freezer, lids were removed from the plates and samples were placed in the lyophilizer chamber of a Unitrap II lyophilizer (Virtis Co., Gardiner, NY). Samples were lyophilized for either 5 or 24 hr under vacuum (0.01 kPa) with the sink temperature at −55 C. After lyophilization was completed, plates were heat-sealed in evacuated polyethylene bags (15.2 cm wide and 0.15 mm thick) with a Sealboy bag sealer (Packaging Aids Corp. San Francisco, CA). Bag evacuation was accomplished by cutting the corner of the plastic bag to create a hole to insert a Pasteur pipet vacuum line. As the vacuum developed, indicated by a tightening of the polyethylene bag around the plate, the bag was heat-sealed in front of the Pasteur pipet tip. Sealed plates were tested for leaks with a vacuum leak detector/tesla coil (Electro-Technic Products, Chicago, IL). Samples were stored either at room temperature or at −73 C. Bacterial viability was tested for three replicates of each bacterial strain at each storage temperature for each lyophilization period after 1, 2, 6, 8, and 12 mo of storage. A 0.2-ml volume of sterile distilled water was slowly added to each well and the contents were carefully mixed with the pellet. When the pellet was well suspended, loopfuls were removed and streaked onto appropriate media. Plates were incubated at 30 C, and bacteria that grew were identified.

RESULTS AND DISCUSSION

The lyophilizer chamber of the Unitrap II could accommodate 32 microwell plates, each plate capable of holding 96 samples. Theoretically, more than 3,000 samples could be lyophilized at once. The identity of each culture is readily maintained by recording the species and the alphabetical and numerical position.

Although the microwell plate lids had to be removed during the lyophilization process, there was no contamination in any of the samples during the test period. All cultures stored equally well at room temperature or at −73 C. Over time, however, some of the polyethylene bags lost their vacuum. Although no discernible effects were observed during the test period, loss of vacuum could affect long-term storage. Bags maintained in the ultra-low-temperature freezer at −73 C were less likely to regain positive pressure. Alternate methods of storing plates under a vacuum such as in a vacuum jar or by double-bagging could alleviate this problem.

A makeshift lyophilizer chamber was evaluated for use if a regular chamber was not available. A vacuum desiccator jar was used as the chamber and connected to one of the ports of a 12-port manifold on the lyophilizer. The constructed chamber method proved unsuccessful because sublimation was too slow and the bacterial samples thawed (J. D. Schmidt, *personal communication*). The small diameter (2–3 mm) of the outlet port connecting the chamber to the manifold and the long (>1 m) path from the chamber to the cold sink are most likely what prevented sublimation. In addition, the makeshift glass chamber could allow a buildup of heat radiated from lights, equipment, and personnel in the laboratory. Packing the chamber in dry ice, covering it with aluminum foil, and increasing the outlet port diameter to at least 1–2.5 cm could create conditions for proper lyophilization.

A regular lyophilizer chamber and the microwell plate method of lyophilization were found convenient for rapid processing of a large number of strains over a short time. Retention of bacterial viability was excellent for at least 1 yr (the maximum length of the test period). In all cases, all replicates of both bacterial strains, XV 83-38 and CM 84-1, survived the initial lyophilization process of either 5 or 24 hr. There was no apparent loss of viability with a 24-hr period of lyophilization as previously reported for certain lactic-acid bacteria (5). In addition, using canned evaporated milk as a cryoprotective agent was more convenient than preparing and "sterilizing" skim milk. Several samples of lyophilized strains have been successfully sent to co-workers around the United States on microwell plates, which have proven to be a convenient and safe means of transporting large numbers of cultures.

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Populations of *Agrobacterium* in Vineyard and Nonvineyard Soils and Grape Roots in Vineyards and Nurseries

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ABSTRACT

Burr, T. J., Katz, B. H., and Bishop, A. L. 1987. Populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and nurseries. Plant Disease 71:617-620.

Higher populations of biovar 3 of *Agrobacterium tumefaciens* and *A. radiobacter* were isolated from roots of grapevines with crown gall than from roots of noninfected vines or from nonrhizosphere soils sampled from infected and noninfected vineyards. Biovar 3 was not detected by plating serial dilutions of nonvineyard soils on a selective medium, indicating that populations were less than 100–1,000 colony-forming units per gram of soil. Roots of apparently healthy rootstocks and nongrafted grapevine cultivars sampled from nursery storages were contaminated with tumorigenic and nontumorigenic biovar 3. When specific sections of roots were assayed for biovar 3, it was predominantly isolated from small, dark, sunken lesions on the roots.

