen, additional registrations of efficacious postharvest fungicides are needed and some fungicides have been tested, including difenoconazole (Inspire; Seed Potato Protectant; Syngenta Crop Protection), a sterol biosynthesis inhibitor (1). The objectives of this study were to (i) characterize the *Fusarium* spp. responsible for dry rot of seed potato tubers produced in Michigan and (ii) determine the sensitivity to fludioxonil and TBZ as well as establish baseline sensitivity levels for difenoconazole in *Fusarium* populations recovered from Michigan potato seed lots.

Materials and Methods

***Fusarium* isolation and species identification.** Dry-rot-symptomatic seed tubers were collected from April to May 2009 and 2010. A total of 370 tubers was collected for processing. The potato tubers were sourced from 18 storage facilities located in the Upper and Lower Peninsulas of Michigan, and represented the

Fig. 1. Potato tuber infected with *Fusarium* dry rot and cut open through the dry rot lesion.

entire seed potato production area in Michigan, which comprised 950 ha. On average, seed lots of about 70 public and 60 proprietary potato cultivars are produced in Michigan annually and the fields vary in size from 0.2 to 50 ha (29). The tuber samples collected in this study represented potato harvested from 51 seed lots (total) and comprised eight public cultivars ('Dark Red Norland', 'Russet Burbank', 'Katahdin', 'Atlantic', 'Eva', 'Goldrush-CF9-5', 'Snowden', and 'CFT-6') as well as five proprietary cultivars. The seed tubers all originated in Michigan and were from seed lots that had achieved "blue tag" certification from the MPSA and the Michigan Department of Agriculture. The class year was not specified on the samples. The average number of tubers sampled was 10/seed lot in 2008 whereas, in 2009, the number of tubers in a sample varied from 5 to 10 tubers/seed lot.

All the symptomatic sampled tubers had shallow or sunken and wrinkled necrotic areas that were discolored light to dark brown, indicating the presence of dry rot symptoms. Small pieces (0.5 cm in diameter, six pieces/tuber) were cut from the margins of the tuber flesh showing dry rot symptoms using a sterile scalpel, and then each piece of tuber was surface disinfested in 0.5% sodium hypochlorite for 10 s, rinsed twice in sterile distilled water, and blotted dry with sterile filter paper. The tissue pieces were plated on half-strength potato dextrose agar (PDA; Difco Laboratories) amended with streptomycin at 0.5 g/liter. The petri dishes were incubated at 23°C for 5 to 7 days in alternating 12 h of light and 12 h of darkness.

Cultures resembling *Fusarium* spp. were transferred onto plates of water agar. To generate pure cultures, hyphal tip transfers from each colony to carnation leaf agar and half-strength PDA were done from the margin of actively growing isolates using a sterile probe. The pure cultures were identified to species based on conidial morphology, production of chlamydospores, growth characteristics, and colony pigmentation as described by Leslie et al. (27). To confirm species identity, lyophilized mycelium from each pure culture grown on PDA for 7 days was used for DNA extraction as described by Goodwin et al. (18), followed by amplification and sequencing of the translation elongation factor 1 α gene region (17). The *Fusarium*-ID (17) and the National Center for Biotechnology Information (GenBank) databases were used to obtain the closest match in nucleotide sequences (lowest *e* value, maximum coverage, and highest identity) to the same gene regions of previously sequenced isolates of *Fusarium*.

Pathogenicity test. All 228 *Fusarium* isolates were tested for pathogenicity on 3-month-old, asymptomatic potato tubers of Dark Red Norland. Dark Red Norland was used as a representative of seed potato cultivars produced in Michigan because no cultivar is resistant to dry rot (39). The tubers were each surface disinfested for 10 min in 0.5% sodium hypochlorite, and rinsed twice in sterile water. Three replicate tubers/isolate were each injected with 20 μ l of a conidial suspension (approximately 10^6 conidia/ml as determined by a hemacytometer) of the appropriate *Fusarium* isolate grown on PDA for 7 days; three control tubers were each injected with 20 μ l of sterile distilled water. The tubers (three tubers/temperature/isolate) were incubated in the dark for 30 days at 4 and 10°C. The two storage temperatures were used to compare virulence of all the *Fusarium* isolates recovered. After 30 days of incubation, tubers were cut in half through the point of inoculation and evaluated for the development of symptoms typical of potato dry rot: dry, necrotic areas discolored light to dark chocolate brown on the tuber surface (Fig. 1). Isolates that caused symptoms on all three tubers at both temperatures were considered pathogenic. The fungi were reisolated (as described above) from all the symptomatic potato tubers and compared morphologically with the original isolate used in each inoculation. Isolation from the control tubers was also done to demonstrate that the seed tubers did not have natural infection by *Fusarium* spp.

