Anastomosis Grouping and Variation in Virulence Among Isolates of *Rhizoctonia solani* Associated with Dry Bean and Soybean in Ohio and Zaire

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ABSTRACT

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Anastomosis groupings (AG) of 290 *Rhizoctonia solani* isolates from diseased dry bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) roots and/or hypocotyls and foliage in Ohio (the United States) and Zaire (Africa) were determined. Virulence of 60 isolates, representing 12 isolates each from one of five origin/host/anastomosis-group categories, was studied on dry bean cv. Great Northern and soybean cv. Williams. Variation in virulence was assessed by inoculating roots, hypocotyls, and leaves. Ohio dry bean and soybean hypocotyl/root isolates were AG-2-2 IIIB, Zaire dry bean hypocotyl/root isolates were AG-4, and Zaire dry bean and soybean foliar isolates were AG-1 IB. AG-1 IB isolates caused foliar blight and root and hypocotyl rot in virulence tests. All 24 AG-2-2 IIIB and 11 of 12 AG-4 isolates tested failed to cause foliar symptoms. AG-2-2 IIIB isolates were more virulent on roots than on hypocotyls; AG-4 isolates were more virulent on hypocotyls than on roots. Disease-severity ratings from agar-plate assays and root rot-severity ratings from potted plant assays were correlated ($P \leq 0.05$) for dry bean AG-2-2 IIIB ($r = 0.70$), soybean AG-2-2 IIIB ($r = 0.64$), and soybean AG-1 IB ($r = 0.66$) isolates but not for dry bean AG-4 isolates. These results indicate that agar-plate assays represent an acceptable preliminary method of assessing variation in virulence among anastomosis groups.

In nature, *Rhizoctonia solani* Kühn occurs as an aggregate of strains that differ in cultural appearance, anastomosis groupings, virulence, and physiology (26,32). Likewise, morphology and virulence among *R. solani* isolates varies greatly within the same and among different anastomosis groups. Virulence differences are related to the production and activity of hydrolytic enzymes (4,24). The species is diverse, and many attempts have been made to organize isolates of *R. solani* into groups based on morphological and pathological similarities (6,32) and on anastomosis and intraspecific groupings (21,22,25,35). Concepts such as forma speciales (6), group (36), and type (32) have been introduced;

anastomosis, however, is the most meaningful and currently accepted form of grouping (3,35).

Since the introduction of the anastomosis-group concept in *R. solani* research, by Schultz in 1937, the concept has been expanded greatly and currently includes 11 anastomosis groups (AG) based on hyphal anastomosis (5,21,22,35), cultural morphology (6,32), virulence, disease type (14,21,22), and DNA base-sequence homology (14,17,39). Subdivision of isolates of AG-1 into AG-1 IA , AG-1 IB, and AG-1 IC was based on cultural type, host range, and plant tissue affected (21,22). Isolates of AG-2-2 were divided into AG-2-2 IIIB (rush type) and AG-2-2 IV (sugar beet type) based on pathogenicity on Japanese mat rush (*Juncus effusus* L. var. *dicipiens*) and sugar beet (*Beta vulgaris* L.), respectively (22). AG-2-2 IIIB represents the high-temperature rush types capable of growing at 35 C, and AG-2-2 IV represents the root rot types that will not grow at 35 C (35).

Isolates of AG-1 and AG-4 have been reported worldwide (22). Isolates of AG-1, however, have not been reported in arid areas (25). The occurrence of certain anastomosis groups in a particular location was restricted and related to cultivated crops (23). In Africa, information on the occurrence of anastomosis groups is sparse. Isolates assigned to AG-1 or AG-2 are responsible for cowpea web blight in Africa, whereas those of AG-3 or AG-4 are associated with seed and seedling decay and basal stem rot (2). Isolates of AG-2-2 IIIB virulent on soybean (*Glycine max* (L.) Merr.) occur in Illinois (16,17). In Ohio, the population of *R. solani* on soybeans has not been studied.

Epidemics of Rhizoctonia root and hypocotyl rot of dry bean (*Phaseolus vulgaris* L.) and soybean are favored by wet weather and a mean soil temperature of 18 C (1) and 25–29 C (34), respectively. Yield loss in soybean can occur at temperatures as low as 15–24 C (34). Aerial blights are most severe at mean air temperatures of 25–26 C, with no less than 80% relative humidity (RH), and under rainy conditions (8,34). Several simple and rapid inoculation methods that initiate infection in the field, greenhouse, and laboratory have been used (15,18,19,27,31). The agar-plate method, among others, has been used successfully in testing alfalfa for resistance to *R. solani* (29) and *Colletotrichum trifolii* Bain. & Essary (9). Little information is available on the use of this technique with bean seeds.

Our studies were designed to investigate anastomosis groupings of isolates of *R. solani* recovered from dry beans and soybeans in Ohio (the United States) and Zaire (Africa), differences in virulence among these isolates and specialization to tissue types (leaves, hypocotyls, or roots), and techniques for evaluating virulence among a large number of isolates. A preliminary report of this work has been published (20).

MATERIALS AND METHODS

Sources of isolates and isolation. Test isolates were obtained from diseased soybean and dry bean roots and hypocotyls in Ohio and from diseased soybean and dry bean roots, hypocotyls, and foliage in Zaire. The isolates from Zaire were obtained from diseased plants in 35 field locations in southwestern (Bas-Zaire) and southeastern (Kasai Oriental) Zaire during December 1989 through January 1990. Ohio isolates originated from 51 locations within 20 central and northwestern counties. Diseased plants were either collected from commercial fields during the spring and summer of 1989 or were obtained (especially the dry bean samples) from greenhouse-grown seedlings planted in field soil from the 51 locations.

