

Biotic resistance to invader establishment of a southern Appalachian plant community is determined by environmental conditions

BETSY VON HOLLE

Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN, USA

Summary

1 Tests of the relationship between resident plant species richness and habitat invasibility have yielded variable results. I investigated the roles of experimental manipulation of understorey species richness and overstorey characteristics in resistance to invader establishment in a floodplain forest in south-western Virginia, USA.

2 I manipulated resident species richness in experimental plots along a flooding gradient, keeping plot densities at their original levels, and quantified the overstorey characteristics of each plot.

3 After manipulating the communities, I transplanted 10 randomly chosen invaders from widespread native and non-native forest species into the experimental plots. Success of an invasion was measured by survival and growth of the invader.

4 Native and non-native invader establishment trends were influenced by different aspects of the biotic community and these relationships depended on the site of invasion. The most significant influence on non-native invader survival in this system of streamside and upper terrace plots was the overstorey composition. Non-native species survival in the flooded plots after 2 years was significantly positively related to proximity to larger trees. However, light levels did not fully explain the overstorey effect and were unrelated to native survivorship. The effects of understorey richness on survivorship depended on the origin of the invaders and the sites they were transplanted into. Additionally, native species growth was significantly affected by understorey plot richness.

5 The direction and strength of interactions with both the overstorey (for non-native invaders) and understorey richness (for natives and non-natives) changed with the site of invasion and associated environmental conditions. Rather than supporting the hypothesis of biotic resistance to non-native invasion, my results suggest that native invaders experienced increased competition with the native understorey plants in the more benign upland habitat and facilitation in the stressful riparian zone.

Key-words: biotic resistance, competition, facilitation, invasibility, non-indigenous, overstorey, richness, stress, understorey

Journal of Ecology (2005) **93**, 16–26

doi: 10.1111/j.1365-2745.2004.00946.x

Introduction

Elton (1958) invoked the notion of biotic resistance to non-native invaders to explain the heavy invasion of species-poor systems such as islands and anthropogenically disturbed areas. The term ‘biotic resistance’, first coined by Chapman (1931), is the degree of resistance offered by the resident biota. This resistance, which may be somewhat predictable (Elton 1958), is the key to

the success of an invasion (Simberloff 1986) and resident richness effects may play an important role in biotic resistance, as higher native species richness might be expected to decrease nutrient availability and increase competition, predation, disease and parasites. These factors may, in turn, lower niche opportunities for invaders and contribute to biotic resistance (Simberloff 1986; Shea & Chesson 2002). The relationship between biotic resistance and the invasibility of a system is typically called the ‘diversity-invasibility’ relationship and most studies of this relationship use species richness as the index of diversity. To clarify the hypothesis, I use the term ‘richness-invasibility’ relationship.

Present address and correspondence: Betsy Von Holle, Harvard Forest, Harvard University, PO Box 68, Petersham, MA, USA 01366 (fax +1 978 724 3595; e-mail vonholle@fas.harvard.edu;).

The effect of resident richness on plant invasibility has been most often researched in open systems such as grasslands, with both observational and experimental studies (Von Holle *et al.* 2003). These studies have provided evidence of positive (Robinson *et al.* 1995; Stohlgren *et al.* 1999), negative (Tilman 1997; Levine 2000; Naeem *et al.* 2000) and neutral (Lavorel *et al.* 1999; Dukes 2001) effects of species richness on community invasibility. In a review of the diversity-invasibility hypothesis, Levine & D'Antonio (1999) indicated that observational studies of natural landscapes tended to indicate a positive relationship between diversity and invasibility. However, small-scale invader addition studies that did not manipulate the resident communities and experimental studies that directly manipulated the recipient plant communities both produced mixed results for this relationship. The lack of consistency between landscape and community-scale studies of invasibility may be due to extrinsic factors, such as greater habitat diversity, affecting non-native and native species in the same way. Additionally, intrinsic interactions between non-native invaders and native residents may not be similar to those between native invaders and residents. The intrinsic effects of species interactions on invasibility are revealed by smaller scale studies, where the degree of invasion may depend on interactions with native residents (Lonsdale 1999; Davis *et al.* 2000; Shea & Chesson 2002). Clearly, Elton's hypothesis regarding invasibility has not been resolved. Nevertheless, empirical and theoretical studies of this hypothesis have provided a mandate for future empirical work. Several explanations have been advanced for why empirical work has been so equivocal.

Many factors, such as disturbance, nutrient availability, climate and propagule pressure (e.g. number of seeds introduced) can covary with species richness. These extrinsic factors can influence invaders and residents differently (Siemann & Rogers 2003; Leishman & Thomson 2004) and appear to affect relationships between species richness and invasibility (Levine & D'Antonio 1999; Levine 2000; Naeem *et al.* 2000; Shea & Chesson 2002). Additionally, resident cover and biomass sometimes covary with species richness. It has been suggested that in disturbed environments invasion is more affected by abiotic than biotic factors (Hood & Naiman 2000). In order to elucidate the unique influence of species richness on invasion, species richness must be manipulated *in situ* in order to separate its effects from covarying factors.

My objectives were to explore the roles of biotic factors in resistance to invasion in a closed-canopy system. I tested the predictions that higher understorey species richness and the presence and size of certain overstorey groups (trees and shrubs) would both aid in resisting invasion, using manipulation of understorey plant richness as a proxy for differing biotic resistance of the system. Manipulated plots were located along a flooding gradient to test whether the richness-invasibility relationship holds under disturbance and stress (Moyle &

Light 1996; Hood & Naiman 2000). I transplanted both native and non-native invaders to better understand variation in biotic resistance to plants of different origin (Vermeij 1996). Manipulating plot species richness allowed me to control covarying extrinsic factors and retain original plot densities, and thus to isolate the effect of understorey species richness on invasibility.

Experimental design and methods

My plots were located in the mid-reaches of Big Stony Creek (38°26' N, 81°50' W, 739 m), a second-order (Leopold *et al.* 1964) stream in south-western Virginia, within the Ridge and Valley physiographic province. All plots were located under forest canopy, and comprised the closed forest types of Trianosky (1994) for evergreen and deciduous temperate forests, i.e. *Tsuga canadensis* forest with *Rhododendron maximum* understorey in the streamside regions and *Liriodendron tulipifera*–*Quercus alba*–*Q. rubra*–*Acer saccharum* forest with *Kalmia latifolia*, *Ilex opaca* and *Rhododendron maximum* understorey in the upper terrace sites (Suiter & Evans 1999). Regeneration patterns under *Rhododendron maximum* canopy differ from under other forest types (Clinton & Boring 1994), possibly owing to differences in light, soil nutrients and water (Nilsen *et al.* 2001). The surface soil layer was deeper in the upper terrace areas, as fewer *Rhododendron maximum* roots occupied this space. Because flooding frequency varies with elevation (Menges & Waller 1983), elevation differences are a necessary component of a study of different flooding regimes. However, differences in elevation are also correlated with differences in edaphic factors (Bridge & Johnson 2000) and these differences may influence species composition (Clinton & Boring 1994) and probability of establishment of certain exotic species (McNab & Loftis 2002). The elevational differences among my sites were slight (approximately 2–3 m) but are associated with statistically significant edaphic differences (Table 1).