Agrobacterium tumefaciens biovar 3 (AT3) has been reported throughout the world as an important pathogen of grapevine (2,4,11,15,16,22). Lehoczky (9), Burr and Katz (3), and Tarbah and Goodman (23) have shown that grape scion and rootstock cuttings used for propagation are often systemically infested with the pathogen. The same authors proposed that the disease may be controlled by propagating and planting noninfested vines. Prerequisite to this approach, however, is a clear understanding of the soil ecology of AT3 and the potential for soil populations to function as inoculum. The purpose of this study was to examine populations of *Agrobacterium* in vineyard and nonvineyard soils and grape roots in vineyards and in nurseries.

MATERIALS AND METHODS

Vineyard soil and root samples. Soils and grape roots were collected from the Finger Lakes Region of New York State, from southeastern Washington State, and from the Demming area of New Mexico in the spring and summer of 1985. Samples consisted of soil and grape roots from vineyards with and without crown gall and from nonvineyard sites. The nonvineyard sites in Washington and New Mexico were fields that were proposed for future vineyards and were at least 500 m from existing vineyards. In New York, it was a field that was planted to vegetables for at least 10 yr and was about 5 km from the nearest vineyard. In diseased vineyards, 70–90% of the vines had galls. Soils were sampled to a depth of 20 cm with a small shovel. Several subsamples (about 100 g each) were collected from each of five vines per vineyard. In vineyards, soils were collected within 20 cm of trunks of infected or noninfected vines. Nonvineyard soils were sampled by collecting several samples (about 50 g each) at random from 5 to 20 cm deep for a total of about 1 kg.

To collect roots, soil was first removed from around the bases of the vines to a depth of 20 cm to expose them. About 30 g of lateral roots less than 4 mm in diameter were collected from each vine. Root samples from New York and New Mexico were collected from the same vines around which soil samples were taken, whereas Washington roots and soil were not necessarily collected from the same vines. Soils and roots were stored in plastic bags, transported to the laboratory in an ice chest, and stored in a refrigerator until isolations were made. The soil type, cultivar, and rootstock of samples are given in Table 1.

Isolations were made on a modification of a selective medium that was developed by Roy and Sasser (RS) (18). The medium is composed of (g/L), $MgSO_4$, 0.20; K_2HPO_4, 0.90; KH_2PO_4, 0.70; adonitol, 4.0; yeast extract, 0.14; NaCl, 0.20; boric acid, 1.0; and agar, 15.0. The pH is adjusted to 7.2, and after autoclaving and cooling to 50 C, the following (g/L) are added by filter sterilization: triphenyl tetrazolium chloride, 0.08, D-cycloserine, 0.02, trimethoprim, 0.02, and cycloheximide, 0.25. Cycloheximide replaces the chlorothalonil in the original recipe.

Soils were mixed in plastic bags, and a 50-g subsample was placed in 500 ml of sterile distilled water (SDW). Ten grams of roots were subsampled from each root sample and added to 100 ml of SDW. The water suspensions of soils and roots were then shaken on a reciprocal shaker for 20 min at 125 rpm, and 0.1 ml of serial water dilutions were plated in triplicate on RS. Plates were incubated for 4 days at 28 C, and typical colonies of *Agrobacterium* were counted. Each soil and root sample was assayed at least twice on different dates.

Nursery samples. Roots from grafted and nongrafted vines in storage were collected from a nursery in Washington State (A) and two nurseries in New York State (B and C) during the winter of 1985–1986. All vines had established good root systems the previous season and had no visible galls. Four nongrafted

Table 1. Soil and root samples assayed for biovar 3 strains of *Agrobacterium*

Sample[a]	Cultivar/rootstock/condition[b]	Soil type
NY1S, NY1R	Chardonnay/3309 C/healthy	Sandy loam
NY2S, NY2R	Chardonnay/3309 C/galled	Clay loam
NY3S	Nonvineyard	Clay loam
NM1S, NM1R	Ugni Blanc/5 BB/healthy	Sandy loam
NM2S, NM2R	French Colombard/own/galled	Sandy loam
NM3S	Nonvineyard	Sandy loam
WA1S, WA1R	White Riesling/own/healthy	Sandy loam
WA2S, WA2R	White Riesling/own/galled	Sandy loam
WA3S	Nonvineyard	Sandy loam

[a] The first two letters of the sample represent the abbreviation of the state from which it was collected, and the last letter indicates whether soil (S) or roots (R) were sampled. Soils were collected from around the trunks of vines to a depth of 20 cm by combining several about 100-g samples from five vines per vineyard. About 30 g of small lateral roots were collected from each of the vines.

