Disease ontogeny overshadows effects of climate and species interactions on population dynamics in a nonnative forest disease complex

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ABSTRACT

Biotic threats to trees often arise from interactions among two or more species, frequently insects and fungi, that function together to defeat host defenses, secure resources and colonize new hosts. Feedbacks among plant enemies can have large effects on host population and disease dynamics, either by promoting stabilizing negative feedbacks or contributing to positive feedbacks that can destabilize populations and permit outbreaks. Feedbacks can be rapid and direct (e.g., within trees or among years) or can arise from slowly developing changes in host resource quantity or quality at the scale of forest stands or landscapes. Climate may also influence system dynamics by altering feedbacks within or among species or through density independent effects. We evaluated major drivers of population dynamics of beech bark disease (BBD), an important forest disease in eastern deciduous forests of North America, using data from 28 study sites in the eastern United States monitored for up to 14 years between 1979 and 1992. Both primary causal agents of BBD – the introduced felted beech scale (*Cryptococcus fagisuga*) and native fungi (*Neonectria* spp.) – showed strong simple density dependence in all study populations. Surprisingly, densities of scale insects and fungi had little or no effects on population growth rates of the other, despite their habit of living in close physical relationships. For both insects and fungi, ecologically important features of the density dependent functions (slope, carrying capacity and density independent variance) were variable across sites. Climatic effects on density-dependent functions (and scatter around them) were evident but generally weak and variable. The most striking predictor of patterns in density dependence was duration since establishment of BBD in the region. Apparently BBD alters forests over decades in ways that strengthen self-regulation among causal agents without eliminating or even dramatically reducing host populations.

Keywords: density dependence | climate | biotic feedbacks | beech bark disease | insect-fungal interactions

INTRODUCTION

The global transfer of pests and pathogens has led to numerous devastating examples of forest disease outbreaks in the United States and worldwide, including Chestnut blight, Dutch elm disease, and pine pitch canker, among others (Brasier 1991, Storer et al. 1997, Paillet 2002). Despite comprehensive efforts to limit their introduction and spread, rates of establishment of exotic insects and pathogens have been increasing nearly exponentially for 200 years (Liebhold et al. 1995) and pose increasing threats to forests worldwide (Seppälä et al. 2009). On the other hand, notable pests are only a subset of the many forest organisms that have become established in novel ecosystems following recent introductions and range extensions. Apparently, ecological controls regularly limit the abundance of newly arrived organisms, but general theoretical principles for predicting pestilence have been elusive (Parker and Gilbert 2004, Dukes et al. 2009). One appealing possibility is that species interactions within the newly formed assemblages are crucial (Burdon et al. 2006).

Of the herbivores and pathogens that cause damage to forest trees, many have strong (frequently symbiotic) associations with other organisms that exploit plants (Lombardero et al. 2003, Six and Klepzig 2004, Klepzig et al. 2009). For example, many bark and wood-feeding insects harbor microbial symbionts that aid in nutrition (Hoffstetter et al. 2006, Klepzig et al. 2009). Where such reciprocally beneficial interactions arise, there could be increased tendency for pestilence due to the intrinsic instabilities associated with mutualisms (May 1982, Dean 1983, Bleiker and Six 2007). However, if co-occurring plant-feeding organisms compete or share predators, populations are more likely to be constrained and pestilence therefore less likely (Holt and Lawton 1994, Chesson 2000).

An alternative general explanation for variable tendencies toward pestilence is feedback from changes in host resources. For example, the abundance of introduced organisms may decline with time since occupancy due to decreased quantity and/or quality of host plants. Impacts on forests will be least when this negative feedback arises quickly and with only modest declines in host

abundance. In the absence of rapid stabilizing feedback from declining resources, the transient dynamics in insect abundance can be lengthy and consequential (Tobin and Bjørnstad 2003) and the new state of the system uncertain (Anderson and May 1979).

Climate provides another potential explanation for variable outcomes from new plant-feeding organisms in forest systems (Berryman et al. 1987, Estay et al. 2009). Furthermore, climate warming seems to be exacerbating undesirable invasions by increasing the extent of forests that are climatically suitable for potential new pests (Seppälä et al. 2009). Climate is a frequent example of "density-independent effects" or demographic forces that are not themselves influenced by abundance (Berryman 2002; exogenous effects *sensu* Turchin 2003). Accordingly, analytical treatments of climate effects have commonly subsumed climate within the error term of per capita population growth (R) as a function of abundance. However, climate can also influence intrinsic growth rates (r), carrying capacity (K) and/or variability around the density dependent function (Royama 1992), though studies addressing such impacts are comparatively rare. Furthermore, geographic patterns in climatic effects on insect populations are likely (e.g., increased importance of winter cold in poleward populations; Ungerer et al. 1999). When climate effects are strong, they can synchronize population fluctuations over large areas (Moran 1953, Peltonen et al. 2002). When there are close associations among plant-feeding species (e.g., symbioses), climatic effects on the system could be (1) more pronounced because there are more avenues for autecological impacts on one or the other species, or (2) more buffered because the assemblage is more environmentally tolerant than the species by themselves (Klepzig and Six 2004).

Empirical study of how the abundance of non-native organisms is influenced by species interactions, resource depletion, and climate requires measurements of population dynamics across a climatically variable region, but such data are rarely available. We were able to compare alternative theoretical models of population dynamics via analysis of spatially replicated time series abundance

data for a conspicuous but poorly studied non-indigenous pest assemblage in North America: beech bark disease (BBD). The spread of BBD through northeastern North America, while undesirable, presents a natural experiment for better understanding the population dynamics of newly interacting organisms associated with forest disease.

