Comparison of Thiabendazole-Sensitive and -Resistant *Helminthosporium solani* Isolates from New York

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ABSTRACT

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A recent increase in the severity of silver scurf of potato has caused significant economic losses in many potato-producing areas in the United States. The increase in disease severity appears to be due, at least in part, to the development of thiabendazole (TBZ) resistance in the *Helminthosporium solani* population. We evaluated the TBZ sensitivity, temperature response, virulence, and genotypic variability of *H. solani* isolates from upstate New York (UNY) counties and Suffolk County, New York, (SNY) on Long Island. The growth of TBZ-resistant isolates was inhibited by 50% at a TBZ concentration of 25 μg/ml. Half of the 14 potato tuber samples collected during 1988-1990 in New York (NY) were infected with *H. solani* isolates that were resistant to TBZ at 25 μg/ml. When multiple isolates were obtained from the same tuber lot, all isolates had the same TBZ sensitivity, with the exception of one sample. The average growth rate of TBZ-resistant isolates was 75–94% of the sensitive isolates on an unamended medium, depending on the temperature. Spore production had a bimodal temperature response with peaks at 9 and 21 C, and did not differ between sensitive and resistant isolates. Virulence on mature tubers, as measured by the area covered with sporulating lesions, did not differ among isolates regardless of TBZ sensitivity or geographic origin. Estimated optimum temperature for radial growth (OG) ranged from 21.7 to 24.9 C. TBZ-sensitive and -resistant isolates did not differ in OG. However, isolates collected from UNY and SNY did differ in OG. Restriction fragment length polymorphisms (RFLP), detected with two moderately to highly repetitive sequences from *H. solani,* indicated that TBZ-resistant *H. solani* isolates collected from NY are not clonal. Analysis of similarity coefficients indicated no aggregation of isolates based on TBZ sensitivity or geographic origin.

Additional keywords: benzimidazole fungicides

Silver scurf of potato, caused by *Helminthosporium solani* Durieu & Mont., is a well-known (2) disease of potato that, in the last 5 yr, has caused significant economic losses in New York. Although usually confined to the periderm, the lesions that develop on the tuber surface decrease the market value of tubers intended for tablestock (4–6). Silver scurf is often present on physiologically mature tubers before harvest, but severity increases greatly in storage (6), probably due to secondary disease cycles. Although most of the economic loss associated with silver scurf is due to discoloration and sloughing of the tuber periderm, this disease can also cause increased water loss from potatoes in storage (14).

The development of thiabendazole (TBZ) resistance in the *H. solani* population appears to be the cause, at least in part, of the recent increase in silver scurf severity in New York and many other potato-producing areas. Resistance of *H. solani* to TBZ was first reported in England by Hide et al in 1988 (5) and was documented in the United States in 1990 by Mérida and Loria (10) and Rodriguez et al (15). TBZ has been used as a postharvest treatment on potato for control of Fusarium dry rot in the United States for about 20 yr, but was never registered for silver scurf control. This benzimidazole fungicide appears to have provided nontarget control of silver scurf in storage until TBZ-resistant *H. solani* isolates became widespread (15). Silver scurf is seedborne (2,6), and fungicide-resistant isolates have probably been distributed on seed tubers.

Our long-term goal is to develop a disease management strategy for silver scurf. To accomplish this, a better understanding of the characteristics of the *H. solani* population, particularly those relevant to the development of disease control strategies, was sought. Very little work has been done on the biology of this pathogen in the United States. Information on temperature response and virulence of isolates from the potato-producing counties in New York was particularly needed. To this end, *H. solani* isolates from New York were collected from infected tubers during 1988–1990. Because of the discovery of TBZ-resistant isolates among those collected during 1988 (10), the entire collection was evaluated for TBZ sensitivity.

Fitness costs may be associated with TBZ resistance in *H. solani,* as has been suggested in other fungal pathogens (3). If so, there would be important implications for the management of fungicide resistance. Since a mechanism for genetic recombination has not yet been identified in *H. solani,* the genetic diversity of TBZ-resistant isolates was also of interest.

This paper describes the frequency of TBZ-resistant isolates in New York (from counties in upstate New York [UNY] and Suffolk County [SNY] on Long Island), compares their growth and sporulation at different temperatures, and compares the relative virulence of TBZ-resistant and -sensitive isolates. In addition, we describe the genotypic variability of a group of TBZ-sensitive and -resistant *H. solani* isolates from the two production areas, to determine if TBZ-resistant *H. solani* isolates are clonal and if the population in New York is geographically subdivided.