PLOT DESIGN

The topography of the field site was a typical stream bank and terrace system (Gregory *et al.* 1991). All plots were located on level terraces to control for different plant interactions on sloped and level ground (Fig. 1). All experimental plots were located under forest canopy and each encompassed a 70 × 10 m area. The two 'streamside' plots (adjacent to the creek) were frequently and partially flooded every year from approximately December through to April (Von Holle 2002) and the two 'upper terrace' plots (in the second level of the floodplain) were not flooded during the experimental time frame. Each plot had four rows of 1.5 × 1.5 m subplots: two plots were established in each of the streamside and upper terrace areas; one pair, with 10 subplots per row, was manipulated, and first censused, in 1999, whereas the second pair, with 14 subplots per row, was manipulated in 2000 (Appendix S1 in Supplementary

Table 1 Average soil characteristic \pm SD. Soil characteristics were compared between streamside and upper terrace sites. After comparing the *P*-values to sequential Bonferroni-adjusted alphas, volumetric water content (VWC), carbon, nitrogen, pH, silt and clay were statistically significantly different, according to unpaired *t*-tests of individual soil characteristics. Statistically significant *P*-values are marked in bold. All percentage data were arc-sine square root transformed for normality. Data from 46 streamside and 43 upper terrace plots were used

Site variable	Site location (mean \pm SD)		<i>t</i> -value	<i>P</i> -value	d.f.
	Streamside	Upper terrace			
VWC	11.05 \pm 3.15	19.20 \pm 6.60	8.11	< 0.0001	66
Carbon (%)	2.02 \pm 0.63	3.18 \pm 1.31	5.61	< 0.0001	74
Sulphur (%)	0.05 \pm 0.02	0.04 \pm 0.02	2.28	0.0248	87
Nitrogen (%)	0.09 \pm 0.02	0.13 \pm 0.05	6.77	< 0.0001	64
pH	3.92 \pm 0.22	3.82 \pm 0.17	2.63	0.0103	84
Sand	71.9 \pm 5.67	68.9 \pm 11.7	1.12	0.2677	56
Silt	13.04 \pm 5.98	23.55 \pm 10.70	5.41	< 0.0001	71
Clay	14.74 \pm 5.65	7.53 \pm 7.23	5.09	< 0.0001	67

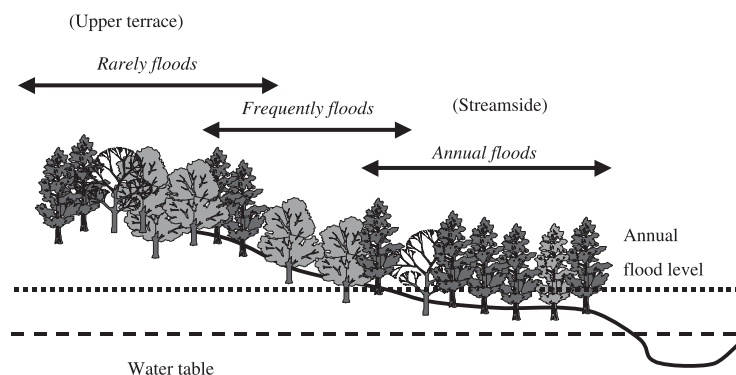


Fig. 1 Site treatment in relation to flooding. The plot treatments are indicated by capital letters. The treatments were assigned to incorporate yearly flooding. Although there were frequent floods, they were not large enough to cover the entire streamside treatment area during the drought winter years that occurred in the southern Appalachians from 1999 through 2001. Thus, only 24 out of 96 streamside subplots were regularly flooded. The upper terrace plots never flooded during the experimental time period.

Material). Native and non-native transplants were introduced 2 weeks after resident community manipulation.

'Site location' indicates the position of the treatment plot (streamside or upper terrace) and 'flooding condition' whether or not a particular subplot was flooded during the previous year. Although there were frequent floods, they were not large enough to cover the entire streamside treatment area during the winter droughts that occurred in the southern Appalachians from 1999 through 2001 and only 24 out of 96 streamside subplots were regularly flooded. Subplot flooding status was confirmed by a covering of debris after the yearly spring floods.

COMMUNITY MANIPULATION

Species richness and stem density were assessed in each subplot, prior to and after manipulation. Community censuses took place in 1999 (1999 experimental plots), 2000 (2000 experimental plots) and 2001 (all plots). The term 'density' is used to indicate the number of individual plants per subplot. I reduced subplot species richness to create low and intermediate richness treatments, with ambient richness (control) subplots serving as high richness treatments (see below; for an explana-

tion of the four morphologically classified functional groupings, see Appendix S1 and Von Holle & Simberloff 2004). All manipulated plants were less than half a metre tall. If the functional group that was chosen randomly for manipulation in that subplot was missing, another plot was chosen randomly and that plot was dropped from the total pool of subplots. Manipulated subplots were left with their original densities, as individuals of the randomly picked species that were to remain in the subplots (either from other subplots that required removal of those individuals or from a nearby, forested area) were inserted into the empty holes left by removed plants. Thus, I manipulated the plot richness while allowing plot density to vary naturally. Richness treatments were applied to randomly chosen subplots.

1. Low richness: subplot species richnesses were manipulated by lowering the number to two randomly picked species in each of the four functional groups, for a total of eight species per subplot. Biomass was maintained by planting an equivalent biomass of the species selected to remain in that subplot.
2. Intermediate richness: one randomly picked functional group was either removed (half the plots) and biomass replaced as randomly chosen species of the remaining functional groups or lowered to one species (other

half) and biomass maintained using the one species of that functional group randomly selected to remain.

3. High richness (control): this treatment was left with the original complement of plants and was undisturbed.

To control for the effect of digging, plants were uprooted and replanted (high richness = control digging): all plants of the randomly chosen functional group per subplot (intermediate) or randomly selected species from all functional groups (low). Any significant difference in invasion success between the digging control and the control plots indicates the effect of digging.

High and intermediate richness treatments were randomly assigned to subplots manipulated in 1999 (Appendix S1). However, because differences in resident richness values between the high and intermediate treatments of the 1999 plots were not significantly different at any time according to student *t*-tests, invader survivorship by richness treatment was not analysed for this cohort. Randomly chosen subplots were randomly assigned low (low richness), intermediate (intermediate richness) and high (control and digging control) species richness treatments for the plots that were manipulated in 2000. In order to reduce transplant stress, I watered all subplots every day for a week and then every other day for another week.

