

The effect of imperfect transmission on the frequency of mutualistic seed-borne endophytes in natural populations of grasses

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We develop a simple mathematical model to explain the lower than expected levels of infection of wild perennial ryegrass populations in France by endophytic *Neotyphodium* fungi (formerly named *Acremonium*). Indeed, seed-borne *Neotyphodium* endophytes are considered as mutualistic symbionts, because they increase survival, growth and flowering rates of their hosts, and should therefore be present at very high frequencies in all host populations. However, recent surveys have shown that 70% of wild populations of perennial ryegrass harbour such endophytes in France. Moreover, most infected populations exhibit a low level of infection. Our simple model, taking into account the life-cycles of the host and the fungus, shows that these patterns can be satisfactorily explained if the vertical transmission of the fungus is imperfect. Such imperfect transmission, though never measured in natural populations, is likely because of the reported mortality of the endophyte in stored seeds. This process, analogous to the mutation-selection balance of classical population genetics theory, may explain the observed patterns even better when we consider random fluctuations of selection coefficients over time and genetic drift.

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Infection of grass species, especially of the genus *Lolium*, by seed-borne fungi was first reported in *Lolium temulentum* by several authors at the end of the last century (Guérin 1898, Nestler 1898, Vogl 1898). Later, Sampson (1935) also found such endophytic fungi in *L. temulentum* and in *L. perenne*. These endophytes were not identified and were neglected until the discovery that they are related to mammal toxicosis caused by the fungal synthesis of alkaloids (Flechler and Harvey 1981, Bacon et al. 1986). Intensive research on endophytes followed and established that endophytes belong to the genus *Neotyphodium* (Glenn et al. 1996), formerly *Acremonium* (*Balanciae*) section *albolanosa* (Morgan-Jones and Gamms 1982). It was shown that endophytes, which probably evolved from parasitic *Epichloë*, are mutualistic symbionts (Clay 1987, 1988,

1990, Leuchtman and Clay 1990) because they enhance tolerance of their hosts to biotic (e.g. herbivorous insects or parasitic nematodes) and abiotic (e.g. drought) stresses and improve host establishment, growth, survival, tillering and seed production (see reviews by Siegel et al. (1987) and Van Heeswijck and McDonald (1992)). These fungi are maternally transmitted (vertical transmission), through seeds, while no horizontal transmission was ever reported.

Several studies investigated the distribution of seed-borne endophytes. White (1987) reported that they are common among festucoid grasses (Poaceae). Latch et al. (1987) showed that infection in *Lolium perenne* cultivars bred in Europe is low whereas several authors (Lewis and Clements 1986, Oldenburg 1994, Lewis et al. 1997) found that most wild populations of perennial

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ryegrass are infected. All the authors who examined wild populations agreed on two points: infection is widespread, in that many populations contain infected individuals, though generally the infection levels within infected wild populations are relatively low. For instance, a survey of 262 wild populations of perennial ryegrass in France (20 seeds per population) showed that the infection rate within population was lower than 0.25 in 47% of infected populations (Lewis et al. 1997). This last result is surprising for a mutualistic association.

Indeed, the beneficial effects of *Neotyphodium* endophytes on infected hosts imply that infected individuals should be favoured by natural selection. As a consequence, and given the vertical transmission of the fungus, only high infection rates should be observed within infected populations at equilibrium, i.e., host populations should be either endophyte-free because founding plants were uninfected or should be heavily infected because plants harbouring endophytes have a higher fitness than endophyte-free plants (Clay 1990, 1993). The intermediate infection rates observed in natural populations would thus reflect that the within-population level of infection has not yet reached equilibrium (Clay 1993). An argument in favour of this hypothesis comes from several studies on agricultural systems which showed that in some cases the frequency of endophyte-infected plants increased over time (Siegel et al. 1985) or that infection rates were correlated to population age (Lewis and Clements 1986).