MATERIALS AND METHODS

H. solani isolates were obtained from tuber samples collected in New York during 1988–1990. Samples of five to 10 tubers were obtained from commercial and seed-production fields located in several potato-producing regions in New York. Tubers with silver scurf symptoms were incubated at 90–100% relative humidity at 22–24 C for 3–7 days. *H. solani* conidia produced on lesions were removed from host tissue with a wire loop and placed on water agar amended with 100 μg/ml streptomycin sulfate. Individual germinating spores were then transferred to V8 agar. Isolates were maintained on V8 agar at 25 C and as spore suspensions in 10% glycerol at −80 C. One to 24 single-spored isolates were obtained from each tuber sample. Individual isolates were identified by tuber sample number, followed by a single-spore isolate designation.

Thiabendazole sensitivity. Eight randomly selected *H. solani* isolates (1-SS3, 2-SS9, 3-SS3, 4-SS5, 5-SS3, 6-SS1, 7-SS3, and 9-SS1) obtained from six potato cultivars and six locations in New York (Table 1) were used in the initial TBZ sensitivity studies. TBZ sensitivity was determined on V8 agar amended with 1, 3.2, 10, 31.2, and 100 μg/ml TBZ. A TBZ stock solution was prepared in acetone (1 g/300 ml) and added to V8 agar cooled to 55 C. Controls were prepared by adding equivalent amounts of acetone to V8 agar cooled to 55 C. Plugs (3 mm) from 30-day-old colonies were

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transferred to amended media, and the radial growth on five replicate plates was measured after 20 and 40 days of incubation at 21 C in the dark. The relative growth of isolates on TBZ-amended and acetone-amended media was measured. TBZ response curves were constructed for the resistant isolates by fitting the data for radial growth to a second-degree polynomial function. The TBZ response curves were used to determine the TBZ concentration that reduced the growth of resistant isolates by 50% (25 μg/ml), which was then used to screen 73 additional *H. solani* isolates from New York (16). Isolates were grown on V8 agar amended with 25 μg/ml TBZ at 25 C in the dark. After 20 days of incubation, the isolates were scored as resistant if growth was observed and sensitive if growth was not observed. Experiments were repeated once.

Temperature response. The eight isolates used in the initial TBZ sensitivity study were evaluated for temperature response. Isolates were grown on V8 agar for 40 days at 25 C in the dark, and plugs (3 mm) from these colonies were transferred to petri plates containing V8 agar (20 ml). Ten replicate plates of each isolate were incubated at 3, 9, 15, 21, 27, and 35 C. Colony diameter was measured 15, 30, and 45 days after inoculation. The growth rate of individual isolates at 9, 15, 21, and 27 C was calculated for five replicate plates selected at random and averaged for each isolate. Optimum temperature for radial growth of each isolate was calculated using a modification of the method described by Keen and Smits (7).

Spore production was estimated as both total spores per plate and spore density per unit colony area in the plates used to determine growth rate. The surface area of 45-day-old colonies was calculated based on colony diameter for five replicate plates of each isolate. A spore suspension was prepared in 10% glycerol (10 ml) by scraping the surface of the colony with a wire loop. Spore concentration for each subsample was counted five times with a hemacytometer, and the average was calculated.

All temperature-response data were compared among isolates by ANOVA, and means were compared by Fisher's protected LSD in pair-wise comparisons of means ranked from highest to lowest (17). TBZ-sensitive and -resistant isolates and isolates from UNY and SNY were compared by contrast analysis. Experiments were repeated once.

Virulence assays. The relative virulence of the eight *H. solani* isolates described above was evaluated by inoculating mature detached potato tubers and visually estimating the proportion of the tuber surface area infected. Disease-free, greenhouse-grown tubers (cv. Norchip) were washed, surface-disinfested with 0.5% NaOCl, and rinsed with sterile distilled water. Tubers were placed in styrofoam boxes with individual cells (cell packs) and sprayed with a spore suspension until runoff (approximately 5 ml). Spore suspensions were prepared in sterile distilled water by scraping the surfaces of 4-wk-old sporulating colonies grown on V8 agar and adjusting the spore concentration, after counting with a hemacytometer, to 10^5 spores per milliliter. Tubers were incubated for 3 wk at 25 C with high relative humidity (>90%) in a growth chamber. Individual tubers were rated visually for percent area of the tuber surface covered with sporulating lesions. Comparisons among isolates were made by ANOVA, and means were compared by Fisher's protected LSD in pair-wise comparisons of means ranked from highest to lowest (17). TBZ-sensitive and -resistant isolates and isolates from UNY and SNY were compared by contrast analysis. Experiments were repeated once.

Genetic variability. The genotypic diversity of 12 TBZ-sensitive and -resistant isolates of *H. solani* from four counties in New York (Table 1) was estimated by restriction fragment length polymorphism (RFLP) analysis using moderately to highly repetitive *H. solani* sequences as DNA probes.

The *H. solani* DNA fragments used as probes were selected from a total genomic DNA library of *H. solani* (isolate 5-SS3). The genomic DNA library was compiled in competent DH5α *Escherichia coli* cells using standard methods of transformation (9). The total genomic DNA was cut with *Eco*RI following manufacturers instructions (Gibco-BRL, Life Technologies, Inc., Gaithersburg, MD) and ligated into pUC19, which was then transformed into *E. coli*. The transformants were screened for ampicillin resistance and disruption of the *lac*Z gene.

