DOES INVASIVE GLOSSY BUCKTHORN (*FRANGULA ALNUS*) HAVE ALLELOPATHIC EFFECTS ON NATIVE NEW ENGLAND WETLAND MARGIN VEGETATION?

by

Amy Mays

A Paper Presented to the
Faculty of Mount Holyoke College in
Partial Fulfillment of the Requirements for
the Degree of Bachelors of Arts with
Honor

Department of Biological Sciences
South Hadley, MA 01075

May, 2009

This paper was prepared
under the direction of
Professor Martha Hoopes
for eight credits.

DEDICATION

to John Sprague, great friend

and to the memory of Kara Lindstrom

ACKNOWLEDGMENTS

I sincerely thank my advisor, Dr. Martha Hoopes, for her mentoring and encouragement throughout this process. I am also grateful for the collaboration of Dr. Kristina Stinson at Harvard Forest in the mulch trial, and for inspiring me with her work on invasive plant disruption of plant-microbe mutualisms. I thank Dr. Amy Frary for sharing her delight in the vegetative kingdom and for laboratory space. Dr. David Benson and Juliana Mastronunzio at the University of Connecticut generously assisted me with *Frankia* work. Dr. George Cobb provided clear statistical advice, Marion Jasmine guided me in *Frankia* inoculation, Sydne Record taught me asceptic technique, and Russell Billings and Ellen Shukis assisted greatly with plant care. I am grateful to Dr. Liza Knapp for sharing useful insights about buckthorn, to Dr. Julie Richburg for pointing me in the direction of her Kampoosa Bog data, and to Bryan Connolly for providing references on swamp birch. Thank you also to Thomas Houle and Dr. Gregory Roman for help with chemistry methodology and supplies, and to Dr. Jill Bubier and Dr. Leszek Bledzki for use of their graphing lab. I deeply appreciate Scott Rasmussen's assistance with many aspects of this process - from whittling buckthorn bark to graphic design. I am grateful to the Clare Boothe Luce Foundation, Dr. Martha Hoopes, Harvard Forest, and the Mount Holyoke College Department of Biological Sciences for funding this project. Finally, I thank John Sprague for his consistent encouragement and care. I am glad the rigors of completing this project did not disrupt our mutualism.