This explanation, however, is unlikely because even relatively small selective advantages of infected plants would lead to high infection rates in relatively few generations (several tens of generations). Grass populations would have to be disturbed extremely often if non-equilibrium dynamics were the only mechanism underlying the observed patterns.

Two other mechanisms could potentially explain the intermediate infection rates. First, the effects of the endophyte could well not be beneficial in all environments (Cheplick et al. 1989). Thus in a heterogeneous environment migration-selection balance could maintain stable intermediate infection rates (Crow and Kimura 1970). An extremely pessimistic version of this hypothesis would be that the beneficial effects of endophytic infection appear only in agricultural systems, while they could be absent in wild populations. Measures of endophyte effects in wild populations are badly needed.

An alternative, and non-exclusive hypothesis, could be that the transmission of the endophyte is not perfect. This could happen either because the endophyte may not be perfectly spread to all tillers of a plant, or because it could not be transmitted to all the seeds of a given inflorescence, or finally because the fungus could not survive within the seeds (Rykard et al. 1985, Welty and Azevedo 1985, Rolston et al. 1986, Welty et al.

1987). All these processes would result in the production of non-infected seeds from infected plants. This "imperfect" transmission of the endophyte could lead to a stable equilibrium characterized by relatively few infected plants within a population. This process is analogous to the mutation-selection balance in population genetics (Crow and Kimura 1970), where selection would favour one form (here, endophyte infection) while recurrent mutation towards deleterious forms (here "imperfect" maternal transmission of the endophyte) would maintain stable polymorphism within each population. In this paper, using a simple mathematical model incorporating characteristics of the life cycle of the host plants and their endophytes, we illustrate how this process can indeed explain the intermediate (or low) frequencies of infected plants in natural populations.

A simple mathematical model

We consider the frequency of endophyte-infected (EI) and endophyte-free (EF) plants (respectively p_1 and p_2 ; in what follows subscript 1 will always refer to infected plants, while subscript 2 will refer to endophyte-free plants). We assume that the population size (N) remains constant over time and large enough that stochastic processes can be neglected. The life-cycle we considered is depicted in Fig. 1: plants flower during the summer (relative reproductive rates r_1 and r_2). After flowering some plants die (relative mortality rates d_1 and d_2). Seeds germinate in the autumn and seedlings compete for resources (relative competition coefficients w_1 and w_2). The endophyte dies in some of the infected seeds (or is not transmitted to some of them when seeds are formed) at a rate μ .

This life-cycle may seem oversimplified in that we assume no seed dormancy. However, this seems realistic because such grasses rarely show long dormancy periods. In the worst case (our simplification is wrong), the infected seeds staying in the soil for many months before germination would more likely lose their endophyte which would yield higher values of μ .

We can now write recursion equations for the change of the frequency of infected and non-infected individuals between successive generations (prime denoting frequency at generation $t + 1$):

$$p'_1 = p_1(1 - d_1) + (1/\pi)(p_1 r_1 w_1)(1 - \mu)(d_1 p_1 + d_2 p_2) \quad (1)$$

$$p'_2 = p_2(1 - d_2) + (1/\pi)(w_2)(p_2 r_2 + r_1 p_1 \mu)(d_1 p_1 + d_2 p_2) \quad (2)$$

where $\pi = w_1 r_1 p_1 (1 - \mu) + w_2 (r_2 p_2 + r_1 p_1 \mu)$. The first part of each equation represents the proportion of individuals which survive from one year to the next, while the second part represents the proportion of the population resulting from recruitment.