[b] Soil and roots were collected from vineyards that had high incidence of crown gall or were apparently healthy.

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recovered from roots from healthy and galled New Mexico vineyards.

AT3 was recovered from apparently healthy grape roots from both nongrafted and rootstock vines from all three commercial nurseries (Table 3). Galls were not apparent on any of the vines sampled. Up to 80% of the strains tested from a single sample were tumorigenic, but the percentage was usually much lower. Nontumorigenic AT3 was recovered from all 12 root samples, and nontumorigenic biovar 1, from two.

Tumorigenic and nontumorigenic AT3 were isolated from the Washington nursery samples regardless of method used. Surface disinfestation of roots with sodium hypochlorite did not noticeably affect isolations. AT3 was recovered from surface washes before and after this treatment, indicating that it may be present in adhering rhizosphere soil, on root surfaces, or in cracks on the surfaces of roots as well as systemically within them.

Necrotic lesions were observed on roots of all three cultivars sampled from Washington State. The lesions were black and sunken and ranged up to 5 mm long and occasionally girdled the entire root. They were dispersed along the entire length of the roots and frequently extended into vascular tissues. By streaking cut ends of roots on RS, it was shown that tumorigenic and nontumorigenic AT3 were concentrated at locations where necrotic lesions occurred on roots (Table 4) and may coexist in the same lesions. The New York samples were not inspected for the presence of root lesions.

Table 3. Assays of roots of grapevines from nursery storages for *Agrobacterium*

Sample[a]	Log_{10} cfu/g root[b]	No. T strains/ no. tested[c]	Biovars of T strains[d]	Biovars of NT strains[c]
WA/A/Chenin Blanc	5.40	1/10	3	3
WA/A/Merlot, 1	5.46	1/10	3	3
WA/A/Merlot, 2	4.11	1/10	3	3
WA/A/Sauvignon Blanc	5.46	5/10	3	3
NY/B/3309 C/1	5.70	0/10	...	3
NY/B/3309 C/2	5.15	0/6	...	3
NY/B/5 BB	5.00	0/10	...	3
NY/B/SO 4	5.26	0/6	...	3
NY/B/5 A	5.82	8/10	3	3
NY/C/3309 C/1	3.98	6/29	3	1,3
NY/C/3309 C/2	4.08	0/24	...	1,3
NY/C/3309 C/3	4.08	1/35	3	3

[a] State from which sample was collected / nursery designation cultivar or rootstock from which the roots were collected / sample number.
[b] Ten grams of roots were blended in 100 ml of water, and serial dilutions were plated on RS.
[c] Tumorigenicity was determined on *Nicotiana glauca*. T = tumorigenic strains and NT = nontumorigenic strains.
[d] Biovars were determined using standard schemes (7,12,19) and or by growth on selective media (11,15,16).

DISCUSSION

AT3 strains were consistently isolated from roots of grapevine. Although nontumorigenic biovar 1 strains were common from all samples, the only tumorigenic strains recovered were AT3. This and other reports (2,14,17) illustrate the ecological specialization of AT3 for grape. AT3 was most frequently isolated from roots of crown gall-diseased vines. In contrast, relatively few AT3 were detected in nonrhizosphere soils, indicating that, like other agrobacteria, they survive preferentially in the rhizosphere (7,20,21).

The failure to detect AT3 strains in nonvineyard soil is significant because it supports the strategy of planting pathogen-free vines in such soils for control of grape crown gall. The production of *Agrobacterium*-free vines has been proposed by Tarbah and Goodman (23) and Burr and Katz (3). The success of this strategy will depend on the rate of reinfestation of the vines. Indexing methods (3,9,23) have demonstrated widespread contamination of propagation material, and it is likely that root residues in old vineyards and nurseries may harbor the bacteria systemically (10) or superficially for years. Preliminary tests have shown that when AT3-free vines are planted into artificially infested soils, they rapidly become reinfested (A. L. Bishop, *unpublished*). It may be possible, however, to establish clean plantings for sources of propagation material by planting AT3-free vines in noninfested soils. Further investigations on the population dynamics of AT3 are needed. The sensitivity threshold of the assay methods used was between 100 and 1,000 cfu/g soil, and the possibility of nondetectable levels of the pathogen surviving in soil exists.

