

# **Neotyphodium endophyte infection frequency in annual grass populations: relative importance of mutualism and transmission efficiency**

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Persistence and ubiquity of vertically transmitted *Neotyphodium* endophytes in grass populations is puzzling because infected plants do not consistently exhibit increased fitness. Using an annual grass population model, we show that the problems for matching endophyte infection and mutualism are likely to arise from difficulties in detecting small mutualistic effects, variability in endophyte transmission efficiency and an apparent prevalence of non-equilibrium in the dynamics of infection. Although endophytes would ultimately persist only if the infection confers some fitness increase to the host plants, such an increase can be very small, as long as the transmission efficiency is sufficiently high. In addition, imperfect transmission limits effectively the equilibrium infection level if the infected plants exhibit small or large reproductive advantage. Under frequent natural conditions, the equilibrium infection level is very sensitive to small changes in transmission efficiency and host reproductive advantage, while convergence to such an equilibrium is slow. As a consequence, seed immigration and environmental fluctuation are likely to keep local infection levels away from equilibrium. Transient dynamics analysis suggests that, when driven by environmental fluctuation, infection frequency increases would often be larger than decreases. By contrast, when due to immigration, overrepresentation of infected individuals tends to vanish faster than equivalent overrepresentation of non-infected individuals.

**Keywords:** endophyte–grass symbiosis; matrix population model; infection frequency; vertical transmission

## **1. INTRODUCTION**

Symbiotic associations between microbes and animals or plants are widespread in nature and may influence both organism evolution and ecosystem functioning (Douglas 1994; Clay & Schardl 2002). Successful persistence of these associations is related to the effects microbial symbionts have on their hosts and to the way and effectiveness of their transmission (Douglas 1998; Herre *et al.* 1999; Bronstein 2001; Saikkonen *et al.* 2002; Darby & Douglas 2003). In particular, symbiotic microorganisms whose transmission is exclusively vertical (i.e. from parents to offspring) have motivated a discussion about whether they can persist without conferring reproductive advantage to their hosts (Clay 1993; Douglas 1998; Herre *et al.* 1999; Bronstein 2001; Saikkonen *et al.* 2002). Among such vertically transmitted symbionts are the endophyte fungi of the genus *Neotyphodium* (Ascomycota: Clavicipitaceae), which live in association with cool-season grasses (Siegel *et al.* 1984a; Clay & Schardl 2002; Selosse & Schardl 2007).

The main argument invoked to explain the persistence and abundance of *Neotyphodium* symbiotic fungi is the alleged mutualistic character of the endophyte–grass

interaction (Clay 1993; Clay *et al.* 2005; Selosse & Schardl 2007). This notion is based on the fact that while *Neotyphodium* fungi are obligated endosymbionts, which obtain nutrition, protection, multiplication and dispersion opportunities from the grass, infected grasses sometimes exhibit traits that might increase fitness. For example, the infected grasses have been found to contain fungal alkaloids that deter herbivores and seed predators or to exhibit higher tolerance to drought, heavy metals and herbicides, than non-infected plants (Bush *et al.* 1997; Malinowski & Belesky 2000; Vila-Aiub *et al.* 2003; Clay *et al.* 2005; Gundel *et al.* 2006). In addition, modelling the dynamics of endophyte infection as driven only by the relative fitness of infected and non-infected plants predicts that an endophyte reducing the fitness of its host would tend to go extinct (Clay 1993). The frequency of infected plants in grass populations has been often linked to the relative fitness of infected versus non-infected plants, and used as a proxy for the degree to which the endophytes enhance the fitness of their hosts (e.g. Lewis *et al.* 1997; Clay & Schardl 2002; Vila-Aiub *et al.* 2003; Jensen & Roulund 2004; Bazely *et al.* 2007; Novas *et al.* 2007).

Linking endophyte infection frequency directly with the degree of host fitness enhancement, however, contradicts both data and theory. First, relatively high infection frequencies have been observed in natural grass

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populations exhibiting little or no evidence that the fungus confers a reproductive advantage to its host (Saikkonen *et al.* 1998; Faeth & Hamilton 2006). In fact, experimental evidence of a positive effect of endophytes on the fitness of their hosts has not been consistent (Cheplick *et al.* 1989; Saikkonen *et al.* 1998; Faeth 2002; Faeth & Sullivan 2003; Cheplick 2004; Lewis 2004; Faeth & Hamilton 2006); and modelling has demonstrated that local persistence of endophyte infection should be possible in the absence of such mutualistic effects, as a result of metapopulation dynamics (Saikkonen *et al.* 2002). Second, natural populations often exhibit intermediate infection levels in contrast to modelling results showing that the frequency of infected plants in a population should equilibrate at 100% if the only driver was host fitness enhancement (Clay 1993; Ravel *et al.* 1997). These discrepancies between the observed infection levels and the relative advantage of infected plants might be accounted for by endophyte transmission failures between plant generations or by non-equilibrium dynamics (Clay 1993; Ravel *et al.* 1997; Saikkonen *et al.* 1998).

Vertical transmission of *Neotyphodium* endophytes from an infected mother plant to its offspring depends on a close connection between grass and fungus throughout their life cycles. To infect the seeds, the endophyte must produce a massive vegetative growth of hyphae into the developing embryos (Philpson & Christey 1986). Once the endophyte reaches the seeds, it has found the way to multiply and disperse, and its persistence becomes dependent on its ability to remain alive during the seminal stage of the host. After seed germination, the endophyte has to follow the development of the plant, growing hyphae into new plant tissues as they develop to finally infect the seeds during the reproductive stage (Clay & Schardl 2002). Endophyte transmission failures can either occur during pre- or post-zygotic stages of the host plant's life cycle. Pre-zygotic failure occurs when the endophyte fails to infect seedlings or colonize tillers, spikes or panicles, spikelets and ovaries during flowering (Welty *et al.* 1994; Ravel *et al.* 1997; Schulthess & Faeth 1998). Post-zygotic failure occurs when the endophyte dies in mature seeds (Rolston *et al.* 1986; Welty *et al.* 1987). Both types of failure would be affected by environmental variation (Do Valle Ribeiro 1993; Hill *et al.* 2005; Ju *et al.* 2006). When they occur, infected plants produce a proportion of non-infected offspring, influencing the dynamics of infection within the population (Ravel *et al.* 1997; Saikkonen *et al.* 2002).