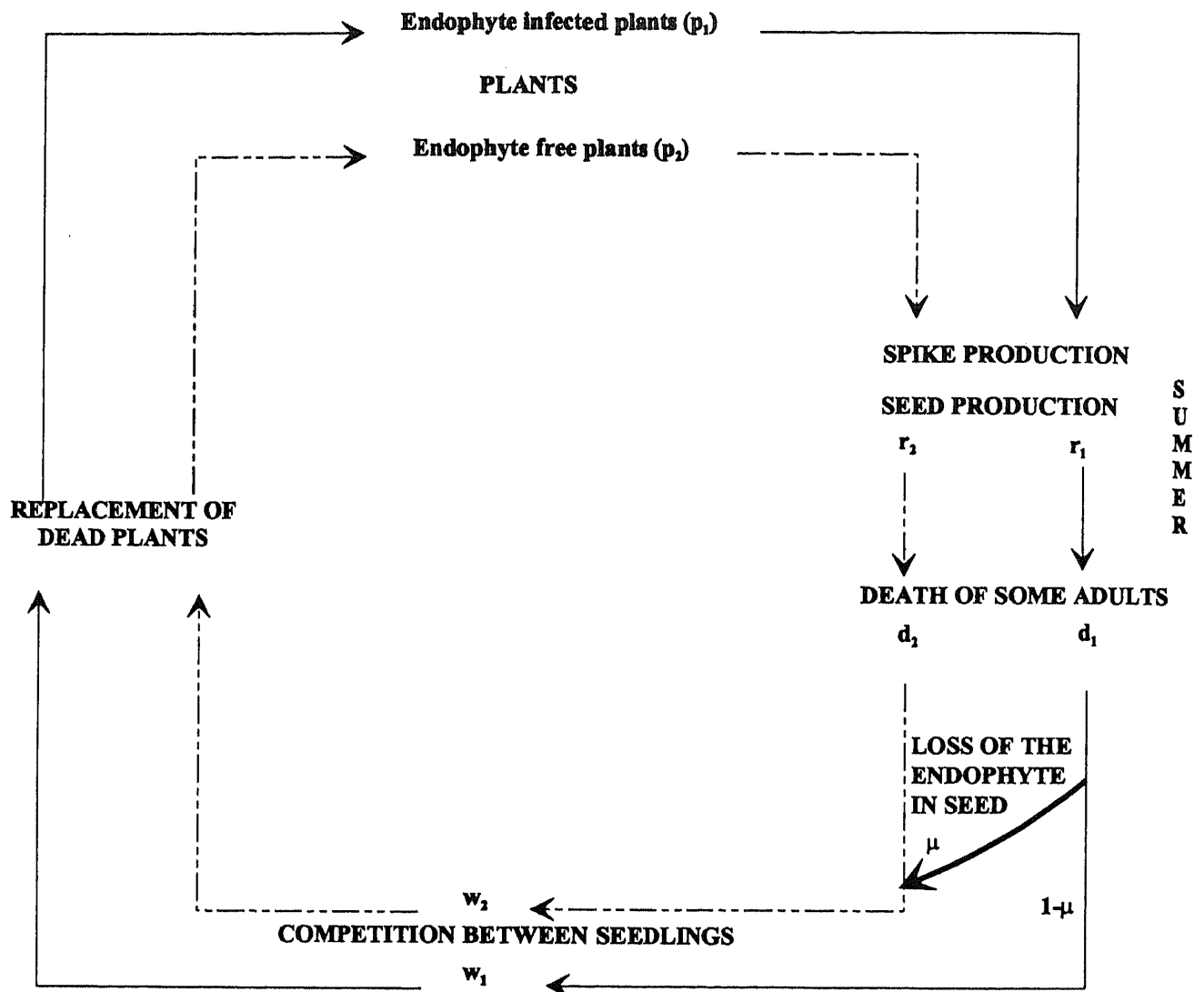


Fig. 1. Life-cycle of endophytes and host plants. Subscript 1 corresponds to endophyte-infected plants, while subscript 2 corresponds to endophyte-free plants. The relative reproductive rates of plants are r_1 and r_2 ; their relative mortality rates are d_1 and d_2 and their relative competition coefficient are w_1 and w_2 . μ corresponds to the rate of loss of the endophyte.