INVADER INPUT

Widespread native and introduced species commonly found throughout the floodplain forests of Big Stony Creek were used as 'invaders', indicating their addition to the community, even though many are native species (Appendix S2). I used plants growing in the watershed, rather than seeds, to avoid introduction of novel genetic variation and because seeds are more likely than transplants to be swept downstream with flooding. These invaders were removed along 50-m transects, which were laid perpendicular to Big Stony Creek in forested areas upstream and downstream from the experiment. Every 5 m a non-native species was systematically searched for, dug up and soil gently removed from the root system; if none was found, a native species was collected. I identified 133 native species and 36 non-native species in study site and collection areas, using the nomenclature of Gleason & Cronquist (1991), and 76 and 28 of these formed the pool of invaders (Appendix S2).

After a 2-week period to allow for rerooting, 1.5 × 1.5 m subplots were planted with one adult or seedling plant (< 0.5 m) of each of 10 randomly chosen invader species, inserted into the subplot as equidistantly as possible, following three parallel, diagonal lines, with the middle line the hypotenuse of two equilateral triangles comprising the inner 1 × 1 m of the subplot. Each plant was marked with a metal tag and watered daily for 1 week and then once every other day the following week, which resulted in 2-week survivorship values of 83% in 1999 (*n* = 800) and 92% in 2000 (*n* = 1120).

Invasion success was measured by invader survivorship and growth after 1 and 2 years in the plots. All

invaders were monitored 2 weeks after transplantation, and then every year for 2 years. Survivorship was measured by death at a census period. A plant was counted as dead if it had no chlorophyll or was missing from the spot where it was planted. If the marker tag was absent the plant was counted as missing, and removed from the analyses. In this closed-canopy riparian forest, very few individuals reproduced by seed (3 out of 835 living individuals in 2002), thus precluding statistical analysis by reproductive status. However, many expanded vegetatively, and this growth was approximated with measurements of plant characteristics over time. Stem diameter at ground level, canopy length, first internode length, and the number of leaves were measured in the field upon collection and at 2-week and yearly censuses to measure growth over time (see Von Holle & Simberloff 2004 for monitoring details).

OVERSTOREY COMMUNITY CHARACTERISTICS

To investigate overstorey impact on invader survival, I censused the distance, size and canopy cover of adult overstorey trees and shrubs near the subplot. I measured the diameter at breast height (d.b.h.) of the largest tree and shrub within 3 m of the edge of each subplot. I also recorded the distances of this tree and shrub from the subplot. If the largest tree or shrub was within the 1.5-m subplot, then the distance was recorded as negative, from the closest edge of the subplot. To estimate tree and shrub canopy cover, I measured the diffuse penetration coefficient, or measurement of sky visible beneath the overstorey canopy (Norman & Campbell 1989), of each subplot with a digital camera equipped with hemispheric lens (CID-110, CID Inc., Camas, WA, USA) at 1.5 m above ground level. All censuses occurred in August 2001.

DATA ANALYSIS

The relationship between invasibility and biotic and environmental characteristics was explored by analysing the percentage of invaders that survived various time periods and flooding in the experimentally manipulated subplots in the two sites. All invader survivorship relationships were analysed separately by plant origin (native and non-native), unless otherwise indicated. The data were the percentages of invaders, by plant origin, that survived from the previous census. For example, if five natives survived 2 weeks after transplantation and three of these survived the following year, the 1-year native survivorship would be 60%. All survivorship data were transformed with Anscombe's arcsine transformation (Zar 1999). All other percentage data were arc-sine square root transformed to increase normality. Annuals were removed from all analyses. I tested each cohort separately to understand the effect on invasibility relationships of the differing environmental conditions associated with these years. Due to the levels of uncontrolled variation

that resulted from the manipulation of natural communities, I used an initial alpha value of 0.1. To correct for multiple testing, I used the sequential Bonferroni technique within each census period (Rice 1989). All analyses were performed with SAS version 8.2 (SAS 2001).

Results

UNDERSTOREY COMMUNITY CHARACTERISTICS

There were no significant differences in native or non-native survivorship between control and digging control plots for any cohort or census period, as indicated by two-way analyses of variance. This result validates my method of directly manipulating resident plot richness by transplanting plants. Immediately after manipulation, species richness treatments of the 2000-manipulated plots differed significantly between high (control and digging control subplots = HR) and low species richness (low richness subplots = LR) treatments (Student *t*-test, $t = 3.59$, $P = 0.0006$, d.f. = 65, means \pm SE: LR = 7.61 ± 0.38 , HR = 11.02 ± 0.87). However, owing to germination of buried seeds or resprouting from roots accidentally left behind during manipulations, differences were no longer significant 1 year after manipulation ($t = 1.09$, $P = 0.28$, d.f. = 78, means \pm SE: LR = 9.30 ± 0.69 , HR = 10.44 ± 0.69). The newly recruited individuals added to the subplot species richness although they were rarely abundant. Of the 24 out of 96 streamside subplots that were regularly flooded, none of these were intermediate richness treatment subplots and this treatment was therefore removed from analyses of flooding and richness treatment effects.

PLOT SPECIES RICHNESS AND FLOODING EFFECTS ON INVADER SURVIVORSHIP AND GROWTH

The understorey species richness of a plot had variable effects on plant invasibility, depending on the site loca-

tion and origin of the plant. I tested the relationships between invader survivorship of the 2000 cohort and three main treatments (plant origin, resident richness and the flooding condition of the sites they were planted into) and their interactions with three-way analyses of variance. There were statistically significant three-way interactions in both yearly censuses (Table 2). Survivorship of native and non-native species depended on richness treatment and flooding condition (Fig. 2). For example, native invaders tended to have greater survivorship in high than in low richness treatments in the streamside plots, but greater survivorship at low richness in the non-flooded upper terrace. These trends were manifested both 1 and 2 years following transplant (Fig. 2a,b). In both years native survivorship at low richness on the upper terrace was statistically significantly higher than for both equivalent non-natives and non-natives in every richness treatment in non-flooded streamside plots. Additionally, native survivorship in low richness treatments differed significantly between non-flooded subplots in streamside and upper terrace locations for both years following transplant (Fig. 2a,b). After 1 year, natives in high richness subplots in the non-flooded streamside plots had significantly higher survivorship than non-natives in both richness treatments in the same site (Fig. 2a,c). The richness-invasibility relationship varied significantly by plant origin and site for both years after transplant (origin \times site \times richness interaction, Table 2). After 2 years in the plots, non-natives in the low richness treatment experienced greater survivorship in the flooded streamside subplots than in non-flooded streamside or upper terrace plots (Fig. 2d), i.e. the opposite of the trend for natives (Fig. 2b), although this trend was not statistically significant and was not evident after 1 year (Fig. 2c). There were insufficient numbers of non-natives within flooded high richness treatment subplots in the second year to analyse their richness-invasibility relationship (Fig. 2d). The interaction terms of the three-way analysis of variance may have been compromised by the missing cell in the three-way analysis of variance