TABLE OF CONTENTS

Page

LIST OF FIGURES vi

LIST OF TABLES viii

ABSTRACT.. ix

CHAPTERS

I. OVERVIEW: RELATIONS BETWEEN INVASIVE GLOSSY BUCKTHORN AND NATIVE SHRUBS ON NEW ENGLAND WETLAND MARGINS 1

II. GLOSSY BUCKTHORN CHEMICAL SUPPRESSION OF INDIGENOUS SHRUBS 6

Introduction...6
Methods...19
Results...27
Discussion..31

III. INDIGENOUS SPECKLED ALDER FACILITATION OF GLOSSY BUCKTHORN 36

Introduction...36
Methods...38
Results...39
Discussion..39

IV. SYNTHESIS & MANAGEMENT IMPLICATIONS 42

BIBLIOGRAPHY 45

LIST OF FIGURES

Figure Page

1. Hypothetical relationship between glossy buckthorn and speckled alder .59

2. Percent alder seeds germinated in buckthorn extract or water 60

3. Percent alder seeds germinated in buckthorn and native extracts or water 61

4. Alder seedling basal diameter in buckthorn extract or water 62

5. Alder seedling basal diameter in buckthorn and native extracts or water 63

6. Initial meadowsweet height in all mulch treatments.................................64

7. Alder basal diameter in buckthorn and native root or leaf mulch.............65

8. Alder basal diameter in all mulch treatments 66

9. Meadowsweet height in buckthorn and native mulch...............................67

10. Meadowsweet leaf production in buckthorn and native mulch 68

11. Meadowsweet height in leaf or root mulch...69

12. Meadowsweet leaf production in leaf or root mulch 70

13. Meadowsweet leaf production in buckthorn and native root or leaf mulch...71

14. Meadowsweet leaf production in all mulch treatments 72

LIST OF FIGURES

Figure Page

15. Meadowsweet height in all mulch treatments...73

16. *Frankia* dry weight in positive and negative controls 74

17. *Frankia* dry weight in buckthorn extract concentrations and controls 75

18. Buckthorn seedling basal diameter in alder extract or water.....................76

LIST OF TABLES

Table Page

1. ANOVA for impacts of buckthorn and native extracts or water on alder seedling growth 77

2. Ammonium and nitrate content of buckthorn and native plant extracts 77

3. ANOVA for initial size of indigenous shrubs in all mulch treatments 77

4. ANOVA for effects of buckthorn and native root or leaf mulch on indigenous shrub growth 78

5. ANOVA for effects of all mulch treatments on indigenous shrub growth 79

6. Carbon to nitrogen ratios of buckthorn and native root or leaf mulches ...79

7. Student's t-tests for impacts of alder extract or water on buckthorn seedling growth 79

ABSTRACT

DOES INVASIVE GLOSSY BUCKTHORN

(*FRANGULA ALNUS*) HAVE ALLELOPATHIC EFFECTS ON

NATIVE NEW ENGLAND WETLAND MARGIN VEGETATION?

AMY MAYS

Resource managers in New England express concern about glossy buckthorn, an invasive shrub that reduces the growth and survival of native tree saplings and alters the structure of indigenous plant communities. Since identifying the mechanisms behind buckthorn's success may assist in restoration, I tested the possibility that allelopathic effects on native wetland shrubs were driving this invasion. In particular, I explored buckthorn chemical suppression of speckled alder (*Alnus incana* ssp. *rugosa*) and its nitrogen-fixing symbiont, *Frankia*. I hypothesized that alder facilitation promotes buckthorn invasion due to elevated plant-available nitrogen in alder-rich wetlands and buckthorn's strong growth response to nitrogen. Testing buckthorn chemical effects on native shrubs indicated mild allelopathic inhibition of alder at several life stages. Surprisingly, buckthorn also appeared to facilitate native meadowsweet (*Spiraea latifolia*), indicating that chemical and nutrient dynamics play a role in buckthorn's restructuring of native plant communities. Since buckthorn extract inhibited *Frankia* growth *in vitro*, further work should be done to explore buckthorn disruption of the alder-*Frankia* mutualism. If evidence presented here for alder facilitation of buckthorn is supported by future study, buckthorn removal should be prioritized where it co-occurs with alder and thus may exclude rare native plants.

CHAPTER I

OVERVIEW: RELATIONS BETWEEN GLOSSY BUCKTHORN AND NATIVE SHRUBS ON NEW ENGLAND WETLAND MARGINS

Invasive plants, those which humans introduce from one ecosystem to another, cause extensive ecological and economic damage – leading to species endangerment and costing over 24 billion dollars in the United States annually (Wilcove et al. 1998; Pimentel et al. 2005). Due to their worldwide reach, plant invasions fit under the rubric of profound anthropogenic environmental change, along with global warming and chemical pollution (Vitousek et al. 1996). Only a small percentage of introduced species, however, become invasive, after passing through initial stages of establishment, spread, and eventual impact (Williamson & Fitter 1996). Investigating controls on the invasibility of ecosystems (Lonsdale 1999; Davis et al. 2000), as well as the characteristics of good invaders (Rejmanek & Richardson 1996; Radford & Cousens 2000; Mitchell et al. 2006), has engaged researchers in multidisciplinary work in evolutionary biology, ecology, genetics, soil microbiology and botany (Klironomos 2002; Lee 2002; Bossdorf et al. 2008).

Biologists have developed many hypotheses for why invasion occurs, beginning with Darwin (1859) and Elton (1958), who proposed that invaders are freed to become better competitors as they escape from their natural predators and pathogens. This ‘enemy release’ hypothesis has since been tested in a range of environments and has garnered ambiguous support (Maron & Vila 2001; Keane &

Crawley 2002; Mitchell & Power 2003; Reinhart & Callaway 2004; Lockwood et al. 2007).

