Relationships Between Inoculum Density of *Rhizoctonia solani*, Wirestem Incidence and Severity, and Growth of Cabbage

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ABSTRACT

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Rhizoctonia solani AG-4 was grown on cornmeal sand and added to nonsterile sandy loam soil at rates of 0, 0.5, 1.0, and 2.0% vol/vol. Ten 2-week-old seedlings of cabbage cv. Gourmet were transplanted individually into infested soil in cells of plug trays. Incidence and severity of wirestem increased nonlinearly (monomolecular model) and fresh plant weight decreased nonlinearly (exponential decay model) as the inoculum density was increased. Experiments to simulate cabbage seedbed conditions were conducted in nonsterile soil in flats in the greenhouse and in fumigated soil in the field. Sclerotia produced on autoclaved green beans were added to soil at 0, 1.25, 12.5, 125, and 1,250 sclerotia/kg. In two greenhouse trials, emergence was reduced significantly at the highest inoculum density. Both incidence of wirestem and area under the disease progress curve increased linearly with the base-ten logarithm of the inoculum density. In field trials in the spring and fall, emergence and plant fresh weight decreased quadratically and incidence of wirestem increased linearly with the base-ten logarithm of the inoculum density.

Additional keywords: *Brassica oleracea* var. *capitata*, preemergence damping-off.

Rhizoctonia solani Kühn reduces emergence (preemergence damping-off) of direct-seeded crucifers and other vegetables in seedbeds and production fields (9,10,14,23,25,26). This soilborne pathogen also reduces stands through postemergence damping-off and stunts surviving plants by causing wirestem and other hypocotyl rots on older seedlings and transplants (9,15,19,20,21,23,25). As a result, plant maturity is delayed (25), yield is reduced (20), and, in addition to decreased returns, production costs may increase because uneven maturation often requires multiple harvests.

Rhizoctonia preemergence damping-off of crucifers has been well characterized. Preemergence damping-off of radish increased as the level of *R. solani* added to the soil increased, and it plateaued at 8,000 ppm of cornmeal-sand inoculum (14). In another study, preemergence damping-off of radish was 50% at 18 propagules per 100 g of soil (10). Preemergence damping-off of canola increased and reached a maximum near 5,000 viable propagules per liter of soilless medium (26). In those studies, however, no information was reported on postemergence damping-off or wirestem (10,14,26). In some cases, very high levels of inoculum, e.g., > 20,000 colonized rye grain fragments per liter of medium, were used (26). Because these levels of inoculum far exceed the highest field levels, information is needed about the activity of *R. solani* at inoculum densities more representative of field conditions.

In general, as the soil inoculum density of *R. solani* is increased, incidence and severity of root rot increase. For example, incidence and severity of Rhizoctonia root rot on snap bean increased as the ratio of infested/noninfested soil was increased (21). In a field study, root rot incidence and severity on snap bean increased to a maximum level of 250 to 300 sclerotia per kilogram of fumigated soil (25). Root rot on canola (*Brassica napus* L. and *B. rapa* L.) seedlings was 8 to 22% at 1,000 viable propagules per liter of soilless medium (9). The *Brassica c*-genome of *B. oleracea* L. has been associated with increased susceptibility to *R. solani* (9). It is not known whether the relationship between inoculum density and disease incidence for vegetable Brassicas, primarily *B. oleracea* varieties, is similar to that reported for canola and snap bean.

The objective of this study was to determine the response of cabbage (*B. oleracea* var. *capitata*) to different inoculum densities of *R. solani* AG-4. Emergence, incidence and severity of wirestem, and plant growth were quantified in greenhouse and field experiments.

MATERIALS AND METHODS

Pathogen culture. *R. solani* AG-4 isolate SP-1 was obtained from a diseased spinach root in Bamberg County, SC, in December 1991, and stored on soil-wheat bran at –20°C (4). Cornmeal sand (3% wt/wt) in 250-ml flasks was autoclaved for 1 h on three successive days. Cultures of *R. solani* on potato-dextrose agar (PDA) (7 to 10 days old) were flooded with sterile distilled water, gently scraped to make a mycelial suspension, and 5 ml of suspension was added to each flask of cornmeal sand. Flasks were incubated at ambient temperature (22 to 25°C) for 1 week. Colony-forming units were determined by suspending 0.1 g of colonized cornmeal sand in 9.9 ml of sterile distilled water and plating dilutions on one-quarter-strength PDA. Sclerotia were produced on autoclaved green beans (24) and sieved to separate those of 710- to 1,000-μm diameter. Sclerotia were stored at –4°C for 1 to 6 months before use. Germinability was tested before each experiment by placing 100 sclerotia on water agar and counting the number which had germinated after 1 and 2 days.