MATERIALS AND METHODS

Beech bark disease in North America

Beech bark disease (BBD) in North America is a bark cankering disease of *Fagus grandifolia* Ehrh. arising from the interaction between an introduced scale insect (the felted beech scale -- *Cryptococcus fagisuga* Lind.) and two species of ascomycete fungi of the genus *Neonectria* (*N. faginata* [Lohman et al.] Castl. & Rossman and *N. ditissima* [Tul. & C. Tul.] Samuels & Rossman; Castlebury et al. 2006). Scale insects feed on cell contents of periderm cells. *Neonectria* spp. only infect beech in the presence of scale insects, exploiting the feeding behavior of the insects to gain access to phloem resources (Houston 1994). Although the effects of *Neonectria* on the scale insects are less clear, it has been generally assumed that cankers and wound callous caused by *Neonectria* infection provide microsites in which scale insects can feed, overwinter, and avoid being displaced by stemflow during rains (Shigo 1964, Houston et al. 1979). Both species are obligatorily sedentary after a brief dispersal opportunity by propagules (ascospores or crawlers). Individual host trees typically harbor persistent populations for many years, frequently of both species living in intimate association within the same bark wounds. While impacts have been dramatic (approximately 50% mortality of adult trees during the first 10 years of infection; Houston et al. 2005), beech remains one of the most abundant forest trees, even in the longest affected regions (Garnas 2012).

Study sites and sampling design

We analyzed time series abundance data of the two BBD agents that were collected with the same protocol for 14 years across 28 sites distributed across about 200,000 km^2 (Supplementary

materials Appendix, Fig. A1). Sites were selected by one of us (DRH) and colleagues in 1978-83 to encompass the geographic range of BBD at the time, and to capture a gradient of temporal development of the disease (Table A1). Based on county-level estimates of the year of initial scale insect colonization (Morin et al. 2007), plots closest to the initial point of scale insect establishment in Halifax, Nova Scotia had been affected for about 44 years at the time of initial sampling, while others had yet to become infested (duration [$\bar{x} \pm SD$] = 19 ± 15 years). All plots retained in the analysis became colonized during the course of the study. Preliminary analyses using a subset of these data were reported in Houston et al. (2005).

Field crews established plots by finding and marking 50-266 trees per plot, and revisited them each summer through 1992 (though not all plots were continuously monitored, there were few missing years; Table A1). All sites were approximately 1.5 hectares, which allowed us to use the number of trees sampled as a proxy for beech stem density. Population estimation and sampling protocols were identical across sites and years (described in detail in Houston et al. 2005). Briefly, each year field crews measured diameter at breast height (DBH) and crown class (Avery and Burkhart 2002), and estimated tree-specific population densities of insects and fungi using visual assessments of waxy secretions produced by scale insects and red fruiting bodies (perithecia) produced by *Neonectria*. Wax is produced by all feeding beech scale insects and is a reliable proxy for insect population density (Ehrlich 1934). Hyphal growth within phloem tissue could not be assessed, but external perithecia are produced annually on most infected trees so the abundance of perithecia is a reasonable proxy for fungal abundance (Houston et al. 2005). Separate estimates of population densities using the same ordinal scales (0-5 for insects and 0-4 for *Neonectria*; Table A2) were made at three heights on the bole of each tree (0-2, 2-4 and 4+ meters above the ground). We also quantified "tarry spots," areas of bark in early stages of infection identified by dark, weeping

and/or stained spots which in many cases indicate incipient *Neonectria* infection. Tarry spots were quantified using four abundance classes: 0 (none); 1 (1-5); 2 (6-10); or 3 (> 10).

Climate data acquisition

Records of daily precipitation, snowfall, maximum and minimum temperatures were obtained for 1978 to 1992 from the National Oceanic and Atmospheric Administration (NOAA) (http://cdo.ncdc.noaa.gov/CDO/cdo). We initially considered all stations within 0.5 degrees of latitude from each plot. All plots (with the exception of NY610 which was excluded from analyses involving climate variables) were within 8 to 55 km of a climate station ($\bar{x}$ = 24 km) with generally complete records during our study years. In cases of missing years for one or more variables at an otherwise ideal nearby climate station, we substituted estimates based on records from the next closest station. Climate stations with >3 consecutive days of missing records for any variable were excluded; we used linear interpolation from surrounding days to estimate temperatures for occasional missing records of 1-3 days. We corrected for adiabatic effects by adjusting daily temperatures (up or down) by 0.5 °C per 100 m (Tran et al. 2007) of difference in elevation between plots and weather stations (maximum difference = 636 m, mean = 194 m).

Data analyses

We began by examining our data with respect to its generic temporal and spatial autocorrelational structure. For temporal patterns, we evaluated the autocorrelation (ACF) and partial correlation (PACF) functions for each population at each site as well as cross-correlations between the scale insects and fungus. Our time series were short for such analyses (Turchin 2003), but we could take advantage of the spatial replication of time series to test for patterns in the correlation structure that were consistent among sites. We evaluated spatial auto- and cross-correlations among sites with spline correlograms from spatially explicit population growth rates for both insects and fungi (Bjørnstad 2009).

146 More explicit tests of our biological hypotheses were permitted by analyses of population growth
147 rate (R) for both insects and fungi using the formula $R_t = \ln(N_{t+1}/N_t)$, where N_t and N_{t+1} correspond
148 to mean population estimates (across height zones) on each tree in the focal and following year
149 respectively. Analyses were based on tree-specific population estimates because this is the scale at
150 which we hypothesize demographic effects (Ylioja et al 1999). This approach assumes that
151 immigration and emigration rates are balanced, or that dispersal among trees has a negligible effect
152 on population dynamics (Royama 1992). Spatially explicit sampling conducted in 2006-2008 at
153 multiple spatial scales throughout the eastern United States indicated a very limited role for dispersal,
154 as extinction at the scale of trees and sites is very rare, and re-colonization from the same tree from
155 year to year typically swamps effects of among-tree dispersal (Garnas 2012). Because population
156 densities were estimated using ordinal classes, and because there are no clear *Neonectria* "individuals",
157 we treat R as an analog to traditional per capita rates. Abundance classes correspond roughly to the
158 natural logarithm of percent cover of scale insects and fungi on trees (as confirmed in post hoc
159 examination in the field) and so scale approximately to continuous variables. Nonetheless, we
160 interpret growth rate parameters cautiously as influencing transitions among abundance classes. Our
161 interpretations emphasize the relative contribution of various biotic and abiotic forces in driving
162 interannual variation in population growth rate, and in how density dependent functions vary
163 spatiotemporally. We excluded tree-year combinations where N_t or $N_{t-1} = 0$ because sampling was
164 sufficiently thorough such that zeros represent the absence of a population on that tree in that year
165 (frequently from local extinction events). Scale insects were common in most sites, present on 3,202
166 trees across 24 plots, each sampled between 6 and 14 times. *Neonectria* was considerably rarer but we
167 were still able to calculate R_t for 629 trees in 18 sites. Preliminary analyses included tarry spots in
168 estimates of fungal abundance but this variable had little or no effect and was dropped from
169 analyses.