Virulence test. To assess the virulence level of the *Fusarium* isolates, images of the symptomatic areas on the inoculated tuber surfaces were generated from a method adapted from Niemira et al. (31), described below. The freshly cut tuber sections were

placed on a piece of glass (30 cm by 40 cm by 2 mm), with the cut tuber surface facing down. A ruler was placed underneath the glass and used for measurements during image analysis. The glass and ruler were transferred to a flatbed scanner (HP Scan-Jet 4c; Hewlett Packard Co.) for image processing. Scanner control software (DeskScan II version 2.4; Hewlett Packard Co.) generated an image of the cut tuber surfaces against a black background (30). The image files created were first loaded into Adobe Photoshop CS3 (version 10.01, 2007; Adobe Systems Incorporated), from which the lesions were selected on each image and “painted” white using the “fill” tool. The images were then loaded into SigmaScan Pro 5 (version 1987-1999; SPSS Inc.) to determine the area of the lesion as described by O’Neal et al. (36), with modifications as follows. The lesion length and width (in millimeters) were measured to calculate the dimension and area (in square millimeters) of the lesions, and calibrated to convert image pixels to a unit of measurement using the ruler within the image. The measurement “fill” was then adjusted to a threshold so that the lesion was a lighter color than the rest of the tuber surface. The area of the lesion was then measured according to the SigmaScan manufacturer’s protocol.

Fungicide sensitivity assays. All the *Fusarium* isolates identified were screened for fungicide sensitivity using two screening methods described below. Two *F. sambucinum* isolates, thiabendazole-resistant (R-09271-Desjardins YG-1 U7200A) and thiabendazole-sensitive (R-00738, R. C. Cetas), were used as standard isolates for comparison with the 228 isolates in the thiabendazole-sensitivity assay. A conidial suspension (approximately 10^6 conidia/ml) of each isolate was prepared as described above. Three fungicides were used as formulated products in aqueous suspensions: fludioxonil (0.5% a.i., formulated as Maxim; Syngenta Crop Protection), thiabendazole (42.3% a.i., formulated as TBZ or Mertect; Syngenta Crop Protection), and difenoconazole (23.2% a.i., formulated as Inspire; Syngenta Crop Protection). A stock concentration of 10,000 mg/liter was prepared for each fungicide by dissolving the fungicide in sterile distilled water. Aliquots were made each day of the experiment for the spiral gradient dilution method. Serial dilutions from 0 to 100 mg/liter were also prepared for the serial dilution plate (SDP) method.

Spiral gradient dilution method. The fungicide spiral gradient dilution (SGD) method was used to estimate the effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) values as described by Förster et al. (14). PDA (50 ml) was poured into each 15-cm-diameter petri dish at least 24 h before each fungicide suspension was applied. A 50- μ l fungicide suspension from each of the stock suspensions was applied with a spiral plater (SGE; Spiral Biotech, Inc.) using the exponential deposition mode. The plates were incubated for 3 h to allow the fungicides to diffuse into the agar medium and form a gradient of concentration along the radius of the plate. Each plate was placed on the template provided by SGE. The template was a transparent polyester sheet marked as a circle with internal concentric radial lines at 2-mm intervals from 20 to 64 mm with 15 marked polygons (6 by 45 mm running from the outside to the center of the circle at equally spaced positions from 0 to 315°, cutting across the radial lines for positioning the samples). Droplets of the appropriate conidial suspension (10 μ l each) were spread across the radial lines in predetermined plate positions using a sterile plastic pestle. Three replicate plates per isolate were used for each fungicide and the experiment was conducted twice. Control treatments consisted of PDA plates without fungicides added, to which the appropriate conidial suspension was applied with a sterile plastic pestle. The plates were incubated at 25°C for 3 days. Radial growth of the fungal isolate on each replicate plate was measured and the values averaged. The radial distance between the center of the plate and the location on the plate where growth was inhibited 50% compared with the control plate was used in the SGE software to calculate the EC_{50} values of each *Fusarium* isolate.