The DNA library was screened for repetitive DNA fragments by hybridizing the *E. coli* cell lines with radiolabeled total genomic DNA of isolate 3-SS5, using a dot blot technique (1). Six frag-

Table 1. *Helminthosporium solani* isolates collected from New York

Sample no.	No. of isolates[w]	Cultivar[x]	County	TBZ sensitivity[y]	Year collected
1	3	Rosa	Tompkins	Sensitive	1988
2	11	Hampton	Suffolk	Sensitive	1988
3[z]	8	NY72	Suffolk	Resistant/sensitive	1988
4	5	Katahdin	Suffolk	Sensitive	1988
5	6	Katahdin	Suffolk	Sensitive	1988
6	5	Monona	Orleans	Resistant	1988
7	5	Katahdin	Suffolk	Sensitive	1988
9	1	A74114-4	Tompkins	Sensitive	1988
10	1	Kanona	Franklin	Resistant	1989
11	4	Not known	Ontario	Sensitive	1989
12	3	Katahdin	Suffolk	Resistant	1990
15	24	Norchip	Franklin	Resistant	1990
18	3	Allegany	Franklin	Resistant	1990
19	3	Yukon Gold	Franklin	Resistant	1990

[w] Isolates were identified by a number indicating the samples from which they were obtained, followed by a second number which designates single-spored isolate.
[x] Potato cultivar from which isolate was obtained.
[y] Thiabendazole sensitivity at 25 μg/ml.
[z] All isolates are TBZ-resistant except for isolate 3-SS1.

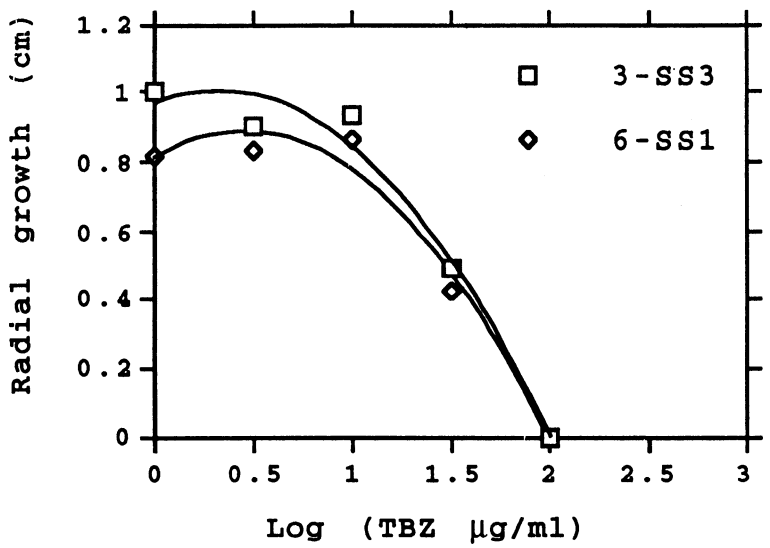

Fig. 1. Thiabendazole response curves for two resistant isolates of *Helminthosporium solani*.

Table 2. Calculated growth rates and calculated optimum temperature for radial growth of eight *Helminthosporium solani* isolates at four temperatures

Isolate[w]	Growth rate (mm/day)[x]				
	9 C	15 C	21 C	27 C	Optimum temperature
1-SS3[y]	0.21 ± 0.02 b[z]	0.74 ± 0.04 cd	1.43 ± 0.01 a	1.42 ± 0.04 a	24.9 ± 0.20 a
2-SS9	0.55 ± 0.06 a	1.31 ± 0.03 a	1.65 ± 0.02 a	1.39 ± 0.04 a	22.1 ± 0.13 d
3-SS3	0.34 ± 0.04 b	0.90 ± 0.10 bcd	1.19 ± 0.02 b	0.99 ± 0.02 b	22.5 ± 0.35 cd
4-SS5	0.24 ± 0.01 b	0.76 ± 0.05 cd	1.45 ± 0.05 a	1.15 ± 0.04 b	24.0 ± 0.09 c
5-SS3	0.55 ± 0.03 a	1.41 ± 0.02 a	1.55 ± 0.11 a	1.43 ± 0.03 a	21.7 ± 0.24 e
6-SS1	0.20 ± 0.03 b	0.65 ± 0.06 d	1.20 ± 0.04 b	1.16 ± 0.02 a	24.2 ± 0.25 ab
7-SS3	0.32 ± 0.02 b	1.02 ± 0.05 b	1.49 ± 0.04 a	1.36 ± 0.06 a	23.8 ± 0.34 abc
9-SS1	0.33 ± 0.04 b	0.91 ± 0.06 b	1.01 ± 0.02 a	1.49 ± 0.05 a	24.8 ± 0.05 ab
LSD ($\alpha = 0.05$)	0.01	0.16	0.14	0.11	0.66

