Inoculum Density of *Phialophora gregata* Related to Severity of Brown Stem Rot and Yield of Soybean in Microplot Studies

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ABSTRACT

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Field microplots were used to determine the relationships among inoculum density of *Phialophora gregata,* severity of brown stem rot and yield of soybean. In the fall, microplots were infested with *P. gregata* by introducing naturally colonized soybean residue either confined in nylon mesh bags or unconfined in corresponding microplots. Different sources of residue were placed on the soil surface or buried in an attempt to establish a range of inoculum densities of the fungus. In the spring, residue confined in nylon mesh bags was weighed and assayed for *P. gregata* on a selective medium. Inoculum density of *P. gregata* was expressed as colony-forming units (cfu) per square meter. Severity of brown stem rot and yield data were recorded from a paired microplot by a bioassay of unconfined residue using the cv. Corsoy 79. Inoculum density of *P. gregata* (cfu/m^2) in confined residue was positively correlated with severity of either foliar or stem symptoms of brown stem rot in all experiments ($P < 0.005$, $r = 0.596$ to 0.646). The severity of stem or leaf symptoms of brown stem rot was low when the inoculum density was less than 1.0×10^7 cfu/m^2. The severity of stem or leaf symptoms of brown stem rot was negatively correlated with yield ($P < 0.001$, $r = -0.741$ to -0.853) in 3 of 4 yr of the study. There was a negative correlation between inoculum density and yield ($P < 0.001$, $r = -0.692$) in an experiment with buried residue, as well as in an experiment with residue exposed on the soil surface and buried ($P = 0.036$, $r = -0.512$). Placement of soybean residue, either buried or on the soil surface, did not affect the relationship between inoculum density and severity of stem or leaf symptoms of brown stem rot.

Additional keywords: area under the disease progress curve, conventional tillage, *Glycine max,* no-till

Phialophora gregata (Allington & D. W. Chamberlain) W. Gams (6) (syn. *Cephalosporium gregatum* Allington & D. W. Chamberlain [4]), the causal agent of brown stem rot (BSR) of soybean (*Glycine max* L. Merr.), overwinters saprophytically as mycelium in soybean residue that is colonized parasitically during the growing season (8). The association of *P. gregata* with soybean residue has been investigated in terms of colonization, sporulation and population dynamics (1,2,8,12,18). Although its biomass has been estimated in residue, there are no reports linking biomass of *P. gregata* in soybean straw to disease severity and effects on soybean productivity. Inoculum density–disease severity relationships and dispersal of inoculum constitute major informational gaps for BSR.

There have been numerous studies on the effects of crop management and environment on BSR severity and influence on yield (3,5,7,11,14,15,17, 19–22,25). However, it is not known how inoculum density of *P. gregata* relates to severity of BSR and yield loss. The purpose of this research was to determine how inoculum density of *P. gregata* relates to the severity of BSR and soybean productivity. Knowledge of inoculum density–disease severity relationships is important to the understanding of how cultural practices and abiotic factors influence the severity and agronomic effect of BSR. Knowledge of the magnitude of change in inoculum density necessary for significant change in yield is needed to develop crop management plans that are responsive to economic thresholds of *P. gregata.*

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MATERIALS AND METHODS

Establishment of microplots. Soybean residue was collected from plants (cv. Corsoy 79) that expressed a range of low to high symptom severity of BSR. Residue was placed in microplots in the fall in an attempt to establish a range of inoculum densities of *P. gregata* the following spring (18). Forty grams (±0.5 g) of the lower 25 cm of mature soybean stems of *P. gregata* were placed in a 30 × 30 cm microplot. The residue from each source (low or high symptom severity) was divided equally among 32 microplots each as paired plots of unconfined and confined residue for experiments 1 and 2. There were 16 microplots, paired as above, for each year of experiment 3; four from each of four sources ranging from low to high densities of *P. gregata.*

Residue was placed between two layers of nylon screen (1.25-mm openings) that was stapled together (30 × 30 cm). This screen size was selected to limit physical removal of organic matter from residue bags by earthworms, and to prevent loss of residue when the bags were handled (13,24). The 1.25-mm openings are large enough to allow movement into the residue bags of soil microorganisms and soil fauna important to the decomposition of plant debris (13). This amount of residue is equivalent to 4,300 kg per ha, which is near the highest amount of soybean residue found in fields after harvest (9,23).