We tested for the strength and importance of density dependent effects and of feedback from associated disease agent populations using the general linear model:

$$R_t = F(\text{Site}, N_t, N_{t-1}, A_t, A_{t-1}, Site \times [N_t, N_{t-1}, A_t, A_{t-1}]) + \varepsilon \quad [1]$$

where N is the population density of the focal species (either scale insects or *Neonectria*) and A is the density of the associated disease agent (*Neonectria* in the case of scale insects, and vice versa) at time t and $t-1$. *Site* was treated as a main categorical effect and crossed with all other terms; no additional interactions or higher order terms were considered. The relationship between R and N_t for each population showed some nonlinearity due to frequent cases of trees with stable, low population densities. We explored various transformations of the dependent variable (e.g., $\ln[N_t]$, $N_t^{1/2}$) as well as a nonlinear model (May 1976), but neither approach substantially improved the model fit. Because our data did not strictly meet the assumptions of the statistical model, we treated our F-tests as approximations and relied primarily on model selection to identify important variables with the potential for strong and/or broad-scale effects. We employed Pollard's randomization test as a distribution-free assessment of density dependence relative to a random walk of population abundance over time (Pollard et al. 1987, Woiwod and Hanski 1992).

For each site independently, and for both insects and fungi, we estimated the slope, carrying capacity (K, the model x-intercept), and the mean squared error (MSE) from the model $R = b_0 + b_1 N_t + \varepsilon$. These measures varied considerably by site, and preliminary analyses suggested latitudinal patterns and spatially autocorrelated dynamics. We analyzed each of three parameters from the density dependent function: slope (b_1), K ($-b_1/b_0$) and variability unexplained by density (MSE) independently for insects and fungi in two complementary ways. First, we compared models containing explanatory variables from four distinct categories in an order corresponding to our *a priori* understanding of the relative importance of each in driving BBD dynamics (Table A3). All model comparison was performed using Akaike's Information Criteria, employing a correction for

194 small sample size (Anderson 2008). We retained variables with parsimonious explanatory power
195 within each of four categories of theoretical possibilities: densities of the associated population
196 (fungi for insects and insects for fungi), resource quality and abundance, climatic effects, and disease
197 ontogeny (duration of regional infection with BBD). Table A3 lists and justifies the variables that
198 we considered. Our model selection was hierarchical in that once a predictor was determined to be
199 important (significant p-value [because null hypotheses were plausible], biologically relevant effect
200 size, and a reduction in AIC_c of >2), we retained it in the model unless there was strong evidence
201 from subsequent analyses of a superior alternative (Anderson 2008). To be certain that we had not
202 missed any conspicuously better models, we also evaluated all possible regressions. Since null
203 hypotheses embedded within the model selection exercise were nontrivial (e.g., minimum winter
204 temperature may or may not influence insect or fungal survival) we favored models where regression
205 coefficients were statistically distinguishable from zero.

206 To assess the relative importance of exogenous effects contributing to the variation around the
207 density dependent relationship across sites, we repeated the model selection process using the
208 residuals (ε_i) from the following regression model: $R_t = Site + N_t + Site \times N_t + \varepsilon$, again for both
209 insects and fungi. In this case only predictor variables that varied interannually were relevant, so the
210 candidate drivers were climate and the abundance of the associated species (fungal densities in the
211 case of scale insect population growth rate, and vice versa). Because the effects of climate may also
212 vary geographically, we also tested models of exogenous effects both alone and crossed with
213 latitude. The inclusion of this interaction term frequently improved overall model fit but gains were
214 modest at best (<2% increase in variance explained) so we only report models containing main
215 effects.

216 Model selection, including comparisons of all possible models, was performed using JMP 5.1
217 and R 2.6.2 (Giraudoux 2008, R Development Team 2008, JMP® v. 5.1). Residuals from all models

were assessed for both univariate and mulitvariate normality and homogeneity of variance. We avoided multicollinearity by avoiding models that included correlated variables.

RESULTS

Simple density dependence

There was strong evidence for simple density dependence for scale insects (Fig. 1, left) and *Neonectria* (Fig. 1, right) across all sites and for all sites combined. Insect population density at time *t* was by far the best predictor of tree-specific population growth rate for scale insects, alone explaining 17% of the variation (Table A4). *Neonectria* contributed little to estimates of insect abundance (0.5%) and its effects were small and variable in direction across sites (slope estimate, all sites = -0.03 ± 0.008 for *Neonectria*[N_t] versus -0.29 ± 0.004 for Insect[N_t]). Lagged effects from either scale insects or *Neonectria* contributed even less and were dropped from models of insect *R*. Autocorrelation functions (ACF) and partial correlation functions (PACF) likewise showed no evidence of lagged density dependence for either insects or fungi (Fig. A2). There was also no evidence for lagged cross-correlations between scale insect and *Neonectria* populations, though there was a modest positive correlation coefficient of 0.20 ± 0.13 at time 0 (no lag; Fig. 3S). Of the best supported models for insect *R*, the simplest also contained Site and the Site×Insect(N_t) interaction in addition to Insect(N_t) main effect, indicating that the density dependent relationship differed in slope and relative position across sites (full model: $F_{49, 25603} = 185.0$; $P < 0.0001$; $R^2 = 0.26$). Pollard's randomization test validated the statistical case for simple density dependence in scale insects ($P < 0.01$ for all sites individually, and for all sites combined).

