Sensitivity to Thiabendazole in *Fusarium* Species Associated with Dry Rot of Potato

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ABSTRACT

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Fusarium species associated with dry rot of potato tubers during 1992 and 1993 were characterized. We isolated fungi from wounds or preexisting lesions on randomly collected samples of seed, tablestock, and processing tubers, primarily from the northeastern United States. Of 154 samples, 99 yielded one or more *Fusarium* isolates, 98% of which were pathogenic on potato tubers. The most frequently recovered pathogenic species were *F. sambucinum*, *F. solani*, and *F. oxysporum*, but pathogenic isolates of *F. acuminatum*, *F. avenaceum*, *F. crookwellense*, *F. culmorum*, and *F. equiseti* also were isolated. Using logistic regression analysis, significant relationships were found between the *Fusarium* species isolated and factors such as tuber use, method of isolation, year of isolation, and state of origin of the sample. Of the 200 *Fusarium* isolates, 82 grew at ≥5 mg/liter of thiabendazole (TBZ) in V8 agar and were considered resistant to TBZ. These included isolates of *F. sambucinum*, *F. solani*, *F. oxysporum*, *F. acuminatum*, and *F. culmorum*. TBZ-resistant isolates were obtained from most locations and all tuber types. The effective dose for 50% reduction in growth differed among isolates of *F. sambucinum* and *F. solani*, suggesting that there may be multiple beta-tubulin mutations that confer resistance.

Additional keywords: *Fusarium coeruleum*, *Fusarium sulphureum*, *Gibberella pulicaris*, *Nectria haematococca*, *Solanum tuberosum*.

Fusarium dry rot is a postharvest disease of potato (*Solanum tuberosum* L.) of worldwide significance (11). Although comprehensive figures are lacking, average annual crop losses attributed to dry rot have been estimated at 6 to 25% (5), with reports that more than 60% of tubers in storage can be affected (4). The greatest losses occur during long-term storage of potato tubers, after growers have incurred most of the production costs. The fungi that cause dry rot are spread readily among tubers during handling and planting, and are an important cause of seed tuber rot and poor stands (11).

Fusarium dry rot is also of great importance to the consumer, because some of the *Fusarium* species that cause dry rot also produce mycotoxins (2,7,21). One major group of mycotoxins produced are trichothecenes, which are inhibitors of eukaryotic protein synthesis (30) and can pose serious health problems, both acute and chronic, for animals and humans (21,30,34).

Many species of *Fusarium* cause dry rot. The predominant causal agents are *F. solani* (Mart.) Sacc. (11,31) (teleomorph: *Nectria haematococca* Berk. & Broome), also called *F. coeruleum* (Libert) Sacc. (24); *F. oxysporum* Schlecht. (29,31); and *F. sambucinum* Fuckel (11) (teleomorph: *Gibberella pulicaris* (Fr.) Sacc.), orange isolates of which are also called *F. sulphureum* Schlecht. (24). Other species also have been reported to cause dry rot, including *F. acuminatum* Ellis & Everh. (teleomorph: *G. acuminata* Wollenweb.) (28,29,31), *F. avenaceum* (Fr.) Sacc. (teleomorph: *G. avenacea* R. J. Cook) (10,11,28), *F. crookwellense* L. W. Burgess, P. E. Nelson & T. A. Toussoun (9,31), *F. culmorum* (Wm. G. Smith) Sacc. (10,28), *F. equiseti* (Corda) Sacc. (teleomorph: *G. intricans* Wollenweb.) (29,31), *F. graminearum* Schwabe (teleomorph: *G. zeae* (Schwein.) Petch) (28,31), *F. scirpi* Lambotte & Fautrey (31), *F. semitectum* Berk. & Ravenel. (29), *F. sporotrichioides* Sherb. (28), and *F. tricinctum* (Corda) Sacc. (28). However, these species are usually considered to be of lesser importance.

Because all potato cultivars grown commercially in the United States are susceptible to dry rot (20), control of Fusarium dry rot has been accomplished primarily by postharvest applications of thiabendazole (TBZ), a benzimidazole fungicide. This is the only fungicide registered for postharvest control of dry rot in the United States, and has been used extensively in potato production here since the early 1970s (19). After about 20 years of use on seed and stored potatoes, dry rot control failures were reported (27). Desjardins et al. (6) reported a shift in the TBZ sensitivity of *F. sambucinum* isolates. All isolates collected between 1963 and 1986 were TBZ sensitive, while the majority of isolates collected in 1990 and 1991 were resistant to TBZ at 10 mg/liter (6).

Reports of TBZ resistance in *Fusarium* species in the United States were preceded by reports of resistance in Europe. TBZ-resistant isolates of *F. sambucinum* were first discovered in Europe in 1986 (33). Resistant isolates of *F. solani* were found in Germany in 1990 (17) and *F. culmorum* with TBZ resistance was found in the United Kingdom in 1991 (10). TBZ resistance in species other than *F. sambucinum* has not been reported outside Europe.