SDP method. Plates of PDA were amended with each of the fungicides listed above at concentrations of 0, 0.1, 1.0, 10.0, or

100.0 mg/liter. Colonized agar plugs (5 mm in diameter) were cut for each isolate from the margin of colonies and placed on the amended agar medium with the colonized-side of the agar plug in direct contact with the agar, and incubated for 5 days in the dark at 25°C. Colony diameter was measured with a caliper after 5 days and the diameter of the inoculation plug subtracted. Fungal growth was expressed as percent inhibition of the fungal growth compared with colony diameter on the control plates without fungicide. Three replicate plates per treatment were used, and the experiment was conducted twice. EC_{50} values for each isolate were calculated by regression analysis of percent growth inhibition versus the log fungicide concentration using Sigmaplot (version 9.01; Systat Software, Inc.). Fludioxonil insensitivity was defined as lack of growth inhibition of an isolate on PDA containing fludioxonil at 100 mg/liter (38). Insensitivity for difenoconazole and TBZ were defined as $EC_{50} \geq 5$ mg/liter based on previous thresholds set for these fungicides (19,34). Each isolate was classified as sensitive or resistant to each of the three fungicides based on these thresholds. Each experiment was arranged in a completely randomized design and data analysis was completed as described below.

Statistical analysis. All data analyses were conducted with JMP (version 8.0; SAS Inc.), except for the regression analyses. Data for the repeated experiments were first tested for homogeneity of variances using Bartlett’s test of homogeneity. The data were also tested for significance of the main effects and interactions. Because variances were homogeneous for each experiment and no significant differences were measured between the repeats of any of the experiments, the data were combined and analyzed statistically using analysis of variance. Means separation was performed using Tukey’s honestly significantly difference test.

Results

***Fusarium* isolation and identification.** Over the 2-year survey, 228 *Fusarium* isolates were recovered and identified to 11 species (Table 1). The proportion of the recovered isolates identified per species remained markedly consistent over the 2-year period despite a smaller sample size in 2010 (110 potato tubers) compared with 2009 (260 potato tubers), with the exception that *F. sporotrichioides* and *F. graminearum* each were isolated in only 1 of the 2 years. *F. oxysporum* was the most commonly isolated species, comprising >25% of the isolates both years (Table 1). *F. equiseti* was the second most commonly isolated species in both years (Table 1). *F. sambucinum* and *F. avenaceum* were third in prevalence, each comprising 13.6% of the isolates averaged over both years (Table 1). Less prevalent *Fusarium* spp. (4 to 10% incidence) included *F. cerealis*, *F. solani*, and *F. acuminatum*. Other *Fusarium* spp. (3% each) included *F. sporotrichioides*, *F. torulosum*, *F.*

Table 1. Relative frequencies of *Fusarium* spp. isolated from symptomatic seed potato tubers sampled during 2009 and 2010 from seed lots produced in Michigan

<i>Fusarium</i> spp.	Relative frequency (%) of isolated species ^z		
	2009	2010	Total
<i>Fusarium oxysporum</i>	28.8	25.9	30.3
<i>F. equiseti</i>	23.0	19.0	19.3
<i>F. sambucinum</i>	14.9	13.3	13.6
<i>F. avenaceum</i>	11.1	17.1	13.6
<i>F. solani</i>	9.5	5.2	7.5
<i>F. cerealis</i>	6.3	5.1	6.1
<i>F. acuminatum</i>	4.1	6.9	4.4
<i>F. torulosum</i>	1.4	3.1	2.2
<i>F. tricinctum</i>	0.9	3.1	1.8
<i>F. sporotrichioides</i>	0.9	0.0	0.9
<i>F. graminearum</i>	0.0	1.7	0.4
Total number of isolates	169	59	228
Total number of tubers sampled	260	110	370

^z Relative frequency = percentage of isolates of a given species relative to the total number of isolates recovered in 2009 and 2010 (169 and 59 isolates), respectively.

tricinctum, and *F. graminearum*. The majority of isolates from dry rot lesions were identified as *Fusarium* spp. (61.6%). The bulk of the remaining fungal isolates were *Alternaria* spp. that were not identified to species. Of the 38.4% of the isolates not identified to species, 5% were *Fusarium*-like but not *Fusarium*, because they produced white to cream-colored, dense mycelia, with no spore formation.

Pathogenicity. All the *Fusarium* isolates recovered were pathogenic to potato tubers of Dark Red Norland at both 4 and 10°C (Fig. 2). The inoculated tubers developed typical potato dry rot symptoms of a brown and dry decay regardless of which isolate of *Fusarium* was inoculated. No dry rot symptoms were observed on the noninoculated control potato tubers.

Virulence. No significant differences in virulence were measured between the two executions of the experiments at each of the temperatures (Table 2); therefore, data were combined for subsequent analyses. The average lesion diameters on inoculated tubers were 52.99 and 55.65 mm² from experiments 1 and 2, respectively, at 10°C; and 43.79 and 43.67 mm², respectively, at 4°C (Table 2).

