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Experimental Removal of the Non-indigenous Shrub *Rhamnus frangula* (Glossy Buckthorn): Effects on Native Herbs and Woody Seedlings

BRIAN FRAPPIER^{1,*}, ROBERT T. ECKERT¹, AND THOMAS D. LEE¹

Abstract - Effects of the non-indigenous shrub *Rhamnus frangula* L. (glossy buckthorn) on tree recruitment, herb cover, forest floor plant species richness, and *R. frangula* recruitment were tested in two southeastern New Hampshire *Pinus* forests using a randomized complete-block field experiment. The treatment, applied in January of 2000, was the presence of well-established *R. frangula* populations with three levels: *R. frangula* absent prior to experiment (“uninvaded”), > 90% *R. frangula* cover (“*Rhamnus* present”), and removal of > 90% *R. frangula* cover (“*Rhamnus* removed”). After 2 years of measurements, *Rhamnus* present had significantly lower first-year native tree seedling density than *Rhamnus* removed and uninvaded plots (0.11, 0.40, and 0.40 seedlings/m² respectively). First-year native tree seedling density in the *Rhamnus* removed and uninvaded treatments were similar. Neither percent herb cover nor plant species richness were significantly affected by the removal of *R. frangula* in the two years following treatment. We believe these results indicate that the presence of dense *R. frangula* inhibits the establishment of tree seedlings. *Rhamnus* removed plots sampled one year after removal had five-fold greater first-year *R. frangula* seedling density than the other treatments. However, after two years first-year *R. frangula* seedling density was similarly low in all treatments (< 0.5 *R. frangula* seedlings/m²). Control efforts for *R. frangula* may need to focus on conspecific seedling emergence for at least two years following initial control.

Introduction

Rhamnus frangula L. (Rhamnaceae), a shrub native to Europe (Gleason and Cronquist 1991), has become a widespread invasive plant in the northeastern United States and adjacent Canada (Catling and Porebski 1994, Converse 1984). Its range will likely continue to expand in North America, becoming abundant in open and semi-open wetlands and some upland woodlands (Catling and Porebski 1994). *Rhamnus frangula* inhabits a wide range of soil and soil moisture conditions (Converse 1984). The fruit is a small, black berry with 2–3 seeds; production is abundant, ranging between 430 and 1804 fruit per genet in one study (Medan 1994). Besides humans who plant for landscaping purposes, birds are the most likely long-range dispersal vectors, possibly including European Starlings, Grosbeaks, Cedar Waxwings, and American Robins (Catling and Porebski 1994).

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Rhamnus frangula cover was negatively associated with herbaceous cover in Pennsylvania savanna areas of the Allegheny Plateau (Possessky et al. 2000). Frappier et al. (2003) found *R. frangula* basal area was inversely associated with tree seedling number, percent total herb cover, and ground-level species richness in several southeastern New Hampshire forests. Only a direct experimental manipulation, however, can establish the causality of the observed negative associations between basal area of *R. frangula* and woody seedling density, herb cover, and forest-floor plant species richness.

Sinclair and Catling (1999) experimentally removed *R. frangula* cover from Ontario wetland plots and found increased native plant species cover in plots in which the *R. frangula* had been removed. They also found *R. frangula* reduced plant species richness (Sinclair and Catling 1999). This contrasts with the findings of Possessky et al. (2000) who observed higher plant species richness under *R. frangula* cover in savanna areas of Pennsylvania. Different ecosystems appear to respond differently to *R. frangula* invasion.

In cases where a non-indigenous species occurs in substantial numbers or biomass, the additional effort of performing field experiments to confirm that a non-indigenous species is causing an alteration in community structure or function is fully justified and should be more widely practiced for three reasons. First, a non-indigenous species may be an “add-on” in a community, accruing biomass but hardly altering native species composition and relative abundances (e.g., Anderson 1995). Second, control efforts are very costly and may adversely impact native species or encourage the spread of another non-indigenous species. Third, acceptance of the individualistic paradigm of species distributions necessitates that even closely related species may have very different effects on native species, requiring independent study (Westman 1990). However, where the non-indigenous species in question is new to an area or exists in small isolated populations, that species should be eradicated as a precautionary measure against future impacts because small populations are much easier to control than larger populations (Simberloff 1997).

Some managers have tested the efficacy of several control methods on *R. frangula*, with good immediate results (Post et al. 1989, Reinartz 1997). However, the response of *R. frangula* seeds in the seed bank to the eradication of overstory *R. frangula* was not measured in these studies. Mature stands of *R. frangula* are usually associated with very high fruit production and high numbers of conspecific seedlings (Converse 1984, Godwin 1943). Thus, the potential establishment of high densities of new *R. frangula* seedlings might require repeated follow-up efforts to assure lasting control. Furthermore, there has been no indication whether the plant community would attain pre-invasion structure after control efforts.

