Shifts in morphological traits, seed production, and early establishment of *Desmodium nudiflorum* following prescribed fire, alone or in combination with forest canopy thinning

Jianjun Huang and Ralph E.J. Boerner

**Abstract:** Reintroduction of periodic dormant-season fire and overstory thinning are currently being employed for forest ecosystem management in deciduous forests of eastern North America. These manipulations usually alter the flux of light and the availability of soil nutrients to the perennial herbaceous plants that dominate the understory. We utilized Bayesian statistical methods to examine the effects of prescribed burning (B) and the combination of burning and overstory thinning (T+B) on the morphology, seed production, and early establishment of *Desmodium nudiflorum* (L.) DC. (Fabaceae) in mixed-oak (*Quercus* spp.) forests in southern Ohio. During the fourth growing season after the first fire, plants from thinned and burned (T+B) plots were 62% larger than plants from control plots (C). Both burning alone (B) and T+B treatments decreased specific leaf area (SLA). T+B also resulted in significantly decreased root mass ratio (RMR), and increased leaf mass ratio (LMR), and specific root length (SRL). During the first growing season after a second fire, both B and T+B resulted in significantly increased plant biomass, LMR, individual seed mass, and total seed production, as well as decreased SLA and plant height; in contrast, neither B nor T+B had significant impacts on leaf area ratio or seedling establishment. Prescribed fire, especially when combined with thinning, can result in increases in total biomass, seed size, and seed production, and hence enhance the fitness of this perennial herb in these mixed-oak forests.

**Key words:** Bayesian, biomass, herb, mass ratio, nodule, specific leaf area.

Introduction

Mixed-oak (*Quercus* spp.) and oak–hickory (*Quercus–Carya*) forests have dominated the Appalachian region of eastern North America for millennia. Analysis of pollen and charcoal deposits in the region indicates that fire has been a primary driver in shaping the forests of the region (Delcourt and Delcourt 1997). However, since the implementation of effective fire suppression in early 1900s in this region (Sutherland 1997), the forests have become denser with more shade-tolerant and fire sensitive species (Lorimer 1984). In response to the perception that such changes are undesirable, dormant-season fire and mechanical thinning in various combinations have been reintroduced on an experimental basis to determine whether such practices are sufficient to restore pre-fire-suppression forest structure and ecological processes.

Herbaceous plants may serve a special role in indicating ecosystem health and maintaining the functional integrity of
forest ecosystems (Gilliam and Roberts 2003); however, there have been few studies examining the fitness of individual herbaceous species, although some studies have quantified fire (thinning) effects on herbaceous communities (Hutchinson et al. 2005). Prescribed burning and canopy thinning may increase the richness and abundance of herbaceous species by scarifying seeds present in the persistent seed bank, by providing favorable micro-environments for germination and establishment, and by decreasing competition with the shrub and sapling strata (Kauffman 1990; Wroblewski and Kauffman 2003). In addition, herbaceous perennials may exhibit phenotypically plastic responses to the changes in resources afforded by burning and (or) thinning that allow for greater fitness in the altered environments (e.g., Knapp 1985).

From the perspective of an understory herb, a lengthy period of fire exclusion results in decreased light flux and altered soil nutrient availability. Similarly, ecological restoration treatments, such as reintroduction of dormant-season fire and canopy thinning to return the woody plant assemblage to pre-suppression characteristics, may both increase light, soil temperature, and also alter soil moisture and nutrient availability (National Wildfire Coordinating Group 2001). Therefore, the ability of herbaceous plants to survive, reproduce, and establish in these different environments depends on their exhibiting an integrated response in morphology, physiology, and reproduction.