In this paper, we attempt to clarify the relative importance of transmission efficiency and the increased reproductive rates of infected plants as determinants of the endophyte infection frequency in grass populations. To highlight the effects of these two drivers, we concentrate on the relatively simple case of a closed population (i.e. a population not subjected to immigration or selective emigration) of an annual grass infected with *Neotyphodium*. Our approach focuses on the fitness of the host plant together with the fitness of the endophyte fungus. Whereas the fitness of a host plant to its ecological scenario is related to the total number of seeds it produces, the fitness of the endophyte depends on the number of *infected* seeds produced by the host, which results from the product of the fecundity of the plant and the endophyte transmission efficiency. Our thesis is that the frequency of infected plants in a population is a poor indicator of the degree to

which the endophyte increases the fitness of its host, as differences in transmission efficiency would often largely account for the differences in infection frequency (cf. Ravel *et al.* 1997). To formalize our argument, we develop a mathematical model of endophyte infection dynamics in an annual grass population and derive the conditions for long-term endophyte persistence. Because environmental variation in the endophyte transmission efficiency and the relative fitness of infected plants is likely to induce relevant patterns of transient dynamics, we use our model to establish which conditions would keep the infection frequency from effectively approaching equilibrium, and we examine the patterns of transient dynamics associated with departures from equilibrium infection frequency.

## 2. DEVELOPMENT OF AN INFECTED POPULATION MODEL

We modelled the dynamics of endophyte infection in a closed annual grass population by means of a stage-based, density-independent, periodic, non-stochastic matrix model (Caswell 2001). This model assumes an environment with deterministic seasonality, in which grass and endophyte populations grow following a series of steps associated with their annual life cycle. In this model, both the grass' vital rates and the endophyte transmission efficiencies associated with all life cycle transitions of the grass remain constant from year to year. We use our model to formally analyse the asymptotic and transient dynamics of the infected plant frequency in a grass population. First, we establish how transmission efficiency and ratio of reproductive rates between non-infected and infected plants determine the equilibrium frequency of infected plants. Second, we characterize how the interplay of transmission efficiency, reproductive rate ratio and initial infection frequency drive changes in infection frequency over relatively short periods.

### (a) *Model structure: endophyte and grass population cycle coordination*

Our model mimics the life cycle of an annual grass and its endophytic fungus in a temperate climate with four well-defined seasons (figure 1a). In this model, specific grass life cycle stages and transitions occur at each season (figure 1b). Seeds (S) are produced and dispersed in summer, and germinate producing seedlings (Sd) in autumn. Seedlings develop vegetative tillers (VT) throughout the winter, and some of them flower to produce reproductive tillers (RT) in spring. Individuals at each stage are either infected ( $E^+$ ) or non-infected ( $E^-$ ). By making this distinction, this model allows the basic vital rates of the grass to vary with the endophyte infection status of the individuals. These vital rates are defined as follows: *germination* ( $G$ ), the proportion of the seeds produced in summer that produce seedlings in autumn; *tillering* ( $T$ ), the number of VT developed in winter per seedling produced in the previous autumn; *flowering* ( $B$ ), the number of RT produced in spring per VT produced in the previous winter; and *fecundity* ( $F$ ), the number of seeds produced in summer per RT produced in the previous spring. Endophyte transmission and its failure are built into the model by means of the transmission efficiencies  $t_g$ ,  $t_t$ ,  $t_b$  and  $t_f$ , respectively, defined as the fraction of all seedlings produced by

