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Population Dynamics of Soilborne Fungi in a Field Multicropped to Rye and Soybeans Under Reduced Tillage in Florida

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ABSTRACT

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Soil sampled to a depth of 5 cm in a reduced-tillage, multicropped field in Florida was assayed for plant pathogenic and nonpathogenic fungi. During a 4-yr period prior to the start of the study, plots in the field were not tilled and were either subsoiled or not subsoiled; a multicropping sequence of winter rye followed by soybeans in the summer was maintained. At the beginning of the study, subsoiled and nonsubsoiled plots were either tilled to a depth of 15 cm or not tilled before the rye crop was planted. Soil samples were collected on 22 days over a period of 27 mo. Of the 16 genera of fungi identified, species of *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Trichoderma* accounted for up to 75% of the total fungal population detected. Plant pathogens in the genera *Rhizoctonia* and *Pythium* accounted for a much lower proportion of the total fungal population detected in the soil. Anastomosis group four (*R. solani* AG 4) and a binucleate anastomosis group of *Rhizoctonia* (CAG 3) were the predominant members of *Rhizoctonia*, and *Pythium irregulare* and *P. acanthicum* were the most common species of *Pythium* isolated from soil. Propagule densities of most of the fungi that were monitored were significantly higher ($P = 0.05$) in no-till plots than in plots tilled to 15 cm, regardless of subsoiling treatment. Differences in detected populations in no-till plots and plots tilled to 15 cm were usually greatest during the rye crop. Although tillage had a significant effect on propagule densities of *Rhizoctonia* spp., propagule densities of *R. solani* AG 4 were not affected by tillage. Instead, propagule densities of this pathogen were influenced by the presence or absence of a susceptible host (rye or soybean seedlings).

Additional key word: minimum tillage.

Certain plant pathogens are known to survive in crop residues (2,4). Due to the amount of crop debris found on soil managed with reduced tillage, one might expect this type of crop management to foster certain pathogens (3,21). Disease has been observed to be more severe under reduced tillage than under conventional tillage in some studies but not in others (3,6,21).

Reduced tillage is a commonly used alternative to conventional tillage in the United States (15,21). Reduced tillage includes minimum tillage and no tillage. Minimum tillage is defined as the least tillage essential and timely for producing a crop, and no tillage is defined as the planting of a crop in previously unprepared soil by opening the soil only wide enough for proper seed coverage (19). Compared to conventional tillage, reduced tillage results in an increase in soil retention of water, nutrients, and organic matter while soil erosion by wind or water is reduced (15,18). In 1982, reduced tillage was used on 32% or 39 million hectares of this country's cropland, and a total of 64 million hectares of land managed with reduced tillage is predicted by the year 2,000 (18).

Land managed with reduced tillage is frequently multicropped. Multicropping is defined as harvesting more than one crop per year from the same plot of land (19). Crop management systems combining reduced tillage and multicropping are only possible in regions with a long growing season and an adequate water supply. In such areas, these combinations provide efficient ways to use land, equipment, and labor.

Crop management systems incorporating reduced tillage and multicropping generally have been proposed only recently. Therefore, limited research regarding the utility of these cropping systems has been conducted. Additional studies on the effect of these nonconventional crop-management systems on plant pathogens is warranted.

The purpose of the research reported here was to identify and quantitate populations of pathogenic and nonpathogenic fungi

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recovered from soil in a reduced-tillage experiment multicropped to rye (*Secale cereale* L.) and soybean [*Glycine max* (L.) Merr.] in Florida. Portions of this work have been published previously (16).