As part of a larger study of the response of the long-lived herbaceous perennial Desmodium nudiflorum (L.) DC. to ecosystem restoration treatments in Ohio mixed-oak forests, this study examined the effects of prescribed burning (B) and the combination of burning and thinning (T+B) on the morphology and reproduction of this species. The specific objectives include (i) to test the hypothesis that forest manipulation activities such as prescribed burning and the combination of burning and thinning will increase the fitness of this moderately shade-tolerant perennial herb (e.g., enhancing plant biomass, seed size, total seed production), (ii) to examine the effects of prescribed fire alone or combined with thinning on morphological traits and biomass distribution at 1 year and 4 year post-burn, and (iii) to evaluate the effects of altered environmental conditions following forest management activities on early seedling establishment.

Materials and methods

Study sites

This study was conducted in the mixed-oak forests of Zaleski State Forest (82°37’W, 39°35’N) and the Raccoon Ecological Management Area (REMA) (39°20’N, 82°39’W) on the unglaciated Allegheny Plateau of southern Ohio. These two study sites are part of the Ohio Hills – Central Appalachian Plateau site of the National Fire and Fire Surrogate (FFS) Study (frames.nbii.gov/FFS).

The climate of the region is cool, temperate, and continental with a mean annual temperature of 11.3 °C and mean annual precipitation of 1024 mm (Sutherland and Hutchinson 2003). The sites are underlain predominantly by sandstones and shales of Pennsylvanian Age. The soils were formed in place from residuum and colluvium, and are dominated by Steinsburg and Gilpin series silt loams (typic ha-pludalfs) (Lemaster and Gilmore 1993). The topography of these sites is dissected and often steep; strong microclimatic gradients of temperature, moisture, and solar radiation are common across this landscape (Wolfe et al. 1949).

The forests of these study sites regenerated between 1850 and 1900, after the cessation of cutting for the charcoal and iron industry (Hutchinson et al. 2003). The most abundant species in the current canopy are white oak (Quercus alba L.), chestnut oak (Quercus prinus L.), hickories (Carya spp.), and black oak (Quercus velutina Lam.) (Yaussy et al. 2003).

Study species

Desmodium nudiflorum, a native perennial herb, was selected because it was abundant in the study areas (frequency > 40%, Hutchinson et al. 2005). It occupied habitats across the light and nutrient gradients produced by the strongly dissected landforms, but with greater frequency on drier sites.

The aboveground parts senesce during autumn, and new leaves typically do not emerge until May. Dormant-season (March–April) fire generally has no direct effect on D. nudiflorum plants, since little or no heat produced by the fires penetrates into the mineral soil to the 5–10 cm depth where the overwintering buds and rootstocks lie (Raison 1979). Mature plants produce a whorl of trifoliate leaves, each of which is 5–10 cm long. Adult plants of D. nudiflorum usually have one infertile stem (foliar stem) and one (or two) fertile stem (flowering stem).

Flowering begins in early August. Each flower is short-lived, but within an inflorescence flowers develop sequentially over several weeks. Loments (seed pods) form in late August, usually producing 2 to 3 seeds/loment. Many of the seeds commonly have under-developed embryos (the embryo is flat and shorter than half of the seed length). Fruit coats are covered with abundant sticky trichomes, which facilitate seed dispersal by sticking to animal fur.

Field methods

The overall FFS experimental design is a randomized complete block with four treatments (control, dormant season prescribed burning, thinning of the canopy from below to presettlement basal area and species composition, and thinning plus burning) randomly assigned to treatment units of 20–30 ha in three areas of southern Ohio (Tar Hollow State Forest, Zaleski State Forest, and the Raccoon Ecological Management Area (REMA)). Each treatment unit included the full range of topographic relief. In each treatment unit, 10 permanent 0.1 ha (20 m x 50 m) plots were randomly established for long term monitoring. The positions of the sample plots were established by GPS, mapped on a digital elevation map, and assigned a GIS-based integrated moisture index (hereinafter, IMI) score developed as a measure of landscape-scale variation in potential soil moisture by Iverson et al. (1997) for this region. IMI scores were achieved through integration of elevation, aspect, hill shade profile, solar radiation potential, downslope flow accumulation, soil depth, soil water holding capacity, and curvature profile (Iverson et al. 1997).