The microplots were spaced 0.9 m apart. In experiments 1 and 2, soybean residue was placed on the soil surface or buried 20 cm below the soil surface. Experiments 1 and 2 each consisted of 64 microplots, 32 of both confined and unconfined residue. The residue was placed in the field on 7 November 1989 for experiment 1, and 7 November 1990 for experiment 2. All residue was buried in experiment 3, which consisted of the same experiment performed two different years. The residue was placed in the field 7 November 1990 for the first year of experiment 3, and 6 November 1991 for the second year of experiment 3. There were a total of 64 microplots, 32 per year, equally divided into confined and unconfined residue in experiment 3. Four microplots with no residue were included as controls in the bioassay portion of experiment 2 and in both years of experiment 3.

Plot location and soil characteristics. Experiment 1 was conducted at the Walnut St. research gardens, University of Wisconsin-Madison campus (Colwood silt loam). Soil test results from Walnut St. were: pH 7.4, organic matter 2.4%, phosphorus 90 ppm, potassium 145 ppm, total nitrogen 0.16%. Experiments 2 and 3 were conducted at the Arlington Agricultural Research Station, Arlington, WI (Plano silt loam). Soil test results from Arlington were: pH 7.5, organic matter 2.4%, phosphorus 65 ppm, potassium 125 ppm, total nitrogen 0.14%. Sweet corn (*Zea mays* L.) was the previous crop grown at both experimental sites.

Soybean culture. In the spring, the unconfined residue was bioassayed to determine the severity of BSR and related to the inoculum density of *P.*

gregata measured in confined residue of the paired plot at planting. It was assumed that the inoculum density measured from the confined subsample could be compared to disease severity and yield in the assay of the paired subsample of unconfined residue. Soybean (cv. Corsoy 79) was planted and thinned to 10 plants per microplot to bioassay *P. gregata* in each microplot with unconfined residue. Planting was done in a manner to keep the disturbance of the residue to a minimum. Microplots were planted 30 May 1990 for experiment 1, 21 May 1991 for experiment 2 and the first year of experiment 3, and 19 May 1992 for the second year of experiment 3.

Soybean plants were removed from plots at growth stage R7 on 24 October 1990 for experiment 1, 17 September 1991 for experiment 2, and 8 October 1991 and 14 October 1992 for experiment 3. Plants were stored in burlap and threshed with an Almaco (Allen Machine Co., Nevada, IA) LPT bundle thresher after the grain had dried to <13% moisture. Soybean yields are expressed as grams of seed per microplot.

Pathogen detection. Residue bags were recovered to determine residue weight and biomass (population density) of *P. gregata* from confined residue on 29 May 1990 for experiment 1, 21 May 1991 for experiment 2 and the first year of experiment 3, and 19 May 1992 for the second year of experiment 3. Residue was rinsed to remove adhered soil, and air-dried 24 hr in the residue bags before being removed for sampling and weighing. A subsample of 1–2 g, comprised of 10 2.5-cm stem pieces taken from either end of random stem pieces, was assayed for *P. gregata*. Mycelia and conidia of *P. gregata* were observed in the residue (personal observation). The population density of *P. gregata* was determined by counting cfu of *P. gregata* per gram of soybean stem residue by the method of Mengistu et al (18) with the following modifications. Residue of 85–95% dry matter was ground through a 0.5-mm or 0.39-mm sieve with a Wiley mill. The Wiley mill was not effective grinding residue with less than 85% dry matter and recovery of the fungus was reduced if the residue was drier than 95% dry matter. The mill was rinsed with 95% ethanol and dried between samples. Ground residue was contained in glass test tubes sealed with two layers of parafilm and was stored at 4 C for less than 48 hr before being processed. A 100 mg (±5 mg) subsample of ground residue was added to 9 ml of sterile distilled water. After agitation, 0.5 ml of a 10^{-2} or 10^{-3} dilution was dispensed onto each of two to three plates per dilution and spread evenly with a sterile glass rod. The dilutant on the surface of the semiselective medium was allowed to dry prior to incubation and the medium was kept out of direct light. It was observed pre-

