HortScience 28(4):327-329. 1993.

Field Tests for Cucumber Resistance to Gummy Stem Blight in North Carolina

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Additional index words. Ascochyta cucumis, Colletotrichum orbiculare, Cucumis anguria, Cucumis myriocarpus, Cucumis sativus, Cucumis zeyheri, Cucurbitaceae, *Didymella bryoniae,* disease resistance, *Mycosphaerella citrullina, Mycosphaerella melonis, Phoma cucurbitacearum, Phyllosticta cucurbitacearum,* vegetable breeding

Abstract. **Gummy stem blight *[Didymella bryoniae* (Auersw.) Rehm] is the second most important pathogen of field-grown cucumbers *(Cucumis sativus* L.) in North Carolina and a severe problem for greenhouse-grown cucumbers worldwide. To determine whether resistance exists under North Carolina field conditions, 83 cultigens [cultivars, breeding lines, and plant introduction (PI) accessions] were evaluated in the field for 4 years for their resistance to a mixture of *D. bryoniae* isolates. Plants were inoculated at the vine tip-over stage and rated for foliar lesion size and number. Cultigens identified as resistant in Wisconsin and The Netherlands were not resistant in North Carolina. When averaged over years and locations, the most resistant *C. sativus* cultigens were PI 164433, 'Slice', PI 390264, M 17, and M 12. Several accessions of related *Cucumis* species were highly resistant: PI 299568 *(C. myriocarpus* Naud.), PI 282450 *(C. zeyheri* Sond.), PI 299572 *(C. myriocarpus),* and PI 233646 (C. *anguria* L.). The most susceptible cultivars were 'Colet', 'Meresto', 'Supergreen', 'Dura', 'Pioneer', 'Marketmore 76', 'Pickmore', and 'Addis'. 'Calypso' and 'Dasher II', popular cultivars in North Carolina, were moderately susceptible.**

Gummy stem blight of cucumber is caused by *Didymella bryoniae* [synonyms: *Mycosphaerella citrullina* (C.O. Sm.) Gross. and *Mycosphaerella melonis* (Pass.) Chiu and Walker] and its anamorph *Phoma cucurbitacearum* (Fr.: Fr.) Sacc. (Farr et al., 1989) (synonyms: *Ascochyta cucumis* Fautr. and Roum. and *Phyllosticta cucurbitacearum* Sacc.). Didymella blight and phoma blight have similar symptoms and control practices and are referred to as gummy stem blight. Gummy stem blight causes severe defoliation in late production stages and is the second most important cucumber pathogen in North Carolina, following the root-knot nematode (St. Amand and Wehner, 1991). Gummy stem blight is a serious disease of greenhouse-grown cucumbers in The Netherlands, where it causes fruit rot (Van Steekelenburg, 1985a). Chemical control is available but is ineffective under certain environmental conditions, such as extended rainy periods. Genetic resistance is usually less affected by environmental conditions than chemical control and is preferable to reduce pesticide inputs.

Received for publication 22 May 1992. Accepted for publication 14 Dec. 1992. Research was supported in part by a grant from the North Carolina Pickle Producers Assn. We gratefully acknowledge the research advice of the late Samual F. Jenkins, Jr., and the technical assistance of R.R. Horton, Jr. Thanks to William Van Der Arend, Nunhems Zaden, for supplying breeding lines that were tested. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

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Disease severity was increased by volatile compounds contained in *Cucumis* spp. and *Cucurbita* spp. (Pharis et al., 1982). Cucumber beetles *(Diabrotica undecimpunctata howardi* Barber and *Acalymma vittatum* Fabricus) and powdery mildew *[Erysiphe cichoracearum* DC. or *Sphaerotheca fuliginea* (Schlect.) Poll.] may predispose the plant to infection or contribute to disease spread (Bergstrom et al., 1982). Infection caused by *D. bryoniae* seemed to depend on relative humidity, with more infection occurring at 95% than at 50%. The most severe infection was produced by free-standing water on leaves. Wounding was essential for infection of older leaves (Van Steekelenburg, 1985b). More lesions on the fruit and main stem were also apparent in plants that were grown for extended periods in high humidity or with free water on fruit (Van Steekelenburg, 1985a).

Using field screening methods in Wisconsin, 'Homegreen #2' and plant introduction (PI) 200818 were reported to be resistant (Wyszogrodzka et al., 1986). In The Netherlands, greenhouse screening methods were used to identify several PI accessions as resistant, including PI 2008 18 (Van Der Meer et al., 1978). In Dutch greenhouse trials, several breeding lines were identified as resistant (William Van Der Arend, Nunhems Zaden, personal communication).

