Individual-Based Approach to Modeling Hyphal Growth of a Biocontrol Fungus in Soil

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ABSTRACT

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Fungi in soil perform beneficial roles that include biological control of soilborne plant pathogens. However, relatively little predictive information is available about the growth and activity of fungal hyphae in soil habitats. A stochastic computer simulation model ("Fungmod") was developed to predict hyphal growth of the biocontrol fungus *Trichoderma harzianum* ThzID1 in soil. The model simulates a fungal colony as a population of spatially referenced hyphal segments, and is individual-based, in that records of spatial location and branching hierarchy are

maintained for individual hyphal nodes. In this way, the entire spatial structure of the fungal colony (hyphal network) can be explicitly reconstructed at any point in time. Also, the soil habitat is modeled as a population of spatially referenced 1-mm³ soil cells, allowing for the simulation of a spatially heterogeneous environment. Initial hyphal growth parameters were derived from previously published results, and the model was tested against new data derived from image analysis of hyphal biomass accumulation in soil. The ability to predict fungal growth in natural habitats will help to improve the predictability of successful mycoparasitic events in biological control systems.

Additional keywords: microbial ecology.

Many soilborne fungi perform beneficial roles, such as biological control of plant pathogens or remediation of soil pollutants, but relatively little predictive information is available about their growth and activity in soil habitats. Perhaps the best-known biological control fungus, *Trichoderma harzianum*, is a mycoparasite of several soil fungi, including the plant pathogen *Sclerotinia sclerotiorum* (1–3,10,11,24,39,46). Colonization of the resting form (sclerotia) of *S. sclerotiorum* may result in biological control. A variety of formulation methods has been described for biocontrol fungi including *T. harzianum*, and these may improve attributes of storage or growth in soil. Biocontrol efficacy is likely to increase with increasing growth of the biocontrol agent, suggesting that quantitative studies of abiotic and biotic factors affecting growth and proliferation of biocontrol agents in soil are necessary, and that ability to predict fungal growth in natural habitats may improve the predictability of biological control efforts (9,10,14,15,23,29,43,44).

To date, there are relatively few studies that deal with predictive methodology for plant disease biological control, although there is a considerable volume of work on different aspects of modeling fungal growth. A number of fungal growth models deal primarily with aspects of physiology and morphogenesis (16,17,44,47) and may not be directly applicable to predicting fungal proliferation in natural habitats, while others deal with colony pattern formation in two- and three-dimensional space. In one early study, Cohen (12) described a two-dimensional simulation approach, in which hyphal tip extension and branching were dependent on biomass density, and growth direction corresponded to the biomass density gradient, resulting in circular colony growth. Hutchinson et al. (21) extended this approach by including experimentally measured values for internode lengths, branch angles, and hyphal extension

rates. Stack et al. (45) and Knudsen and Stack (29) introduced the idea of a simulation model for hyphal growth of a fungal hyperparasite through soil, and use of the model to predict the incidence of hyperparasitism of sclerotia of certain soilborne plant pathogens. Lejeune and Baron (31) and Lejeune et al. (32) simulated the three-dimensional growth of the filamentous fungus *T. reesei*, based on properties of mycelial growth (total hyphal length and total number of tips). Cross and Kenerley (13), using a combination of the Ratkowsky and Arrhenius equations, modeled colony growth of *T. virens* at different temperatures.

Models developed for fungal growth in homogeneous environments, such as on agar surfaces or in culture media, may not be applicable for predicting colony expansion in a heterogeneous environment. One potential way to deal with problems of spatial heterogeneity is the development of an "individual-based" model that tracks spatial attributes of the organism of interest as well as those of the local environment. With an individual-based model, information about individual organisms and/or habitat patches is retained in a database, and the model can then aggregate attributes of individuals to generate dynamics of the larger population. Thus, individual-based models represent an inductive approach to spatial population modeling, in that population statistics are derived from collective attributes of individuals (20). For example, we have used this approach to predict epizootics caused by the entomopathogen *Beauveria bassiana*, in populations of Russian wheat aphid on uniform versus nonuniform host plant distributions (27,40). The objective of this study was to develop a modeling framework, in the form of an individual-based computer simulation model, that is suitable for exploration of the spatial dynamics of hyphal growth of a biocontrol fungus in a three-dimensional soil habitat.

MATERIALS AND METHODS

***T. harzianum* strains ThzID1 and ThzID1-M3.** Initial hyphal growth parameters, described below, were derived primarily from

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previously published experiments using *T. harzianum* isolate ThzID1 (5,6,10,24,25). Isolate ThzID1 originally was obtained from Palouse silt loam soil at the University of Idaho Plant Science Farm near Moscow, ID. ThzID1 can act as a mycoparasite of sclerotia of the plant pathogen *S. sclerotiorum* (10,24). Additional experiments, including model validation, were performed using the engineered strain *T. harzianum* ThzID1-M3, which is a derivative of ThzID1 that we previously transformed with marker genes encoding hygromycin B resistance, β -glucuronidase (GUS), and green fluorescent protein (GFP) (5–7,35). For all experiments, calcium alginate pellets of either ThzID1 or ThzID1-M3, containing a mixture of hyphal segments and spores, were formulated with wheat bran as described previously (18,23,33). Pellets were air-dried on waxed paper and stored at 4°C, and were used within 2 to 4 weeks from time of formulation.

