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Exotic Invasive Shrub Glossy Buckthorn Reduces Restoration Potential for Native Forest Herbs

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Abstract: Invasive glossy buckthorn could reduce restoration potential for understory native forest herbs by compromising their growth and biodiversity. Few studies of glossy buckthorn's effects on forest herbs exist, and none were done in early-successional, partially open hardwood forests. This study was conducted in a mature hybrid poplar plantation invaded by buckthorn, located in southeastern Québec. We tested the effect of buckthorn removal on the growth of three forest herb species, whether this effect varied among species, and if canopy type (two poplar clones) influenced this effect. Forest herbs were planted in herbicide (buckthorn removed) and control treatments in the plantation understory, an environment similar to that of early-successional hardwood forests. Over the first two growing seasons, species showed specific reactions to buckthorn cover. Mean relative growth rate (RGR) for *Asarum canadense* and *Polygonatum pubescens* was increased in the herbicide treatment (48% and 33%, respectively) and decreased in the control treatment (−35% and −33%, respectively). *Sanguinaria canadensis* growth was the highest among species, with no difference between treatments. No effects of canopy type were detected. Results suggest that planting forest herbs for restoration purposes may be unsuccessful if buckthorn is present. Important changes in understory flora biodiversity are likely to occur over the long term in forests invaded by buckthorn.

Keywords: *Frangula alnus*; introduced species; eastern North America; southeastern Québec; understory biodiversity; early-successional forests; poplar plantation; bloodroot; wild ginger; Solomon's seal

1. Introduction

Exotic species represent from a quarter to a third of the plant species of the eastern Canadian provinces and American states [1]. Although most exotic plants have integrated into native ecosystems without producing major changes [2,3], a small number have become invasive, resulting in losses in biodiversity in native ecosystems and causing serious ecological and economic consequences [4,5]. However, the abundance of an exotic species is not necessarily indicative of its impact on native species [6]. Furthermore, an exotic species can be a driver of change in its new environment, or it can also be a "passenger" simply taking advantage of the current state of an ecosystem, such as following a disturbance [7]. In the latter case, control efforts would not be justified because the opportunistic species is not the primary source of the negative effects and its eradication will probably not restore the ecosystem and its biodiversity. In addition, control efforts themselves risk resulting in negative impacts on native species or even foster new invasions by other species [8]. Given that resources for control are limited and that control actions are expensive, labour-intensive, and imply long term commitments, prioritization of the invasive exotic species requiring control is essential. Therefore, it is necessary to experimentally test the effects of invasive exotic plants to verify causal links between the effects

observed and their presence and abundance, and then prioritize the monitoring and control of species for which a causal link has been established [9].

One species of concern throughout eastern North America is glossy buckthorn (*Frangula alnus* Miller, syn. *Rhamnus frangula*), an exotic shrub that is now naturalized and dominant in many ecosystems. Buckthorn may be a threat to biodiversity because it is very efficient at outcompeting native tree species in the colonization of canopy gaps, where it often forms a dense pure cover, reducing light availability to the forest floor [10–12]. Following buckthorn invasion, native species biodiversity may decline and forest productivity may be reduced [13,14].

Although most studies examining the effect of buckthorn have focused on the response of native tree seedlings, only a few have included forest herbs. However, Mason et al. [15] observed that the effect of woody invasive species is generally stronger on herbaceous plants than on other plant forms. Moreover, buckthorn is capable of invading humid and low-nutrient sites, where it may adversely affect the large number of endangered plant species that often occur in these environments [16]. The study of the herb stratum could therefore provide a more accurate and comprehensive assessment of the effect of buckthorn on the entire forest understory. This stratum, which can include around fifty species in southern Québec's rich maple stands [17], is one of the most important components of plant biodiversity in eastern North American hardwood forests. Buckthorn may threaten this biodiversity, and perhaps other ecological processes essential to the sustainability of these forests.