An extension of the enemy release hypothesis is the notion that invaders need to allocate less energy to defense against co-evolved specialist predators and can therefore grow and reproduce more prolifically, out-competing their new neighbors (Blossey & Notzold 1995; Lockwood et al. 2007). Some research supports this hypothesis (Fenner & Lee 2001), but since many introductions of biocontrol species have failed, other factors are also likely important for determining which organisms thrive in new environments (Hierro & Callaway 2003).

The 'empty niche' concept further expands upon the 'enemy release' hypothesis, and posits that invaders not only escape harmful biotic interactions, but also benefit from unused resource opportunities (Shea & Chesson 2002). Other explanations for successful invasion include repeated organism introductions (Tilman 2004), accidental or purposeful selection for fitness by human transporters (Forsyth et al. 2004; Marchetti et al. 2004), and invasive preadaption to the new environment (Mack 2003) due to similar climate or soil regimes in native and introduced ranges (Wiens & Graham 2005; Alpert 2006; Bossdorf et al. 2008).

Once exotic species become invasive, there are multiple mechanisms by which they alter natural ecosystems (Vivrette & Muller 1977; D'Antonio & Vitousek 1992; Levine et al. 2003). Understanding these mechanisms can lead to better restoration of invaded areas as the most detrimental processes are identified

and ameliorated. These include accumulation of burn-prone biomass, modified nutrient cycling, novel interaction with soil biota, and allelopathy (Levine et al. 2003).

Invasion biologists in the past twenty years have expressed renewed interest in allelopathy, the competitive chemical suppression of one plant species by another (Hierro & Callaway 2003; Prati & Bossdorf 2004; Vivanco et al. 2004) and have devised more ecologically-relevant methodologies (Callaway & Aschehoug 2000; Bais et al. 2003) than those employed by earlier researchers. These new techniques include realistic delivery of allelochemicals and the use of native bioassay plants that co-occur with the candidate allelopath in the invaded range (Hierro & Callaway 2003). An important theory that has guided this work is the notion that some invasives are successful due to their possession of biochemicals with which native species have not evolved (Callaway & Aschehoug 2000). This is called the 'novel weapons' hypothesis and testing it ultimately requires assessing differences in candidate allelopath effects on neighbors both in home and introduced ranges (Callaway & Ridenour 2004; Callaway et al. 2008).

Allelochemicals are secondary metabolites (compounds not needed for rudimentary plant functioning) that are transmitted to the environment via leaf litter or through root exudation (Bertin et al. 2003; Inderjit & Duke 2003). Examples of allelochemicals from invasive plants include (-)-catechin from spotted knapweed (*Centaurea maculosa*), which increases oxidative stress and leads to root death (Bais et al. 2003), and gallic acid from common reed

(*Phragmites australis*), which disrupts plant cell microtubule assembly (Rudrappa et al. 2007). Allelochemicals not only directly harm plants but may also affect plant-microbe interactions belowground (Klironomos 2002; Bais et al. 2002). Invasive, allelopathic garlic mustard (*Alliaria petiolata*), for example, inhibits arbuscular mycorrhizal fungi upon which native North American trees depend for salubrious growth (Stinson et al. 2006).

Glossy buckthorn (*Frangula alnus*), an invasive shrub from Eurasia, colonizes both upland and mesic areas in northeastern North America but thrives particularly well along river, pond and wetland margins (Reinartz & Kline 1988; Catling & Porebski 1994; Possessky et al. 2000). Resource managers in the region express concern about buckthorn (Post et. al 1989; Reinartz 1997; C. Mattrick, pers. comm.), which diminishes the growth and survival of native tree saplings (Fagan & Peart 2004) and changes the composition of indigenous plant communities (Frappier et al. 2004). Buckthorn grows in dense, monospecific stands typical of allelopaths (Hierro & Callaway 2003) and contains several potential inhibitory chemicals, including emodin (Sydiskis et al. 1991; Francis et al. 1998), which is implicated in knotweed (*Polygonum*) allelopathy (Inoue et al. 1992; Izhaki 2002) and is lethal to amphibians (Sacerdote 2008).