Characterization of the fusaria causing dry rot and a quantitative assessment of the sensitivity of *Fusarium* populations to TBZ are needed to determine the potential impact of fungicide resistance on dry rot control, and to aid development of management strategies, including fungicide recommendations. Information on

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the factors that affect tuber infection can also be important in the development of disease management strategies. Our major objectives were to determine which *Fusarium* species are associated with dry rot in the northeastern United States and to evaluate their sensitivity to TBZ. We also examined the effects of tuber type, state of origin, method of isolation, and previous exposure of samples to benzimidazoles on TBZ sensitivity and species distribution.

MATERIALS AND METHODS

Isolate collection. Potato tubers were collected in 1992 and 1993 from cooperators throughout the northeastern United States. Tubers were collected without regard to Fusarium dry rot symptoms, to obtain an unbiased estimate of the frequency of TBZ-resistant isolates in the *Fusarium* population associated with commercially produced tubers in the northeastern United States. The 154 samples of 10 to 20 tubers each included seed, tablestock, and processing potatoes. Data on year, location, tuber use, potato cultivar, and postharvest fungicide treatment were catalogued.

In order to maximize the number of *Fusarium* isolates recovered from tubers, three methods of isolation were used. Up to six tubers with existing lesions were arbitrarily selected from each sample, and additional symptomless tubers were selected for a total of 10 tubers per sample. From tubers with existing lesions, fungi were isolated by plating necrotic tissue excised from the edge of lesions in interior tuber tissues onto 2% water agar (WA) amended with streptomycin (300 mg/liter) and penicillin G (100 mg/liter). Isolates were transferred to WA and V8 agar (200 ml of V8 strained through two layers of cheesecloth, 5 g of calcium carbonate, and 14 g of agar per liter of media) plates for short-term storage. Isolates were grown on V8 agar slants for 4 to 5 days and stored at 4°C. Symptomless tubers were separated into two groups. Approximately half of the tubers were cut with a sterile knife and shaken for about 10 s in a paper bag with any soil carried with the initial sample (32). The remaining tubers were bruised by dropping from a height of 1.5 m onto a cement floor that had been surface disinfested with 0.5% sodium hypochlorite. Tubers were placed in paper bags, incubated at 24 to 28°C for 1 to 2 weeks, and examined for lesions. Isolates were obtained from lesions that developed from wounds, as described previously. Isolations were also made from nonwounded control tubers and wounded tubers without lesions.

Isolate characterization. The species of each *Fusarium* isolate was determined using techniques described by Nelson et al. (24). Single macroconidia were transferred to potato-dextrose agar slants (24) to check pigment production, and to carnation leaf agar (24) for sporulation. Cultures were examined weekly for up to 4 weeks for pigment production and spores. Each isolate was examined three times, from three separate macroconidia on each medium.

All isolates were tested for pathogenicity on disease-free potato tubers, cv. Sebago. Tubers were surface disinfested for 15 min in 0.5% sodium hypochlorite and rinsed twice in sterile distilled water. Tubers were cut in half with a sterile knife. One cut surface was pressed for 30 s onto a petri plate on which a *Fusarium* isolate had grown to a colony diameter of 90 to 100 cm. The cut surfaces of the tubers were placed together, loosely held together with tape, incubated at 22 to 26°C for 1 week, and then examined for symptoms. Control tubers were pressed onto plates of sterile WA or a nonpathogenic *F. oxysporum* isolate for negative controls, and onto a culture of a known pathogen as a positive control. Isolates that produced lesions on tuber surfaces after 2 weeks were considered pathogenic. Isolations were made from tubers inoculated with each species to confirm the presence of *Fusarium* in lesions. Two tubers were inoculated per isolate in each of two experiments.

Methods for assessment of benzimidazole resistance are not standardized (6,10,33,35). We chose to use radial growth in vitro (6,10,17,33,35) at 5 mg/liter (10) as our criterion for TBZ resistance in *Fusarium* species. Technical grade TBZ (Sigma Chemical Co., St. Louis) was dissolved in ethanol and added to molten V8 agar to give TBZ concentrations of 0, 5, 10, 25, and 50 mg/liter and an ethanol concentration of 1% (vol/vol). A plug of mycelium from the edge of a rapidly growing culture was placed on a plate and incubated at 22 to 26°C for 5 days. Three replicate plates of each concentration were used for each isolate. Known TBZ-sensitive (RN-5) and TBZ-resistant (RN-1) isolates of *F. sambucinum*, generously provided by A. E. Desjardins (USDA-ARS, Peoria, IL), were used as controls in the TBZ-sensitivity assays. Colony diameters on fungicide-amended and control plates were measured and compared. Isolates that grew away from the agar plug at 5 mg/liter of TBZ or higher were designated as resistant to TBZ.

A dose-response curve, based on average radial growth at each of the TBZ concentrations, was prepared for each TBZ-resistant isolate. Curves were transformed to log growth-log concentration, and the effective dose for 50% reduction in growth (ED_{50}) was determined from this curve.

The growth rate for each isolate was determined on control plates (V8 agar with 1% ethanol). At 5 days, the colony diameter, minus the diameter of the agar plug, was determined as an average of three plates for each isolate and divided by the number of days, to give average growth rate.

Statistical analyses. A multiple logistic regression model (12) was used to analyze the data from the isolates to identify predictor variables strongly associated with TBZ sensitivity.

