Eight Isolates of *Didymella bryoniae* from Geographically Diverse Areas Exhibit Variation in Virulence but No Isolate by Cultivar Interaction on *Cucumis sativus*

P. C. St. Amand, Former Graduate Research Assistant, and **T. C. Wehner,** Professor, Department of Horticultural Science, Box 7609, North Carolina State University, Raleigh 27695-7609

ABSTRACT

St. Amand, P. C., and Wehner, T. C. 1995. Eight isolates of *Didymella bryoniae* from geographically diverse areas exhibit variation in virulence but no isolate by cultivar interaction on *Cucumis sativus*. Plant Dis. 79:1136-1139.

Eight isolates of *Didymella bryoniae* from geographically diverse areas were tested for differences in virulence on nine genotypes of cucumber (*Cucumis sativus*) in two greenhouse experiments. Cucumber genotypes tested represent the range of resistance currently available. Isolates were collected in Arizona, California, The Netherlands, North Carolina, South Carolina, Sweden, and Wisconsin. The original host for one isolate was unknown, six were from cucumber, and one from muskmelon (*C. melo*). No significant isolate by cultivar interaction was detected in either experiment. Ranks of isolates were usually consistent across cultivars and experiments, and ranks of cultivars were usually consistent across isolates and experiments. Thus, resistance in cucumber to *D. bryoniae* appears to be nonspecific in nature. Single degree of freedom contrasts showed that the two foreign isolates (from The Netherlands and Sweden) were significantly more virulent than the U.S. isolates. Regression analysis indicated that the variance among cultivar ratings was not significantly correlated with mean isolate rating and that the variance among isolate ratings was not correlated with mean cultivar rating, indicating that an additive model of host-pathogen interaction may control resistance. The finding that resistance to *D. bryoniae* in cucumber is nonspecific suggests that breeders can use a single virulent isolate of *D. bryoniae* to screen for resistance.

Additional keywords: Cucurbitaceae, gummy stem blight

Gummy stem blight of cucumber (*Cucumis sativus* L.) is caused by *Didymella bryoniae* (Auersw.) Rehm and its anamorph *Phoma cucurbitacearum* (Fr.: Fr.) Sacc. (1,4). Symptoms of the disease are severe defoliation and stem necrosis in the late stages of cucumber production. It is one of the most important pathogens in North Carolina for field-grown cucumbers (11). Also, it is a serious disease of greenhouse-grown cucumbers in The Netherlands, where it causes fruit rot (14).

A small to moderate degree of resistance does exist in cucumber germ plasm (12, 17), but no gene for resistance has yet been identified (8). Breeding efforts to improve resistance to *D. bryoniae* would be aided by a better understanding of the host-pathogen relationship, yet there are no published reports on the relative virulence or pathogenicity of U.S. isolates on cucumber. Also, isolates from the U.S. have not been compared with those collected from other areas. Several reports, however, have indicated variation in virulence or pathogenicity of *D. bryoniae* isolates. Chiu and Walker (3) reported that 10 single-spore isolates varied in their ability to kill squash (*Cucurbita pepo* L.) cultivar Table Queen, and isolates of *D. bryoniae* from Florida were more able to infect watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) seedlings than were isolates from Alabama and New York (10). Van Steekelenburg (14) reported variation in the virulence of 11 isolates of *D. bryoniae*, probably from The Netherlands, on cucumber fruits and seedlings.

There are no reports indicating physiologic specialization of *D. bryoniae* for any host (9). However, Lee et al. (6) reported data suggesting that physiologic specialization of *D. bryoniae* may occur. Isolates from pumpkin (*Cucurbita* spp.) and cucumber were pathogenic to cucumber, oriental melon (*Benincasa hispida* (Thunb.) Cogn.), pumpkin, and watermelon, but some of the hosts showed more severe symptoms during the initial stages of infection when inoculated with an isolate collected from the same species of host (6).

Our objectives for this study were to compare isolates from the U.S. with each other and with foreign isolates for their relative virulence and to determine if resistance to *D. bryoniae* in cucumber is specific or nonspecific.