Table 2 Three-way analyses of variance of invader survivorship as dependent on site, richness treatment, plant origin and their interactions.* Analyses were conducted on the yearly censuses of the 2000 cohort. The first sequential Bonferroni-adjusted α -value of each census was 0.01. Significant *P*-values that are below the sequentially ranked α -values are marked in bold. See Fig. 2 for native and non-native species survivorship values by richness and site treatments

Source	1 year				2 years			
	d.f.	SS	<i>F</i>	<i>P</i>	d.f.	SS	<i>F</i>	<i>P</i>
Origin	1	8.94	19.5	< 0.0001	1	7.57	16.9	< 0.0001
Site	2	6.57	7.18	0.001	2	3.43	3.82	0.0235
Richness	2	0.01	0.01	0.9885	2	0.01	0.02	0.9845
Origin \times site	2	0.18	0.20	0.8225	2	1.12	2.49	0.0858
Origin \times richness	2	0.22	0.24	0.7890	2	0.26	0.57	0.5665
Site \times richness	3	3.13	2.28	0.0807	3	1.47	3.28	0.0220
Origin \times site \times richness	3	6.37	4.64	0.0037	3	1.82	4.05	0.0080
Model	15	40.5	5.90	< 0.0001	15	42.7	6.34	< 0.0001

*Site variables for the yearly censuses were flooded streamside, non-flooded streamside, and upper terrace plots. Type three sum of squares are reported.

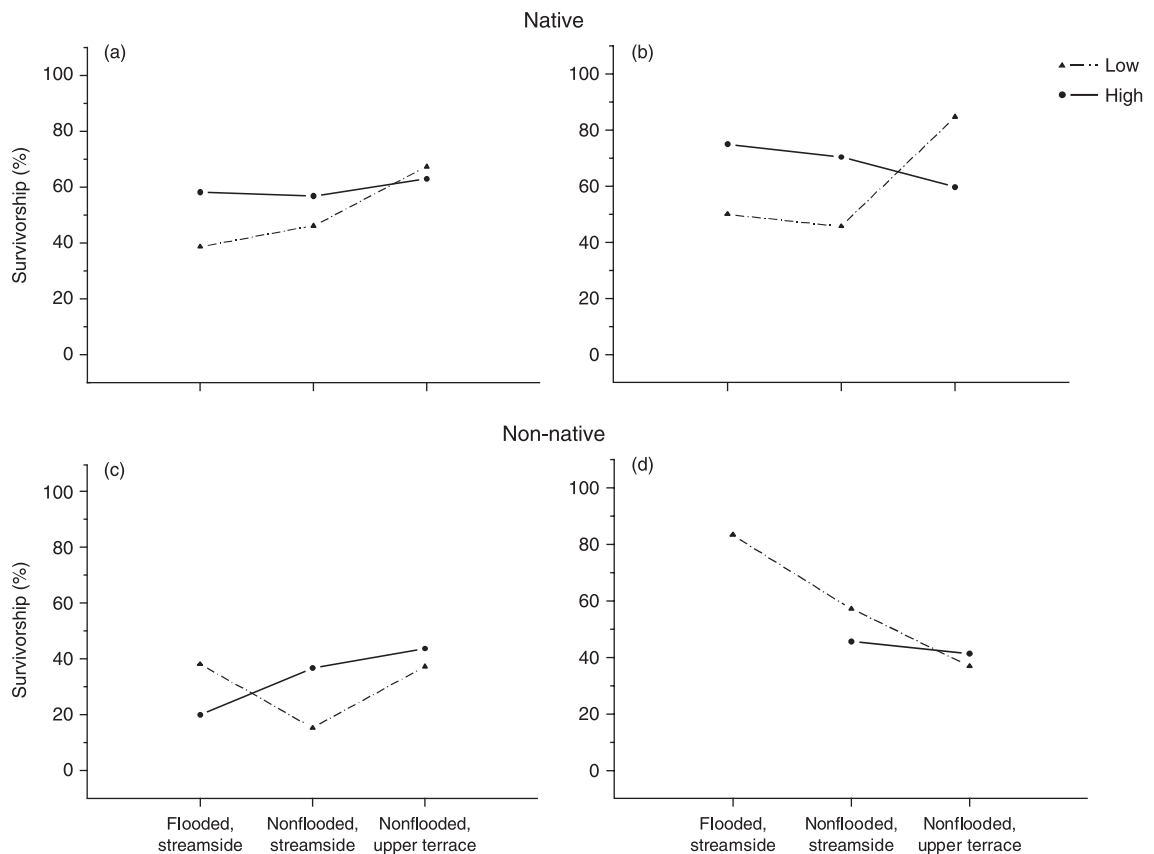


Fig. 2 Native (a, b) and non-native (c, d) survivorship by site (and flooding condition) and richness treatment (low, high). One-year (a, c) and 2-year (b, d) survivorship values from the 2000 cohort are presented.

of the second year (Underwood 1997). However, the strongly significant three-way interaction after the first year of invasion and the consistent trends across both years are strong evidence for differences in native and non-native invasion that are affected by plot richness and environmental condition. The different edaphic characters of the streamside and upper terrace plots might have caused the differences in richness-invasibility relationships experienced by the native and non-native species (Table 1). These streamside plots had lower soil water content, carbon, nitrogen and silt with higher pH and percentages of clay in the soils. Additionally, the soil layer was not as deep as in the upper terrace areas. It is clear that the interaction between understorey residents and site environmental conditions has significantly different effects on native and non-native invader survivorship.

Growth of native invaders depended on the understorey richness treatments while non-native invader growth was unrelated to richness treatments. I analysed stem diameter, canopy length, internode length and leaf number growth of the 2000 cohort from years 2000–02. I conducted two-way analyses of variance of growth of plants of different origins and species, as dependent on richness, site and flooding, and on the interaction of richness with either site or flooding. Species with 10 or more individuals alive at the end of the experiment were analysed separately to investigate individual species

responses to richness treatments. No relationships were found between any growth parameter of native species and richness treatment. However, natives in the upper terrace plots had significantly greater increases in the number of leaves at high than at low richness ($F = 7.48$, $P = 0.0095$, d.f. = 1, means: HR = 0.71 ± 0.36 , LR = -0.67 ± 0.25). Additionally, *Aster divaricatus* had significantly greater stem diameter growth at intermediate than at low richness ($F = 6.44$, $P = 0.0099$, d.f. = 4, means: INT = 0.36 ± 0.10 , LR = -0.55 ± 0.13). Nevertheless, neither non-native species as a group (or *Lysimachia nummularia*, *Rosa multiflora* and *Veronica officinalis* individually) nor the native species *Acer rubrum*, *Parthenocissus quinquefolia*, *Potentilla simplex* and *Viola septentrionalis* had statistically significant differences in any of the measured characteristics with richness treatment or site.