To investigate the factors associated with TBZ sensitivity, the samples in which infection occurred were included in the analyses. The data consisted of the binary response Y_i, which was TBZ resistant or TBZ sensitive, and values for a set of possible predictor variables for the ith infected sample. The possible predictors examined were indicator variables representing the isolate species (*F. sambucinum*, *F. solani*, or *F. oxysporum*), tuber use (seed, tablestock, or processing), the state of origin (New York, Maine, Pennsylvania, or other), the method of isolation (existing lesion or wound), year (1992 or 1993), exposure of the sample to benzimidazoles, and two-factor interactions of these.

The goal of the logistic regression analysis was to determine which predictors were associated significantly with the presence or absence of TBZ resistance. Best subsets (also known as all-possible subsets) regression (12) was used to find good models containing one predictor, two predictors, etc., which were examined individually in detail. Two kinds of models were considered: models containing only main effects, and models including two-factor interactions as well as main effects. The computer analysis was performed using the procedure LOGISTIC in the Statistical Analysis System, version 6.07 (SAS Institute, Cary, NC).

The goodness-of-fit of models derived from logistic regression was assessed by using log likelihood (LL) statistics (12). A model or model improvement was considered statistically significant if the corresponding G attained the 0.05 level of statistical significance.

Classification tables (12) were used to supplement the LL measures of model fit. The observed responses were cross-classified with the responses predicted by the estimated model. Good predictive accuracy, i.e., a high proportion of responses correctly predicted, constituted evidence that prediction of response based on the logistic regression model gave improved results over prediction of response without this model. The LL tests were used as the primary analyses, supplemented by the classification tables.

The same logistic regression approach was used to examine the factors associated with infection of tubers by *F. sambucinum*, *F. solani*, and *F. oxysporum*. Each species was analyzed separately, because different factors may influence the infection process for

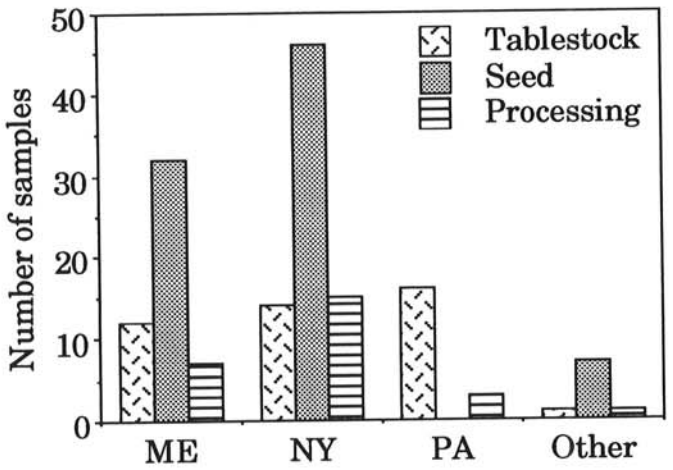

Fig. 1. Distribution of potato tuber samples by state of origin and tuber type. The seed tuber samples included in the "other" category were collected in the Northeast, but produced in Michigan, Nebraska, South Dakota, or New Brunswick. Tablestock and processing tuber samples in the "other" category were collected from Connecticut or New Jersey.

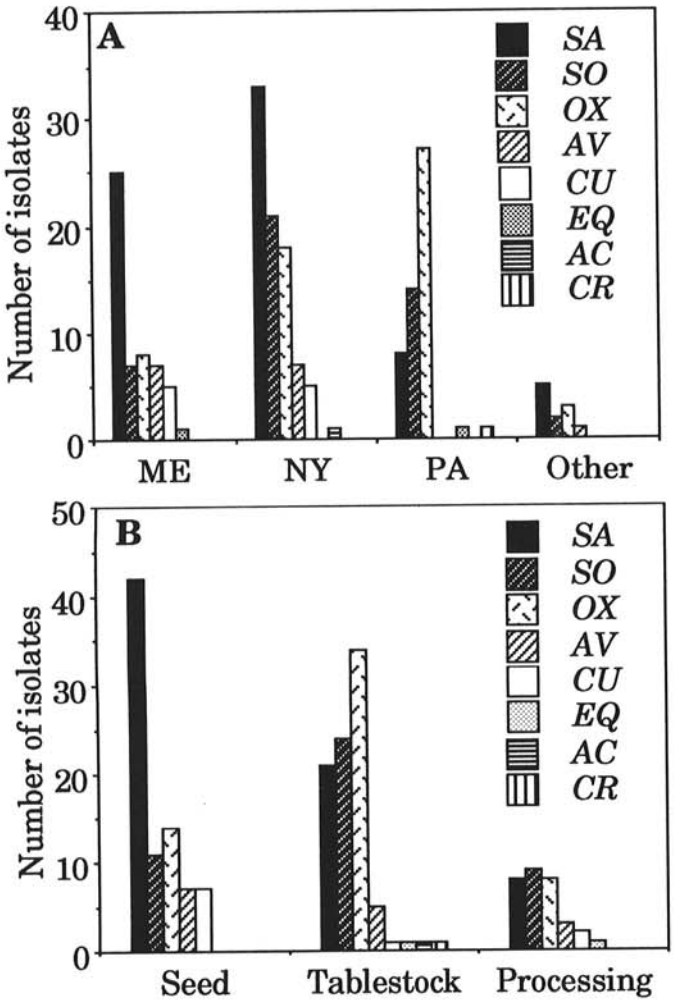

Fig. 2. A, Distribution of *Fusarium* species by state of origin of the sample. The "other" category included Connecticut, Michigan, Nebraska, New Jersey, South Dakota, or New Brunswick. **B,** Distribution of *Fusarium* species by tuber type. AV = *F. avenaceum*, CU = *F. culmorum*, AC = *F. acuminatum*, OX = *F. oxysporum*, SA = *F. sambucinum*, SO = *F. solani*, EQ = *F. equiseti*, and CR = *F. crookwellense*.