Forests in eastern North America that were cut for agriculture, and allowed to recover post-agriculture, showed very slow establishment of small-seeded ant-dispersed forest herbs, such as *Sanguinaria canadensis*, *Asarum canadense*, *Trillium* and *Viola* species [18]. These forest herbs took nearly a century to recolonize the second growth forests [18]. *Sanguinaria canadensis* (bloodroot) and *Asarum canadense* (wild ginger) are two of the three experimental forest herbs used in this study. Along with *Polygonatum pubescens* (Solomon's seal, likely bird dispersed), these deciduous forest species are wide ranging in eastern North America. They are typical of mature deciduous forests, growing on rich soils of Eastern Canada and the US. Wild ginger (food and medicinal uses) and bloodroot (medicinal uses) are known non-timber forest products (NTFPs). However, there is considerable evidence that forest understory herbs cannot withstand any harvesting in natural forests because of slow population growth, especially those species that are sought for their roots, thus killing the harvested plants [19]. In Québec, bloodroot and wild ginger are legally designated as "vulnerable" because they were potential targets of harvesting to supply nurseries with native shade garden plants [20]. The cultivation of wild ginger and bloodroot in forest farming systems, as an environmentally sound alternative to harvesting in the wild, has been investigated in Québec [21].

Full restoration of the biodiversity of the forest herb layer of young forests will require active restoration by transplantation. Natural second growth forests that are 50 years old are quite suitable for this purpose [22], as are productive hybrid poplar plantations that are only 10 years old [22,23]. Such hybrid poplar plantations can be used to create forested corridors linking natural woodlots (which are mostly second growth). Over time, native tree species will colonize these hybrid poplar corridors, as was observed for several deciduous species within 10 years, whereas native forest herb species will not colonize for perhaps a 100 years [18]. Forest herbs are planted for restoration purposes [15] and some species are cultivated as NTFPs. Both activities often take place in disturbed or managed forests, or in plantation understories [22], where buckthorn may be especially likely to invade. Whether buckthorn affects the success of these practices is a question that also needs answering.

In several New Hampshire pine-mixed hardwood forests and plantations, high basal areas of glossy buckthorn have been associated with a lower herb cover and a lower ground level plant species biodiversity [24]. In contrast, no effect of buckthorn removal on herb cover and herb species richness was detected in two experimental manipulations done in the same region and forest types, one of them testing results five years after buckthorn removal [11,25]. In riparian savannahs in Pennsylvania, Possessky et al. [14] observed a reduced percent herb cover in plots invaded by buckthorn compared to control savannah plots, but no effect on herb species richness. A causal link between glossy buckthorn

presence and a reduction in forest herb growth has therefore not yet been clearly established. Moreover, the observation of a reduction in understory species richness by Frappier et al. [24] suggests that different forest herb species may be differently affected by buckthorn. Information on differences in susceptibility to buckthorn among forest herb species would help conservationists identify species that are more at risk, as well as guide the choice of less sensitive species to be transplanted in biodiversity restoration projects.

Another consideration is that regional and ecosystem-specific studies are often necessary to evaluate the effects of invasive species, which can vary depending on these factors [6,8,11,12]. Susceptibility to invasion by buckthorn was observed to vary depending on the forest type [25] and the effect of buckthorn on the forest understory may vary depending on the composition of the canopy. In Canada, southern Québec is a region that is particularly vulnerable to invasive species because of its relatively warm climate and fertile soils, and because of its location near sources of new invasive species (Ontario and USA). In southern Québec, early-successional, partially open hardwood forests seem to be especially susceptible to invasion by buckthorn, but no research on how buckthorn affects forest herbs has been conducted in that region and forest type. This type of forest covers a vast area in the region, where human settlements and agricultural activities have highly fragmented the landscape and modified ecosystems. Baeten et al. [26] observed that environmental constraints can amplify the delay in colonization of forest herbs when these are slow-dispersers. Knowing if buckthorn is a threat to forest herb biodiversity, especially in fragmented landscapes where colonization is more difficult, and if it interferes with their growth in early-successional, partially open hardwood forests in southern Québec (Canada), is important for answering the question of whether the control of buckthorn is necessary.

To verify the presumed negative effect of buckthorn on forest herbs, we planted three species of forest herbs in the understory of a mature hybrid poplar plantation invaded by buckthorn. The objectives of this study are: (1) to test the effect of glossy buckthorn on the growth of three forest herb species; (2) to determine if this effect varies among the species tested; and (3) to determine if the overhead tree canopy type (two hybrid poplar clones tested) influences the effect of buckthorn. This experiment was done under two different hybrid poplar clones than the five clones used in a similar experiment, within the same plantation, in which we conducted an experiment on transplanted tree seedlings [27]. Increased knowledge of the effects of buckthorn on forest herbs will help include glossy buckthorn control in management, restoration or cultivation plans if justified, in order to preserve biodiversity, ecosystem services and sustainable forest use.

