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A spatially-structured stochastic model to simulate heterogenous transmission of viruses in fungal populations

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Abstract

A spatially explicit, interacting particle system model was developed to simulate the heterogeneous transmission of viruses in fungal populations. This model is based primarily on hypoviruses in the chestnut blight fungus, *Cryphonectria parasitica*, which debilitate their hosts and function as biological control agents. An important characteristic of this system is that virus transmission occurs freely between individuals in the same genetically defined vegetative compatibility (vc) type, but is restricted among individuals in different vc types, resulting in heterogeneous transmission. An additional source of heterogeneity is spatial structure in host populations; viruses are dispersed by fungal spores which disperse relatively short distances. The model showed that vc type diversity is highly correlated to the horizontal transmission rate and therefore significantly affects virus invasion. The probability of virus invasion decreased as the diversity of vc types increased. We also demonstrated that virus transmission would be overestimated if we assumed virus transmission was homogeneous, ignoring both genetic and spatial heterogeneity. Genetic and spatial heterogeneity are not independent because both are affected by the reproductive biology of the fungus. In asexual populations, restricted fungus dispersal resulted in nonrandom spatial patterns of vc types, increasing the chance of contact between vegetatively compatible individuals, and promoting virus transmission. In contrast, virus transmission was poor in sexual populations due to spatial randomization of vc types by long distance dispersed sexual spores. Finally, this model was used to evaluate the release of genetically engineered virus-infected strains for disease management. The release of transgenic strains resulted in only marginally greater virus establishment than for non-transgenic strains. Virus invasion was still restricted by vc type diversity in the resident fungus population. Simulation of inundative releases of transgenic virus-infected strains slightly improved virus establishment, but viruses did not persist after treatment was terminated. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Contact process; Hypovirulence; Stochastic model; Vegetative incompatibility

1. Introduction

The interactions between host and pathogen (or parasite) populations, and their importance to

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population dynamics, transmission of infections and spread of diseases, have received much attention in population biology. Mathematical models have been developed to analyze the effects of various factors on host-pathogen systems. Most models of host-pathogen interactions assume mass-action contact of healthy and infected host individuals, i.e., homogeneous mixing. However, the dynamics of host-pathogen interactions may be driven by various factors that violate assumptions of homogeneous mixing, resulting in different patterns of establishment, spread and persistence of disease (Lively and Apanius, 1995; Grenfell and Harwood, 1997; Lannou and Mundt, 1997; Torres-Sorando and Rodríguez, 1997; Caraco et al., 1998).

Heterogeneity in host-pathogen systems may result from variation in susceptibility among host individuals (Levin and Pimentel, 1981; Anderson and May, 1984, 1986; Rouse, 1991; Shaw, 1994). Variation may be either developmental or genetically determined, as in the well documented examples such as varying susceptibility to measles in different age classes (Anderson and May, 1984) and the host-pathogen interaction between the myxoma viruses in their rabbit hosts (Levin and Pimentel, 1981). Additional heterogeneity can occur when host and pathogen individuals are spatially or behaviorally isolated (Anderson and May, 1986; Hassell et al., 1991; Tilman, 1994; Lipsitch and Nowak, 1995; Lipsitch et al., 1995; Shaw, 1994). Spatial structure may have marked effects on diversity (Levin, 1974), competition (Pacala, 1986), or population dynamics in general (Hasting and Higgins, 1994; Ruxton, 1995). Different types of heterogeneity can be generated by various processes, and an epidemiological pattern might be attributed to a combination of several types of heterogeneities (Read et al., 1995). The combined effects of genetic and spatial heterogeneities on plant-pathogen interaction provide a good example. Empirically, mixtures of genetically different crop plants that are resistant to different pathogen genotypes function as spatial barriers to reduce disease severity (Burdon and Jarosz, 1990). Furthermore, the level of disease reduction is markedly affected by the dispersal ability of pathogens and the spatial configuration of host genotypes (Goleniewski and Newton, 1994).

In this paper, we consider the dynamics of virus invasion in fungal populations. Invasion of viruses in fungal populations has received attention because of the potential for biological control of fungal plant diseases. The potential for biological control is recognized most clearly for diseases such as chestnut blight (Van Alfen et al., 1975; MacDonald and Fulbright, 1991; Nuss, 1992) and Dutch elm disease (Brasier, 1986; Webber, 1993; Sutherland and Brasier, 1997), both of which caused devastating epidemics. The biological control potential for other fungal plant diseases has been explored in several other systems also (Dodds et al., 1988; Nuss and Koltin, 1990; Milgroom, 1999). In all these cases, the spread of viruses is determined by the dispersal of the fungal individuals, which is often spatially localized (Madden, 1989; Madden and Hughes, 1995; Real and McElhany, 1996). Perhaps equally important, the dynamics of virus transmission are also genetically dependent. The transmission of viruses is governed by a self-nonself recognition system in the fungus, termed vegetative incompatibility, which restricts the exchange of cytoplasmic elements (including viruses) between genetically distinct fungal individuals (Caten, 1972; Hartl et al., 1975; Nauta and Hoekstra, 1994; Leslie, 1996; Milgroom, 1999). Therefore, both genetic and spatial heterogeneities in these fungus-virus systems need to be modeled simultaneously.

We developed a model for the invasion of viruses in fungal populations, specifically designed for the chestnut blight system. We used a type of spatially explicit, individual-based model known as an interacting particle system (Durrett and Levin, 1994). This type of model was chosen because of its capacity to incorporate complex biological features and spatial relationships. Interacting particle systems have been considered more powerful tools for investigating interacting populations than other spatially explicit models [e.g. patch-occupancy models (Levin et al., 1993)] because they can account for a complete configuration of the states of individuals and are more sensitive to perturbations and heterogeneity at various spatial scales. Our objective was to ad-

dress the importance of heterogeneous transmission on the dynamics of host (fungus)–pathogen (virus) interactions. We clearly demonstrate that both spatial and genetic contributions to transmission heterogeneity significantly affect the dynamics of this system. Our second objective was to use this model to evaluate the release of genetically modified strains for the control of chestnut blight (Anagnostakis et al., 1998). We found that engineered strains have only a marginal impact compared to conventional biological control treatments.

2. Biology of the chestnut blight fungus and hypoviruses

Cryphonectria parasitica is a haploid, ascomycete fungus that causes chestnut blight on species of *Castanea* (reviewed in Griffin, 1986; Anagnostakis, 1987; Milgroom, 1995). This fungus infects chestnut trees and forms cankers on the bark. Infected trees die when the canker expands and girdles the tree. After the introduction of *C. parasitica* into North America from Asia in ca. 1900, the disease spread throughout the natural range of American chestnut (*Castanea dentata*) and destroyed most of the mature trees (Anagnostakis, 1987). Healthy sprouts continue to grow from the root systems but are invariably killed by blight before reaching maturity. A similar epidemic occurred after the introduction of *C. parasitica* into Europe where it caused severe epidemics on European chestnuts (*Castanea sativa)* (Heiniger and Rigling, 1994).