MATERIALS AND METHODS

This study was conducted in a field on the Green Acres Research Farm of the University of Florida at Gainesville. For 4 yr prior to the start of the study, field plots were not tilled and either subsoiled at a depth of 45 cm to break compacted subsurface layers or not subsoiled. Soybeans (cultivar Bragg) were planted in May and harvested in October, and rye (cultivar Wrens Abruzzi) was planted in November and harvested in April as grain. At the beginning of the present study, plots subsoiled and not subsoiled were either tilled to a depth of 15 cm or not tilled; in the split-plot design, subsoil treatments were the main plots, and tillage treatments were subplots. Tillage and subsoiling treatments were imposed before the rye crop was planted each year, and both crops were drill-planted. Treatments were replicated four times.

On 22 sample dates, soil samples in each plot were taken to a depth of 5 cm. Sample dates spanned a period of 820 days at intervals of approximately 5 wk, and all 16 treatment plots were sampled on each date. In each plot, approximately 40 subsamples were taken from within plant rows with a 2.5-cm-diameter soil core sampler and then pooled for one sample per plot.

Pooled samples were assayed for fungi within 16 hr of recovery from the field. Percent soil moisture (grams water per gram of oven-dried soil) was obtained by weighing 5- to 10-g subsamples of pooled samples before and after drying at 100 C. For each pooled sample, the equivalent of 300 g of oven-dried soil was suspended in 125 ml of 0.25% water agar by mixing in a Waring blender at low speed for 15 sec; 1 ml of these suspensions contained 1.0 ± 0.1 g of soil. Soil suspensions were then used immediately in assays on selective agar media.

Ko's (11) medium amended with 0.5 mg of benomyl per liter (8) was used for the isolation of *Rhizoctonia* spp. for the first six sampling dates, and Flowers' (9) medium amended with 0.5 mg of benomyl per liter (8) (FB medium) was used for isolating *Rhizoctonia* spp. for the last 16 sampling dates. Soil suspensions were added to wells produced in either medium by a circular 1-cm-diameter die attached to a vacuum pump; 10 wells were made in each 9-cm-diameter petri plate and 10 plates were used for each sample. After 48 and 72 hr of incubation at 25 C without light, media were observed for growth of *Rhizoctonia* spp. Isolates of *Rhizoctonia* spp. were then transferred to Difco potato-dextrose agar for identification. The nuclear condition of hyphal cells of isolates of *Rhizoctonia* spp. was determined after staining cultures grown on ICN agar (ICN Nutritional Biochemicals, Cleveland, OH) with aniline blue in lactophenol or glycerine. The anastomosis group to which isolates belonged was determined after pairing unidentified isolates with tester isolates on 3% Difco water agar (17). Incidence of *Rhizoctonia* spp. in soil was recorded as a proportion of 100 total wells (for each pooled sample) from which *Rhizoctonia* spp. grew. Arcsine square root transformations were perfomed on the data before analyses.

Soil was assayed for *Pythium* spp. with a selective medium consisting of 10 mg of pimaricin (Delvocid, Gist-Brocades N. V., Delft, Holland), 250 mg of ampicillin (Polycillin-N, Bristol, Syracuse, NY), 10 mg of rifamycin SV (Rifampicin, Sigma Chemical Co., St. Louis, MO), 100 mg of PCNB, and 20 g of Difco cornmeal agar per liter of deionized water (10). Soil suspensions diluted 10- to 100-fold with sterile 0.25% water agar were used. Dilution rates at each assay date were dependent on the season and treatment. One milliliter of a dilution was applied to the surface of the solidified medium in a 9-cm-diameter petri dish and spread evenly over the medium surface with the blunt end of a test tube disinfested with ethanol. Ten plates were used for each pooled sample. Plates were incubated at 25 C without light for 48 hr before examination for growth of *Pythium* spp. Pure cultures of isolates of *Pythium* spp. were identified to species by examining isolates after growth on boiled grass blades placed in sterile pond water. Due to the consistent growth rate and colony morphology of isolates of *P. irregulare* Buisman on the selective medium, isolates of this species were routinely identified solely on these bases.