2. Materials and Methods

2.1. Study Site

The study site is a mature experimental hybrid poplar plantation, planted in 2000 and located at Sainte-Catherine-de-Hatley, in southeastern Québec (Lat. 45.27 N, Long. 72.05 W). The plantation covers approximately 0.5 ha and is surrounded by a buffer row of hybrid poplars to reduce the edge effect. It has a randomized block design, with three blocks each containing nine 12 m × 12 m main plots, randomly attributed to 9 different hybrid poplar clones. Each main plot had 12 planted hybrid poplars, for an initial density of 833 stems/ha [28]. Two of the 9 clones were selected for this experiment because of their greatest differences in parentage. These two clones (DxN-3333 and MxB-915303) have dissimilar ecology, yield, physiology, structure and biomass allocation (stem, branches and roots) which may influence light availability, and possibly available soil nutrients after many growing seasons. Following the plantation's establishment in May 2000, buckthorn has naturally invaded the plantation understory (Figure 1), most likely from large buckthorn thickets located in the adjacent open abandoned field. Buckthorn was the only woody vegetation present in the plantation understory, except for a few small tree seedlings (mostly white ash). A layer of low herbaceous vegetation, mostly continuous throughout, was also present. See Results Section for buckthorn density and biomass data from control plots.
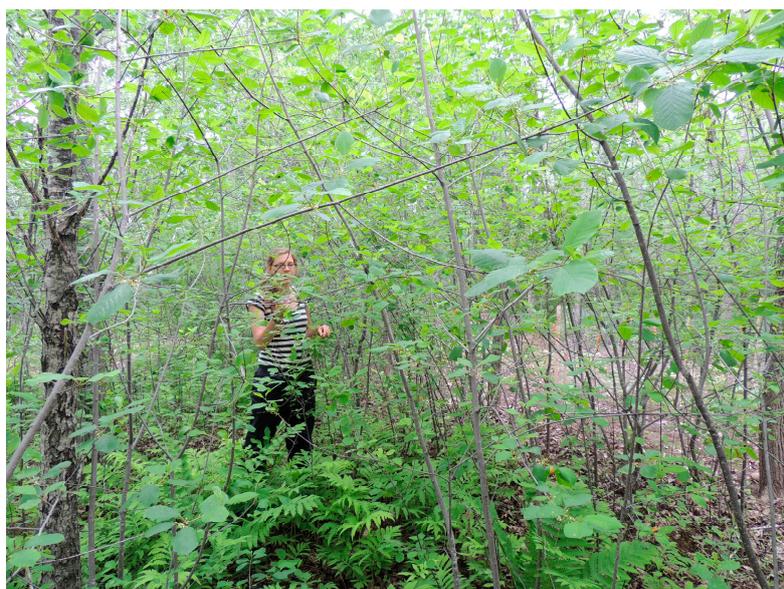
Figure 1. Untreated plot (control) of the hybrid poplar plantation, showing several 2- to 3-m-high stems of glossy buckthorn (40,000 stems/ha) and the understory vegetation (June 2014).

The plantation used for this experiment offers an experimental design that reduces confounding factors because the tree stem sizes and distribution (structure), as well the nature of the canopy (species composition) are uniform, especially because the trees are all hybrid poplar clones (genetically identical individuals). In addition, the understory conditions of the studied plantation are comparable to those of natural early-successional forests that are common throughout the Northern Hardwoods forest region. The understory environment is also comparable to that of partially open forests regenerated by under-planting and of tree plantations on abandoned farmland [22], two practices which are becoming more widespread in the region.

Each main plot of the experiment (3 blocks × 2 hybrid poplar clones = 6 plots) was split in two (1st split-plot level), an herbicide treatment was randomly attributed to one half (9 m × 4 m) and the other half remained untreated (control) (9 m × 4 m) (12 subplots; 6 for each treatment). In October 2013, double the minimal dose (7.52 kg a.i. ha^{-1}) of glyphosate ("Round-up" by Monsanto) was applied in the herbicide subplots using a backpack sprayer, with its nozzle aimed (1–2 m above ground) at the leaves of buckthorn stems. In late April 2014, dead buckthorn stems were cut and removed from herbicide subplots. No soil disturbance, weeding or additional herbicide treatments were done afterwards during the whole duration of the experiment. In spring 2014, a 2.4 m plastic mesh fence was attached to the hybrid poplar buffer row to exclude deer.

