

There are many ways to be a mutualist: Endophytic fungus reduces plant survival but increases population growth

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Abstract. One of the challenges to quantifying the costs and benefits of symbiosis is that symbionts can influence different components of host fitness. To improve understanding of the ecology of inherited symbionts, we developed general theory for a perennial host–hereditary symbiont interaction, in which symbionts can have independent and potentially opposing effects on host regeneration and survival. The model showed that negative effects on one component of fitness may be outweighed by positive effects on another, leading to a net positive impact of symbiosis on population growth. Model predictions depended on the availability of suitable patches, which influenced the relative contributions of survival vs. regeneration to host fitness. We then used experimental symbiont removal to quantify effects of a hereditary, fungal endophyte on a grass host. Endophyte presence strongly reduced host survival but increased regeneration. Application of the model revealed that negative effects on plant survival were overwhelmed by beneficial effects on regeneration, resulting in stable endophyte persistence at 100% frequency, consistent with field observations. Our work demonstrates the utility of a demographic perspective for predicting the dynamics of symbioses and supports the hypothesis that symbionts function as mutualists when host and symbiont fitness are coupled through vertical transmission.

Key words: *Cinna arundinacea; demography; fungal endophyte; herbivory; mutualism; Neotyphodium schardlii; reproductive parasite; symbiosis; vertical transmission.*

INTRODUCTION

Hereditary symbioses, which are vertically transmitted from parent to offspring, are common and important in nature. Vertical transmission was central to the evolution of chloroplasts and mitochondria (Margulis 1991, McFadden 2001), and many other microbial symbionts in animals, plants, and fungi are transmitted vertically (Bonfante and Anca 2009, Chaston and Goodrich-Blair 2010, Schardl 2011). Hereditary symbionts can supply host organisms with nutrients and novel defenses, leading to niche expansions and range shifts (e.g., Moran and Telang 1998, Stewart et al. 2005b, Nobre et al. 2010).

Hereditary symbionts vary in their reliance on alternative modes of transmission, with some symbionts spreading both vertically and contagiously and others transmitting only vertically. Exclusively vertical transmission appears to be a derived state resulting from the loss of contagious potential within horizontally transmitted lineages (e.g., Selosse and Schardl 2007, Engelstadter and Hurst 2009). The transition to vertical transmission is hypothesized to select for reduced

virulence in the symbiont and to favor the evolution of mutualism via partner fidelity feedbacks (Bull and Rice 1991, Sachs et al. 2004). Vertical transmission creates feedbacks by extending species interactions through successive host generations and stabilizes mutualisms by reducing the fitness of partners that fail to cooperate (Ewald 1987, Foster and Wenseleers 2006). Some observations support a positive relationship between the rate of vertical transmission and the strength of mutualism across host organisms (Wulff 2008, Rudgers et al. 2009). In addition, experimental tests, while rare, have shown that benefits conferred by symbionts increase when vertical transmission can occur (Sachs and Bull 2005, Stewart et al. 2005a).

Despite the expectation that exclusively vertically transmitted symbionts should evolve toward mutualism, differences in the reproductive strategies of symbionts and their hosts can generate conflicts of interest that limit or dissolve beneficial relationships. First, symbionts inherited through just one parent can increase their fitness by acting as reproductive manipulators, shifting host resources toward the sex through which they propagate. Such manipulations, manifested as male killing, cytoplasmic incompatibility, feminization of male embryos, and induction of parthenogenesis, are well documented in animals, particularly arthropods and crustaceans that host maternally transmitted *Wolbachia* bacteria (Engelstadter and Hurst 2009).

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Second, it is possible that parasitic symbionts become trapped in hosts when their contagious transmission has been lost, taking a slow path toward extinction (Faeth 2002). For example, vertically transmitted fungal endophytes (*Neotyphodium* spp.) of grasses may arise via hybridization, if chimeras form during rare co-infection events and subsequent genetic incompatibilities prevent sexual reproduction and contagious spread (Selosse and Schardl 2007). The rate of extinction of maladapted symbionts would depend on the magnitude of costs to the host and the rate of vertical transmission to offspring. The persistence of vertically transmitted parasites has also been explained by meta-population processes. For example, if the outcome of a host-symbiont interaction depends on the environmental context, then dispersal from locations where symbionts are strongly beneficial may maintain symbiosis in environments where symbionts' effects on hosts are predominantly negative (Saikkonen et al. 2002).

Understanding the ecological dynamics and evolutionary trajectories of hereditary symbionts requires careful quantification of their costs and benefits to host organisms. One of the challenges to this task is that symbionts can influence different components of host fitness (e.g., growth, survival, fecundity). Making matters more complicated, symbiont effects can vary in direction among these rates. For example, Faeth (2009) reported positive effects of vertically transmitted fungi on grass seed production, but hypothesized that symbiotic hosts may suffer reduced survival due to elevated costs of reproduction. Does this make the symbiont a mutualist or a parasite? Because not all demographic rates contribute equally to fitness or to population growth, this question is best answered within a population dynamics framework, where effects of the symbiont can be integrated over the host life cycle (Rudgers et al. 2010).

Epichloid fungal endophytes (family Clavicipitaceae) provide tractable systems for applying a population dynamics approach. These endophytes are facultative symbionts for the host plant, making them particularly amenable to experimental removal under natural conditions. In a small number of agronomic grass species, endophytes are well known for their deterrence of insect and mammalian herbivores via the production of fungal alkaloids (Schardl 2011).