In addition to the previously reported systemic survival and spread of AT3, we determined that the bacterium may be transported with rhizosphere soil or on the rhizoplane of apparently healthy nursery vines. This means of dissemination has also been suggested for *Agrobacterium* on other hosts (6).

Although RS was very useful in our studies, we could not rely totally on colony morphology and color for identifying biovars or tumorigenicity. Of 194 potential AT3 colonies selected for further testing from vineyard soil and root samples, only about 10% were identified as AT3. Most of the nontumorigenic strains belonged to biovar 1.

The association of AT3 with lesions on grape roots suggests a means by which the pathogen may invade the plant. Some lesions extended into the vasculature of roots and may thus be a point of entry for the pathogen into the vascular system of the vine. Subsequent testing of AT3 strains from root lesions and from our culture collection has demonstrated the ability of all of them to cause nontumorigenic root infections (T. J. Burr, *unpublished*) on grape. The effects of root decay by AT3 on root development, vine growth, and pathogen establishment in the plant are under investigation.

Table 4. Association of *Agrobacterium tumefaciens* biovar 3 with lesions on grape roots

Cultivar	No. AT3 recovered/ no. lesions assayed[a]	No. AT3 recovered/ no. healthy areas assayed
Sauvignon Blanc	2/11	0/18
Sauvignon Blanc	1/3	0/18
Sauvignon Blanc	5/14	0/27
Chenin Blanc	0/11	0/12
Merlot, 1	6/10	1/10
Merlot, 2	1/11	0/9

[a] Soil was washed from the surface of root segments with running tap water; they were soaked in 1.05% sodium hypochlorite for 1 hr and rinsed thoroughly. Cuts were made through lesions or apparently healthy areas of roots with a sterile scalpel, and the cut ends were streaked once across RS medium. Typical colonies were subcultured and tested for tumorigenicity and biovar.

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Seedborne *Diaporthe phaseolorum* var. *caulivora* in Iowa and Its Relationship to Soybean Stem Canker in the Southern United States

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ABSTRACT

McGee, D. C., and Biddle, J. A. 1987. Seedborne *Diaporthe phaseolorum* var. *caulivora* in Iowa and its relationship to soybean stem canker in the southern United States. Plant Disease 71:620-622.

Soybean pods collected from fields in Iowa in 1981 and 1982 were extensively colonized by *Diaporthe phaseolorum* var. *caulivora*, the cause of soybean stem canker. Plants with symptoms of stem canker, however, were not found in the fields. Twenty-three isolates of *D. phaseolorum* var. *caulivora* from seeds and stems of soybeans grown in different locations in Iowa in 1983 and 16 isolates of *D. phaseolorum* from stem-cankered plants from Mississippi, Georgia, and Florida were tested for pathogenicity against seedlings of soybean cultivars Bragg, Tracy-M, Harosoy, Hawkeye, Williams 82, and BSR 201 under laboratory conditions. All Iowa isolates were moderately virulent on all six cultivars. Ten southern isolates were highly virulent on Bragg and avirulent on the other cultivars, and six were moderately virulent on Bragg and BSR 201 and avirulent on the others. Cultural tests, made by growing isolates for 5 wk on acid PDA plates at 25 C under constant light, showed that isolates from Iowa and southern states were easily distinguishable by mycelial texture, chlamydospore production, stromatal size, shape, and distribution, presence of pycnidia or perithecia, and thickness of perithecial necks. Iowa isolates were extremely uniform in cultural characters. Southern isolates showed considerable variability in the degree of chlamydospore production but were uniform for other traits.

Soybean stem canker, caused by *Diaporthe phaseolorum* Cke. & Ell) Sacc. var. *caulivora* Athow & Caldwell (*D. p.* var. *caulivora*) has been known for many years in soybean (*Glycine max* (L.) Merr.) production areas of the northern United States. Apart from a period in the late 1940s and early 1950s when severe losses were sustained (1,5) it has been a minor disease. In recent years, stem canker has become a serious problem in the southeastern United States, with losses estimated at $37 million in 1983 (2). Recent studies indicating that the southern disease differs from the northern disease in symptomatology (6), pathogenicity (4,8,10), and growth of the pathogen in culture (6,13,14) suggest that it should be referred to as southern stem canker (6). It is recognized that the pathogen is similar to *D. p.* var. *caulivora*, but may be a different forma specialis of *D. phaseolorum* (2) (southern *D. phaseolorum*). McGee and Biddle (*unpublished*) have shown that southern *D. phaseolorum* is seedborne. It, therefore, could easily be introduced into northern soybean production areas. Whether it already is present is an important question in assessing the threat it poses to this region. This study characterizes the present population of *D. p.* var. *caulivora* in Iowa with respect to its distribution in the state and relationship to isolates of southern *D. phaseolorum* obtained from stem-cankered plants in southern states.