This paper reports the results of a two-year field experiment manipulating the presence of *R. frangula* in two *Pinus strobus* L.-*P. resinosa* Soland. stands in southern New Hampshire. The experiment was designed to test the following null hypotheses: > 90% *R. frangula* cover does not suppress first-year native tree seedling density; > 90% *R. frangula* cover does not reduce herb cover; > 90% *R. frangula* cover does not affect ground level plant species richness; and removal of *R. frangula* cover does not increase the number of germinating *R. frangula* seeds.

Methods

A randomized complete-block field experiment with six replicates was performed. The treatment was the presence of well-established *R. frangula* populations with three levels: uninvaded by *R. frangula* ("uninvaded"), > 90% cover ("*Rhamnus* present"), and removal of > 90% *R. frangula* cover ("*Rhamnus* removed"). The experiment was performed in and blocked by two forest stands, College Woods (43.1334°N, 70.9425°W) and MacDonald Lot (43.1248°N, 70.9275°W), both woodlands on University of New Hampshire property. College Woods is a 2.5 ha *Pinus strobus* - *P. resinosa* stand planted in the 1930s. MacDonald Lot is a 3 ha *P. strobus* stand approximately 70 years old, that originated in an old field. Except for *R. frangula*, there was no apparent shrub-sapling layer in the forests studied, perhaps due to forest management activities or canopy suppression.

In December 1999, areas of > 90% *R. frangula* cover in each forest were delineated by visually estimating cover of stems and branches. Three 4 x 6 m control plots were randomly located within three separate areas that had 0% *R. frangula* cover (uninvaded). Two additional 4 x 6 m plots were randomly established within the first 20 m of an invaded area near each uninvaded treatment plot. One of those plots was randomly given the *Rhamnus* removed treatment wherein all *R. frangula* cover was cut at the soil level, the stems removed, and the stumps swabbed with a 50% glyphosate solution per the methods suggested by Reinartz (1997). Because the height of the *R. frangula* canopy in the study areas was approximately 2–3 m, any *R. frangula* stems in an additional 2-m swath around these plots were similarly removed to minimize shading effects from adjacent shrubs at the study site's latitude of 42° N. The *R. frangula* in the other plot was allowed to remain (*Rhamnus* present). Three complete blocks were established in each stand for a total of 18 plots. All treatments were completed by January of 2000. Because the ground was frozen, we do not believe there was substantial soil disturbance due to the cutting. Thus, we made no effort to trample the other plots as well.

The plots were surveyed in the first two weeks of June of 2000 and again in June of 2001. In each survey, first-year native tree seedling

density and first-year *R. frangula* seedling density were recorded; first-year seedlings were determined by the presence of cotyledons. Percent cover of each herb species was visually estimated in each plot and classified into cover categories of 0–1%, 1–3%, 3–10%, and incremental categories of 10% cover thereafter. Species richness was the combined number of species recorded for herbs and seedlings. Additionally, in June of 2000, the diameter at breast height (dbh) was measured for all trees in the plots larger than 5 cm in dbh.

To test the null hypotheses that 90% *R. frangula* cover does not affect first-year tree seedling density, herb cover, or ground-level plant species richness, a MANOVA was performed on the collected data. The independent variables were the treatment, sampling year, and a treatment x sampling year interaction term. Sampling year was included in the model as a treatment because germination is affected by yearly changes in weather conditions and seed availability. The analysis was blocked by site. The dependent variables were first-year tree seedling density, first-year *R. frangula* seedling density, total herb cover, and ground-level plant species richness. Scheffe's tests were used for post-hoc pairwise comparisons.

Potential differences in overstory composition between treatments were investigated using a MANOVA. The fixed factor was treatment and site was used as a blocking factor. The dependent variables were canopy tree dbh measurements converted to basal area (m²/ha) by species.

Results

The variables used in the MANOVA analyses were evaluated for normality using skewness and kurtosis values. First-year tree seedling density and *R. frangula* seedling density had absolute skewness values greater than 2 (2.1 and 2.3 respectively). However, log₁₀ and square-root transformations, the recommended transformations for positive skewness and Poisson distributions, respectively (Zar, 1996), did not improve the distributions enough to warrant the complications in interpreting transformed data. Thus, all variables were used in their original form.

Table 1. Overstory composition of each forest. Values are diameters at breast height (m²/ha) calculated from the trees found within the experimental plots in each forest.