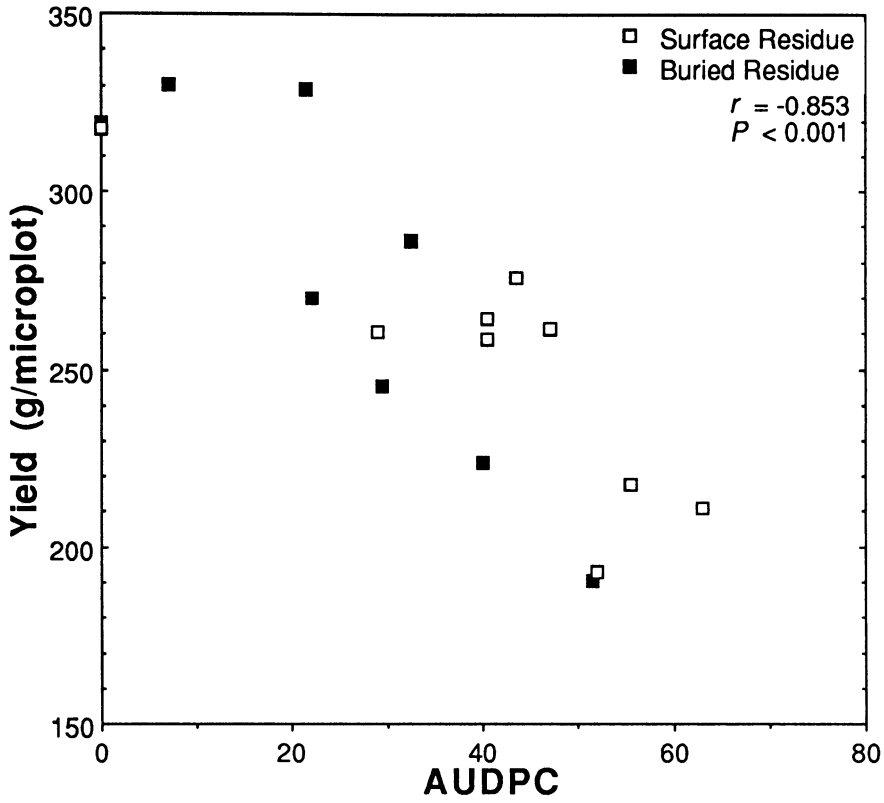

Fig. 1. Correlation of area under disease progress curve (AUDPC) of foliar symptoms of brown stem rot (BSR) with yield of cv. Corsoy 79 in bioassay of microplots with soybean residue (experiment 1).

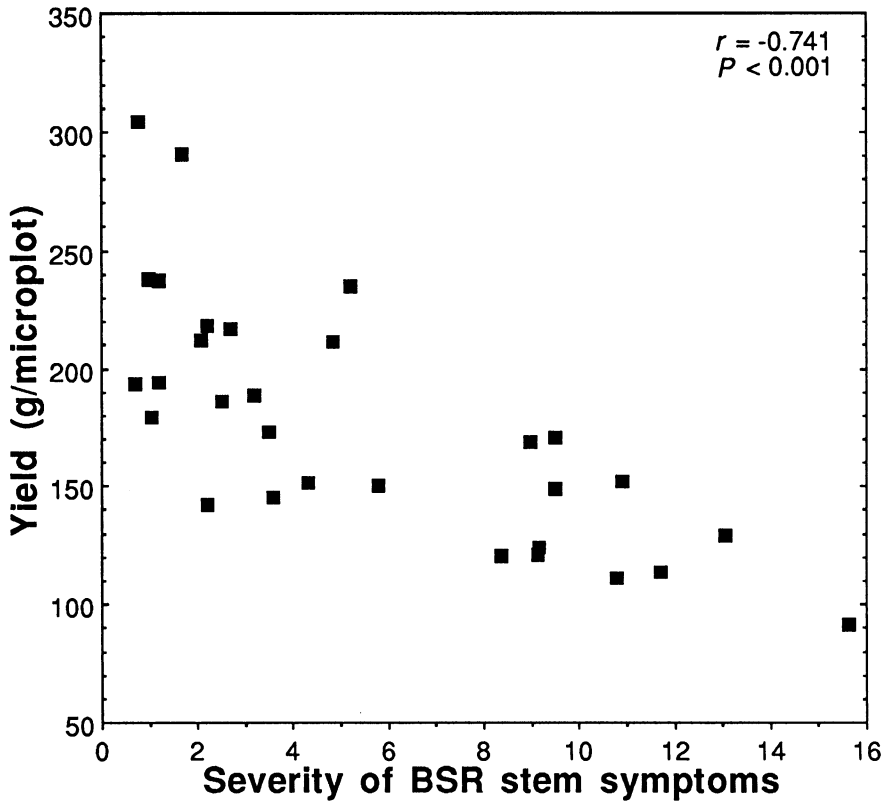

Fig. 2. Correlation of severity of stem symptoms of brown stem rot (BSR) with yield of cv. Corsoy 79 in bioassay of microplots with soybean residue (experiment 3). At growth stage R7 ratings for proportional height of internal stem discoloration (0–5 scale: 1 = symptoms at lowest nodes and 5 = symptoms to highest nodes of plant) and intensity of stem discoloration (0–5 scale: 0 = no discoloration and 5 = continuous discoloration of pith tissue) were multiplied to arrive at an overall stem severity value.