Double-stranded (ds) RNA viruses in the genus *Hypovirus* (Hillman et al., 1995) reduce the virulence of *C. parasitica* to chestnut trees (Van Alfen et al., 1975; Hillman et al., 1992; Nuss, 1992); this phenomenon is known as hypovirulence. Hypovirulence has contributed substantially to the control of chestnut blight in European chestnuts (Heiniger and Rigling, 1994). Despite repeated introductions, hypoviruses have failed to establish and spread in *C. parasitica* populations in eastern North America (Anagnostakis, 1987; MacDonald and Fulbright, 1991).

2.1. Heterogeneities in fungus-virus interactions

2.1.1. Vegetative incompatibility

One hypothesis to explain the limited spread of hypoviruses in North American populations of *C. parasitica* is that vegetative incompatibility inhibits horizontal transmission of viruses (Anagnostakis and Day, 1979; Anagnostakis and Waggoner, 1981; Liu and Milgroom, 1996). Viruses can be transmitted horizontally from the cytoplasm of virus-infected individuals to virus-free individuals through hyphal anastomosis (Van Alfen et al., 1975; Anagnostakis and Day, 1979). However, vegetative incompatibility limits hyphal anastomosis and restricts virus transmission (Newhouse and MacDonald, 1992; Liu and Milgroom, 1996). Vegetative incompatibility is controlled by at least six vegetative incompatibility (*vic*) loci (Cortesi and Milgroom, 1998). Individuals are vegetatively incompatible, i.e., in different vegetative compatibility (vc) types, unless they share the same alleles at all *vic* loci. Laboratory studies have shown that viruses are transmitted at high frequencies ($\approx 100\%$) between individuals in the same vc type; however, transmission occurs at significantly lower frequencies between individuals in different vc types (Anagnostakis and Day, 1979; Huber and Fulbright, 1992; Liu and Milgroom, 1996). Therefore, *vic* allele frequencies and *vic* gene (and genotype) diversities will affect the average horizontal transmission rate and are likely to be important in determining the invasion of viruses in fungal populations.

2.1.2. Reproductive biology of C. parasitica

The chestnut blight fungus reproduces both sexually and asexually (Anagnostakis, 1987; Milgroom et al., 1992, 1993; Milgroom, 1995). When the fungus reproduces asexually, it produces spores mitotically (conidia), which function as vegetative propagules and male gametes (spermatia). In sexual reproduction, the fungus produces ascospores meiotically. There are two mating types in *C. parasitica*, controlled by a single locus with two alleles (Anagnostakis, 1988; Marra, 1998); outcrossing occurs only between individuals with opposite mating types, resulting in recombinant offspring. *C. parasitica* can also

self-fertilize and produce offspring genetically identical to the parent (Milgroom et al., 1993; Marra, 1998). Populations of *C. parasitica* in North America are predominantly sexual and randomly mating (Milgroom et al., 1992; Liu et al., 1996; Milgroom and Cortesi, 1999), although a significant amount of selfing often occurs in nature (Milgroom et al., 1993; Milgroom, 1995).

Viruses can be transmitted vertically into the asexual spores (conidia) of infected individuals (Russin and Shain, 1985; Enebak et al., 1994). However, viruses inhibit female fertility; virus-infected individuals can only mate as male (spermatial) parents, but viruses are not transmitted to offspring (ascospores) (Elliston, 1985a; Anagnostakis, 1988). Therefore, virus transmission in sexual populations may be limited because of the lack of vertical transmission in ascospores. Furthermore, vc type diversity in outcrossing populations can be maintained or generated by recombination of *vic* genes (Liu et al., 1996; Milgroom and Cortesi, 1999), resulting in low frequency of horizontal transmission.

2.1.3. Dispersal of fungal spores

Since viruses can be transmitted to asexual spores (conidia) but not to sexual spores (ascospores), the dispersal of conidia is essential for virus dissemination (Day, 1979). Conidia can be dispersed by rain, insects, birds and small mammals (Griffin, 1986; Anagnostakis, 1987). Only limited dispersal of conidia between trees has been documented (Garrod et al., 1985), therefore, conidia are more likely to disperse viruses on the same tree than on different trees. Spatial analyses of vc types (Milgroom et al., 1991), DNA fingerprints and RFLPs (Milgroom and Lipari, 1995), showed aggregations of genotypes on the same trees, but not among different trees. Aggregations were presumably caused by short-distance dispersal of conidia in asexual reproduction. In contrast, ascospores are wind-dispersed and have been reported to have relatively long-distance dispersal (on the order of 10–100 m), which can be described by a negative exponential distribution (Heald et al., 1915). The lack of aggregation of vc types among trees may be due to the dispersal of ascospores in diverse vc types generated by recombination (Milgroom et al., 1991). Therefore, the dispersal of conidia and ascospores are likely to affect the distribution of vc types and virus transmission.

3. Spatially structured model

3.1. Basic model structure

We used a spatially structured, interacting particle model following the basic structure described by Durrett and Levin (1994). We assume the population is represented by a grid of 50×50 cells, in which each cell can be occupied by a single chestnut tree. This grid size roughly corresponds to the number of chestnut stems found in the understorey of ≈ 5–6 ha of forest in the eastern US (Milgroom and Lipari, 1995). To incorporate multiple infections on the same tree, each cell (tree) is divided into six sites, each of which allows an infection by a virus-free or virus-infected fungus. Therefore, this model is formulated on a finite 3-dimensional lattice with a total of $50 \times 50 \times 6 = 15\,000$ sites.

At any given time, each of the sites can be in one of four states: (i) vacant (no tree segment present); (ii) uninfected tree segment; (iii) infected by a virus-free fungus, or (iv) infected by a virus-infected fungus. If a site is occupied by a fungus, it is also represented by genetic characters for *vic* genes and mating type. Transitions in the state of each site depend on its current state, states of its neighbors, genetic and spatial characteristics of the site and its neighbors, and the transition probability, which is described in more detail below and in Table 1. The neighborhood in this model is an extension of the two dimensional 'Moore neighborhood' (Durrett and Levin, 1994), in which neighbors are defined as a set of cells within a distance d of the origin. This distance (d) is determined by the dispersal function of the fungal spores, which therefore determines the spatial scale of interactions. For example, if $d = 1$ then occupants of a cell interact only with their immediate neighbors.

3.2. Transitions and parameters

Our model is formulated in continuous time, on a finite lattice; transitions of states are based on the concept of the basic contact process. This type of model is described in detail by Durrett and Levin (1994). In continuous time, time, t, is indexed by non-negative real numbers rather than by non-negative integers as in discrete time models. The model is described in terms of transition rates. To illustrate this type of model we first describe a simple birth and death process for the fungus, assuming no viruses are present. Assuming all sites are occupied by trees, the host-pathogen interaction (tree-fungus only) can be formulated as:

1. a fungus gives birth at rate β, and dies at rate γ;
2. a fungus born at site x disperses to a site y, which is chosen at random from N neighbors, where N is determined by the dispersal function of the fungus;
3. if y is occupied by another fungus, the birth is suppressed; otherwise, the fungus colonizes site y.