Isolates were recovered as mycelia on 2% water agar plus metalaxyl (20 g of Difco agar [Difco Laboratories, Detroit, MI] and 5 mg of a.i./L of metalaxyl [Ridomil 2E, 25% a.i., Ciba Geigy Corp., Greensboro, NC] in 1 L of distilled water). Tissue sections, 1- to 1.5-cm long, were cut from the margins of lesions, washed under tap water, rinsed with an antibiotic solution (10 mg of chloramphenicol and 100 mg of neomycin per 1 L of distilled water), and transferred aseptically to 2% water agar plus metalaxyl in petri plates. After 48 h at 21 ±2 C in the dark, cultures were examined at 40× for mycelia of *R. solani*. Hyphal tips were transferred to test-tube slants of Difco potato-dextrose Agar (39 g/L of distilled water) supplemented with Difco yeast extract (1 g/L of distilled water) (PDA-YE) (33). Isolates (350) representing five origin/host/disease-type categories were obtained: Ohio/dry bean/hypocotyl-root, Ohio/soybean/hypocotyl-root, Zaire/dry bean/hypocotyl-root, Zaire/dry bean/foliar, and Zaire/soybean/foliar. These categories were represented by specific anastomosis groups; as a result, isolates will be referenced to certain origin/host/anastomosis-group categories hereafter.

Nuclear condition. Numbers of nuclei in vegetative hyphae of isolates were determined by the aniline-blue rapid-staining technique (10). The number of nuclei was checked later with the HCl-Giemsa method on 12 isolates randomly selected from each of the five origin/host/anastomosis-group categories (28,37).

Anastomosis groups. Anastamosis-group determinations (25) were conducted on sterilized microscope slides aseptically coated with 1.5% water agar at approximately 80 C. Tester strains of AG-1 IC, AG-2-2, AG-4, and AG-5 were obtained from L. J. Herr (The Ohio State University, Wooster). The cultural characteristics of isolates that anastomosed with AG-1 IC were compared with AG-1 IA and AG-1 IC tester strains obtained from R. Vilgalys (Duke University, Durham, NC). Isolates were grown in 9-cm-diameter petri plates containing 14 ml of PDA-YE and were incubated in continuous darkness for 14 days at room temperature. Comparisons of sclerotial size on foliage of inoculated plants were made also (12). Isolates that anastomosed with the AG-2-2 tester isolate were grown on PDA-YE at 35 C in the dark (35). AG-2-2 isolates that grew at 35 C were assigned to AG-2-2 IIIB (35).

Agar-plate virulence assay on roots and hypocotyls. Twelve randomly selected isolates from each of the five origin/host/anastomosis-group categories were used in virulence tests. Two hosts, dry bean cv. Great Northern and soybean cv. Williams, were used. Seeds were washed in a 0.3% sodium hypochlorite solution in deionized water for 5 min, rinsed in deionized water, and air-dried before use.

Ten seeds of each host were placed in a circle 1 cm from the edge of a 15-cm-diameter sterile, disposable petri plate (Lab-Tek, Nunc Inc., Naperville, IL) containing 20 ml of 1.5% water agar. One 13-mm-diameter mycelial disc from the edge of a 2- to 3-day-old 1.5% water-agar culture of each of the 60 test isolates was transferred aseptically to the center of each plate (one isolate per plate). Control plates received a noncolonized agar disc.

Two days after inoculation, 3–5 drops (approximately 30–50 μl) of sterile distilled water were dispensed aseptically onto each seed. Plates were sealed at two points with clear adhesive tape and incubated in continuous darkness at room temperature for 5 days. Plates were then placed on a laboratory bench under fluorescent light (approximately 280 μmol m^{-2} s^{-1}) for 12 h of each 24-h period. The percentage of seedlings with infected roots and/or hypocotyls and disease severity on individual seedlings was recorded 9 days after inoculation. Disease severity was rated based on a 1–5 scale: 1 = no symptoms, normal root development; 2 = localized tissue discoloration without necrosis, near-normal root development; 3 = localized lesions with extensive tissue discoloration, near-normal root development; 4 = nearly complete root necrosis, partially restricted root length; and 5 = complete root rot, root length severely restricted. The experiment was designed as a randomized complete block, with each plate, each containing 10 seedlings, representing one replication. The experiment was conducted four times, with each repetition in time representing one block.

Potted plant virulence assay on roots and hypocotyls. Ten seeds of soybean cv. Williams or dry bean cv. Great Northern were planted in a 474-ml polystyrene container (Dart Container Corp., Mason, MI) containing 300 g of a soil/peat mixture of Wooster silt loam/peat (5:1 v/v), 3.3 g of lime, and 0.6 g of ammonium nitrate. The soil/peat mixture was autoclaved at approximately 116 C for 6 h. Using the inoculum-layer technique (30), each pot was infested with one 2- to 3-day-old culture of one of the 60 test isolates cultured on 10 ml of 1.5% water agar. Control pots received a noncolonized water-agar layer. Tests were con-

ducted at 24 C with 20% RH and under 12 h of illumination (approximately 415 μmol m^{-2} s^{-1}) provided by metal halide lamps. Pots were watered to saturation after planting and were watered lightly once a day thereafter. The experiment was designed as a randomized complete block, with each pot representing one replication of each treatment. The experiment was conducted four times, with each repetition in time representing one block.