Difco potato-dextrose agar amended with 1,000 mg of Tergitol NP-10 plus 50 mg of chlortetracycline per liter was used for estimating the propagule densities of common spore-forming fungi in soil (20). Dilutions of soil ranging from 2 to 10×10^3 in sterile deionized water were used at various times throughout the season. Molten medium, cooled to 45 C, was added to soil dilutions in 9-cm-diameter petri plates (1 ml per plate); dishes were agitated to disperse the soil dilution evenly throughout the medium. Ten plates were used for each pooled sample. After the medium solidified, plates were incubated 2–3 wk at 25 C without light. Plates were

TABLE 1. Effects of tillage, subsoiling, and time after initiation of the experiment on propagule densities of fungi recovered from soil in a reduced-tillage field multicropped to rye and soybean in Florida

Fungi	Source[t]	d.f.[u]	Mean-square	Probability of exceeding *F*-value
Total fungi	Subsoiling (sub)[v]	1	0.7388	0.6122
	Tillage (till)[w]	1	177.3067*[x]	0.0006
	Sub × till	1	0.1661	0.8492
	Time[y]	19	39.1445*	0.0001
	Time × sub	19	2.7531	0.4865
	Time × till	19	7.2001*	0.0006
	Time × sub × till	19	3.7279	0.1683
Rhizoctonia spp.	Sub	1	0.0141	0.3926
	Till	1	0.4990*	0.0008
	Sub × till	1	0.0019	0.7146
	Time	20	0.2727*	0.0001
	Time × sub	20	0.0163	0.0573
	Time × till	20	0.0272*	0.0001
	Time × sub × till	20	0.0105	0.3983
R. solani AG 4	Sub	1	0.0030	0.7201
	Till	1	0.0157	0.4169
	Sub × till	1	0.0216	0.3455
	Time	14	0.0541*	0.0001
	Time × sub	14	0.0097	0.3147
	Time × till	14	0.0134	0.0866
	Time × sub × till	14	0.0080	0.5018
CAG 3[z]	Sub	1	0.0237	0.2426
	Till	1	0.1068*	0.0048
	Sub × till	1	0.0212	0.1001
	Time	11	0.0092	0.0575
	Time × sub	11	0.0086	0.0790
	Time × till	11	0.0031	0.8248
	Time × sub × till	11	0.0081	0.1105
Pythium spp.	Sub	1	23.2819	0.1154
	Till	1	35.3339*	0.0477
	Sub × till	1	0.8017	0.7214
	Time	19	47.6284*	0.0001
	Time × sub	19	2.7927	0.6303
	Time × till	19	7.8067*	0.0012
	Time × sub × till	19	2.8496	0.6084
P. irregulare	Sub	1	20.1581	0.1798
	Till	1	65.0666*	0.0028
	Sub × till	1	1.2068	0.5316
	Time	19	20.2470*	0.0001
	Time × sub	19	2.7129	0.5494
	Time × till	19	3.7788	0.1892
	Time × sub × till	19	2.5618	0.8146

[t] Source of variability.
[u] Degrees of freedom.
[v] Main plots in the split-plot design were either subsoiled at a depth of 45 cm to break compacted subsurface layers of soil or were not subsoiled.
[w] Subplots in the split-plot design were either tilled to a depth of 15 cm or were not tilled.
[x] Asterisk (*) denotes statistical significance, $P = 0.05$.
[y] Time = number of days after the initiation of the experiment when samples were recovered from the field.
[z] Binucleate anastomosis group of *Rhizoctonia* (5).

examined for fungal growth after 2–3 wk or exposed to fluorescent light for an additional 1–3 days before examination.

The data for spore-forming fungi and those for *Pythium* spp. were square-root transformed before analysis. Data for all fungi were analyzed with an SAS (Statistical Analysis Systems; SAS Institute Inc., Cary, NC) GLM (General Linear Models) program.