2.2. Transplantation of Forest Herbaceous Plants

Three understory herbaceous species (2nd split-plot level) were planted in this experimental design (*Sanguinaria canadensis*, *Asarum canadense*, *Polygonatum pubescens*). Transplants of *Sanguinaria canadensis* and *Asarum canadense* were studied previously in a hybrid poplar plantation network (that includes the plantation used in this study) and both species showed good potential for cultivation or conservation in plantation understories [23]. The cultivation of these species under a canopy could provide a sustainable alternative supply of NTFPs, and they could serve as well for biodiversity restoration purposes [22].

This experiment used nursery-produced forest herbs to simulate large plants, which has never been done previously in field studies of glossy buckthorn. Using transplants removes part of the heterogeneity in age and development that exists in natural forest understory herbs, because transplants all had a similar age and cultivation history at the time of plantation. The use of more

mature herbaceous plants, rather than seedlings, can better reflect the effect of glossy buckthorn than a response measured at earlier growth stages, where environmental variables have a greater effect on plant growth [29]. Using transplants allowed us to measure the response to buckthorn directly on individual plants, which is a more sensitive measurement than overall understory herb cover. The transplants also more accurately represent a biodiversity restoration or cultivation reality.

　　The plants we used were obtained from Horticulture Indigo, a local nursery (Melbourne, QC, Canada) producing native plants from seed, with no harvesting impacts on native forests. All plants were planted on 8 and 9 May 2014. A screening based on plant size was done prior to plantation to ensure that the distribution of plants differing in size was as homogeneous as possible among subplots, main plots and blocks. A central area of 3 m by 4 m in each subplot (herbicide and control) was amended with lime (calcium hydroxide) in October 2013, in order to increase the soil pH and improve soil conditions for the calcicolous species [23]. An amount of 166 g of lime per m^2 was added to this area (2 kg for 12 m^2) of each subplot.

　　This experimental design is a split-split-plot design [30] with 36 experimental units (3 blocks × 2 clones × 2 treatments × 3 species = 36 sub-subplots). In each experimental unit, the content of 20 pots of each species, which included a varying number of plants, were planted 30 cm apart along two rows that were spaced 20 cm apart. Each pot contained on average from 2 to 6 plants, depending on species. Two rows of the transplanted content of 10 pots for each species formed a rectangular species unit, and 3 such species units (one sub-subplot for each species) were placed side by side in a random order in each subplot (Figure 2).

Figure 2. View of an herbicide treatment subplot after one growing season (June 2015). Transplants of wild ginger are located on the left side (bottom of photo), with Solomon's seal in the middle, and bloodroot located on the right. Control treatments, containing buckthorn, are visible in background.

2.3. Measurements of Transplanted Forest Herbs

　　Initial measurements (time 1; data collected 27 May–13 June 2014), taken shortly after planting, after full plant stem and leaf development, and measurements taken two years later (time 2; data collected 13–16 June 2016) were used to calculate relative growth rates (RGR) based on percent biomass gain for each experimental unit, all individual plants/ramets combined. RGRs were calculated using the following formula: RGR = $(LnW_2 - LnW_1)/(t_2 - t_1)$, and were expressed in g/g/year [31]. Data at time 1 represent growth produced from the resources stocked in the rhizomes of the plants during the previous growing season, that is to say the last one in the nursery. Data taken at time 2

represent growth from resources stocked after transplantation, during the plant's first two growing seasons (2014 and 2015) in the plantation understory.