The percentage of seedlings that emerged and the percentage of seedlings with root and/or hypocotyl infections were recorded 14 days after infestation. Individual seedlings were evaluated for root- and hypocotyl-disease severity on a 1–5 scale. For roots, 1 = no symptoms; 2 = discrete light- or dark-brown superficial necrotic lesions; 3 = adventitious and/or tap-root necrosis and decay; 4 = extensive root rot; and 5 = damping-off. For hypocotyls, 1 = no symptoms; 2 = discrete reddish- or dark-brown superficial necrotic lesions; 3 = discrete reddish- or dark-brown deep necrotic lesions without stem girdling; 4 = extensive hypocotyl rot with stem girdling; and 5 = pre- or postemergence damping-off.

Detached leaf virulence assay. Leaves from 21-day-old plants of dry bean cv. Great Northern and soybean cv. Williams grown in the greenhouse were used for the detached leaf assay. Apparently healthy leaves were washed in a 0.3% aqueous sodium hypochlorite solution for 3 s and rinsed twice with deionized water. One trifoliate leaf was placed on filter paper (grade 362, Baxter Healthcare Corp., McGaw Park, IL) moistened with sterile distilled water in a 15-cm-diameter sterile, disposable petri plate. A 4-mm-diameter mycelial plug from the margins of 2- to 3-day-old cultures of one of the 60 test isolates grown on 1.5% water agar was placed aseptically in the center of the abaxial surface of each leaflet. Control leaves received noncolonized agar discs.

Each leaflet was subsequently sprayed with approximately 3–4 ml of sterile distilled water from a low-pressure, hand-operated atomizer to wet leaflet surfaces and to increase humidity. Plates were completely sealed with Parafilm (American National Can, Greenwich, CT) and maintained at room temperature (21 ±2 C) under 12 h of illumination, as described previously for laboratory experiments. Five days later, each leaflet was evaluated for disease symptoms based on a 1–5 rating scale: 1 = no symptoms; 2 = 1–25% leaf area blighted; 3 = 26–50% leaf area blighted; 4 = 51–75% leaf area blighted, and 5 = 76–100% leaf area blighted. Each petri plate represented one replication, and the assay was conducted three times as a randomized complete-block design, each time representing one block.

Whole-plant virulence assay on leaves. Test plants were grown in the autoclaved soil/peat mixture described above. Ten seeds each of dry bean cv. Great Northern and soybean cv. Williams were planted per pot, and the number of plants was thinned to five per pot after emergence.

Inoculum consisted of 3- to 4-day-old PDA cultures of the 60 test isolates. Each plate was flooded with 20 ml of deionized water and was gently scraped. The mycelial suspension was poured into a low-pressure, hand-operated atomizer and vigorously mixed by shaking. Final volume was increased to 40 ml, and a drop of Tween 20 (Sigma Chemical Co., St. Louis, MO) was added. Ten 21-day-old plants (five plants per pot) were inoculated to run-off (7). Control plants were sprayed with deionized water mixed with a drop of Tween 20. Plants were maintained at 100% RH in moistened, clear-plastic bags for 48 h, in a growth chamber at 25 C with a 12-h photoperiod (approximately 544 μmol m^{-2} s^{-1}).

The experimental design was a split-split plot in which the isolate anastomosis group was the main plot, isolates were the subplots, and cultivars were sub-subplots. The split-split plot design was used to reduce contact between plants inoculated with different isolates and to prevent cross contamination by the mycelial web. The study was conducted four times, and each repetition in time represented one block. Five days after plants were inoculated, the incidence of diseased plants per treatment was recorded, and individual plants were evaluated for disease severity using a 1–5 scale: 1 = no symptoms; 2 = small, discrete, and irregular, water-soaked lesions without leaf blight; 3 = coalescent water-soaked and necrotic lesions with leaf blight, no mycelial web; 4 = leaf drooping with extensive mycelial web coverage; and 5 = top dead.

Statistical analyses. In each virulence test, individual seeds, seedlings, plants, or leaflets were evaluated for disease incidence and disease severity. Treatment means were calculated from evaluated plant material in each petri plate or pot, and each time the test was conducted represented a replication for statistical analysis. Analysis of variance (ANOVA) was performed on incidence, and severity data and means were separated with Fisher's least significant difference (LSD) test at the 5% level of probability. SAS (SAS Institute, Inc., Cary, NC) computer software was used for analysis. Data in percentages were transformed using arcsine transformation before analysis. ANOVA indicated no significant ($P = 0.05$) block effect for each assay; thus, data from interaction effects were presented. Using Minitab 7.0 software (Minitab, Inc., State College, PA), correlation analysis was performed among anastomosis groups, between disease incidence and severity, between agar-plate and potted plant assays for root/hypocotyl rot severity, and between detached leaf and whole-plant assays for leaf blight.