RESULTS

Fungi in the genera *Aspergillus*, *Fusarium*, *Penicillium*, *Rhizopus*, and *Trichoderma* accounted for 36–75% of all fungi recovered from a given plot on a given sampling date. Low populations of species of the following genera were detected: *Laetisaria*, *Mortierella*, *Myrothecium*, *Mucor*, *Neocosmospora*, *Neurospora*, *Paecilomyces*, *Phoma*, *Pyrenochaeta*, *Pythium*, and *Rhizoctonia*. Several other species were detected but not identified. The following fungal species are listed in descending order of frequency of recovery for a given genus. Species of *Penicillium* recovered from the field included *P. citrinum* Thom, *P. purpurogenum* Stoll, and two other species that were not identified. *Aspergillus ochraceous* Wilhelm, *A. clavatum* Desm., *A. flavus* Link ex Gray, and *A. niger* van Tieghem constituted the total detectable population of *Aspergillus* in field soil. Only two species of *Trichoderma* were isolated routinely during these

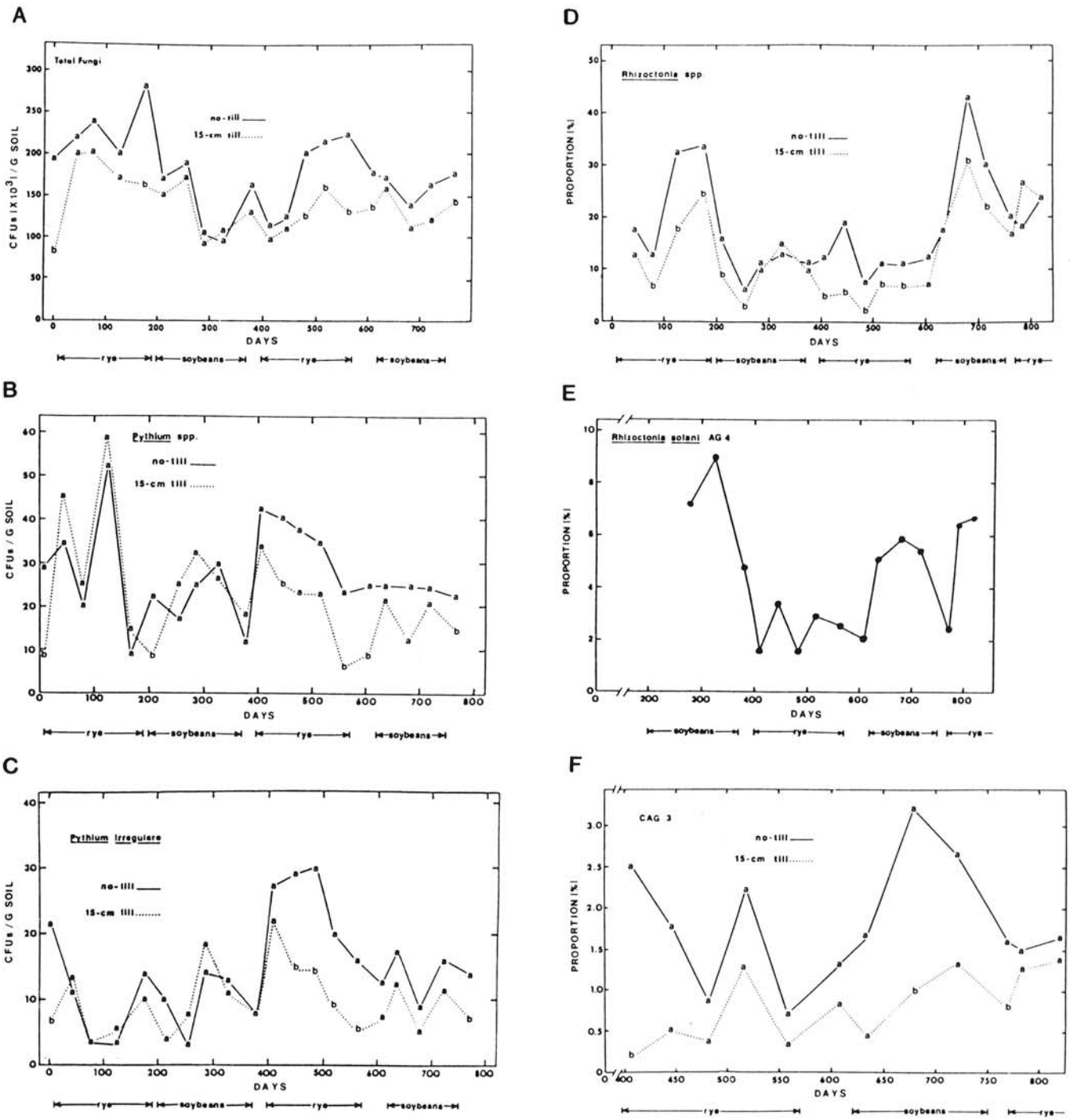

Fig. 1. Propagule densities of fungi recovered from soil in a reduced-tillage experiment in Florida multicropped to rye (cultivar Wrens Abruzzi) and soybeans (cultivar Bragg). Rye was planted in November and harvested as grain in May, and soybeans were planted in May and harvested in October. At the beginning of this study, plots in the field were either tilled to a depth of 15 cm or not tilled prior to planting the rye crop each year. Data represented in D, E, and F are the proportion (percentage) of 0.1-g samples of soil placed on a selective medium from which species of *Rhizoctonia* were isolated. Tillage data points for A, B, C, D, and F for a given sampling date that differ significantly ($P = 0.05$) are represented by different letters.

studies: *T. harzianum* Rifai aggr. and *T. hamatum* (Bon.) Bain. aggr. Isolates of *Fusarium* and *Rhizopus* were not identified to species.