2.4. Forest Herbs Allometric Relationships

To calculate forest herb biomass using non-destructive measurements, predictive equations of total dry biomass were built for each species using specific size measurements as predictor variables. Because the species planted have different vegetative propagation modes, our equations for *Asarum* and *Sanguinaria* were built for each leaf, in order to represent a proportion of the total biomass of a ramet. We regressed the dry weight of one ramet, or part of a ramet (one leaf and its associated rhizome and root material) on various single leaf metrics, such as leaf width. To estimate the total weight of one of these species in one replicate, we then used the regression equation to estimate biomass of each ramet, or part of a ramet, in the replicate and then summed up these estimates of ramet biomass. *Asarum* produces each year several new rhizomes producing new ramets, which may remain connected for several years, creating expanding patches. *Sanguinaria* can also produce some new ramets from dividing rhizomes, but in far less abundance than *Asarum*. In the case of *Polygonatum*, biomass estimation was simpler because each stem is a ramet attached to a single rhizome, growing a few cm each year, and producing at its end a new larger aerial stem each year. The method used was aimed at measuring the total biomass produced by all the plants transplanted in each experimental unit through non-destructive methods. Total biomass estimates for all plants/ramets within each individual replicate plots were made using individual measurements of all *Polygonatum* stem lengths in the individual plots, and of all leaves of *Asarum* and *Sanguinaria* in the individual plots.

To obtain these allometric relationships a destructive subsample, consisting of the planted content of 12 initial pots per species, each pot containing several individual plants, was harvested in 2014 (from 27 May to 13 June) to determine total dry biomass (aboveground and belowground). These harvested subsamples were distributed over the entire experimental design. Plant size measurements depended on individual species' morphology (e.g., length and width of leaves, stem length, number of flowers, etc.), and the size measurements that were the best predictors of dry biomass were determined by regression analyses (single variable regression using the R program package [32]). Regression model selection was based on the Akaike Information Criterion (AIC) and on the fit of the model (R^2). All regression models are power functions.

Table 1 provides information on the allometric equations developed for each forest herb species. Three outlier data points were removed from the *Polygonatum pubescens* dataset in order to meet the requirements for a reliable model.

Table 1. Allometric relationships between leaf and stem size measurements, as predictor variables (*x*), and total dry biomass, as response variable (*Y*), for three forest herb species planted in the understory of a mature hybrid poplar plantation.

Species	Size Variable	Plants/Ramets Harvested (*n*)	Model *x* = Size Measurement (cm) *Y* = Dry Biomass (g)	R^2
Asarum canadense (biomass of part of a ramet)	Leaf width (cm)	76	$Y = 0.0236x^{1.3282}$	0.80
Sanguinaria canadensis (biomass of part of a ramet)	Leaf width (cm)	25	$Y = 0.01819x^{1.7095}$	0.88
Polygonatum pubescens (biomass of one plant)	Stem length, first to last leaf (cm)	56	$Y = 0.1181x^{1.1091}$	0.77

2.5. Measurement of Environmental Variables

Canopy openness (proxy for light availability), soil humidity, soil nutrient availability, buckthorn stem and seedling density, buckthorn biomass, understory vegetation biomass (other than buckthorn)

and canopy poplar biomass and volume were measured. Canopy openness, a proxy for light availability, was estimated through the analysis of two hemispherical photographs per treatment plot (at 1 m above ground level, below most of the buckthorn cover and above planted forest herbs height, and at 3 m, above most of the buckthorn cover), using the Gap Light Analyzer software v.2.0 (Simon Fraser University, Burnaby, BC, Canada). To determine soil nutrient availability, four sets of soil Plant Root Simulator probes (developed by Western Ag Innovations, Saskatchewan) were buried in each of the 12 treatment plots during 40 days (from 2 June 2014 to 11 July 2014). Soil was collected in metal containers for gravimetric measurements in each treatment plot (sub-subplots), on a single day in July 2014 (no rain 48 h prior to sampling), to measure soil humidity. All glossy buckthorn stems were counted and their basal diameters measured in each treatment plot for subsequent conversion into biomass values using an allometric relationship developed for this experiment (described in another publication) [33]. Buckthorn seedlings were counted in four 50 cm × 50 cm microplots located within each treatment plot. All understory vegetation besides buckthorn was harvested in the same four 50 cm × 50 cm microplots used for buckthorn seedlings, to obtain biomass data. DBH of hybrid poplars included in plots were measured for conversion to aboveground biomass and volume, using predictive equations developed in the plantation network for 13-year-old hybrid poplars [34].

3. Results

3.1. Results of the Forest Herb Experiment

Over the two first growing seasons in the plantation understory, the mean relative growth rate for the three forest herb species combined was higher in the herbicide subplots than in the control subplots (Table 2). Although it was not detected as statistically significant at $p = 0.05$, the treatment was significant at $p = 0.097$. In addition, confidence intervals for the two treatments did not overlap. We consider this result to be biologically significant, providing some support to the hypothesis of an overall negative effect of buckthorn on forest herb growth.