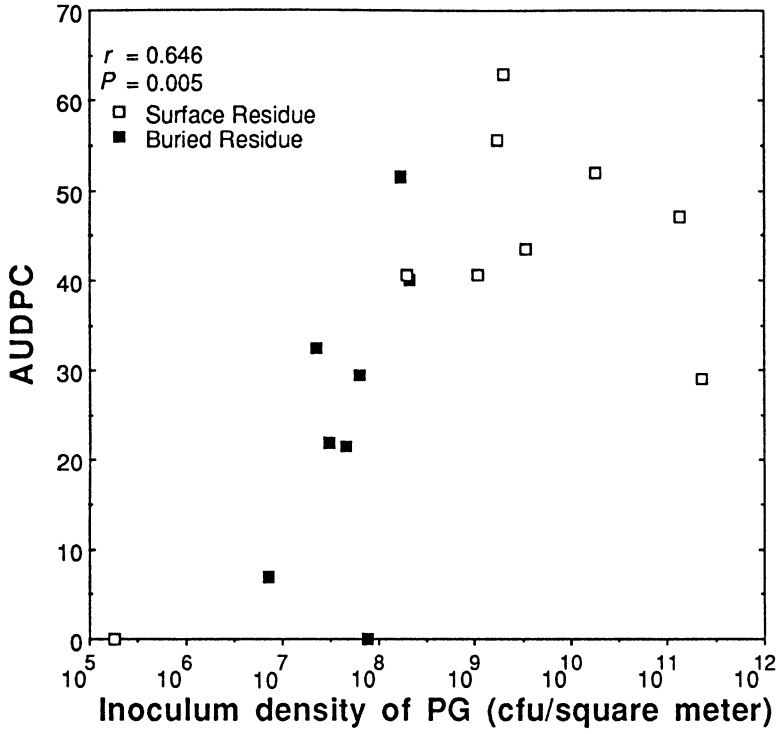

Fig. 3. Correlation of colony-forming units (cfu per m^2) of *Phialophora gregata* (PG) in residue on the soil surface and buried (20 cm) with an area under disease progress curve (AUDPC) of foliar symptoms of brown stem rot in plants in microplots with unconfined residue of the same treatments (experiment 1). Data are from microplots with detectable population densities of *P. gregata*.

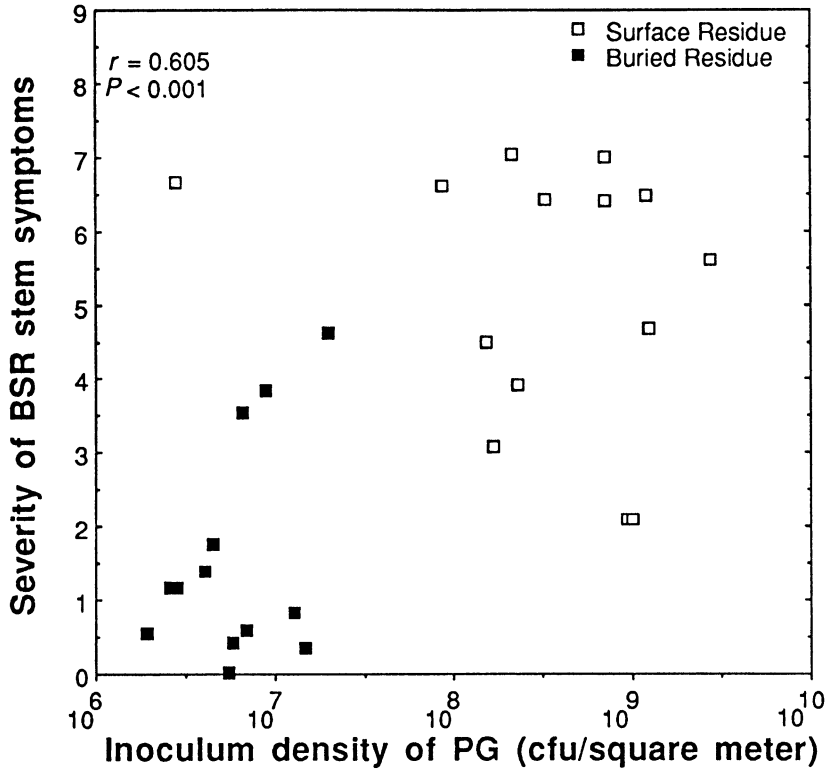

Fig. 4. Correlation of colony-forming units (cfu) (inoculum density = cfu per gram × weight of residue and extrapolated to per m^2) of *Phialophora gregata* (PG) per m^2 with severity of stem symptoms of brown stem rot (BSR) in plants in microplots with unconfined residue of the same treatments (experiment 2). Residue was contained in residue bags on the soil surface or buried (20 cm). At growth stage R7 ratings for proportional height of internal stem discoloration (0–5 scale: 0 = no symptoms, 1 = symptoms at lowest nodes and 5 = symptoms to highest nodes of plant) and intensity of stem discoloration (0–5 scale: 0 = no discoloration and 5 = continuous discoloration of pith tissue) were multiplied to arrive at an overall stem severity value. Data are from microplots with detectible population densities of *P. gregata*.