To implement this system in the computer we did the following:We picked a site x at random and let $\xi(x)$ represent the current state of x, where:

$\xi(x) = 0$ if x is empty (uninfected tree),

$\xi(x) = 1$ if x is occupied (fungus-infected tree).

Table 1
Transition rates and probability that determined the change of different states

Transition	Rate (year^{-1})	Probability	References[a]
Tree growth	1.0	–	
Disease-induced tree mortality	0.4	–	Kuhlman, 1983
Fungal asexual reproduction			
Virulent	1.5	–	Elliston, 1985a; Anagnostakis, 1987; Anagnostakis and Kranz, 1987
Hypovirulent	0.75	–	Elliston, 1985a; Anagnostakis, 1987; Anagnostakis and Kranz, 1987
Fungal sexual reproduction			
Virulent	1.5[b]	–	Elliston, 1985a; Anagnostakis, 1987; Anagnostakis and Kranz, 1987
Hypovirulent	0.0[c]		Elliston, 1985a; Anagnostakis, 1987
Hypovirulent canker loses virus	–	0.01	
Hypovirus transmission			
Vertical transmission	–	0.75	Russin and Shain, 1985; Enebak et al. 1994
Horizontal transmission			
0 *vic* genes different	–	1.00	Liu and Milgroom, 1996
1 *vic* gene different	–	0.45	Liu and Milgroom, 1996
2 *vic* genes different	–	0.15	Liu and Milgroom, 1996
>2 *vic* genes different	–	0.03	Liu and Milgroom, 1996

[a] Transition rates were estimated from published data as indicated below. Estimates without specified references were made arbitrarily, with little or no data available.

[b] Ascospores were assumed to be the major source of inoculum of virulent isolates (Heald et al. 1915; MacDonald and Double 1978). The number of ascospores produced in each sexual reproduction was determined by a random number generated from a Poisson distribution with a mean of four. Self-fertilization occurs at rate of 25% when sexual reproduction occurs (Milgroom et al. 1993).

[c] Virus-infected fungi produce only conidia (Elliston 1985a; Anagnostakis 1987).

Asssuming $\beta \geq \gamma$:

if $\xi(x) = 1$ then it becomes 0 with probability $\frac{\gamma}{\beta}$,

if $\xi(x) = 0$ then it becomes 1 with probability $\frac{n(x,\ \xi)}{N}$, where $n(x,\ \xi)$is the number of neighbors of x that are occupied in ξ.

In other words, each site has an equal probability of being chosen; then another random number (between 0 and 1) is drawn to determine which transition, if any, occurs at that site. Each state has a defined set of possible transitions. The probability of each transition is determined by its transition rate in relation to the maximum rate among different sets of transitions in the system (Durrett and Levin, 1994). In the example above, β is greater than γ so transition rates are divided by β to determine the probabilities of transitions. Thus, the probability of a death is $\gamma\ /\beta$. The probability of a birth, however, is determined by the fraction of neighboring sites occupied since β cancels out ($\frac{\beta}{\beta} \bullet \frac{n(x,\ \xi)}{N} = \frac{n(x,\ \xi)}{N}$).

The transition process in the chestnut blight system is more complicated than the simple birth-and-death process described above, especially when viruses are introduced into the model. The interacting process in our model has the following possible transitions, with their transition rates and probabilities listed in Table 1:

1. A vacant site becomes occupied by an uninfected tree at a constant rate with the birth of a chestnut tree in an unoccupied cell, or when an existing tree grows an additional site (maximum of six sites per tree).
2. A site occupied by an uninfected tree may become: (a) occupied by a virus-free fungus by the arrival of either an asexual or a sexual spore from a neighbor, or (b) occupied by a virus-infected fungus by the arrival of an asexual spore from a virus-infected neighbor.
3. A site occupied by a virus-free fungus may become virus-infected by the arrival of a virus-infected spore from a virus-infected neighbor, followed by the successful transmission of virus into the resident virus-free fungus.
4. A site occupied by a virus-infected fungus becomes occupied by a virus-free fungus if the virus is lost.
5. Any occupied site may become empty if (a) the site is occupied by a virus-free fungus that causes tree mortality in this site, or (b) a virus-free fungus in any other site on the same tree (same cell) causes tree mortality. Mortality caused at one site can cause the loss of any distal site on the same tree, each with an independent probability of 0.20. This probabilistic approach was used to avoid having to keep track of canker age and position. Cankers caused by virus-infected fungus do not cause any tree mortality.

The transition rates for these events were determined from data reported from field and laboratory studies when available (Table 1). However, because of the lack of data, some transition rates were assigned arbitrarily.

3.3. Genetic and spatial heterogeneities

To incorporate genetic and spatial heterogeneities in this system, we assumed there are up to five polymorphic *vic* loci, each with two alleles, in the fungus population. Alleles were initially assigned at random for each *vic* locus to represent a range of vc type diversity. The probability of virus transmission (horizontal transmission) is based on the numbers of *vic* genes in common between individuals (see Table 1) (Liu and Milgroom, 1996). Fungal individuals may reproduce both sexually and asexually. Self-fertilization occurs at a rate of 25% when sexual reproduction occurs (Milgroom et al., 1993). When outcrossing occurs, progenies are assigned with vc types determined by the random recombination of parental *vic* genes. The number of offspring produced by each fungus per site was assumed to follow a Poisson distribution. Virus-free fungi reproduce sexually and asexually, producing, on average, four times as many ascospores as conidia. Virus-infected fungi produce only conidia. Dispersal of asexual spores followed a discretized negative exponential distribution with a mean of 0.5 cells, while the air-borne sexual spores are assumed to disperse for a relatively longer distance following a dis-

cretized negative exponential distribution with a mean of 2.5 cells, which was found to approximate random dispersal in the 50×50 simulated area. These parameters were estimated based on the relative distances these spores are known to disperse (Heald et al., 1915; Garrod et al., 1985). We calibrated dispersal distances, and verified the spatial structure of this model by generating spatial patterns of vc types that were similar to observations from field studies (Milgroom et al., 1991) (results not shown).

Initial conditions used in most simulations are as follows. All individuals are randomly assigned in the spatial grid. The population was initialized so that 1/3 of the sites were occupied by uninfected trees. Into this tree population, virus-free and virus-infected fungi, with the same vc type frequencies, were randomly assigned to tree-occupied sites (250 sites for each). Since each simulation could result in one realization of the stochastic behavior of virus transmission, at least 10 simulations were performed for each set of parameter values and means of the simulations were presented as results. Unless otherwise specified, simulations were conducted with the above conditions. Simulations were run for 3000 time units in an attempt to approximate conditions approaching equilibrium. Shorter times were used (e.g. 100 time units) to simulate conditions more relevant to biological control. Each time unit is approximately equal to 1 year.

