Plant pathogens drive density-dependent seedling mortality in a tropical tree

Abstract

One explanation for the extraordinary diversity of tropical forest trees is that density-dependent mortality from herbivores or pathogens puts locally rare species at an advantage. Density-dependent mortality of seeds and small seedlings is particularly intense in tropical forests, but its causes remain uncertain. Here, we show experimentally that pathogens from the Oomycota are associated with intense mortality in seedlings of a neotropical tree, *Sebastiana longicuspis*. Seedlings in untreated plots experienced eight times higher mortality compared with seedlings in plots treated with fungicide. Mortality was strongly density dependent: in fungicide-treated plots survival was unaffected by density, but survival in unsprayed plots was over three times higher at low density. Density-dependent mortality observed in a simultaneous, non-manipulative study was highly transient, suggesting that short-term observational studies may underestimate the intensity and form of pathogen-induced mortality. If such effects are widespread, plant pathogens may play a key role in maintaining and structuring tropical diversity.

Keywords


INTRODUCTION

A single hectare of humid tropical forest can support over 300 tree species (Valencia et al. 1994), but the processes that allow so many species to coexist remain unclear (Givnish 1999; Wright 2002). One possibility is that density-dependent mortality puts locally rare species at an advantage, preventing anyone species from reaching high abundance (Janzen 1970; Connell 1971; Harms et al. 2000). Recent studies have provided strong evidence that density-dependent processes play a role in the maintenance of plant diversity in the tropics (Gilbert et al. 1994; Wills et al. 1997; Wills & Condit 1999; Harms et al. 2000; Peters 2003), and more widely (Lambers et al. 2002; Lambers & Clark 2003), and that the seed-to-seedling transition is a critical stage in determining the diversity of plants in larger size classes (Harms et al. 2000; Lambers et al. 2002; Connell et al. 2005; Wright et al. 2005). The precise causes of density dependence remain less certain.

Density-dependent mortality and growth can be generated by intraspecific competition and by the action of specialized natural enemies (herbivores and pathogens). Observational studies are consistent with the hypothesis that natural enemies drive density dependence, with seeds and seedlings thought to occur at insufficiently high abundance for competition to generate the patterns observed (Wright 2002). Insect herbivores and seed predators, mammal herbivores and plant pathogens have all been suggested as possible causes of density dependence, but data confirming their role are lacking. Pathogens, particularly fungus-like oomycetes which cause 'damping off' disease in small seedlings, have long been suspected as agents of density-dependent mortality in tropical forests (Augspurger 1984; Augspurger & Kelly 1984; Augspurger & Kitajima 1992; Dalling et al. 1998; Gilbert 2002; Hood et al. 2004; Gilbert 2005), and there is growing evidence for density-dependent effects of plant pathogens in temperate ecosystems (e.g. Klinkomoos 2002; Packer & Clay 2000; Reinhart et al. 2003). However, the impact of plant pathogens may have been underestimated or misrepresented in previous observational studies where confounding effects cannot be excluded. For example, resource competition at high densities may make
individuals more prone to attack by pathogens, so pathogens may be a symptom rather than a cause of density dependence (Burdon 1982; Dobson & Crawley 1994). In contrast, experimental manipulations of both density and pathogen levels offer an unambiguous demonstration of the underlying interactions.

Here, we describe a manipulative field experiment in moist tropical forest in Belize, Central America, which demonstrates a key role for plant pathogens in inflicting density-dependent mortality of newly germinated seedlings. We simultaneously conducted an observational study on the same system and show how the observational approach would have underestimated or failed to identify the nature of the interaction between density and pathogen-induced mortality.

**MATERIALS AND METHODS**

**Study site**

The field site was in moist tropical forest near the Las Cuevas Research Station in the 170,000 ha Chiquibul Forest Reserve, Cayo District, in south-west Belize. This forest is classified as deciduous forest and deciduous/semievergreen forest (Wright et al. 1959). The climate is seasonal, with a humid season typically from June to January and a dry season from February to May. The Chiquibul Forest is affected by hurricanes, and much of the vegetation in the study area has grown up following extensive wind and fire damage resulting from Hurricane Hattie in 1961.

**Fungal exclusion experiment**

We treated plots containing germinating seedlings of a locally common neotropical tree, *Sebastiana longicuspis* Standl. (Euphorbiaceae), with a selective fungicide, Ridomil Gold® (Syngenta Ltd, Basel, Switzerland). This metalaxyl-based, systemic fungicide is targeted at fungus-like pathogens in the Oomycota, including species of *Pythium* and *Phytophthora*, which cause damping-off diseases in young seedlings (Cohen & Coffey 1986). This fungicide has low toxicity to organisms other than fungi, and has been found to have minimal inhibitory effects on arbuscular mycorrhizae in agricultural systems (e.g. Afek et al. 1990; Seymour et al. 1994). The experiment comprised six 1 m² blocks, each divided into four 25 × 25-cm plots. The location of each block was selected to maximize the number of newly germinated (cotyledon stage) *S. longicuspis* seedlings. All blocks were within 5 m of each other, and were immediately below a cluster of mature *S. longicuspis* trees.