Here, we develop general models to evaluate how the independent effects of symbionts on different components of host fitness translate into net effects on host population dynamics. We then integrate the general theory with empirical studies of a hereditary, hybrid fungal endophyte (*Neotyphodium schardlii* [Ghimire et al. 2010]) and its host grass (*Cinna arundinacea*) under natural conditions. We used experimental symbiont removal to ask: (1) Does endophyte symbiosis affect different components of host fitness, including survival and fecundity? (2) What mechanisms underlie the symbiont's effects on host fitness; specifically, does

endophyte symbiosis reduce herbivory, as reported for other grass-endophyte associations? Finally, we apply our general model to the *Cinna-Neotyphodium* system using field-based parameter estimates to ask: (3) Are the effects of endophyte symbiosis on components of *C. arundinacea* fitness more consistent with host-symbiont mutualism or parasitism?

METHODS: MODEL FOR POPULATION DYNAMICS OF HEREDITARY SYMBIOSIS

We developed a general demographic model to evaluate the net effects of vertically transmitted symbionts on the rate of increase of a host population. Our model builds upon that of Gundel et al. (2008), who considered hosts with a simple, annual life history. For annuals, the effect of vertically transmitted symbionts can be captured with a single parameter, fecundity, which drives host population dynamics. However, for iteroparous hosts, symbiont effects can be multidimensional and may differ in magnitude and sign among demographic rates of the host. Our model accounts for iteroparous life histories in which symbionts can independently affect survival and fecundity. We first examine host-symbiont dynamics in a nonspatial, density-independent context. Then, we ask whether and how expectations for symbiont persistence are modified when host population density is regulated by the availability of suitable space.

Model structure

Let the vector \mathbf{N}_t represent the total host population at time t , with elements E_{t-} and E_{t+} representing symbiont-free and symbiotic hosts, respectively. The population vector is updated in discrete time according to \mathbf{A} , a 2×2 transition matrix: $\mathbf{N}_{t+1} = \mathbf{A}\mathbf{N}_t$. Discrete-time dynamics are appropriate for hosts that reproduce in seasonal bouts, such as grasses. Matrix elements consist of the lower-level demographic parameters f (fecundity: new reproductive hosts/host) and s (probability that a host survives a reproductive bout), both of which are specific to symbiont status (+ or -). Note that f incorporates both propagule production and establishment processes. The rate of increase of each host type is simply $f + s$ (Charnov and Schaffer 1973). The transition matrix is

$$\mathbf{A} = \begin{bmatrix} f^- + s^- & 0 \\ 0 & f^+ + s^+ \end{bmatrix}. \quad (1)$$

Model assumptions

Our model makes several simplifying assumptions. First, demographic rates are constant in time and space, and there is no migration. Lacking information about migration rates and spatiotemporal variation in demographic parameters for the *Cinna* system, these assumptions are a reasonable place to start. In our model, horizontal transmission does not occur, and both vertical transmission and retention of symbionts by surviving hosts are perfect (100%), in which case the off-

diagonal elements of **A** are zero. The host population is structured only by symbiont status and not by size or life stage. These assumptions are appropriate for the *Cinna* system because stromata formation has not been observed (no horizontal transmission), vertical transmission was perfect, and there was little size variation in our experimental populations (see following section).

Incorporating variation in host life history

Under these simple rules, a host population closed to migration that includes both symbiont-free and symbiotic individuals will eventually be dominated by either host type at 100% frequency, depending upon which has the greater rate of increase. To isolate symbiont effects on each host demographic rate and to explicitly examine the importance of host life history variation, we define the following:

$$F = \frac{f^+}{f^-} \tag{2a}$$

$$S = \frac{s^+}{s^-} \tag{2b}$$

$$\rho = \frac{f^-}{s^-} \tag{2c}$$

Symbiont-free and symbiotic hosts experience identical rates of fecundity and survival when $F=1$ and $S=1$, respectively. The symbiont-free host life history strategy is represented by the ratio ρ . As the probability of surviving a reproductive bout becomes small, ρ approaches ∞ , corresponding to a semelparous (and, because there is no stage structure, annual) life history where population dynamics are dominated by regeneration. At the other extreme, values of ρ close to zero represent long-lived hosts for which survival probability is high but recruitment of new reproductive hosts is very rare. Note that different values for fecundity and survival rates can yield the same value of ρ ; the ratio of regeneration to survival is useful because it captures continuous variation in the relative magnitudes of these rates. Using the substitutions in Eq. 2, we solved for the conditions under which symbiotic hosts achieve a greater rate of increase than symbiont-free hosts.

Patch-dynamic model

We next allowed host population density to be regulated by the availability of suitable patches. In this spatial, density-dependent context, the demographic parameters play different dynamic roles. The production of propagules (e.g., seeds) allows hosts to capture patches that are available for regeneration, whereas survival allows hosts to retain patches already occupied. We consider an environment that contains a finite number of patches suitable for the host species, with one host individual per patch. The host population vector now consists of three patch types: occupied by a symbiont-free (E^-) host, occupied by a symbiotic (E^+)

host, or vacant. The projection matrix describes dynamic transitions among patch types, holding constant total patch number. Patches become vacant if a host in an occupied patch dies “naturally” or is killed by an extrinsic disturbance event (with symbiont-independent probability of disturbance δ). For example, in a plant population, extrinsic disturbance could include soil turnover caused by animal burrowing. Vacant patches may remain vacant if redisturbed or become occupied by E^- or E^+ hosts at rates determined by the joint probability of the patch not being redisturbed, being colonized by an E^- (or E^+) propagule, and the E^- (or E^+) colonist surviving to reproduction (see Appendix A). Thus, E^- and E^+ hosts engage in lottery competition for suitable patches. Because this version of the demographic model is nonlinear, we analyzed it by numerical simulation. We projected the patch model to obtain the frequency of each patch type at equilibrium, and we examined the influence of disturbance rate (δ) on the equilibrium frequencies. Appendix A gives further details, including the dynamic equations.