MATERIALS AND METHODS

Plant materials. Nine genetically diverse cucumber genotypes were selected based on their widely differing ratings for field resistance to *D. bryoniae* (17). Since race-differentiating hosts are not known to exist (9), cucumber genotypes were chosen to represent the range of resistance currently available. Cultivars Slice, Poinsett 76, Straight 8, and Marketmore 76 are monoecious inbred fresh market (slicer) types; Wisconsin SMR 18 and NCSU M 17 are monoecious inbred processing (pickle) types. Dasher II is a gynoecious hybrid slicer, and Colet is a gynoecious hybrid pickle. PI 164433 is an open-pollinated plant introduction from India.

Plants of each cultivar were grown in standard, 100-mm-diameter (500-ml-volume) round, plastic pots filled with a soilless mix of peat, vermiculite, and perlite (Metro Mix 220, Grace/Sierra, Milpitas, Calif.) in a greenhouse at Raleigh, North Carolina. Greenhouse temperatures ranged from 20 to 34°C, and the relative humidity ranged from 45 to 95%. Plants were watered as needed and no supplemental light was given. A soluble complete fertilizer, Peters 20-20-20 (Grace/Sierra), was applied as a soil drench 1 week and 2 weeks after seeding at a rate of 10 g per liter of water. Plants were tested when the third true leaf had a width of 6 ± 1 cm.

Fungal isolates and inoculum production. Eight isolates of *D. bryoniae*, collected from diverse geographic locations (Table 1), were evaluated in this study. Interstate shipment and greenhouse testing of plant pathogenic isolates were approved by the USDA Animal and Plant Health Inspection Service (Plant Protection and Quarantine permit no. 927281). To minimize variation caused by laboratory culturing, all isolates were individually inoculated on 2-week-old, disease-free plants of the susceptible cultivar Straight 8. Reisolations were made from lesions, and the resulting single-conidium cultures were maintained and used in both experiments.

Each isolate of *D. bryoniae* was grown on petri plates containing 10 ml of cucumber malt extract agar containing 250 ml of cucumber puree and 750 ml of distilled water with 30 g of malt extract agar (Difco, Detroit, Mich.). Inoculated plates were incubated for 10 to 14 days at 24 ±

The research reported herein was funded in part by the North Carolina Agricultural Research Service, and by a grant from the North Carolina Pickle Producers Association. Use of trade names in this publication does not imply endorsement by the NCARS or the products named, nor criticism of similar ones not mentioned.

Corresponding author: P. C. St. Amand
E-mail: PST@KSU.KSU.Edu

Accepted for publication 4 August 1995.

2°C, under alternating periods of 12 h florescent light (40 to 90 μE s^{-1} m^{-2} PPFD) and 12 h darkness, until spore-producing pycnidia formed. Conidia were collected by flooding plates with 5 to 10 ml of acidified, sterile, distilled water (pH of 3.5 to 4.5 using lactic acid with 20 drops per liter Tween 80) and scraping the surface of the agar with a rubber spatula. The suspension was filtered through four layers of cheesecloth to remove mycelia, pycnidia, and dislodged agar and standardized to a concentration of approximately 1×10^6 spores/ml using a hemacytometer. The final pH of the inoculum was unadjusted and ranged from 5.4 to 6.8. The inoculum was held at 5°C for approximately 15 h until use. Immediately prior to inoculation, Tween 80 (10 drops per liter), casein hydrolysate (0.05% wt/vol, Sigma, St. Louis, MO) and sucrose (0.1% wt/vol) were added to the inoculum (12,13).

Inoculation procedure. Plants were inoculated at the third true leaf stage beginning on 30 October 1992 (experiment 1) and on 27 November 1992 (experiment 2). Between 5 and 7 a.m., inoculum was sprayed on the entire plant, nearly to the point of runoff, as a fine mist using a Badger air brush model 100 (Badger Air Brush Co., Franklin Park, Ill.) at approximately 140 kPa (20 lb/in^2) and at a distance of approximately 20 cm. To prevent cross-contamination of isolates, flats of plants were individually inoculated in a separate greenhouse.