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Greenhouse experiments. Cabbage cv. Gourmet (Ferry-Morse Seed Co., San Juan Bautista, CA), which is very susceptible to wirestem (19), was used in all experiments. Seed were sown in 60% vermiculite–40% peat (Fafard #2 commercial potting mix; Piedmont Nursery Supply, Spartanburg, SC) in 128-cell plastic trays in a greenhouse. Two-week-old seedlings were transplanted into infested soil in 72-cell plastic trays. The experiment was a randomized complete block design with four replications and five treatments. Each replication consisted of 10 transplants. The treatments included noninfested sandy loam soil, soil infested with 0.5, 1, and 2% colonized cornmeal sand (vol/vol), and soil amended with noncolonized cornmeal sand (2% vol/vol) as a control. The experiment was performed twice.

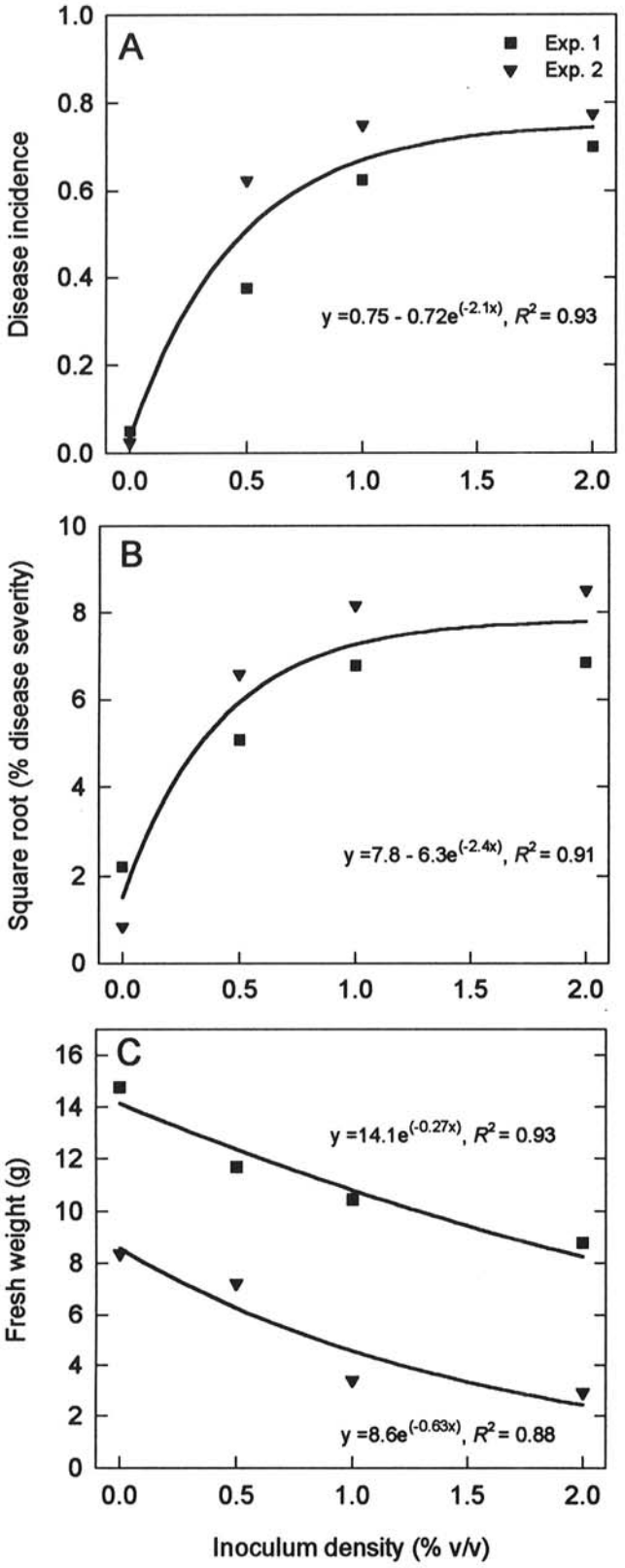

Fig. 1. Relationships between inoculum density (expressed as percent [vol/vol] colonized cornmeal sand in nonsterile soil) of *Rhizoctonia solani* AG-4 and **A,** wirestem incidence; **B,** wirestem severity; and **C,** fresh weight of individual cabbage plants 2 weeks after inoculation. Each data point is the mean of four replications, 10 plants per replication.