RESULTS

Anastomosis groups. Of the 350 isolates originally recovered from diseased plant tissues, 60 had binucleate and 290 had multinucleate vegetative hyphal cells. Pairing of test isolates and tester strains revealed that the 290 multinucleate isolates collected represented three anastomosis groups. Ohio dry bean and Ohio soybean isolates anastomosed with the AG-2-2 tester isolate. Zaire dry bean isolates anastomosed with the AG-4 tester isolate, and all foliar isolates anastomosed with the AG-1 IA tester isolate. Considerable variability in color of vegetative hyphae, zonation, and sclerotial formation and distribution on agar was observed among the 290 isolates after a 14-day growth period on PDA-YE at room temperature in continuous darkness. All isolates that anastomosed with the AG-2-2 tester isolate were characterized by light- to dark-brown vegetative hyphae, had definite concentric rings of dark-brown hyphae with few sclerotia, and grew on PDA-YE at 35 C. These isolates were assigned to AG-2-2 IIIB. All isolates, except one, that anastomosed with the AG-4 tester isolate were characterized by the absence of apparent zonation and sclerotia and were morphologically identical to the AG-4 tester isolate. The isolates that anastomosed with the AG-1 IA isolate were characterized mainly by the presence of brownish mycelial patches that radiated from the center of the agar plate, whitish vegetative hyphae, and moderate sclerotial development scattered over the agar surface. All of these isolates were assigned to AG-1 IB because they produced small sclerotia on inoculated soybean and dry bean plants in growth-chamber virulence tests.

Agar-plate virulence assay on roots and hypocotyls. Infection of seedlings was observed 5 days after inoculation. Soybean AG-2-2 IIIB isolates mainly caused root rot, AG-1 IB isolates caused root as well as hypocotyl necrosis, and dry bean AG-2-2 IIIB and AG-4 isolates caused more disease on hypocotyls than on roots. Although all AG-1 IB isolates and soybean AG-2-2 IIIB isolates caused root lesions, the latter induced complete root decay, whereas root length of seedlings inoculated with the former isolates was near normal. Soybean and dry bean seedlings inoculated with dry bean AG-2-2 IIIB isolates and with AG-4 isolates had well-developed roots without apparent disease symptoms.

The anastomosis group by host-interaction effect was significant ($P < 0.001$) for hypocotyl- and root-disease incidence and for disease severity (Fig. 1). AG-4 and AG-1 IB isolates caused hypocotyl symptoms on significantly more dry bean seedlings than did the AG-2-2 IIIB isolates, and no infection was recorded on soybean hypocotyls after inoculations with soybean AG-2-2 IIIB isolates (Fig. 1A). The dry bean AG-2-2 IIIB and AG-4 isolates caused root lesions on significantly more dry bean than on soybean seedlings (Fig. 1B). The soybean AG-2-2 IIIB and AG-1 IB isolates caused more disease on each host than did the

dry bean AG-2-2 IIIB and AG-4 isolates (Fig. 1C). Soybean seedlings had significantly lower disease severities than had the dry bean seedlings, regardless of the isolate used to inoculate seedlings.

Within each anastomosis group, the interaction between isolate and host was significant for all the response variables measured ($P = 0.05$) (data not presented). Out of 12 isolates tested in each anastomosis group, nine dry bean AG-2-2 IIIB, 10 soybean AG-2-2 IIIB, and one AG-4 isolate did not cause lesions on soybean hypocotyls. Conversely, only two dry bean AG-2-2 IIIB isolates did not cause lesions on soybean roots.

Potted plant pathogenicity assay on roots and hypocotyls. Various disease symptoms were observed on seedlings 8 days after seeds were planted in the infested soil/peat mix. On hypocotyls, infections started as superficial, irregular, reddish lesions that later developed into deep, light- or dark-brown lesions that sometimes girdled the stem. Infected roots had light-brown necrotic lesions at first. Root-tip necrosis and extensive root decay were observed later. Other symptoms included pre- and postemergence damping-off and cotyledon necrosis.

The anastomosis group-host interaction was significant for all the response variables measured (Fig. 2). Inoculation with the