Mechanical thinning was done during late fall 2000 and early spring 2001. Thinning from below removed an average
of 27.9% of the basal area and left an average of 20.9 m²/ha in residual basal area (D. Yaussy, USDA Forest Service, personal communication). Prescribed fire was applied in early April 2001 and again in March–April 2005. Fire intensity was heterogeneous within and across sites and among years. In 2001, fires burned across the units completely, but dry sites tended to burn more intensely (Iverson et al. 2004); these fires caused very little overstory tree mortality and consumed primarily unconsolidated litter and fine woody fuels. However, in 2005, fires were more intense at both sites, particularly at REMA, where flame lengths were often 1.5–3 m, resulting in significant patches of overstory mortality and the consumption of coarse woody fuels (T. Hutchinson, USDA Forest Service, personal communication).

We sampled three treatment units [control (C), burn (B), thin+burn (T+B)] in two of the study sites (Zaleski and REMA). From mid-July through late August 2004, we sampled plants in 7–9 randomly chosen plots of the 10 permanent plots in each treatment unit, and from early June through late July 2005, we sampled plants in 6 randomly chosen plots of the 10 permanent plots in each treatment unit. In total, 49 and 36 plots were sampled in 2004 and 2005, respectively. Within each plot, 3–5 plants of *D. nudiflorum* were selected on each sampling date using the following criteria: (i) to avoid cluster effects, plants were at least 8 m apart; and (ii) newly emerged seedlings and individuals with two or more infertile stems were avoided. After the height of the infertile stem was measured in the field, each whole plant was excavated with care to preserve the integrity of all root tissues. Extracted plants were returned to the laboratory whole.

Loment of *D. nudiflorum* form in late August. Individual seeds within loments develop sequentially with the seeds farthest from the pedicel being the first to mature. Full loment development can take ≥4 weeks. During this time, some seeds can be lost due to gravity, sticking to animal fur, and fragmentation. Therefore, to estimate seed number and seed weight, we bagged (starting in late August) the fertile stems of at least four randomly selected plants per plot using 1 mm mesh nylon bags. Seeds were harvested in early November 2005.

In summer 2006, we sampled 5–6 *D. nudiflorum* in 6 randomly chosen plots of the 10 permanent plots in each treatment unit. Plants were carefully excavated and the number of root nodules was recorded in the field.

**Lab methods**

Leaves (lamina), stems (stem and rachis), coarse roots (≥ 2 mm), and fine roots (<2 mm) of each individual plant were separated. Leaf area was determined using LI-COR 3100 leaf area meter (LI-COR Inc., Lincoln, Nebr.). Fine roots were scanned and the root length was estimated using Root Edge software (Kaspar and Ewing 1997). Coarse root length was measured using a ruler. All parts were oven-dried at 70 °C for 2–3 d, and then weighed. Specific leaf area was calculated as the ratio of leaf area to dry leaf mass. Specific root length was calculated as the total root length (coarse root length + fine root length) divided by the total root mass.

**Seedling establishment**

We had planned to determine the effects of both seed characteristics (from different treatments) and environmental conditions (different light availability and soil moisture and nutrient conditions) on seedling establishment in a full factorial experiment; however, owing to limited seed production by plants in the control plots, we used seeds from the burn-only plots in the Zaleski study area to evaluate the effects of altered environmental conditions on seedling establishment.

Seeds were collected in October 2005 and stored in a refrigerator at 4–5 °C over winter. In late April 2006, we randomly established three 50 cm × 50 cm subplots within 6 randomly chosen plots of the 10 permanent plots in each treatment unit. The top 10 cm of soil in each subplot was excavated, sieved (to remove roots and seeds), and replaced in the subplot. Then, 25 seeds were evenly sown approximately 1.0 cm below the soil surface in the center 30 cm × 30 cm area of the subplot. Four red flags were placed at the four corners of each subplot to facilitate later recovery. Established seedlings were recorded in early August 2006, approximately 4 months after sowing.