Here, I test the possibility that allelopathy is important to buckthorn's invasive success on several native shrubs of New England wetland margins, including speckled alder (*Alnus incana* ssp. *rugosa*), a prominent constituent both spatially (Huenneke 1987) and functionally (Hurd et al. 2001) of these areas (Figure 1). Alder is an actinorhizal plant that lives mutualistically with *Frankia,*

an endosymbiotic bacterium that fixes nitrogen (Torrey 1978), a nutrient that is often limiting for vegetation (Haynes 1986). Alder-dominated wetlands contain significantly more plant-available nitrogen than do non-alder dominated wetlands (Hurd & Raynal 2004), and plant neighbors of alder show improved growth over non-neighbors (Jutras et al. 2006). Buckthorn, meanwhile, is a strong nitrogen responder (Knapp 2006), and I hypothesize that alder is facilitating its growth (Figure 1).

Recent evidence demonstrates that invasive vegetation can disrupt mycorrhizal relationships (Stinson et al. 2006), but there has been little exploration of invasive plant disturbance of actinorhizal mutualisms (Richardson et al. 2000). By testing the prediction that buckthorn suppresses alder-infective *Frankia*, I investigated this possibility. Chapter II examines buckthorn chemical inhibition of alder and *Frankia* as well as buckthorn allelopathic effects on other native shrubs, while Chapter III explores alder facilitation of buckthorn. Synthesis and management recommendations for buckthorn-invaded alder wetlands follow in Chapter IV.

CHAPTER II

GLOSSY BUCKTHORN CHEMICAL SUPPRESSION

OF INDIGENOUS SHRUBS

Introduction

Glossy buckthorn belongs to the family Rhamnaceae, which comprises 52 temperate and tropical genera, most of them woody (Heywood et al. 2007). This shrub attains a height of two to seven meters and has shiny, ovate leaves (Converse 1985). In buckthorn's native Europe and western Asia, high-quality charcoal is made from its stems for use in gunpowder and its bark is employed medicinally as a cathartic (Serdar et al. 2007). In the early 1800s, glossy buckthorn was introduced to North America for ornamental purposes and wildlife cover (Wyman 1971; Webster et al. 2006).

Buckthorn flowers are insect-pollinated, hermaphroditic, and self-incompatible, and in the southern part of the shrub's native range, blossom from May to early June (Medan 1994). From July to October, these blooms develop into plentiful, blue-black drupes, each of which contains two to three seeds. Buckthorn's prolificacy is evidenced by Medan's (1994) observation that buckthorn genets produce between 403 and 1804 fruit each year. Primarily bird-dispersed, the laxative property of the drupes promote their spread (Hampe 2004). In Spain, at least 12 avian species eat buckthorn fruit (Hampe 2004), while in North America grosbeaks, starlings, robins, and waxwings, among other birds, do so (Catling & Porebski 1994). Water dispersal is an important secondary mechanism for the spread of buckthorn seeds, which can float for several weeks,

thus promoting the species' establishment in mesic environments, along with the seeds' high rate of germination (Howell & Blackwell 1977; Hampe 2004).

Buckthorn produces foliage earlier in the spring than most native North American plants and retains it longer in the autumn (Harrington et al. 1989), growing rapidly (Sanford et al. 2004; Knapp 2006). In Ontario, buckthorn comprises up to 90% of the vegetation in certain wetlands, and creates dense shaded thickets (Catling & Porebski 1994) that bias plant communities toward shade-tolerant species (Converse 1984). Glossy buckthorn resprouts well after cutting and burning, thus requiring consistent, multi-year efforts for successful eradication (Reinartz 1997; Upper Thames River Conservation Authority 2007).