These recursion equations allow us to calculate the rate of change in frequency of infected individuals, Δp_1

$$\Delta p_1 = -\frac{p_1}{w} [d_1 w_2 r_2 p_2 + d_1 w_2 r_1 \mu (1 - p_2) - p_2 d_2 w_1 r_1 (1 - \mu)] \quad (3)$$

and solving this equation for $\Delta p_1 = 0$, obtain the equilibrium frequency of non-infected plants (p_2^*). There is only one non trivial equilibrium (i.e. $0 < p_2 < 1$) defined by:

$$p_2^* = \mu / [(1 - \mu)(d_2 w_1 - d_1 w_2)(1/d_1 w_2) + (1/r_1)(r_1 - r_2)] \quad (4)$$

The biological significance of eq. (4) can be better understood by defining $S_r = (r_1 - r_2)/r_1$ as the reproduction differential, $S_d = (d_2 - d_1)/d_1$ as the survival differential and $S_w = (w_1 - w_2)/w_2$ as the competition differential. We can furthermore group the survival and competition differentials because of the order in which

they occur in the life cycle to obtain $S_{dw} = S_d S_w - (S_d + S_w)$. Eq. (4) can thus be rewritten as:

$$p_2^* = \mu / [S_r - S_{dw}(1 - \mu)] \quad (5)$$

This solution is similar to the classical solution of population genetics for mutation-selection balance, and indeed reduces to it if the competition-survival differential is 0 (i.e. $p_2^* = \mu/s$, where μ is here the mutation rate to deleterious alleles and s the selective disadvantage of deleterious alleles). The differences arise from the specific hypotheses that we made on the life cycle. Whenever $p_2^* > 1$ the endophyte goes extinct in the population. When $S_r < S_{dw}(1 - \mu)$ the denominator becomes negative. As will be discussed below, these cases correspond to parameter values favouring the non-infected individuals and lead to the loss of the endophyte.

The range of biologically relevant values of the various parameters can be restricted due to the results of several experimental studies which are supposed to reflect as well as possible which could happen in nature.

$1 \geq S_r \geq 0$ (i.e. $r_1 \geq r_2$) because EI plants tend to produce more fertile tillers and seeds than EF ones (Rice et al. 1990).

$S_w \geq 0$ (i.e. $w_1 \geq w_2$) in general. Indeed, Marks et al. (1991) and Hill et al. (1991) reported that endophyte infection enhances the competitive ability of tall fescue seedlings, as well as early survival and germination rate (Clay 1990). However, opposite results were obtained with perennial ryegrass (Marks et al. 1991). Furthermore, Cheplick et al. (1989) suggested that endophytes could in some cases be "costly" to their hosts due to competition for nutrients or photosynthate. Because of these contrasting results we will consider cases where $w_1 \leq w_2$ as well (and therefore $S_w \leq 0$).

$S_d \geq 0$ ($d_1 \leq d_2$) because EI plants have a better survival rate than EF plants (Lewis and Clements 1986, Clay 1990).

In theory the upper limit of both S_w and S_d could be infinity (i.e. $w_1 \gg w_2$ and $d_1 \ll d_2$). In the following illustrations, however, we will arbitrarily limit their upper values to 1 (i.e. infected plants are at maximum twice as competitive than non-infected plants and survive at a maximum twice as much). Within these limits the compound differential of competition and survival, S_{dw} , lies within the interval -1 and 0 , and decreases as S_w and S_d increase. Since S_{dw} never admits positive values under these conditions, p_2^* is always positive.

It should also be noted that when $S_w < 0$, i.e. non-infected plants are more competitive, and $|S_d| < |S_w|$, S_{dw} becomes positive. As a consequence the denominator of eq. (5) may become negative, if $S_r < S_{dw}(1 - \mu)$. Under these parameter value combinations, non-infected plants are overall favoured by selection: they are more competitive ($S_w < 0$) while their survival and reproductive rates are not too much inferior to those of infected plants.