Simulation model description. Knudsen and Stack (29) and Stack et al. (45) described preliminary versions of a stochastic individual-based spatial model for hyphal growth in soil. In an individual-based model, information about organisms or their components in localized habitats is retained rather than averaged over the larger population, and thus, the model aggregates known biological attributes of individuals to generate dynamics of a population. Our approach in this study was to simulate a fungal colony as a population of individual, spatially referenced hyphal segments, within a three-dimensional grid of individual, spatially referenced soil cells.

In the model, which we call “Fungmod”, records of spatial location and branching hierarchy are maintained for individual hyphal nodes, for one or more fungal colonies. Thus, each hyphal segment is spatially referenced (coordinates in three dimensions) in terms of its ending node position, with its starting location referenced as the ending position of its parent node (Fig. 1). In this way, the entire spatial structure of the fungal colony can be explicitly reconstructed from the data set at any point in time. The model proceeds at 1-day time steps, and allows prediction of colony diameter, fungal biomass, and the spatial distribution of hyphae over time. Although fungal growth is a continuous process, the 1-day time step was chosen in part for computational efficiency, and also represents a time scale at which incremental hyphal growth and/or branching are readily observable. A maximum value for daily hyphal segment extension, *MAXGROWSTEP*, was estimated by testing a range of candidate values against experimentally derived results (described below). Actual hyphal extension (*Length* = daily growth increment, in millimeters) for any given node, up to the maximum value, is proportional to the relative nutrient status value (*NutrStatus*) maintained for each node: $Length = MAXGROWSTEP \times NutrStatus$. Pellets made with bran initially provide a source of nutrients for hyphal growth (25). To simulate energy-dependent growth (43) in the model, a nutrient decay (depletion) parameter (*NutrDecayVal*) was used to determine the relative nutrient status of each node compared with its parent node; thus, the decline in nutrients available for growth follows first-order kinetics: $NutrStatus_{node} = NutrStatus_{parent} \times NutrDecayVal$. Also, for simulations reported here, hyphal nodes each were assigned a life span of 3 days, so that individual nodes older than 3 days did not contribute to the predicted total active hyphal biomass. This value corresponds to an estimated longevity of hyphal segments in nonsterile soil, prior to lysis, as observed microscopically (23; unpublished data). At each 1-day time step, hyphal elongation is initiated at each terminal node. Hyphal segments elongate in the same direction as their parental segments, plus/minus (arbitrary) deviation of up to 22° as determined by simple Monte Carlo selection (38,41) of a random number between 0 and 22. Also at each time step, a branch node may be initiated if the value of a Monte Carlo random variable is less than a value (*BranchProp*) representing branching frequency (Fig. 1). For each simulation presented here, a constant value (0.5) was specified for *BranchProp*. If a branch is generated, the model

apportions available nutrients equally between the extension node and the branch node. Because hyphal extension (including branching) occurs in three-dimensional space, the vector representing growth is determined using standard spherical coordinates, determined by the scalar value *Length*, and angles θ (rotation about the vertical axis) and ϕ (rotation about the horizontal axis). For simulations presented here, constant values of $\theta = 45^\circ$ and $\phi = 45^\circ$ were used. Calculated spherical coordinates of the new node position then are converted to Cartesian coordinates as follows:

$$X_{node} = X_{parent} + Length \times \sin(\phi) \times \cos(\theta)$$

$$Y_{node} = Y_{parent} + Length \times \sin(\phi) \times \sin(\theta)$$

$$Z_{node} = Z_{parent} + Length \times \cos(\phi)$$

The model divides the soil habitat into a three-dimensional array of soil cells, each of which is 1 mm³ in size. In this way, the model is also individual-based in terms of the soil cells, which are treated as individuals. This model structure allows hyphal growth to potentially be a function of locally encountered environmental conditions (i.e., those in specific soil cells), since records of temperature, moisture content, nutrient availability, etc. may be maintained for each soil cell and thus may vary among cells. However, in the simulations presented here we did not incorporate spatial variability in the soil environment. Although not presented here, the model is designed to allow prediction of the frequency at which target propagules, such as sclerotia of *S. sclerotiorum*, would be contacted by hyphae of the growing biocontrol agent over time. The model is written in the language C++ and is implemented on a desktop microcomputer. The number of nodes and the size of the soil cell array that can be simulated depend on available computer memory; e.g., on a microcomputer with 64 MB of memory, about 10⁶ nodes can be simulated in a spatial array containing 5 × 10⁴ soil cells. A copy of the source code is available from G. R. Knudsen.