RESULTS: MODEL PREDICTIONS

We found that vertically transmitted symbionts have net positive effects on host population growth and are therefore expected to stably persist at 100% frequency when

$$F > \frac{1 - S + \rho}{\rho} \tag{3}$$

As ρ becomes very large, effects of symbionts on survival (S) become unimportant and Eq. 3 reduces to $F > 1$ (Fig. 1), which is precisely the condition for symbiont persistence in annual hosts with perfect vertical transmission (Gundel et al. 2008). As ρ approaches 0, survival becomes a more important driver of host population dynamics and Eq. 3 reduces to $S > 1$ (Fig. 1). The persistence of exclusively vertically transmitted symbionts is guaranteed when they positively affect both demographic pathways ($F > 1, S > 1$), and extinction is guaranteed when they negatively affect both ($F < 1, S < 1$) (Fig. 1). More interestingly, symbionts can also stably persist when they have negative effects on one pathway, provided that they have sufficiently compensatory positive effects on the alternate pathway. The magnitude of effect necessary for sufficient compensation is determined by the life history (ρ), which controls the slope of the symbiont persistence/extinction boundary. As the slope becomes steeper (host population dynamics driven by survival), stronger positive effects on fecundity would be required to offset a given negative effect on survival (Fig. 1). Conversely, as the slope becomes shallower (host population dynamics driven by regeneration), weaker positive effects on fecundity would be required to offset a given negative effect on survival. Thus, there are many possible combinations of positive and negative effects that can result in host-symbiont mutualism.

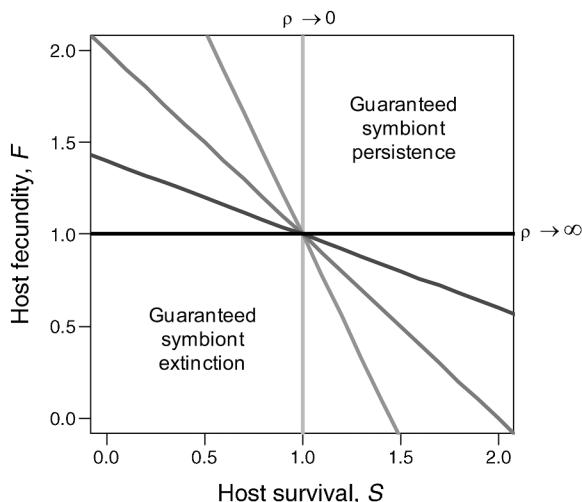


FIG. 1. Theoretical predictions for the persistence of vertically transmitted symbionts based on their effects on host survival (S) and fecundity (F) and the host life history strategy (ρ). The S axis represents the ratio of $E+$ to $E-$ survival, and the F axis represents the ratio of $E+$ to $E-$ fecundity, where $E+$ and $E-$ represent symbiotic and symbiont-free hosts, respectively. Lines separate regions of net negative (below, left) and positive (above, right) demographic impacts in which symbionts are expected to go extinct and persist, respectively. Darker line colors indicate larger values of ρ , corresponding to a more annual-like life history. As ρ increases, the symbiont extinction/persistence boundary shifts from vertical to horizontal.

In the patch-dynamic model, regardless of the disturbance rate and regardless of the host life history, occupied patches were always either 100% symbiotic or 100% nonsymbiotic when symbionts had positive effects on both host demographic pathways ($F > 1, S > 1$) or negative effects on both ($F < 1, S < 1$), respectively. Thus, when symbiont effects were consistent in sign across components of host fitness, explicit consideration of competition for space did not modify expectations relative to the simple, density-independent model.

However, when symbionts had contrasting effects across components of host fitness, patch dynamics produced outcomes that differed from the simpler model. Specifically, if symbionts increased fecundity but decreased survival, a high rate of disturbance allowed symbiotic hosts to disproportionately capture newly vacant patches and thus experience a fitness benefit over nonsymbiotic hosts (Fig. 2a). In contrast, a stable, low-disturbance environment selected for nonsymbiotic hosts, which were better able to retain occupied patches through survival, leading to symbiont extinction. Alternatively, if symbionts decreased fecundity but increased survival, stable environments favored symbiont persistence, but high-disturbance environments led to symbiont extinction (Fig. 2b). Fig. 2 shows outcomes using combinations of positive and negative symbiont effects that, based on Eq. 3, are expected to result in stable symbiont persistence. We obtained similar results with combinations of positive and

negative effects expected to lead to symbiont extinction. We present these particular examples to demonstrate that inclusion of patch dynamics and environmental perturbation *can* modify expectations for the stability of symbiosis relative to the simple, density-independent model, but it does not necessarily do so. Our numerical analyses indicated that disturbance-induced tipping points between persistence and extinction were less likely to occur as the components of fitness for symbiotic vs. nonsymbiotic hosts became more different.