Fig. 2 shows the equilibrium frequency of uninfected plants as a function of S_r and S_{dw} for several values of μ . As expected the frequency of uninfected individuals increases as μ increases. Not surprisingly, it decreases as the reproduction differential increases (i.e. infected plants reproduce more than uninfected ones) and decreases as the competition-survival differential decreases (i.e. when infected plants survive better and are more competitive than non-infected plants).

The equilibrium is reached relatively rapidly, as illustrated by Fig. 3. The time necessary for the dynamics to stabilise depends on the specific values of the various parameters. For the range of parameters we used in our numerical examples the dynamics stabilise within approximately 90 to 200 yr.

Finally, we looked at how temporal variability in the values of the selection coefficients and finite population

size might affect the results. To do so, random variates of r_1 , r_2 , d_1 , d_2 , w_1 and w_2 were generated each year from a normal distribution. The probability of each individual to reproduce or to die, was determined by these coefficients. Fig. 4 shows that the results are not significantly altered by such stochastic processes. The frequencies of infected and non-infected individuals oscillate around the equilibrium values through time. As expected, when parameter values are such that the frequency of non-infected plants is high at equilibrium, the endophyte can disappear from populations of relatively small size (Fig. 4).

Discussion

Our mathematical model, despite its simplicity, captures several features of the distribution of *Neotyphodium* endophytes in natural populations of their host plants. Indeed, even though these endophytes are generally considered to have beneficial effects on major fitness components of their hosts, such as reproductive output, competitive ability and survival at various demographic stages, not only are several host plant populations not infected but generally the within-population frequency of infected plants is far lower than the expected value of 1.

Considering that the maternal transmission of the endophyte is not perfect, due to the loss of the endophyte within seeds of infected plants is sufficient to explain the observed patterns to a large degree. The value of μ , the proportion of uninfected seeds produced by an infected plant, has never been measured in natural populations. Measuring the survival of endophytes in stored seeds, Welty and Azevedo (1985), Rolston et al. (1986) and Welty et al. (1987) showed that endophyte survival in the seeds depends on their storage conditions: it is favoured by low temperatures and hygrometry. For example, Welty et al. (1987) showed that the endophyte dies in 50% of infected seeds of perennial ryegrass if seeds are stored for 18 months at 10°C and 19% relative humidity. Similarly, Rolston et al. (1986) found that less than 15% of perennial ryegrass seeds were *Neotyphodium* infected after 12 months of storage in ambient temperature at 13% seed moisture, though initially 84% of these seeds were infected. In natural conditions, seeds are produced in early summer and are generally submitted to dry and hot conditions which could kill the endophytes. Thus, the results of these studies indicate that the potential rate of "imperfect transmission" of the endophyte in natural populations could be of the order of magnitude used in the illustrations of Fig. 2.

Another biologically plausible mechanism leading to "imperfect transmission" of the endophyte, could result from the failure of the endophyte to propagate in all