	College Woods		MacDonald Lot	
	Mean	St. dev.	Mean	St. dev.
<i>Pinus strobus</i>	201.729	190.3042	480.481	268.4485
<i>Quercus rubra</i>	20.145	38.0225	0.330	0.9902
<i>Pinus resinosa</i>	192.027	321.0101	0	0
<i>Carpinus caroliniana</i>	0	0	0.665	1.9955
<i>Fraxinus americana</i>	1.335	2.6954	0.067	0.1333
<i>Prunus serotina</i>	0.410	1.2310	0.458	1.3745
<i>Ostrya virginiana</i>	0	0	0.261	0.7858
<i>Betula lenta</i>	0.111	0.2346	0.699	1.6246

Although the canopy of College Woods had more *P. resinosa* than MacDonald Lot (Table 1), the MANOVA investigating potential differences in canopy composition between the sites and treatments did not differ significantly ($F_{7,6} = 1.355$, $p = 0.364$). The canopy composition also did not differ significantly with treatment ($F_{14,12} = 0.837$, $p = 0.629$).

Site, *R. frangula* treatment, year, and treatment x sampling year all significantly affected the combined dependent variables (Table 2). Wilks' Lambda multiplicatively combines the percent of unexplained variances

Table 2. Summary of MANOVA and subsequent ANOVA's of four variables, percent herb cover, ground level plant species richness, first-year tree seedling density, and first-year *Rhamnus frangula* seedling density, in response to manipulation of *R. frangula* presence at two sites in southern New Hampshire. Scheffe's tests were used to test significance in the subsequent ANOVA's.

(A) MANOVA

Source	df	Wilks' lambda	F-ratio	p-value
Site	4,26	0.346	12.293	< 0.001
Sampling year	4,26	0.429	8.634	< 0.001
<i>Rhamnus</i>	8,52	0.342	4.620	< 0.001
<i>Rhamnus</i> x year	8,52	0.527	2.451	0.025

(B) Univariate ANOVA's

Source	df	Type III sum of squares	F-ratio	p-value	Partial coefficient of determination ^(a)
Herb cover					
Block	1	55,696.000	45.703	< 0.001	0.612
Year	1	576.000	0.473	0.497	0.016
<i>Rhamnus</i>	2	5359.292	2.199	0.129	0.132
<i>Rhamnus</i> x year	2	396.375	0.163	0.851	0.011
Error	29	35,341.083			
Plant species richness					
Block	1	300.444	28.056	< 0.001	0.492
Year	1	11.111	1.038	0.317	0.035
<i>Rhamnus</i>	2	10.167	0.475	0.627	0.032
<i>Rhamnus</i> x year	2	5.722	0.267	0.767	0.018
Error	29	310.556			
First-year native woody seedlings					
Block	1	49.000	1.178	0.287	0.039
Year	1	1089.000	26.179	< 0.001	0.474
<i>Rhamnus</i>	2	382.889	4.602	0.018	0.241
<i>Rhamnus</i> x year	2	222.000	2.668	0.086	0.155
Error	29	1206.333			
First-year <i>Rhamnus</i> seedlings					
Block	1	299.175	1.826	0.187	0.059
Year	1	2210.880	13.495	< 0.001	0.318
<i>Rhamnus</i>	2	3860.525	11.782	< 0.001	0.448
<i>Rhamnus</i> x year	2	2966.023	9.052	< 0.001	0.384
Error	29	4750.952			

^aThe partial coefficient of determination multiplied by 100 approximates the relative percentage of variation attributable to the effect.

across the treatments to express an approximation of the amount of unexplained variance (Tabachnik and Fidell 2001). The *R. frangula* treatment displayed the strongest effect on the combined response variables as indicated by Wilks' Lambda. Site, sampling year, and the sampling year x treatment interaction displayed decreasing respective effect sizes (Table 2). The residual correlations of the dependent variables were generally low ($R < 0.3$), allowing confident interpretation of the univariate F-ratios for each main effect and the interaction (Tabachnik and Fidell 2001). The coefficient of determination was calculated to measure the percent variance in the dependent variable explained by each treatment.