Significant differences in virulence among isolate of the 11 *Fusarium* spp. were evident (Table 3). Overall, *F. sambucinum* was the most virulent species, causing significantly ($P = 0.0001$) larger

lesions compared with that of the other species at both 4 and 10°C storage temperatures. The rest of the species did not differ significantly with respect to diseased area on inoculated tubers at either temperature. There was a significant ($P = 0.0001$) difference in virulence within isolates of *F. sambucinum* but not within isolates of the rest of the species (*data not shown*). The same fungal species was reisolated from the diseased area of the inoculated tubers, thus completing Koch's postulates for each species. The lesion area observed on inoculated tubers incubated at 10°C was not significantly ($P = 0.6774$) different from that of tubers stored at 4°C.

Fungicide sensitivity of dry rot *Fusarium* spp. No significant differences in fungicide sensitivity were measured between the two executions of the experiments for any of the fungicides tested or the two methods used for testing sensitivity (SGD and SDP analyses; Tables 2 and 4); therefore, data across repeat experiments were combined for subsequent analyses within each method of testing for fungicide sensitivity. The SGD method of testing fungicide sensitivity gave slightly lower EC₅₀ values compared with the SDP method for the 228 isolates of *Fusarium* tested; however, the two methods were not significantly different ($P = 0.7752$) and the EC₅₀ values were within similar ranges for both methods with each of the three fungicides tested (Table 5).

Fig. 2. Pathogenicity of *Fusarium* isolates obtained from Michigan potato seed pieces with dry rot symptoms and inoculated onto seed potato tubers ('Dark Red Norland'). Isolates of the following species were tested: **a**, *Fusarium sambucinum*; **b**, *F. avenaceum*; **c**, *F. tricinctum*; **d**, *F. acuminatum*; **e**, *F. cerealis*; **f**, *F. sporotrichioides*; **g**, *F. solani*; **h**, *F. equiseti*; **i**, *F. oxysporum*; **j**, *F. torulosum*; **k**, *F. graminearum*; and **l**, noninoculated control tubers.

Table 2. Results (P values) for the main effects in the analyses of variance for virulence and effective fungicide concentration that caused 50% inhibition of mycelial growth (EC₅₀) values for each of three fungicides calculated by the spiral dilution gradient (SGD) and serial dilution plate (SDP) methods for sensitivity of 11 species of *Fusarium* isolates, obtained from dry rot-symptomatic tubers in Michigan potato seed crops for a repeated experiment (experiments 1 and 2)

Dependent variables	Temperature, fungicide [†]	F ratio [‡]	Prob < F [‡]	Lesion area (mm ²)			EC ₅₀ (mg/liter) [§]		
				Exp 1	Exp 2 [‡]	HSD [¶]	Exp 1	Exp 2 [‡]	HSD [¶]
Virulence ^x	10°C	0.2059	0.6501	52.99	55.65	11.502	...	...	...
	4°C	0.0007	0.9791	43.79	43.67	9.722	...	...	...
SGD [‡]	Difenoconazole	1.6551	0.1989	...	...	...	1.68	1.77	0.133
	Fludioxonil	0.0000	1.0000	...	...	...	1.99	1.99	0.141
	Thiabendazole	1.9641	0.1619	...	...	...	2.74	2.89	0.201
SDP [‡]	Difenoconazole	2.7876	0.0957	...	...	...	1.88	1.70	0.213
	Fludioxonil	1.3436	0.2474	...	...	...	2.77	2.93	0.263
	Thiabendazole	0.0319	0.8583	...	...	...	3.73	3.71	0.225

[§] EC₅₀ was calculated by regression analysis of percent growth inhibition versus the log fungicide concentration using Sigmaplot.

[†] Storage temperature or fungicide tested.

[‡] Model mean square divided by the error mean square and Prob < F lists the P value for the test. Values of 0.05 or less were considered evidence that there was at least one significant effect in the model.

[‡] Exp = experiment; Exp 2 is a repeat of Exp 1.

[¶] HSD = Tukey's Honestly significant difference of the comparison between repeats of the experiments for each measured variable.

^x Virulence was estimated as the mean area of the lesion after inoculation of potato tubers (cv. Dark Red Norland).

[‡] SGD method (14,21).

[‡] SPD method (14,21).

Difenoconazole. All the *Fusarium* isolates were sensitive to difenoconazole using both screening methods based on the criterion of an EC₅₀ value <5 mg/liter (Table 5). *F. equiseti* had a significantly greater EC₅₀ compared with that of *F. graminearum*, *F. sporotrichioides*, *F. torulosum*, *F. acuminatum*, *F. avenaceum*, and *F. sambucinum* based on the SGD method but not the SPD method.