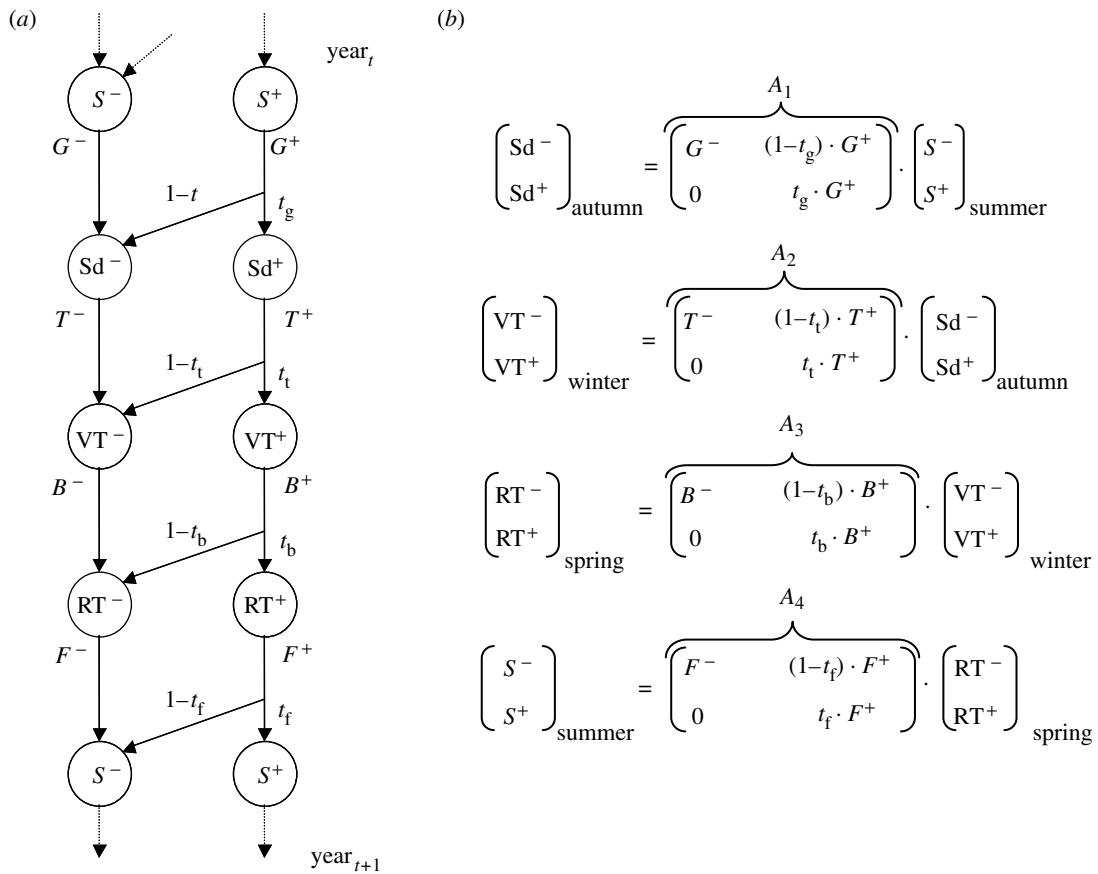


Figure 1. (a) Schematic depicting the annual dynamics of an annual grass population infected with *Neotyphodium* endophytes and (b) its corresponding seasonal transition matrices.  $S$  (seed),  $Sd$  (seedling),  $VT$  (vegetative tiller) and  $RT$  (reproductive tiller) indicate the consecutive stages of life of the annual grass population occurring every year, and  $G$  (germination),  $T$  (tillering),  $B$  (bloom) and  $F$  (fecundity) are the corresponding vital rates among consecutive stages. Each life stage is associated with a specific season of the year; thus, the vital rates connect seasons. Superscript ‘+’ and ‘-’ indicate endophyte-infected and non-infected plants, respectively.

infected seeds that are infected,  $VT$  produced by infected seedlings that are infected,  $RT$  originated from infected  $RT$  that remained infected through flowering, and seeds produced from infected  $RT$  that are infected. This model is similar in concept to the model by Ravel *et al.* (1997). In comparison, our model considers only the case of an annual grass but adds detail to its population dynamics allowing for both pre- and post-zygotic transmission failures and for non-equilibrium trends in the grass population.

In this model, vital rates and transmission efficiencies are organized into four seasonal transition matrices (figure 1b) allowing computation of the numbers of grass individuals in each life cycle stage and their infection status over successive seasons. By consecutively multiplying the four seasonal matrices, we obtain an annual transition matrix of the form

$$\mathbf{M} = r \begin{bmatrix} \phi & (1 - \tau) \\ 0 & \tau \end{bmatrix}, \tag{2.1}$$

corresponding to the annual life cycle of the infected population (figure 2). The elements of  $\mathbf{M}$  are functions of:  $r$ , the annual multiplication rates of infected individuals;  $\phi$ , the ratio between the annual multiplication rates of non-infected and infected individuals; and  $\tau$ , the annual endophyte transmission efficiency. The parameters  $r$  and  $\phi$  are non-negative, whereas  $0 \leq \tau \leq 1$  (see appendix A for expressions of  $\phi$ ,  $\tau$  and  $\mathbf{M}$  in terms of the seasonal vital

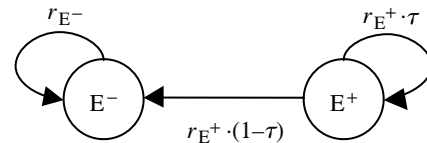


Figure 2. Schematic of the annual life cycle of an annual grass population infected with *Neotyphodium* endophytes.  $r$  represents the constant annual multiplication rates of endophyte-infected ( $E^+$ ) and non-infected ( $E^-$ ) plants and  $\tau$  represents the constant annual endophyte transmission efficiency.

rates). Matrix  $\mathbf{M}$  is upper triangular because this model does not contemplate the possibility of horizontal endophyte transmission. This model for a closed population becomes isomorphic to the model for a meta-population consisting of identical patches presented by Saikkonen *et al.* (2002).

The matrix product

$$r \begin{bmatrix} \phi & (1 - \tau) \\ 0 & \tau \end{bmatrix} \cdot \begin{bmatrix} n_1(t) \\ n_2(t) \end{bmatrix} = \begin{bmatrix} n_1(t+1) \\ n_2(t+1) \end{bmatrix}_{t+1}, \tag{2.2}$$

projects the vector with the numbers of individuals non-infected,  $n_1(t)$ , and infected,  $n_2(t)$ , over a 1-year time step. The dominant eigenvalue of  $\mathbf{M}$  gives the asymptotic annual growth rate of the grass population, and the elements of the corresponding right hand side eigenvector are proportional to the equilibrium percentages of  $E^-$  and  $E^+$  individuals.

**(b) Analysis of asymptotic dynamics**

From our model, we derived the expressions for the asymptotic growth rate and the equilibrium proportions of  $E^+$  individuals in the grass population. Based on these results, we derived the necessary condition for long-term persistence of the endophyte in the host population and the sensitivities of the equilibrium frequency of infected individuals to small changes in  $\phi$ , the ratio between the multiplication rates of non-infected and infected individuals, and in  $\tau$ , the annual endophyte transmission efficiency (see appendix A for demonstrations).

Under the assumptions of our model, the necessary condition for long-term persistence of endophyte infection is

$$\tau > \phi. \tag{2.3}$$

The annual endophyte transmission efficiency must be larger than the ratio between the multiplication rates of non-infected and infected individuals. In other words, endophyte infection tends to persist in the long term only if the annual rate at which infected individuals produce new infected individuals (i.e. the multiplication rate of the endophyte itself is higher than that of the non-infected individuals). This condition implies that, in a closed population, the endophyte can only persist in the long term if the infection results in some increase in the host-plant fitness (because  $\tau \leq 1$ ). However, such an increase could be very small ( $\phi \approx 1$ ), as long as the transmission efficiency is large enough for equation (2.3) to hold.