**Statistical analysis**

Bayesian methods of statistical inference, which are based on a different philosophy about the nature of truth and probability than are analysis of variance-based methods, are becoming more frequently used in ecology (Calder et al. 2003; Gelfand et al. 2006; Williams et al. 2006, and reviews by Ellison 2004; Clark 2005). Bayesian inference is particularly useful in answering many important ecological questions because Bayesian approaches incorporate both existing data and the uncertainty and complexity of ecological processes (Ellison 2004). In addition, implementing many models is often easier in a Bayesian framework (Clark 2005).

All plant variables were averaged for each sample plot, and plot means were then analyzed as response variables. We considered 2004 as the fourth growing season after the 2001 fire, and 2005 as the first growing season after the 2005 fire. We analyzed the responses to the two fires independently, rather than as a repeated measures design, as prior studies in neighboring sites have demonstrated that fires 3–4 years apart in this ecosystem type are statistically independent events (Boerner et al. 2004). Study site (REMA, Zaleski) was considered to be a random factor and treatment was included as fixed effect. The 0.1 ha plots (*n* = 12–17/treatment, rather than individual plants) were considered to be the experiment unit in this study and were therefore considered replicates. The spatial independence of sample plots and the appropriateness of this statistical design have been demonstrated in these study sites (Boerner and Brinkman 2004; Boerner et al. 2005).

Since many morphological variables (e.g., leaf mass ratio and root mass ratio) may follow allometric trajectories and, therefore, change with plant size (Müller et al. 2000; Shipley and Meziane 2002; Weiner 2004), the direct comparison of the average of each parameter would confound the effects of treatment with that of plant size, and thus provide a biased estimation of the treatment effects. Therefore, we first analyzed plant biomass as a response variable as an additive function of site, treatment, and IMI score; then we analyzed all other responses by including plant biomass as
covariate. Since IMI score was correlated with plant biomass, and adding this term in the regression model did not yield smaller Aikake’s information criterion (AIC; Burnham and Anderson 2002), IMI score was therefore removed from the model (see also Table 1 for definition of abbreviations and units of measurement).

The model was described as:

Data level (eq. 1): $Y_{ijk}/\mu_{ijk}, \sigma \sim N(\mu_{ijk}, \sigma^2)$ where $Y_{ijk}$ is the response variable in the $k$th plot in the $i$th treatment at the $j$th site, $\mu_{ijk}$ is the expected mean response, $\sigma^2$ is the variance related with $\mu$.

Process level (eq. 2): $\mu_{ijk} = \alpha_i + \beta_1 \times B_{ijk} + \beta_2 \times TB_{ijk} + \beta_3 \times TSB_{ijk}$ where $\alpha_i$ is the random effects of the $i$th site, $\beta_1$ is the burning effect, $\beta_2$ is the effect of burning combined with thinning, and $\beta_3$ is the effect of plant biomass. $B_{ijk}$ represents burning treatment and is coded as $B = 1$ if the plots are from the burning treatment, otherwise $= 0$. $TB_{ijk}$ represents burning combined with thinning treatment and it is coded as $TB = 1$ if the plots are from burning combined with thinning treatment, otherwise $= 0$. $TSB$ (plant biomass) is included as a covariate. (Wilk 2003)

Parameter level: prior distribution of $1/\sigma^2$ is from gamma distribution $\sim \Gamma(0.001, 0.001)$

$\beta_1, \beta_2,$ and $\beta_3$ are from normal distribution $\sim N(0, 10^6)$
$\alpha_i$ is from normal distribution $\sim N(0, \sigma^2)$, where $\sigma_i^2$ is the variance associated with $\alpha_j$ and $1/\sigma^2 \sim \Gamma(0.001, 0.001)$.