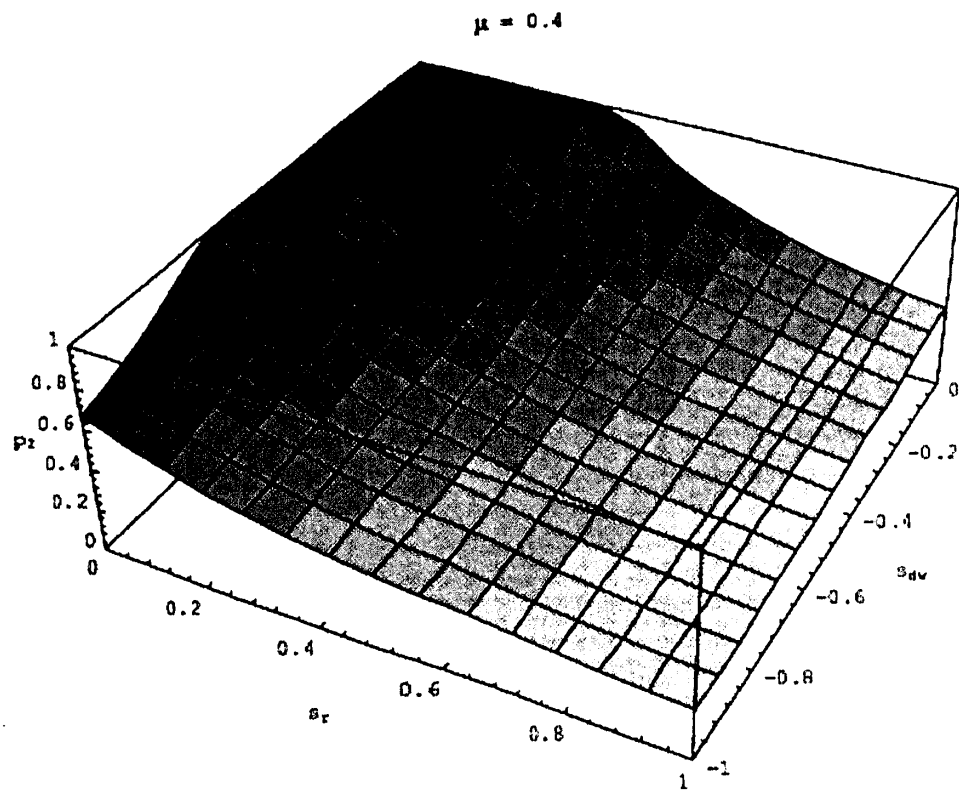
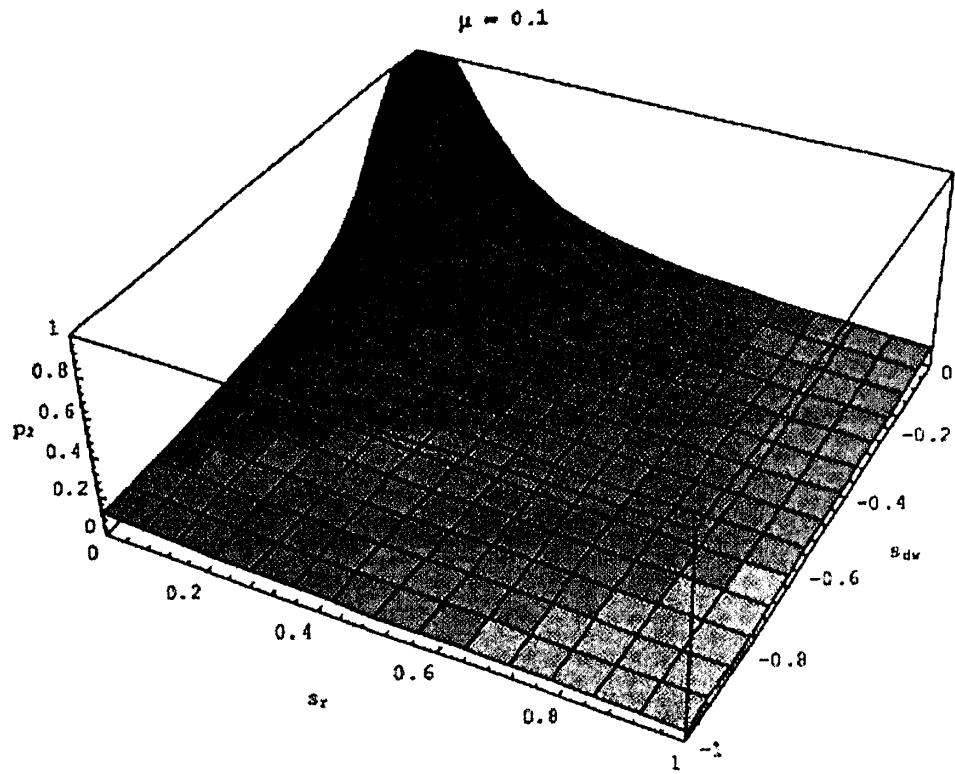