In its wide native range, buckthorn exhibits high phenotypic and genetic diversity (Hampe & Bairlein 2000). Spanish populations, for example, grow abundantly before reproduction and have long lifespans, while central European populations reproduce early and senesce more quickly (Hampe & Bairlein 2000). Fruit from these two regions also evidence significant differences in the quantity of sugar, protein, and phenolic substances they contain (Hampe & Bairlein 2004). This variation is likely due to movement of buckthorn populations from Mediterranean glacial refugia into diverse environments in the Quaternary Period, with subsequent local adaptation (Hampe & Bairlein 2000). In Eurasia, as in North America, buckthorn inhabits drained mires, gallery forests, mesic woodlands, forest edges, wet meadows, riparian edges and fens (Converse 1984; Taft & Solecki 1990; Catling & Porebski 1994; Hampe 2004; Serdar et al. 2007). Woody genera that co-occur with buckthorn in both its native and introduced

ranges include *Salix* (willow), *Alnus* (alder), *Betula* (birch), *Populus* (poplar), and *Corylus* (hazel) (Janiszewski et al. 2006; Cohen & Kost 2007). The ectomycorrhizal ascomycete *Cenoccocum graniforme*, among other fungal species, forms a symbiosis with glossy buckthorn (Trappe 1962; Harley & Harley 1987; McDermott & Berry 2003).

Chemicals in buckthorn include the anthraquinones physcion and the aforementioned emodin, which occur in complex primary glycosides that are responsible for the plant's cathartic qualities (Gunton 1921; Izhaki 2002). Rhamnose is a common sugar found in these compounds. Emodin, present in 17 plant families including the Fabaceae, Polygonaceae, and Rhamnaceae, exhibits a host of functions, from herbivore deterrence to the prevention of premature fruit dispersal (Izhaki 2002). North American insects, for example, avoid feeding upon buckthorn leaves (Upper Thames River Conservation Authority 2007) while European beaver (*Castor fiber*) forage less frequently on buckthorn than other vegetation (Janiszewski et al. 2006).

Emodin's previously-mentioned allelopathic properties are exemplified by the capacity of root exudates from giant knotweed (*Polygonum sachalinense*) and the emodin-containing soil in which it persists, to inhibit amaranth (*Amaranthus viridis*) and lettuce (*Lactuca sativa*) seedling growth (Inoue et al. 1992

Physcion and emodin also alter soil pH and change the balance of soil inorganic ions and other chemicals (Inderjit & Nishimura 1999). Soil amended with these compounds is more acidic, demonstrates higher Na^+ availability, and possesses greater amounts of phenolic compounds than does non-amended soil

(Inderjit & Nishimura 1999). Phenolics, which are produced as emodin degrades, reduce PO_4^{3-} (phosphate) uptake by both vegetation and soil microbes (Glass 1973; Inderjit & Nishimura 1999).

Emodin additionally demonstrates antimicrobial properties, inhibiting at least 17 species of fungi (Singh et al. 1992), hindering the proliferation of viruses (Schinazi et al. 1990; Sydiskis et al. 1991), and suppressing the growth of many bacterial species, including those that dwell in the soil (LeVan 1984; Levin et al. 1988).

Speckled alder, a presumed recipient of emodin's detrimental effects, is a member of the family Betulaceae, which contains six genera and 130 species, most of which grow in temperate regions. The genus *Alnus* belongs to the subfamily Betuloideae and includes both trees and shrubs (Heywood et al. 2007). Alders are important to primary and secondary succession due to their colonization of low-nutrient and disturbed sites impacted by volcanic activity, floods, landslides, fires, and windfalls (Anderson et al. 2004;Yamanaka & Okabe 2006; Roy et al. 2007). Since alders, in association with *Frankia*, fix atmospheric nitrogen, the genus is also useful for mine reclamation, intercropping in agroforestry and timber applications, and phytoremediation of hydrocarbon-polluted soils, which are characteristically nutrient-poor (Baker & Schwintzer 1990; Roy et al. 2007). Due to their clonal growth, alders are also useful as windbreaks and for erosion control (Nesom 2003). Alders, together with *Frankia*, vesicular-arbuscular fungi, and ectomycorrhizal fungi such as *Cenococcum*

graniforme, form tetrapartite symbioses which synergistically promote alder growth (Trappe 1962; Jha et al. 1992; Beccera et al. 2005).