The parameters were estimated using WinBUGS version 1.4, which implements Markov chain Monte Carlo (MCMC) methods using a Gibbs sampler (Spiegelhalter et al. 2003). To ensure the target “convergent” posterior distribution was sampled, parameter estimates were based on 10,000 sampling iterations after initial “burn in” of 10,000 iterations. The 2.5th and 97.5th percentiles were calculated to describe the 95% Bayesian credible interval.

**Results**

In 2004 (the fourth growing season after first fire), prescribed fire combined with thinning had a significant positive effect on total plant biomass (Fig. 1A). Total plant biomass was negatively correlated with SLA, LAR, LMR, SRL, RLR and positively correlated with the number of lamina (Table 2). When this biomass effect was included as a covariate in the analysis of the effects of the manipulative treatments, prescribed fire alone (B) or combined with thinning significantly (T+B) resulted in decreased SLA (Table 2, Fig. 1B). Fire combined with thinning (but not prescribed burning alone) resulted in significantly decreased RMR and increased LMR and SRL (Table 2).

In 2005 (the first growing season after second fire), both B and T+B resulted in increased total plant biomass. Mean total plant biomass of C plants was only 55% and 47% of those in B and T+B plants, respectively (Fig. 2A). Plant biomass was positively correlated with number of lamina and plant height, and negatively correlated with SLA and LAR (Table 2). Both manipulative treatments resulted in decreased SLA (Fig. 2B) and plant height (Table 2). In contrast, the number of lamina, LAR, and RMR were not affected significantly by the manipulative treatments when biomass was considered as covariate (Table 2). Plants from T+B plots had more root nodules than plants from C plots (Table 2).

In 2005, ranges in individual seed mass were 3.7–13.4 mg/seed in C, 4.3–16.6 mg/seed in B, and 7.6–16.2 mg/seed in T+B treatments. Individual seed mass size was significantly greater in T+B and B plots than in C plots (Fig. 3A). Plants from the manipulated plots produced sig-

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**Table 1. Definition of abbreviations and units of measurement.**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
<th>Unit</th>
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<tbody>
<tr>
<td>TPB</td>
<td>Plant biomass per individual</td>
<td>g</td>
</tr>
<tr>
<td>NL</td>
<td>Number of lamina</td>
<td>n</td>
</tr>
<tr>
<td>SLA</td>
<td>Specific leaf area</td>
<td>cm².g⁻¹</td>
</tr>
<tr>
<td>LAR</td>
<td>Leaf area ratio</td>
<td>cm².g⁻¹</td>
</tr>
<tr>
<td>LMR</td>
<td>Leaf mass ratio</td>
<td>g/g</td>
</tr>
<tr>
<td>SRL</td>
<td>Specific root length</td>
<td>cm.g⁻¹</td>
</tr>
<tr>
<td>RLR</td>
<td>Root length ratio</td>
<td>cm.g⁻¹</td>
</tr>
<tr>
<td>RMR</td>
<td>Root mass ratio</td>
<td>g/g</td>
</tr>
<tr>
<td>TSB</td>
<td>Total seed biomass per plant</td>
<td>mg</td>
</tr>
<tr>
<td>SW</td>
<td>Individual seed mass</td>
<td>mg</td>
</tr>
<tr>
<td>SE</td>
<td>Seeding establishment rate</td>
<td>%</td>
</tr>
<tr>
<td>NN</td>
<td>Number of root nodules</td>
<td>n</td>
</tr>
</tbody>
</table>

**Note:** TSB was log transformed and indicated as ln TSB in all subsequent data analysis, while all other variables were left untransformed.

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Fig. 1. (A) Total plant biomass and (B) specific leaf area responses of Desmodium nudiflorum to different forest manipulative treatments in 2004 (fourth growing season after fire) in two southern Ohio forests. Boxplots with means (broken line) are presented. Whiskers indicate the 10th and 90th percentiles. Boxes with different letters are significant different at p < 0.05.
nificantly more seeds per plant and greater total seed bio-
mass than did control plants (Fig. 3B).