Fig. 2. Equilibrium frequency of endophyte-free plants (p_2) as a function of S_r (the reproduction differential) and S_{dw} (survival and competition differentials) for two values of μ (rate of loss of the endophyte in seed).

the tillers of a growing plant. Thus some tillers of a given plant would produce only endophyte-free seeds, while tillers infected by the endophyte would produce both kinds of seeds. This mechanism would lead to even larger rates of loss of the endophyte, and conse-

quently to lower equilibrium frequencies of endophyte-infected plants.

Adding temporal variation to the various selection coefficients results in an even better match with the observed patterns (Fig. 3). Such stochastic fluctuations

appear realistic, and result in a variance in the frequency of infected individuals around the predicted equilibrium values. Larger variation in selection coefficients or smaller population sizes could even result in the loss of the endophyte. Such a process would be equivalent to the fixation of a deleterious allele due to genetic drift. This phenomenon should not be neglected in plant breeding programs. However, its relevance in natural populations at least of ryegrass is more questionable because isozyme variability data indicate that the effective population size in ryegrass ranges from 10^3 to 10^4 individuals (Charmet et al. 1993).

Spatial variability in fitness components could contribute to explain the observed patterns. Indeed, Cheplick et al. (1989) suggested that under some circumstances infection by endophytes could incur a cost to the host. Such variation in the costs and benefits of endophyte infection across different environmental conditions could offer an alternative and complementary explanation of the observed relatively low levels of infection in natural populations. Taking spatial heterogeneity of selection coefficients into account typically dramatically increases the complexity of models. Therefore we consider it premature to develop these complex models until a clear empirical demonstration of such heterogeneity in selection is available. To this end we have initiated a long-term experiment involving water

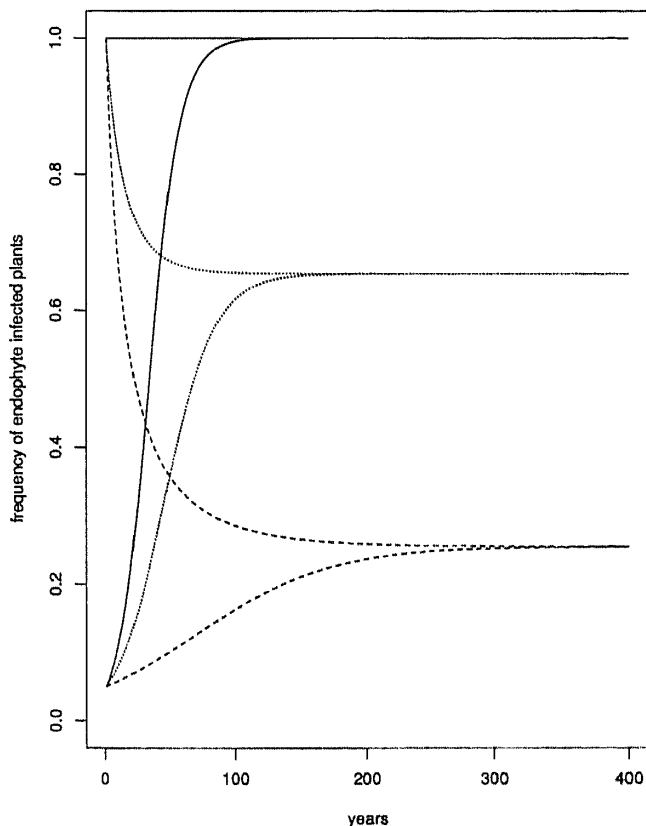


Fig. 3. Frequency of endophyte-infected plants in a population over time. Solid, dotted and dashed lines correspond to $\mu = 0, 0.1, 0.2$, respectively. In this simulation, $S_r = S_d = S_w = 0.1$.

