Competition between strains of a pathogenic fungus in plant leaves

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ABSTRACT

We have developed mathematical models for intra- and inter-strain competitive interactions among multiple localized infections of the biotrophic plant parasite *Puccinia graminis* in wheat leaves. In the infection model, the leaf is composed of a defined number of potential lesion sites, in which only one infection per site can mature and sporulate. In the sporulation model, all infections in a leaf compete for a pool of resources within the leaf for spore production. In inoculations with single parasite strains, we measured the following parameters: infection efficiency, maximum number of infections per leaf, parasite sporulation efficiency per lesion, and maximum sporulation supported by leaves. From mixed-strain inoculations, we determined competition coefficients for effects of each strain on the other during infection and spore production. Strain SR22 showed superior infection efficiency, carrying capacity for infections per leaf, and maximum sporulation supported per leaf in single-strain tests. The fitness advantage of SR22 was tempered, however, by the greater competitive ability of strain SR41, which reduced both infection by SR22 spores and sporulation by SR22 infections in leaves with mixed parasite populations. In the model, superior infection efficiency produces a fitness advantage that is independent of density, but fitness advantages associated with other parameters are density dependent. Fitness effects for competition coefficients not equal to 1.0 are both density and frequency dependent. Our methods for analyzing competitive ability are applicable to other plant parasites.

Introduction

Although the impacts of intra- and inter-specific competition on population dynamics have been extensively analyzed [2, 6, 11], competition in microbial systems has received relatively little attention until recently, partly because populations of most microbes are difficult to quantify [1, 10]. Many plant pathogenic rust fungi do not share this difficulty. For example, *Puccinia graminis*, cause of wheat stem rust, infects wheat leaves and stems producing pustules (known as uredinia) that release asexual spores (urediniospores) that can infect the same or other wheat plants. A heavily infected plant may have hundreds of individual rust pustules, each resulting from infection by a different spore. Even at high densities, the pustules do not coallesce. Thus, the spore bearing mycelium of each pustule is a discrete individual in the fungus population [7]. With rust fungi, crowding can reduce both their efficiency in forming sporulating pustules and the numbers of spores they produce per pustule [4, 5, 9, 12]. Rust infection cycles can be controlled experimentally, because urediniospores germinate only in a film of water and require several hours of

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continuous wetness to penetrate the host epidermis. For these reasons, *P. graminis* and other rust fungi make good models to study microbial population ecology. We studied the effects of competition between strains of *P. graminis* on infection and urediniospore production on wheat seedlings. Our objectives were to measure intrinsic fitness components as well as specific competitive interactions between *P. graminis* strains and to model density and frequency dependent influences on fitness.

Materials and Methods

We analyzed pathogen density effects on competition between two strains of *P. graminis*, SR22 an orange spore mutant, and SR41 a gray-brown spore mutant from a different asexual lineage. We inoculated primary leaves of seedlings of the rust-susceptible wheat cultivar McNair 701 with either SR22 alone, SR41 alone, or a 1:1 mixture of SR22 and SR41 by spraying the leaves with spore suspensions at densities of 5 x 10^5, 5 x 10^6, 2.5 x 10^7, and 5 x 10^7 urediniospores per ml [7]. Numbers of spores delivered to the leaves were estimated by counting spores of each strain on transparent plastic strips cut to the size of leaves and inserted into each pot of four seedlings. Nine days after inoculation, the numbers of orange and gray-brown uredinia (pustules) were counted on each leaf. Beginning 10 days after inoculation, urediniospores were collected from the leaves every 2 days for 2 weeks and counted in a hemacytometer.

Numbers of uredinia formed on the leaves increased with increasing inoculum density in the general pattern of a saturation curve. The same was true for the increase in spores produced per leaf with increasing numbers of uredinia per leaf. The data for numbers of uredinia vs.spores applied per leaf fit the model [7]

$$U_1 = K_1\left[\left(1-\left(1-\frac{E_1}{K_1}\right)^{s_1}\right)\left(1-\frac{E_2}{K_1}\right)^{s_2} + \left(\frac{E_1}{E_1+\alpha_2 E_2}\right)\left(1-\left(1-\frac{E_1}{K_1}\right)^{s_1}\right)\left(1-\left(1-\frac{E_2}{K_1}\right)^{s_2}\right)\right]$$

in which U_1 = number of uredinia of strain 1 in the mixture; K_1 = maximum number of uredinia of strain 1 that a leaf can support; E_1 = infection efficiency of strain 1 spores (proportion of strain 1 spores applied to a leaf that produce mature uredinia when there are no competiting spores on the same leaf); S_1 = number of spores of strain 1 applied to the leaf; S_2 = number of spores of strain 2 applied; and α_2 = the competition coefficient for the effect of strain 2 on ability of strain 1 to establish uredinia. For numbers of uredinia formed by strain 2, the subscript numbers are reversed. In this model, which is based on the assumptions of the multiple infection transformation [3], competition is conceived as being a localized contest among spores initiating infections within a single potential site on the leaf for development of a sporulating pustule (uredinium). Only a single infection can be successful within a site; no mixed genotype uredinia were observed in our experiments.