the three species. In the first of these analyses, the response was either "infected by *F. sambucinum*" or "not infected by *F. sambucinum*"; the other two species were analyzed the same way. The factors considered were tuber use, state, method of isolation, exposure of samples to benzimidazoles, and year. Again, both main effect models and interaction models were examined to determine significant associations of these factors with infection.

RESULTS

Of the 154 tuber samples obtained, 99 yielded one or more *Fusarium* isolates, for a total of 200 isolates. Of the 1,540 individual tubers sampled, 194 yielded *Fusarium* isolates. No *Fusarium* isolates were obtained from any wounded or nonwounded tubers without lesions. The samples included tubers intended for use as seed (85 samples), tablestock (43 samples), and processing (26 samples). Most of the samples were procured from New York (75 samples), Maine (51 samples), and Pennsylvania (19 samples) (Fig. 1). However, tubers also were collected from New Jersey and Connecticut. Some seed samples came from tuber lots that were grown in other states (Michigan, Nebraska, and South Dakota) or a Canadian province (New Brunswick) and shipped to the Northeast. Most of the seed samples were obtained from New York and Maine (Fig. 1). Thirty-five (23%) of the tuber samples had been treated with a benzimidazole fungicide. Fourteen (9%) of the samples were from tuber lots with economically significant levels of dry rot, based on documentation received from growers.

Eight *Fusarium* species were isolated (Fig. 2). *F. sambucinum*, *F. oxysporum*, and *F. solani* were the most common species, comprising 35.5, 28, and 22% of the isolates, respectively, and were widely distributed (Fig. 2A). *F. avenaceum* and *F. culmorum* were less common, comprising approximately 7.5 and 5% of the isolates, respectively. The other species were infrequent: *F. equiseti* (1%), *F. acuminatum* (0.5%), and *F. crookwellense* (0.5%). *F. sambucinum*, *F. solani*, *F. oxysporum*, *F. avenaceum*, and *F. culmorum* were found in all tuber types (Fig. 2B). The infrequent species were found only in the tablestock and processing samples (Fig. 2B).

Ninety-eight percent of the isolates were pathogenic on cut potato tubers. This included all eight species isolated and both TBZ-resistant categories. The three nonpathogenic isolates included two TBZ-sensitive *F. oxysporum* isolates and one TBZ-resistant *F. sambucinum* isolate.

One hundred sixty isolates were obtained from preexisting lesions. These included all eight of the species identified: *F. sambucinum* (41%), *F. solani* (22%), *F. oxysporum* (20%), *F. avenaceum* (8.5%), *F. culmorum* (6%), *F. acuminatum* (0.7%), *F. crookwellense* (0.7%), and *F. equiseti* (0.7%). Thirty-eight percent of the isolates obtained from preexisting lesions were resistant to TBZ.

Forty of the two hundred isolates were obtained from experimentally wounded potatoes. These included six of the eight species identified: *F. solani* (37.5%), *F. oxysporum* (35%), *F. sambucinum* (17.5%), *F. avenaceum* (5%), and one isolate each of *F. culmorum* and *F. equiseti*. Eight TBZ-resistant isolates were obtained from wounded tubers (20% of the isolates). No significant differences were found between the proportions of *Fusarium* species recovered from bruised and cut tubers ($P = 0.86$).

We investigated the best logistic regression models for predicting infection of tubers with *Fusarium* species from state, tuber use, benzimidazole exposure, isolation method, and year. The models that showed a significant logistic regression relationship between predictors and infection frequency differed markedly among the three species. Up to two one- and two-predictor models with a P value of 0.0001 and the highest values of the LL statistic G for each species are shown in Table 1 as examples of the factors that showed significant responses. Other models were also

found that were statistically significant, but these contained similar predictors and thus are not shown. For *F. sambucinum*, the only significant main effect model was the one with method of isolation as the sole predictor ($G = 41.07$, $P = 0.0001$, Table 1). Thus, *F. sambucinum* was significantly less common from experimentally wounded potatoes than from existing lesions. This model was not improved significantly by adding any other main effect predictors. Significantly improved models were obtained by adding interaction predictors, as shown in Table 1. The interaction predictors that appeared most frequently in these models were interactions of tuber use, benzimidazole use, and state.

Significant main effect models with one to three predictors were found for *F. solani*. The most commonly appearing predictors were indicators of tuber use (seed and tablestock). However, indicators of state, year, and method of isolation also were present in the models shown in Table 1. Significant interaction models with one to three predictors were also found. For two predictors (Table 1) and three predictors (models not shown), the best fitting interaction models have higher G values than the best fitting main effect models. Among the best interaction models, the most commonly occurring interaction term was that of Pennsylvania with the method of isolation.