Nine anastomosis groups or species of *Rhizoctonia* were isolated from field soil; isolates of anastomosis group four (AG 4) (14) of *R. solani* Kühn and a binucleate anastomosis group of *Rhizoctonia* (CAG 3) (5) were the most commonly isolated of these and were included in statistical analyses. *P. irregulare*, *P. acanthicum* Drechsler, and several other unidentified species of *Pythium* were isolated from the field; only *P. irregulare* was included in statistical analyses.

In general, fungal propagule densities were influenced significantly by tillage and time after initiation of the experiment (time); subsoiling effects were not significant ($P = 0.05$, Table 1). The effects of tillage and time on propagule densities of total fungi were highly significant, as was the tillage × time interaction. *Rhizoctonia* spp., *Pythium* spp., *Penicillium* spp., and *Rhizopus* spp. responded to these influences on variability in similar manners. Tillage and time influenced significantly the propagule densities of *P. irregulare*, *Aspergillus* spp., and *Fusarium* spp.; tillage × time interactions were not significant for these fungi. Propagule densities of *R. solani* AG 4 and *Trichoderma* spp. were affected significantly by time, and propagule densities of CAG 3 were affected significantly by tillage.

Subsoiling had no significant effect on the propagule densities of any of the fungi monitored. Therefore, data from subsoiled and nonsubsoiled plots were combined for no-till and 15-cm-till treatments when by-date contrasts ($P = 0.05$) of tillage data were made for the following categories of fungi: total fungi, *Pythium* spp., *P. irregulare, Rhizoctonia* spp., and CAG 3 (Fig. 1A–D and F). Because tillage had no significant effect on propagule densities of *R. solani* AG 4, data for this fungus for each sampling date were combined when illustrating trends of propagule densities over time (Fig. 1E).