METHODS: EXPERIMENTAL POPULATIONS

Study system

Cinna arundinacea (stout woodreed) occurs in mesic forest understory habitats in the USA, from Maine to Georgia and westward to Texas. It is commonly

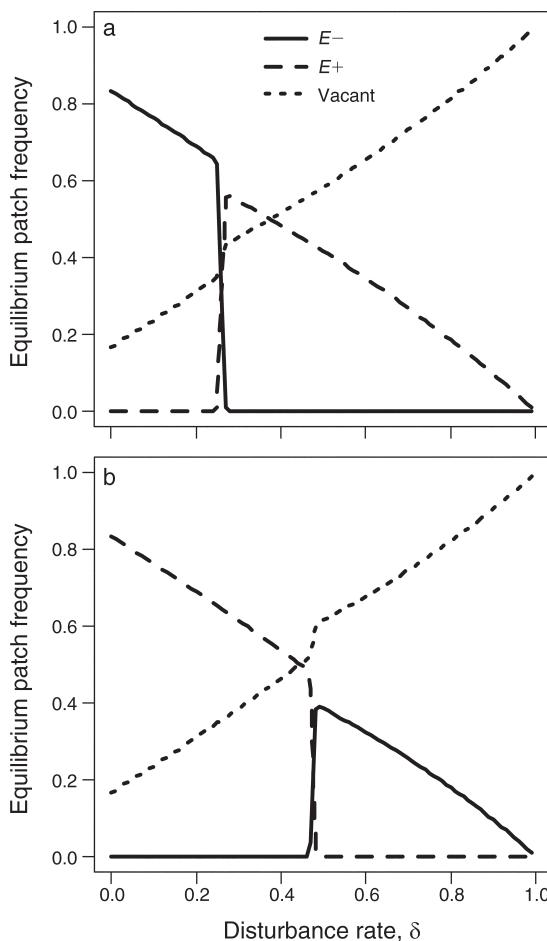


FIG. 2. Equilibrium frequencies of patches occupied by nonsymbiotic hosts ($E-$, solid line), patches occupied by symbiotic hosts ($E+$, dashed line), and vacant patches (dotted line) in relation to the symbiont-independent environmental disturbance rate (δ). In panel (a), symbionts increase fecundity but decrease survival ($F=1.36, S=0.75, \rho=1.36$). In panel (b), symbionts decrease fecundity but increase survival ($F=0.73, S=2.0, \rho=3.75$). For both panels, the nonspatial model (Eqs. 1 and 3) predicts symbiont persistence.

symbiotic with *Neotyphodium schardlii*, a fungal endophyte of hybrid origin (Ghimire et al. 2010). Seeds were collected from 30 randomly chosen plants from a 100% endophyte-infected population at the Indiana University Research and Teaching Preserve at Lilly Dickey Woods, Nashville, Indiana, USA (39°14'54" N, 86°13'05" W) on 3 October 2006. The endophyte in this population appears to be exclusively vertically transmitted through host seeds; we have never observed stromata (the sexual structures for horizontal transmission).

Endophyte removal

Seeds were heat treated for 8–14 days in a convection oven at 60°C. Both heat-treated and control seeds were surface sterilized by dipping in 50% bleach and rinsing with sterile water, then placed on 2% water agar in 10-cm petri plates for cold stratification at 4°C. After 28 d, we transferred the plates to the greenhouse (maximum temperature 25°C, minimum temperature 13°C). We transplanted emergent seedlings into 115-mL pots (Conetainers, Stuewe and Sons, Canby, Oregon, USA) filled with ProMix BX potting soil (Premier Horticulture, Quakertown, Pennsylvania, USA) and watered daily. Plants were split to produce equal-sized clones consisting of one tiller, and then returned to 115-mL pots. To evaluate endophyte status, we applied aniline blue-lactic acid stain to thin sections of the inner leaf sheath (Bacon and White 1994). Stained tissue was examined under a compound brightfield microscope at 200–400×. The heat treatment reduced endophyte frequency from 98% (sample size $n = 186$ seedlings) to 29% (sample size $n = 247$ seedlings) (log-linear model, $\chi^2 = 252.9$, $P < 0.0001$), but did not significantly reduce the probability that a seed germinated (log-linear model: $N = 894$, $\chi^2 = 2.1$, $P = 0.15$).

Field experimental design

We planted two cohorts of field plots consisting of 100% endophyte-symbiotic plants ($E+$) or 100% endophyte-free plants ($E-$, endophyte-removed treatment). Plots were located at the Lilly Dickey Woods Preserve near, but not overlapping with, sites where *C. arundinacea* occurred naturally. The first cohort was planted during 27–29 September 2007, and the second cohort was planted in a nearby location during 7–10 April 2008. Each plot (3 × 2.5 m) included 20 plants (0.5 m apart) uniquely tagged and arranged in a 4 × 5 grid. For each cohort, we planted five replicate $E+$ and five replicate $E-$ populations, for a total of 400 plants. Plots were positioned ~5 m apart to prevent seed dispersal, and the endophyte treatment was assigned to plot location at random. Within a plot, each of the 20 plants had the same endophyte status but represented a unique genotype; thus, all plots had the same level of initial genotypic diversity. Plants were added to the natural matrix of vegetation with minimal disturbance. Due to high mortality in 2007, which we suspected was caused

by deer, we fenced the second set of plots (2008 cohort) using 1.8 m tall T-posts, wrapped in plastic netting (2.5-cm openings). Thus, the two cohorts differed in the planting date and presence of fencing.

Response variables and analyses

Survival, growth, and reproduction.—We recorded plant survival on 13 September 2008 and on 15 September 2009. We counted the number of tillers per plant during the peak of growth (20–25 August 2008, 15 September 2009) and determined the proportion of tillers that produced inflorescences (5–19 October 2008, 15 September 2009). From each plant, we removed a single, randomly chosen inflorescence just prior to seed dehiscence. In the laboratory, we counted the number of spikelets per inflorescence and the proportion of filled seeds per spikelet. In *C. arundinacea*, each spikelet produces a single flower. Thus, to estimate total seed production, we multiplied the proportion of filled seeds per spikelet × the number of spikelets per inflorescence × the number of inflorescences. We included zero values for plants that were alive but failed to reproduce. Each year, collected seeds were redistributed within the plot from which they were collected.