Table 2. Relative growth rates expressed in terms of total biomass (g/g/year or %) of three forest herb species planted in herbicide and control treatments in the understory of a hybrid poplar plantation (SAC: *Sanguinaria canadensis*, ASC: *Asarum canadense*, POP: *Polygonatum pubescens*).

	SAC	ASC	POP	Treatment Mean	Treatment Standard Error	Treatment *p* Value
Herbicide	66%	48%	33%	49%		
Control	79%	−35%	−33%	4%	15%	0.097
Species mean	72%	6%	0%			
Species standard error		19%				
Species *p* value		0.029 *				

* Significant at $p < 0.05$.

The ANOVA did not detect a significant Treatment × Species interaction ($p = 0.1951$), However, the results do show a trend towards a differential effect of the herbicide treatment which removed buckthorn from the understory vegetation. Buckthorn shrubs alone represented 88% of total understory biomass, the other 12% consisting of herbaceous plants. In the herbicide treatment, the three species had positive growth (biomass gain) with overlapping confidence intervals between species. In the control treatment, both *Polygonatum pubescens* and *Asarum canadense* had markedly reduced and negative RGRs (biomass loss) (Table 2). These negative RGRs were of the same order of magnitude as the positive RGRs observed in the herbicide treatment, representing a completely opposite growth trend between treatments. In contrast, *Sanguinaria canadensis* had marked positive RGRs under both treatments, with overlapping confidence intervals. *Sanguinaria canadensis* had the highest growth of the three species in both treatments and the smallest difference between treatments. Differences in RGR between the three

species (treatments combined) were significant (Table 2), probably because of the diverging responses to buckthorn. After two growing seasons, *Sanguinaria canadensis* had the highest RGR of the three species by far, *Polygonatum pubescens* had the lowest RGR, and *Asarum canadense* had an intermediate RGR. Confidence intervals around RGRs for *Polygonatum pubescens* and *Asarum canadense* were overlapping. The superior growth of *Sanguinaria canadensis* in the herbicide treatment is amplified when looking at overall growth (treatments combined), because the growth of *Sanguinaria canadensis* remained similar under both treatments, while the other two species had similar, marked growth reductions under the control treatment, resulting in an overall RGR close to zero for both *Polygonatum pubescens* and *Asarum canadense*.

3.2. Environmental Variations between Clone Types and between Treatments

Hybrid poplar clone type canopy did not significantly differ in biomass or volume between clone types (Table 3). It also did not have a significant effect on any environmental variable, except on canopy openness at 1 m above ground level, under most of the buckthorn cover. This appears to be a spurious effect, because clone type canopy did not have an effect on canopy openness at 3 m above ground level, above most of the buckthorn cover, and no other related variable, such as buckthorn density or biomass, followed a similar trend. Because no effects of canopy type on forest herb growth were detected, the poplar canopy type does not seem to influence buckthorn or its effect on forest herbs. The hypothesis for our third research question about the potential of hybrid poplar clone canopy influencing buckthorn or its effect on forest herbs is therefore rejected. At the time the experiment was conducted, hybrid poplar density was 691 stems/ha (2 of 12 stems/plot initially planted were harvested to build allometric equations), volume was 170 m^3/ha, and dry biomass was 70,000 kg/ha (or 7000 g/m^2 for comparing with buckthorn biomass).

Table 3. Results from ANOVAs (*F* and *p* values) comparing environmental and vegetation variables of two canopy hybrid poplar clone types (DxN-3333 and MxB-915303) and two treatments (herbicide and control) of a mature hybrid poplar plantation.