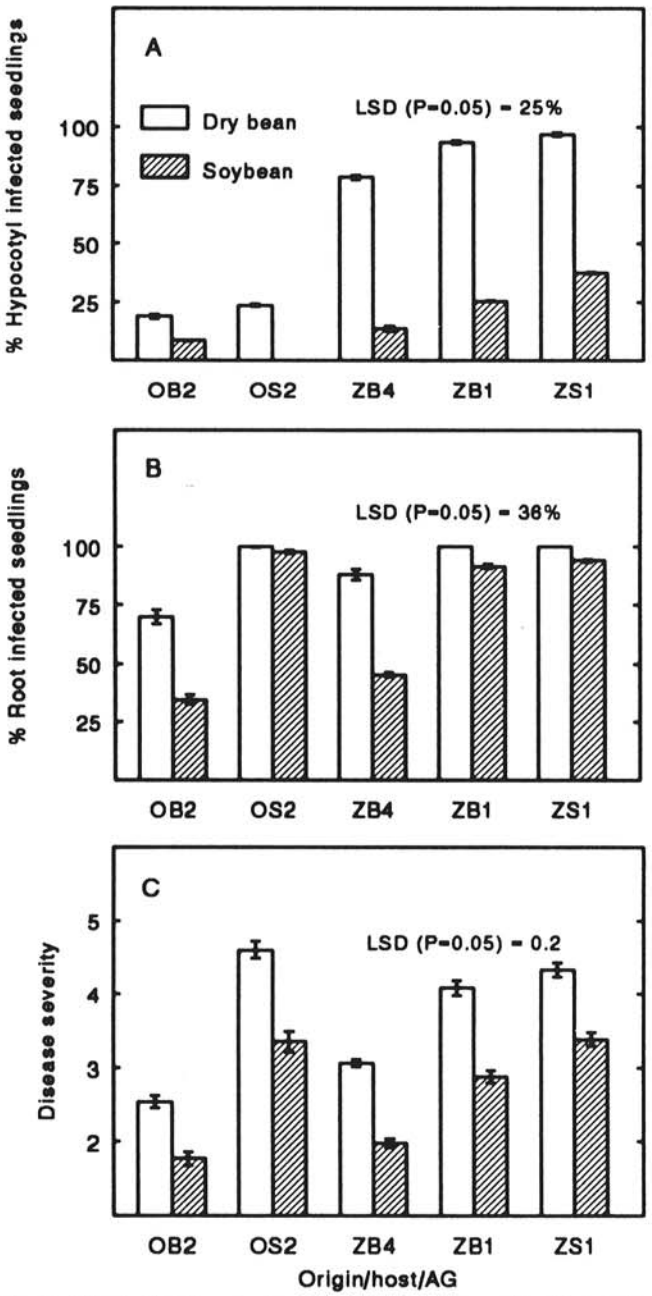

Fig. 1. Effect of *Rhizoctonia solani* isolates, representing five origin/host/anastomosis-group categories, and host species on **A,** hypocotyl- and **B,** root-disease incidence and **C,** disease severity on soybean cv. Williams and dry bean cv. Great Northern seedlings in an agar-plate virulence assay. Disease severity was based on a 1–5 scale: 1 = no lesions, normal root development and 5 = complete root rot, root length greatly restricted. Data are pooled means of 12 isolates for each origin/host/anastomosis-group category. OB2 = Ohio/dry bean/AG-2-2 IIIB, OS2 = Ohio/soybean/AG-2-2 IIIB, ZB4 = Zaire/dry bean/AG-4, ZB1 = Zaire/dry bean/AG-1 IB, and ZS1 = Zaire/soybean/AG-1 IB isolates. Bars indicate standard errors.

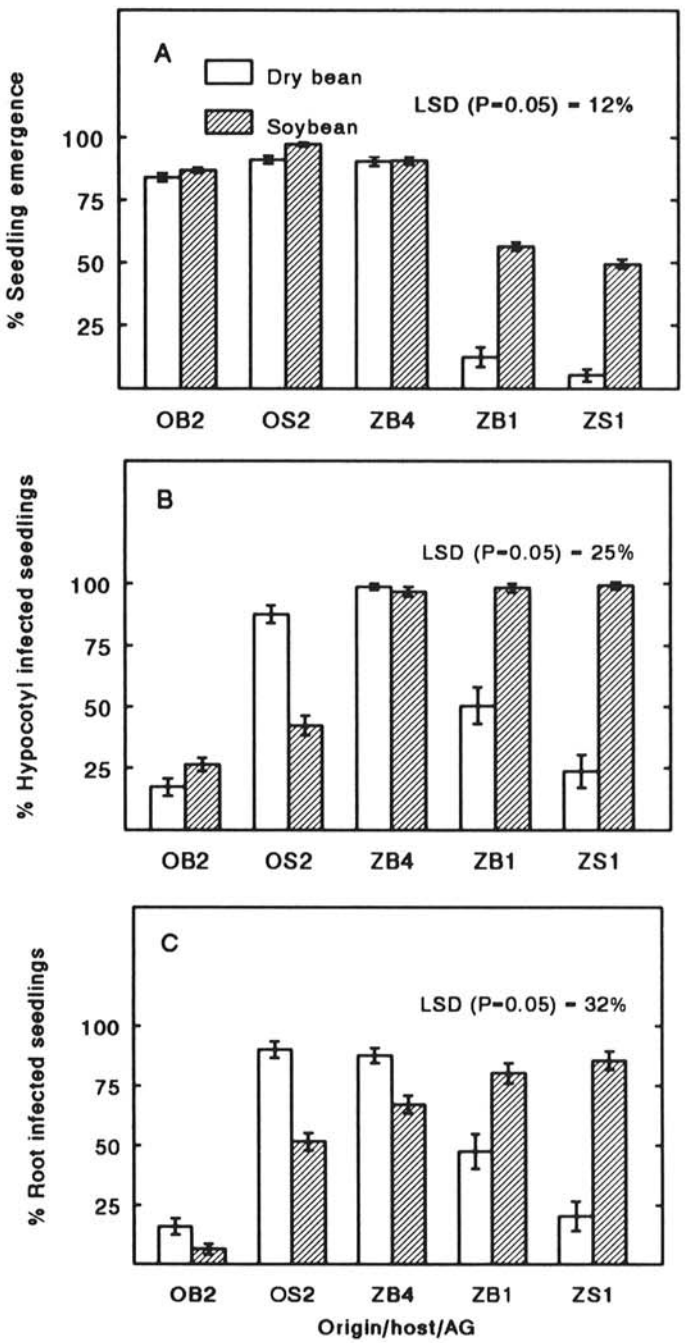

Fig. 2. Effect of *Rhizoctonia solani* isolates, representing five origin/host/anastomosis-group categories, and host species on **A,** percent emergence and **B,** hypocotyl- and **C,** root-disease incidence on soybean cv. Williams and dry bean cv. Great Northern seedlings in a potted plant virulence assay. Data are pooled means of 12 isolates for each origin/host/anastomosis-group category. OB2 = Ohio/dry bean/AG-2-2 IIIB, OS2 = Ohio/soybean/AG-2-2 IIIB, ZB4 = Zaire/dry bean/AG-4, ZB1 = Zaire/dry bean/AG-1 IB, and ZS1 = Zaire/soybean/AG-1 IB isolates. Bars indicate standard errors.