4. Results

4.1. Effect of vc type diversity on horizontal transmission rates and virus invasion

We simulated the invasion of viruses in fungal populations with different vc type diversities. Diversity of vc types was expressed by the Shannon diversity index (Hutcheson, 1970), ranging from 0 (a single vc type) to 3.5 (32 vc types, each at equal frequency). In general, the probability of successful virus invasion was high when the initial vc type diversity was low, but virus invasion did not occur at high diversities; stochastic behavior of virus invasion was evident at intermediate vc type diversities (see 'spatial model' in Fig. 1). The horizontal transmission rate was estimated from the initial vc type distribution by calculating the average transmission probability between each pair of individuals in the population. Average horizontal transmission rates are negatively correlated to vc type diversity (Fig. 2). Therefore, viruses would be less likely to invade when the horizontal transmission rate is decreased.

We evaluated the sensitivity of the model to different values of disease-induced tree mortality rate, vertical transmission rates, and reproductive rates of virus-free and virus-infected fungus. When there was an intermediate level of vc type diversity, all of these parameters were important in determining virus invasion. Virus invasion was more likely with lower fungus-induced tree mortality, lower reproductive rates of virus-free fungi, higher reproductive rates of virus-infected fungi, and higher vertical transmission rates (results not shown). At low vc type diversities, these parameters had little effect on virus invasion. Virus invasion appeared to be high even at high vc type diversities when the reproductive rate of virus-infected fungi was as great as virus-free fungi. However, roughly equal reproductive rates for virus-free and virus-infected fungi implies little or no virulence of the viruses towards their hosts. When this occurs, the effect of viruses on fungal fitness becomes insignificant, and successful virus invasion will have little effect on fungal populations.

We also evaluated how the model responded to dispersal abilities of different spore types. The dispersal distance of sexual spores (ascospores) and asexual spores (conidia) showed large effects on virus establishment only at intermediate vc type diversities; however, the effects were opposite for the two types of spores (Fig. 3). Virus invasion decreased as ascospores, which are virus-free, dispersed farther, presumably escaping local virus outbreaks. In contrast, as dispersal distance increased for conidia, which disseminate viruses, virus invasion increased.

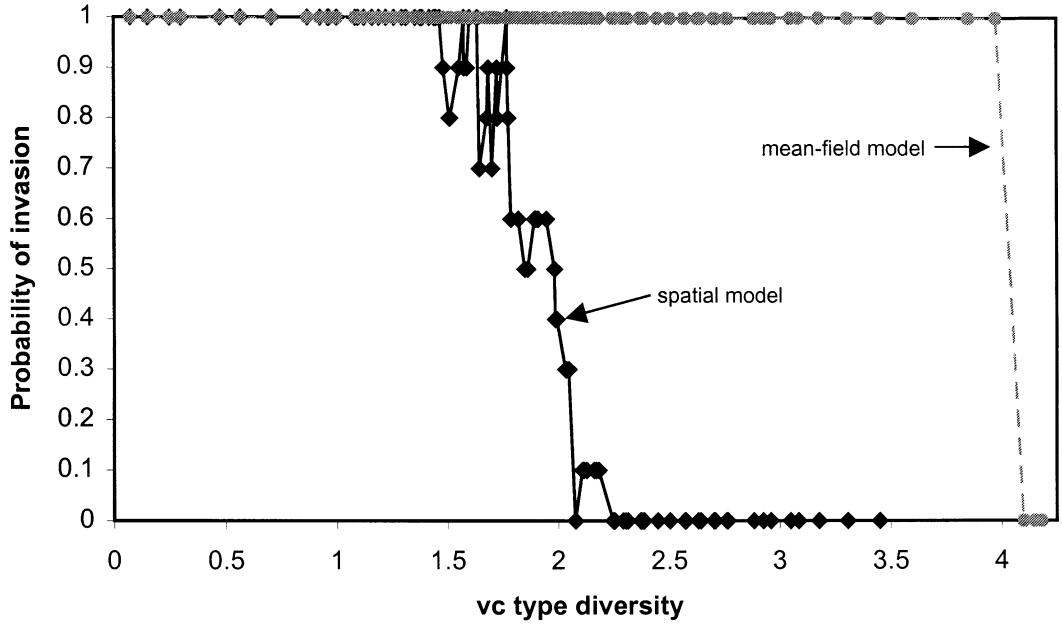

Fig. 1. The probability of virus invasion in relation to initial vegetative compatibility (vc) type diversity. Each point is the proportion of ten replicate simulations in which the number of virus-infected individuals exceeded 2/3 of the total sites after 3000 time units. The spatial model assumed heterogeneous dispersal of sexual and asexual spores. The mean-field model assumed homogeneous mixing of individuals. Initial vc type diversity was calculated as the Shannon diversity index (Hutcheson, 1970): $D = -\Sigma p_i \ln p_i$, where p_i is the frequency of i^{th} vc type. The horizontal transmission rates used in simulations of the mean-field model were calculated as a function of vc type diversity (Fig. 2). Virus invasion was simulated in populations with vc type diversities >3.5 in the mean-field model to estimate the invasion threshold.

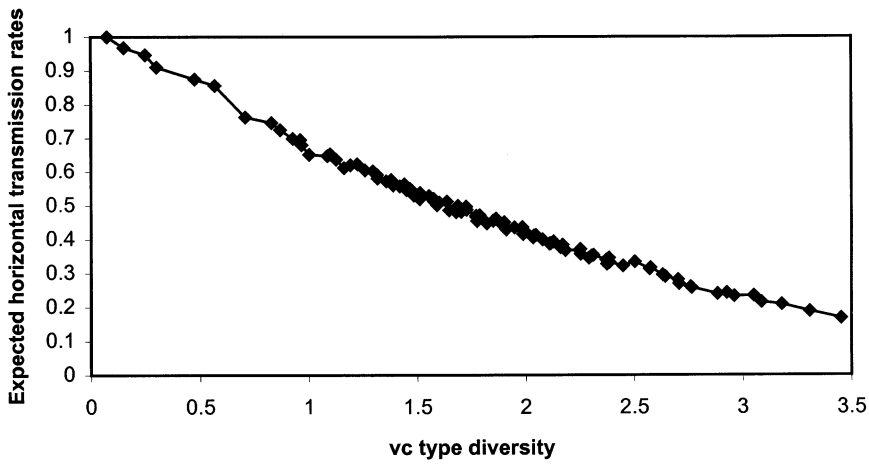

Fig. 2. The relationship between vegetative compatibility (vc) type diversity and expected horizontal transmission rate. Vc type diversity was calculated as the Shannon diversity index (Hutcheson, 1970): $D = -\Sigma p_i \ln p_i$, where p_i is the frequency of i^{th} vc type. The expected horizontal transmission rate was calculated based on all possible pairs of individuals in the initial distribution of vc types in the fungal population and the probability of transmission between vc types (Table 1).