Acer rubrum L., *Fraxinus americana* L., *P. strobus*, and *Quercus rubra* L. accounted for 51%, 16%, 13%, and 9%, respectively, of the first-year tree seedlings recorded in the experiment. The density of first-year tree seedlings was affected by the presence of *R. frangula* ($F = 4.602$, $p = 0.018$). Scheffe's post-hoc tests indicated that *Rhamnus*-present plots had significantly lower first-year tree seedling density than either the *Rhamnus*-removed or uninvaded plots ($p = 0.048$ and $p = 0.042$ respectively, Fig. 1a). Furthermore, first-year tree seedling densities in the *Rhamnus*-removed plots were similar to the uninvaded plots ($p = 0.998$, Fig. 1a). The

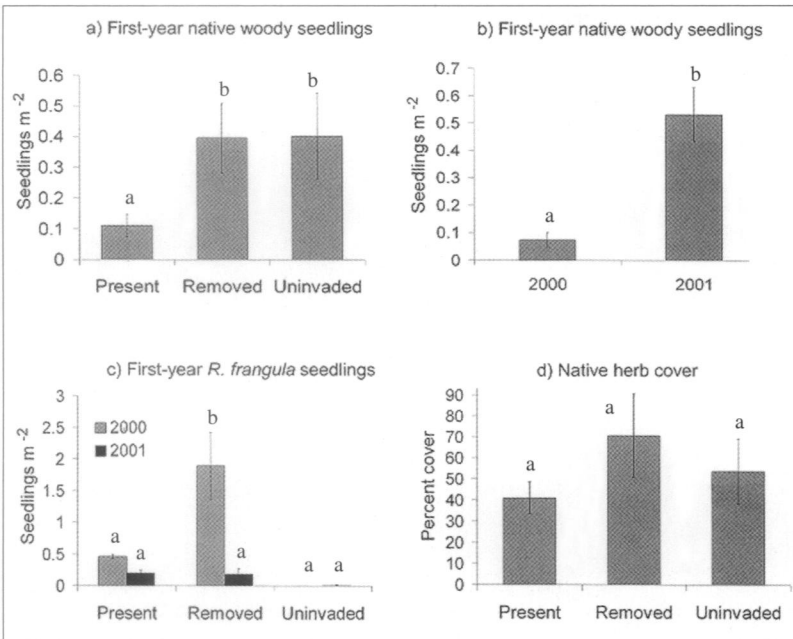


Figure 1. Responses of ground level plant species to manipulation of *Rhamnus frangula* presence ($n = 6$) at two sites in southern New Hampshire. Error bars represent standard error for each treatment level and letters denote significant differences between treatments at the $p < 0.05$ level as determined using Scheffe's test.

presence of *R. frangula* accounted for 24.1% of the variation in first-year tree seedling density. Sampling year also significantly affected first-year tree seedling density ($F = 26.179$, $p < 0.001$) and accounted for 47.4% of the explained variance (Table 2). There were many more first-year tree seedlings in 2001 than in 2000 (Fig. 1a).

Multiple ANOVAs of individual tree species first-year seedling densities were performed to investigate the possibility that some species are more impacted than others. The alpha value for significance in each comparison was adjusted by dividing the conventional alpha of 0.5 by the number of comparisons, 10, to yield an alpha of 0.005. This keeps the overall alpha for this round of tests at 0.05. After adjusting alpha values, no species first-year seedling densities differed significantly with treatment (Lowest $p = 0.02$ for *Q. rubra*).

First-year *R. frangula* seedling density was significantly affected by a sampling year \times treatment interaction ($F = 9.052$, $p < 0.001$; Table 2). *Rhamnus*-removed plots sampled in 2000 had five-fold greater densities of first-year *R. frangula* seedlings than the other plots that year. However, in 2001, the *Rhamnus*-removed treatment had similarly few *R. frangula* germinants as the uninvaded and *Rhamnus*-present treatments (Fig. 1c).

The presence of *R. frangula* did not affect percent herb cover ($F = 2.199$, $p = 0.129$; Table 2 and Fig. 1d) or plant species richness ($F = 0.475$, $p = 0.627$; Table 2 and Fig. 1b). However, study site significantly affected both variables and accounted for the largest amount of explained variance in each (61.2% and 49.2% respectively, Table 2).

Discussion

This field experiment supports the hypothesis that $> 90\%$ *R. frangula* cover inhibits tree species first-year seedling recruitment. Canopy composition of the plots did not appear to be a factor in producing these results. In addition, the suppression appears to impact equally all first-year tree species seedlings regenerating in the two stands in which this experiment was performed. These results are consistent with the inverse relationship between seedling number and *R. frangula* basal area found by Frappier et al. (2003). Given that both pattern and experiment agree, it is reasonable to assume that *R. frangula* is causing a reduction in tree seedlings in stands it invades. The reductions in first-year tree seedling density due to *R. frangula* were very similar to reductions observed by Collier et al. (2002) in their removal of *Lonicera maackii* (Robr.) Maxim, another exotic shrub invading the northeastern United States, within an Ohio forest. We recorded reductions of 74% in tree seedling density below *R. frangula* compared to 68% reductions for *L. maackii* (Collier et al. 2002). The exact mechanism for the inhibition of tree seedling density by *R. frangula* is not known. Resource competition for light, space, nutrients, water, or some combination of those resources is possible.