Model parameterization and calibration. Initial estimates of growth rate and branching frequency parameters for *T. harzianum* ThzID1 were obtained from published data (10,23) and preliminary experiments to estimate initial numbers of hyphae originating from pellets, and branching angles, as described below. In experiments described by Knudsen and Bin (23), alginate pellets containing hyphae of *T. harzianum* and wheat bran were buried beneath nylon mesh in steamed silt loam soil, at –0.1 MPa soil matric potential, and then incubated at 25°C. The extent of hyphal growth was observed and mapped after 0, 1, 2, 3, 5, 7, and

Fig. 1. Diagram of the basic fungal growth model, showing generation of hyphal nodes and branches. Hyphal extension and branching occur in three-dimensional space; the vector representing growth is determined using standard spherical coordinates, determined by the scalar value *Length*, and angles θ (rotation about the vertical axis) and ϕ (rotation about the horizontal axis). For simulations presented here, constant values of $\theta = 45^\circ$ and $\phi = 45^\circ$ were used for branching angles.

14 days, and mean colony radii were calculated from digitized maps. Since the dried alginate pellets containing *T. harzianum* typically require about 1 day of imbibition before hyphae grow from them, an initial 1-day delay in hyphal growth was incorporated into the model. Values of daily hyphal segment extension were estimated from these published results and then incorporated into the simulation model. Additional hyphal growth parameters (mean branching frequency and branching angle) were determined for *T. harzianum* growing from alginate pellets on agar surfaces. Individual pellets were placed on water agar in plastic petri dishes, and incubated at 20°C for 48 h. Then, individual pellets were viewed using light microscopy at $\times 40$ magnification. Digital images were captured using an attached black and white video camera and video capture board. Digitized images were saved as tagged image format (TIFF) files at a resolution of 400×500 pixels. Using image analysis software (ImagePro Plus, Media Cybernetics, Inc., Silver Spring, MD), we generated initial statistical summaries of hyphal segment lengths and branching frequencies.

An estimate of the mean number of hyphae originating from each alginate pellet was made as follows. Pellets as described above (mean pellet diameter approximately 4 mm) were incubated in soil as reported previously (23). Pellets first were placed into sterile Palouse silt loam soil and overlaid with two layers of 1-mm² pore-sized nylon mesh, which then were covered with additional soil. After incubation (5 days, 25°C), pellets were carefully retrieved and sliced with a microtome to a thickness of 2 μ m. Sections were observed at $\times 250$ magnification, and 20 images of the outer edges of each of four pellets were systematically captured with a video camera, each image representing an area of approximately 0.05-mm². From each image, the number of hyphae protruding from the pellet was determined, per unit surface area. The mean surface area of a pellet was estimated by measuring the volume displacement of a sample of pellets and calculating surface area assuming a spherical shape for the pellets. Pellets were estimated to generate a mean of 1,896 (± 257) hyphae from their surfaces.

The initial model parameters described above were refined (model calibration) using the data of Knudsen and Bin (23). In a first set of simulation experiments, the model's values predicting radial growth were compared with the experimental data for radial growth of *T. harzianum* from alginate pellets with bran, at 25°C. Values for the model parameters *MAXGROWSTEP*, *NutrDecayVal*, and *BranchProp* were chosen to minimize the variance between the reported values and the model's predicted values for average colony radial growth over time. Initial value pairs for the parameters *MAXGROWSTEP* and *NutrDecayVal* were chosen by comparing model predictions for mean daily colony radial growth with the experimental data of Knudsen and Bin (23), for each nutrient decay value (for day n , model variable $Length = MAXGROWSTEP \times (NutrDecayVal)^n$). Using those solutions as initial values, the model was run for 25 initial parameter pairs, varying first the growth step parameter and next the nutrient decay parameter. The best-fit parameter pair was chosen based on minimum mean variance over 100 runs for each trial pair. Overall best-fit values for these parameters were *NutrDecayVal* = 0.6 and *MAXGROWSTEP* = 13.3 (millimeters per day). Simulations then were similarly run to determine the best-fit value for branching frequency, *BranchProp* = 0.5.

Model validation using image analysis of hyphal biomass accumulation in soil. Palouse silt loam soil was obtained from the University of Idaho Plant Science Farm near Moscow, Idaho. Soil was sieved through a 2-mm mesh, allowed to air dry, and then adjusted to a matric potential of -50 kPa (determined from a standard curve) prior to use. Soil (20 g dry weight) was placed into plastic containers measuring $8 \times 6.3 \times 6.3$ cm in size (318 cm³), to a depth of approximately 8 mm. Pellets of the GFP-transformant *T. harzianum* ThzID1-M3 were formulated with