While Equation 1 is satisfactory for 1:1 mixtures of competing strains, it does not apply when frequencies of strains in the mixture vary from 1:1 [8]. In those cases, we use the equation

$$U_1 = K_1[\mathrm{EXP}(pr_1)]$$

in which $\mathrm{EXP}(pr_1)$ is the expected probability that a strain 1 uredinium will develop in a site. This is equal to the expected value of $x_1/(x_1 + \alpha_2 x_2)$ over the distribution of all possible combinations of numbers of infections of strain 1, x_1, and strain 2, x_2, in a site that can result in a strain 1 uredinium. The value of x_1 can range from 1 to S_1, and x_2 can range from 0 to S_2. This expectation, U_1, can be calculated as the summation

$$U_1 = \sum_{x_1=1}^{S_1} \sum_{x_2=0}^{S_2} P(x_1)\, P(x_2) \left(\frac{x_1}{x_1 + \alpha_2 x_2} \right)$$

in which $P(x_1)$ is a binomial

$$\left(S_i, \frac{E_i}{K_1} \right)$$

probability.

Sporulation data from our experiments fit the equation

$$Q_1 = \left(\frac{U_1}{U_1 + \beta_2 U_2} \right) M_1 \left[1 - \left(1 - \frac{A_1}{M_1} \right)^{(U_1 + \beta_2 U_2)} \right]$$

in which Q_1 = number of spores of strain 1 produced per leaf; U_1 = number of uredinia of strain 1 on the leaf; U_2 = number of uredinia of strain 2; M_1 = the maximum numbers of spores produced by strain 1 per leaf; A_1 = the sporulation efficiency of strain 1 (numbers of spores produced per strain 1 uredinium when there is no competition); and β_2 = the competition coefficient for the effect of strain 2 uredinia on the ability of strain 1 uredinia to produce spores. Since each uredinium sets up a metabolic sink that draws leaf photosynthates to it, we view competition during sporulation as a contest among all uredinia on a leaf to capture as much of a common pool of resources as possible.

Results

In our experiments [7], SR22 exhibited significantly greater infection efficiency and greater maximum numbers of uredinia and sporulation per leaf, whereas SR41 had significantly greater competition coefficients for both infection and sporulation (Table 1).

Table 1. Fitness components of two strains of *Puccinia graminis* on wheat seedlings

	Strain	
Parameter	SR22	SR41
Max. no.pustules/leaf (K)	433*	384
Infection efficiency (E)	0.08*	0.03
Max. no. spores formed/leaf (M)	239,000*	167,000
Sporulation efficiency (A)	5,670	4,610
Competition coefficient, infection (α)	0.52	1.99*
Competition coefficient, sporulation (β)	0.62	1.52*

* indicates significantly greater value ($P < 0.05$).

The two strains did not differ significantly in sporulation efficiency. In our models, the parameters E, K, A, and M can be measured for each strain in single strain tests and, therefore, can be regarded as intrinsic components of fitness. The competition coefficients, however, can be determined only by comparing crowding effects in single strain tests with those in mixed strain tests. The competition coefficients, therefore, quantify specific abilities of each strain to compete with the other strain in the mixture. It is important to recognize that when competitive ability is defined in this narrow sense, it is not equivalent to relative fitness but rather is just one component of fitness. We calculated relative fitness in terms of the proportion of total spores produced by a strain in relation to the proportion of spores of that strain applied in the initial inoculum. For SR22, relative fitness was defined as

$$w_{22}=\frac{\left(\dfrac{Q_{22}}{Q_{22}+Q_{41}}\right)\left(\dfrac{S_{41}}{S_{22}+S_{41}}\right)}{\left(\dfrac{Q_{41}}{Q_{22}+Q_{41}}\right)\left(\dfrac{S_{22}}{S_{22}+S_{41}}\right)}=\frac{Q_{22}S_{41}}{Q_{41}S_{22}}$$

in which S_{22} and S_{41} are the numbers of spores of SR22 and SR41 applied per leaf, and Q_{22} and Q_{41} are the numbers of spores of SR22 and SR41 produced per leaf in the resulting infections. In our experiments, SR22 displayed greater fitness in mixtures with SR41 at all inoculum densities, although the difference in fitness was less at high densities than at low densities. The diminished fitness advantage of SR_{22} at high densities was due to its lower competitive ability (smaller competition coefficients) relative to SR_{41} (Table 1).