Fludioxonil. In both experiments, sensitive and insensitive isolates of *F. sambucinum* and *F. oxysporum* were identified. The insensitive isolates for each species had EC₅₀ values >100 mg/liter and represented 8.9% of the *F. sambucinum* isolates and 20.4% of the *F. oxysporum* isolates, respectively (Fig. 3). In all, 6 isolates of *F. sambucinum*, 14 isolates of *F. oxysporum*, and all isolates of the other nine species were sensitive to fludioxonil, with EC₅₀ values <5 mg/liter (Table 5). *F. sambucinum* isolates had a significantly greater EC₅₀ compared with isolates of *F. avenaceum* and *F. cerealis* when the SGD method was used (Table 5).

Thiabendazole. Insensitivity to thiabendazole was observed for some of the *Fusarium* isolates tested based on growth on PDA containing TBZ at 5 mg/liter. All isolates of *F. sambucinum* and the known TBZ-insensitive *F. sambucinum* isolate (R-09271) were insensitive to TBZ, with EC₅₀ values >100 mg/liter. However, isolates from the rest of the species and the TBZ-sensitive *F. sambucinum* isolate (R-00738) were sensitive to TBZ, with EC₅₀ values <5 mg/liter (Table 5). *Fusarium* spp. isolates with EC₅₀ values from

2.9 to 2.2, 2.6 to 1.6, and 2.3 to 1.0 mg/liter and 4.4 to 3.0, 3.6 to 2.3, 3.6 to 1.8, and 3.1 to 1.6 mg/liter were not significantly different when the SGD and SPD methods, respectively, were used to determine different degrees of sensitivity to TBZ (Table 5).

Discussion

F. sambucinum has been the predominant causal agent of potato tuber dry rot in storage and seed piece decay in the northern United States, including Michigan (25,42). However, in this 2-year survey, 11 *Fusarium* spp. were recovered from diseased seed tubers produced in Michigan. The most frequently isolated species was *F. oxysporum*, which concurs with similar reports from other areas of the United States (19,34). All of the isolates of *F. oxysporum* were pathogenic to potato tubers of Dark Red Norland. The prevalence of *F. oxysporum* may pose a challenge to potato growers because the isolates of *F. oxysporum* have also been found to cause wilting of potato plants (28,42). It is not known whether the isolates that cause dry rot can also cause wilt; therefore, this warrants further investigation (46).

F. equiseti was the second most commonly isolated species. Leslie et al. (27) indicated that *F. equiseti* can be divided into biological species using molecular techniques, thus making it a species complex. This species complex has not been reported previously from potato tubers in Michigan. However, Hanson et al. (19)

Table 3. Virulence of *Fusarium* isolates on potato tubers ('Dark Red Norland') based on the area (in square millimeters) of the dry rot lesion on cut tubers after 30 days of incubation of the inoculated tubers at 4 and 10°C^z

<i>Fusarium</i> spp.	Number of isolates	Incubation temperature			
		4°C		10°C	
		Range	Mean	Range	Mean
<i>Fusarium sambucinum</i>	31	12.9–589.9	234.2 a	6.9–1186.8	250.6 a
<i>F. tricinctum</i>	4	9.7–17.5	14.3 b	27.6–85.2	47.3 b
<i>F. sporotrichioides</i>	2	8.9–20.9	14.5 b	17.6–46.7	33.4 b
<i>F. cerealis</i>	17	13.0–20.9	17.2 b	1.3–351.4	31.9 b
<i>F. torulosum</i>	5	12.0–12.7	15.4 b	5.4–64.8	30.2 b
<i>F. equiseti</i>	44	8.1–31.1	17.6 b	8.7–52.9	29.6 b
<i>F. solani</i>	13	9.5–26.1	17.4 b	7.5–64.8	29.0 b
<i>F. oxysporum</i>	69	9.9–30.3	17.4 b	8.2–89.4	28.6 b
<i>F. avenaceum</i>	31	8.3–35.0	17.2 b	3.0–194.8	19.8 b
<i>F. acuminatum</i>	11	6.4–32.5	14.6 b	4.2–29.5	11.3 b
<i>F. graminearum</i>	1	1.5–1.6	1.6 b	2.2–2.4	2.3 b
Noninoculated control	...	0.0	0.0 b	0.0	0.0 b
HSD	...	...	144.3	...	157.3
Prob <i>F</i> (<i>P</i> < 0.05)	...	...	<0.0001	...	<0.0001

^z Mean area of the lesion for all isolates within a species (mean of three replicates per isolate) and the experiment was conducted twice. Data were pooled, because there were no significant differences between the two experiments at either temperature (Table 2). Numbers followed by the same letter within a column are not significantly different at *P* = 0.05 by Tukey's honestly significantly difference (HSD) test.