bran, as described above, and 10 pellets per container were placed randomly in the soil at a depth of approximately 2 mm. Containers were kept closed over the course of the experiment. Additional containers of soil were sampled over the course of the experiment to monitor soil moisture, which remained constant (data not shown). Sampling was done after 0, 3, 5, 7, and 14 days. At each sample time, the soil within each container was homogenized by suspending it in 200 ml of 50 mM sodium phosphate buffer, and then placing on a rotary shaker (150 rpm) for 1 h. Subsamples (1 ml) were filtered through nonfluorescent membrane filters (Millipore MF Black Filter, 0.8 μ m; Millipore, Billerica, MA) and viewed at $\times 100$ with epifluorescence to observe GFP activity, using a Nikon Eclipse E1000 Microscope (Nikon, Melville, NY) with a multiband DAPI-FITC-Rhodamine filter cube (excitation wavelengths of 380 to 400, 470 to 490, and 545 to 565 nm; dichroic mirror wavelengths of 425 to 470, 500 to 540, and 570 nm; and barrier wavelengths of 450 to 465, 510 to 540, and 580 to 620 nm). Two membrane filters per sample were prepared and examined entirely for presence of hyphal segments, which then were captured using a Nikon DXM 1200 Digital Camera and ACT-1 Imaging Software (Nikon). To determine the conversion factor from pixels to micrometers at $\times 100$, a standard hemacytometer (50 μ m per smallest division) was used. With transmitted illumination, the image of the hemacytometer was focused on the monitor screen and an electronic aperture was used to adjust the pixels to a known length. For each image, the total length of visible hyphae was measured and recorded using ImagePro Plus imaging software, and ThzID1-M3 biomass was estimated using standard techniques and conversion factors (described previously). The fungal biomass (B), derived from the fungal volume, was calculated as $B = \pi \times r^2 \times l \times d$, where r is the radius of fungal hyphae, l is the length of fungal hyphae, and d represents a typical value for specific gravity of fungal hyphae (0.33 g/cm³) (48). There were three replicate containers for this experiment, and the experiment was performed four times. The Fungmod simulation model then was initialized to correspond to the experimental starting conditions, and 14 days of hyphal growth and biomass accumulation were simulated (100 simulation runs) and compared with the experimental results. At each 1-day time step, the simulated biomass of each active hyphal segment was calculated using the formula above, assuming a hyphal radius of 5.2 μ m (35), and this value was apportioned to the soil cell in which the segment's terminal node was located. Mean cumulative simulated biomass values, at 1-day time steps, are reported here.

RESULTS

Sample simulation model output after 1, 5, 9, and 14 days is shown in Figure 2. Although the model simulates and records hyphal density in three spatial dimensions, results through a single planar sample are shown for ease of visualization. Figure 2 clearly shows the initial expansion of colony radius and increase in total active hyphal density that lasted for approximately 3 to 5 days, followed by a reduction in colony expansion rate and a gradual reduction in viable hyphal biomass, due to nutrient depletion and death of older hyphal segments.

Representative sensitivity analysis simulation results are shown for the parameters of maximum growth step (*MAXGROWSTEP*; Fig. 3A), nutrient decay (*NutrDecayVal*; Fig. 3B), and branching frequency (*BranchProp*; Fig. 3C). In each case, the two extremes of the range tested are shown, along with the best-fit value. Additional intermediate parameter values were tested but are not shown. Sensitivity analysis results showed that, at least at the values tested, the model was relatively more sensitive to changes in nutrient decay value, less sensitive to changes in the growth step value, and relatively insensitive to small changes in branching frequency (Fig. 3A to C). This result was expected, since

incorporation of the nutrient decay factor effectively results in an exponential reduction of endogenous nutrients over time. Lower values of this parameter represent a smaller proportion of parental nutrient reserves that are available to "progeny" hyphal segments. Figure 3D shows the results of the best-fit model parameters ($MAXGROWSTEP = 13.3$ mm, $NutrDecayVal = 0.6$, $BranchProp = 0.5$) compared with the experimental data of Knudsen and Bin (23). Simulation of the best-fit parameters slightly overestimated the initial colony expansion rate and slightly underestimated the final colony size, but was overall a very close fit to the experimental results.

Model validation experiments. Figure 4A to D show results of the simulation model, as run with the above best-fit parameters, plotted against hyphal biomass data obtained by image analysis of ThzID1-M3 in soil, for the four validation experiments. Colony growth rate of ThzID1-M3 was previously shown to be nearly identical to that of the parental strain ThzID1 (5), so that it is unlikely that differences in growth rates of the two strains would account for differences between expected and observed results. Experimentally, an increase in recoverable biomass over several days, followed by a decline, was observed, and model predictions conformed to this pattern. However, there was considerable experimental variability, especially in the maximum levels of biomass observed in the different experiments (Fig. 4A to D). Standard errors for simulation results were quite small (Fig. 4A to D), due in part to the large number of values at each time step ($n = 100$). For the first experiment (Fig. 4A), the simulation results provided a close fit to the experimental data, predicting the increase in fungal biomass which peaked approximately on day 3, and the subsequent decline in biomass for the remainder of the experiment. For the second and third experiments (Fig. 4B and

C), observed hyphal biomass was lower and below model predictions, although the same pattern of initial biomass increase followed by reduction was observed in both simulation and experimental results. Results of the fourth validation experiment are shown in Figure 4D, and simulation predictions again were a good fit to the observed biomass data. Thus, although patterns of biomass increase and subsequent decline were generally consistent and well predicted by the model, the magnitude of increase varied experimentally to a greater degree than it did in simulations.