[w] Isolates were incubated on V8 agar in the dark for 45 days.
[x] Growth rate was not calculated at 3 or 35 C since no growth was observed at these temperatures; however all data were used to calculate optimum temperature.
[y] Sources of isolates are listed in Table 1.
[z] Means (n = 5) in the same column followed by a different letter are significantly different ($\alpha = 0.05$).

ments of 1.1, 7.1, 8.3, 9.2, 11.2, and 13.7 kb (A2-11, A3-39, A4-43, A3-70, A2-59, and A2-92B, respectively) were selected to be used as probes based on a strong hybridization signal.

The *H. solani* isolates were grown in 100 ml of M3 minimal liquid media ($MgSO_4$, 2.5 g; KH_2PO_4, 2.7 g; peptone, 1 g; yeast extract, 1 g; sucrose, 10 g) containing ampicillin (100 μg/ml). Total genomic DNA was isolated using a modification of a minipreparation procedure (8). DNA was restricted to completion overnight with *Eco*RI and electrophoresed in 0.6% agarose in 1× TBE (Tris-borate-EDTA) buffer (9) at 70 volts for 16 hr. Digested DNA was transferred to a Nytran membrane using Southern transfer procedure (9) and probed with the six moderately to highly repetitive DNA sequences from *H. solani* that were identified previously (1).

DNA fragments used as probes were ^{32}P-labeled by the random hexamer method (Boehringer Mannheim, Indianapolis, IN). The membranes were prehybridized at 65 C overnight in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhart's reagent, 0.5% sodium dodecyl sulfate (SDS), and 100 μg/ml denatured salmon sperm DNA. The membranes were hybridized overnight at 65 C in 6× SSC and 0.05× BLOTTO (9). The membranes were washed twice at room temperature with 6× SSC + 0.5% SDS for 15 min each, twice at 65 C for 15 min with 1× SSC + 1% SDS each, and once at 65 C with 0.1× SSC + 1% SDS. The membranes were exposed to X-ray film at −80 C for 24–48 hr.

Isolates were scored for the presence or absence of hybridizing bands. The data were analyzed using NTSYS-PC (version 1.40). A similarity matrix with percentage of bands shared among isolates was generated for each data set using the Dice coefficient, and dendrograms were generated using the unweighted pair-group method (12). The null hypothesis that the percentage of