Table 4. Results (*P* values) of the analyses of variance (ANOVAs) for the main effects of *Fusarium* spp. and isolates of each species, and interactions between these two factors on effective fungicide concentration that caused 50% inhibition of mycelial growth (EC₅₀) values when the isolates were tested for sensitivity to difenoconazole, fludioxonil, and thiabendazole using the spiral gradient dilution (SGD) and serial dilution plate (SDP) methods^y

Fungicide	ANOVA factors	<i>P</i> values ^z			
		SGD experiments		SDP experiments	
		1	2	1	2
Difenoconazole	Species	<0.0001	<0.0001	<0.0001	<0.0001
	Isolate	0.0722	0.7222	0.9991	0.9992
	Species × isolate	0.8939	0.8939	0.5636	0.5665
Fludioxonil	Species	<0.0001	<0.0001	<0.0001	<0.0001
	Isolate	0.9928	0.9929	0.4677	0.4677
	Species × isolate	0.1897	0.1897	0.8829	0.8829
Thiabendazole	Species	0.6992	0.7024	<0.0001	<0.0001
	Isolate	1.0000	1.0000	0.9994	0.5994
	Species × isolate	0.0041	0.0041	0.0481	0.0481

^y EC₅₀ was calculated by regression analysis of percent growth inhibition versus the log fungicide concentration using Sigmaplot. The experiment was conducted twice.

^z Probability value (values of 0.05 or less were considered evidence that there was at least one significant effect in the model). For SGD and SDP, three replicates of each isolate were tested independently with each fungicide and the experiment conducted twice (14,21). Experiment 2 was a repeat of experiment 1.

reported the *F. equiseti* species complex associated with potato tubers in the northern United States, although at a low frequency (0.7%) in comparison with the incidence observed in this Michigan study (19.3%). *F. equiseti* has also been reported in other areas of the world (44,45) and is a potential human pathogen capable of producing mycotoxins (27). A high prevalence in potato tubers may be of concern for human food safety because *F. equiseti* is also allergenic to humans.

F. sambucinum and *F. avenaceum* were third most prevalent in this Michigan potato seed tuber survey. *F. sambucinum* has been reported as a commonly isolated *Fusarium* sp. from dry-rot-symptomatic tubers in the northeastern United States (19) and the Columbia Basin of Oregon and Washington (34) but is less common than *F. oxysporum* (19,34). Although *F. sambucinum* was not the most prevalent species in this study, isolates of this species were the most virulent, causing significantly larger lesions than isolates of the other 10 species evaluated at both 4 and 10°C tuber incubation temperatures. These results are in agreement with those of Ocamb et al. (34), who reported that *F. sambucinum* isolates caused relatively larger areas of tuber decay compared with isolates of *F. oxysporum* and *F. solani*. Several authors have reported that *F. sambucinum* is the major dry rot pathogen of potato tubers in the northern United States (10,42). However, this study indicated there were at least four other *Fusarium* spp. which caused the development of tuber lesions >30 mm², although three of these four species were found at a low frequency (<4%) over the 2 years of this Michigan study. The four species included *F. tricinatum*, *F. sporotrichioides*, *F. torulosum*, and *F. cerealis* but, compared with the relatively small areas of tuber decay these isolates caused compared with isolates of *F. sambucinum*, they may not be major potato pathogens.

A preliminary report was published in 2011 on the presence of *F. torulosum* as a potato pathogen in the United States (15). However, *F. torulosum* was only separated from *F. sambucinum* in 1995 (32) and, therefore, may have been present in potato tubers previously but not recognized. Although *F. avenaceum* is most commonly reported as a cereal pathogen (27), the prevalence of this species in this study was the same as that of *F. sambucinum*. In the northern U.S. potato regions, *F. avenaceum* has been less commonly reported from potato tubers than *F. oxysporum*, *F. solani*, and *F. sambucinum* (19) whereas, in the United Kingdom, *F. avenaceum* has been recovered at greater frequencies (8,37), and the species has been reported in other northern European countries

such as Finland (43). The prevalence of *F. avenaceum* isolates in Michigan potato seed lots may have an implication for crop rotations because potato is usually rotated with cereal crops. *Fusarium* spp. being both seed- and soilborne indicates that the primary sources of inoculum may be infected seed tubers or infested soil (42). The presence of inoculum in the soil or in seed tubers could lead to infection of the tubers through wounds inflicted during harvesting and dry rot development during storage. All Michigan isolates of *F. avenaceum* tested in this study were pathogenic to seed tubers of Dark Red Norland at both 4 and 10°C, although the virulence of the isolates was low at both temperatures. In contrast, Aprasad et al. (4) found that isolates of *F. avenaceum* were slightly more virulent at 5 than at 10°C. In Scotland, isolates of *F. avenaceum* caused more dry rot compared with isolates of *F. solani* var. *coeruleum* (7,8).