DISCUSSION

A large number of models have been derived to describe growth of fungi either on agar or in broth (4,8,12,19,31,32,34,37,50), but relatively few are available to predict fungal growth in soil. Paustian and Schnurer (36) developed nonspatial, physiologically based models for fungal growth in soil, which predicted increases in fungal biomass and total hyphal length as functions of carbon and nitrogen inputs, assuming a steady-state conversion into fungal biomass. Other models of fungal growth (16,17,47,49) are useful for studying fungal physiology and morphogenesis but do not have an explicit spatial component (i.e., they assume spatial homogeneity), and thus may not be applicable to predicting colony expansion in a heterogeneous environment.

There are important reasons why spatially homogeneous models of microbial growth kinetics and interactions are inadequate for some purposes, especially for analysis of mechanisms that generate different spatial dynamics as populations develop. If a bio-control system is made more complex with patchily distributed agents and/or target pathogens, the spatial dimensions of the

Fig. 2. Sample simulation model output, showing density of active hyphae (Z axis; values are linear millimeters of viable hyphae per millimeters soil volume) in a horizontal $50 \times 50 \times 1$ mm³ cross-section (x, y axes) through the center of the simulated soil space, after 1, 5, 9, and 14 days. Initial parameters were set at $MAXGROWSTEP = 13.3$ mm; $NutrDecayVal = 0.6$; $BranchProp = 0.5$. Data were gridded and plotted using Surfer version 7.00, Surface Mapping System Software, 1999 (Golden Software, Inc., Golden, CO) (inverse distance to a power gridding method; weighting power = 1, smoothing factor = 1).

system become central to effective analysis and comprehensive understanding. We contend that description of spatially complex biological processes may most effectively be accomplished by modeling individuals. With an individual-based model, information about organisms (or their components) in localized habitats is retained rather than averaged over the larger population (22). However, the concept of an individual is ambiguous for clonal organisms such as fungi. Numerically, physiologically, and often genetically, a single fungal colony is best described as a network of individual hyphal segments (29,37). The model that we developed in this study is explicitly designed to predict spatial as well as temporal development of one or more fungal populations, and is based on the premise that variability within populations can best be approached by modeling growth responses of individuals (e.g., hyphal segments) and then assembling populations from large numbers of such individuals. Two advantages of this approach are as follows: first, the spatial structure of the fungal

colony (hyphal network) can be explicitly reconstructed at any point in time; second, the approach has the flexibility to potentially accommodate an environment that is spatially heterogeneous with respect to physical and biotic conditions, and for phenotypic heterogeneity among individuals. Previously, we successfully used a similar approach to model insect populations and spread of a biocontrol fungus within them (27,40), and also described an individual-based model that predicted dispersal and deposition patterns for aerosolized bacteria in field trials (22).

A few simulation models have been used for biocontrol studies. Knudsen and Hudler (26) described a simulation model that predicted population dynamics of antibiotic-producing strains of the bacterium *Pseudomonas fluorescens* on conifer foliage, and subsequent effects on conidial germination of the fungal pathogen *Gremmeniella abietina*. Spurr and Knudsen (42) and Knudsen and Spurr (28) described how population models for antagonistic phylloplane bacteria might be incorporated into predictive models

Fig. 3. Simulated (dashed line, triangles) versus experimental (solid line, circles) colony radius of *Trichoderma harzianum* ThzID1 in soil, over time. Experimental results are from Knudsen and Bin (23); error bars represent ± 1 standard error, $n = 3$. Initial colony radius of 2 mm represents the average radius of a *T. harzianum*/alginate pellet. **A**, Selected model sensitivity results for the daily maximum growth step parameter, *MAXGROWSTEP*, versus colony radius; growth step values are 11.3 (▲), 13.3 (*), and 15.3 (◆) (*NutriDecayVal* = 0.6, *BranchProp* = 0.5). **B**, Selected model sensitivity results for the nutrient decay parameter, *NutriDecayVal*, versus colony radius; nutrient decay values are 0.4 (▲), 0.6 (*), and 0.8 (◆) (*MAXGROWSTEP* = 13.3, *BranchProp* = 0.5). **C**, Selected model sensitivity results for the branching frequency parameter, *BranchProp*, versus colony radius; branching frequency values are 0.3 (◆), 0.5 (*), and 0.7 (▲) (*MAXGROWSTEP* = 13.3, *NutriDecayVal* = 0.6). **D**, Simulation with best-fit parameter set of *MAXGROWSTEP* = 13.3 mm, *NutriDecayVal* = 0.6, and *BranchProp* = 0.5.

for foliar fungal diseases. However, these studies did not address spatial aspects of growth and proliferation of the putative control agents. For control of sclerotia-forming pathogens in soil, the ability of the biocontrol agent to effectively explore the three-dimensional soil space via hyphal growth probably is more important than spatially localized proliferation, e.g., via sporulation. During the initial stage of colony development in soil, hyphae of biocontrol fungi will obtain their energy from the formulation matrix and/or from substrata located within the soil volume in which they are growing. The extent of mycoparasitism thus would depend on the density and spatial arrangement both of target sclerotia and of hyphae of the biocontrol agent. Quantification of patterns and pattern changes in hyphal growth should enhance prediction of the likelihood of successful mycoparasitic events.