Immediately after inoculation, plants were placed in a moist chamber maintained at 95 to 100% relative humidity and 22 to 26°C with no light for 48 h. The chamber doors were opened after 48 h and the plants remained for an additional 24 h. Before and after inoculation, plants in all treatments were watered daily using overhead sprinklers.

Experiment design. The effects of isolates, cultivars, and their interactions on foliar necrosis were tested using a split-block design with three replications. Isolates were randomly assigned to flats of cucumber plants as main plots, subplots were cultivars randomly assigned to positions within flats. The experiment was conducted twice. Leaf necrosis was rated 10 days after inoculation using a linear 0 to 9 scale. Each increment of the scale was approximately equal to an 11% increase in the necrotic area, with zero representing no symptoms. The rating scale data were checked for violations of the assumptions required for the analysis of variance using residual plot analysis (5). Residuals were randomly distributed in both experiments. Therefore, transformation of the rating scale data was not necessary (7). Effects due to experiments and replications were considered to be random, and isolate and cultivar effects were considered fixed. Analysis of variance was performed on data from each experiment separately and on combined data using PROC GLM of SAS (Statistical Analysis System, SAS Institute, Cary, N.C.). A preplanned comparison between U.S. and non-U.S. isolates was tested using a single degree of freedom contrast. Regression was performed using PROC REG of SAS on the combined data from both experiments.

RESULTS

Data analysis. Analysis of variance indicated that the effects of isolates and cultivars were significant, and that there was no significant isolate by cultivar (I × C) interaction in either experiment (Table 2) or when data from both experiments were combined (Table 3). The I × C interaction was less than 4% of total treatment variance in either experiment or when experiments were analyzed together. Experiments were significantly different and interacted with cultivars, but not with isolates (Table 3). Variance among cultivar ratings was not correlated with mean isolate rating, nor was the variance among isolate ratings correlated with mean cultivar rating (data not shown).

Effect of isolates. All eight isolates of *D. bryoniae* were pathogenic to all nine cucumber cultivars tested (Fig. 1). However, there were differences in virulence (Table 4). Even though there was no significant I × C interaction, isolates did change rank over cultivars (Fig. 1). For example, isolate number 7 (North Carolina) was ranked second, and isolate number 4 (North Carolina) was ranked third on NCSU M 17, but they were ranked seventh and second respectively, for Dasher II (Fig. 1). Changes in isolate rank from experiment 1 to experiment 2 were minor, with most changes occurring among the less virulent isolates. Generally, the four most virulent isolates (when averaged over cultivars) were usually the most virulent on each individual cultivar. Isolate numbers were assigned based on mean ratings for isolates averaged over cultivars and experiments. The most virulent isolate was from The Netherlands and the least virulent isolate was from Arizona (Table 4).

Table 1. Isolate number, designation, location of collection, host, and source for eight isolates of *Didymella bryoniae* tested for pathogenicity on nine cucumber cultivars in greenhouse tests

Isolate number	Isolate designation	Location of collection	Host	Source
1	DB-H-NL	The Netherlands	*Cucumis sativus*	ATCC (#56275)
2	DB-H-22	California, U.S.A.	*Cucumis sativus*	Petoseed
3	DB-H-SW	Sweden	*Cucumis sativus*	ATCC (#36934)
4	DB-H-23	North Carolina, U.S.A.	*Cucumis sativus*	C. Averre
5	DB-H-SC	South Carolina, U.S.A.	*Cucumis sativus*	M. Havey
6	DB-?-WI	Wisconsin, U.S.A.	Unknown	M. Havey
7	DB-H-K/H	North Carolina, U.S.A.	*Cucumis sativus* var. *hardwickii*	P. St. Amand
8	DB-C-AZ	Arizona, U.S.A.	*Cucumis melo*	M. Havey

Table 2. Analysis of variance and contrast of foliar resistance rating for nine cultivars of cucumber inoculated with eight isolates of *Didymella bryoniae* in two greenhouse experiments

	Experiment 1		Experiment 2	
Source of variation	df[x]	MS[y]	df	MS
Replication	2	65.05	2	22.55
Isolate (I)	7	12.88 **[z]	7	15.21 **
Main Plot Error	14	2.95	14	2.63
Cultivar (C)	8	39.55 ***	8	32.28 ***
I × C	56	2.14 NS	56	1.63 NS
Subplot Error	123	.87	125	2.23
Total	210	...	212	...
Contrast				
U.S. vs. foreign isolates	1	22.50 **	1	77.89 ***

[x] Degrees of freedom.
[y] Mean square.
[z] NS, **, or *** indicate nonsignificant or significant at the 0.01, or 0.001 level, respectively.