Only one isolate of *F. graminearum* was isolated from Michigan seed lots in this study, and caused small dry rot lesions when

Fig. 3. Frequency of *Fusarium sambucinum* and *F. oxysporum* isolates sensitive (gray bars) and insensitive (black bars) to fludioxonil, obtained from Michigan potato seed tubers with dry rot symptoms. The frequency was calculated relative to the total number of *F. sambucinum* and *F. oxysporum* isolates tested for fludioxonil sensitivity ($n = 14$ and 6 , respectively). Each data point represents the mean and standard deviation of the mean.

Table 5. Effective fungicide concentration that caused 50% inhibition of mycelial growth (EC_{50}) of *Fusarium* spp. causing potato dry rot as determined by the spiral gradient dilution (SGD) and serial dilution plate (SDP) methods^v

<i>Fusarium</i> spp.	N ^x	EC_{50} for inhibition of mycelial growth (mg/liter) ^w					
		Difenoconazole		Fludioxonil		Thiabendazole	
		SGD	SDP	SGD	SDP	SGD	SDP
<i>Fusarium oxysporum</i>	68	1.7 abc	1.6 a	2.3 ab	4.3 a ^y	2.6 ab	2.6 bcd
<i>F. equiseti</i>	44	2.3 a	2.4 a	1.6 ab	3.5 a	2.2 abc	1.8 cd
<i>F. sambucinum</i>	31	1.4 bc	1.3 a	2.7 a ^y	3.3 a ^y	...	...
<i>F. avenaceum</i>	31	0.8 c	1.5 a	1.5 b	2.7 a	2.2 abc	3.6 abc
<i>F. cerealis</i>	18	1.9 abc	1.1 a	1.2 b	1.9 a	2.4 ab	4.4 a
<i>F. solani</i>	14	2.2 ab	3.1 a	2.4 ab	2.9 a	2.3 abc	3.1 abcd
<i>F. acuminatum</i>	11	1.1 bc	1.9 a	1.7 ab	1.3 a	2.4 ab	4.0 a
<i>F. tricinatum</i>	4	1.5 abc	2.3 a	2.9 a	2.9 a	2.9 a	2.3 bcd
<i>F. torulosum</i>	4	0.9 bc	0.6 a	1.6 ab	1.5 a	1.6 bc	3.6 abc
<i>F. sporotrichioides</i>	2	0.8 c	0.9 a	2.6 ab	2.8 a	1.1 c	1.6 d
<i>F. graminearum</i>	1	0.4 c	0.9 a	1.7 ab	2.3 a	1.0 c	1.9 cd
R-00738 ^z	1	...	...	...	...	2.6 ab	3.0 abcd

^v SGD and SDP methods (14,21).

^w EC_{50} was compared with control plates without fungicides (14). All values are means of two experiments, with three replicate petri dishes per experiment. Numbers followed by the same letter within a column are not significantly different at $P = 0.05$ by Tukey's honestly significantly difference test; ... indicates that data were not included for thiabendazole-insensitive *F. sambucinum* isolates or for isolates not from Michigan (R-00738, R. C. Cetas) = a thiabendazole-sensitive standard isolate. These data are shown in Figure 3.

^x Number of isolates.

^y Mean EC_{50} values of 14 isolates of *F. oxysporum* or 6 isolates of *F. sambucinum* were sensitive to fludioxonil. The rest of the isolates of *F. oxysporum* ($n = 54$) and *F. sambucinum* ($n = 25$) were insensitive to fludioxonil with $EC_{50} > 100$ mg/liter (Fig. 3).

^z R-00738 = thiabendazole-sensitive isolate of *F. sambucinum* (R-00738, R. C. Cetas).

inoculated onto healthy tubers of Dark Red Norland that were stored either at 4 or 10°C. This is in contrast to recent reports from the north-central United States, where *F. graminearum* has been reported to be the predominant dry rot pathogen on tubers stored at 10°C, although less virulent than isolates of *F. sambucinum* (13). *F. culmorum*, although previously reported from potato tubers (19,24,43), was not isolated in this study. This was the only species previously reported on potato in the United States that was not found on Michigan seed lots in this survey. The ability of isolates of all *Fusarium* spp. to cause dry rot at both 4 and 10°C storage temperatures indicated a potential for all of these species to infect both seed and chipping potato (9) but this should be investigated further.