Non-native survival was weakly associated with the original diversity values of the subplots, prior to manipulation. I conducted multiple regression analyses of native and non-native survivorship as dependent upon the pre-manipulated subplot richness and density, to understand whether the extrinsic factors that contributed to the original subplot diversity values were important to invader survival. There were no relationships between the original values and survivorship of native invaders of the 2000 cohort at any census period, but non-native survivorship was significantly positively related to original

richness and negatively related to original density values 1 year after transplant (non-native survivorship = $1.06 + 0.03 \text{ richness} (P = 0.0063) - 0.0001 \text{ density} (P = 0.2916)$; Model $r^2 = 0.08$, $P = 0.0129$, d.f. = 2). However, this weak relationship does not explain much variance in non-native survivorship. The lack of relationship of natives with original subplot diversity indicates that observed patterns of native plant survivorship and growth from this experiment were due to the richness and site treatments, rather than extrinsic factors that covary with original plot richness. Non-native species did not respond to the manipulated understorey richness treatments; however, they were weakly related to original subplot diversity values, suggesting that factors that covary with plot richness, such as light, disturbance and nutrients, may be more important to non-native than native survival in this habitat.

OVERSTOREY COMMUNITY CHARACTERISTICS

The overstorey significantly affected understorey invasibility by non-native species. I evaluated percentage invader survivorship and invader growth as dependent variables and shrub and tree diameter at breast height (d.b.h.) and distance from subplot, subplot flooding condition, and the interaction of flooding condition with overstorey composition of the recipient subplots as the independent variables in multiple regression analyses. I checked all independent variables for interactions with flooding condition by seeking significant flooding-by-independent variable interactions. If these interactions were not statistically significant, I lumped flooded and unflooded treatment plots together for 2-week and yearly analyses. There were no significant relationships between size and distance of the overstorey plants and native plant survivorship, for either cohort in any census period (Table 3a,b). Additionally, there were no relationships between overstorey composition and growth of native or non-native species from 2000 to 2002. The interaction between the overstorey composition and flooding condition on 1999 non-native survivorship was significant at 1 and 2 years after transplantation, so each flooding condition was analysed separately for the overstorey composition effect on invader survivorship. Non-native plants in the flooded subplots had significantly increased survivorship near larger trees at the 2-year census (Table 3a). The most abundant overstorey trees in these flooded subplots were *Tsuga canadensis* and *Nyssa sylvatica*.

I investigated the relationship between canopy cover and invader survivorship as a mechanism for the overstorey effect on invasibility. There were no significant relationships between the diffuse penetration coefficient and native plant survivorship, for either cohort in any census period, using simple regression analyses (Table 4). One and 2-year survivorship of non-native plants of the 2000 cohort indicated a dependence of these species on light for survival (Table 4). The 1-year

survivorship of the 1999 cohort was negatively related to light levels (Table 4). These relationships suggest that the understorey conditions for non-native invasion are the result of environmental mediation by the overstorey. The non-native plants with greater light availability had greater 1- and 2-year survivorship. It should be noted that the level of variance in invader survivorship accounted for by the overstorey composition was much greater than that accounted for by light penetration alone.

Discussion

UNDERSTOREY RICHNESS AND INVASIBILITY

Contrary to Elton's (1958) hypothesis that high diversity areas resist invasion by non-native species through decreased resource availability and the increased probability of a negative interaction occurring between an invading species and a resident species, non-natives were facilitated by native trees and did not interact with the understorey resident natives as strongly as the native invaders did. Understorey richness clearly modified site effects on invader establishment success and these effects depended on whether the invading plant was a native or non-native species, as indicated by significant origin-by-site-by-treatment interactions in both censuses of the 2000 invaders. Native and non-native species had categorically different survivorship responses to the richness treatments across sites, and these trends were maintained both years after transplant. The richness-invasibility relationship appeared to be affected by the environmental conditions of the plots, rather than by flooding, although this result may be due to the dearth of flooded subplots in this drought period rather than a lack of relationship. It is possible that the strength and direction of interactions with resident plants differs with plant origin and that these interactions vary with environmental conditions. The significantly higher levels of carbon and nitrogen in the non-flooded upper terrace site may make this habitat more benign for plant survivorship. The occurrence of facilitation between plants in stressful and disturbed environments and competition between plants in more benign environments is a well-known phenomenon (Bertness & Callaway 1994; Bertness & Leonard 1997; Callaway & Walker 1997; Bruno *et al.* 2003). Thus the positive resident richness effects on yearly native invader survival might have been a result of the importance of facilitative interactions under the stressful conditions of the streamside areas, whereas biotic resistance to native invaders may have occurred through increased understorey competition in the more benign upper terrace plots. Nevertheless, native leaf growth in the upper terrace increased with higher resident richness treatments. Growth values of non-natives as a group and three individual non-native species were unrelated to subplot richness treatments. The complex, idiosyncratic interaction between stress and understorey biodiversity

Table 3 Multiple regression for the size and distance of the nearest large tree and shrub to invader survivorship, for invaders that were planted in 1999 (a) and 2000 (b). All analyses are multiple regressions, conducted with percentage invader survivorship transformed using Anscombe's arcsine transformation (Zar 1999). Each model has a degree of freedom value of four. Cell numbers are parameter estimates of five multiple regressions carried out on invader survivorship vs. the independent variables denoted as the column headings. All individual parameter *P*-values were sequentially compared with a Bonferroni-adjusted alpha of 0.025 to adjust for multiple tests. Statistically significant individual parameter estimates are indicated in bold

(a)		Native		Non-native, flooded plots		Non-native, non-flooded plots	
Census time	Variable	Sign	Parameter estimate	Sign	Parameter estimate	Sign	Parameter estimate
1-year census							
	Shrub d.b.h.	+	0.02	+	0.02	–	0.007
	Shrub distance	+	0.002	–	0.004	–	0.001
	Tree d.b.h.	–	0.007	+	0.03	–	0.004
	Tree distance	–	0.001	+	0.001	–	0.002
		Model $r^2 = 0.0426$, $P = 0.5865$		Model $r^2 = 0.5670$, $P = 0.0822$		Model $r^2 = 0.1098$, $P = 0.3254$	
2-year census							
	Shrub d.b.h.	–	0.05	+	0.05	–	0.004
	Shrub distance	+	0.001	+	0.0003	–	0.0003
	Tree d.b.h.	–	0.001	+	0.05	–	0.004
	Tree distance	+	0.0001	+	0.003	+	0.0001
		Model $r^2 = 0.0625$, $P = 0.3801$		Model $r^2 = 0.5032$, $P = 0.0452$		Model $r^2 = 0.0550$, $P = 0.6874$	