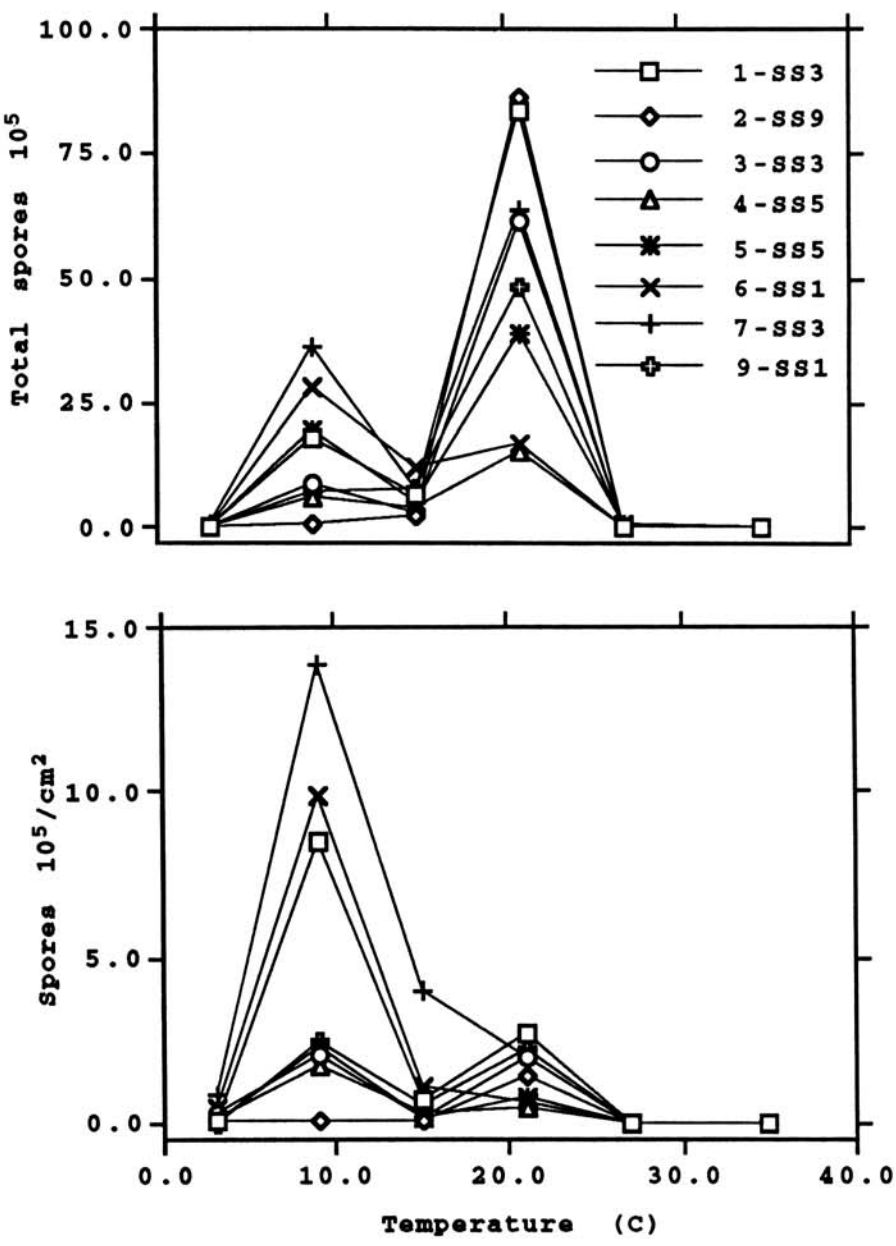

Fig. 2. Spore production of eight *Helminthosporium solani* isolates at five temperatures measured as (top) total spores/colony and (bottom) spore density, after 45 days of incubation in the dark.

shared bands between UNY and SNY isolates is not different than that if the isolates were assigned at random was tested using the model described by Milgroom et al (11). The null hypothesis that the percentage of shared bands between TBZ-sensitive and -resistant isolates was not different than random assignment of isolates was also tested.

RESULTS

Thiabendazole sensitivity. Six of the eight isolates tested were inhibited at TBZ concentrations above 1 μg/ml after 20 days of incubation. Growth was observed in all isolates at 1 μg/ml, but sensitive isolates showed reduced growth. Two *H. solani* isolates (3-SS3 and 6-SS1) grew at concentrations up to 31.2 μg/ml TBZ, but did not grow at 100 μg/ml (Fig. 1). Results were similar after 40 days of incubation at 21 C. The TBZ concentration that reduced radial growth of resistant isolates by 50% was determined by interpolation to be approximately 25 μg/ml. The same response curves were observed when the experiment was repeated. Of the 81 isolates tested at 25 μg/ml, 45 were resistant to TBZ (Table 1). Resistant isolates were obtained from three counties and seven potato cultivars. When muitiple isolates were obtained from the same tuber lot, all isolates had the same TBZ sensitivity, except for sample 3, which yielded seven resistant isolates and one sensitive isolate.