The equilibrium frequency of infected individuals in the grass population ( $y_{eq}$ ) is

$$y_{eq} = \begin{cases} \frac{\tau - \phi}{1 - \phi} & \text{if } \phi < \tau, \\ 0 & \text{otherwise.} \end{cases} \tag{2.4}$$

This equilibrium frequency of infected individuals does not depend on whether the grass population tends to increase, decrease or remain stable, and depends on both the annual endophyte transmission efficiency and the ratio of multiplication rates between non-infected and infected individuals.

Given  $\phi < \tau \leq 1$ , the sensitivity of the equilibrium frequency of infected individuals to small changes in  $\phi$ , the ratio between the multiplication rates of non-infected and infected individuals is given by the equation

$$\frac{\partial y_{eq}}{\partial \phi} = \frac{-(1 - \tau)}{(1 - \phi)^2}. \tag{2.5}$$

Therefore, under conditions determining long-term persistence of the endophyte ( $\phi < \tau \leq 1$ ), the equilibrium frequency of infected individuals decreases with increasing  $\phi$  at a rate which becomes faster as  $\tau$  decreases or as  $\phi$  approaches 1 (see slopes of the curves in figure 3). If the endophyte transmission efficiency is low, the relationship between the equilibrium infection levels and the ratio between the multiplication rates of non-infected and infected individuals is approximately linear. However, the higher the transmission efficiency, the more nonlinear this relationship becomes (figure 3).

The sensitivity of the equilibrium frequency of infected individuals to small changes in  $\tau$ , the annual endophyte

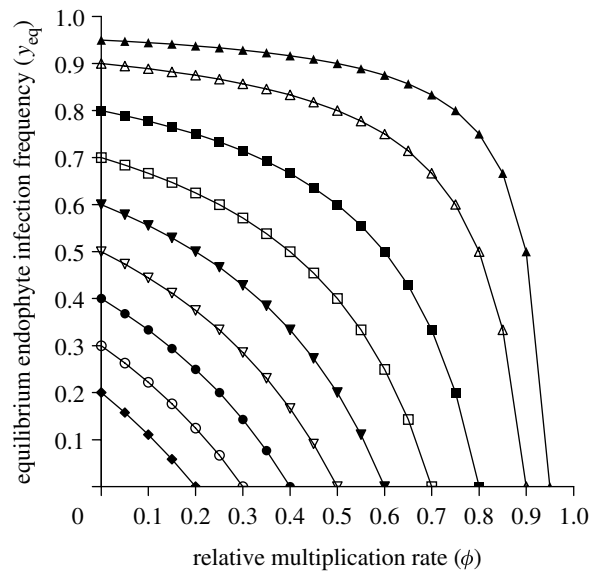


Figure 3. Relationship between equilibrium endophyte infection frequency in the population and relative multiplication rates of non-infected and infected plants ( $\phi$ ) for different endophyte transmission efficiencies ( $\tau$ ).  $\tau$  values: 0.95 (filled triangles); 0.9 (open triangles); 0.8 (filled squares); 0.7 (open squares); 0.6 (filled down triangles); 0.5 (open down triangles); 0.4 (filled circles); 0.3 (open circles); 0.2 (filled diamonds).

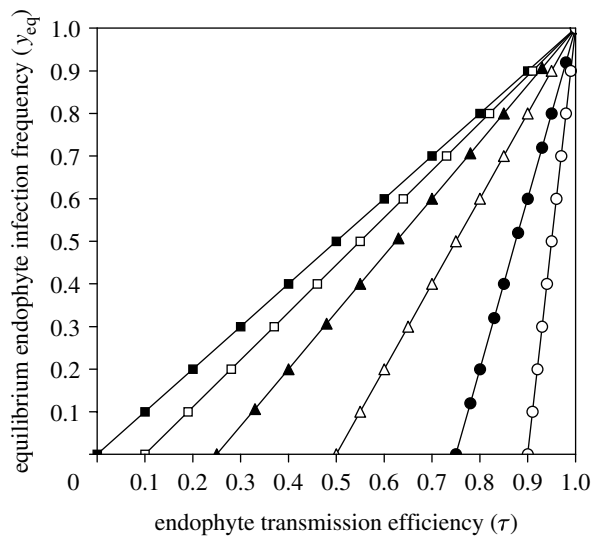


Figure 4. Relationship between equilibrium endophyte infection frequency in the population and efficiency of endophyte transmission ( $\tau$ ) for different relative multiplication rates of non-infected and infected plants ( $\phi$ ).  $\phi$  values: approximately 0 (filled squares); 0.1 (open squares); 0.25 (filled triangles); 0.5 (open triangles); 0.75 (filled circles); 0.9 (open circles).

transmission efficiency, is

$$\frac{\partial y_{eq}}{\partial \tau} = \frac{1}{1 - \phi}. \tag{2.6}$$

Therefore, given any ratio between the multiplication rates of non-infected and infected individuals (smaller than  $\tau$ ), the equilibrium endophyte infection level of the grass population is linearly related to the transmission efficiency (figure 4). The equilibrium frequency of infected individuals increases with the transmission efficiency at a rate tending to infinity as the multiplication rates of

non-infected and infected individuals tend to become equal (see slopes of lines in figure 4).

This sensitivity analysis reveals that if the endophyte has a high transmission rate and the infected grass individuals have only a small reproductive advantage (both  $\tau$  and  $\phi$  are close to 1), the equilibrium infection frequency becomes extremely sensitive to both small changes in  $\phi$  and  $\tau$  (figures 3 and 4).

(c) Analysis of transient dynamics

Based on our model, we derived formulae for the damping ratio, i.e. the rate at which the frequency of infected individuals converges to equilibrium (Caswell 2001), and for the infected plant frequency at any time after an initial time with known infection frequency  $y_0$ . Our formulae cover all possible scenarios, namely the endophyte depressing or not affecting the reproductive rate of its host ( $1 \leq \phi$ ), the endophyte enhancing the reproductive rate of its host but still tending to go extinct as a result of insufficient transmission ( $\tau \leq \phi < 1$ ) and the endophyte tending to persist in the long term as a result of mutualism and sufficient transmission ( $\phi < \tau \leq 1$ ). With these formulae, we examined the patterns of transient change in infection level, which would be expected to occur in response to departures from equilibrium associated with changes in  $y_0$ ,  $\tau$  or  $\phi$  (see appendix A for demonstrations).