Net seedling establishment of *D. nudiflorum* over 4
months in the field was low (mean 5.2%). The altered envi-
ronmental conditions caused by burning alone or combined
with thinning had no significant impacts on seedling estab-
lishment rate (Table 2).

### Discussion

#### Biomass, seed size, nodules, and seed germination rate

Plants from manipulated sites were significantly larger
than plants in control sites. Two factors likely contributed
to this difference. First, fires in oak forests leave a black-
ened litter layer on the forest floor, which has been shown
to increase soil temperatures, particularly in early spring
prior to canopy leaf-out (Iverson and Hutchinson 2002).
Higher spring soil temperatures during a period when light,
moisture, and nutrient availability are high may trigger an
earlier onset of growth. Second, burning alone or combined
with thinning increases light penetration and reduces compe-
tition by top-killing shrub and small tree stems, and hence
enhances the photosynthetic rate of this species (Huang et
al. 2007).

Since seed size and production are closely linked with
both plant biomass and photosynthesis, it is not surprising
that plants from manipulated sites produced significantly
larger seeds. In contrast to previous reports that seed mass
and seed number are negatively correlated owing to plastic
responses to resource stress (Werner and Platter 1976), we
found that plants in the manipulated sites had both larger
seeds and more seeds than control plants. In addition, within
a given treatment there was no significant relationship be-
tween individual seed mass and seed number, thus our re-
results do not support the hypothesis that these plants are
faced with an energetic trade-off between individual seed
mass and total seed number. In the control areas, resources
(especially light during the seed maturation period) are so
limited that only a very few, small seeds can be supported.
In turn, in the manipulated sites, resource availability is en-
hanced to such a degree as to reduce resource-based con-
straint on reproductive output.

### Table 2. 95% credible intervals for posterior effects of burning, burning combined with thinning, and biomass in eq. 2.

<table>
<thead>
<tr>
<th></th>
<th>( \beta_1 ) (Burn alone)</th>
<th>( \beta_2 ) (Thin and burn)</th>
<th>( \beta_3 ) (Biomass)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LCB</td>
<td>UCB</td>
<td>LCB</td>
</tr>
<tr>
<td><strong>2004</strong> (fourth year after fire)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>-1.29</td>
<td>1.92</td>
<td>0.06</td>
</tr>
<tr>
<td>SLA</td>
<td>-88.13</td>
<td>-12.04</td>
<td>-122.3</td>
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<td>LAR</td>
<td>-19.14</td>
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</tr>
<tr>
<td>LMR</td>
<td>-0.02</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>SRL</td>
<td>-23.85</td>
<td>41.78</td>
<td>2.7</td>
</tr>
<tr>
<td>RLR</td>
<td>-14.28</td>
<td>14.4</td>
<td>-3.01</td>
</tr>
<tr>
<td>RMR</td>
<td>-0.08</td>
<td>0.01</td>
<td>-0.16</td>
</tr>
<tr>
<td><strong>2005</strong> (first year after second fire)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NL</td>
<td>-2.79</td>
<td>3.32</td>
<td>-2.5</td>
</tr>
<tr>
<td>SLA</td>
<td>-253.8</td>
<td>-116.7</td>
<td>-244.8</td>
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<tr>
<td>LAR</td>
<td>-28.56</td>
<td>3.61</td>
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<tr>
<td>LMR</td>
<td>0.01</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>RMR</td>
<td>-0.09</td>
<td>0.01</td>
<td>-0.1</td>
</tr>
<tr>
<td>height</td>
<td>-10.08</td>
<td>-5.51</td>
<td>-11.78</td>
</tr>
<tr>
<td>lnTSB</td>
<td>1.36</td>
<td>3.29</td>
<td>1.36</td>
</tr>
<tr>
<td>SW</td>
<td>2.47</td>
<td>8.88</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>2006</strong> (2nd year after second fire)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE (%)a</td>
<td>-2.50</td>
<td>8.41</td>
<td>-0.32</td>
</tr>
<tr>
<td>NNa</td>
<td>-0.46</td>
<td>5.20</td>
<td>2.95</td>
</tr>
</tbody>
</table>

Note: LCB and UCB represent the lower and upper credible band, respectively. 95% credible intervals not including zero are indicated in bold type.