Variable	Clone Types		Treatments	
	F	*p* Value	*F*	*p* Value
Glossy buckthorn				
Stem density	0.93	0.437	31.68	<0.001 ***
Seedling density	0.38	0.600	1.46	0.294
Total aboveground biomass	1.91	0.301	9.64	0.0361 *
Soil nutrients				
NO$_3$	1.67	0.325	0.00	0.978
P	0.67	0.500	0.57	0.493
K	1.35	0.365	0.62	0.475
Ca	0.37	0.607	0.02	0.905
Mg	0.46	0.566	1.45	0.295
Soil humidity	1.49	0.347	0.24	0.651
Canopy openness				
1 m aboveground	26.05	0.0363 *	0.35	0.584
3 m aboveground	1.09	0.405	0.72	0.445
Understory vegetation biomass (without buckthorn)	0.51	0.549	16.5	0.0154 *
Hybrid poplar				
Volume	3.17	0.217		
Biomass	7.89	0.107		

*** Significant at *p* < 0.001. * Significant at *p* < 0.05.

Unsurprisingly, buckthorn stem density (stems >50 cm in height) and total biomass were significantly higher in control treatment subplots than in herbicide subplots (Table 4). Understory vegetation (biomass) was also significantly more abundant in control subplots than in herbicide

subplots. However, in all control subplots, where both buckthorn and other understory vegetation are present, buckthorn biomass was much higher than that of the other understory vegetation (mean per block). The non-buckthorn understory vegetation biomass represented 24% of the total understory biomass (non-buckthorn vegetation + buckthorn) in block 1, 7% in block 2 and only 4% in block 3. On average, over all blocks, the non-buckthorn understory vegetation biomass was 12% of the total understory biomass (understory vegetation + buckthorn), while buckthorn biomass accounted for 88% of it (Table 4). Differences in canopy openness or soil conditions between treatments were not significant, nor were all possible interactions between clone types, treatments and species.

Table 4. Results from ANOVAs (*p* values) comparing environmental and vegetation variables between herbicide and control treatments in the understory of a mature hybrid poplar plantation.

Variable	Unit	Herbicide	Control	Standard Error	*p* Value
Glossy buckthorn					
Stem density	n/m^2	0	4	1	0.0049 **
Seedling density	n/m^2	66	127	36	0.294
Total aboveground biomass	g/m^2	2	503	114	0.0361 *
Soil humidity	%	20	20	1	0.651
Canopy openness					
1 m aboveground	%	31	32	1	0.584
Understory vegetation biomass (without buckthorn)	g/m^2	6	68	11	0.0154 *

* Significant at $p < 0.05$. ** Significant at $p < 0.01$.

4. Discussion

The most important effect of the herbicide treatment was buckthorn removal (reduced from 503 to 2 g/m^2; 99.6% removal). Buckthorn biomass in the control plots, at an average of 503 g/m^2, made up 88% of total understory biomass (571 g/m^2 = 503 + 68) (Table 4). Herbicide effects can thus be viewed primarily as buckthorn effects. However, other understory species were also reduced by herbicide, although not quite to the same extent (91% removal), and they initially represented only 12% of the total understory biomass. Their loss may also have had effects, but if so, they were slight.

The high relative rate of growth and the small differences between treatments observed for *Sanguinaria canadensis* are consistent with the observations of this species as particularly successful in transplantation experiments, and capable of coping with competition. In Massachusetts, in a transplantation experiment including eight perennial plant species, *Sanguinaria canadensis* was one of only two species which remained after 15 growing seasons, and it was the only species which had succeeded in the establishment of reproducing populations [35]. In our experiment, we observed *Sanguinaria canadensis* seedlings in June 2016, from seeds produced in situ. In another study, *Sanguinaria canadensis* was observed to have certain competitive abilities against *Alliaria petiolata*, an invasive biennial plant [36]. *Sanguinaria canadensis* does not seem to be as substantially affected as the other two species by the herbicide treatment, the major effect of which was to remove buckthorn.

The responses of the two other species (*Asarum canadense* and *Polygonatum pubescens*) may accurately represent the reaction to buckthorn that most eastern North American forest herb species are more likely to have. Non-significant differences between all three species in the herbicide treatment could be due to specific growth patterns or differences in transplantation success. Uncontrolled factors, such as micro-topography (small depressions in two herbicide treatments in block 1 were often waterlogged), could be responsible in part for the large variations observed in transplant growth. The differences in the results for the three species in the control treatment, in turn, seem to show specific reactions to buckthorn cover.

From a conservation and restoration perspective, it is expected that buckthorn will strain forest herb populations in natural habitats, and negatively affect their growth and biodiversity. The lack

of significant differences in relative growth rate between species in this study may be due in part to the fact that only two years have passed since transplantation. With additional growing seasons, differences could become more pronounced.