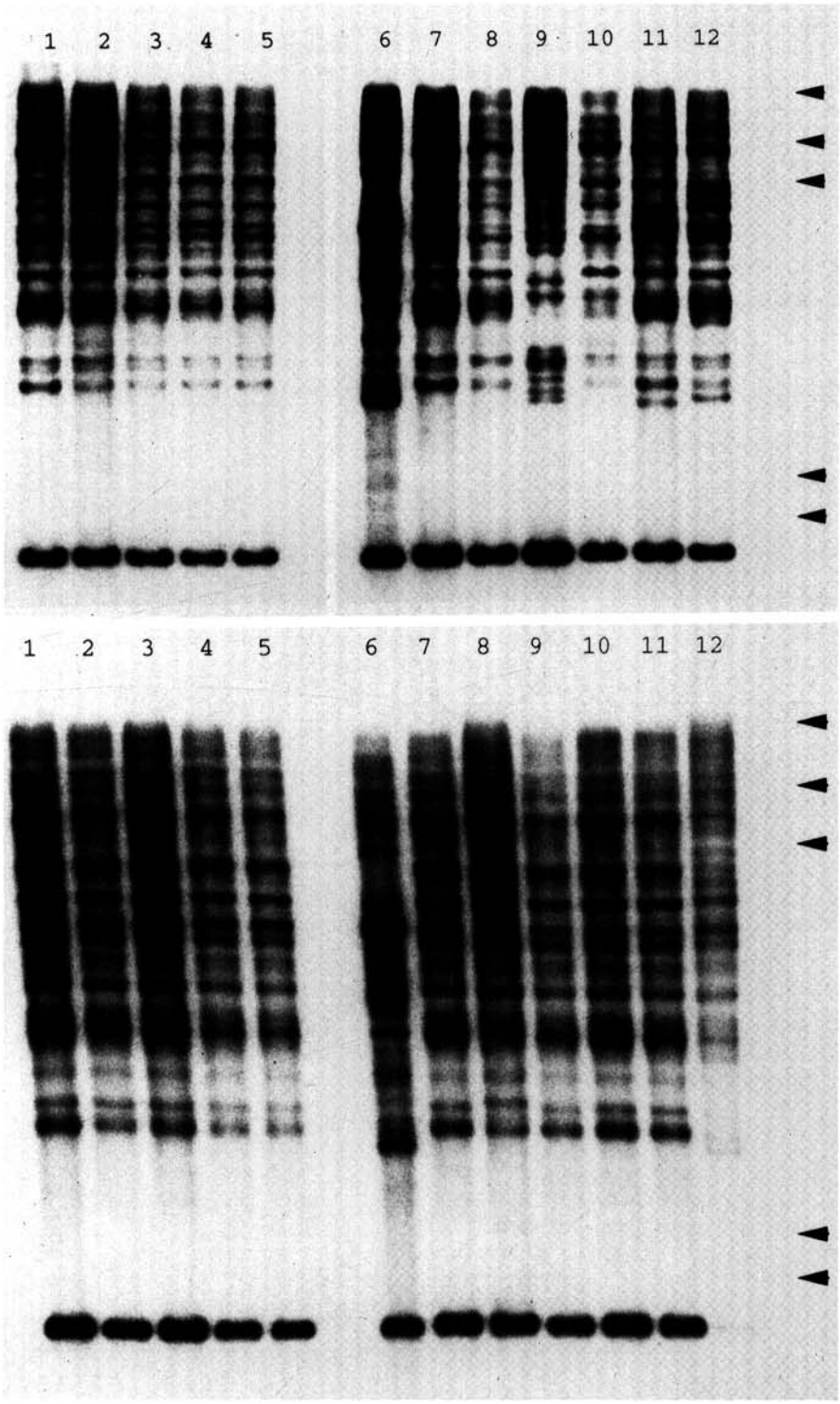

Fig. 3. Autoradiographs of *Eco*RI-restricted total genomic DNAs from 12 New York *Helminthosporium solani* isolates hybridized with (top) probe A2-59 and (bottom) probe A4-43. Lane 1 = 1-SS3, Lane 2 = 9-SS1, Lane 3 = 11-SS1, Lane 4 = 6-SS1, Lane 5 = 10-SS1, Lane 6 = 2-SS9, Lane 7 = 3-SS1, Lane 8 = 4-SS5, Lane 9 = 5-SS5, Lane 10 = 7-SS3, Lane 11 = 3-SS3, and Lane 12 = 12-SS2. Sources of isolates are listed in Table 1. Arrows indicate molecular weights of bacteriophage lambda DNA restricted with *Hin*dIII.

Temperature response. All of the eight isolates tested had the greatest radial growth at either 21 or 27 C. None of the isolates grew at 3 or 35 C. Estimated optimum temperatures for radial growth ranged from 21.7 to 24.9 C (Table 2). Significant ($P < 0.001$) differences in mean optimum temperature were found among isolates. Growth rates were calculated for the temperatures at which growth was observed (Table 2). Significant differences ($P < 0.001$) in growth rate were found among isolates within each temperature.

Using contrast analysis, it was determined that isolates from UNY and SNY had significantly different optimum temperatures for radial growth. No significant difference in optimum temperature for radial growth was observed between TBZ-resistant and -sensitive isolates. Resistant isolates had significantly ($P < 0.001$) slower growth rates than the sensitive isolates at all temperatures. The mean growth rates of six sensitive isolates were 0.36, 1.02, 1.43, and 1.37 mm/day, and mean growth rates of two resistant isolates were 0.27, 0.77, 1.20, and 1.29 mm/day at 9, 15, 21, and 27 C, respectively. Isolates from UNY had significantly ($P < 0.001$) slower growth rates than SNY isolates at 9, 15, and 21 C. Isolates from SNY had significantly ($P < 0.001$) slower growth rates at 27 C. The mean growth rates of three UNY isolates were 0.25, 0.76, 1.21, and 1.35 mm/day, and the mean growth rates of five SNY isolates were 0.40, 1.08, 1.46, and 1.26 mm/day at 9, 15, 21, and 27 C, respectively. Differences between UNY and SNY isolates, and sensitive and resistant isolates, were repeatable except that the mean growth rate of TBZ-resistant isolates was not significantly different than that of TBZ-sensitive isolates at 27 C in the second experiment.

Both estimates of sporulation (total number of spores and spore density) for individual isolates had peaks at 9 and 21 C, intermediate values at 15 C, and very low values at 3 and 27 C (Fig. 2A and B). Significant differences were found among isolates in total spores produced at 15, 21, and 27 C. However,

total spore production was greatest at 21 C for all isolates except 6SS-1. Spore density was greatest at 9 C for all isolates except 2SS-9. Significant ($0.002 < P < 0.001$) differences were found among isolates in spore density at 9, 15, and 21 C, although differences were greatest at 15 C. These differences were repeatable in a second experiment.