The objective of this study was to evaluate pickling and slicing cucumbers, including cultigens identified as resistant in Wisconsin and The Netherlands, for resistance to gummy stem blight under North Carolina field conditions.

Field tests were run in 1981, 1982, 1983, and 1986. In 1981, we evaluated 1165 cultigens (PI accessions, breeding lines, cultivars, and related *Cucumis* spp.) available in the U.S. Dept. of Agriculture and North Carolina State Univ. germplasm collections. The most resistant and most susceptible cultigens were chosen from the 198 1 test for further study. Additional cultigens were examined in 1983 and 1986 to duplicate the results of studies done in The Netherlands and include standard cultivars as controls. 'Homegreen #2', which was identified as resistant in Wisconsin, was not available at the time of testing and was reported to be resistant after our research was completed.

Plants in field plots were rated for foliar lesions using a scale, where 0 = no foliar symptoms, 1 to 2 = trace, 3 to 4 = slight, 5 to 6 = moderate, 7 to 8 = advanced, and 9 = plant dead. The rating system was modeled after the categories developed by Thompson and Jenkins (1985). In 1984, after inoculation and irrigation were applied, plants failed to develop disease due to an apparently unfavorable environment.

Plots were inoculated with an equal number of spores from 12 *D. bryoniae* isolates. Also, noninoculated fields near the tests usually had a high incidence (≈80%) of gummy stem blight. Plants were sprayed at the vine tip-over stage (four to six true leaves) to runoff using a back-pack sprayer (Solo, Newport News, Va.) at 103 to 138 kPa (15 to 20 psi). Overhead irrigation was used (25 to 38 $mm \cdot week^{-1}$) to spread the inoculum and encourage uniform disease development. Every third row (fourth row in 1986) was planted with susceptible Wisconsin SMR 18 to enhance the uniformity of disease spread.

Inoculum preparation. Twelve *D. bryoniae* isolates, collected from cucumber fields in Arizona, Florida, North Carolina, and Wisconsin, were increased on petri plates containing 10 ml of malt extract agar using mycelial plug inoculation. Inoculated plates were incubated for 10 days at 24 ± 2C under alternating periods of 12 h of fluorescent light (40 to 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux) and 12 h of darkness, which promoted the formation of spore-producing pycnidia. Inoculum was prepared by flooding plates with 15 ml of sterile distilled water, scraping the surface of the agar with a rubber spatula, and collecting the liquid. The spore suspension was standardized to a concentration of 1×10^6 spores/ml using a hemacytometer and kept at 5C for ≈15 h until use. The surfactant Tween-80 was added ($0.5\ ml \cdot liter^{-1}$) to the spore suspension before inoculating plants.

Experiment design. Plots were 6 m long (1981) with 40 plants each or 3 m long (1982, 1983, 1986) with 30 plants each and planted on raised, shaped beds 1.5 m apart (center to center) and separated at each end by 1.5-m alleys. Guard rows surrounded each test. Standard cultural practices were used for crop production (Hughes et al., 1983).

A randomized complete-block design was used for all tests. Each test was conducted at the Horticultural Crops Research Station, Clinton, N.C., with three (1982) or six (1983, 1986) replications, except for the 1981 test,

which was conducted at the Horticultural Crops Research Station, Castle Hayne, N.C., without replication. For each plot, one rating was given 7, 14, and 21 days after inoculation, except in 1981, when ratings were given only 7 days after inoculation. The 1981 test was treated as a germplasm survey to identify cultigens to use in further resistance studies.

Data were analyzed using PROC GLM (for analysis of variance) and PROC STANDARD from SAS (SAS Institute, Cary, N.C.). As expected, cultigens changed rank over years andlocations (e.g., M 12 and M 17 in Table 1). Most changes in rank generally were not large. To reduce variability over years and locations, data for each environment were standardized to a mean of 4.5, SD 1.5. However, this procedure resulted in greater changes in rank for most cultigens (data not presented) than actual (not standardized) means; therefore, the latter are presented (Table 1).

Cultigens were ranked on mean rating over years and locations. Analysis of variance was used to test treatment effects. Mean separations were performed using Fisher's LSD. The 1981 test was conducted as a germplasm survey to identify cultigens that would be useful for further testing. Therefore, only the most resistant and most susceptible cultigens from the 1165 tested were used in subsequent tests. Due to the large number of cultigens examined in 198 1, only one plot (without replication) of each cultigen was tested, therefore, cultigen rank for that test should not be interpreted as a final rank (Table 1).