AG-1 IB isolates caused a greater percentage of dry bean than soybean seedlings to die from preemergence damping-off (Fig. 2A). The interaction effect on the percentage of seedlings with hypocotyl and root infections was the result of AG-1 IB isolates causing more soybean than dry bean seedlings to develop lesions and soybean AG 2-2 IIIB isolates causing fewer soybean than dry bean seedlings to develop lesions (Fig. 2B and C). Likewise, hypocotyl- and root-disease severity was greater on dry bean than on soybean seedlings in treatments with soybean AG-2-2 IIIB and AG-1 IB isolates ($P < 0.001$) (Fig. 3). No statistical difference occurred between hosts for hypocotyl- or root-disease severity when inoculated with dry bean AG-2-2 IIIB and AG-4 isolates.

As in agar-plate virulence assays on roots and hypocotyls, some variation in the ability to cause symptoms occurred among isolates of the same anastomosis group and origin (data not presented). Variation in the percentage of seedling emergence was greatest among soybean AG-1 IB isolates, soybean AG-2-2 IIIB isolates causing hypocotyl symptoms, dry bean AG-2-2 IIIB isolates causing root disease, and soybean AG-2-2 IIIB and AG-4 isolates causing root-disease severity. Only two isolates, one each of dry bean AG-2-2 IIIB and soybean AG-2-2 IIIB, caused no symptoms on either host.

Detached leaf virulence assay. Leaflets developed disease symptoms 2 days after inoculation. The first symptoms were small, irregular, water-soaked lesions around the inoculum. Discrete lesions later coalesced, and depending on the host and the anastomosis group, as much as the entire leaflet became blighted by 4 days after inoculation.

AG-2-2 IIIB isolates did not cause leaf blight on detached leaves; thus, results from AG-2-2 IIIB isolates were excluded from statistical analyses, and data are not presented. The interaction between anastomosis group and host was significant ($P = 0.05$) for severity of leaf blight (Fig 4). All AG-1 IB isolates caused leaf lesions and exhibited less variation in their ability to colonize leaves than did AG-4 isolates. Generally, soybean had lower leaf-blight severities than had dry bean when inoculated with either the AG-1 IB or AG-4 isolates; however, the AG-4 isolates caused much less disease than did the AG-1 IB isolates on soybean than on dry bean. Dry bean AG-1 IB isolates 38 and 57 caused the least amount of disease of the AG-1 IB isolates tested, with disease-severity scores of 3.7, and AG-4 isolate 54 did not cause lesions on leaves.

Whole-plant virulence assay on leaves. Leaf lesions were observed 48 h after inoculation with AG-1 IB isolates. Symptoms appeared as water-soaked irregular lesions, but symptom expression varied with the isolate tested. Leaf blight, mycelial webs, and small, brown sclerotia were observed on infected leaves by 3–4 days after inoculation. Leaf death was recorded from 4 to 5 days after inoculation.

All AG-2-2 IIIB isolates and all but one AG-4 isolate failed to cause symptoms on plants under growth-chamber conditions. AG-4 isolate 39 caused water-soaked lesions (disease-severity score = 2) by the end of the incubation period (5 days). All nonpathogenic isolates were excluded from statistical analysis, and data are not presented.

ANOVA detected no statistical differences ($P = 0.05$) among soybean AG-1 IB isolates for foliar-disease severity or for the soybean AG-1 IB isolate-host interaction. Dry bean AG-1 IB isolates, however, varied in their ability to colonize leaf tissues on both hosts (Fig. 5). Isolates 38 and 54 caused the least amount of disease among the 12 isolates tested from dry bean.

Correlations among assays. Correlation analyses indicated a significant relationship between root disease-severity ratings in agar-plate assays and root-disease severity in potted plant assays for dry bean AG-2-2 IIIB ($r = 0.70$, $P \leq 0.01$), soybean AG-2-2 IIIB ($r = 0.64$, $P \leq 0.05$), and soybean AG-1 IB isolates ($r = 0.66$, $P \leq 0.01$). This relationship was not significant for dry bean AG-4 isolates. The relationship between root disease-severity ratings in agar-plate assays and hypocotyl disease-severity ratings in potted plant assays was significant for dry bean AG-