(b)		Native		Non-native	
Census time	Variable	Sign	Parameter estimate	Sign	Parameter estimate
1-year census					
	Shrub d.b.h.	–	0.007	+	0.007
	Shrub distance	+	0.002	+	0.0007
	Tree d.b.h.	–	0.01	+	0.009
		–	0.001	+	0.001
	Tree distance	Model $r^2 = 0.0925$, $P = 0.1172$		Model $r^2 = 0.0843$, $P = 0.1529$	
2-year census					
	Shrub d.b.h.	–	0.007	–	0.004
	Shrub distance	+	0.002	+	0.0007
	Tree d.b.h.	–	0.012	+	0.006
	Tree distance	–	0.001	+	0.0001
		Model $r^2 = 0.0690$, $P = 0.2393$		Model $r^2 = 0.0421$, $P = 0.5281$	

is undoubtedly important to plant establishment and growth in this system.

Interactions between native communities and non-indigenous invaders as mediated by stress or disturbance gradients have not been well studied (Higgins & Richardson 1998). Positive, linear relationships between native plant richness and percentage of non-native species have been found at the whole-river and smaller patch scales in forested upland (100 m²) and mixed canopy flooded sites (50 m²) on rivers in south-western France and in western Oregon (Planty-Tabacchi *et al.* 1996). Observations in a riparian habitat in the south-eastern United States revealed negative relationships between non-native and native plant richness in mixed canopy flooded areas at small scales (0.01 m²) and

positive relationships at larger scales (0.1–100 m²), whereas no richness-invasibility relationships were found in adjacent, forested upland areas at any scale (Brown & Peet 2003). A series of observations of vernal pools in the Central Valley of California led Gerhardt & Collinge (2003) to infer that abiotic constraints limited non-native invasion in stressful environments and interactions with native plants limited invasion into more benign environments. In a field experiment in northern California, Williamson & Harrison (2002) found that invasion success of a non-native grass, *Dactylis glomerata*, was positively correlated with richness in abiotically harsh serpentine meadows and negatively correlated with understorey richness in the more benign oak woodlands. Taken together, these studies suggest a need

Table 4 Individual regressions of available light values of each subplot with invader survivorship, for invaders planted in 1999 and 2000. All analyses are single regressions, conducted with percentage invader survivorship transformed using Anscombe's arcsine transformation (Zar 1999). Cell numbers are parameter estimates of 12 individual regressions carried out on invader survivorship vs. the independent variable of diffuse penetration coefficient, a measurement of light available to each plot. Each model has a degree of freedom value of one. Statistically significant individual parameter estimates are indicated in bold

Census time	Invader input	Native		Non-native	
		Sign	Parameter estimate	Sign	Parameter estimate
1 year	1999	–	0.35	–	1.27
			Model $r^2 = 0.0021$, $P = 0.6852$		Model $r^2 = 0.0537$, $P = 0.0553$
	2000	+	0.41	+	1.18
			Model $r^2 = 0.0039$, $P = 0.5248$		Model $r^2 = 0.0552$, $P = 0.0169$
2 years	1999	+	0.54	–	0.72
			Model $r^2 = 0.0052$, $P = 0.5283$		Model $r^2 = 0.0251$, $P = 0.1810$
	2000	+	0.72	+	0.85
			Model $r^2 = 0.0104$, $P = 0.2963$		Model $r^2 = 0.0408$, $P = 0.0429$

for future tests of the richness-invasibility hypothesis to be carried out across environmental gradients.

In this study, the experimental manipulation of resident species richness had the effect of decoupling native richness levels from extrinsic factors, such as soil nutrient, light or disturbance status. Native species survival and growth responded to resident richness manipulations and were unrelated to original subplot diversity values, while non-native species survival and growth were unrelated to richness treatments and weakly related to the original subplot diversity values. Intrinsic effects of understorey interactions may be more important for native establishment and growth while interactions with understorey natives do not appear to govern non-native establishment in this habitat.

OVERSTOREY COMMUNITY CHARACTERISTICS

One of the strongest predictors of non-native invader survival was overstorey composition. There is a strong overstorey influence on understorey species growth and density in southern Appalachian forests (Runkle & Yetter 1987). Non-native plants had higher survival rates under larger trees in the flooded subplots. This result may simply be a function of the higher survival rates of those plants that were raised above the flooding zone by the elevated microhabitats provided by these root systems. Additionally, the edaphic characteristics surrounding the root zones of these trees may be more suitable for non-native species survivorship. It is possible that the soil biota associated with trees may facilitate invasion by non-native species (Richardson *et al.* 2000; Klironomos 2002). I have observed high recruitment of such species as *Veronica officinalis*, *Prunella vulgaris* and *Cerastium* spp. on the raised, mossy ground immediately surrounding tree roots. Non-native invasion of this forested riparian system is facilitated by trees, whereas native establishment does not seem to depend on overstorey characteristics. Mutualisms between native resident species and invaders can have

stronger influence on community invasibility than the long-held competition-based paradigm that increasing species richness decreases invasibility (Richardson *et al.* 2000). Ultimately, it is important to account explicitly for environmental conditions of the study habitat to understand better the interaction between native biodiversity and invasion by non-indigenous species.

Additional factors other than light availability need to account fully for this overstorey effect. Communities made up of overstorey dominants that cast deep shade and have dense root systems, such as *Tsuga canadensis* and *Rhododendron*, exert powerful microenvironmental effects on the recruiting understorey. Nilsson *et al.* (1999) found in experiments across Europe that leaf litter was a major predictor variable of riparian species density and biomass. Non-native species survival increased under larger trees in the flooded zones, where floods effectively removed the thick leaf litter layer that might otherwise have smothered non-native invaders left under it. In contrast, in the non-flooded zones, where leaf litter was allowed to accumulate, non-native species survival decreased near larger overstorey trees. This indirect overstorey effect has implications for understanding the mechanisms of biotic resistance under a closed-canopy system. It is possible that conditions created by the overstorey are far more important to non-native invader survival than understorey species richness (Von Holle *et al.* 2003).