For *F. oxysporum*, the most highly significant single-predictor main effect model was based on the indicator for Pennsylvania. Significantly improved models containing two (Table 1) or three predictors (models not shown) were obtained by adding indicators for method, year, and tuber use. Interaction models as a group did not perform better than main effect models; as Table 1 shows, the one-predictor model with the highest G value contained only main effects. The two-predictor model with the highest G contained only one interaction term, the interaction of method and tuber use, and the model with the second highest G was a main effect model.

Of the 200 *Fusarium* isolates obtained, 82 grew at 5 mg/liter of TBZ and, thus, were resistant by our criteria. The control *F. sambucinum* isolates, RN-5 and RN-1, were sensitive and resistant, respectively. Resistant isolates were obtained from all locations and from all tuber types. The proportion of TBZ-resistant isolates varied greatly by species (Fig. 3). Most of the resistant isolates were *F. sambucinum* (67 isolates). The remainder of the resistant isolates were *F. solani* (8 isolates), *F. oxysporum* (5 isolates), or *F. culmorum* (1 isolate). The one isolate of *F. acuminatum* also grew at 5 and 10 mg/liter of TBZ, and, thus, would be considered resistant by our criteria.

We investigated the best logistic regression models for predicting TBZ sensitivity from species, state, tuber use, isolation method, benzimidazole use, and year. Several models were found that showed a highly significant logistic regression relationship between predictors and TBZ sensitivity. Among main effect models, the predictor with the strongest effect was species (*F. sambucinum*). The model with this as the sole predictor was significant ($G = 141.79$, $P = 0.0001$). Significantly improved two-predictor models included indicators for *F. sambucinum* and the state of Maine ($G = 152.13$) or an interaction term for the state of Maine with tablestock use ($G = 156.54$). Previous use of benzimidazoles did not appear in any of the best one-, two-, or three-predictor models. All of the models demonstrated the presence of strong associations between TBZ sensitivity and predictor variables based on species and state, with year and tuber use appearing in some models. However, the most powerful predictor, both by itself and in combination with others, was the indicator for *F. sambucinum*. Predictors that appeared in many of these models were the indicators for Maine (a negative association for TBZ sensitivity) and for Pennsylvania (a positive association for TBZ sensitivity).

According to the best main effect one-predictor model, all *F. sambucinum* isolates were predicted to be TBZ resistant, and all other species were predicted to be TBZ sensitive. The correct prediction rate among those isolates observed to be TBZ resistant was 85/(85 + 2) = 97.7%; the correct prediction rate among those isolates observed to be TBZ sensitive was 67/(67 + 11) = 85.9%. Although the models had very high correct prediction rates, they should be interpreted with caution, because they were all based on the very strong relationship between TBZ resistance and *F. sambucinum*.

These results were obtained from analyses that excluded the few (29) isolates that were not *F. sambucinum*, *F. solani*, or *F. oxysporum*. When data from these species were included in the analysis, amalgamated as a fourth species category, the results differed only in minor details.

TABLE 1. Logistic regression models with the highest G values for predicting infection of tubers with *Fusarium* species from state, tuber use, benzimidazole use, isolation method, and year

G of model[a]	Predictor model type	Predictors[b]
F. sambucinum		
41.07	One-predictor	Method (–)[c]
54.38	Two-predictors[d]	Method (–), U:table*Benz (+)
49.41	Two-predictors	Method (–), St:NY*U:table (+)
F. solani		
17.06	One-predictor	U:seed (–)
16.78	One-predictor	U:table*Year (+)
30.75	Two-predictors	Method (–), St:PA*Method (+)
29.41	Two-predictors	St:PA*Method (+), Method*U:seed (–)
F. oxysporum		
47.55	One-predictor	St:PA (+)
39.90	One-predictor	St:PA*U:table (+)
60.48	Two-predictors	St:PA (+), U:seed*Method (–)
58.83	Two-predictors	St:PA (+), Year (+)

[a] For all models in the table, the P value corresponding to G is 0.0001.

[b] Predictor notations are as follows: indicators for states are St:NY (New York), St:ME (Maine), and St:PA (Pennsylvania); indicators for use categories are U:seed, U:table (tablestock), and U:proc (processing); indicator distinguishing existing lesion from wound is Method; indicator distinguishing 1992 from 1993 is Year; indicator distinguishing no benzimidazole use from benzimidazole exposure is Benz. Interactions are denoted by *.

[c] Sign of the predictor indicates the direction of the effect. (+) indicates that levels of infection were higher than with other predictive indicators, (–) indicates that levels of infection were lower than with other predictive indicators.

[d] Two-predictors models listed showed significantly improved fit over the best model with fewer predictors, as measured by G for model comparison.