viously that light altered the medium color and that colonies of *P. gregata* were reduced in size and intensity of pigmentation (personal observation). The plates were incubated without light at 12 C for 2 wk. A 100 mg (±5 mg) subsample of ground residue was dried at 105 C for 1 wk to determine the dry matter for each sample. The cfu of *P. gregata* per m^2 of plot area (inoculum density of *P. gregata*) was calculated by multiplying weight of residue remaining by cfu of *P. gregata* per gram of residue and extrapolated to cfu/m^2.

Disease severity. Foliar symptoms of BSR were rated weekly after the initiation of symptoms, from 27 August to 17 September 1990 for experiment 1, by the Horsfall-Barratt scale (10). An area under the disease progress curve (AUDPC) was calculated for each microplot (15). Foliar symptoms were confounded with other leaf symptoms and were not distinctive for experiments 2 and 3, thus the severity of internal stem symptoms was used as a measure of BSR. All soybean stems were split longitudinally and rated for severity of internal browning at growth stage R7 (physiological maturity) on 17 September 1991 for experiment 2, and 8 October 1991 and 14 October 1992 for the respective years of experiment 3. The rating system was based on the proportional height of internal stem discoloration; 0–5 scale: 0 = no symptoms, 1 = symptoms <25%, 2 = 25–40%, 3 = 41–60%, 4 = 61–80% of plant height, and 5 = symptoms to highest nodes of plant. Also, intensity of stem discoloration was measured on a 0–5 scale: 0 = no discoloration, 1 = faint discoloration of pith at nodes, 2 = distinct discoloration of pith at nodes, 3 = discoloration of pith into internodes, 4 = discoloration of majority of pith in internodes, and 5 = continuous discoloration of pith tissue. Ratings for height and intensity were multiplied to arrive at an overall disease severity value for stems.

Data analysis. Correlation analysis was performed on the data by use of MSTAT-C (MSTAT-C, Michigan State University, East Lansing, MI). Data from paired microplots that did not have detectible densities of *P. gregata* were not included in the analyses. It is feasible that the density of *P. gregata* was not zero and the lack of detection was more likely due to the sensitivity of the assay procedure. Since there were no significant qualitative interactions between factors, data from both years of experiment 3 were combined for analysis.

RESULTS and DISCUSSION

The severity of BSR was correlated with yield of soybean. An inverse correlation between AUDPC (foliar symptoms) of BSR and yield of soybean ($P < 0.001$, $r = -0.853$) (Fig. 1) was observed in experiment 1 and supports research done