We analyzed data from each year separately. For survival, we used a log-linear model with the fixed effect of the endophyte treatment, and the random effect of plot (nested within endophyte treatment) (Proc GENMOD, SAS Institute 2009). For growth and reproduction, we used mixed model ANOVA with the fixed effect of the endophyte treatment and the random effect of plot (Proc MIXED, SAS Institute 2009). For tiller number, analyses met model assumptions following square-root transformation. To reduce Type I error, we first examined whether the endophyte affected the total number of spikelets per plant; if significant, we then decomposed this composite measure with separate analyses of the probability that a plant flowered, the relative reproductive effort (ratio of flowering tillers to total tillers), the number of spikelets per inflorescence, and the proportion of filled seeds per spikelet. For reproduction data, the distributions of the residuals were non-normal, and normality could not be achieved through transformations. Therefore, we used distribution-free randomization tests with 10 000 iterations (Manly 1991) by encompassing mixed-model ANOVA within a SAS randomization macro (Cassell 2002, SAS Institute 2009).

Germination and recruitment.—To test germination rates, we removed a subset of three seeds from each collected inflorescence during October 2008 and 2009, retaining a minimum of 10 seeds per plot per year (total seeds $E+ = 105$, $E- = 110$). Seeds were placed on 2% water agar in 10-cm petri plates for 28 days of cold stratification at 4°C, then transferred to the greenhouse, where germination was scored weekly. Germination data were combined across the cohorts to improve the ability

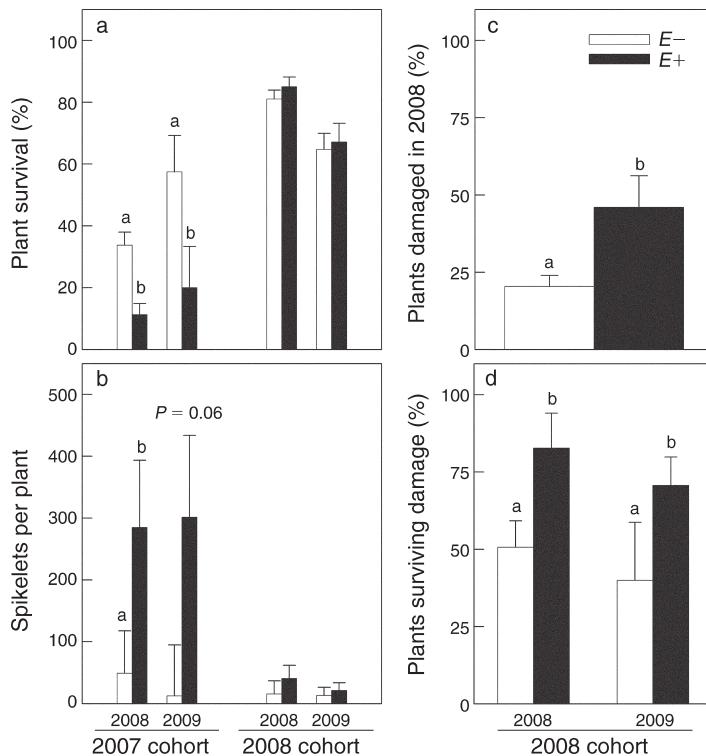


FIG. 3. Responses of the host grass (*Cinna arundinacea*) to endophyte (*Neotyphodium scharidii*) treatment (E^- or E^+) in experimental field plots planted during 2007 or 2008 (cohorts) and surveyed in years 2008 and 2009, including (a) survival of plants and (b) per-plant production of spikelets. For the 2008 cohort, panel (c) shows the percentage of plants per plot damaged by mammals, mainly voles, and panel (d) shows the percentage of plants that were damaged in 2008 and survived through 2008 and through 2009. Significant differences ($P < 0.05$) within a graph are indicated by different letters within each cohort and year, and the marginally significant P value is also shown. Bars show means + SE.

to detect an endophyte effect and because all seeds were treated under identical laboratory conditions. To assess vertical transmission rates, we examined seedlings for endophyte presence using rose bengal stain following Belanger (1996). In September 2009, we tagged all new recruits and calculated the rate of recruitment = the number of recruited seedlings \div the estimated total number of seeds produced by parent plants in the plot, adjusted for the number of seeds removed for germination trials in 2008. For germination and recruitment data, we applied randomization test equivalents of ANOVA with plot as the unit of replication.

Herbivory.—We scored the presence/absence of damage by mammals for the 2008 cohort during 13 September 2008 and 15 September 2009. Damage by small mammals was characterized by a 45° cut at the base of tillers, often accompanied by a vole burrow. Deer browse was rare during 2008–2009, but evident from shredded leaves and tillers. We used a log-linear model including a binary response (damaged/undamaged), the fixed effect of the endophyte treatment, and the random effect of plot (nested within endophyte treatment) (Proc GENMOD; SAS Institute 2009). We also examined the relationship between herbivory and plant survival using a log-linear model with the response of plant survival (alive/dead) and herbivory (damaged/undamaged) included as an independent variable along with endophyte treatment, year (2008/2009), the interaction term, and the random effect of plot.