Separate experimental units were harvested 1 and 2 weeks after inoculation. Plants were gently removed from the trays and washed with a stream of water to remove adhering soil. Disease severity on the stem and roots was rated on a pretransformed scale of 1 to 4: 1 = no symptoms (0%); 1.1 to 2 = ≤ 33% of the stem cortex with small (≤ 2-mm diameter), discrete lesions; 2.1 to 3 = 34 to 67% of the stem cortex girdled with coalesced lesions; and 3.1 to 4 = 68 to 100% of the stem and root cortex and lateral roots decayed. Disease incidence was the percent plants with symptoms, i.e., those rated ≥ 2. Plant fresh weight was measured.

To simulate cabbage seedbeds, nonsterile sandy loam soil was infested with sclerotia at rates of 0, 1.25, 12.5, 125, and 1,250 per kilogram of soil. Cabbage cv. Gourmet seed were sown in plastic trays (32 × 65 cm) at one seed per 1.2 cm of row in three rows per tray spaced 10 cm apart. The experiment, a randomized complete block design with four replications, was performed twice. Emergence was counted at 7 days after planting and the number of diseased seedlings was determined five times (5). Plants were considered diseased if a purplish black, sunken lesion was visible on the hypocotyl at the soil line (5).

Five to 6 weeks after planting, 20 plants were dug from the center row of each flat. Symptoms differed from those on transplants in previous experiments, so a 1 to 7 scale was used to assess wirestem severity: 1 = no symptoms, 2 = superficial cracking of the stem cortex, 3 = 0 to < 25% of the stem circumference girdled ≥ 1-mm deep, 4 = 25 to 75% of the stem circumference girdled ≥ 1-mm deep, 5 = > 75 to 100% of the stem circumference girdled ≥ 1-mm deep, 6 = stem girdled through the endodermis, and 7 = lower stem decayed through the stele and lateral roots absent. Disease incidence was calculated as the percent plants with wirestem, i.e., those rated ≥ 3.

Field experiments. Field plots were established at the Coastal Research and Education Center, Charleston, SC, in Yonges loamy fine sand (Typic Albaquult). Soil was fumigated by injecting 121 kg/ha of 98% methyl bromide–2% chloropicrin 15-cm deep and covered with black polyethylene mulch for 3 weeks to reduce the level of indigenous *R. solani*. Before planting, plots were fertilized with 168 kg/ha of N, 75 kg/ha of P, and 140 kg/ha of K (1680 kg/ha of 10-10-10 N-P-K fertilizer) and treated with the herbicide trifluralin (Treflan 4EC, 0.67 kg/ha). The experimental design was a split plot with four replications. Cabbage cultivar, either Gourmet or Fortuna (PetoSeed, Saticoy, CA), was the main plot treatment and the inoculum density was the subplot treatment. Each subplot was 0.9-m wide and 1.0-m long. Sclerotia were spread over the surface of each subplot at 0, 1.25, 12.5, 125, or 1,250 per kilogram of soil (based on a soil weight of 2.2×10^6 kg of soil/ha to a depth of 15 cm) and incorporated 15-cm deep with a hand-held rotary tiller. Plots were raked smooth and seeded on 2 March and 21 September 1993. Seed were sown by hand at one seed per 1.2 cm of row in three rows per plot spaced 15 cm apart. Emergence was counted at 21 days after planting in the spring and at 14 and 21 days after planting in the fall. The number of diseased seedlings was determined twice for each trial. Plants were considered diseased if a purplish black, sunken lesion was visible at the soil line.

Twenty plants were dug from the center row of each plot on 23 April (52 days after planting) and 27 October 1993 (36 days after planting). Disease incidence and severity were determined as described previously for the greenhouse experiments. The fresh weight of healthy and diseased plants in each treatment was

measured. Stem segments with lesions were surface disinfested in 0.5% NaOCl solution for 30 s, rinsed in sterile distilled water, and incubated on water agar plus 100 mg/liter of streptomycin sulfate. Hyphae growing from diseased tissue were transferred to PDA to verify identification as *R. solani*. Hyphae of randomly selected colonies were stained with 3% KOH and alkaline safranin O to determine the number of nuclei per cell (2). The identity of isolates recovered in the fall of 1993 was determined by pairing them with *R. solani* SP-1 on glass slides coated with water agar (13). Pairings were checked for C3 anastomosis points (fusions between walls and membranes in which the diameter of the hyphae at the point of anastomosis is not less than the average hyphal diameter), which indicate that the two isolates belong to the same anastomosis group, the same clone, or are the same isolate (12).