To test the density and frequency dependence of each of the six components of fitness, we [8] calculated relative fitness for two hypothetical strains of *P. graminis* identical in all components except one in which strain 1 has a 50% greater value. Baseline parameter values were E = 0.05, K = 400, M = 200,000, $\alpha = 1.0$, and $\beta = 1.0$. In the model, a 50% advantage in infection efficiency conferred a fitness advantage of approximately 50% at all population densities and strain frequencies. The fitness advantage gained from a 50% advantage in either K or M was negligible at low population densities but approximately 50% at the highest densities; the fitness advantage of greater K or M was not frequency dependent. The fitness advantage from 50% greater sporulation efficiency was about 50% at the lowest population densities but essentially nil at high densities; as with K and M the fitness advantage conferred by greater A was not frequency dependent.

Advantages in the competition coefficients α or β gave fitness advantages that were both density and frequency dependent, with greatest fitness advantages at high population density. For $\alpha_1 = 1.5$ and $\alpha_2 = 1.0$ or $\beta_1 = 1.5$ and $\beta_2 = 1.0$, the fitness advantage of strain 1 increased with increasing frequency of strain 1 in the mixture, whereas the reverse was true for $\alpha_1 = 1.0$ and $\alpha_2 = 0.67$ or $\beta_1 = 1.0$ and $\beta_2 = 0.67$. Even when strains 1 and 2 had identical values for all six fitness components, relative fitness of the two strains in mixture was both density and frequency dependent if values of α or β differed from 1.0. For example, if $\beta_1 = \beta_2 = 1.5$, the more frequent strain in the mixture had an advantage in relative fitness that increased with increasing frequency of that strain and with increasing population density. On the other hand, if $\beta_1 = \beta_2 = 0.67$, a stable equilibrium existed with either strain having greater fitness when it occurred at frequencies below the equilibrium

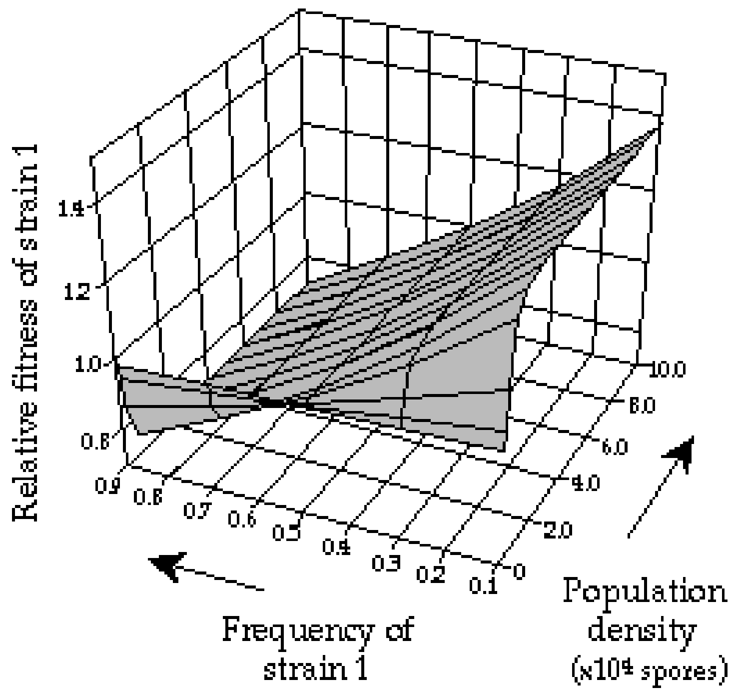

Fig. 1.

value. The fitness differentials at any given strain frequencies were greater at higher population densities (Fig. 1).

Discussion

Competitive ability as we have defined it in our models is not synonymous with fitness. Instead it is just one of the components of fitness of plant pathogenic fungi. Other components of fitness include such intrinsic characters as infection efficiency and sporulation efficiency and maximum numbers of uredinia and spore production that strains of the fungus can attain per leaf. These intrinsic characters can be measured for each strain independently of any competitors, but the competition coefficients for infection and sporulation must be determined in mixtures with a competing strain in the host plant. Competition coefficients $\neq 1.0$, which may be expected to be the rule, should lead to frequency dependent selection as a rule in mixed strain populations of plant pathogenic fungi that reach high population densities in severe epidemics. Whether such frequency dependent selection promotes or diminishes diversity in populations depends upon the magnitude of the competition coefficients. If they generally have values less than 1.0, we may expect frequency dependent selection to favor a diversity of strains. However, if the competition coefficients are generally greater than 1.0, frequency dependent selection will act against rare strains in mixed populations and will, therefore, tend to reduce pathogen diversity.

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