4.2. Effect of spatial heterogeneity on virus invasion

We compared the dynamics of virus invasion in our spatially-structured model with a mean-field model that was modified from the spatial model by assuming homogeneous mixing of all individuals at all times. In the mean-field model, sexual recombination of the fungus and spatial processes were ignored. The occurrence of fungus infection of trees and virus transmission in fungi was independent of the state and genetic characters of the near neighbors; instead, these transitions were formulated based on mean-field equations (Anderson and May, 1984), with the notation of Taylor et al. (1998), as follows:

1. an uninfected tree, X, becomes infected by a virus-free fungus at a rate $\beta_f Y_f$ or by a virus-infected fungus at a rate $\beta_v Y_v$, where β_f and β_v are the infection rates of virus-free and virus-infected fungi, respectively, and Y_f and Y_v are the proportions of sites occupied by virus-free and virus-infected fungi, respectively.
2. a virus-free fungus was infected with viruses at a rate $s\beta_v Y_v$, where s is the expected horizontal transmission rate of viruses based on the mean vc type diversity of the fungal population as shown in Fig. 2.

The mean-field model showed that viruses may successfully invade the fungal population even at a very high vc type diversity (Fig. 1). Compared to the mean-field model, virus invasion in the spatial model was more sensitive to vc type diversity (Fig. 1). The results derived from the spatial model are consistent with the general observation of virus establishment in the field. Hypoviruses have been well established in fungal populations where vc type diversity is low, as in Europe (Heiniger and Rigling, 1994; Cortesi et al., 1996) or in Michigan (Fulbright et al., 1983, Liu et al., 1996). However, the spread of viruses has been poor in most of the US populations where vc type diversities are relatively high (MacDonald and Fulbright, 1991; Milgroom and Cortesi, 1999).

4.3. Reproductive mode and spatial structure

The effects of fungal reproduction on virus transmission is not simple because: (1) viruses inhibit sexual reproduction; (2) viruses are not transmitted through ascospores; (3) recombination of *vic* genes by sexual reproduction can generate and maintain vc type diversity, and (4) asexual and sexual spores disperse differently. To investigate the effects of different reproductive

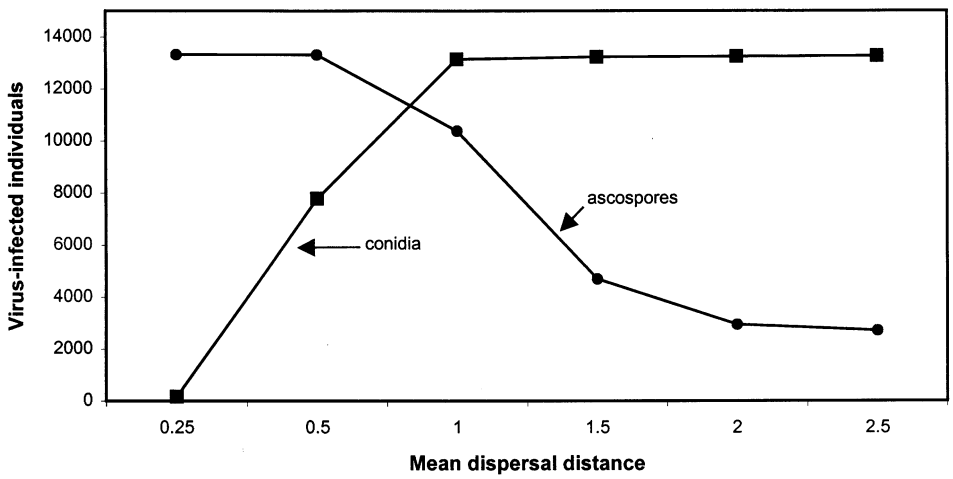

Fig. 3. The effect of spore dispersal distances on virus establishment in a population with initial vc type diversity of 0.67. The establishment of viruses is expressed as the mean number of virus-infected individuals in ten simulations after 1000 time units. Mean dispersal distance is expressed in units of cells in the spatial grid, assuming a negative exponential distribution. Conidia are asexual spores in which viruses are dispersed; ascospores are sexual spores that are always virus-free.

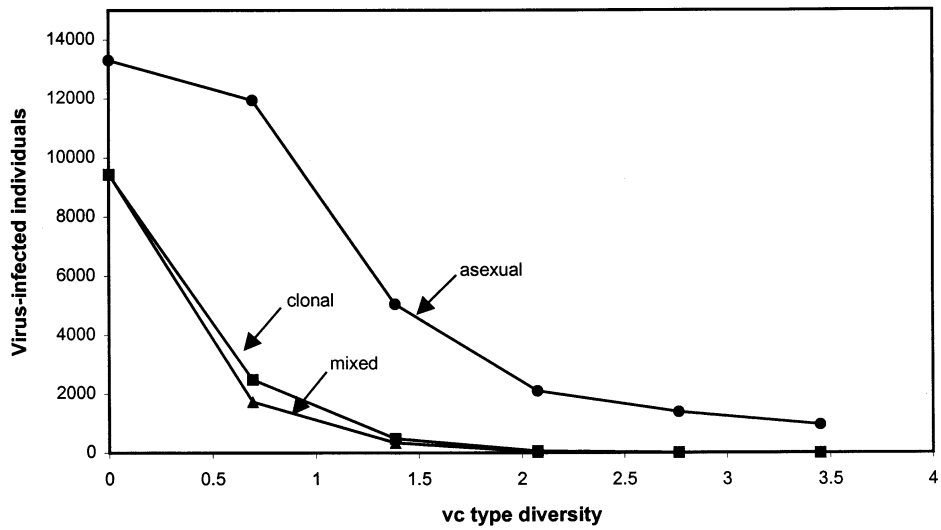

Fig. 4. The effect of the reproductive biology of *C. parasitica* on the establishment of viruses as a function of the initial vc type diversities. The establishment of viruses was compared when the fungal population was completely asexual, clonal (with both asexual reproduction and self-fertilization), or mixed (reproducing both sexually and asexually). Establishment of viruses is represented by the mean number of virus-infected individuals in ten replicate simulations at the end of 100 time units.

modes on virus establishment, we used the spatial model to simulate three different reproductive regimes:

1. Asexual: individuals could only reproduce by conidia.
2. Clonal: a combination of asexual reproduction (as above) and sexual reproduction by self-fertilization. This is called clonal because no recombination of *vic* genes and mating types occurred.
3. Mixed reproduction: a combination of asexual and sexual reproduction by outcrossing and selfing (as above). This situation most closely approximates populations in eastern North America (Milgroom et al., 1992, 1993; Liu et al., 1996; Milgroom and Cortesi, 1999).

Viruses were more likely to invade asexual populations (Fig. 4). However, in clonal populations, which were genetically equivalent to asexual populations, viruses only invaded when vc type diversity was low (Fig. 4). Virus invasion was further restricted when mixed reproduction occurred (Fig. 4). The difference between the asexual and clonal populations was that virus-free fungi dispersed as poorly as virus-infected fungi in asexual populations since only conidia were produced. However, in clonal populations, the majority of reproduction is from virus-free ascospores produced by self-fertilization, which disperse longer distances than conidia. In Fig. 5 we plot the spatial configuration of vc types and the corresponding spatial pattern of virus distribution for asexual and clonal populations. There was a significant aggregation of vc types in the asexual population, where viruses established as connected patches. In contrast, vc types were randomly distributed in the clonal population in which small numbers of sites were occupied by virus-infected individuals; similar patterns were found in a population with mixed reproduction (result not shown).