Table 3. Analysis of variance and contrast of foliar resistance rating for nine cultivars of cucumber inoculated with eight isolates of *Didymella bryoniae* in two greenhouse experiments

Source of variation[w]	df[x]	MS[y]
Experiment (E)	1	143.60 ***[z]
Isolate (I)	7	25.68 **
E × I	7	2.68 NS
Cultivar (C)	8	66.79 ***
E × C	8	5.35 **
I × C	56	1.94 NS
E × I × C	56	1.84 NS
Error	280	2.71
Total	423	...
Contrast		
U.S. vs. foreign isolates	1	96.16 ***

[w] Analysis performed on data combined from experiments 1 and 2.
[x] Degrees of freedom.
[y] Mean square.
[z] NS, **, or *** indicate nonsignificant or significant at the 0.01, or 0.001 level, respectively.

The mean rating of the two foreign isolates was significantly higher than the mean rating of the isolates from the U.S. in both experiments (Table 2).

Effect of cultivars. Changes in cultivar rank from experiment 1 to experiment 2 were minor and usually involved changes in rank between adjacent cultivars. The one exception was PI 164433, which ranked the second most resistant cultivar in experiment 1, but was ranked sixth in experiment 2. When averaged over experiments and fungal isolates, Slice was the most resistant cultivar; Poinsett 76, PI 164433, NCSU M 17, Dasher II, and Wisconsin SMR 18 were intermediate; and Straight 8, Marketmore 76, and Colet were the most susceptible (Table 5).

DISCUSSION

Race specificity in a host-pathogen system is usually implied by a significant I × C interaction. However, Vanderplank (15, 16) suggested that nonparametric ranking methods be used to verify race specificity, and that a non-significant I × C interaction can verify the lack of race specificity. In our study, there was no significant I × C interaction in either experiment or when data from both experiments were combined. Also, ranks of isolates were usually consistent across cultivars and experiments, and ranks of cultivars were usually consistent across experiments. Together, these results suggest that resistance in cucumber to *D. bryoniae* is nonspecific.

Single degree of freedom contrasts showed that isolates from The Netherlands and Sweden were significantly more virulent than U.S. isolates. However, comparisons among geographic regions using a small number of isolates should be considered with caution. This finding does point out the need for a more exhaustive survey of the differences among isolates of *D. bryoniae*.

Cultivar rankings for greenhouse tests in this study were similar to the cultivar rankings for field resistance reported by Wehner and St. Amand (17), in which PI 164433 and Slice were resistant, NCSU M 17 and Poinsett 76 were intermediate, Dasher II, Wisconsin SMR 18, and Straight 8 were susceptible, and Marketmore 76 and Colet were highly susceptible to *D. bryoniae*. However, a major difference between the two studies was that, in the field, PI 164433 was more resistant than Slice. In our study, PI 164433 changed rank greatly between experiments, suggesting an environmental component.

Didymella bryoniae has a broad host range within the Cucurbitaceae. Lee et al. (6) noted that some isolates of *D. bryoniae* were more virulent on their original host during the early stages of infection, but the differences did not persist throughout the infection process. The least virulent isolate on cucumber in this study was isolated from muskmelon, which may indicate that some degree of host preference may exist in isolates of *D. bryoniae*. Results from this study agree with others indicating that isolates of *D. bryoniae* vary in virulence (3,6,14).