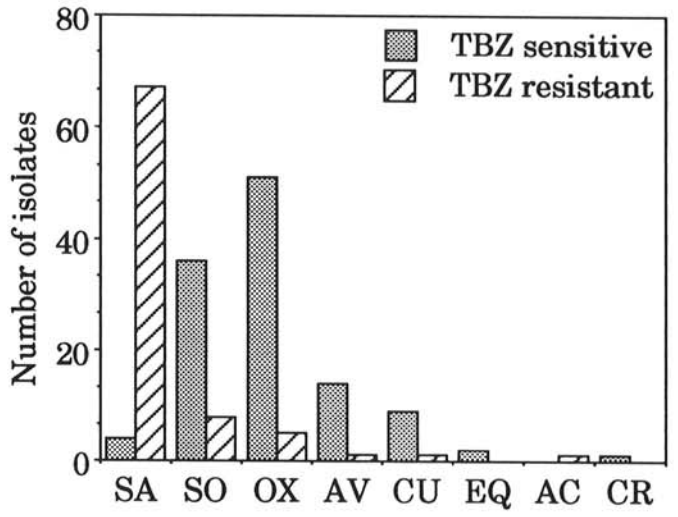

Fig. 3. Distribution of thiabendazole (TBZ)-resistant and -sensitive isolates by *Fusarium* species. AV = *F. avenaceum*, CU = *F. culmorum*, AC = *F. acuminatum*, OX = *F. oxysporum*, SA = *F. sambucinum*, SO = *F. solani*, EQ = *F. equiseti*, and CR = *F. crookwellense*.

F. sambucinum isolates that grew at 5 mg/liter of TBZ or above were clustered into three categories, based on radial growth relative to the unamended control (Fig. 4). The corresponding ED_{50} levels for these categories were high (ED_{50} > 30 mg/liter), medium (ED_{50} = 8 to 20 mg/liter), and low (ED_{50} < 5 mg/liter) (data not shown). Most of the *F. sambucinum* isolates (57) had a high level of TBZ resistance. However, four isolates had a moderate level of resistance and six isolates had a low level (Fig. 4). The TBZ-resistant control *F. sambucinum* isolate, RN-1, placed in the high level resistance category.

F. solani isolates were variable in their dose response to TBZ (Fig. 5). Two isolates had a high level of TBZ resistance (ED_{50} > 30 mg/liter) and six had a low level of resistance (ED_{50} < 5 mg/liter). All of the other *Fusarium* isolates that grew at 5 mg/liter of TBZ (*F. culmorum*, *F. acuminatum*, and *F. oxysporum*) had a low level of resistance to TBZ (ED_{50} < 5 mg/liter).

Growth rate of TBZ-resistant isolates of *F. sambucinum* and *F. oxysporum* was not significantly different from growth rate of TBZ-sensitive isolates (P = 0.76 and P = 0.97, respectively). However, the average growth rate of TBZ-resistant isolates of *F. solani* was less than that of TBZ-sensitive isolates (probability of being equal, P = 0.035), and one had malformed hyphae and spores.

DISCUSSION

Worldwide, 12 species of *Fusarium* have been reported to cause dry rot of potato (9,10,11,28,29,31). We found eight of these species in the northeastern United States.

Forty-two percent of the isolates in our study were resistant to TBZ, based on growth at 5 mg/liter of TBZ. The fungicide concentration used to distinguish between resistant and sensitive isolates varies among studies from 5 to 10 mg/liter of TBZ (6,10,33). Hide et al. (10) also used 5 mg/liter of TBZ to distinguish between TBZ-sensitive and TBZ-resistant isolates of *F. sambucinum*, *F. culmorum*, and *F. solani*. Using the more stringent criterion of growth at 10 mg/liter, 39% of our isolates still would be classified as resistant. Three isolates of *F. sambucinum*, two isolates of *F. oxysporum*, and one isolate of *F. solani* had minimum inhibitory concentrations between 5 and 10 mg/liter of TBZ (data not shown).

The use of ED_{50} to determine resistance seemed inadequate for identifying resistance in some *Fusarium* species that had a low ED_{50} but could continue to grow at TBZ concentrations two to five times greater than the minimum inhibitory concentration of sensitive isolates. For example, five of the six *F. solani* isolates with low ED_{50} levels could grow at the highest level of TBZ (50 mg/liter) tested (Fig. 5), and isolates of *F. oxysporum* and *F. culmorum* with low ED_{50} values could grow at 25 mg/liter of TBZ (data not shown), while sensitive isolates could not grow at 5 mg/liter.

The proportion of *F. sambucinum* isolates resistant to TBZ in our survey (97%) was consistent with the results of a study by Desjardins et al. (6) in the United States. In that study, 24 out of 25 (96%) of the *F. sambucinum* isolates collected from dry rot lesions during 1990 and 1991 were resistant to TBZ at 10 mg/liter (6). However, other dry rot-causing *Fusarium* species were not included in this study (6). The proportions of *F. sambucinum* isolates resistant to 5 mg/liter of TBZ found in the United Kingdom (68%) (10) and in Canada (60%) (14) were lower than reports from the United States.

We also identified TBZ-resistant isolates of *F. culmorum*, *F. oxysporum*, and *F. solani*. Resistance has been reported in *F. solani* (17) and *F. culmorum* (10) isolates from potato in Europe, but this was the first report of TBZ resistance in these species in North America. Benzimidazole resistance has been reported in isolates of *F. oxysporum* recovered from other hosts (8), but not previously in isolates from potato. While the *F. acuminatum* isolate would be considered resistant by the criteria we chose, and by the more stringent criteria of growth at 10 mg/liter, no data on TBZ sensitivity was available for this species.