5. Model application — evaluation of genetically engineered virus-infected strains

We used our model to evaluate the use of genetically engineered virus-infected strains, which were developed recently (Choi and Nuss, 1992) and deployed in small scale field trials (Anagnostakis et al., 1998). These strains contain a full-length cDNA copy of the viral genome integrated into a nuclear chromosome (Choi and Nuss, 1992)

and are referred to as transgenic strains. Based on their biology, we made the following modifications to our model to simulate transgenic strains. Transgenic strains produce viral dsRNAs in the cytoplasm and are phenotypically similar to non-transgenic virus-infected strains (Choi and Nuss, 1992). However, transgenic strains produce conidia that are virus-infected at a frequency of 100% because they contain the viral transgene (Chen et al., 1993). This vertical transmission rate is significantly greater than in non-transgenic virus-infected strains, which ranges from 2 to 100% (Russin and Shain, 1985; Enebak et al., 1994). Furthermore, in marked contrast to non-transgenic virus-infected strains, in which viruses are not transmitted to ascospores, 50% of ascospore progeny inherit viral transgenes when conidia of transgenic strains function as male gametes in sexual crosses (Chen et al., 1993). When mating occurs between strains that are

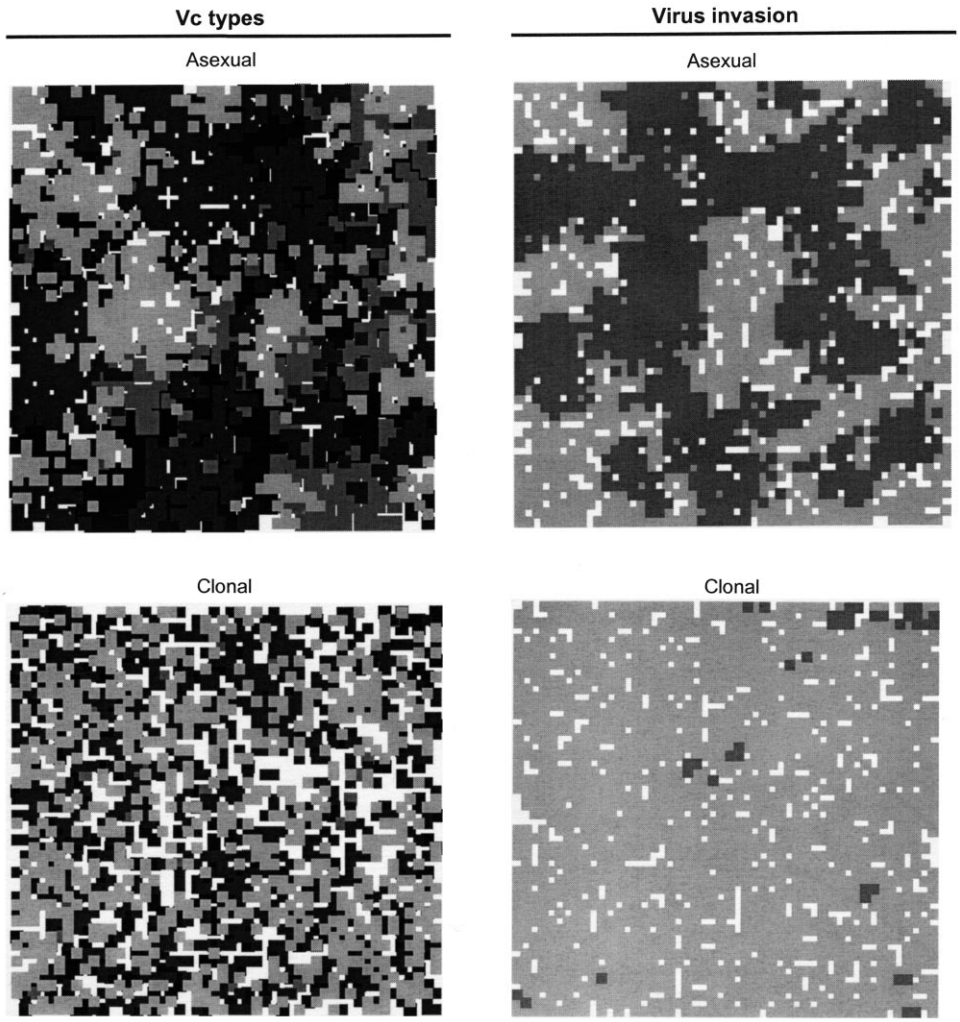

Fig. 5. Spatial patterns of vc types and virus-infected individuals in completely asexual and clonal (asexual and self-fertilization) populations at the end of 100 time units. Vc types are shown as different shades of gray in the left column. Virus-infected (dark gray) and virus-free individuals (light gray) are shown in the right column. In both populations, there were initially four vc types at equal frequencies randomly assigned in space.

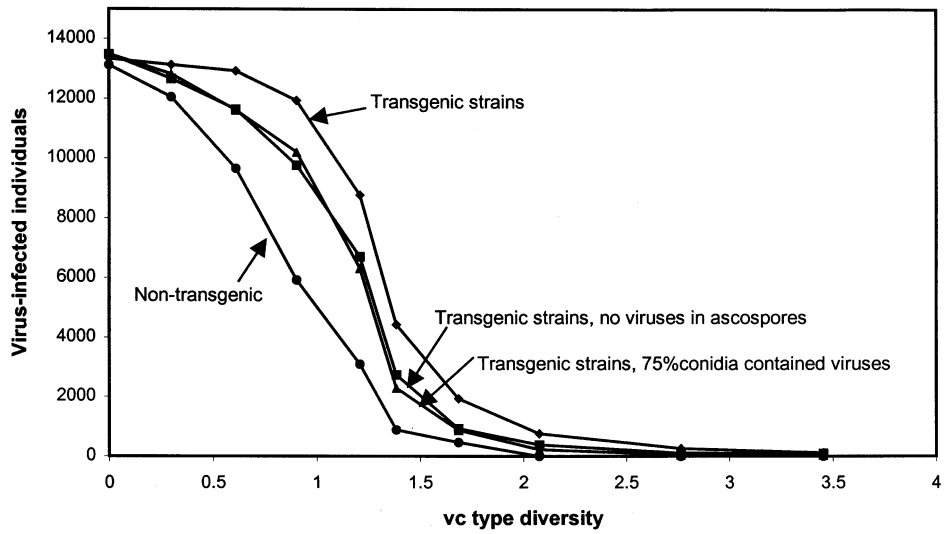

Fig. 6. Effects of releasing transgenic strains and non-transgenic virus-infected strains as a function of the initial vc type diversity. All parameter values were the same for transgenic and non-transgenic virus-infected strains, except that transgenic strains could transfer the viral transgene to sexual progeny and had greater rates of vertical transmission to asexual progeny. The effects of vertical transmission into conidia and ascospores were also compared (see text for details).

vegetatively incompatible, viruses are transmitted to ascospore progeny, including those with recombinant *vic* genotypes (Chen et al., 1993). When transgenic strains come in contact with virus-free fungi, only the cytoplasmic viruses are transmitted resulting in non-transgenic (cytoplasmic) virus-infected strains. Therefore, with the release of only transgenic strains, non-transgenic virus-infected strains would also appear in the population once horizontal transmission occurred.