Statistical analysis. Analysis of variance and regressions were performed with PROC GLM of SAS (release 6.10; SAS Institute Inc., Cary, NC). All data sets were tested for equality of variance and normality before analysis, and transformed when necessary. Data from individual trials were pooled and analyzed together when error variances were equal, according to two-tailed F tests (22). Area under the disease progress curve (AUDPC) was calculated from the disease incidence ratings with standard iterative procedures (18). Linear and nonlinear regression analyses (1) were used to examine the effect of inoculum density and cultivar on emergence, percent disease incidence, disease severity, AUDPC, and plant fresh weight. Analyses of disease incidence and total plant weight were weighted by the inverse of the number of plants per plot (22). When the monomolecular model was used, the asymptote was estimated from the data (17). Least-squares means *t* tests were used to compare inoculum levels to the noninfested control or cultivars at a given inoculum level after a regression model had been selected (7).

RESULTS

Greenhouse experiments. The proportion of cabbage transplants with wirestem increased at a decreasing rate as the inoculum density (percent [vol/vol] cornmeal sand colonized by *R. solani* added to the soil) increased ($P < 0.005$) (Fig. 1A). Wirestem severity (degree of hypocotyl infection, girdling, and root rot expressed as a square-root percent) also increased at a decreasing rate as the inoculum density was increased (Fig. 1B). Because there was no significant difference between harvests at 1 and 2 weeks after inoculation, only data from 2 weeks are shown. Data from the two experiments were pooled, based on equality of error variances. The monomolecular (asymptotic) growth model provided the best fit to the data for both incidence and severity, which were correlated highly with each other ($r = 0.95$, $P = 0.0001$) and increased at similar rates (2.1 and 2.4, respectively).