Carson (2) suggested that regression methods may be useful to determine the type of host-pathogen interaction model for a given pathosystem. Regression analysis indicated that the variance among cultivar ratings was not correlated with mean isolate rating, and that the variance among

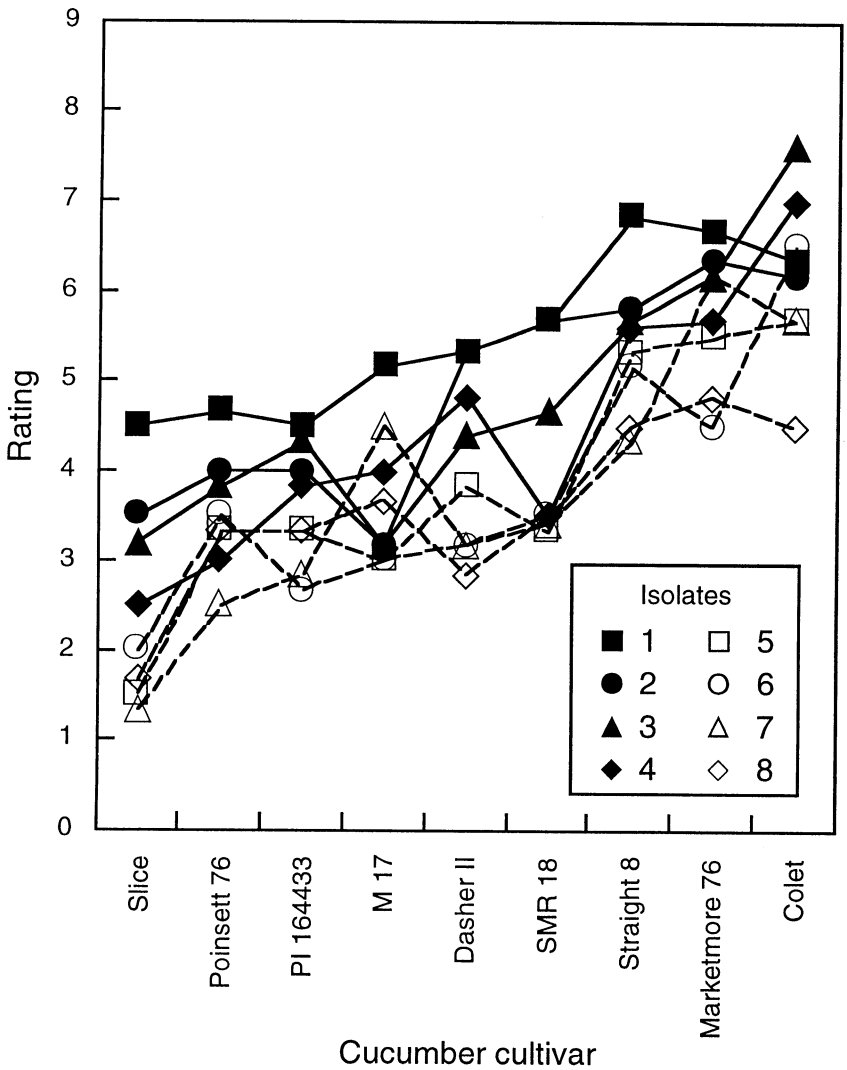

Fig. 1. Isolate by cultivar means for foliar rating of disease symptoms on nine cucumber cultivars inoculated with eight isolates of *Didymella bryoniae*. Means are averages of two greenhouse experiments. See Table 1 for isolate descriptions.

Table 4. Means for foliar virulence rating of eight isolates of *Didymella bryoniae* averaged over cucumber cultivars and two greenhouse experiments[y]

Isolate (number)	Mean[z]
Netherlands (1)	5.52 a
California (2)	4.87 ab
Sweden (3)	4.76 b
North Carolina (4)	4.42 bc
South Carolina (5)	3.89 cd
Wisconsin (6)	3.78 cd
North Carolina (7)	3.77 cd
Arizona (8)	3.57 d

[y] Leaf necrosis rated using a 0 to 9 scale, with 0 representing no symptoms.
[z] Means followed by the same letter are not significantly different, least significant difference ($P = 0.05$).