*Plant biomass was not included as a covariate in the regression model (eq. 2).
inspection from both 2004 and 2005 suggests plants from T+B plots had considerably more nodules than did plants in C plots (Huang 2007). Thus, in 2006, as expected, T+B resulted in more nodule production. Enhanced photosynthetic rate, resulting from increased light availability, and increased soil temperature and moisture may have contributed both additional fixed C and additional N uptake needs that resulted in more intensive nodule development in plants from T+B plots.

Altered light, nutrient availability, and soil temperature and moisture usually have an important influence on seed germination and establishment rate (Silvertown 1980; Baskin and Baskin 1988; Isselstein et al. 2002). In our study, thinning increased the percentage of open sky from approximately 8% (C and B plots) to approximately 15% (T+B plots) (T. Hutchinson and D. Yaussy, USDA Forest Service, personal communication.). In addition, soil total inorganic N and plant available P were lower in B and T+B plots than in controls (Huang and Boerner 2007). We found that the enhanced light penetration and decreased nutrient availability that resulted from these forest management activities had no significant impacts on early establishment rate of *D. nudiflorum*. It should be noted, however, that *D. nudiflorum* exhibited a low rate of net germination and establishment over the 4 month growing period. The limited number of seeds that we were able to deploy in this study, inefficient breaking of seed dormancy during the overwintering refrigeration, and the length of time between sowing the seeds and enumerating established seedlings might have combined to limit the generalization of the effects of altered environment conditions (e.g., light availability, soil temperature, moisture, and nutrient availability) on the seedling establishment rate of this herb.

The positive influence of fire alone and in combination with thinning on plant biomass, seed size, and total seed production should increase the abundance of this herbaceous species in the post-fire years. Since larger biomass and seed size are often positively related with plant fitness (Harper 1977), we expect fire, especially combined with thinning, to increase the fitness of this herbaceous species. Therefore, a re-introduction of periodic fire in this ecosystem should result in a long-term increase in the abundance of *D. nudiflorum* in the mixed-oak forest ecosystem type.

**Morphological traits and biomass distribution**

A plant’s specific leaf area (SLA) is assumed to reflect an integrated response to the light, soil water, and soil nu-
tient conditions under which those leaves are produced (Lambers et al. 1998). Our results showed that burning and thinning+burning decreased SLA of this herb. We feel there are two factors at work here. First, summer soil moisture was significantly lower on burned than control plots (Iverson and Hutchinson 2002) and this may have led to greater midsummer water stress in B than in C plots. To acclimate to this, Desmodium plants may have reduced SLA to minimize water loss. Second, increased canopy light penetration after thinning leads to thicker leaves and therefore reduced SLA (Lambers et al. 1998). Although this plastic response appears to be present in this perennial herb, it should not be assumed that this is a general response of all plants growing in the forest understory. For example, previous studies have found that burning may either increase or not affect SLA in oak seedlings (Boerner et al. 1988; Reich et al. 1990).

Greater SRL is often assumed to be an advantage in nutrient-poor habitats (Lambers and Poorter 1992). Consistent with that assumption, we found that burning, combined with thinning, both resulted in lowered nutrient availability and in increased Desmodium SRL.