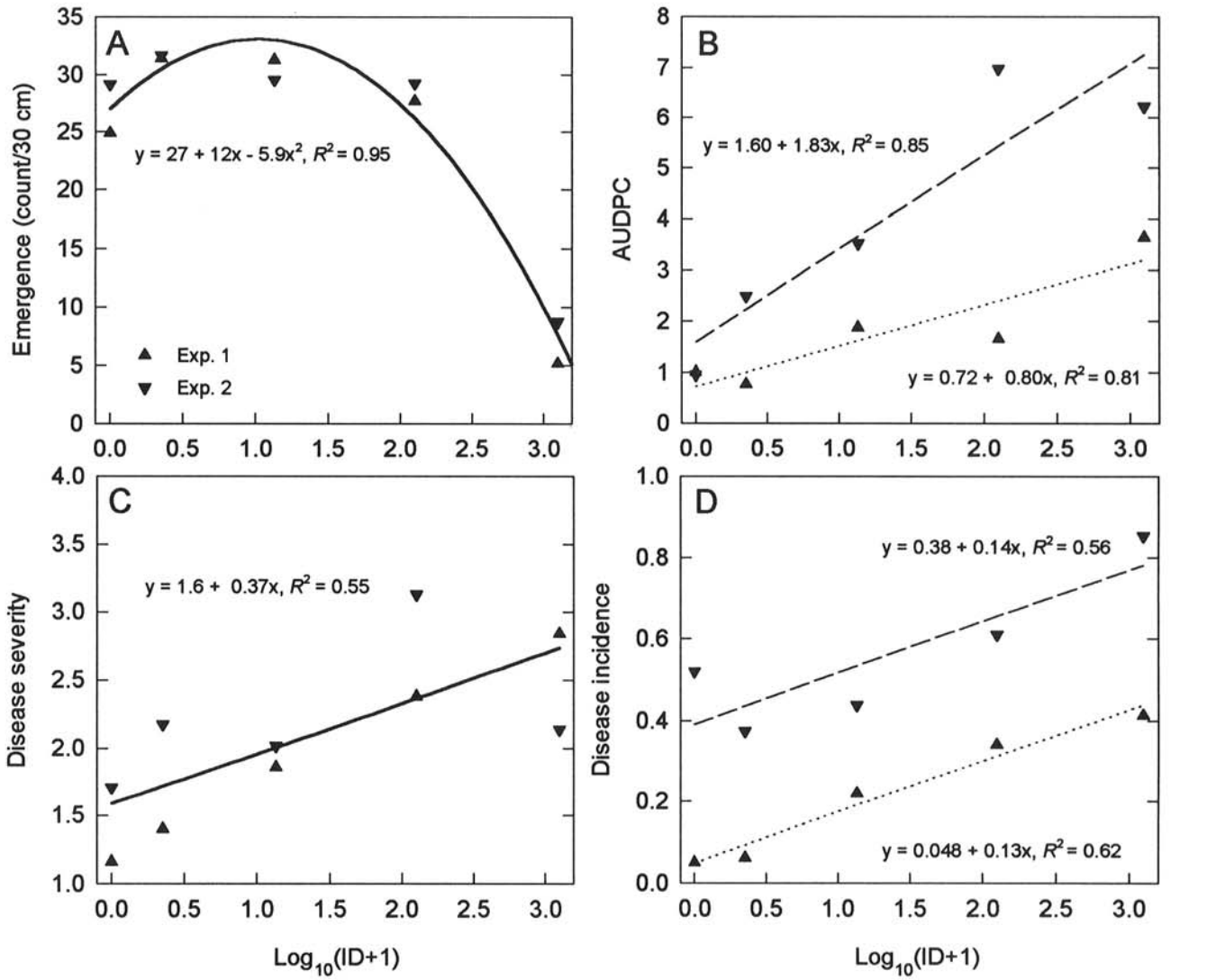

Fig. 2. Relationships between inoculum density ($\log_{10}$[sclerotia per kilogram of nonsterile soil]) of *Rhizoctonia solani* AG-4 and **A,** emergence of cabbage seedlings; **B,** area under the disease progress curve (AUDPC); **C,** wirestem severity (rated on a 1 to 7 scale); and **D,** incidence of wirestem at harvest in greenhouse experiments. Data points are the means of four replications.

The predicted maximum incidence and severity (asymptotes) were 76 and 61%, respectively, based on means in the two trials. The predicted incidence and severity in noninfested soil, y-intercepts of 3.6 and 2.3%, respectively, were not significantly different from 0 (95% confidence intervals). There were no significant differences between noninfested soil and soil amended with noncolonized cornmeal sand.

The fresh weight of cabbage plants harvested 2 weeks after inoculation decreased exponentially as the inoculum density increased ($P < 0.005$) (Fig. 1C). Because of overall plant growth, weights after 2 weeks were significantly different from weights after 1 week, and plants were significantly larger in the second trial than in the first. Increasing the inoculum density of *R. solani* from 0 to 2% cornmeal sand reduced the mean fresh weight of an individual cabbage plant by 6 g in both trials.

Preemergence damping-off of cabbage seedlings in flats in the greenhouse was severe at the highest inoculum density, 1,250 sclerotia per kilogram of nonsterile soil (Fig. 2A). Emergence in this treatment was significantly less than in noninfested soil (t test, $P = 0.0001$). However, at lower inoculum levels, emergence was not significantly different from the noninfested control. A quadratic equation gave the best fit to mean emergence and the base-ten logarithm of the inoculum density. Data from the two experiments were pooled, based on equality of error variances.

Mean AUDPC between 2 and 7 weeks after planting increased linearly as the base-ten logarithm of the inoculum density increased (F significant at $P < 0.05$) (Fig. 2B). AUDPC was higher in the second trial than in the first trial. Disease severity at harvest was moderate, similar in both experiments, and increased linearly as the base-ten logarithm of the inoculum level increased (F significant at $P \leq 0.01$) (Fig. 2C). Incidence of wirestem on harvested plants increased linearly as the base-ten logarithm of the inoculum increased in both trials (F significant at $P \leq 0.0002$) (Fig. 2D). Slopes, 0.13 and 0.14 in trials one and two, respectively, did not differ, but the y-intercept was significantly higher in trial two than in trial one. Incidence and severity on harvested plants were correlated with each other ($r = 0.86$, $P = 0.0001$).

Field experiments. In both the spring and fall plantings of 1993, preemergence damping-off of cabbage seedlings became greater as the rate of sclerotia added to the soil increased from 0 to 1,250/kg (Fig. 3A). At the highest inoculum level, 86 to 93 and 100% of the seedlings failed to emerge in the spring and fall plantings, respectively. In the spring at 27 days after planting, quadratic equations described the relationship between emergence and the base-ten logarithm of the inoculum density for both cultivars (Fs significant at $P = 0.0001$). Within each cultivar, emer-