Because the two eigenvalues of the transition matrix are real numbers under any setting (see appendix A), given fixed  $\phi$  and  $\tau$ , the frequency of endophyte infection always converges smoothly and without oscillations either to a positive equilibrium or to endophyte local extinction (Caswell 2001). In addition, the damping ratio  $\rho$ , defined as the quotient between the first eigenvalue and the absolute value of the second eigenvalue (Caswell 2001), is

$$\rho = \begin{cases} \frac{\tau}{\phi} & \text{if } \phi < \tau, \\ \frac{\phi}{\tau} & \text{otherwise.} \end{cases} \quad (2.7)$$

Convergence to equilibrium frequency is slower as  $\phi$  and  $\tau$  become close to each other. In particular, if both  $\tau$  and  $\phi$  are close to 1, the condition under which equilibrium infection frequency is most sensitive to small changes in  $\phi$  or  $\tau$ , convergence to such an equilibrium is slow. Under this condition, impacts of small changes in  $\phi$  or  $\tau$  and of seed immigration on the dynamics of infection should be long lasting. In addition, given  $y_0$ , moderate changes in either  $\tau$  or  $\phi$  tend to result in faster convergence to equilibrium if the new equilibrium is above  $y_0$  than if it is below  $y_0$ . Specifically, given  $y_0$  and  $\tau$  (or  $\phi$ ), the frequency of infected individuals approaches a positive equilibrium  $y_{eq} = y_0 + k$  faster than a positive equilibrium  $y_{eq} = y_0 - k$ , where  $0 < k < \min(y_0, 1 - y_0)$  (figure 5).

Under the assumptions of our model, the infection frequency at any time  $t$ , after an initial time when the grass population had an infection frequency  $y_0$ , is

$$y_t = \begin{cases} \left[ \frac{1}{y_0} + \left( \frac{1-\tau}{\tau} \right) t \right]^{-1} & \text{if } \phi = \tau, \\ \left[ \frac{1-\phi}{\tau-\phi} + \left( \frac{1}{y_0} - \frac{1-\phi}{\tau-\phi} \right) \left( \frac{\phi}{\tau} \right)^t \right]^{-1} & \text{otherwise.} \end{cases} \quad (2.8)$$

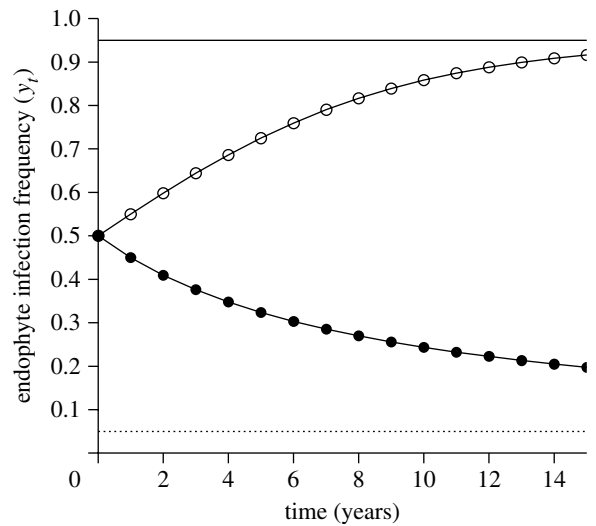


Figure 5. Transient changes in endophyte infection frequency over time, given  $y_0 = 0.5$  and  $\phi = 0.8$  towards two different equilibria, respectively, determined by  $\tau = 0.99$  (solid line) and by  $\tau = 0.81$  (dotted line). The upper equilibrium is approached faster (open circles) than the lower equilibrium (filled circles).

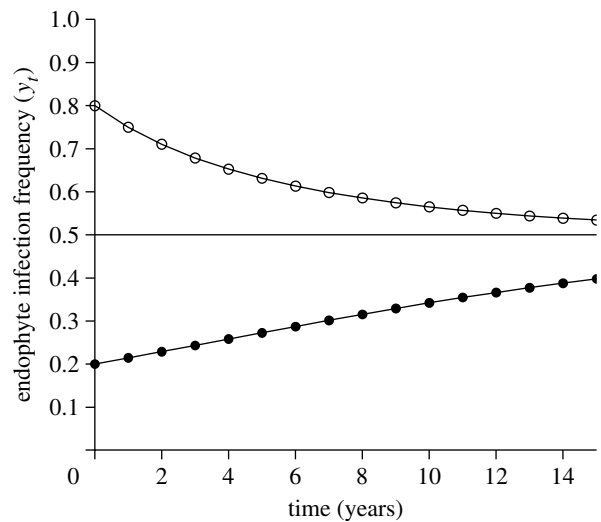


Figure 6. Transient changes in endophyte infection frequency over time given towards a single equilibrium endophyte infection frequency of 0.5 determined by  $\phi = 0.8$  and  $\tau = 0.9$  given  $y_0 = 0.8$  (open circles) and  $y_0 = 0.2$  (filled circles). In this case, the change is faster when the endophyte-infected individuals are overrepresented than when they are underrepresented.

The magnitude of transient change in infection frequency depends on the length of the interval  $t$  and on  $y_0$ ,  $\phi$  and  $\tau$  in a nonlinear idiosyncratic way. However, given  $\tau$  and  $\phi < \tau$  so that the endophyte tends to approach a positive equilibrium infection frequency  $y_{eq} > 0$ , transient changes in infection frequency tend to be larger if the initial infection frequency is above the equilibrium ( $y_{eq} < y_0$ ), and therefore decreases, than if it is below equilibrium ( $y_0 < y_{eq}$ ) and therefore increases. Specifically, given a local environment determining  $\tau$  and  $\phi < \tau$ , an overrepresentation of infected individuals decreases faster than an equivalent overrepresentation of non-infected individuals (figure 6). Therefore, arrival of non-infected seed into a local population in which  $\phi$  is smaller than  $\tau$  and the infection frequency is at equilibrium

should have a longer lasting effect on infection frequency than an equivalent arrival of infected seed. Such a difference should become apparent when the damping ratio is low.