The relative biomass allocation to roots and aboveground plant parts presumably reflects the light and soil nutrient environments to which the plant is exposed. Balanced-growth theory predicts that plants allocate more biomass to leaf production to gain maximum growth rate when light becomes more limited; allocation is shifted to roots if belowground resources, such as water or N, are relatively more limited than light (Shipley and Meziane 2002; Reich 2002). Although our study did not directly measure biomass allocation to each plant part within a given period, we hypothesized that (at any given plant biomass) larger LMR, coupled with smaller RMR would be more advantageous for plants in C plots because such sites are characterized by low light and relatively high nutrient availability (Huang and Boerner 2007). In contrast to that expectation, when total plant biomass was factored into the analysis as a covariate, the increased light and decreased nutrient availability present in T+B plots (relative to C) resulted in either decreased RMR or increased allocation to leaves. In addition, the allometric relationship (Reich 2002; Shipley and Meziane 2002) between log-transformed root biomass and log-transformed leaf biomass also revealed the same pattern. Previous studies that have compared plants under varying light conditions reported that plants had allocated a greater fraction of biomass to leaf mass in low light conditions (Olff et al. 1990; Lei and Lechowicz 1998; reviews by Poorter and Nagel 2000; Reich 2002). We suggest that N-fixation by rhizobial symbionts of Desmodium might play an important role in explaining this apparent contradiction. The root systems of Desmodium plants from burned plots (especially T+B) had significantly more nodules than did those from the C site, and the resultant N-fixation may serve to reduce any soil nutrient limitation on growth. In addition, T+B plots should experience increased soil water availability as a result of the decreases in both canopy interception and plant transpiration following thinning of the canopy (Swift et al. 1993; Brêda et al. 1995). Since N-fixation, and perhaps enhanced mycorrhizal colonization, effectively remove potential sources of belowground resource limitation, plants from T+B plots can spend more biomass on leaves to fix more carbon.

At the same time, we observed greater SLA and larger plant height in the C plants than in plants from B or T+B plots. Thus, our results show that in unmanaged control sites where light flux density was low, this herb tended to maximize carbon gain by increasing SLA and plant height, instead of by increasing leaf mass ratio. It is clear, then, that as resource availability changes, these plants can alter their carbon gain and allocation by modifying combinations of SLA, LMR, and plant height to maximize growth potential.

A comparison of the morphological responses of these plants in the first year after a (second) fire with those in the fourth year after a (first) fire showed that morphological differences between control and manipulated sites tended to decrease with time after fire. Plants from manipulated plots exhibited a greater range of variation in their morphological traits shortly after fire, and that variation tended to decrease with time. We suggest that the large morphological variation immediately after the 2005 fires resulted from the highly variable fire intensities across these watersheds, which in turn resulted in heterogeneous effects on the forest floor microclimate (e.g., litter depth, soil temperature) and canopy transmission of light. It should be noted, however, that interannual variations in weather and in fire intensity across the sampling years may have also contributed to the decreasing treatment effects with time after fire; unfortunately our experimental design does not allow us to separate these random effects from the effects of time post-fire.

Since most of the morphological traits we examined were associated with plant biomass, considering biomass as a covariate can minimize the bias that can be introduced by total plant size, although the observation that the ranges of plant sizes present in the three treatments in 2005 were quite distinct indicates that such adjustment may not have been sufficient to test for treatment effects in 2005. However, among all the morphological traits we examined, SLA was consistently larger in the C plants than plants from manipulated sites for both the fourth season after fire and the first season after repeated fire, and we suggest that SLA can be considered as an important indicator or "functional marker" (as suggested by Garnier et al. 2004) for this perennial herb; this hypothesis needs further testing to determine its generality.

In conclusion, this study provided evidence for acclimative differentiation in response to light and soil resource changes following fire (with or without thinning). Among all the morphological traits examined, SLA was the most powerful functional marker to describe treatment effects. If seed size and seed production can be considered as performance measures related to fitness, we can also conclude that either fire alone or in combination with thinning can increase the fitness of this herbaceous species in the mixed-oak forest ecosystem.

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