The primary objective of this study was development of a modeling paradigm, in the form of an individual-based computer simulation model, that is suitable for exploration of the spatial dynamics of hyphal growth of fungi, including biocontrol fungi, in a three-dimensional soil habitat. Thus, the model we present is not intended to be a complete integration of the factors determining growth of *T. harzianum* in soil, but rather as a framework to explicitly accommodate physiological spatial heterogeneity in the organism, and resource spatial heterogeneity in the habitat. We did observe that while patterns of biomass increase and sub-

sequent decline were generally consistent with model predictions, experimental variability was greater than predicted by the model. One likely cause of this may be variability in the surrounding microbial community, since our validation experiments were carried out in nonsterile soil. We have previously described effects of specific antagonistic bacteria, as well as the soil microbial community in general, on growth and biocontrol efficacy of *T. harzianum* (7,10). We are hopeful that use of the simulation model concurrently with microbial community analysis will help to further elucidate these relationships.

Experimental determination of spatial patterns of fungal growth in a three-dimensional matrix is extremely challenging, and arguably no currently available methods are adequate to fully validate the spatial distributions predicted by this model. For that reason, we used an aggregated measure of hyphal biomass to compare with model predictions. Our method (35) of using fluorescence microscopy and image analysis to quantify the biomass of our GFP-transformed organism in nonsterile soil is a novel approach to model validation, but it still fails to capture the full spatial information that the model predicts. One promising direction for future research will be to use the model to predict the efficiency with which hyphae of a biocontrol fungus contact propagules (e.g., sclerotia) of a plant pathogen, which may be nonrandomly distributed in soil. We anticipate that this model will

Fig. 4. Model validation experiments 1 to 4 (A to D). Simulated (dashed line) versus experimental (solid line) hyphal biomass (per 20 g of soil) of *Trichoderma harzianum* ThzID1-M3 in soil. Simulation results based on the best-fit parameter set of $MAXGROWSTEP = 13.3$ mm, $NutrDecayVal = 0.6$, and $BranchProp = 0.5$. Error bars represent ± 1 standard error of the mean, $n = 100$ (simulated) and $n = 3$ (experimental). Error bars around simulated means are small and hidden by symbols.

also prove useful to evaluate factors which may be key determinants of biocontrol efficacy, for example absolute levels of nutrient availability in soil, and spatial heterogeneity of those nutrients, some of which may be amenable to management, for example through tillage or nutrient addition.

Some aspects of the soil habitat that can affect fungal growth, such as structure and macropore distribution, will be more difficult to address, and may require that habitat cells be modeled at a smaller scale than presented here. Although model parameter values were obtained where possible from independent experiments, in some instances parameter values (e.g., values for hyphal segment longevity and nutrient decay) were based on best estimates or other unpublished observations. These are areas where future biological research should improve the robustness of the general model. Experimentally, habitat heterogeneity will be challenging to manipulate and also to measure, and we limit the results reported here to predictions about the homogenous state. However, the modeling framework presented should provide the necessary flexibility for addressing questions about spatially heterogeneous environments, especially in conjunction with other more physiologically based approaches. For example, Lamour et al. (30) described a nonspatial fungal growth model in which substrate with a defined C:N ratio was supplied to a fungus at a constant rate, then broken down and taken up by the fungal mycelium, stored in internal metabolic pools, and finally incorporated into structural fungal biomass. Lamour et al. (30) then solved their model numerically for overall steady-states of the variables. A promising future direction would be the use of the mathematical approach of Lamour et al. (30) in conjunction with the spatially explicit approach we present here.

The model described in this study is able to accommodate more than one initial source of fungal growth, and a larger soil volume than presented here, subject to computer memory constraints. A logical next step will be to use the model to generate three-dimensional maps of hyphal density in soil, and then to test and quantify the functional relationship between biocontrol agent density and spatial arrangement in soil, target pathogen spatial arrangement, and probability of effective contact and resulting biological control. This approach should be useful for making biological control of soilborne plant pathogens a more predictable and successful endeavor.