Neonectria populations likewise showed evidence for simple endogenous regulation that varied by site, with little impact of co-occurring scale insect densities. Interannual variation in population growth rate for fungi were best explained with a model containing *Neonectria*(N_t) and Site ($F_{40,1658} = 25.37$; $P = <0.0001$; $R^2 = 0.38$; Fig. 1 right). The contribution of Insect(N_t) was small and highly

variable, and while statistically significant in the full model (eq. 1; $F_{1,1418}$; $P < 0.0001$), added little explanatory power to overall fungal dynamics. The same was true for year *t-1* lags for both insects and fungi. Models containing Site×*Neonectria*(N_t) interaction were nearly indistinguishable from the simpler model without the term ($\Delta AIC_c = 2.67$), though the preferred model containing the interaction was over three times as likely as a ratio of AIC weights. Empirically, estimates of the density dependent slopes did in fact differ by site, ranging from -1.45 and -0.35 ($\bar{x} \pm SE = -0.66 \pm 0.07$) and the interaction was significant ($F_{17,1418} = 1.9$, $P = 0.009$), so we chose to retain it in the final model. For *Neonectria*, Pollard's test confirmed the existence of density dependence in 13 of 16 sites as well as for all sites combined. Regression slopes failed to differ from random in one site each in Maine, Connecticut and Vermont ($P = 0.25$, 0.07 and 0.50 respectively). In all three of these sites, *Neonectria* was comparatively rare and small sample size was likely a factor; given the overall trend, we included estimates of density dependent parameters for fungi from all 16 sites in the remainder of our analyses.

Biotic and abiotic effects on density dependent functions across sites

The variability among sites in the parameters of density dependent functions (DD slope, equilibrium abundance [K] and MSE) revealed by the above analyses were explained reasonably well by subsequent analyses, described below. For both BBD organisms, density dependent slope and carrying capacity (K) were the most generally predictable; simple models containing 1-3 predictors explained 41-79% of variation in these two parameters. For scale insects, the top model also explained a large proportion of variation among sites in MSE (R^2=0.62). Following our ordered modeling approach, we treat each broad category of biotic and abiotic effects below.

Disease agent associates

Variability in the population density of insects and *Neonectria* showed little to no influence on any of the aspects of density dependence we considered. We therefore rejected the hypothesis that

associated species influence the strength or shape of population regulation in the BBD system. *Neonectria* densities did not affect strength or form of the scale insect density dependent relationship, or vice versa. When entered alone into models predicting the slope of insect density dependence, carrying capacity and MSE, in no case was the "*Neonectria*" predictor significant, and explained only 3%, 1% and 0.1% of variation respectively. *Neonectria* also failed to appear as a predictor in any of the top models identified using the all possible models approach (Table A4). Scale insect density was a similarly poor predictor of fungal dynamics, explaining very little variation in the slope, carrying capacity and MSE for *Neonectria* (<1%, <1% and 2% respectively). Accordingly, scale insect density was virtually absent from top models for *Neonectria*, with the single exception being that predicting MSE where the explanatory power of top models was low in general ($R^2 = 0.03$).

Resource availability

Variation in resource availability influenced various aspects of the density dependence for both scale insects and fungi. Mean tree size (diameter at breast height, or DBH) and the total number of trees (a proxy for density in plots of approximately equal size) were present among the top three models for several response variables for both scale insects and *Neonectria* (Table A4). Both were positively associated with density dependent slope for scale insects (parameter estimates: DBH = 0.02 ± 0.005; tree count = 0.001 ± 0.0005). Though later displaced by better performing variables, the model containing only these two factors was highly significant and had reasonable predictive power ($F_{2,21} = 8.8$, $P = 0.001$, $R^2 = 0.46$). DBH was a component of all three top models predicting scale insect K, and was the dominant predictor in the selected model (Table 5, Fig. 3c). Tree count appeared as a weakly negative predictor (-0.002 ± 0.001) in a candidate model identified by all possible regressions predicting the density dependent slope for *Neonectria,* but was displaced by simpler models with higher AIC_c weights ($w_i = 0.68$ versus 0.19; simpler model ~ 3.5 times as likely; Table A4). Finally, DBH was the single best predictor of MSE for *Neonectria* ($F_{1,13}=0.87$; $P=0.37$;

290 R^2= 0.06 Fig. 3d), though explanatory power was low and several other models were effectively
291 equivalent ($\Delta AIC_c < 2$; Table A4 and S5).

292 Climate effects

293 Climate moderately influenced aspects of the density dependent function for scale insects and
294 *Neonectria.* Of the ten climate variables considered, several showed some explanatory power.
295 Among them, four – early precipitation, late precipitation, thermal sum, and the number of days
296 with snow cover > 10 cm (herein, "snow cover") – appeared in a subset of the final models. For
297 scale insects, higher early season precipitation correlated negatively with density dependent slope
298 (mean parameter estimate [± SE] = -0.02 ± 0.01; Fig. 2b). Inclusion of early precipitation in this
299 model along with the duration of BBD infection (with its squared term) marginally improved fit and
300 explained an additional 9% of the variation, though models with or without the variable were not
301 easily distinguishable based on information theory ($\Delta AIC_c = 2.04$; Table A4). Scale insect carrying
302 capacity was also negatively associated with early precipitation (-0.05 ± 0.3; Fig. 3d). Variation in
303 MSE for scale insects was best predicted by early precipitation, thermal sum and duration of
304 infection ($F_{3,19} = 10.5$; $P = 0.0002$; $R^2 = 0.62$). In this model, thermal sum was negatively associated
305 with MSE (-0.0003 ± 0.0001; Fig. 2e) while early precipitation showed a positive association (0.007
306 ± 0.003; Fig. 3f).