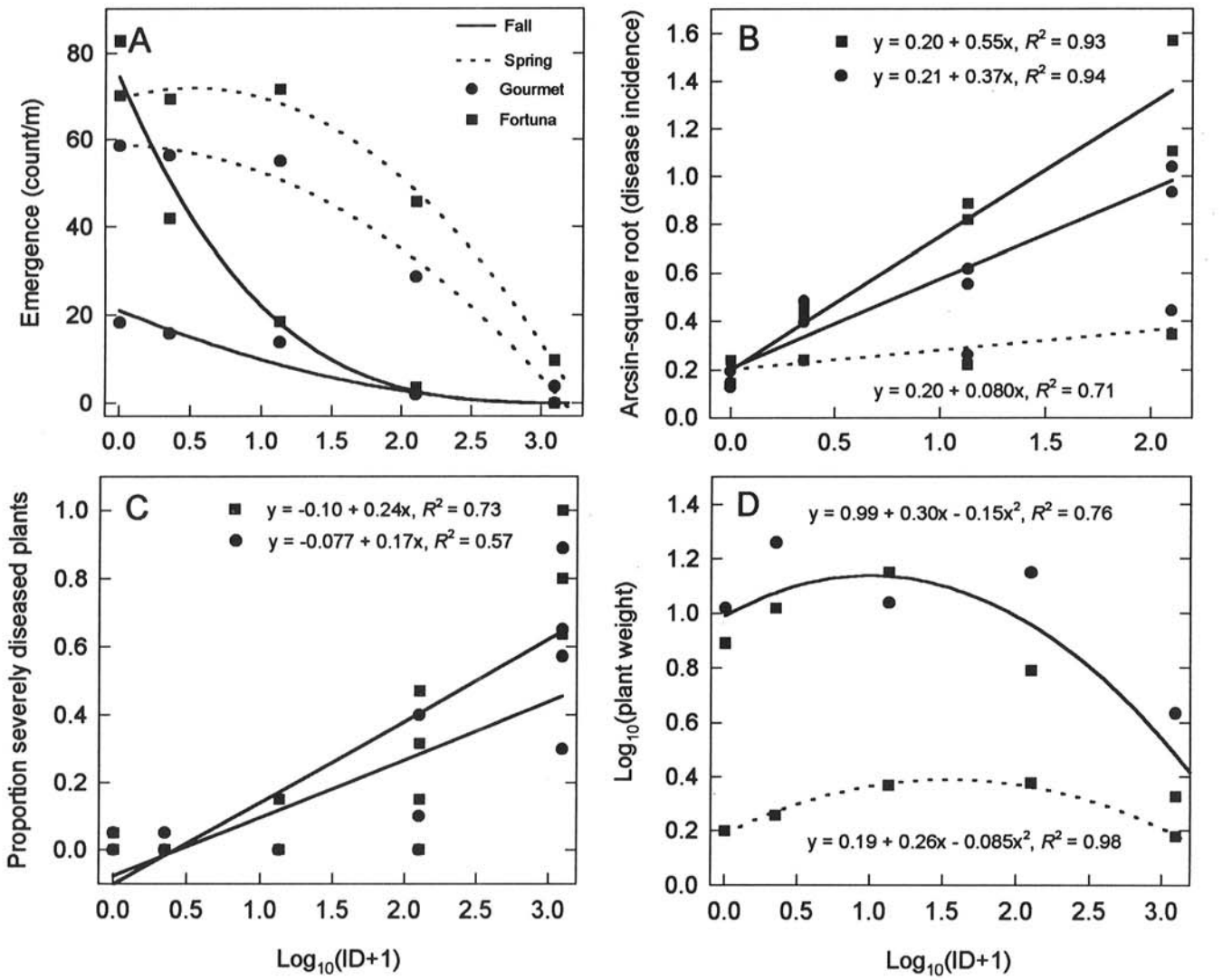

Fig. 3. Relationships between inoculum density ($\log_{10}$[sclerotia per kilogram of soil]) of *Rhizoctonia solani* AG-4 and **A,** emergence of cabbage seedlings; **B,** wirestem incidence assessed at 27 days after planting in the spring and at 21 and 29 days after planting in the fall; **C,** proportion of severely diseased plants (rated ≥ 6 on a scale of 1 to 7); and **D,** fresh weight of individual plants in field plots. **A, B,** and **D** data points are the means of four replications; **C** data points are individual observations. **A,** Spring, Gourmet: $y = 59 - 0.64x - 5.6x^2$, $R^2 = 0.96$; Fortuna: $y = 70 + 9.6x - 9.4x^2$, $R^2 = 0.95$; and Fall, Gourmet: $y^{0.5} = 4.6 - 1.4x$, $R^2 = 0.88$; Fortuna: $y^{0.5} = 8.6 - 4.5x + 0.57x^2$, $R^2 = 0.94$.

gence was lower in plots infested with 125 and 1,250 sclerotia per kilogram of soil than in noninfested plots (t tests, $P = 0.0001$). In the fall, emergence did not increase significantly between 14 and 21 days after planting. The relationship between emergence and inoculum density (base-ten logarithm) was linear for cultivar Gourmet ($P = 0.0001$) but quadratic for Fortuna ($P = 0.01$). For cultivar Gourmet, emergence again was lower in plots infested with the two highest inoculum densities than in noninfested plots, but for cultivar Fortuna, emergence was reduced in all infested plots (t tests, $P = 0.0001$).