5.1. Comparison of transgenic and non-transgenic virus-infected strains

We first compared the effects of transgenic and non-transgenic virus-infected strains. One-thousand randomly distributed virus-free fungi were assigned to occupied sites before treatment with viruses; 1000 transgenic strains or non-transgenic virus-infected strains were then randomly released into the field, with the same vc type frequencies as the virus-free population. Virus establishment was evaluated after 100 time units.

The relative importance of vertical transmission in conidia or ascospores in transgenic strains was evaluated by varying each factor separately. The effect of transmission in ascospores was analyzed assuming viruses were transmitted into conidia of the transgenic strains at the same frequency (75%) as the non-transgenic virus-infected strains, while 50% of ascospores were virus-infected because of inheriting the viral transgene from the transgenic male parent. The influence of greater vertical transmission rate in conidia was evaluated by assuming viruses were not transmitted into ascospores but 100% of conidia contained viruses.

Virus establishment was greater after releasing transgenic strains than after releasing non-transgenic virus-infected strains (Fig. 6). However, as with non-transgenic virus-infected strains, the number of virus-infected individuals decreased as vc type diversity increased (Fig. 6). The transmission of viruses into ascospores and greater vertical transmission rate into conidia of transgenic strains had equal effects on virus establishment. Virus establishment under both of these two assumptions was intermediate between transgenic and non-transgenic virus-infected strains (Fig. 6).

5.2. Inundative release of transgenic strains

To enhance disease control in populations where vc type diversity is high, as in most populations in the North America, one potential strategy is to repeatedly and intensively release a mixture of transgenic strains with both mating types but only one vc type (Anagnostakis et al., 1998). The transgenic strains are expected to function mainly as sources of male gametes in sexual crosses in order to enhance the production of ascospores that carry the viral transgene. Furthermore, by producing an abundant source of conidia (male gametes) in a single vc type to mate repeatedly with the resident population, the diversity of vc types could conceivably be reduced over time (analogous to recurrent backcrossing). A decrease in vc type diversity may enhance horizontal virus transmission by increasing relatedness of vc types (Fig. 2).

To investigate the persistence of transgenic strains in the treated area and their dissemination to untreated areas, virus establishment was examined by inundatively releasing transgenic strains in the center 20×20 cells of the plot for 20 time units. For comparison, virus establishment was also examined under the same condition except non-transgenic virus-infected strains were released. Since the novelty of transgenic strains lies in their ability to transmit viruses through ascospores, we assumed that 50% of outcrosses occurred by mating with the released virus-infected strains when viruses were intensively released.

Transgenic strains persisted in the field for a short period of time after the treatment was terminated ($t = 20$); non-transgenic virus-infected strains that were derived from horizontal transmission behaved similarly. However, virus-infected individuals were distributed mainly within the treated area (Fig. 7A). Although the effect of inundative release of transgenic strains was greater than non-transgenic strains (Fig. 7A), virus establishment could not be maintained over time (Fig. 7A). Contrary to expectation, the vc type diversity in the treated area did not decrease despite intensive release of a single vc type to promote recurrent backcrossing at a population scale (Fig. 7B). The initial vc type diversity in the treated area was reduced only because a large number of the released strains were assumed to form cankers. Diversity of vc types increased after releases stopped and virus-infected individuals declined in number.

6. Discussion

The goal of this study was to investigate the importance of both genetic and spatial heterogeneity on virus invasion in fungal populations. We demonstrated that a spatially structured, interacting particle model is able to reasonably simulate the qualitative behavior of virus transmission, despite limited data for parameter estimation. This model showed that the establishment of viruses is regulated by the diversity of vc types in fungal populations, which agrees with conclusions from empirical studies (Anagnostakis and Day, 1979; Anagnostakis and Waggoner, 1981; Liu and Milgroom, 1996). The increase of host genetic diversity results in lower probability of contacts between individuals of the same vc type and therefore a decrease in the horizontal virus transmission rate.

The spatial process of virus transmission in this system is important. The success of pathogen invasion is usually determined by a key parameter defined as the potential number of secondary infections per infectious individual. In mean-field models, pathogen invasion will succeed if this parameter has a value greater than one (Anderson and May, 1984). But in systems where individuals are not mixing homogeneously, the critical value of this parameter can be significantly higher because there is a chance of 'waste infections' (Mollison and Levin, 1995), e.g. contacts with individuals that are already infected. The transmission of viruses is basically a contact process. Horizontal transmission of viruses can be overestimated in mean-field models in which virus-free and virus-infected individuals are assumed to come in contact randomly. We showed that in a mean-field model viruses are likely to successfully invade fungal populations regardless of vc type diversity. A similar prediction was made by a

mass-action, non-spatial model of a similar fungus-virus system in which virus can always invade as long as the horizontal transmission is greater than zero (Taylor et al., 1998). However, in the spatial model presented here, virus transmission is governed by strong genetic and spatial heterogeneities, resulting in significantly different host-pathogen dynamics. Ignoring these sources of heterogeneity in horizontal transmission could lead to erroneous conclusions.

The different outcomes of virus invasion in populations with different reproductive modes clearly demonstrated that genetic heterogeneity interacted with the spatial distribution of viruses and their fungal hosts in determining the invasion dynamic. The spread of viruses is likely to be