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MATERIALS AND METHODS

Survey of *D. p.* var. *caulivora* in Iowa. One hundred pods were detached from soybean plants at growth stage R7 (3) in 12 and 18 fields in different parts of Iowa in 1981 and 1982, respectively. These were surface-sterilized in 1.3% sodium hypochlorite for 3 min, washed in sterile water, and plated on potato-dextrose agar (PDA) adjusted to pH 4.5 with lactic acid. After incubation at 25 C in the dark for 14 days, the pods from which *D. p.* var. *caulivora* grew were counted.

Comparative tests of northern and southern isolates. Soybean pods were collected at harvest maturity from soybean fields near Ames, Halbur, Jefferson, and Keystone, IA, in 1983. Seed infection by *D. p.* var. *caulivora* was induced from naturally occurring inoculum on pods by placing pods on wire racks over free water in sealed plastic boxes. After incubation for 7 days in the dark at 25 C, seeds were removed, surface-sterilized in 0.5% sodium hypochlorite for 1 min, rinsed in sterile water, and plated on acid PDA. After incubation at 25 C in the dark for 14 days, isolates of *D. p.* var. *caulivora* were obtained from seeds. Five isolates of *D. p.* var. *caulivora* also were obtained from stem canker lesions on plants in a soybean field in Scotch Grove, IA, in 1983 by surface-sterilizing stem sections and plating on acid PDA as described. Isolates of southern *D. phaseolorum* from stem-cankered soybean plants in southern states were obtained

from F. Shokes (Florida), D. V. Phillips (Georgia), and B. L. Keeling and W. D. Moore (Mississippi).

Twenty-three isolates of *D. p.* var. *caulivora* and 16 of southern *D. phaseolorum* were tested for pathogenicity against six differential cultivars. These included Bragg and Tracy-M, selected because they were susceptible and resistant, respectively, to southern stem canker (8,9); Hawkeye and Harosoy, selected because they were susceptible and resistant, respectively, to stem canker in the north in the 1950s (5); and Williams and BSR 201, selected because they represented diverse genotypes of modern northern cultivars. Pathogenicity tests were carried out by using a modification of Keeling's (7) greenhouse test, which enabled large numbers of isolates to be tested under uniform environmental conditions in the laboratory. Seeds were planted in trays (40 × 20 cm) containing acid-washed sand (6 cm deep) and grown in seed germination incubators set at 25 C, 85–90% relative humidity, and a 3-hr-light 3-hr-dark cycle. Each incubator contained six trays with each tray containing 16 rows of 10 seedlings of a cultivar. Eight days after planting, seedlings were inoculated by inserting toothpicks infested with an isolate into the hypocotyl, as described by Keeling (7). Four isolates and a control, in which seedlings were inoculated with sterile toothpicks, were tested in each incubator. Two adjoining rows of 10 seedlings of each cultivar were inoculated with each isolate. A single-row barrier of seedlings existed between inoculated sets. Ten days after inoculation, lengths of lesions on hypocotyls were rated on a scale of 0–5, where 0 = 0 mm, 1 = 1–20 mm, 2 = 21–40 mm, 3 = 41–60 mm, 4 = 61–80 mm, and 5 = greater than 80 mm (and dead plants). The complete test was replicated three times over time.

Cultural characteristics of each isolate were tested by incubating cultures on acid PDA plates at 22–25 C under continuous light. After 5 wk, mycelial texture, stromatal size, shape, and distribution, pycnidial and perithecial formation, and neck width of perithecia were examined. There were four replicate plates of each isolate.