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LITERATURE CITED

- Adams, P. B., and Ayers, W. A. 1979. Ecology of *Sclerotinia* species. *Phytopathology* 69:896-899.
- Ahmad, J. S., and Baker, R. 1987. Rhizosphere competence of *Trichoderma harzianum*. *Phytopathology* 77:182-189.
- Ayers, W. A., and Adams, P. B. 1980. Mycoparasitism and its application to biological control of plant diseases. Pages 91-103 in: *Biological Control in Crop Protection*. G. C. Papavizas, ed. BARC Symposium No. 5. Allanhead Osmun, Totowa, NJ.
- Aynsley, M., Ward, A. C., and Wright, A. R. 1990. A mathematical model for the growth of mycelial fungi in submerged culture. *Biotechnol. Bioeng.* 35:820-830.
- Bae, Y.-S., and Knudsen, G. R. 2000. Cotransformation of *Trichoderma harzianum* with β -glucuronidase and green fluorescent protein genes provides a useful tool for monitoring fungal growth and activity in natural soils. *Appl. Environ. Microbiol.* 66:810-815.
- Bae, Y.-S., and Knudsen, G. R. 2001. Influence of a fungus-feeding nematode on growth and biocontrol efficacy of *Trichoderma harzianum*. *Phytopathology* 91:301-306.
- Bae, Y.-S., and Knudsen, G. R. 2005. Soil microbial biomass influence on growth and biocontrol efficacy of *Trichoderma harzianum*. *Biol. Control* 32:236-242.
- Bartnicki-Garcia, S., Hergert, F., and Gierz, G. 1989. Computer simulation of fungal morphogenesis and the mathematical basis for hyphal (tip) growth. *Protoplasma* 153:47-57.
- Beagle-Ristaino, J. E., and Papavizas, G. C. 1985. Survival and proliferation of propagules of *Trichoderma* spp. and *Gliocladium virens* in soil and in plant rhizospheres. *Phytopathology* 75:729-732.
- Bin, L., Knudsen, G. R., and Eschen, D. J. 1991. Influence of an antagonistic strain of *Pseudomonas fluorescens* on growth and ability of *Trichoderma harzianum* to colonize sclerotia of *Sclerotia sclerotiorum* in soil. *Phytopathology* 81:994-1000.
- Chet, I., Hadar, Y., Elad, Y., Katan, J., and Henis, Y. 1979. Biological control of soil-borne plant pathogens by *Trichoderma harzianum*. Pages 585-591 in: *Soil-Borne Plant Pathogens*. B. Schippers and W. Gams, eds. Academic Press, New York.
- Cohen, D. 1967. Computer simulation and biological pattern generation processes. *Nature* 216:246-248.
- Cross, D., and Kenerley, C. M. 2004. Modeling the growth of *Trichoderma virens* with limited sampling of digital images. *J. Appl. Microbiol.* 97:486-494.
- Danielson, R. M., and Davey, C. B. 1973. Nonnutritional factors affecting the growth of *Trichoderma* in culture. *Soil Biol. Biochem.* 5:495-504.
- Eastburn, D. M., and Butler, E. E. 1988. Microhabitat characterization of *Trichoderma harzianum* in natural soil: Evaluation of factors affecting population density. *Soil Biol. Biochem.* 20:541-545.
- Edelstein, L. 1982. The propagation of fungal colonies: A model for tissue growth. *J. Theor. Biol.* 98:679-701.
- Edelstein, L., Hadar, Y., Chet, I., Henis, Y., and Segel, L. A. 1983. A model for fungal colony growth applied to *Sclerotium rolsfii*. *J. Gen. Microbiol.* 129:1873-1881.
- Fravel, D. R., Marois, J. J., Lumsden, R. D., and Connick, W. J., Jr. 1985. Encapsulation of potential biocontrol agents in an alginate-clay matrix. *Phytopathology* 75:774-777.
- Georgiou, G., and Shuler, M. L. 1986. A computer model for the growth and differentiation of a fungal colony on solid substrate. *Biotechnol. Bioeng.* 28:405-416.
- Huston, M., DeAngelis, D., and Post, W. 1988. New computer models unify ecological theory. *BioScience* 38:682-691.
- Hutchinson, S. A., Sharma, P., Clarke, K. R., and MacDonald, I. 1980. Control of hyphal orientation in colonies of *Mucor hiemalis*. *Trans. Br. Mycol. Soc.* 75:177-191.
- Knudsen, G. R. 1991. Models for the survival of bacteria applied to the foliage of crop plants. Pages 191-216 in: *Modeling the Environmental Fate of Microorganisms*. C. J. Hurst, ed. ASM Press, Washington, DC.
- Knudsen, G. R., and Bin, L. 1990. Effects of temperature, soil moisture, and wheat bran on growth of *Trichoderma harzianum* from alginate pellets. *Phytopathology* 80:724-727.
- Knudsen, G. R., Eschen, D. J., Dandurand, L. M., and Bin, L. 1991. Potential for biocontrol of *Sclerotinia sclerotiorum* through colonization of sclerotia by *Trichoderma harzianum*. *Plant Dis.* 75:466-470.
- Knudsen, G. R., Eschen, D. J., Dandurand, L. M., and Wang, Z. G. 1991. Method to enhance growth and sporulation of pelletized biocontrol agents. *Appl. Environ. Microbiol.* 57:2864-2867.
- Knudsen, G. R., and Hudler, G. W. 1987. Use of a computer simulation model to evaluate a plant disease biocontrol agent. *Ecol. Model.* 35:45-62.
- Knudsen, G. R., and Schotzko, D. J. 1999. Spatial simulation of epizootics caused by *Beauveria bassiana* in Russian wheat aphid populations. *Biol. Control* 16:318-326.
- Knudsen, G. R., and Spurr, H. W., Jr. 1988. Management of bacterial populations for foliar disease biocontrol. Pages 83-92 in: *Biocontrol of Plant Diseases*. K. G. Mukerji, ed. CRC Press, Boca Raton, FL.
- Knudsen, G. R., and Stack, J. P. 1991. Modeling growth and dispersal of fungi in natural environments. Pages 625-645 in: *Handbook of Applied Mycology, Vol. I: Soil and Plants*. D. K. Arora, B. Rai, K. G. Mukerji, and G. R. Knudsen, eds. Marcel Dekker, Inc., New York.
- Lamour, A., Van Den Bosch, F., Termorshuizen, A. J., and Jeger, M. J. 2001. Modelling the growth of soil-borne fungi in response to carbon and nitrogen. *Math. Med. Biol.* 18:329-346.
- Lejeune, R., and Baron, G. V. 1997. Simulation of growth of a filamentous fungus in 3 dimensions. *Biotechnol. Bioeng.* 53:139-150.
- Lejeune, R., Nielsen, J., and Baron, G. V. 1995. Morphology of *Trichoderma reesei* QM 9414 in submerged cultures. *Biotechnol. Bioeng.* 47:609-615.
- Lewis, J. A., and Papavizas, G. C. 1987. Application of *Trichoderma* and *Gliocladium* in alginate pellets for control of Rhizoctonia damping-off. *Plant Pathol.* 36:438-446.
- Nielsen, J. 1993. A simple morphologically structured model describing the growth of filamentous microorganisms. *Biotechnol. Bioeng.* 41:715-727.
- Orr, K. A., and Knudsen, G. R. 2004. Use of green fluorescent protein and image analysis to quantify proliferation of *Trichoderma harzianum* in nonsterile soil. *Phytopathology* 94:1383-1389.