The high level of TBZ resistance (ED_{50} > 30 mg/liter) of most of our *F. sambucinum* isolates was similar to the level found in other studies (ED_{50} = 30 to 40 mg/liter) (6,10,33). However, we also found four isolates with a moderate ED_{50} (10 to 20 mg/liter) and six isolates with a low ED_{50} (<5 mg/liter). Four of the low ED_{50} isolates produced a red pigment, similar in color to the pigment produced by *F. avenaceum* and *F. culmorum*, whereas the other TBZ-resistant *F. sambucinum* isolates did not produce a red pigment. One of these red *F. sambucinum* isolates was nonpathogenic on potato tubers; all other *F. sambucinum* isolates were pathogenic. The level of resistance to TBZ in *F. solani* also var-

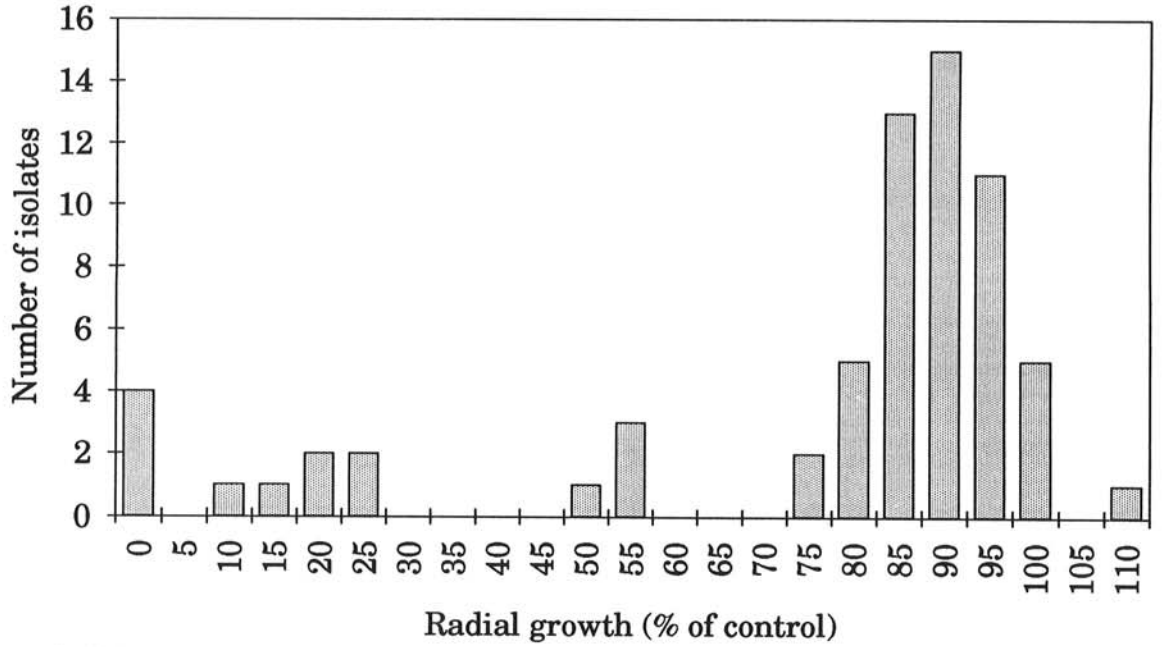

Fig. 4. Frequency distribution of *Fusarium sambucinum* isolates based on radial growth on 5 mg/liter of thiabendazole (TBZ) relative to growth on unamended control. Sensitivity categories are low growth (10 to 25% of control), moderate growth (50 to 55% of control), and high growth (70 to 110% of control).

ied among isolates (Fig. 5). In other filamentous fungi, levels of benzimidazole resistance have been found to correspond to specific mutations in the beta-tubulin gene (15,16). Differences in sensitivity to TBZ among isolates within species, as indicated by ED_{50} values, suggested that TBZ resistance in *Fusarium* also may be conferred by more than one mutation in that gene.

There was no evidence for reduced growth or pathogenicity in TBZ-resistant isolates of *F. sambucinum* or *F. oxysporum*. This was consistent with previous reports (6,10,14), and suggested that control of Fusarium dry rot of potato with postharvest TBZ applications would be compromised. The slow growth rate of the TBZ-resistant *F. solani* isolates in culture, relative to the TBZ-sensitive isolates of *F. solani*, suggested that, in this species, there may be some fitness costs associated with TBZ resistance. However, all of the TBZ-resistant *F. solani* isolates were pathogenic on potato tubers.

The high proportion (56%) of TBZ-resistant isolates recovered from tubers produced for seed suggested that dry rot may be more difficult to control in the future. *Fusarium* species have been shown to spread to new tubers from infected seed tubers (1,18,19). The prevalence of TBZ-resistant isolates in seed could be of particular concern since there is a potential for increased rot severity in seed tubers when tuber lots with TBZ-resistant isolates are treated with TBZ, compared with tubers not treated with TBZ (25).