Determination of baseline sensitivity levels of *Fusarium* isolates to new fungicides used for potato tuber seed treatments, and monitoring for sensitivity of the isolates measured, are important for management of dry rot pathogens (23). Monitoring studies help detect changes in sensitivity of pathogen isolates in the field (40) and become practically feasible when a relatively high frequency of fungicide-resistant isolates (>1%) is reached, according to the Fungicide Resistance Action Committee (6). In vitro testing of the EC₅₀ is a technique commonly used for monitoring shifts in sensitivity of pathogen populations to specific fungicides (40). Use of the SGD fungicide method for fungal pathogens to determine the EC₅₀ has been used widely (14). In this Michigan seed lot study, the SGD method was compared with the traditional SDP method (14,21) for determination of EC₅₀ values. Although significant differences were measured in EC₅₀ among isolates of the *Fusarium* spp. for all three fungicides tested using the SGD method but not the SDP method (for which only TBZ results showed significant differences in sensitivity among isolates), the results showed EC₅₀ values of the same order of magnitude for both methods.

Fludioxonil is a protectant fungicide used effectively to reduce potato seed piece decay and sprout rot (50). In vitro insensitivity to fludioxonil has been reported for isolates of *F. sambucinum*, *F. coeruleum* (39), *F. sambucinum*, and *F. oxysporum* (16). In this study, 20% of *F. oxysporum* isolates and 9% of *F. sambucinum* isolates were insensitive to fludioxonil. All isolates of the other nine species recovered were sensitive to fludioxonil. The discovery of insensitive isolates may pose a challenge to controlling *Fusarium* seed piece decay in seed tuber production in Michigan. Although it is not known whether the isolates tested had previously been exposed to fludioxonil during bulking of certified seed for commercial use, it is important to maintain good management practices during tuber cutting and storage of cut seed (49) prior to planting. This study did not provide compelling evidence to suggest that fludioxonil seed treatments had failed because of insensitivity of *Fusarium* spp. to this fungicide. However, the presence of fludioxonil-insensitive strains necessitates the development and registration of fungicides with different modes of action for use on potato tubers. If possible, fungicides with alternative modes of action should be used more for seed potato production than for commercial potato production.

In this study, resistance to TBZ was found only in isolates of *F. sambucinum*. This is in contrast to other studies in which other *Fusarium* spp. insensitive to TBZ have been reported, including *F. oxysporum*, *F. solani*, and *F. culmorum* (19,34); *F. avenaceum*, *F. equiseti*, and *F. sporotrichioides* (34); *F. acuminatum* (19); and *F. culmorum* (20). It is not clear why previous studies have found TBZ resistance in other species and no such resistance was observed in this Michigan seed lot survey but this indicates that TBZ could still be used to control *Fusarium* dry rot in Michigan seed production as long as the causal agent is not *F. sambucinum*. Regular evaluation of *Fusarium* spp. composition in seed lot production in Michigan is necessary to justify continued use of TBZ. In addition, because no other fungicides have been registered for postharvest use against *Fusarium* dry rot on potato in Michigan, cultural practices should not be neglected.

Although in vitro sensitivities to fludioxonil (38) and TBZ (19,37) have been reported previously for *Fusarium* spp. causing

potato dry rot, this is the first study to present baseline sensitivity of difenoconazole. Difenoconazole is a systemic demethylation inhibitor fungicide that has been recommended by Adaskaveg and Förster (1) for registration against potato tuber rots caused by *Fusarium* spp. Other triazoles, including prothioconazole and tebuconazole, have been tested against *Fusarium* spp. on crops such as wheat, with EC₅₀ values of 0.1 to 3.2 and 1.1 to 5.5 mg/liter, respectively (29). All the isolates tested in this Michigan study came from tubers that had not previously been exposed to difenoconazole because this fungicide had not been used previously in seed potato tuber production in Michigan. All 228 *Fusarium* isolates were sensitive to difenoconazole. However, monitoring for changes in sensitivity to difenoconazole among isolates of *Fusarium* spp. that can cause tuber dry rot is important. Although this study indicated that difenoconazole was effective against *Fusarium* spp. isolated from potato tubers in Michigan, Allen et al. (3) reported that difenoconazole had a limited effect on growth of *Fusarium* spp. isolated from pine seed. Difenoconazole has an additional benefit in the potential for activity against other potato pathogens. For example, Olaya et al. (35) reported that difenoconazole was effective at reducing growth of *Colletotrichum coccodes* and, hence, could be used for control of black dot of potato.

Acknowledgments

This research was funded, in part, by the Michigan AgBioResearch GREEN (Generating Research and Extension to meet Economic and Environmental Needs) Project GR 08/071, Michigan Potato Industry Commission, and supported in principal by the NCERA 211 (Quad State Potato Group), CSREES Hatch Project Number M1CL01966, and the Michigan Potato Industry Commission.

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