36. Paustian, K., and Schnurer, J. 1987. Fungal growth response to carbon and nitrogen limitations: Application of a model to laboratory and field data. *Soil Biol. Biochem.* 19:621-629.
37. Prosser, J. I. 1991. Mathematical modeling of vegetative growth of filamentous fungi. Pages 591-623 in: *Handbook of Applied Mycology*, Vol. I: Soil and Plants. D. K. Arora, B. Rai, K. G. Mukerji, and G. R. Knudsen, eds. Marcel Dekker, Inc., New York.
38. Ripley, B. D. 1981. *Spatial Statistics*. Wiley-Interscience, Inc., Hoboken, NJ.
39. Ruppel, E., Baker, R., Harman, G. E., Hubbard, J. P., Hecker, R. J., and Chet, I. 1983. Field tests of *Trichoderma harzianum* Rifai. aggr. as a biocontrol agent of seedling disease in several crops and Rhizoctonia root rot of sugar beet. *Crop. Prot.* 2:399-408.
40. Schotzko, D. J., and Knudsen, G. R. 1992. Use of geostatistics to evaluate a spatial simulation model of aphid population dynamics. *Environ. Entomol.* 21:1271-1282.
41. Snedecor, G. W., and Cochran, W. G. 1967. *Statistical Methods*. The Iowa State University Press, Ames.
42. Spurr, H. W., Jr., and Knudsen, G. R. 1985. Biological control of leaf diseases with bacteria. Pages 45-62 in: *Biological Control on the Phylloplane*. C. E. Windels and S. E. Lindow, eds. The American Phytopathological Society, St. Paul, MN.
43. Stack, J. P., Kenerley, C. M., and Pettit, R. E. 1987. Influence of carbon and nitrogen sources, relative carbon and nitrogen concentrations, and soil moisture on the growth in nonsterile soil of soilborne fungal antagonists. *Can. J. Microbiol.* 33:626-631.
44. Stack, J. P., Kenerley, C. M., and Pettit, R. E. 1988. Application of biological control agents. Pages 43-54 in: *Biocontrol of Plant Diseases*. K. G. Mukerji, ed. CRS Press, Boca Raton, FL.
45. Stack, J. P., Knudsen, G. R., and Koch, D. O. 1987. A computer simulation model to predict the dispersal of biocontrol fungi in soil. *Phytopathology* 77:1771.
46. Steadman, J. R. 1979. Control of plant diseases caused by *Sclerotinia* species. *Phytopathology* 69:904-907.
47. Trinci, A. P. J. 1984. Regulation of hyphal branching and hyphal orientation. Pages 23-52 in: *The Ecology and Physiology of Fungal Mycelium*. D. H. Jennings and A. D. M. Rayner, eds. Cambridge University Press, Cambridge.
48. van Veen, J. A., and Paul, E. A. 1979. Conversion of biovolume measurements of soil organisms, grown under various moisture tensions, to biomass and their nutrient content. *Appl. Environ. Microbiol.* 37:686-692.
49. Wessels, J. G. H. 1988. A steady-state model for apical wall growth in fungi. *Acta Bot. Neerl.* 37:3-16.
50. Yang, H., King, R., Reichl, U., and Gilles, E. D. 1992. Mathematical model for apical growth, septation, and branching of mycelial microorganisms. *Biotechnol. Bioeng.* 39:49-58.