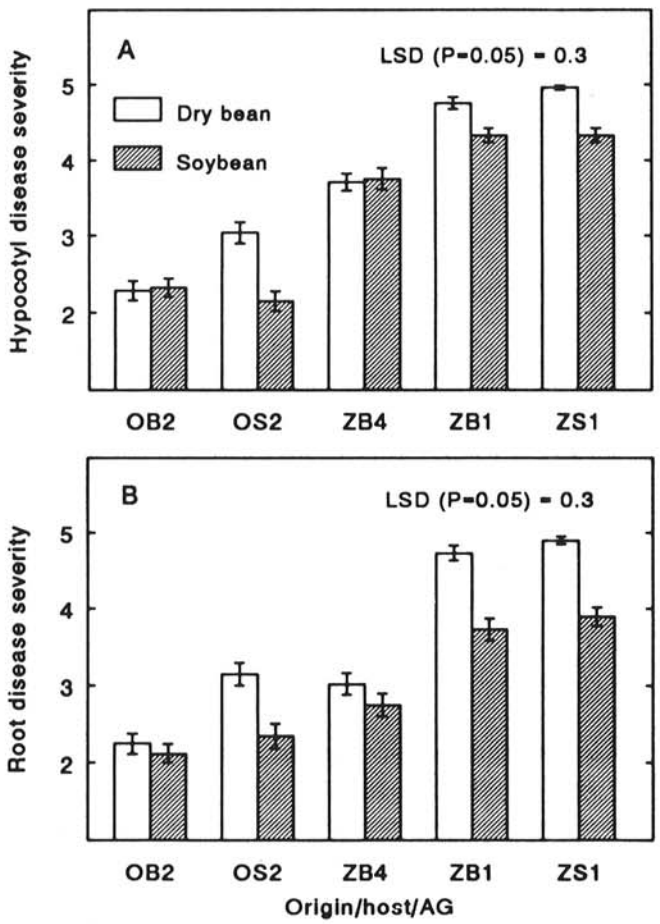

Fig. 3. Effect of *Rhizoctonia solani* isolates, representing five origin/host/anastomosis-group categories, and host species on **A,** hypocotyl- and **B,** root-disease severity on soybean cv. Williams and dry bean cv. Great Northern seedlings in a potted plant virulence assay. Disease severity was based on a 1–5 scale. For hypocotyls, 1 = no symptoms and 5 = damping-off resulting from stem girdling. For roots, 1 = no symptoms and 5 = damping-off resulting from extensive root rot. Data are pooled means of 12 isolates for each origin/host/anastomosis-group category. OB2 = Ohio/dry bean/AG-2-2 IIIB, OS2 = Ohio/soybean/AG-2-2 IIIB, ZB4 = Zaire/dry bean/AG-4, ZB1 = Zaire/dry bean/AG-1 IB, and ZS1 = Zaire/soybean/AG-1 IB isolates. Bars indicate standard errors.

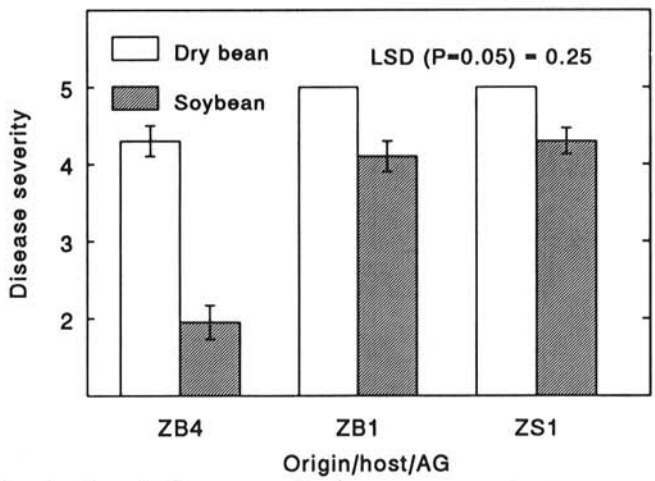

Fig. 4. Effect of *Rhizoctonia solani* isolates, representing three origin/host/anastomosis-group categories, and host species on foliar-disease severity on soybean cv. Williams and dry bean cv. Great Northern seedlings in a detached leaf virulence assay. Disease severity was based on a 1–5 scale: 1 = no symptoms and 5 = 76–100% leaf area blighted. Data are pooled means of 12 isolates for each origin/host/anastomosis-group category. ZB4 = Zaire/dry bean/AG-4 , ZB1 = Zaire/dry bean/AG-1 IB, and ZS1 = Zaire/soybean/AG-1 IB isolates. Bars indicate standard errors.

2-2 IIIB ($r = 0.49$, $P \leq 0.05$) and for soybean AG-1 IB isolates ($r = 0.75$, $P \leq 0.0005$) but not for soybean AG-2-2 IIIB or dry bean AG-4 isolates. In leaf blight virulence assays, disease severity in detached leaf assays was significantly correlated ($r = 0.70$, $P \leq 0.01$) with disease-severity ratings in whole-plant assays for soybean and dry bean AG-1 IB isolates.

DISCUSSION

The population of *R. solani* isolates studied belonged to three anastomosis groups and exhibited a considerable variability in morphological characteristics. Within anastomosis groups, some differences were observed among AG-2-2 IIIB isolates, but on PDA-YE medium, isolates were consistently characterized by sharply concentric zonation and few sclerotia. AG-1 IB isolates produced moderate numbers of sclerotia scattered over the agar surface; AG-4 isolates exhibited faint zones without apparent sclerotia. Zonation in AG-2-2 IIIB isolates was the result of aggregation of small sclerotia, whereas zonation in AG-4 was the result of periodic changes in mycelial density (38). These results indicate relationships between cultural morphology and anastomosis groups and corroborate earlier findings on variation within the species. Standard culture conditions are clearly needed for such studies because the use of different culture media, vessels, and temperature regimes can alter cultural morphology of *R. solani* (6,32).