### 3. DISCUSSION

Standard theory indicates that vertically transmitted symbionts only persist if they confer reproductive advantage to their hosts (Clay 1993; Herre *et al.* 1999; Bronstein 2001; Selosse & Schardl 2007). For *Neotyphodium* endophytes, however, compelling evidence of increased fitness of the host has been elusive despite extensive observational and experimental works (Cheplick *et al.* 1989; Saikkonen *et al.* 1998; Faeth 2002; Faeth & Sullivan 2003; Cheplick 2004; Müller & Krauss 2005; Faeth & Hamilton 2006). Confronted by the paradox of a vertically transmitted symbiont attaining very high frequency but showing no clear positive effect on its host, Saikkonen *et al.* (2002) sought an explanation not requiring the mutualistic effect and even allowing for a parasitic (negative) effect of the endophyte. They found that under quite restrictive conditions (100% transmission) metapopulation dynamics could account for the persistence of the endophyte in the absence of mutualism. In a more realistic metapopulation scenario, including imperfect transmission efficiency, the endophyte may persist locally, even in sites where it behaves as a parasite, but still requires the mutualistic effect to occur somewhere within the landscape (Saikkonen *et al.* 2002). Therefore, the question of how the endophyte persists and attains high infection levels without producing evident benefit to its host appeared to remain unresolved.

Our results suggest that the apparent paradox may in fact arise from the difficulty to detect a small effect against a noisy background. A very small mutualistic effect can account for endophyte persistence in a closed grass population, and even for a high equilibrium infection level, provided that the transmission efficiency is high enough (cf. Saikkonen *et al.* 2002). In a closed grass population, a high frequency of infected plants does not necessarily mean that they have had a large reproductive advantage over non-infected plants. Even if the endophyte increases the fitness of the host so little that the reproductive advantage of infected plants becomes undetectable, infection rate can be high as a result of high transmission rate. Conversely, if the endophyte increases substantially the fitness of the host, the equilibrium infection frequency can still be small due to diminished transmission rate (cf. Ravel *et al.* 1997; Darby & Douglas 2003). These results illustrate how the usual inferences of intensity of endophyte mutualistic effect based on observed frequencies of infected individuals are likely to be misleading (e.g. Lewis *et al.* 1997; Vila-Aiub *et al.* 2003; Jensen & Roulund 2004; Bazely *et al.* 2007; Novas *et al.* 2007).

According to our model, a high frequency of infected plants always means that the transmission efficiency has been high. The equilibrium infection frequency is effectively limited by the reproductive advantage of infected individuals only under specific conditions, namely that such a reproductive advantage is rather small ( $\phi$  is close to 1) or that the transmission efficiency is low. By contrast, imperfect transmission efficiency

always limits the equilibrium infection frequency, even if infected individuals have an overwhelming reproductive advantage (figures 3 and 4). Moreover, if the endophyte has only a small mutualistic effect, small differences in transmission efficiency translate as large differences in the equilibrium infection frequency (see also Ravel *et al.* 1997). Therefore, any existing spatial or temporal variability in transmission efficiency should be expected to drive the patterns of endophyte infection frequency.

Variability in transmission efficiency can result from different rates of either pre- or post-zygotic transmission failure. Current evidence of pre-zygotic failure is limited to observations indicating that the rates at which the endophyte colonizes young seedlings, tillers and developing seeds vary among individual plants. As a consequence, infected plants differ in the proportions of infected seeds they produce (Siegel *et al.* 1984a; Do Valle Ribeiro 1993; Welty *et al.* 1994; Schulthess & Faeth 1998; Hill *et al.* 2005; Ju *et al.* 2006). These differences among plants may be associated with genetic or environmental effects and are likely to translate as transmission efficiency differences among populations with different genetic composition or exposed to different environmental conditions. For post-zygotic transmission failure, the patterns associated with environmental conditions are well documented. The temperature and humidity to which infected seeds are exposed affect the survival of the endophyte mycelia and, therefore, depending on these environmental factors, variable proportions of viable endophyte-infected seeds produce non-infected seedlings (Siegel *et al.* 1984b; Rolston *et al.* 1986; Welty *et al.* 1987). In addition, it has been observed that infected seeds that passed through the digestive tract of steer produce substantially larger proportions of non-infected seedlings that control infected seeds (Siegel *et al.* 1984a). More recently, the differences in the timing of *Lolium perenne* seed harvest were found to result in variation in the degree of post-zygotic transmission ranging from 84.75 to 90.77 (Hill *et al.* 2005). These evidences strongly suggest that variability in transmission efficiency does occur. Our modelling results show that such a variability would account, in combination with the patterns of endophyte effect upon the fitness of the host plants, for actual differences in equilibrium endophyte infection frequency among grass populations (cf. Ravel *et al.* 1997).

Existing data suggest that the most common situations in nature would be those in which the endophyte confers very little reproductive advantage to the host plants but has high transmission efficiency (Clay & Schardl 2002; Müller & Krauss 2005; Faeth & Hamilton 2006). In such situations, convergence to equilibrium infection frequency is slow and slight modifications of either transmission efficiency or intensity of mutualism determine large changes in the equilibrium infection frequency. As a consequence, it is expected that in many populations endophyte infection frequency remains most of the time away from equilibrium and exhibits transient dynamics as a result of either environmental fluctuations or seed immigration (cf. Clay 1993; Saikkonen *et al.* 2002). Such an apparent prevalence of non-equilibrium in the dynamics of infection would contribute to mismatch between observed infection levels and current degree of fitness enhancement of endophyte-infected grasses.