Density (high or low) and fungicide (sprayed or control) were allocated at random to each plot, so each of the four treatment combinations was represented once in each block. In low-density plots, we manipulated the initial density of seedlings to 25 per plot (100 individuals m⁻²) by hand thinning. In high-density treatments, we left seedling density unmanipulated; seedling density in these plots ranged from 416 to 1068 individuals m⁻². Fungicide was applied using a hand-held mister at the manufacturer's recommended concentration of 0.25 g m⁻², with 50 mL of solution applied to each 0.0625-m² plot. Control plots were treated with an identical volume of water. The fungicide treatment was applied weekly from 3 June to 8 July 2005. At the initial census, each seedling was labelled individually. Newly germinating seedlings were counted and marked each week, and the number of seedling fatalities recorded.

The proportion of seedlings dying over the course of the experiment was analysed using a generalised linear model in which block, density, fungicide and the interaction between density and fungicide were entered as fixed factors, assuming a binomial error distribution and logit-link function. The initial model exhibited overdispersion (dispersion coefficient = 1.95). We found that inclusion of a block × fungicide term reduced the over-dispersion to an acceptable level (dispersion coefficient = 0.90).

**Observational survey**

Each week for 4 weeks (10 June to 8 July 2005), we recorded natural *S. longicuspis* density and frequency of infection with damping-off pathogens in 40–50 randomly located 1 m² quadrats (different quadrats each week). Infected seedlings were characterized by obvious necrosis of the stems, and always died in the week that the infection became visible. The proportion of seedlings infected was analysed using a generalised linear mixed model, assuming a binomial error distribution and logit-link function. Week of observation (coded 1–4) was entered as a random effect, and density nested within week was entered as a covariate.

**RESULTS**

**Fungal exclusion experiment**

We found a strong fungicide treatment effect after 5 weeks, with seedlings in untreated plots experiencing eight times the mortality of seedlings in plots treated with fungicide (deviance explained = 655, $P < 0.0001$, d.f. = 1). This shows that pathogens are a key determinant of survival in this species. A high significant density × fungicide interaction term (deviance = 9.53, $P < 0.0001$, d.f. = 1) indicates that the effect of density on mortality is dependent on whether fungicide is applied. Plots sprayed with fungicides showed no significant difference in survival between density treatments.
In contrast, survival was over three times higher in the unsprayed low-density plots compared with the unsprayed high-density plots (Fig. 1).

**Observational survey**

In the non-manipulative survey there was a significant overall effect of density \( (F = 7.16, \text{ d.f.} = 4,182, P < 0.0001) \). However, examination of the model coefficients for the individual weekly surveys indicated that only the first survey yielded a strong relationship between density and pathogen infection \( (t = 4.53, \text{ d.f.} = 182, P < 0.0001) \), with the third yielding a weaker relationship \( (t = 2.44, \text{ d.f.} = 182, P < 0.05) \) and the other two no significant relationship \( (t = 1.54, \text{ d.f.} = 182, P = \text{NS}; t = 1.24, \text{ d.f.} = 182, P = \text{n.s.}) \) (Fig. 2).

**DISCUSSION**

Our results demonstrate that fungal pathogens inflict severe mortality on young seedlings of *S. longicuspis*, and that this mortality is clearly density dependent. The experiment is unique in manipulating both plant pathogen abundance and seedling density *in situ* in a tropical forest, and provides direct evidence for the role of plant pathogens as agents of density-dependent mortality in this species. The approach that we have used - simultaneous experimental manipulation of both pathogens and density in the field - may be useful in determining the role of pathogens in the dynamics of tree populations and communities elsewhere in the tropics, and also in temperate ecosystems where plant pathogens may have a large but cryptic influence on the diversity and structure of ecological communities (e.g. Van der Putten *et al.* 1993; Klironomos 2002; Packer & Clay 2000; Reinhart *et al.* 2003).

Previous studies of pathogen infection in relation to plant density in tropical forests have found mixed results.

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**Figure 1** Proportion of *Sebastiana longicuspis* surviving in an experiment that manipulated seedling density and fungal pathogens. Each data point is the mean of six replicates, and the error bars show standard errors.

**Figure 2** Relationship between the proportion of *Sebastiana longicuspis* seedlings unaffected by fungal pathogens and the density of seedlings, censused at weekly intervals. Each data point is a census from a 1 m\(^2\) quadrat.
with distance from parent tree, and variable results among
moisture and light conditions (Augspurger 1984; Augspurger
is the interaction with pathogen incidence that is of
importance, and only manipulative experiments can fully
reveal the nature of this interaction. For example, our
observational study provided at best an uncertain picture
of the influence of seedling density on survival. In large
part this was because high mortality in high-density
patches resulted in the relationship between density and
infection weakening through time (Fig. 2), as seedling
densities were thinned rapidly to a level at which further
mortality from pathogens was minimal.