All isolates obtained from soybean and dry bean hypocotyl and root lesions in Ohio were in AG-2-2 IIIB. Isolates from the same crops and plant parts in Zaire were in AG-4. The basis for this difference might simply be differences in ecological conditions between the two geographical locations. The geographical distribution of anastomosis groups of *R. solani* has been associated with such factors as soil type (25), altitude (8), and cropping pattern (23). All foliar isolates from either host were assigned to AG-1 IB and exhibited the morphological characteristics of the soybean web blight isolates reported in Texas (12).

Results of virulence assays revealed that AG-1 IB isolates were as virulent on roots and hypocotyls as were AG-2-2 IIIB and AG-4 isolates under our experimental conditions. Earlier reports (7,25,32) on virulence, host-range, and disease-type relationships among anastomosis groups have not indicated any clear-cut delineations among these characteristics. AG-1 isolates are reported to cause seed rot, pre- and postemergence damping-off, root and hypocotyl rot, and foliar blight (25). Our tests using artificial inoculation with AG-1 IB isolates agree with these findings, but under natural conditions, the type of disease incited by AG-1 IB may be limited by host or environmental factors.

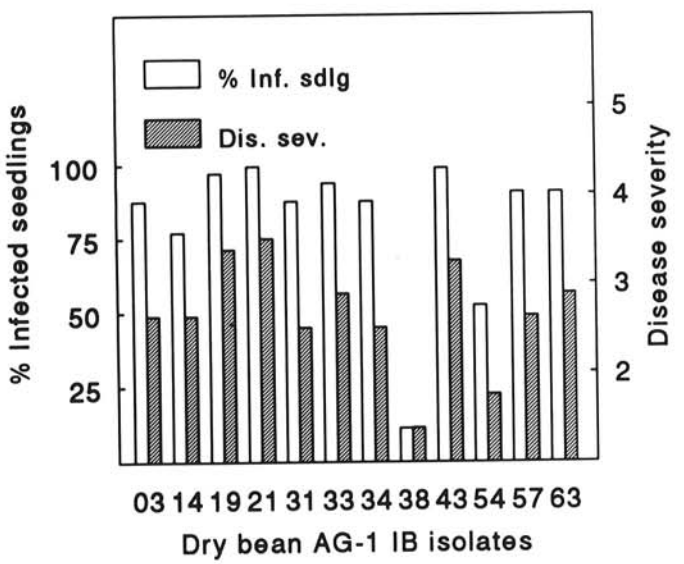

Fig. 5. Effect of *Rhizoctonia solani* dry bean AG-1 IB isolates on percent infected seedlings and disease severity on soybean cv. Williams and dry bean cv. Great Northern seedlings in a whole-plant virulence assay for foliar blight. Disease severity recorded 5 days after inoculation on plants maintained at 25 C and 100% relative humidity. Disease-severity ratings are based on a 1–5 scale: 1 = no symptoms and 5 = plant leaves dead. Data are pooled means for dry bean and soybean. Percent infected seedlings: LSD = 11% and $P = 0.05$. Disease severity: LSD = 0.25 and $P = 0.05$.

The AG-2-2 IIIB isolates tested were not capable of infecting leaves. AG-4 isolates infected detached leaves in the laboratory assays but not intact plants in growth-chamber assays. Liu and Sinclair (16,17) reported that an AG-2-2 IIIB isolate from soybean in Illinois caused both leaf blight and root rot of soybeans in greenhouse studies. Another report indicated that AG-2 isolates had significantly lower foliar disease-severity ratings in inoculated tests than had AG-1 isolates (7). All the isolates we obtained from soybean and dry bean foliage were assigned to AG-1 IB. Our results and those of others (3,21) indicate AG-1 isolates are primarily foliar pathogens, whereas AG-2 and AG-4 isolates are primarily root and hypocotyl pathogens (3,21).

We are unable to explain why dry bean AG-2-2 IIIB isolates were less virulent on roots and hypocotyls than were soybean AG-2-2 IIIB isolates. Isolates from dry bean were baited from potted seedlings in soil previously planted to soybeans, and isolates from soybeans were recovered from diseased plant material from the field. Virulence of *R. solani* isolates from a given farm field depends on the host plants, cropping history, and the disease types previously encountered (11,13,21). Therefore, the use of the baiting technique to obtain dry bean isolates may have had an effect on the type and virulence of isolates recovered.

Considerable variation was detected in virulence within anastomosis groups, even in the original tissue type from which the isolate was obtained. In *R. solani*, virulence is not a determining characteristic of the species (26). In a comprehensive study of the relationship between pectolytic and cellulytic enzyme production and virulence in *R. solani*, pectin methylesterase and polygalacturonase activities were higher in foliar than in root isolates, and enzyme activity was positively correlated with virulence. Thus, differences in enzymatic activity may explain the variation detected in the virulence of tested isolates.

Results from agar-plate and potted plant virulence assays had similar anastomosis-group trends. The relationship between data from agar-plate and potted plant assays for root and hypocotyl rot was positive and significant for dry bean AG-2-2 IIIB, soybean AG-2-2 IIIB, and soybean AG-1 IB isolates but not for dry bean AG-4 isolates. The correlation between the detached leaf and the whole-plant assays for AG-1 IB isolates was also positive and significant. Some variation in ability to cause disease symptoms occurred among individual isolates within anastomosis groups, however. This suggests that agar-plate assays can be used as rapid methods for testing virulence differences among anastomosis groups but should not replace virulence testing with seedlings or whole plants for determining variation in virulence among isolates within anastomosis groups.

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