The logistic regression models demonstrated associations between tuber infection by *F. oxysporum*, *F. sambucinum*, and *F. solani* and predictor variables based on state, tuber use, isolation method, previous benzimidazole use, and year. The prediction accuracy of these models was not high (not substantially greater than 50% in both correctly predicted as positive and correctly predicted as negative categories), in large part because of the small number of infected tubers. However, all of these models were statistically significant and showed that associations were present. Determination of the precise nature of those associations would require further investigation. However, there are some possible explanations for some of the factors that showed a significant relationship with tuber infection.

F. sambucinum isolates were more frequent in preexisting lesions than in wounds created for the purposes of this study. Preexisting lesions were the result of tuber injury during harvest and postharvest handling. We wounded tubers 2 to 3 months after harvest. Differences in *F. sambucinum* frequency may be because of poor survival of this fungus in the soil with the tubers or on tuber surfaces. Other researchers have reported lack of evidence for long-term survival of *F. sambucinum* in soil (18). Potato tubers have been reported to become more susceptible to *Fusarium* infection during storage (22). However, the rate of increased susceptibility varied for different *Fusarium* species. While only *F. solani* and *F. avenaceum* were examined in this study, it did suggest the possibility that differences in tuber susceptibility could also affect which species grew in our wounding tests. There was also a positive relationship between previous benzimidazole use and infection with *F. sambucinum* (though this was not a strong relationship). This may be because of the prevalence of TBZ-resistance in this species, and this might be one reason that we found *F. sambucinum* as the most frequently isolated species, while older reports indicated that *F. solani* was the primary dry rot pathogen (11).

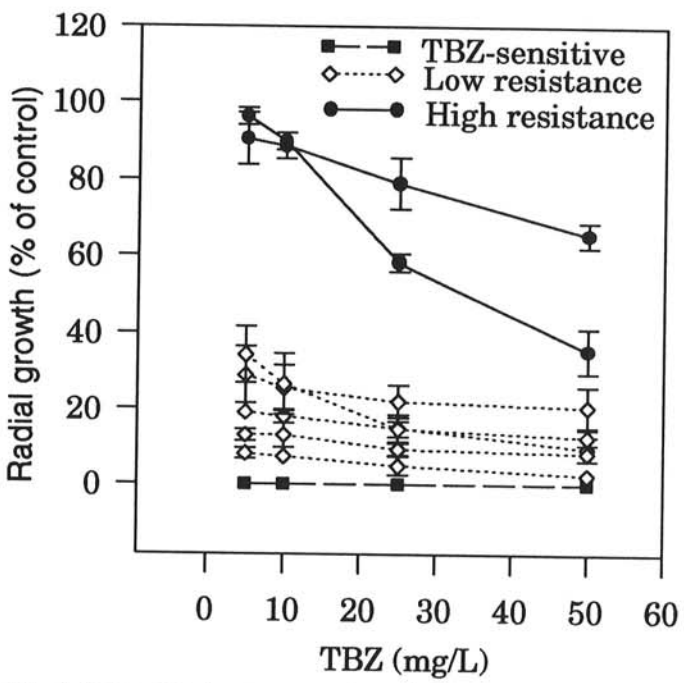

Fig. 5. Radial growth, as a percentage of the unamended control, of *Fusarium solani* isolates in response to increasing thiabendazole (TBZ) concentrations. Each line is an average of three replicate plates of an individual isolate.

No single predictor was associated consistently with *F. solani* infection (Table 1). The possible negative association with seed tubers may be explained by the decreased susceptibility of tubers to infection by *F. solani* at lower storage temperatures (3), since seed tubers are stored at lower temperatures than are tablestock and processing tubers. Previously, *F. solani* was identified as the most frequent and aggressive seed tuber pathogen in the United States, although *F. sambucinum* also was isolated frequently (11). The differences in results between our study and this previous report may be because of the higher proportion of TBZ-resistant isolates in *F. sambucinum* than in *F. solani*, which we reported here.

Our analysis suggested a higher incidence of *F. oxysporum* in tubers grown in Pennsylvania than would be predicted by random chance. This result was surprising, as *F. oxysporum* is reported to be ubiquitous (23). However, differences between Pennsylvania and other states included in the study in soil factors, climate, or crop rotation factors could influence prevalence of *F. oxysporum* associated with tubers (23).

Fusaria infect potato tubers through wounds (26) that occur at harvest or during handling. Minimizing tuber injury at harvest and providing storage conditions that promote rapid wound healing immediately after handling are important control strategies. However, stony soils or poor weather conditions during the growing season or at harvest can greatly limit the effectiveness of these strategies. Dry growing seasons increase the specific gravity of tubers, making them more susceptible to bruising. Dry soil conditions at harvest reduce the quantity of soil carried onto the primary chain of the digger, thus reducing cushioning of the tubers and increasing bruising. Infection can be limited, to some degree, by managing storage temperatures and humidity immediately after harvest, but temperature and humidity control is poor in many potato storages. Potato cultivars with resistance to Fusarium dry rot are not available (20) and Huaman et al. (13) suggested that resistance to different species of *Fusarium* may be genetically independent, which would make the development of resistant cultivars difficult. Thus, the development of TBZ resistance in *Fusarium* is of great concern in the management of dry rot, and alternative control practices are needed.

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