Other researchers have found reductions in herb cover and species richness in response to non-indigenous shrubs. In Ontario wetland thickets, Sinclair and Catling (1999) found lower native plant species cover and richness below dense *R. frangula* cover compared to plots in which the *R. frangula* cover had been removed. Collier et al. (2002) found significantly lower herb species cover and species richness below the crowns of *L. maackii* compared to plots without *L. maackii* cover in Ohio forests. In the Allegheny Plateau of Pennsylvania, Possessky et al. (2000) found significantly lower herbaceous layer cover in plots under *R. frangula* compared to plots in the nearby uninvaded savanna.

The lack of response by percent herb cover and species richness may be because our experimental design did not have adequate power to detect difference due to the high variability in those variables. The low effect size ($sr^2 < 0.06$) of *R. frangula* abundance on herb cover and species richness in a previous correlative study indicate that more experimental replicates may be needed to detect any differential response to *R. frangula* removal (Frappier et al., 2003). **The exceptionally low mean herb cover in the College Woods site or a lag in any herb species growth responses may have also obscured any trends.** However, Possessky et al. (2002) also found no significant differences in herb species richness in plots under *R. frangula* compared to savanna plots. The response of species richness to invasion by woody shrubs may be more complicated than previously thought, possibly differing with the ecosystem under consideration (i.e., forest, wetland, or savanna).

The effect of sampling year on tree seedling density may have been the result of a severe drought in the northeast the year before the experiment was initiated (NOAA 2000). Lower seed production in the drought year may have lowered the number of potential seeds for germination in 2000. The lack of a significant interaction between *R. frangula* and sample year for seedling density indicates that ***R. frangula* reduced tree regeneration regardless of climatic condition in this study.**

The sharp increase in first-year *R. frangula* seedling density in *Rhamnus*-removed plots may be due to germination of seeds in the seed bank or seed rain from adjacent individuals. However, we believe that the increase primarily reflects the seed bank for two reasons. First, Godwin (1936) found that the vast majority of the fruit not taken by birds dropped directly below the parent plant. We believe that the 2-m buffer surrounding the plots where *R. frangula* was removed was enough to greatly reduce seed rain from the surrounding *R. frangula*, though bird droppings from the tree canopy could not be ruled out. Second, first-year *R. frangula* seedling density was greatly reduced in the second year following removal. We interpreted the sharp reduction in the second year as a depletion of the viable seeds in the soil from the first-year to the second. The few *R. frangula* seedlings that managed to

germinate in the second year may reflect the lower rate of seed rain from surrounding *R. frangula*. Thus, initial control efforts need to be followed with some effective, yet non-damaging, technique for destroying the resulting increase in *R. frangula* seedlings.

With respect to control efforts, there are two encouraging aspects of the response by the native plant community to *R. frangula* removal. First, the sharp decline in *R. frangula* seedling density in the second year after removal indicates that the follow-up period for control efforts does not appear to need to last longer than a year or two. Second, the non-significant difference between seedling density in the *Rhamnus* removed plots and the uninvaded plots indicates that these forests may have begun to return to pre-invasion conditions in as little as two years, at least in terms of seedling density independent of species composition. While seedling densities in the removed plots were not significantly different from densities in the uninvaded plots after two years (Fig. 1a), we have no direct data on seedling densities prior to invasion.

The ability of *R. frangula* to inhibit the regeneration of trees could permanently alter the successional path of invaded forests. Silvicultural techniques to encourage tree species regeneration could be slowed or completely thwarted through the suppression of seedling germination and growth by *R. frangula*. It is possible that forest canopy harvesting or thinning in invaded stands could result in a *R. frangula* shrub community dominating for an extended period. However, whether full cover of *R. frangula* will continue to inhibit tree seedling density following forest canopy removal is unclear. The increased resource availability (light, nutrients, water, etc.) following a canopy harvest could promote *R. frangula* growth at the continued expense of native seedlings, slowing succession to a mature forest or possibly maintaining a shrub community. It is instructive that Sinclair and Catling (1999) observed that *R. frangula* suppressed native species cover despite no overstory tree canopy, though this study was done in a wetland ecosystem that might respond differently to *R. frangula* cover than an upland forest. Alternatively, the increased resource availability may alter the response of tree seedlings to *R. frangula* cover and allow regeneration of trees to continue with little impact from the *R. frangula* cover. To develop an appropriate and successful response to the continued spread of this non-indigenous plant, these questions must be answered.

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