307 Climate influenced density dependence in *Neonectria,* again to a moderate degree. Several climate
308 predictors showed explanatory power with respect to site level variation in the parameters of the
309 density dependent function, though only two were retained in the final models (Table A4). Late
310 precipitation was associated with increased carrying capacity for *Neonectria* (slope ± SE = 0.23 ±
311 0.05) and together with duration of infection with its squared term formed the best model ($F_{3,12} =$
312 13.7; $P = 0.0005$; $R^2 = 0.79$; Fig. 3c). Early precipitation was positively correlated (0.014 ± 0.007)

313 with overall variation around the density dependent function, though the relationship was rather
314 weak ($F_{1,87} = 4.2$; $P = 0.046$; $R^2 = 0.05$; Fig. 3e).

315 Our analyses of spatial synchrony in population fluctuations for both insects and fungi provided
316 additional support for a moderate climate signal in the BBD system. The spatial scale of synchrony
317 exceeded that which could be easily explained by dispersal or mobile predators; spatial
318 autocorrelation in population growth was evident out to about 92 km for both scale insects and
319 *Neonectria* (Fig. S4). While long-distance movement via wind currents occurs for both scale insects
320 and fungi, the only comprehensive study on the scale of dispersal found that over 99% of scale
321 insect crawlers dispersed locally, falling from within a meter up to 12-15 m from the inoculum
322 source (Wainhouse 1980). For neither species did we find evidence of anisotropy (directional bias in
323 spatial autocorrelation which – if evident along lines of latitude – would implicate a role for climate).

324 Forest change and disease ontogeny

325 The number of years that stands were colonized by BBD was the strongest and most general
326 predictor of variation in density dependence for insects and fungi (Fig. 2 and 3). There was a clear,
327 nonlinear relationship between duration of infection and density dependent slope for scale insects,
328 with the longest affected stands exhibiting the most negative slope (Fig. 2a). Density dependent
329 slope for *Neonectria* was also best predicted by duration of infection and duration^2 ($F_{2,13} = 4.66$; $P =$
330 0.017; $R^2 = 0.417$; Fig. 3a). Together with late precipitation, duration and duration^2 also provided
331 the best fit for *Neonectria* carrying capacity, with the highest values at intermediate duration of
332 infection, roughly coincident with the center of the range. The best model predicting scale insect
333 MSE also contained duration of infection; in this case the relationship was linear, but rather weak
334 (Fig. 2g). Finally, though not among the top three models based on AIC_c, there was a significant
335 univariate, linear relationship between the duration of infection and scale insect K (not shown).

Scale carrying capacity generally declined with increasing duration of infection ($F_{1,21} = 5.97$; $P = 0.02$; $R^2 = 0.22$, though only marginally after removing two outliers [$P = 0.07$]).

Contribution of climate and associated species densities to exogenous variation

Climate was a moderately weak predictor of variation in population growth rate around the density dependent function for both insects and *Neonectria*, explaining only 8 to 10% of variation (Fig. 2h-i and Fig. 3e). In addition, relationships between residual (exogenous) variation and climate metrics varied unpredictably in strength and direction across sites. Pooling across sites, years with comparatively high spring precipitation were positively associated with population growth rates for both insects (0.014 ± 0.007) and fungi (0.36 ± 0.33; Table A4). For scale insects, the top model containing the early precipitation and snow cover was indistinguishable from slightly more complex models ($AIC_c < 2$), accounting for 7% of the variation in residual error ($F_{2,155} = 5.75$; $P = 0.004$; $R^2 = 0.07$). Top models explaining residual error for *Neonectria* contained various combinations of early precipitation, minimum winter temperature and thermal sum variables (Table A4); of these, we favored the model containing only early precipitation as most ecologically parsimonious (Fig. 3e).

DISCUSSION

Where two or more organisms interact, an understanding of the nature and scale of feedbacks is essential to predicting and understanding dynamics. Our results demonstrate that interannual variation in abundance of both scale insects and *Neonectria* is effectively independent of local, within-tree densities of the associated species within the established range of BBD. Population dynamics for both organisms were best explained with models allowing only for simple density dependence that varied by site, and we therefore reject the hypothesis of coupled dynamics among BBD associates. This result was particularly surprising for *Neonectria*, which depends on scale insect feeding to gain access to host tree tissues, and suggests that either beech trees typically support densities of scale insects sufficiently high relative to the fungus that infection sites are not limiting,

or that trees harbor persistent fungal infection such that interannual fluctuations in the abundance of scale insect are irrelevant to short term *Neonectria* dynamics. This pattern might be different during the first wave of BBD infestations, a stage in the invasion process that was not well represented in our data, but it would be somewhat surprising if scale insects are more limiting for fungi during the years when scale insects are most abundant (Ehrlich 1934). Given the length of our time series, we cannot exclude the possibility that the relevant time scale for the feedback is greater than we were able to evaluate, but there was no temporal signal out to 6 years. In addition, fluctuations in the local abundance of *N. ditissima* (which unlike the dominant *N. faginata* is not obligatorily associated as a forest pathogen either with beech trees or scale insects) may also be driven in part by dynamics on alternate hosts..

Density dependence was clearly variable across sites for both scale insects and *Neonectria*, adding to accumulating evidence that the form and strength of the endogenous relationship is spatially variable for many species (Peltonen et al. 2002, Post 2005). Density dependent slopes and carrying capacities (and MSE for scale insects) were surprisingly well predicted using simple models selected from a pool of variables describing aspects of resource availability, climate and disease history. The most general predictor was the duration of infection with BBD. Such relationships were often nonlinear, indicative of threshold effects. For scale insects, duration of infection predicts a modest, linear decline in carrying capacity, together with a sharp increase in the strength of density dependence in the longest-affected stands (Fig. 4 left). Density dependent functions for *Neonectria* were nearly identical between the most recently and the longest affected stands, while sites of intermediate duration of infection had weaker density dependence and dramatically increased carrying capacity (Fig. 4 right). One possible explanation for the prominence of duration of infection as a predictor of density dependence in BBD populations is that the disease itself has altered the forest and so influenced its own dynamics. Williams and Liebhold (2000) found that