CONFOUNDING FACTORS

The objective of this experiment was to test Elton's original hypothesis regarding invasion as influenced by species richness. I eliminated or reduced representation of functional groups randomly in naturally occurring communities. Had the difference in richness treatments persisted, it is possible that the richness effects on plot invasibility could have been different. The large native species pool (133 species) available for the construction of communities reduced the likelihood that sampling effects (e.g. variance-reduction, selection probability

effects) resulted from construction of the experimental recipient communities (Huston 1997; Wardle 2001). Very different trends in richness-invasibility relationships might have resulted had I not kept subplot vegetation densities at their original levels. An explanation for the variable support (Levine & D'Antonio 1999) for Elton's (1958) biotic resistance hypothesis might be that researchers often did not control density when experimentally manipulating richness of resident communities (Wetzel *et al.* 2003). Because resident cover and biomass can covary with species richness, 'low richness' experimental plots may also indicate a lower density if density has not been controlled. Many researchers have intimated that resident biomass or cover is the most important determinant of community invasibility (Peart & Foin 1985; Burke & Grime 1996; Crawley *et al.* 1999; Lavorel *et al.* 1999).

Biotic resistance to invasion may depend on the type of species entering the recipient habitat. I randomly collected invader species that pre-existed in the watershed. With the exception of *Rosa multiflora* and *Eleagnus umbellata* (Appendix S2), most of the non-native species found in this stream system were herbaceous dicots, monocots and graminoids, species that could be classified as having a ruderal strategy (Grime 1974). However, other riparian systems have experienced variable invasions by non-native woody species. Planty-Tabacchi *et al.* (1996) found few to no alien trees invading riparian areas in western Oregon and south-western France, respectively. Additionally, there were fewer woody alien species in the American sites than the French sites. This study might have yielded different results, had other non-native plant strategies existed in this system. My 3-year study focuses on the establishment of seedling and adult plants. The previous stage, germination, might have very different community dynamics and a study of this phase might have yielded different results. However, the results from this experiment plainly demonstrate that establishment of seedling and adult native plants is categorically different from that of non-native invaders of this system.

With this experiment, I attempted to uncover differences in richness-invasibility relationships across a flooding disturbance and stress gradient. The clear differences in the richness-invasibility relationships of native and non-native invaders with site conditions suggest a need for exploring the effect of the interaction between biodiversity and the environment on invasibility, rather than simple biodiversity effects on invasibility (Elton 1958). Tracking of individual native and non-native plants exposed contrasting behaviours of plants of different origin under different environments and biotic conditions. The results of this study suggest that non-native species invasion patterns cannot always be inferred by native species invasions, as native and non-native species survivorship trends were influenced by different aspects of the biotic community and site of invasion. In this forested riparian system, biotic effects on invasion success were mediated by environmental conditions.

Acknowledgements

D. Simberloff, S.M. Hermann and M.H. Williamson graciously provided extensive suggestions to improve this research and manuscript. I also thank S.W. Bigelow, R.L. Brown, H.R. Delcourt, D.J. Gibson, L. J. Gross, J.M. Levine, F.E. Putz, J.F. Wetzel and anonymous reviewers for their input on a previous version of this manuscript. E. Campbell, B. Ozimec, C. Bergman, K. Joseph, K. Huffman, C. Miller, C. Nacci and J. Pantel provided crucial field assistance. A.M. Ellison provided statistical advice. Funding was granted by the Department of Ecology & Evolutionary Biology of the University of Tennessee, Sigma Xi, Mountain Lake Biological Station, and The Explorer's Club. I thank Mountain Lake Biological Station for use of their facilities and Jefferson National Forest for land-use permission.

Supplementary material

The following material is available from <http://www.blackwellpublishing.com/products/journals/suppmat/JEC/JEC946/JEC946sm.htm>

Appendix S1 Experimental design for the invaders placed in the 192 subplots sampled in this study.

Appendix S2 Plant species in study site, listed by origin and growth form.

References

- Bertness, M.D. & Callaway, R.M. (1994) Positive interactions in communities: a post cold war perspective. *Ecology*, **72**, 125–137.
- Bertness, M.D. & Leonard, G.H. (1997) The role of positive interactions in communities: lessons from intertidal flats. *Ecology*, **78**, 1976–1989.
- Bridge, S.R.J. & Johnson, E.A. (2000) Geomorphic principles of terrain organization and vegetation gradients. *Journal of Vegetation Science*, **11**, 57–70.
- Brown, R.L. & Peet, R.K. (2003) Diversity and invasibility of southern Appalachian plant communities. *Ecology*, **84**, 32–39.
- Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, **18**, 119–125.
- Burke, M.J.W. & Grime, J.P. (1996) An experimental study of plant community invasibility. *Ecology*, **77**, 776–790.
- Callaway, R.M. & Walker, L.R. (1997) Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology*, **78**, 1958–1965.
- Chapman, R.N. (1931) *Animal Ecology: with a Special Reference to Insects*. McGraw-Hill Park, New York.
- Clinton, B.D. & Boring, L.R. (1994) Regeneration patterns in canopy gaps of mixed-oak forests of the southern Appalachians – influences of topographic position and evergreen understory. *American Midland Naturalist*, **132**, 308–319.
- Crawley, M.J., Brown, S.L., Heard, M.S. & Edwards, G.R. (1999) Invasion resistance in experimental grassland communities: species richness or species identity? *Ecology Letters*, **2**, 140–148.
- Davis, M.A., Grime, J.P. & Thompson, K. (2000) Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology*, **88**, 528–534.
- Dukes, J.S. (2001) Biodiversity and invasibility in grassland microcosms. *Oecologia*, **126**, 563–568.