Using field data to parameterize the models

We used the field-based demographic data for *C. arundinacea* to generate point estimates for F , S , and ρ over the 2008–2009 census interval for each cohort (Appendix B). Fecundity estimates (f) were based on the product of mean per capita seed production in 2008 and mean per capita seed recruitment probability from 2008 to 2009 (estimated from the 2007 cohort, as there was no recruitment for the 2008 cohort). Survival estimates (s) reflected the mean per-capita rate of established plant survival from 2008 to 2009. We then examined the combinations of F and S for which Eq. 3 was satisfied, given the life history values (ρ) of *C. arundinacea*. To apply the patch-dynamic version of the model, we decomposed fecundity into lower-level parameters (see Appendix A).

RESULTS: EXPERIMENTAL POPULATIONS

1. *Does endophyte symbiosis affect components of host fitness?*—Endophyte-symbiotic (E^+) plants had ~65% lower survival than endophyte-free (E^-) plants in the 2007 cohort (Fig. 3a, Appendix B). By the second year, only 20% of the symbiotic plants that survived through the first year were remaining, whereas the conditional survival rate for E^- plants was nearly 60%. In contrast, survival for the 2008 cohort was higher overall (>60%) in both years (Fig. 3a) and not significantly reduced by the endophyte (Fig. 3a, Appendix B).

There was little variation in plant size; most plants produced a single tiller, and no plants made more than

three. In the 2007 cohort, tiller number was >20% higher for $E+$ plants during the first year of growth (mean \pm SE; $E- = 1.0 \pm 0.04$ tillers per plant, $E+ = 1.22 \pm 0.07$ tillers per plant; $P = 0.011$; Appendix B) but not during the second year ($E- = 1.14 \pm 0.11$ tillers per plant, $E+ = 1.33 \pm 0.23$ tillers per plant; $P = 0.464$). In the 2008 cohort, plant size was not affected by the endophyte (2008; $E- = 1.21 \pm 0.05$ tillers per plant, $E+ = 1.15 \pm 0.05$ tillers per plant, $P = 0.438$; 2009; $E- = 1.12 \pm 0.04$ tillers per plant, $E+ = 1.09 \pm 0.04$ tillers per plant, $P = 0.636$, Appendix B).

The endophyte increased spikelet production per plant by 480–2300% in the 2007 cohort and by 60–160% in the 2008 cohort; differences were statistically significant for the 2007 cohort (Fig. 3b; Appendix B). When we decomposed this effect, the endophyte increased spikelets per inflorescence ($E- = 82.8 \pm 99.4$ spikelets per inflorescence, $E+ = 362.7 \pm 100.9$ spikelets per inflorescence, $P = 0.026$), but did not alter the probability that a plant flowered (mean percentage of plants flowering, $E- = 70.4\%$, $E+ = 66.7\%$, $\chi^2 = 0.09$, $P = 0.763$), the relative investment in inflorescences vs. tillers ($E- = 0.74 \pm 0.09$, $E+ = 0.67 \pm 0.15$, $P = 0.664$), or the proportion of seeds matured per spikelet ($E- = 0.91 \pm 0.07$, $E+ = 0.88 \pm 0.07$, $P = 0.791$).

Seedling recruitment occurred only in the 2007 cohort. The endophyte increased recruitment by 880%, but this effect was not statistically significant ($E- = 0.002 \pm 0.001$ recruits per seed, $E+ = 0.023 \pm 0.023$ recruits per seed, $P = 0.251$). The endophyte did not affect germination in the laboratory (percentage of seeds germinated, $E- = 70.6\% \pm 20.5\%$, $E+ = 77.6\% \pm 3.6\%$, $P = 0.426$). The rate of vertical transmission in laboratory-grown seedlings was 100%.

2. *Does endophyte symbiosis reduce herbivory?*—Unexpectedly, endophyte symbiosis increased the probability of damage by mammals. In the 2008 cohort, $E+$ plants were 125% more likely to be damaged than $E-$ plants (Fig. 3c, $P = 0.043$; Appendix B). Overall, plants damaged by mammals had reduced survival relative to undamaged plants ($\chi^2 = 3.84$, $P = 0.05$), suggesting a causal role for mammals in plant mortality. However, while $E+$ plants were more likely to be damaged, they also had a 60–80% higher probability of surviving damage than $E-$ plants (Fig. 3d; mammal damage \times endophyte treatment: $\chi^2 = 3.91$, $P = 0.048$). This result explains the lack of a significant endophyte effect on survival for the 2008 cohort, despite the increase in damage.

3. *Are the effects of endophyte symbiosis on components of *C. arundinacea* fitness more consistent with host-symbiont mutualism or parasitism?*—We examined the combinations of F and S for which Eq. 3 was satisfied, given the life history values (ρ) of *C. arundinacea* (Appendix C). In Fig. 4, parameter regions above the thick black lines represent net positive demographic impacts of *Neotyphodium* endophytes, and therefore predicted endophyte persistence. For the 2007 cohort, there was a strong negative effect of endophyte symbiosis