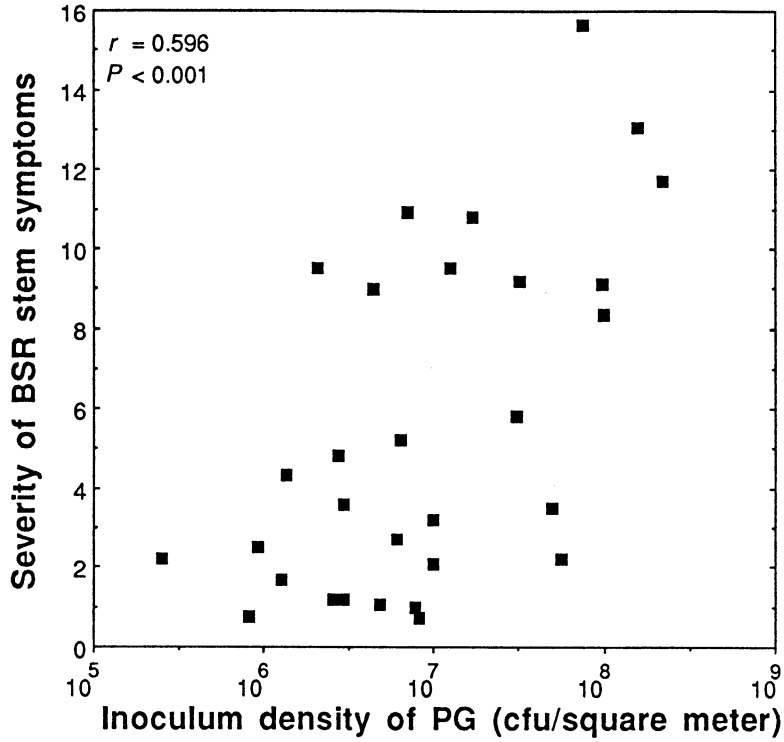

Fig. 5. Correlation of colony-forming units (cfu) (inoculum density = cfu per gram × weight of residue and extrapolated to per m^2) of *Phialophora gregata* (PG) per m^2 with severity of stem symptoms of brown stem rot (BSR) in plants in microplots with unconfined residue of the same treatments (experiment 3). Residue was contained in residue bags that were buried (20 cm). At growth stage R7 ratings for proportional height of internal stem discoloration (0–5 scale: 0 = no symptoms, 1 = symptoms at lowest nodes and 5 = symptoms to highest nodes of plant) and intensity of stem discoloration (0–5 scale: 0 = no discoloration and 5 = continuous discoloration of pith tissue) were multiplied to arrive at an overall stem severity value. Data are combined for 2 yr. Data are from microplots with detectable population densities of *P. gregata*.

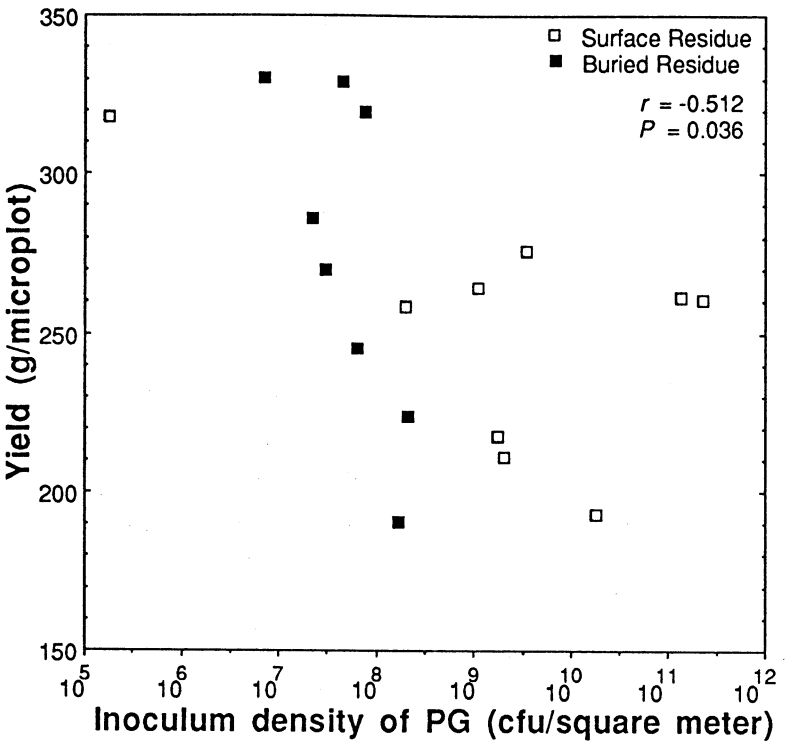

Fig. 6. Correlation of colony-forming units (cfu) (inoculum density = cfu per gram × weight of residue and extrapolated to per m^2) of *Phialophora gregata* (PG) per m^2 with yield of soybean in the bioassay (cv. Corsoy 79) of microplots with unconfined residue of the same treatments (experiment 1). Residue was contained in residue bags on the soil surface or buried (20 cm). Data are from microplots with detectable population densities of *P. gregata*.

in larger field plots (3,7,15). The severity of stem symptoms was negatively correlated with yield ($P < 0.001$, $r = -0.741$) in experiment 3 (Fig. 2), also supporting research done in larger fields plots (7,15). Although both leaf and stem symptoms were highly correlated to yield, our current research supports the concept that the severity of foliar symptoms is a better predictor of yield loss than the severity of internal stem symptoms (15).

Even though a BSR severity-yield relationship has been shown before, our work is the first to demonstrate that severity of BSR is influenced by inoculum density of *P. gregata*. There was a positive correlation between the inoculum density of *P. gregata* measured in confined residue and disease ratings from the bioassay of unconfined residue in the paired microplots. The inoculum density of *P. gregata* was positively correlated ($P = 0.005$, $r = 0.646$) with AUDPC (experiment 1; Fig. 3) and severity of BSR stem symptoms ($P < 0.001$, $r = 0.605$; Fig. 4) in experiment 2. Within the same environment of the buried residue of experiment 3, the inoculum density of *P. gregata* was positively correlated ($P < 0.001$, $r = 0.596$) with the severity of stem symptoms in bioassay plants (Fig. 5).