Density-dependent mortality affecting seeds and small
seedlings may have been overlooked in most previous plot-
based studies in tropical forests, which focus on plants in
larger size categories (Harms et al. 2000). We now need to
identify how widespread such effects are across a diverse
distinct of tropical and temperate plant species. The hypothesis
that density-dependent mortality generates the latitudinal
gradient in plant species richness requires that tropical
forests experience more intense or more widespread
density-dependent mortality that temperate forests (Lambers
et al., 2002), and it seems unlikely that this issue will be
resolved until there have been experimental manipulations
quantifying the strength of density-dependent mortality
using several species in both temperate and tropical
ecosystems.

Our experiment was carried out at high but natural
densities of seedlings. A novel secondary result from our
work is that, in the absence of pathogens, seedling
survivability was uniform (around 50%) over the course of the
experiment, with apparently no direct effects of
seeding density on survivorship. In fungicide-treated plots,
mortality was similar whether seedlings were at high (416-
1068 individuals m-2) or low (100 individuals m-2) density.
This result supports the contention that competition
between seedlings for resources is unlikely to be a major
factor contributing to seedling mortality in tropical forests,
at least in the initial stages of growth (Wright 2002), and
indicates that density-dependent mortality from other
categories of plant natural enemy such as insect herbivores
did not occur during the experiment. The impact of
pathogens on plants may be highly dependent on local soil
moisture and light conditions (Augspurger 1984; Augspurger
& Kelly 1984; O’Hanlon-Manners & Kotanen 2004), perhaps
explaining the significant block effects observed in our
experiment.

An important but unknown factor is the degree of host
specificity of the pathogens causing mortality in S. langsdorffii
and other tropical trees (Qarosz & Davelos 1995). Density
dependence is only expected to have a strong diversity-
Enhancing role when pathogens show high specificity
(Qarosz 1970; Connell 1971). Pythium species known from
agricultural systems typically have low host specificity, while
Phytophthora can be more specialized (Augspurger 1990); but
data from tropical forest environments are lacking. Molecul-
ar and experimental approaches will now be required to
measure the specificity of pathogen species and strains
isolated from seedlings of individual tropical tree species,
and to disentangle the correlated effects of seedling density
and distance from mature conspecific and heterospecific
(Augspurger 1990; Hood et al., 2004). Oomycete
pathogens typically have steep dispersal curves, so that
their incidence is highly heterogeneous over small spatial
grades (Augspurger 1990). Host-specific oomycete
pathogens may build up high inoculum levels in the soil around
parent trees through the input of susceptible seeds and
seedlings whereas they typically exist at low density or
heterogeneously in the soil further from conspecifics

Pathogen host specificity will also be of interest in the
context of wider community composition. If pathogens are
oligophagous at higher taxonomic levels, for example,
infesting con familial plant species (Gentry 1988), density-
mediated indirect effects such as apparent competition (Holt
1977; Connell 1990; Holah & Alexander 1999) among plant
taxa may structure these communities. For example,
pathogen-mediated indirect effects may reduce the like-
lihood that trees from the same family (which are more
likely to share pathogens) rectuit in close proximity. In a
recent study of sapling mortality on Barro Colorado Island,
Panama, 11 of 60 species showed reduced growth in proximity to con familial and congeneric species (Uriarte
et al., 2004). It remains to be determined whether a similar or more pronounced trend occurs in small seedlings, whether
mortality as well as growth is affected, and whether shared
pathogens are driving such effects.

If intense density-dependent pathogen-mediated mortality
of seedlings is widespread, plant pathogens may play a key
role in maintaining and structuring tropical diversity.
However, it will be important to understand the degree to
which seed and seedling dynamics translate into differences
in adult tree composition. This is likely to be influenced by
longer-term interactions among larger seedlings and sap-
lings, and on the outcome of competition for gaps (Denslow
1987). Long-term plot-based studies are thus required to
confirm whether ecological processes involving small
seedlings operating under a closed canopy determine the
template for the next generation of mature trees (e.g.
Connell et al., 2005).
ACKNOWLEDGEMENTS

This study was funded by the UK Natural Environment Research Council (Grant, NE/C515063) with research permission from the Ministry for Natural Resources, Government of Belize. We thank Augustine Howe and the staff of the Las Cuevas Research Station for assistance with fieldwork, Greg Gilbert, Sarah Gur, and Alien Herre for advice on pathogen biology, and the anonymous referee for their helpful comments. OTL and RPF are Royal Society University Research Fellows.

REFERENCES