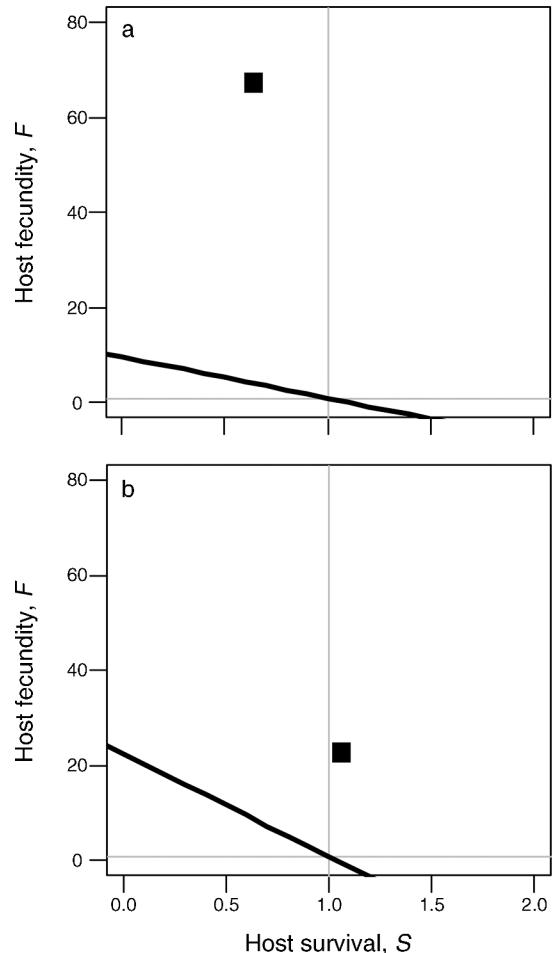


FIG. 4. Predictions for symbiont persistence or extinction using field-based parameter estimates for *Cinna arundinacea*–*Neotyphodium schardlii* (see Fig. 3): (a) cohort established in 2007; (b) cohort established in 2008. F and S axes are defined in Fig. 1. Thin gray lines separate regions of positive and negative effects of the endophyte on each host demographic rate ($F = 1$ or $S = 1$). Thick black lines show endophyte persistence/extinction boundaries (Eq. 3) based on observed life history values (ρ). Above these lines, endophytes are expected to persist with stability in host populations. Squares indicate observed combinations of S and F in experimental plots. These observations fell within a region of predicted endophyte persistence, even when endophytes had negative effects on host survival as in panel (a).

on survival (Fig. 3a). However, parameter values nonetheless fell easily within a region of expected endophyte persistence (Fig. 4a, Appendix C). The strong positive effect on fecundity more than compensated for reduced survival, producing a net positive demographic impact of symbiosis. For the 2008 cohort, endophytes tended to have beneficial effects on both survival and fecundity (Fig. 4b). Although these effects were not, individually, statistically significant (Appendix B), these plots also fell within the region of endophyte persistence (Fig. 4b).

The patch-dynamic model indicated that fecundity benefits may not outweigh survival costs of symbiosis if

the environmental disturbance rate (δ) is too low (Fig. 2). While we lack long-term information about the disturbance frequency in natural populations of *C. arundinacea*, we could determine the level of disturbance required to alter our conclusion that the combination of positive and negative endophyte effects we observed was consistent with host–symbiont mutualism and thus stable symbiont persistence. Given our field-based parameter values (Appendix A), $E+$ patches were predicted to dominate the host population at 100% frequency for all values of δ . Thus, in this particular case, the fecundity advantage was sufficiently great that symbiotic hosts were favored even in extremely low-disturbance environments.

DISCUSSION

Vertical transmission couples host and symbiont fitness and is expected to favor the evolution of mutualism due to partner fidelity feedbacks (Bull and Rice 1991, Sachs et al. 2004). Yet prior studies in native grass populations (reviewed by Cheplick and Faeth 2009) and our new results for *Cinna arundinacea* (Fig. 1) indicate that negative effects of fungal endophytes on particular components of host plant fitness are not uncommon. The persistence of vertically transmitted symbionts with negative effects on their hosts has been previously explained by nonequilibrium processes (e.g., trapped asexual parasites on a path toward extinction [Faeth 2002]), by meta-population dynamics, in which migration processes maintain parasitic symbionts in local populations where they would otherwise go extinct (Saikkonen et al. 2002), or by reproductive manipulation (e.g., by *Wolbachia* [Engelstadter and Hurst 2009]). Here, we show that vertically transmitted symbionts with negative effects on their hosts may persist stably and in the absence of migration. The key to stable persistence is that negative effects on one component of host fitness must be outweighed by positive effects on a different component; the particular balance of positive and negative effects required for symbiont persistence depends on the host life history. Furthermore, our patch dynamic model indicates that, in competitive and disturbance-prone environments, the fate of a symbiont may be influenced by whether it increases the host's ability to capture or retain space. Evaluating the net effects of a symbiont thus requires a demographic perspective that examines how independent and potentially opposing effects translate to host population dynamics. This demographic perspective can aid in classifying the outcomes of a wide variety of symbiotic interactions in which costs and benefits are manifest via different demographic pathways. For example, plant-inhabiting ants may sterilize their hosts but enhance host growth and survival (Frederickson 2009), and bacterial symbionts of insects may protect against viruses but skew offspring sex ratios to the host's detriment (e.g., Hedges et al. 2008). Our results are also relevant for vertically transmitted parasites that may confer benefits to hosts only in the presence of other

parasites (e.g., Haine et al. 2005, Jaenike and Brekke 2011, Jones et al. 2011).

The models developed here provide a starting point for evaluating the net demographic effects of symbiotic interactions and could be elaborated to include stage structure, sex structure, imperfect vertical transmission, horizontal transmission, and other biological details that may influence the conditions for stable host–symbiont mutualism. For example, when vertical transmission is imperfect, the magnitude of positive symbiont effect required to offset a negative effect and maintain symbiosis in the host population is likely to be even greater. In addition, demographic storage stages, such as nonreproductive juveniles or dormant propagules, can increase the complexity of host–symbiont dynamics by creating additional opportunities for positive (and negative) effects of symbionts (e.g., altering the probability that hosts enter or survive storage) as well as new avenues for imperfect transmission (e.g., the probability of symbiont survival during storage). These demographic effects will be the focus of future study.