It was not possible to find an equation that would adequately describe the spore production of *H. solani* isolates in response to temperature, using either total number of spores produced per colony or spores per unit area of colony. Therefore, an optimum temperature for sporulation could not be calculated. No significant difference in total number of spores or spore density was observed between TBZ-sensitive and -resistant isolates or UNY and SNY isolates.

Virulence. Surface areas of tubers covered with sporulating lesions were not significantly different among isolates in either experiment. Means for individual isolates ranged from 14.6 to 19.6% in experiment 1 and 12.5 to 16.6% in experiment 2. No differences in virulence were found between groups of isolates from UNY and SNY or between groups of TBZ-sensitive and -resistant isolates.

Genetic variability. Polymorphisms were detected with two of the six probes used. The number of scorable bands ranged from 20 to 35. Hybridization patterns were stable after repeated subcloning of the isolates by mass transfer of mycelium and conidia. Autoradiographs of *Eco*RI-restricted DNA probed with A2-59 and A4-43 are presented in Figure 3. Dendrograms are presented in Figure 4. Analysis of similarity coefficients indicates that genetic similarity was not greater within groups than between groups of isolates, and that therefore no significant aggregation of TBZ-resistant and -sensitive ($P = 0.88$) isolates or isolates from UNY and SNY (P 0.61) could be identified with probe A2-59 or probe A4-43.

DISCUSSION

Seven of 14 samples collected in New York during 1989–1990 had *H. solani* isolates resistant to TBZ, and isolates obtained from the same tuber lot usually had the same TBZ sensitivity. Two of the eight tuber samples collected during 1988 were infected by TBZ-resistant *H. solani* isolates, while all four of the samples collected during 1990 had resistant isolates. TBZ-resistant *H. solani* isolates may be displacing TBZ-sensitive isolates because of the strong selection pressure provided by benzimidazole fungicides used as postharvest and seed treatments. However, a collection of *H. solani* isolates appropriate for testing this hypothesis is not available.

TBZ-resistant *H. solani* isolates are probably fit enough to be maintained in the population, as has been documented for other fungi which cause postharvest diseases (3). This inference is supported by the results from in vitro studies on growth rate, sporulation, and virulence of a small group of isolates. The growth rates of TBZ-resistant isolates were 75–94% of the sensitive isolates, depending on the temperature. Although these differences were statistically significant, it is doubtful that the reduction in growth rate, should it occur in vivo, would greatly reduce the fitness of the TBZ-resistant isolates. Further, TBZ-resistant isolates have temperature response curves for growth and sporulation similar to those of TBZ-sensitive isolates. Virulence did not differ among TBZ-sensitive and -resistant isolates, also suggesting that resistant isolates were not greatly reduced in fitness. This is consistent with findings on benzimidazole resistance in other plant pathogenic fungi that cause postharvest diseases (3,13).

TBZ-resistant *H. solani* isolates have a large selective advantage in the presence of benzimidazole fungicides and, once they are introduced onto a tuber lot, probably compete very well with sensitive isolates. Therefore, one would expect that TBZ-resistant isolates would continue to exist in the New York population for many years, even if TBZ is no longer used. The proportion of TBZ-resistant isolates might decrease over time in the absence of benzimidazoles, but would likely rebound quickly if benzimidazoles were reintroduced.

Increased losses to silver scurf have been reported by potato growers in northern production areas in the United States and in Canada, and TBZ-resistant *H. solani* isolates have been obtained from these locations (15). *H. solani* is seedborne (2,6), and distribution of TBZ-resistant isolates on infected seed tubers is probably the primary mechanism for