All 11 accessions belonging to related species *(C. africanus* L. f., *C. anguria, C. dipsaceus* Ehrenb. ex. Spach, *C. ficifolius* A. Rich., *C. myriocarpus,* and *C. zeyheri)* tested in the 1981 field screening were highly resistant. In the 1981 and 1982 field tests, the four most resistant related *Cucumis* spp. were (in order of resistance): PI 299568 *(C. myriocarpus),* PI 282450 *(C. zeyheri),* PI 299572 *(C. myriocarpus),* and PI 233646 *(C. anguria)* (Table 1). However, none of those species (or any others) is sexually compatible with *C. sativus.*

In the 1983 field test, 'Slice', 'Commanche 7', 'Poinsett 76', M 17, and 'Tablegreen' were the most resistant cultigens; in 1986, 'Slice', 'Poinsett 76', and M 12 were the most resistant. When averaged over years and locations, no *C. sativus* cultigens were as resistant as the related *Cucumis* spp. tested in 1981 and 1982 (Table 1). 'Calypso' pickle and 'Dasher II' slicer, cultivars commonly grown in North Carolina, were moderately susceptible in all tests, as expected. 'Addis' was susceptible in the 1982 and 1983 tests, but was less susceptible in the 1986 test, possibly because cultigen means had a small range (3.6-7.2) for the test in 1986. PI 164433, 'Slice', PI 390264, M 17, and M 12 were the most resistant cucumber cultigens over years and locations. The most susceptible cultivars were 'Colet', 'Meresto', 'Supergreen', 'Dura', 'Pioneer', 'Marketmore 76', 'Pickmore', and 'Addis' (Table 1). Several breeding lines from Nunhems Zaden, RS 78038, and PI 351139 were the most susceptible of all cultigens tested.

Table 1. Resistance of 83 cultigens of *Cucumis sativus* (unless otherwise specified) to foliar symptoms of gummy stem blight in inoculated fields during 1981 at Castle Hayne, N.C., and 1982, 1983, and 1986 at Clinton, N.C. (cultigens ranked by mean rating).