In both seasons, emergence in noninfested soil was greater for cultivar Fortuna than for Gourmet (t test, $P \leq 0.01$) (Fig. 3A). In the spring, emergence was greater for cultivar Fortuna than for Gourmet at all inoculum densities except 1,250 sclerotia per kilogram of soil (t test, $P \leq 0.01$). In the fall, emergence was greater for cultivar Fortuna than for Gourmet only at the two lowest inoculum densities, 0 and 1.25 sclerotia per kilogram of soil (t test, $P = 0.0001$).

At 27 days after planting in the spring of 1993, incidence of wirestem (arcsin-square root transformation) increased linearly as the inoculum density increased from 0 to 125 sclerotia per kilogram of soil, but did not differ significantly for the two cultivars (Fig. 3B). At 41 days after planting, mean incidence of wirestem, 20.4% ± 1.1% standard error, was not related to inoculum density. In the fall of 1993, no diseased seedlings were observed at 14 days after planting. Incidence of wirestem increased rapidly during the following 7 days, but then did not change significantly between 21 and 29 days after planting. For both cultivars, incidence was higher in all infested treatments than in the noninfested control (t tests, $P \leq 0.05$). Y-intercepts of the linear equations describing the increase in incidence of wirestem did not differ between the cultivars, but the slope (0.55) for cultivar Fortuna was greater than the slope (0.37) for Gourmet (cultivar × inoculum, F significant at $P \leq 0.03$). Wirestem was greater on Fortuna than on Gourmet at 125 sclerotia per kilogram of soil (t test, $P = 0.02$).

In the fall of 1993, there was a linear relationship between the inoculum density (base-ten logarithm) and the proportion of severely girdled plants at harvest (those rated 6 or 7 on a scale of 1 to 7) (Fig. 3C). Intercepts for both cultivars were not significantly different from 0, but the slope for cultivar Fortuna, 0.24, was greater than for Gourmet, 0.17 (cultivar × inoculum, F significant at $P = 0.03$). Fortuna suffered more wirestem at the highest inoculum density than Gourmet (Fig. 3C). In the spring, percent severely girdled plants was lower (mean 13% ± 1.6%) than in the fall, and was not related to the inoculum density. Incidence and severity of wirestem on harvested plants were correlated with each other in both plantings ($r = 0.81$, $P = 0.0001$). In general, incidence of wirestem on harvested plants increased with the inoculum density, but no consistent relationship was found at this sampling time in either trial (data not shown).

The total weight of plants per plot was lower at 1,250 than at 0 sclerotia per kilogram of soil for both cultivars in both seasons (t tests, $P \leq 0.01$), while weights in the other inoculum levels were not significantly different from that in the noninfested control. Mean fresh weight of 20 plants per plot at 0 and 1,250 sclerotia per kilogram of soil was 42.6 and 21.4 g in the spring and 183.4 and 39.6 g in the fall, respectively. In the fall, the base-ten logarithm of mean weight of individual plants decreased with the increasing inoculum density (base-ten logarithm) (F significant at $P = 0.02$) (Fig 3D). There was no significant difference between the two cultivars. In the spring of 1993, mean plant weight, 2.1 g, was much lower than in the fall, 8.5 g. The base-ten logarithm of mean plant weight changed quadratically as the inoculum increased ($P < 0.01$) for cultivar Fortuna (Fig. 3D), but did not change significantly for Gourmet, 2.1 ± 1.1 g.

In the spring of 1993, 52 of 83 isolates recovered from diseased Gourmet and Fortuna plants in infested plots were checked to determine the number of nuclei per cell. All 52 were multinucleate (*R. solani*). In the fall of 1993, 47 of 136 isolates recovered from diseased plants in infested plots were checked for the number of nuclei per cell and C3-type anastomosis with *R. solani* SP-1. All 47 isolates were multinucleate. At least three C3 anastomoses per pairing were observed when 46 of the 47 isolates, plus the SP-1 control, were paired with SP-1, indicating that these isolates were very likely identical to SP-1 (12). The remaining isolate also was multinucleate and AG-4, but not identical with SP-1. In the spring and fall trials, 11 and one isolates, respectively, were recovered from plants in noninfested plots.