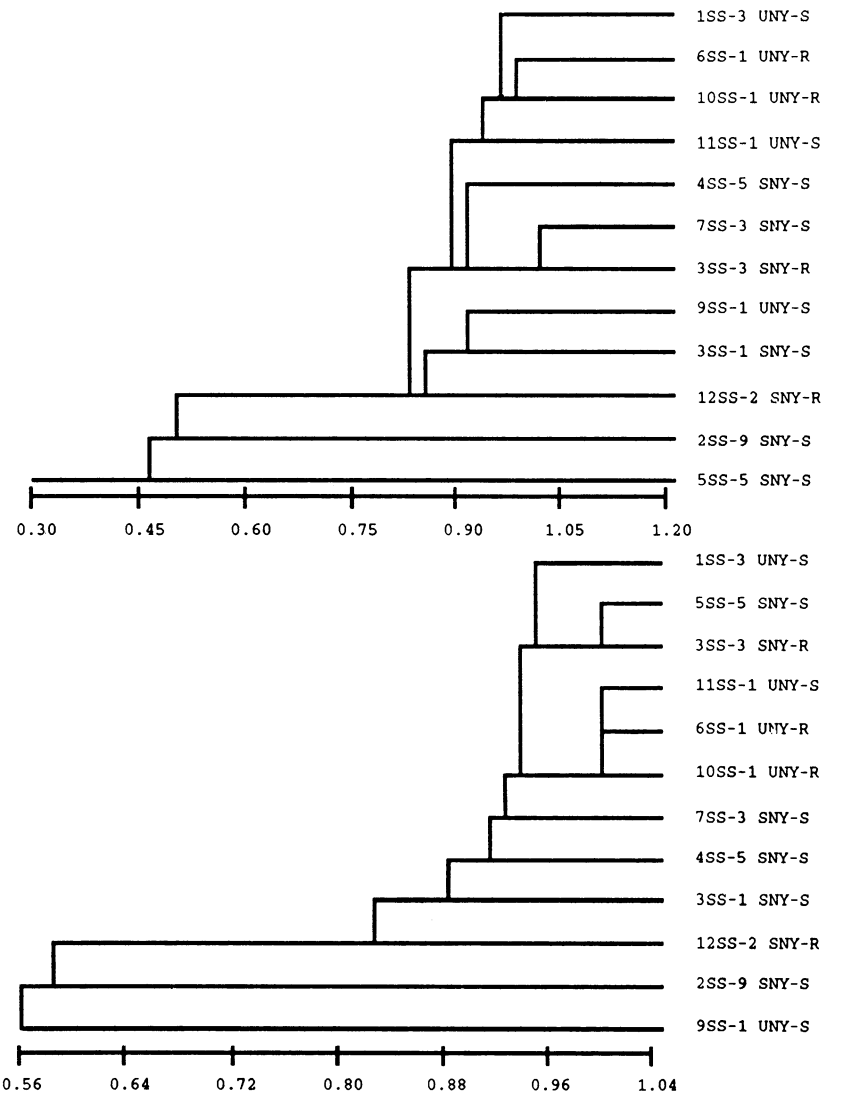

Fig. 4. Visual representation of similarity among 12 *Helminthosporium solani* isolates (top) with probe A2-59 and (bottom) probe A4-43. Dendrograms were generated with NTSYS-PC (version 1.40) using the unweighted pair-group method (UPGMA). Letters indicate geographical origin of the isolates and TBZ sensitivity: SNY-S = Suffolk County, New York-sensitive; SNY-R = Suffolk County, New York-resistant; UNY-S = Upstate New York-sensitive; and UNY-R = Upstate New York-resistant.

long-distance dispersal of benzimidazole-resistant isolates. TBZ is applied to tubers when they are placed in storage, and both TBZ and thiophanate-methyl, another benzimidazole fungicide, have been used as seed treatments. Multiple applications of these fungicides on successive generations of seed tubers before they reach the commercial grower have provided heavy selection pressure for benzimidazole resistance. The seed tuber is probably the most important source of inoculum for primary infection. Some of the TBZ-resistant isolates characterized in this study were obtained from seed tubers.

The TBZ-resistant isolates examined here are not identical clones. At this time, there is no evidence for a perfect stage in this fungus, and other mechanisms of genetic recombination are not known. Therefore, because the genotypic diversity observed among TBZ-resistant isolates was not different from the diversity observed between resistant and sensitive isolates, it seems reasonable to suggest that TBZ-resistant genotypes may have been selected or introduced more than once in the New York population. However, since the genetic markers used in the RFLP analyses have not been characterized and nothing is known about their mutation rate in the *H. solani* population, we cannot rule out the possibility that all of the TBZ-resistant isolates are of a clonal lineage.

Based on the significant differences in optimum temperature for growth and the growth rates between UNY and SNY isolates, there appears to be a geographic subdivision of the New York State *H. solani* population. The analyses of polymorphisms observed with probes A2-59 and A4-43 did not indicate a significant aggregation of UNY and SNY isolates. Since seed tubers produced in UNY are commonly grown in SNY, geographic subdivision of *H. solani* isolates from these two regions would be surprising.

The optimum temperatures for radial growth of *H. solani* ranged from 21.7 to 24.9 C and were comparable to those reported by Burke (2) for at least one *H. solani* isolate obtained from New York in 1938. This study demonstrates that low temperature does reduce the growth of *H. solani* in vitro and suggests that cooler temperatures will significantly slow the rate of disease development in storage. However, some growth did occur as low as 9 C. If these data are predictive of the effect of temperature on lesion expansion in vivo, disease can be expected to increase in storage, even at 5 C, the lowest temperature used for storage of potato tubers.

Sporulation had a bimodal temperature distribution with peaks at 9 and 21 C, a result which was unexpected. Maximum total spore production is expected at temperatures at which the growth rate is greatest, and this did occur. However, there was a secondary peak in total spores produced at about 9 C, a temperature at which radial growth is inhibited. Spore density had a similar distribution, but densities were greater for most isolates at 9 C than at 21 C, which probably reflects reduced radial growth at 9 C. The large number of spores produced at 9 C may explain the increase in disease severity that typically occurs in potato storages, since temperatures are usually maintained at 5–12 C with high relative humidity (>90%).

ACKNOWLEDGMENT

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