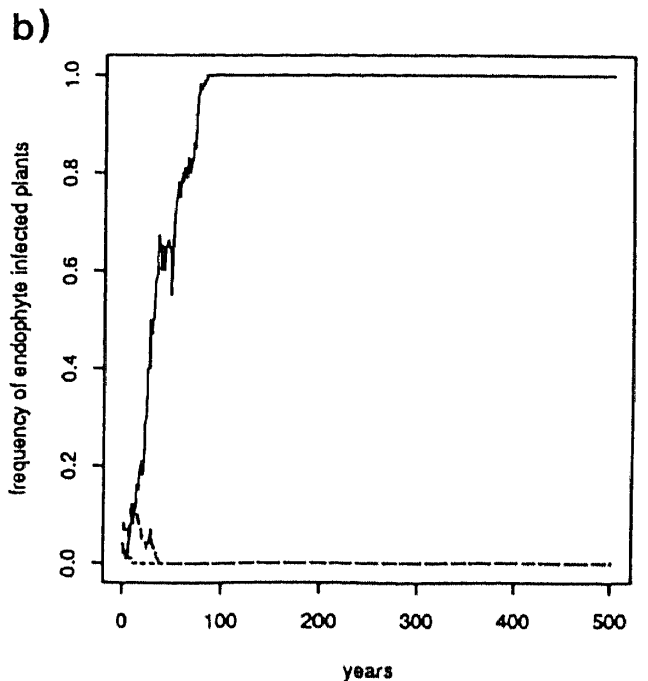
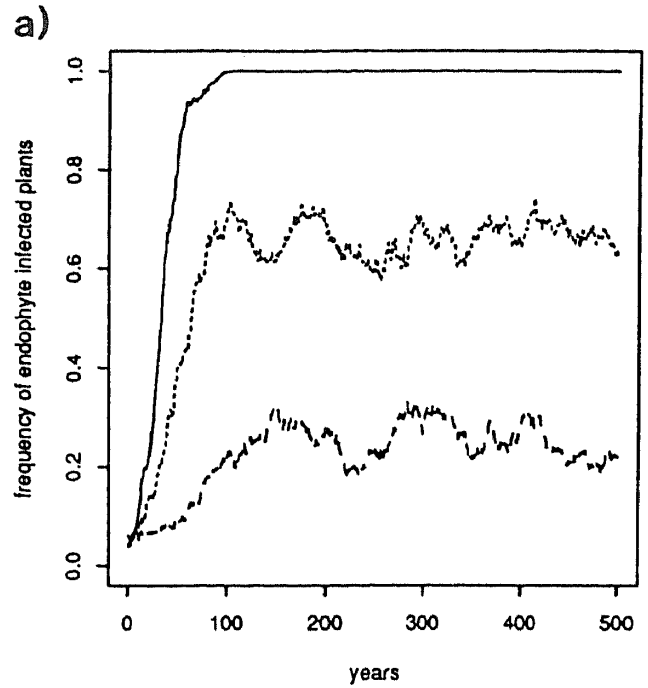


Fig. 4. Evolution of endophyte-infected plants in a population over time with stochastic processes. a) $N = 1000$, b) $N = 100$. Solid, dotted and dashed lines correspond to $\mu = 0, 0.1, 0.2$, respectively. In this simulation, $S_r = S_d = S_w = 0.1$.

availability on the relative success of infected and uninfected plants of perennial ryegrass and on the rate of loss of the endophyte from the plants. We chose a drought stress because several studies showed that such a stress could increase differences between endophyte infected and endophyte free plants (see review by West 1994).

The simple model presented here, nonetheless, explains both intermediate infection frequencies in populations that have co-occurred with *Neotyphodium* for a

long time, as well as the occurrence of uninfected populations. Imperfect vertical transmission can lead to populations stably polymorphic for infection. We hope that this result will stimulate more detailed investigations on transmission efficacy as well as on spatially heterogeneous selection.

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