Table 1. Number of soybean pods infected by *Diaporthe phaseolorum* var. *caulivora* in soybean seed fields at different Iowa locations in 1981 and 1982[a]

Location	Percent pods infected[b] 1981	1982
Perry	10	13
Council Bluffs	8	2
Beaman	6	2
Creston	7	1
Belle Plaine	8	1
West Point	5	7
Williams	7	8
Jefferson	16	2
Vincent	10	5
Alexander	10	3
Ames	47	5
Oskaloosa	31	...
Dewitt	...	8
Keosaqua	...	4
Lynnville	...	11
Fremont	...	4
Johnston	...	6
Carroll	...	5
Harlan	...	37

[a] Pods collected at the R7 growth stage, surface-sterilized in 1.3% sodium hypochlorite for 3 min, washed in sterile water, plated on acid PDA (pH 4.5), and incubated at 25 C in the dark for 14 days.

RESULTS

Soybean pods from different locations in Iowa in 1981 and 1982 were extensively infected by *D. p.* var. *caulivora* (Table 1). A considerable range in infection level existed in each year.

Pathogenicity tests on six differential cultivars indicated three obvious groupings of isolates (Table 2). In group 1, one Mississippi, two Georgia, and seven Florida isolates of southern *D. phaseolorum* were highly virulent (lesion rating 2.5-5.0) on Bragg and avirulent (lesion rating < 1.0) on all the other cultivars. In group 2, four Mississippi and two Georgia isolates of southern *D. phaseolorum* were moderately virulent (lesion rating 1.0–2.5) on Bragg and BSR 201 and avirulent on the others. In group 3, all Iowa isolates of *D. p.* var. *caulivora* were moderately virulent on all six cultivars.

Clear differences were observed between *D. p.* var. *caulivora* and southern *D. phaseolorum* in the cultural characteristics, mycelial texture, chlamydospore production, stromatal size, shape, and distribution, pycnidial and perithecial production, and width of perithecial necks (Table 3). Chlamydospores were found only in isolates of southern *D. phaseolorum*. They were brown with thick cell walls and appeared as brown strands in otherwise white mycelium. In some cultures, the whole surface was brown; in others, only a few strands were seen. Differences in chlamydospore production were not related to the two pathogenicity groups described for southern *D. phaseolorum* (Table 2). All other cultural characteristics were uniform for southern *D. phaseolorum* isolates. *D. p.* var. *caulivora* isolates, whether they were from seeds or stems, were extremely uniform for all cultural traits.

DISCUSSION

The isolates of *D. p.* var. *caulivora* in Iowa on soybean seeds and stems differred physiologically from *D. phaseolorum* that causes stem canker in southern states. This study confirms previous work (4,6,8,13,14) but is the first report differentiating isolates on both pathological and cultural characteristics. Furthermore, sufficient isolates were tested to allow characterization of the population on a regional basis. The uniformity of the Iowa isolates suggests that they constitute one physiological race of *D. p.* var. *caulivora*. Southern *D. phaseolorum* isolates were more variable both in pathogenicity and in cultural traits and may consist of several races. Keeling (10), using a different set of differential cultivars, reached a similar conclusion.

The seedling pathogenicity test clearly distinguished three groups of isolates. Results were not, however, consistently related to adult-plant responses to these diseases. The virulence and avirulence of southern *D. phaseolorum* on Bragg and

Table 2. Mean infection ratings of isolates of *Diaporthe phaseolorum* var. *caulivora* (DPC) from Iowa and *D. phsaeolorum* from southern states (SDP) on six differential cultivars of soybeans

Group	Fungus	Source and number of isolates	Seedling infection ratings[a] on differential cultivars Bragg	Tracy-M	Hawkeye	Harosoy	Williams	BSR 201
1	SDP	Mississippi (1) Georgia (2) Florida (7)	3.2 ± 0.6[b]	0.9 ± 0.2	0.9 ± 0.2	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.1
2	SDP	Mississippi (4)	1.1 ± 0.2	0.9 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	1.4 ± 0.1
3	DPC	Iowa (23)	1.9 ± 0.6	1.5 ± 0.2	1.6 ± 0.4	1.7 ± 0.4	1.4 ± 0.3	1.7 ± 0.3

[a] Seedlings grown in sand trays in seed germination chambers at 25 C and 85–90% relative humidity for 8 days, then inoculated using toothpicks infested with the fungus. Ten days later, lesion length was rated on the scale 0 = 0 mm, 1 = 1–20 mm, 2 = 21–44 mm, 3 = 41–60 mm, 4 = 61–80 mm, and 5 = greater than 80 mm (and dead plants).

[b] Values are the mean (± standard error) of the average infection ratings for three replicates of each isolate in the group.