We were also able to show the relationship between inoculum density of *P. gregata* and yield of soybean. Inoculum density was inversely correlated with yield in experiment 1 ($P = 0.036$, $r = -0.512$) (Fig. 6) and experiment 3 ($P < 0.001$, $r = -0.692$) (Fig. 7). The severity of stem symptoms and soybean yield ($P = 0.287$), and inoculum density and yield in experiment 2 were not significantly correlated ($P > 0.50$), probably due to wildlife damage to the bioassay crop.

These correlations provided an indication of inoculum densities needed for significant development in the severity of both foliar and internal stem symptoms of BSR, in spite of many environmental differences among the microplots. The severity of BSR in all experiments was low in microplots when inoculum was less than 1.0×10^7 cfu/m^2. In experiment 3, the yields in plots which had less than 1.0×10^7 cfu/m^2 (Fig. 7) were similar to the yields from the control microplots that had no residue and low levels of stem symptoms (Table 1).

The inoculum densities in plots with surface residue tended to be greater than those with buried residue (1.9×10^9 and 4.97×10^7 cfu/m^2 of *P. gregata*, respectively, for experiment 1; 3.3×10^8 and 5.9×10^6 cfu of *P. gregata* /m^2, respectively, for experiment 2). However, the range of inoculum densities at both soil positions was not great enough to determine if the inoculum density and BSR severity relationship is the same when the residue is at the different soil positions.

Phialophora gregata was not detected

in all plots that received colonized residue. In microplots where *P. gregata* was undetectable (experiments 1 and 2) the paired plots with unconfined residue had a low incidence of BSR and the yields were among the highest or equal to the yields in the control microplots (Table 1). In experiment 1, soybeans in paired microplots with undetectable densities of *P. gregata* did not express foliar symptoms of BSR and averaged 279 g of seed per microplot, with one exception that had a very low AUDPC of 19 and yield of 231 g. In experiment 2, the average severity of BSR stem symptoms was 2 in paired microplots with no measurable *P. gregata*, and averaged 202 g per microplot compared with 199 g for the control microplots. These results indicate the microplots of unconfined residue corresponding with those of confined residue having undetectable densities of the fungus were consistent in the expected disease severity and yield. There were two microplots where *P. gregata* was undetectable in experiment 3. The soybeans in the corresponding microplots had moderately low levels of BSR (stem symptom severity = 6) and yields (140 g per microplot), which are similar to results from microplots having an inoculum density of 7.9×10^6 cfu/m^2. The control microplots in this experiment had a low severity rating of BSR (stem symptom severity ≤ 1) and yielded 181 g. The disease ratings and yields from plots having undetectable densities of the fungus in experiment 3 may indicate a failure of the assay procedure to detect the fungus, or there were higher inoculum densities of the fungus in the corresponding microplots that were bioassayed. Therefore, we believed that it was not appropriate to include data from microplots that had population densities of *P. gregata* below the detection densities of this assay procedure. Use of these data would be extrapolating beyond the capabilities of the assay and could skew the correlations among inoculum, disease severity, and yield.