Our empirical results further emphasize the importance of tracking the effects of symbiosis on multiple components of host fitness. Applying the nonspatial, density-independent model to our field data, we found that two cohorts of *Cinna arundinacea* populations benefited from symbiosis with the fungal endophyte *N. schardlii*. Specifically, in the 2007 cohort, the endophyte significantly reduced plant survival but strongly increased fecundity via the production of more spikelets per inflorescence and enhanced recruitment of new seedlings. When combined, these effects predicted long-term persistence of the endophyte, which was consistent with the high prevalence of the endophyte in naturally occurring plants (100% of plants were symbiotic). In the 2008 cohort, the endophyte had no significant effect on survival but marginally increased reproduction. Applying the 2007 recruitment rates, this analysis also predicted long-term persistence of the endophyte.

Our patch-dynamic model indicated that a high disturbance rate may be necessary for the persistence of symbionts that confer a fecundity benefit but survival cost to their host. However, applying our demographic field data, we predicted dominance of patches at 100% endophyte frequency for all rates of external disturbance. Thus, consideration of patch dynamics did not modify our conclusions for the *C. arundinacea* system. Nonetheless, our results suggest that disturbance regimes and competition for space warrant greater attention in field studies of host–symbiont interactions. For example, Faeth (2009) showed positive effects of endophytes on the fecundity of *Festuca arizonica*, but hypothesized that hosts experience reduced survival, making the endophyte a parasitic reproductive manipulator. Our patch-dynamic model suggests an alternative hypothesis: In a high-disturbance environment, a combination of fecundity benefits and survival costs may have net positive effects for both symbiont and host. Studies of host–symbiont interactions that measure or, ideally,

manipulate environmental disturbance would be valuable. In addition, while our model allowed only for lottery competition for space and thus was insensitive to the total density of patches, it may be interesting to incorporate intraspecific competition for resources, which would intensify with the density of patches.

Why did the *C. arundinacea* cohorts show different responses to endophyte symbiosis? Survival was strongly reduced by endophyte presence for the 2007 cohort, but unaffected in the 2008 cohort. We suggest two possible causes for this difference. First, planting during the fall may have exposed the 2007 cohort to winter weather that increased the mortality of newly planted, symbiotic plants. For example, a study of naturally symbiotic and symbiont-free *Lolium pratense* (meadow fescue) in the subarctic suggested weaker benefits of a *Neotyphodium* endophyte when the duration of snow cover was longer (Wali et al. 2008). Second, the 2007 cohort was not protected from mammalian herbivores, particularly deer, which may have preferred symbiotic plants, explaining their higher mortality. Small mammals (*Microtus* spp.) were not likely responsible for differences between the cohorts because the fenced 2008 cohort experienced significant vole herbivory (Fig. 2). Perhaps the 2007 cohort was less able to recover from herbivory than the 2008 cohort, thereby explaining the higher mortality of symbiotic plants.

What mechanisms underlie the effects of endophyte symbiosis on *C. arundinacea*? First, we suggest that production of peramine, which has been linked to reduced insect herbivory in other grass species (Bush et al. 1997), may increase reproduction by symbiotic *C. arundinacea*. Consistent with this hypothesis, profiling of alkaloid biosynthetic genes from 23 isolates of *N. schardlii* (including isolates from our population) revealed the presence of the peramine biosynthetic gene (*perA*) (Ghimire et al. 2010). In another study, we found reduced insect preference for and reduced damage on *E+* relative to *E-* *C. arundinacea* in both the laboratory and the field (Crawford et al. 2010). Second, our results suggest that endophytes could mediate tolerance to mammalian herbivores: In the 2008 cohort, *E+* plants were more likely to survive consumption by mammals than were *E-* plants. Small mammals may prefer symbiotic plants because of their larger size or increased nutritional value. For example, the endophyte in tall fescue grass can increase plant nitrogen content (Lyons et al. 1990). A lack of resistance to mammals is not surprising given that *N. schardlii* lacks genes for the production of ergot alkaloids and indole-diterpenes, the primary mammalian toxins produced by grass endophytes (Ghimire et al. 2010). Experiments imposing a fixed amount of damage to plants would be necessary to confirm this tolerance hypothesis. Tolerance traits, by mitigating the costs of interactions, may be central to the transition from parasitism to mutualism, and ultimately to the stability of mutualisms (Edwards 2009).

In summary, a population dynamics perspective that explicitly considers separate components of host fitness reveals many ways in which symbionts can function as mutualists. As we have shown with the *Cinna-Neotyphodium* interaction, even when symbionts have negative effects on one host demographic pathway (survival), this can be overwhelmed by positive effects on others (seed production and recruitment). Our empirical results confirm that integrating potentially opposing effects of symbionts over the host life cycle and accounting for differences among demographic rates in their contribution to population growth provide an improved method for classifying species interactions as mutualistic, commensal or parasitic.

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SUPPLEMENTAL MATERIAL

Appendix A

Further details on the construction and analysis of the patch model of host–symbiont dynamics (*Ecological Archives* E093-050-A1).

Appendix B

A table showing results from statistical analyses of the responses of *Cinna arundinacea* to the endophyte treatment in experimental field plots (*Ecological Archives* E093-050-A2).

Appendix C

A table of parameters and empirical point estimates for the host–symbiont population dynamics of *Cinna arundinacea*–*Neotyphodium schardlii* (*Ecological Archives* E093-050-A3).