Analysis of our model shows that infection frequency always tends to converge smoothly to the equilibrium determined by the current transmission efficiency and the relative fitness of infected plants. Therefore, any oscillations of infection frequency must be the result of either environmental fluctuations driving the equilibrium away from current infection levels or immigration of seeds with a different infection level than the local population. Transmission efficiency fluctuations might occur, for example, as a result of year-to-year changes in summer weather conditions. Because high temperatures and humidity increase the mortality of the endophyte in seeds, transmission efficiency would decrease when the summer is wet and hot (see Rolston et al. 1986; Welty et al. 1987). Fluctuations in reproductive advantage of infected individuals might result, for example, from year-to-year oscillations in the activity of seed predators consuming preferentially non-infected seed, and therefore transiently increasing the relative fitness of infected individuals (Bush et al. 1997; Clay & Scharld 2002). Grass-seed immigration into local populations would be critically dependent on agents of secondary dispersal such as animals, flooding water or humans, as primary dispersal distance is often rather limited (Williams & Bartholomew 2005). Our model shows that the size of transient infection frequency changes resulting from these drivers is largely idiosyncratic. Yet, environmentally driven increases in either transmission efficiency or reproductive advantage of infected plants would often drive larger changes in infection frequency than equivalent decreases in these drivers. By contrast, overrepresentations of infected individuals due to immigration of infected seed into a local population tend to vanish more rapidly than equivalent overrepresentations of non-infected individuals due to immigration of non-infected seed. This contrast suggests that high levels of endophyte infection in grass populations might be more dependent on endophyte adaptation to local environments than on long-distance connections between local populations.

Our modelling analysis of the dynamics of endophyte infection in an annual grass population yielded results that might provide insight into other cases of vertically transmitted microbial symbionts (Douglas 1998). First, although to persist in the long term, microbial symbionts must confer some fitness advantage to their hosts, an exceedingly small advantage, which may be very difficult to measure, is sufficient to maintain high rates of infection provided the rates of vertical transmission are high. Second, the infection frequency is affected by differences in the degree of reproductive advantage of infected individuals only under specific conditions, whereas imperfect transmission effectively limits infection under any circumstances. Third, environmentally driven variability in transmission efficiency is, therefore, likely to account for spatial or temporal patterns of infection level. Fourth, in local populations where the reproductive advantage of infected host individuals is small and the transmission efficiency of the symbiont is high, the infection levels are likely to be away from equilibrium and subject to transient dynamics. Fifth, any fluctuations in the population infection level are driven either by immigration or by environmental fluctuation. Sixth, environmental fluctuation and migration may have different significance for the maintenance of high levels of symbiosis.

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## APPENDIX A

### A.1 Expression of the annual transition matrix in terms of low-level parameters

Multiplication of the four seasonal matrices yields the following expression for the annual transition matrix:

$$\mathbf{M} = \begin{pmatrix} F^-B^-T^-(1-t_g)G^+ + F^-B^-(1-t_t)T^+t_gG^+ & \\ F^-B^-T^-G^- + F^-(1-t_b)B^+t_tT^+t_gG^+ + (1-t_f)F^+t_bB^+t_t & \\ 0 & \begin{matrix} \times T^+t_gG^+ \\ t_fF^+t_bB^+t_tT^+t_gG^+ \end{matrix} \end{pmatrix}$$

where  $F^-$ ,  $B^-$ ,  $T^-$  and  $G^-$  are the rates of fecundity, flowering, tillering and germination of non-infected plants or seeds, respectively;  $F^+$ ,  $B^+$ ,  $T^+$  and  $G^+$  are the rates of fecundity, flowering, tillering and germination of infected plants or seeds, respectively; and  $t_f$ ,  $t_b$ ,  $t_t$  and  $t_g$  are the respective endophyte transmission efficiencies at these four grass life-history transitions. Comparison of this expression of matrix  $\mathbf{M}$  with that in equation (2.1) yields the expressions for  $r$ ,  $\phi$  and  $\tau$  in terms of the seasonal vital rates.

The annual multiplication rate of infected seeds is

$$r = F^-B^-T^-(1-t_g)G^+ + F^-B^-(1-t_t)T^+t_gG^+ + F^-(1-t_b)B^+t_tT^+t_gG^+ + F^+t_bB^+t_tT^+t_gG^+.$$

Because the annual multiplication rate of non-infected seeds is

$$r\phi = F^-B^-T^-G^-,$$

we get

$$\phi = \frac{F^-B^-T^-G^-}{r}.$$

Finally, the annual endophyte transmission efficiency is

$$\tau = \frac{t_fF^+t_bB^+t_tT^+t_gG^+}{r}.$$

### A.2 Endophyte-persistence condition and equilibrium infection frequency

Because the annual transition matrix  $\mathbf{M}$  is upper triangular (see equation (2.1)), its eigenvalues are the elements along the diagonal. Therefore, the asymptotic growth rate of the grass population is  $\lambda = \max(r\phi, r\tau)$ . If  $\phi > \tau$ , the equilibrium number of infected individuals is zero; therefore, the condition for long-term persistence of the infection is  $\tau > \phi$  as stated in equation (2.3).

Representing the equilibrium proportions of non-infected and infected individuals in the grass population as the vector  $\begin{pmatrix} x_{eq} \\ y_{eq} \end{pmatrix}$ , we have that  $x_{eq} + y_{eq} = 1$  and

$$\mathbf{M} \begin{pmatrix} x_{eq} \\ y_{eq} \end{pmatrix} = \tau r \begin{pmatrix} x_{eq} \\ y_{eq} \end{pmatrix}.$$

Therefore, we have the following equation system:

$$\begin{cases} \phi x_{\text{eq}} + (1 - \tau)y_{\text{eq}} = \tau x_{\text{eq}}, \\ x_{\text{eq}} + y_{\text{eq}} = 1 \end{cases}$$

with solutions  $x_{\text{eq}} = 1 - y_{\text{eq}}$  and  $y_{\text{eq}} = (\tau - \phi)/(1 - \phi)$ , where  $y_{\text{eq}}$  is the equilibrium proportion of infected plants in the population as stated in equation (2.4).