DISCUSSION

The monomolecular (asymptotic growth) model provided the best description of the relationship between the inoculum density of *R. solani* AG-4 and incidence of wirestem on cabbage in this study. There was a linear relationship between the logarithm of the inoculum density (number of sclerotia per weight of soil) and disease incidence. This was consistent with a previous study, in which an asymptotic growth model also described the relationship between the inoculum density of *R. solani* and the proportion of infected bean plants (25). In another study, the preplant density of *R. solani* AG-1 IA sclerotia correlated positively with the incidence of diseased tillers and disease foci in rice (6). Also, the density of viable sclerotia of *R. solani* AG-2-2 correlated positively with damping-off and root rot of sugar beet (8).

Emergence of direct-seeded cabbage decreased at an increasing rate (quadratic regression coefficient < 0) in the greenhouse and in the March field planting, when air temperatures averaged 21 and 18.8°C, respectively, but decreased at a decreasing rate (quadratic regression coefficient > 0) in the September field planting, when mean air temperature was 30.4°C. At moderate temperatures, the most rapid change in the rate of preemergence damping-off was between 125 and 1,250 sclerotia per kilogram of soil, but at high temperatures, between 0 and 12.5 sclerotia per kilogram of soil. Likewise, slopes for the increase in incidence of wirestem were greater in the fall than in the spring planting. A similar effect of temperature was reported with Rhizoctonia preemergence damping-off of radish; disease progressed significantly faster at 30°C than at 20°C (3).

Preemergence damping-off on cabbage in this study reached a maximum at 1,250 sclerotia per kilogram of soil, an inoculum level approximately one-fourth of that reported to cause maximum damping-off on canola (26). Incidence of wirestem averaged 76% on 4-week-old cabbage plants grown in nonsterile soil infested with 2% cornmeal sand. In a previous study, *R. solani* reduced by ≤ 94% the emergence of collard, turnip, mustard, and especially cabbage seeded in autoclaved soil infested with 2% cornmeal sand, and as many as 88% of the surviving plants had wirestem (23). The level of disease in the current study was slightly lower, possibly because nonsterile soil was used, plants were 2 weeks older, and plant-to-plant spread of the pathogen was prevented.

In general, low inoculum densities had a more pronounced effect on incidence or severity of wirestem than on preemergence damping-off. Emergence was reduced consistently at 1,250 sclerotia per kilogram of soil in the greenhouse and in the field. Wirestem incidence, however, increased linearly over all levels of sclerotial inoculum. At low inoculum densities, wirestem may occur more frequently than damping-off on cabbage, and perhaps on other vegetable Brassicas.

The two cabbage cultivars used in this study differed in susceptibility to *R. solani* as indicated by preemergence damping-off and incidence of wirestem. In general, cultivar Fortuna had better emergence than cultivar Gourmet, but also had a higher incidence of wirestem. Four cultivars of mustard (*B. juncea*) reacted similarly to *R. solani* AG-2-1; the two cultivars with the lowest per-

cent preemergence damping-off had the highest percent postemergence seedling root rot (9). Fortuna may be less susceptible to preemergence damping-off but more susceptible to wirestem than Gourmet. It is also possible that sclerotia germinate more rapidly in response to root exudates (16) from Gourmet than from Fortuna and attack Gourmet seedlings sooner, causing preemergence damping-off instead of wirestem.

Reductions in plant weight in response to infection by *R. solani* have not been previously quantified for vegetable Brassicas. The quadratic response of individual plant weight to the inoculum density in this study was similar to the reduction in shoot weight of tomato infected with Southern root knot nematode (7). In a previous study, *R. solani* reduced root, stem, and leaf weight of snap bean in infested field plots, but this reduction was related linearly to the inoculum level (25). Whether reduced growth of crucifer seedlings is directly correlated with reduced head size or delayed sizing of heads remains to be determined.

Gliocladium virens and *Trichoderma* spp. have been used successfully to control postemergence damping-off of cabbage in the greenhouse (11) and radish in the field (15), respectively. Nonsterile soil infested with colonized cornmeal sand or sclerotia could be used to evaluate other biocontrol agents or new fungicides for control of wirestem on vegetable Brassicas. Such a screening system also could be employed to evaluate resistance to *R. solani* in *B. oleracea*. Based on the curves relating wirestem incidence and plant weight to inoculum density, 0.5% cornmeal sand or 125 sclerotia per kilogram of soil should provide a reproducible level of disease incidence near 50%. These curves also can be used to predict to what extent sclerotial density or viability must be reduced to realize a reduction in wirestem or preemergence damping-off.

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