Mean propagule densities of total fungi on a given sample date in no-till plots were often significantly greater than those recovered from plots tilled to a depth of 15 cm; this trend was most pronounced during the rye crop (Fig. 1A). Propagule densities of *Rhizoctonia* spp. also were usually greater in no-till plots than in plots tilled to 15 cm (Fig. 1D). In general, these differences were greatest and often significant following tillage treatments (imposed on days 0 and 400). Propagule densities of CAG 3 were always higher in no-till plots than in plots tilled to a depth of 15 cm (Fig. 1F). Due to high variability among these data, however, these differences were seldom significant when by-date contrasts of tillage data were made.

Propagule densities of *R. solani* AG 4 fluctuated in response to the presence of the seedling (susceptible) stage of rye and soybean hosts in the field. During complete growth cycles of rye and soybean (days 408–770, Fig. 1E), propagule densities of *R. solani* AG 4 increased shortly after planting and reached peak levels during a given growth cycle within 55 days of planting. These maximum propagule densities decreased as the rye or soybean crop matured. During growth of the last three crops in this study (after day 408, Fig. 1E), propagule densities of *R. solani* AG 4 were significantly greater about 5 wk after planting than at the time of planting (contrasts, $P = 0.01$).

During the third and fourth crops of this study (days 380–770), propagule densities of *Pythium* spp. and *P. irregulare* in no-till plots were higher than those in plots tilled to 15 cm (Fig. 1B and C). However, these differences were usually significant only during the rye growth cycle. During the first and second crops of this study (days 0–380), there were few significant differences between propagule densities of these fungi in soil tilled to 15 cm or not tilled.

In plots tilled to 15 cm, propagule densities of *Pythium* spp. and *P. irregulare* apparently varied with the presence of rye and soybean crops in the field. Increases in propagule densities of these fungi were noted for sample dates from 7–55 days after planting when compared to propagule densities detected before planting (Fig. 1B and C). These changes were always significant for *Pythium* spp., but were significant for *P. irregulare* only at the beginning of the second rye crop (days 375–408; contrasts, $P = 0.05$). In no-till plots, a similar increase after planting was noted for propagule densities of *Pythium* spp. and *P. irregulare* only during the second rye crop.

DISCUSSION

Soil microbial changes in cropping systems with reduced tillage as compared to those with conventional tillage have been reported by others (7,12,22,23). In soils planted to winter wheat, Lynch and Panting (12) described greater soil biomass in no-till soils than in tilled soils. They attributed this difference to an increase in fungal biomass. Doran (7) studied surface soils from several different cropping systems and found consistently higher populations of three groups of microorganisms in no-till soils than in conventionally tilled soils. In a multicropping study, Sumner et al (22) detected higher population densities of *R. solani* (predominantly AG 4) and *Pythium* spp. in surface soil from reduced-tillage systems than from conventional-tillage systems when soils were assayed shortly after planting. Wacha and Tiffany (23) studied a 4-yr rotation of corn and soybeans. They found no significant quantitative differences between total fungal populations from no-till and conventional-till soil. However, their soil samples were taken at the end of the soybean season and after plant debris and thatch had been removed from the soil surface. These factors probably obscured any quantitative differences that may have existed after plowing and during the growing season in soil in their experiment.

Our results with detected propagule densities of total fungi are in agreement with earlier reports (7,12,22). In the present study, propagule densities of several groups of fungi were generally higher in soil not tilled than in soil tilled to a depth of 15 cm. When propagule densities of total fungi were divided into their component genera and species, however, a positive influence of no tillage on populations of fungi was not always detected. For example, although higher propagule densities of *Rhizoctonia* spp. were more often recorded in soils not tilled than in soils tilled to 15 cm, this trend did not occur with propagule densities of *R. solani* AG 4. *R. solani* AG 4 is a seedling pathogen (1). Propagule densities of this pathogen in our studies were influenced significantly by the presence of a susceptible host (rye or soybean seedlings, Fig. 1E), but not by tillage (Table 1). In the barley (*Hordeum vulgare* L.)-*R. solani* AG 3 pathosystem, Murray (13) reported higher propagule densities of the pathogen during the seedling stages of the host than during later growth stages.

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