**A.3 Changes in either  $\phi$  or  $\tau$  and convergence to equilibrium infection frequency**

We prove that given an initial infection frequency  $y_0$  and given  $\tau$  (or  $\phi$ ), convergence towards a positive equilibrium is faster when  $y_{\text{eq}} = y_0 + k$  than  $y_{\text{eq}} = y_0 - k$ , with  $0 < k < \min(y_0, 1 - y_0)$ .

First, consider  $y_0$  and  $\tau$  are given. In this case,  $y_{\text{eq}} = (\tau - \phi)/(1 - \phi) = y_0 + k$  implies that the ratio between the multiplication rates of non-infected and infected individuals is  $\phi = (\tau - y_0 - k)/(1 - y_0 - k) = \phi_1$ . In addition,  $y_{\text{eq}} = y_0 - k$  implies that  $\phi = (\tau - y_0 + k)/(1 - y_0 + k) = \phi_2$ . The difference  $\phi_2 - \phi_1 = 2k(1 - \tau)/(1 - y_0 + k)(1 - y_0 - k) > 0$ . Therefore,  $\phi_2 > \phi_1$  and  $(\tau/\phi_1) > (\tau/\phi_2)$ ; the damping ratio is larger for  $y_{\text{eq}} = y_0 + k$  than  $y_{\text{eq}} = y_0 - k$ .

Second, consider  $y_0$  and  $\phi$  are given. In this case,  $y_{\text{eq}} = y_0 + k$  implies that the transmission rate is  $\tau = \phi + (1 - \phi)(y_0 + k) = \tau_1$ . By contrast,  $y_{\text{eq}} = y_0 - k$  implies that  $\tau = \phi + (1 - \phi)(y_0 - k) = \tau_2$ . The difference  $\tau_1 - \tau_2 = 2k(1 - \phi) > 0$ . Therefore,  $\tau_1 > \tau_2$  and  $(\tau_1/\phi) > (\tau_2/\phi)$ ; the damping ratio is larger for  $y_{\text{eq}} = y_0 + k$  than for  $y_{\text{eq}} = y_0 - k$ . This completes the proof.

**A.4 Transient infection frequency**

Caswell (2001) showed that the vector with the number of individuals in each class of a structured population at time  $t$  is

$$\mathbf{n}(t) = \sum_k \lambda_k^t \mathbf{w}_k \mathbf{v}_k' \mathbf{n}(0)$$

where  $\lambda_k$ ,  $\mathbf{w}_k$  and  $\mathbf{v}_k'$  are the  $k$ th eigenvalue,  $k$ th right eigenvector and the  $k$ th left eigenvector of the transition matrix, respectively. In our case, application of this formula for  $\tau \neq \phi$  yields

$$\begin{aligned} \mathbf{n}(t) &= \begin{bmatrix} n_1(t) \\ n_2(t) \end{bmatrix} \\ &= \begin{bmatrix} r^t \left( n_1(0)\phi^t + n_2(0)\frac{1-\tau}{\tau-\phi}(\tau^t - \phi^t) \right) \\ r^t n_2(0)\tau^t \end{bmatrix}, \end{aligned} \tag{A 1}$$

where  $n_1(0)$  and  $n_2(0)$  are the initial numbers of non-infected and infected individuals, respectively. For the particular case in which  $\tau = \phi$ , mathematical induction proves that

$$\mathbf{M}^t = r^t \begin{pmatrix} \tau^t & t\tau^{t-1}(1 - \tau) \\ 0 & \tau^t \end{pmatrix},$$

and therefore,

$$\begin{aligned} \mathbf{n}(t) = \mathbf{M}^t \mathbf{n}(0) &= \begin{bmatrix} n_1(t) \\ n_2(t) \end{bmatrix} \\ &= \begin{bmatrix} r^t (n_1(0)\tau^t + n_2(0)t\tau^{t-1}(1 - \tau)) \\ r^t n_2(0)\tau^t \end{bmatrix}. \end{aligned} \tag{A 2}$$

The frequency of infected individuals at time  $t$  is  $y_t = (n_2(t))/(n_1(t) + n_2(t))$ . Replacing  $n_1(t)$  and  $n_2(t)$  from equations (A 1) and (A 2) and rearranging yield equation (2.8).

**A.5 Infection frequency changes**

We prove that for a local environment determining  $\tau$  and  $\phi < \tau$  so that  $y_{\text{eq}} = (\tau - \phi)/(1 - \phi) > 0$ , the transient infection frequency change resulting from  $|y_{\text{eq}} - y_0| = \Delta$  is faster if  $y_0 > y_{\text{eq}}$ , and the infection frequency decreases, than if  $y_0 < y_{\text{eq}}$ , and the infection frequency increases.

If  $y_{\text{eq}} = (\tau - \phi)/(1 - \phi) > 0$ , the infection frequency at time  $t$  can be written as

$$y_t = \frac{y_{\text{eq}}}{1 + \frac{y_{\text{eq}} - y_0}{y_0} \left(\frac{\phi}{\tau}\right)^t}.$$

Therefore, the absolute change in infection frequency is

$$|y_t - y_0| = |y_{\text{eq}} - y_0| \frac{\left\{1 - \left(\frac{\phi}{\tau}\right)^t\right\}}{1 + \frac{y_{\text{eq}} - y_0}{y_0} \left(\frac{\phi}{\tau}\right)^t}. \tag{A 3}$$

We now examine the two cases in which  $|y_{\text{eq}} - y_0| = \Delta$  with  $\Delta < y_{\text{eq}}$ . First, consider  $y_0 > y_{\text{eq}}$  so that the infection frequency decreases with time. In this case, the denominator at the left hand side of equation (A 3) is smaller than 1, and therefore  $|y_t - y_0| > \Delta\{1 - (\phi/\tau)^t\}$ . Second, consider  $y_0 < y_{\text{eq}}$  so that the infection frequency increases with time. In this case, the denominator at the left hand side of equation (A 3) is larger than 1, and therefore  $|y_t - y_0| < \Delta\{1 - (\phi/\tau)^t\}$ . This completes the proof.

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