The two hybrid poplar clone types tested did not result in significantly different understory environments, and no effect on any buckthorn or forest herb growth variables was detected. Similarly, differences among clones were also not observed in a study on buckthorn effects on tree seedlings conducted under five other hybrid poplar clone canopies within the same plantation used in this study [27]. Likewise, Boothroyd-Roberts et al. [22] also did not detect significant differences in environmental variables between 10-year-old hybrid poplar clone type canopies.

The environments in the herbicide and control treatments were significantly different in terms of vegetation. Light, soil nutrient and soil humidity conditions were similar between treatments, but vegetation (buckthorn and other understory vegetation) was significantly more abundant in control plots than in herbicide plots. The soil probes were inserted for a month at the beginning of the spring, which is likely to be the moment when the nutrients would have been released in the herbicide treatment, but not captured by the removed buckthorns. Moreover, four replicate pairs of soil probes were distributed in each subplot, which exceeds the recommended quantity. The absence of a difference in soil nutrient availability and soil humidity between treatments, after the removal in the herbicide plots of 500 g of buckthorn per m^2 on average, perhaps indicates that buckthorn does not take up large amounts of nutrients and water. Eliminating buckthorn had no significant impact on soil nutrients and water. These results would be consistent with the description of glossy buckthorn as a generalist species with low edaphic requirements. Another hypothesis would be that the hybrid poplars, which are known to have high soil water and nutrient uptakes [37,38], and which represent 94% of the biomass of the plantation (all vegetation strata included) collected most of the soil resources made available by the removal of buckthorn.

However, although no differences between treatments were observed in this experimental design for canopy openness, light seems to be the resource that is limited by buckthorn according to the results obtained from a larger experimental design (including five clone types) within the same plantation [27]. Because buckthorn is far more abundant than the other understory vegetation in terms of biomass (representing on average 88% of total understory biomass), and because other environmental variables are quite constant between treatments, treatment effects on transplanted forest herb growth can plausibly be mainly attributed to buckthorn abundance. This hypothesis of buckthorn's main effect being through reduced light availability is also supported by previous studies [24].

The results of this study are probably generally applicable to early-successional, partially open, hardwood forests dominated mostly by intolerant hardwoods (poplars, birches, ashes, etc.). Support for this generalization comes from a study by Boothroyd-Roberts et al. [22], who observed that 10-year old productive hybrid poplar plantations had many environmental conditions similar to young second growth woodlots (31 to 58 years in age), such as basal area, canopy closure and leaf litter accumulation. The 15-year-old hybrid poplar plantation used for the experimental design probably has environmental conditions that are comparable to those of early-successional forests found throughout southeastern Canada and the Northeastern United States.

5. Conclusions

Our results indicate that buckthorn appears to be interfering with forest herb growth, and the different species tested seem to be differently affected. If this trend of a differential effect of buckthorn on forest herb species is confirmed in the future, important changes in understory herbaceous flora biodiversity will likely occur over the long term.

Considering that transplantation studies have yielded contradictory results [29,35,39], and that success rate seems to decline when observed over a period which is closer to the long life expectancy of forest herbs [35], our results bring support for a removal of buckthorn prior to establishing transplanted

forest herb populations, for a choice of species with ecological requirements matching those of planting sites, and of transplants that are larger in size to maximize the potential for success.

Although our results are short-term, they may have important implications considering the bottleneck pattern of forest herb population establishment [26]. The lag in growth that buckthorn may cause could significantly reduce the future development capacity of forest herb populations in eastern North America and thus reduce hardwood forest understory biodiversity.

Many eastern North American forest herb species are slow dispersers, such as *Asarum canadense* and *Sanguinaria canadensis*, the seeds of which are dispersed by ants [40]. Therefore, this additional constraint by buckthorn may further increase their already lengthy time lag in colonizing new habitats, which can exceed a 100 years [18]. Although some slowly-dispersed species such as *Sanguinaria canadensis* could be transplanted to overcome dispersal issues and establish successful populations under buckthorn (this needs to be confirmed over a longer period of time), other species, such as *Asarum canadense* and *Polygonatum pubescens* may not be able to succeed in establishing a population when buckthorn is present. These results have important implications for the planting of these two forest herb species, for biodiversity restoration purposes or for non-timber forest products, because it appears unproductive to plant them when buckthorn is present in the forest or plantation understory.

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