Table 5. Means for foliar resistance rating of nine cultivars of cucumber averaged over isolates of *Didymella bryoniae* and two greenhouse experiments[y]

Cultivar	Mean[z]
Slice	2.51 a
Poinsett 76	3.52 b
PI 164433	3.60 b
NCSU M 17	3.72 b
Dasher II	4.11 b
Wisconsin SMR 18	4.17 b
Straight 8	5.39 c
Marketmore 76	5.73 cd
Colet	6.15 d

[y] Leaf necrosis rated using a 0 to 9 scale, with 0 representing no symptoms.
[z] Means followed by the same letter are not significantly different, least significant difference ($P = 0.05$).

isolate ratings was not correlated with mean cultivar rating. A lack of correlation indicated that an additive model of host-pathogen interaction explained resistance (2), and that all isolates should be equally effective in discriminating between resistant and susceptible genotypes.

The finding that resistance to *D. bryoniae* in cucumber is nonspecific to isolates implies that breeders can use a single, virulent isolate of *D. bryoniae* when screening for resistance.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the advice of E. Echandi and the technical assistance of R. R. Horton, Jr.

LITERATURE CITED

1. Boerema, G. H., and Van Kesteren, H. A. 1972. Enkele bijzondere schimmelaantastingen. IV. Gewasbescherming 3:65-69.
2. Carson, M. L. 1987. Assessment of six models of host-pathogen interaction in horizontal pathosystems. Phytopathology 77:241-246.
3. Chiu, W. F., and Walker, J. C. 1949. Physiology and pathogenicity of cucurbit black-rot fungus. J. Agric. Res. 78:589-615.
4. Farr, D. F., Bills, G. F., Chamuris, G. P., and Rossman, A. Y. 1989. Fungi on Plants and Plant Products in the United States. American Phytopathological Society, St. Paul, Minn.
5. Fernandez, G. C. J. 1992. Residual analysis and data transformations: Important tools in statistical analysis. HortScience 27:297-300.
6. Lee, D., Mathur, S. B., and Neergaard, P. 1984. Detection and location of seed-borne inoculum of *Didymella bryoniae* and its transmission in seedlings of cucumber and pumpkin. Phytopathol. Z. 109:301-308.
7. Little, T. M. 1985. Analysis of percentage and rating scale data. HortScience 20:642-644.
8. Pierce, L. K., and Wehner, T. C. 1990. Review of genes and linkage groups in cucumber. HortScience 25:605-615.
9. Punithalingam, E., and Holliday, P. 1972. Commonwealth Mycological Institute Descriptions of Pathogenic Fungi and Bacteria. No. 332. *Didymella bryoniae*. The Eastern Press Ltd., London.
10. Rankin, H. W. 1954. Effectiveness of seed treatment for controlling anthracnose and gummy-stem blight of watermelon. Phyotopathology 44:675-680.
11. St. Amand, P. C., and Wehner, T. C. 1991. Crop loss to 14 diseases of cucumber in North Carolina from 1983 to 1988. Cucurbit Genet. Coop. Rep. 14:15-17.
12. St. Amand, P. C., and Wehner, T. C. 1995. Greenhouse, detached-leaf, and field testing methods to determine cucumber resistance to gummy stem blight. J. Am. Soc. Hortic. Sci. 120:673-680.
13. Svedelius, G., and Unestam, T. 1978. Experimental factors favoring infection of attached cucumber leaves by *Didymella bryoniae*. Trans. Br. Mycol. Soc. 71:89-97.
14. Van Steekelenburg, N. A. M. 1982. Factors influencing external fruit rot of cucumber caused by *Didymella bryoniae*. Neth. J. Plant Pathol. 88:47-56.
15. Vanderplank, J. E. 1978. Genetic and Molecular Basis of Plant Pathogenesis. Springer-Verlag, Berlin.
16. Vanderplank, J. E. 1988. Specificity, interaction and additivity in host-pathogen systems. Plant Pathol. 37:165-167.
17. Wehner, T. C., and St. Amand, P. C. 1993. Field tests for resistance to gummy stem blight of cucumber in North Carolina. HortScience 28:327-329.