density dependence was strongest at the edge of an outbreak for the spruce budworm, which they attributed to reduced predation effects in the higher quality habitat at the epicenter. At least for scale insects, it is very likely that habitat quality has been degraded in the longest-affected regions. Mean tree diameter correlates negatively with duration of infection at the landscape scale (Garnas 2012), and was the dominant predictor of scale insect carrying capacity. While the positive correlation between mean tree diameter and scale insect K does not demonstrate causation, this is an attractive interpretation because it has been commonly noted that larger trees have higher susceptibility to scale insect attack because increased fissuring of bark creates suitable microhabitats (Gove and Houston 1996). An alternative interpretation is that the relative frequency of susceptible genotypes has declined over time. Habitat suitability for *Neonectria* appears to peak approximately 2-3 decades after the arrival of scale insects. Other work has suggested that *Neonectria* infection trails the arrival of scale insects by approximately 1-10 years (Ehrlich 1934, Houston 1994). It is not difficult to imagine that the buildup of *Neonectria* takes some time after it first appears and that conditions remain optimal or improve for a period once fungal infection begins. In the longest-affected stands, however, habitat quality appears to deteriorate for both scale insects and *Neonectria*.

Overall, the contribution of climate to population dynamics was minimal within the core range of BBD where plots were monitored (though recent work suggests that historically, scale insects have been limited by low winter temperatures in northwestern Maine; M. Kasson, personal communication). Spring precipitation was associated with stronger density dependence and reduced carrying capacity for scale insects and with increased total variability around the density function (MSE), but effect sizes were low. Sites with higher late Summer/Fall precipitation had higher carrying capacities for *Neonectria*. Predictors that relate directly to insect or fungal growth rate (thermal sum) or to overwintering survival (minimum winter temperature) were conspicuously absent from most top models. Similarly, resource related predictors showed some association with

aspects of the density dependent functions (i.e., DBH positively associated with carrying capacity for scale insects) but overall were overshadowed by the effect of duration of infection with BBD. In fact, duration of infection was negatively correlated with tree size ($r = -0.63$, $P = 0.001$), which provides an alternate model to predict scale K.

The modest contribution of climate to population dynamics was striking because we tested across a large geographic extent and allowed for a broad spectrum of possibilities (including interannual fluctuations around density dependent functions and changes in the density dependent functions themselves). Furthermore, support for the climatic patterns that emerged from the modeling was not necessarily compelling. For example, early precipitation was related to per capita population change in both scale insects and fungi, but the direction of the effect varied among sites and did not conform to hypothesized mechanisms (Houston and Valentine 1988). Snow cover was positively associated with scale insect growth, which we had hypothesized might be related to the role of snowpack as a thermal refuge during cold weather (Houston and Valentine 1988; Dukes et al. 2009), but the case for this theoretical mechanism was weakened by the absence of consistent relationships with minimum winter temperature.

At the outset of this study, we had predicted coupled population dynamics between BBD associates. This is clearly not the case. Important feedbacks likely do exist, but at a much larger spatiotemporal scale than we had originally hypothesized. A plausible hypothesis to explain observed patterns is that temporal patterns in disease development correspond to asynchronous peaks in host tree suitability for scale insects and fungi. In this framework, BBD may best be understood as a system where the density dependent relationship is itself regulated by slow-developing, endogenous feedbacks linked to large scale forest change caused by disease and by management in response to outbreak mortality. Once peaks of high host and habitat suitability have passed (as they have for much of the northeastern forest), both scale insects and *Neonectria* are

apparently regulated at relatively low densities. Whether the current condition represents a new equilibrium or a trough in a very long cycle (as would be the case if beech must simply age into higher susceptibility for a new outbreak to occur; Houston 1975), is an interesting and open question with important consequences to the structure and function of the eastern deciduous forest.

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551

552 Supplementary material (available as Appendix E6938 at www.oikosoffice.lu.se/appendix).

553 Appendix Tables A1-A5, Figures A1-A5

FIGURE CAPTIONS

Figure 1. Density dependent relationships in three sites (left column: scale insects, right column: *Neonectria*). Rows correspond to sites ME103, NY611 and WV821 respectively, randomly selected for illustration. Grey lines are OLS regressions for each site.

Figure 2. Bivariate relationships between population parameters for scale insects (slope, carrying capacity, MSE and error residuals around the density dependent function) and explanatory variables from the top models identified by AIC_c. For models with two or more predictors, dependent variables were corrected for the effects of all other variables in the model; grey lines are 1^{st} or 2^{nd}-order regression lines.

Figure 3. Bivariate relationships between population parameters for *Neonectria* (slope, carrying capacity, MSE and error residuals around the density dependent function) and explanatory variables from the top models identified by AIC_c. For models with two or more predictors, dependent variables were corrected for the effects of all other variables in the model; grey lines are 1^{st} or 2^{nd}-order regression lines.

Figure 4. Density dependent functions by regional duration of infestation for scale insects (left) and infection for fungi (right).