Rank, cultigen	Seed source	Disease test ratings (0–9)[z]				
		1981	1982	1983	1986	Mean
1 PI 299568 (*C. myriocarpus*)	South Africa	1.5	0.8	---	---	1.2
2 PI 282450 (*C. zeyheri*)	South Africa	1.7	0.8	---	---	1.2
3 PI 299572 (*C. myriocarpus*)	South Africa	2.5	0.3	---	---	1.4
4 PI 233646 (*C. anguria*)	Ethiopia	1.5	1.5	---	---	1.5
5 PI 164433	India	1.5	2.2	---	---	1.8
6 Slice	Clemson Univ.	2.5	2.5	2.9	3.6	2.9
7 PI 390264	Japan	2.5	3.7	---	---	3.1
8 M 17	North Carolina State Univ.	2.5	1.7	4.1	4.4	3.2
9 M 12	North Carolina State Univ.	1.5	2.7	4.8	4.1	3.3
10 PSX 10780	PetoSeed	2.5	3.2	4.8	---	3.5
11 Commanche 7	SunSeeds	---	---	3.5	---	3.5[y]
12 Clinton	North Carolina State Univ.	---	3.0	4.3	---	3.6
13 Ark 77-19B	Univ. of Arkansas	2.5	4.7	---	---	3.6
14 Tablegreen	Rogers NK	---	---	4.1	---	4.1[y]
15 PI 200818	Burma	---	4.2	---	---	4.2[y]
16 Poinsett 76	Asgrow Seed	---	4.6	4.0	4.0	4.2
17 PI 357858	Former Yugoslavia	2.5	6.0	---	---	4.2
18 PI 418962	China	2.5	6.0	---	---	4.2
19 PI 432883	China	2.5	6.0	---	---	4.2
20 WI 2757	U.S. Dept. of Agr., Wisconsin	---	---	---	4.3	4.3[y]
21 Cherokee 7	SunSeeds	---	---	4.3	---	4.3[y]
22 PI 172838	Turkey	2.5	6.2	---	---	4.4
23 PI 432893	China	1.5	7.4	---	---	4.4
24 Chipper	Clemson Univ.	---	---	4.3	4.5	4.4
25 Lemon	Rogers NK	2.5	7.0	4.8	---	4.8
26 Calico	North Carolina State Univ.	---	---	5.1	4.4	4.8
27 Sumter	Asgrow Seed	---	---	4.9	4.9	4.9
28 MSU 5802A	Michigan State Univ.	3.4	8.4	---	---	5.0[y]
29 Wautoma	U.S. Dept. of Agr., Wisconsin	---	---	---	5.1	5.1[y]
30 Windermoor Wonder	Stokes Seed	---	---	5.1	---	5.1[y]
31 Slice Mor	Harris–Moran	---	---	5.0	5.3	5.2
32 Guardian	Rogers NK	---	---	4.8	5.5	5.2
33 Dasher	PetoSeed	---	---	5.2	---	5.2[y]
34 Spartan Salad	Rogers NK	---	---	5.2	---	5.2[y]
35 Carolina	Rogers NK	---	---	5.6	4.8	5.2
36 Regal	North Carolina State Univ.	---	---	5.1	5.4	5.2
37 Ashley	Asgrow Seed	---	---	4.8	5.7	5.2
38 Calypso	Asgrow Seed	---	---	5.4	5.2	5.3
39 Raider	Harris–Moran	---	---	5.3	5.6	5.4
40 Palomar	Asgrow Seed	---	---	4.9	6.0	5.4
41 Dasher II	PetoSeed	---	---	---	5.5	5.5[y]
42 Cypress	Ferry–Morse	---	---	5.2	5.8	5.5
43 Marketer	Ferry–Morse	---	---	5.3	5.8	5.6
44 Castlepik	SunSeeds	---	---	5.1	6.0	5.6
45 Bush Champion	Burpee Seed	---	---	5.0	6.1	5.6
46 Triple Crown	Ferry–Morse	---	---	5.8	5.4	5.6
47 Pacer	Harris–Moran	---	---	5.2	6.0	5.6
48 Pikmaster	Rogers NK	---	---	5.6	5.7	5.6
49 Verino	Sluis & Groot	---	---	5.5	5.8	5.6
50 Wis. SMR 18	Univ. of Wisconsin	---	5.0	5.6	6.6	5.7
51 National Pickling	SunSeeds	---	---	5.7	---	5.7[y]
52 PI 103049	China	7.0	4.7	---	---	5.8
53 PI 339241	Turkey	6.0	5.5	---	---	5.8[y]
54 Straight 8	Rogers NK	---	---	5.8	5.9	5.8
55 Earlipik 14	Rogers NK	---	---	5.4	6.3	5.8
56 Coolgreen	Asgrow Seed	---	---	5.6	6.6	6.1
57 NZ 99695[x]	Nunhems Zaden	---	6.2	---	---	6.2[y]
58 Addis	North Carolina State Univ.	---	8.3	6.2	4.5	6.3
59 PI 257487	China	8.5	4.5	---	---	6.5
60 NZ 99702[x]	Nunhems Zaden	---	6.5	---	---	6.5[y]
61 Pickmore	Harris–Moran	8.5	5.5	5.7	---	6.6
62 Marketmore 76	Cornell Univ.	---	9.0	5.3	5.5	6.6
63 Pioneer	Rogers NK	8.5	6.8	5.1	6.1	6.6
64 NZ 99715[x]	Nunhems Zaden	---	6.7	---	---	6.7[y]
65 PI 172848	Turkey	8.0	5.7	---	---	6.8

Continued on next page

Table 1. Continued.

Rank, cultigen	Seed source	Disease test ratings (0–9) 1981	1982	1983	1986	Mean
66 Dura	Rijk Zwaan	9.0	7.8	5.8	6.0	7.2
67 Meresto	Nunhems Zaden	8.5	7.5	5.6	---	7.2
68 Supergreen	Harris–Moran	---	---	---	7.2	7.2[y]
69 NZ 99712[x]	Nunhems Zaden	---	7.3	---	---	7.3[y]
70 PI 137848	Iran	8.5	6.3	---	---	7.4
71 NZ 99721[x]	Nunhems Zaden	---	7.6	---	---	7.6[y]
72 PI 174177	Turkey	8.0	7.3	---	---	7.6
73 PI 274902	Great Britain	8.5	6.8	---	---	7.6
74 PI 360939	Netherlands	8.0	7.7	---	---	7.8
75 PI 205995	Sweden	8.5	7.5	---	---	8.0
76 Colet	Royal Sluis	8.5	8.5	8.2	7.1	8.1
77 NZ 626B-76[x]	Nunhems Zaden	8.5	7.7	---	---	8.1
78 NZ 87195[x]	Nunhems Zaden	8.5	8.3	---	---	8.4
79 NZ 99722[x]	Nunhems Zaden	---	8.6	---	---	8.6[y]
80 RS 78038	Royal Sluis	8.5	8.8	---	---	8.6
81 NZ 99725[x]	Nunhems Zaden	---	8.7	---	---	8.7[y]
82 NZ 87197[x]	Nunhems Zaden	9.0	8.8	---	---	8.9
83 PI 351139	Former Soviet Union	9.0	9.0	---	---	9.0
LSD ($P \leq 0.05$)		1.6	1.0	0.4	0.4	0.6
Mean		5.2	5.8	5.1	5.5	5.5
Minimum		1.5	0.3	2.9	3.6	1.2
Maximum		9.0	9.0	8.2	7.2	9.0
CV (%)		62	42	16	16	33