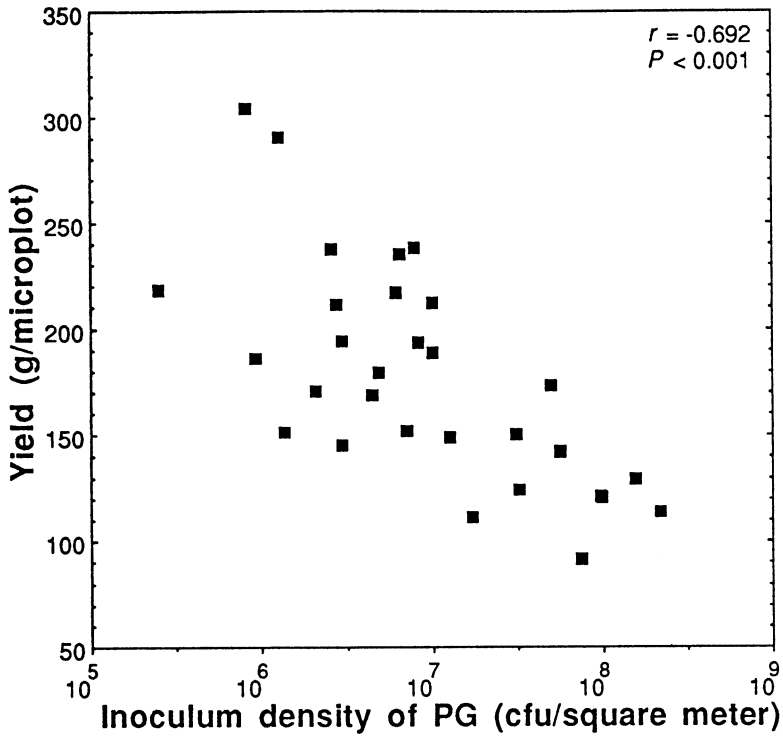

Fig. 7. Correlation of colony forming units (cfu) (inoculum density = cfu per gram × weight of residue and extrapolated to per m^2) of *Phialophora gregata* (PG) per m^2 with yield of soybean in the bioassay (cv. Corsoy 79) of microplots with unconfined residue of the same treatments (experiment 3). Residue was contained in residue bags that were buried (20 cm). Data are combined for 2 yr. Data are from microplots with detectable population densities of *P. gregata*.

Table 1. Frequency of microplots where *Phialophora gregata* was undetectable, average severity of brown stem rot (BSR) and yield in infested and noninfested microplots

Microplots with undetectable densities of *P. gregata*				Noninfested microplots	
Experiment position	#/total	Severity of BSR[w]	Yield (g/microplot)[w]	Severity of BSR[w]	Yield (g/microplot)[w]
		Range (avg.)	Range (avg.)	Range (avg.)	Range (avg.)
1 Surface	7/16	0–18.5 (2.6)[x]	231–362 (284)	...	...
1 Buried	8/16	0 (0)	174–327 (274)	...	...
2 Surface	2/16	1.5–3.6 (2.6)[y]	170–203 (187)	0–1.4 (0.4)[y,z]	159–220 (199)[z]
2 Buried	3/16	0.9–2.0 (1.3)	195–225 (212)		
3 Buried	2/32	2.8–8.5 (5.7)[y]	126–154 (140)	0–2.1 (0.5)[y]	150–220 (181)

[w] Values given are ranges. Numbers in parentheses are averages.
[x] Severity of foliar symptoms of BSR through season are represented as an area under the disease progress curve.
[y] Severity of stem symptoms of BSR. At growth stage R7 ratings for proportional height of internal stem discoloration (0–5 scale: 0 = no symptoms, 1 = symptoms at lowest nodes and 5 = symptoms to highest nodes of plant) and intensity of stem discoloration (0–5 scale: 0 = no discoloration and 5 = continuous discoloration of pith tissue) were multiplied to arrive at an overall stem severity value.
[z] Values for noninfested microplots applicable to surface and buried residue in experiment 2.

This is the first report directly relating the inoculum density of *P. gregata* with the severity of BSR and yield. An application for the relationship of inoculum density of *P. gregata* to severity of BSR and yield loss of Corsoy 79 has been demonstrated in previous work comparing conventional tillage with a moldboard plow and no-till, and second year soybeans with soybean annually alternated with corn in no-till (3). The inoculum density in residue with an initially high concentration of the fungus after overwintering in buried residue (simulated moldboard plowing) was 1.7×10^7 cfu/m^2 compared with 1.4×10^9 cfu/m^2 in surface residue (simulated no-till) (1). This compares with Corsoy 79 grown in conventional tillage with a moldboard plow and no-till, for which the severity of BSR was 32% less and yield 16% greater in conventional tillage than in no-till with the second consecutive year of soybean (3). The inoculum of *P. gregata* in simulated no-till decreased from 1.4×10^9 cfu/m^2 the first spring to 4.5×10^7 cfu/m^2 the second spring the residue was in the field (1). This is equivalent to the comparisons of Corsoy 79 in the second year of soybean to a corn/soybean rotation in no-till in which the severity of BSR was 13% less and the yield 8% greater in the rotation than in the second-year soybean (3). While there is a strong relationship between inoculum density and severity of BSR and yield loss, this relationship can be altered by other factors, such as environment (15,17,20,22), planting date (7), and cultivar selection (16,25).

Establishing the importance of the inoculum density of *P. gregata* to severity of BSR has led to a better understanding of how management practices can be effective in reducing the severity of BSR. Further work could be directed to determine the effectiveness of different types of tillage practices and crop rotations in reducing the inoculum density of *P. gregata*. Additionally, if BSR-resistant cultivars are found to reduce the amount

of fungus in the field for the following soybean crop, it would give growers another management practice to incorporate into an integrated pest management system. The significant correlation between inoculum density and severity of BSR provides guidance to develop an assay procedure to determine the density of *P. gregata* in soybean fields. This knowledge would aid a farmer in determining which fields are at risk and provide guidance as to which cultural practices to implement to reduce yield loss due to BSR.

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