Figure 1

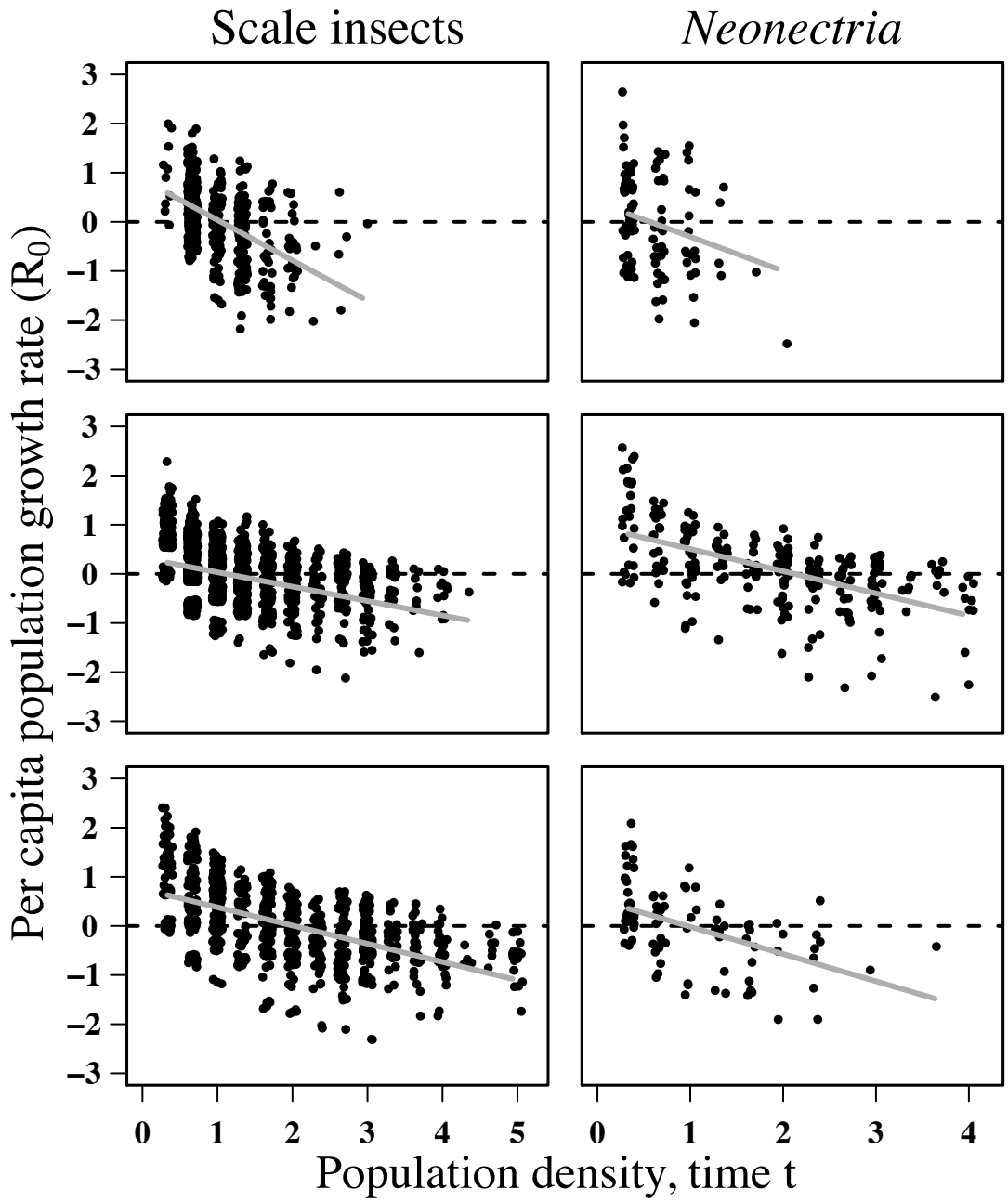

Scale insects
Neonectria
Per capita population growth rate (R_0)
Population density, time t
3
2
1
0
−1
−2
−3
0
1
2
3
4
5

Figure 2

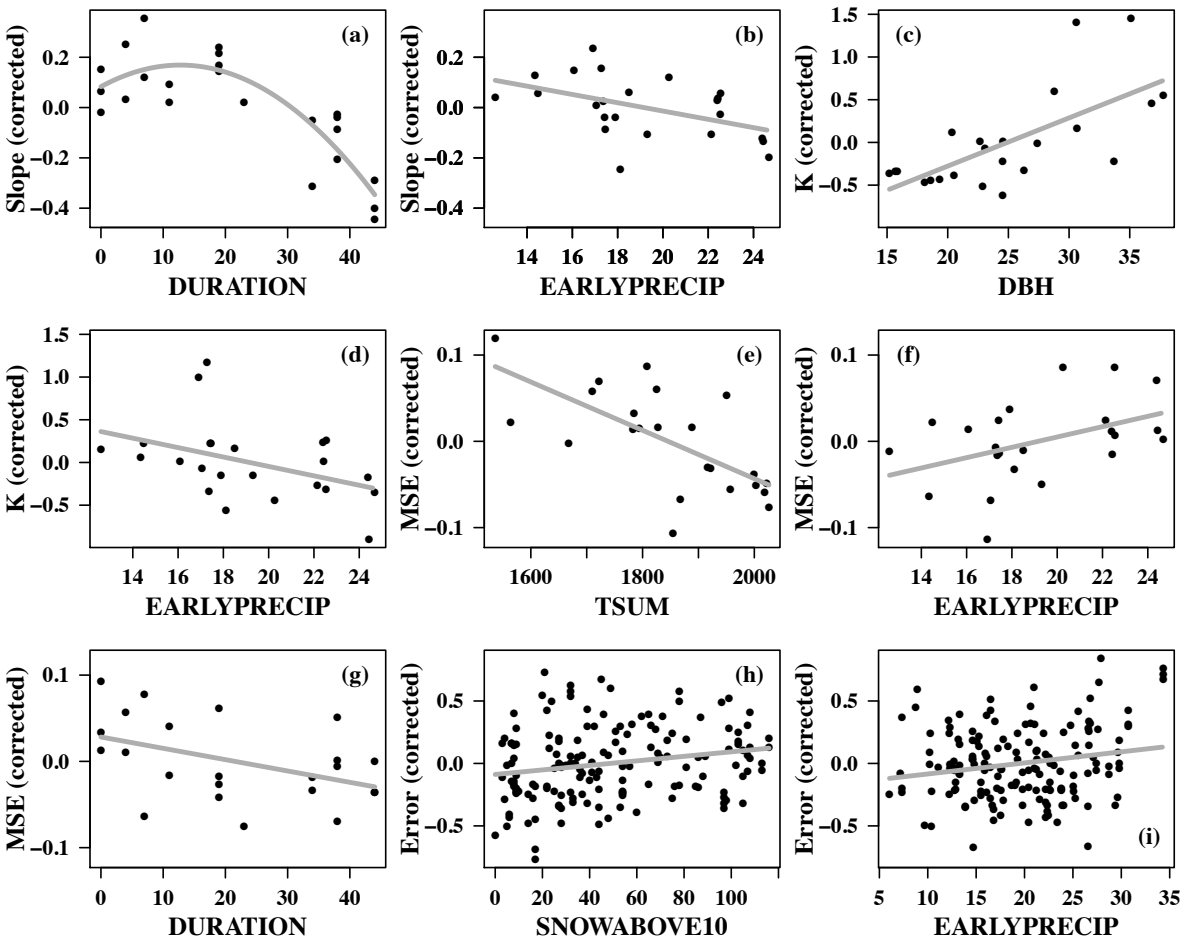

(a)
Slope (corrected)
DURATION
(b)
Slope (corrected)
EARLYPRECIP
(c)
K (corrected)
DBH
(d)
K (corrected)
EARLYPRECIP
(e)
MSE (corrected)
TSUM
(f)
MSE (corrected)
EARLYPRECIP
(g)
MSE (corrected)
DURATION
(h)
Error (corrected)
SNOWABOVE10
(i)
Error (corrected)
EARLYPRECIP