[z]Rated for foliar lesions on a 0 to 9 scale (0 = no foliar symptoms, 1–2 = trace, 3–4 = slight, 5–6 = moderate, 7–8 = advanced, 9 = plant dead). Data are for one rating without replication in 1981, means of three ratings of three replications in 1982, and means of three ratings of six replications in 1983 and 1986.
[y]Indicates cultigens tested in 1 year only.
[x]Indicates gummy stem blight-resistant cultigens from a Dutch breeding program.

Eight cultigens comprise a useful set of standards for testing resistance to gummy stem blight in the field: PI 299568 and PI 164433 (resistant), 'Slice' and M 17 (moderately resistant), 'Coolgreen' and 'Marketmore 76' (moderately susceptible), and 'Supergreen' and PI 351139 (susceptible).

All of the breeding lines reported as resistant in Dutch greenhouse trials (W. Van Der Arend, Nunhems Zaden, personal communication) were susceptible or very susceptible in field studies in North Carolina (Table 1). PI 2008 18, reported as resistant in Wisconsin and The Netherlands, was moderately resistant in the 1982 test (Table 1); however, it was not as resistant as PI 164433, 'Slice', PI 390264, M 17, or M 12 in the same test (Table 1). Possibly, PI 200818 was not homogeneous for resistance to gummy stem blight, a fact that could account for observed differences in resistance over environments. PI 339241, which was reported to be resistant in The Netherlands (Van Der Meer et al., 1978), was susceptible in our tests. 'Homegreen #2', also identified as resistant in Wisconsin, was not tested. Variability over environments also may be due to differences in fungal isolates or to isolate x environment interactions. Variability in virulence has been reported (Van Steekelenburg, 1982). However, it is not known if true *D. bryoniae* races exist. Another possibility for the differing reactions between our tests and others is that our inoculations used several ***D. bryoniae*** isolates, while other tests used only one (Van Der Meer et al., 1978; Wyszogrodzka et al., 1986). The cultigens from Nunhems Zaden were selected for resistance under Dutch greenhouse conditions. Their lack of resistance may have been due to a poor correlation between field and greenhouse reaction, which has been demonstrated in some of our studies (St. Amand and Wehner, unpublished data) and by Wyszogrodzka et al. (1986). Additional studies are needed to determine the important environmental factors controlling resistance.

The coefficient of variability for gummy stem blight ratings varied over environments from 16% to 62%. Also, anthracnose *[Colletotrichum orbiculare* (Berk. and Mont.) Arx] lesions on the plants in 1983 made it more difficult to rate gummy stem blight. There may have been an interaction between the two diseases. However, it is difficult to keep anthracnose out of field tests, since gummy stem blight and anthracnose occur naturally at about the same time in North Carolina cucumber production areas.

Seedling tests for gummy stem blight may be sufficiently reliable as a preliminary selection tool in breeding programs, but field testing will be necessary in later stages. The occurrence of other diseases in field tests may not be a problem for breeding programs, since resistance usually is evaluated for all major diseases in each stage.

In summary, resistance to gummy stem blight under North Carolina field conditions was found among the cucumber cultigens tested. PI 164433, 'Slice', PI 390264, M 17, and M 12 offer a usable level of resistance for plant breeders interested in developing improved cultivars. Cultigens resistant in The Netherlands and Wisconsin were not resistant in North Carolina, a result that may be due to differences in pathogen or environment-subjects for future studies. Developing a rapid and accurate greenhouse screening method or detached leaf test with good correlation to field tests would facilitate selecting resistant cultigens.

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