- Elton, C.S. (1958) *The Ecology of Invasions by Plants and Animals*. Methuen, London.
- Gerhardt, F. & Collinge, S.K. (2003) Exotic plant invasions of vernal pools in the Central Valley of California, USA. *Journal of Biogeography*, **30**, 1043–1052.
- Gleason, H.A. & Cronquist, A. (1991) *Manual of Vascular Plants of Northeastern United States and Adjacent Canada*, 2nd edn. New York Botanical Garden, Bronx, New York.
- Gregory, S.V., Swanson, F.J., McKee, W.A. & Cummins, K.W. (1991) An ecosystem perspective of riparian zones. *Bioscience*, **41**, 540–551.
- Grime, J.P. (1974) Vegetation classification by reference to strategy. *Nature*, **250**, 26–31.
- Higgins, S.I. & Richardson, D.M. (1998) Pine invasions in the southern hemisphere: modelling interactions between organism, environment and disturbance. *Plant Ecology*, **135**, 79–93.
- Hood, W.G. & Naiman, R.J. (2000) Vulnerability of riparian zones to invasion by exotic vascular plants. *Plant Ecology*, **148**, 105–114.
- Huston, M.A. (1997) Hidden treatments in ecological experiments: re-evaluating the ecosystem function of biodiversity. *Oecologia*, **110**, 449–460.
- Klironomos, J.N. (2002) Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature*, **417**, 67–70.
- Lavelle, S., Prieur-Richard, A.-H. & Grigulis, K. (1999) Invasibility and diversity of plant communities: from pattern to processes. *Diversity and Distributions*, **5**, 41–49.
- Leishman, M.R. & Thomson, V.P. (2004) Experimental evidence for the effects of additional water, nutrients and physical disturbance on invasive plants in low fertility Hawkesbury Sandstone soils, Sydney, Australia. *Journal of Ecology*, doi: 10.1111/j.1365-2745.2004.00938.
- Leopold, L.B., Wolman, M.G. & Miller, J.P. (1964) *Fluvial Processes in Geomorphology*. W.H. Freeman, San Francisco.
- Levine, J.M. (2000) Species diversity and biological invasions: relating local process to community pattern. *Science*, **288**, 852–854.
- Levine, J.M. & D'Antonio, C.M. (1999) Elton revisited: a review of evidence linking diversity and invasibility. *Oikos*, **87**, 15–26.
- Lonsdale, W.M. (1999) Global patterns of plant invasions and the concept of invasibility. *Ecology*, **80**, 1522–1536.
- McNab, W.H. & Loftis, D.L. (2002) Probability of occurrence and habitat features for oriental bittersweet in an oak forest in the southern Appalachian mountains, USA. *Forest Ecology and Management*, **155**, 45–54.
- Menges, E.S. & Waller, D.M. (1983) Plant strategies in relation to elevation and light in floodplain herbs. *American Naturalist*, **122**, 454–473.
- Moyle, P.B. & Light, T. (1996) Biological invasions of fresh water: empirical rules and assembly theory. *Biological Conservation*, **78**, 149–161.
- Naeem, S., Knops, J.M.H., Tilman, D., Howe, K.M., Kennedy, T. & Gale, S. (2000) Plant diversity increases resistance to invasion in the absence of covarying extrinsic factors. *Oikos*, **91**, 97–108.
- Nilsen, E.T., Clinton, B.D., Lei, T.T., Miller, O.K., Semones, S.W. & Walker, J.F. (2001) Does *Rhododendron maximum* L. (Ericaceae) reduce the availability of resources above and belowground for canopy tree seedlings? *American Midland Naturalist*, **145**, 325–343.
- Nilsson, C., Xiong, S.J., Johansson, M.E. & Vought, L.B.M. (1999) Effects of leaf-litter accumulation on riparian plant diversity across Europe. *Ecology*, **80**, 1770–1775.
- Norman, J.M. & Campbell, G.S. (1989) Canopy structure. *Plant Physiological Ecology: Field Methods and Instrumentation* (eds R.W. Pearcy, J. Ehleringer, H.A. Mooney & P.W. Rundel), pp. 301–325. Chapman & Hall, London.
- Peart, D.R. & Foin, T.C. (1985) Analysis and prediction of population and community change: a grassland case study. *The Population Structure of Vegetation* (ed. J. White), pp. 313–339. Junk, Dordrecht.
- Planty-Tabacchi, A.M., Tabacchi, E., Naiman, R.J., Deferrari, C. & Decamps, H. (1996) Invasibility of species rich communities in riparian zones. *Conservation Biology*, **10**, 598–607.
- Rice, W.R. (1989) Analyzing tables of statistical tests. *Evolution*, **43**, 223–225.
- Richardson, D.M., Allsop, N., Antonio, C.D., Milton, S.J. & Rejmanek, M. (2000) Plant invasions – the role of mutualisms. *Biological Review*, **75**, 65–93.
- Robinson, G.R., Quinn, J.F. & Stanton, M.L. (1995) Invasibility of experimental habitat islands in a California winter annual grassland. *Ecology*, **76**, 786–794.
- Runkle, J.R. & Yetter, T.C. (1987) Treefalls revisited – gap dynamics in the southern Appalachians. *Ecology*, **68**, 417–424.
- SAS (2001) *SAS System For Microsoft Windows*. SAS Institute, Cary, North Carolina.
- Shea, K. & Chesson, P. (2002) Community ecology theory as a framework for biological invasions. *Trends in Ecology and Evolution*, **17**, 170–176.
- Siemann, E. & Rogers, W.E. (2003) Changes in light and nitrogen availability under pioneer trees may indirectly facilitate tree invasions of grasslands. *Journal of Ecology*, **91**, 923–931.
- Simberloff, D. (1986) Introduced insects: a biogeographic and systematic perspective. *Ecology of Biological Invasions in North America and Hawaii*, Volume 58 (eds H.A. Mooney & J.A. Drake), pp. 3–24. Springer-Verlag, New York.
- Stohlgren, T.J., Binkley, D., Chong, G.W., Kalkhan, M.A., Schell, L.D., Bull, K.A. *et al.* (1999) Exotic plant species invade hot spots of native plant diversity. *Ecological Monographs*, **69**, 25–46.
- Suiter, D.W. & Evans, D.K. (1999) Vascular flora and rare species of New River Gorge National River, West Virginia. *Castanea*, **64**, 23–49.
- Tilman, D. (1997) Community invasibility; recruitment limitation; and grassland biodiversity. *Ecology*, **78**, 81–92.
- Trianosky, P.A. (1994) *A Classification of the Terrestrial Plant Communities of West Virginia*. West Virginia Natural Heritage Program Technical Report 94–2. West Virginia Division of Natural Resources, Elkins, WV, USA.
- Underwood, A.J. (1997) *Experiments in Ecology: Logical Design and Interpretation Using Analysis of Variance*. Cambridge University Press, New York.
- Vermeij, G.J. (1996) An agenda for invasion biology. *Biological Conservation*, **78**, 3–9.
- Von Holle, B. (2002) *Ranking the components of ecological resistance to plant invaders: an experimental manipulation in a Southern Appalachian riparian forest*. PhD thesis, University of Tennessee, Knoxville.
- Von Holle, B., Delcourt, H.R. & Simberloff, D. (2003) The importance of biological inertia in plant community resistance to invasion. *Journal of Vegetation Science*, **14**, 425–432.
- Von Holle, B. & Simberloff, D. (2004) Testing Fox's assembly rule: does plant invasion depend on recipient community structure? *Oikos*, **105**, 551–563.
- Wardle, D.A. (2001) Experimental demonstration that plant diversity reduces invasibility – evidence of a biological mechanism or a consequence of sampling effect? *Oikos*, **95**, 161–170.
- Weltzin, J.F., Muth, N., Von Holle, B. & Cole, P.G. (2003) Genetic diversity and invasibility: a test using a model system with a novel experimental design. *Oikos*, **103**, 505–518.
- Williamson, J. & Harrison, S. (2002) Biotic and abiotic limits to the spread of exotic revegetation species. *Ecological Applications*, **12**, 40–51.
- Zar, J.H. (1999) *Biostatistical Analysis*, 4th edn. Prentice Hall, Englewood Cliffs, New Jersey.

Received 1 April 2004

revision accepted 1 September 2004

Handling Editor: David Gibson