Figure 3

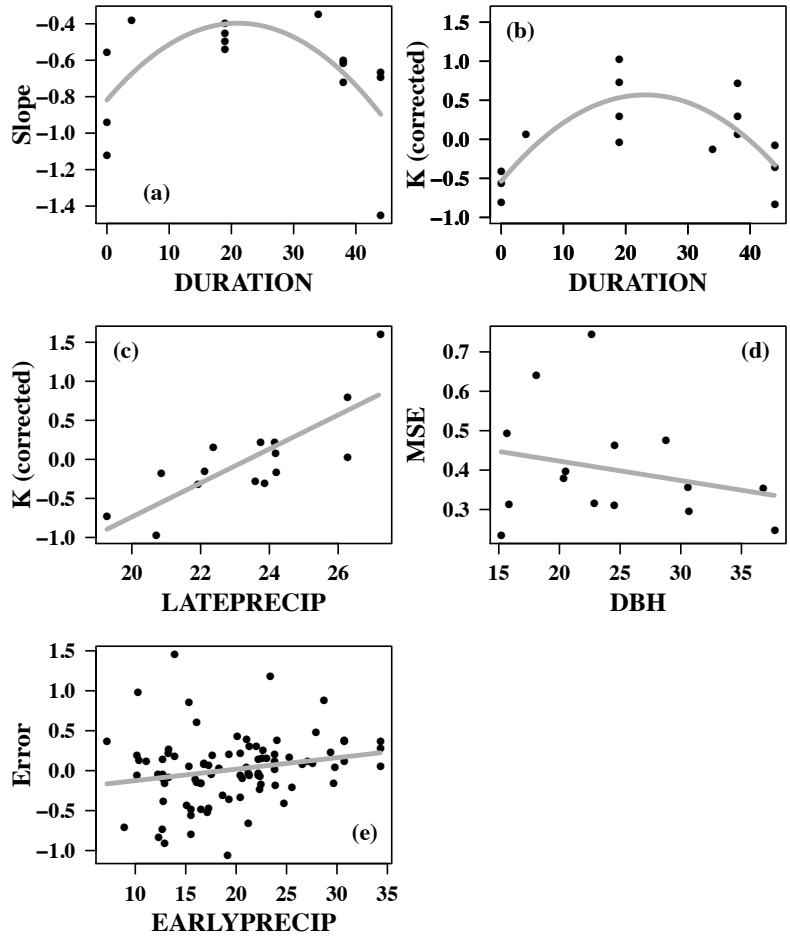
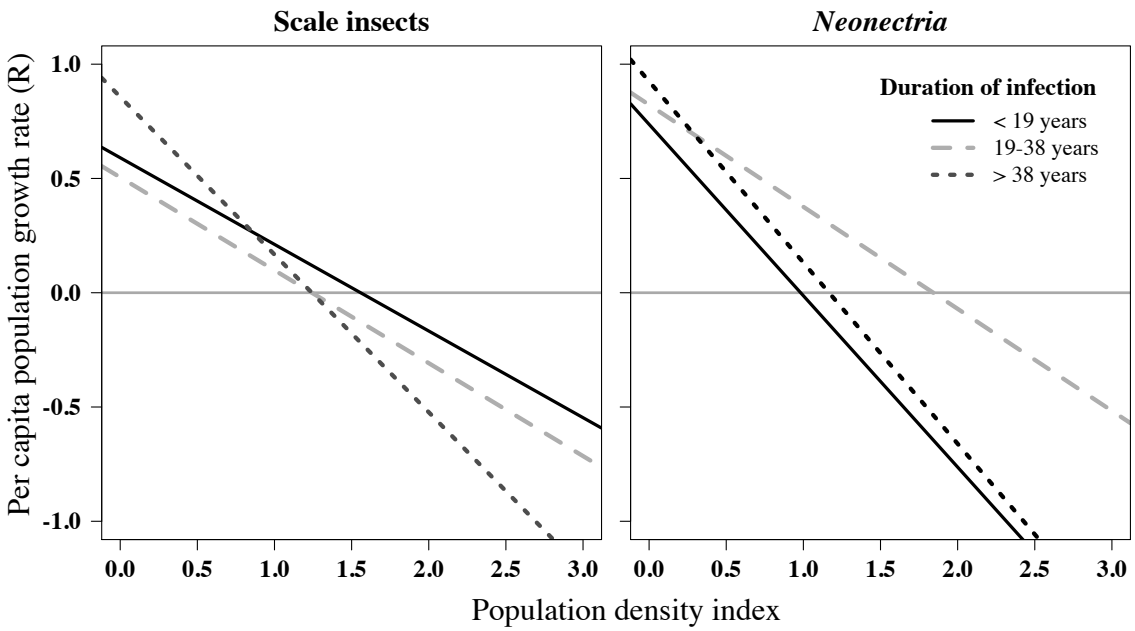

Slope
DURATION
(a)
K (corrected)
DURATION
(b)
K (corrected)
LATEPRECIP
(c)
MSE
DBH
(d)
Error
EARLYPRECIP
(e)

Figure 4


Scale insects
Neonectria
Duration of infection
< 19 years
19-38 years
> 38 years
Per capita population growth rate (R)
1.0
0.5
0.0
-0.5
-1.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Population density index