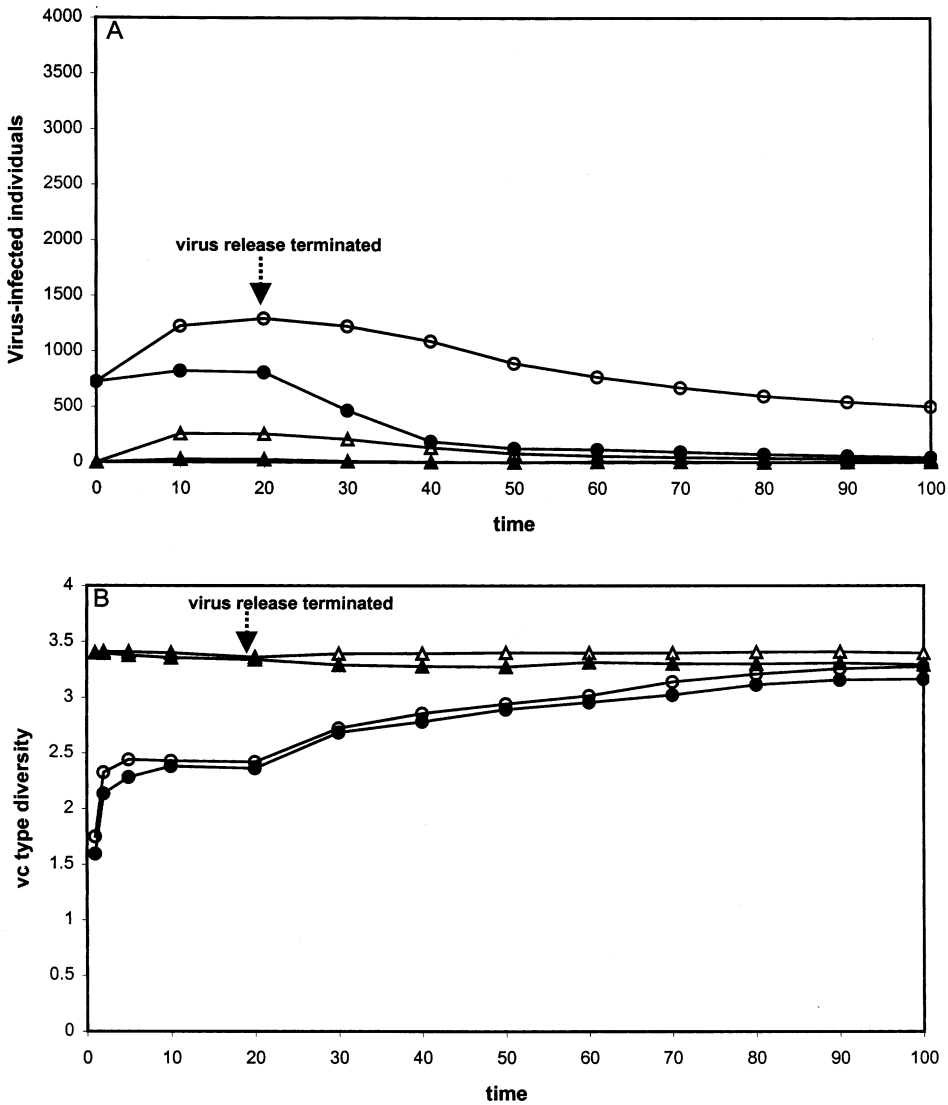

Fig. 7. Effects of intensive treatment with transgenic strains on (A) virus establishment and (B) Changes of vc type diversities. Transgenic (open symbols) or non-transgenic (closed symbols) virus-infected strains were released intensively for 20 time units in the center 20×20 cells in a fungus population with 32 vc types in equal frequencies. Results for the treated area (circles) and the untreated area (triangles) are shown separately.

driven mostly by nearest neighbor contact. The aggregation of vc types in asexual populations resulted in small patches within which viruses increased their chances of spreading and persisting in the fungal population because of the increasing chance of contacts between vegetatively compatible individuals. Previous studies on chestnut blight stressed the correlation between the success of virus invasion and population structure of the fungus, concentrating on the confounding effect of vc type diversity and reproductive biology (Liu et al., 1996). Our model suggests that the spatial structure of the fungal population should be equally if not more important in the virus-fungus interaction. In fact, dispersal of sexual and asexual spores had greater influence on virus invasion than recombination of *vic* genes.

One of the goals of this study was to use the model to evaluate strategies for disease management. We evaluated the potential benefits and limitations of using genetically engineered virus-infected strains, showing that the use of transgenic strains may improve biological control to a limited extent. However, virus establishment still depends strongly on vc type diversity. Inundative release of transgenic strains seems to offer greater chance of virus persistence than non-transgenic virus-infected strains in populations with high vc type diversity. However, to establish long term disease control transgenic strains need to be repeatedly released over time to maintain mating between virus-free and transgenic strains and to maintain virus population size. Furthermore, although mating among virus-free and transgenic strains produced virus-infected ascospores that dispersed outside the treated area, the transgenic strains were not self-sustaining in the untreated area. Once treatments were terminated, the transgenic strains died out due to death of trees caused by the immigration of virus-free individuals from the untreated area, as predicted by Jarosz and Davelos (1995). These results suggest that transgenic strains will need to be repeatedly released over time to establish long term disease control, which is probably unrealistic because of the intensive effort required.

Viruses and other double-stranded (ds)RNA elements have been found associated with a large number of fungal plant pathogens, although only a few have been incorporated into biological control strategies (see Nuss and Koltin, 1990). Similar phenotypic consequences on infected individuals, such as reduced virulence and loss of female fertility, and restrictions on virus transmission by vegetative incompatibility have been observed in several plant pathogenic fungi. Previous models for these types of systems, based on deterministic equations, focused on the mode of virus transmission, concluding that vertical transmission could be a more robust factor than horizontal transmission to effect virus invasion (Swinton and Gilligan, 1999; Taylor et al., 1998). Swinton and Gilligan (1999) further used a deterministic model with a stochastic process and patch-occupancy spatial structures to show the potential effects of spatial heterogeneity and stochastic complexity on biological control. When individual-based spatial interactions were taken into account, our model suggested that horizontal transmission might be as important as the other factors. Furthermore, we demonstrated that investigation of the explicit reproductive behavior of the fungus, which was overlooked by previous studies (Taylor et al., 1998; Swinton and Gilligan, 1999), is critical in studying the dynamics of virus invasion.

The implications of heterogeneous transmission on the evolution of both hosts and pathogens require further study. Recent literature on the evolutionary dynamics of diseases has focused on the relationship between the transmission of pathogens and the evolution of virulence (reviewed by Bull, 1994; Lipsitch et al., 1995, 1996). Spatial and temporal structures of host populations have been demonstrated as important determinants for evolution of virulence (Lipsitch et al., 1995; Lannou and Mundt, 1997). For the transmission of hypoviruses, transmission efficiency is greatly affected by genetic relatedness of vc types (Liu and Milgroom, 1996). This type of dynamic could lead to frequency-dependent selection for *vic* alleles or vc types because rare genotypes will have lower probabilities of becoming infected (Hartl et al., 1975; Nauta and Hoekstra, 1994). Additionally, different hypoviruses have been found in natural populations of *C. parasitica* (Chung et al., 1994; Enebak et al., 1994; Peever et

al., 1997). Super-infection of viruses has recently been observed among field isolates of *C. parasitica* (Peever et al., 1997, 1998). Studies on super-infection in natural and laboratory strains suggested that different viruses could have additive effects on virulence (Elliston, 1985b; Smart and Fulbright, 1995). Studies of genetic diversity and the evolution of virulence of viruses in structured populations will be important and interesting directions for future research in this system.

7. Conclusions

We used a spatially structured, interacting particle model, incorporating complex biological and spatial features, to simulate the process of virus invasion in fungal populations, with special reference to the chestnut blight system. This model demonstrated strong effects of host genetic heterogeneity and spatial structure on virus transmission. Virus invasion is restricted when their host populations have increased vc type diversities and reproduce sexually. We investigated the release of genetically engineered virus-infected strains and demonstrated that disease control would not be established due to the poor persistence of transgenic strains.

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