Speckled alder's range extends from British Columbia and the Yukon Territories in Canada through the midwestern and northeastern United States (Nesom 2003). The shrub reaches ten meters in height, has elliptical to ovoid doubly toothed leaves and gray bark with copious white lenticels (Nesom 2003). Native Americans and early European settlers used speckled alder bark to make black dye and to staunch blood flow from wounds (Erichsen-Brown 1989). Similar to buckthorn, speckled alder grows in thick clumps along stream banks and lakeshores as well as in bogs, fens and wet meadows. It is intolerant of shade, which is likely due to the high levels of photosynthate that nitrogen-fixation requires (Roy et al. 2007).

Speckled alder catkins are wind-pollinated and bloom from March to May, before the surrounding foliage becomes too thick to allow pollen passage (Nesom 2003). From August to September, the resultant woody cone-like infructescences grow to contain many small, winged nuts that are dispersed via wind and water in the winter and spring (Huenneke 1987). Speckled alder shrubs are monoecious (i.e., possess both male and female flowers) and reach sexual maturity by five to ten years of age (Nesom 2003). In addition to sexual reproduction, speckled alder also proliferates through vegetative growth from its root collar and underground runners (Huenneke & Marks 1987). Interestingly, however, in a three-year speckled alder demography study at four sites in New York State, Huenneke (1987) observed neither root suckers, offsets, nor seed germination. This suggests

either very low regeneration in the species or only periodic pulses of regeneration (Huenneke 1987).

Speckled alder communities often occur in post-glacial areas with acid to alkaline seasonally-flooded soils (Cohen & Kost 2007). These sites are classified variously as 'northern shrub thickets,' 'alder swamps,' '*Alnus incana* swamp shrublands,' '*Alnus incana* seasonally-flooded shrubland alliances,' and 'Laurentian-Acadian wet meadow shrub swamps' (Cohen & Kost 2007). The alder-*Frankia* symbiosis contributes significant quantities of nitrogen to these areas – approximately 60-320 kg/hectare annually, of which 48-185 kg/hectare is plant-available (Dawson 1990). This nutrient input is due in part to alder's inefficient reabsorption of foliar nitrogen before autumnal leaf abscission (Bischoff et al. 2001). Thus, nitrogen fixed by alder is recycled in plant communities, which translates into improved growth for co-occurring vegetation (Hurd et al. 2005). Jutras et al. (2006), for example, found eastern larch (*Larix laricina*) and black spruce (*Picea mariana*) in a Canadian peatland to develop thicker trunks when grown within three meters of speckled alder. Due to alder's capacity to fix nitrogen, it is considered a keystone species on floodplains (Anderson et al. 2004).

Speckled alder wetlands, as a function of their nitrogen content, exhibit high levels of primary productivity, and accumulate significant biomass, thus functioning as carbon storage sites (Tilton & Bernard 1975). These ecosystems also improve water quality by absorbing floodwater and catching sediment (Cohen & Kost 2007).

In addition to biogeochemical services, speckled alder wetlands provide cover and browse for many animal species, including moose (*Alces alces*), deer (*Odocoileus virginianus*), rabbits (*Silvilagus* spp.), beavers (*Castor canadensis*), muskrats (*Ondatra zibethicus*), mink (*Mustela vison*), river otters (*Lutra canadensis*), lynx (*Lynx canadensis*), and rare Blanding's turtles (*Emydoidea blandingii*) (Cohen & Kost 2007). Birds such as American woodcocks (*Philohela minor*), ruffed grouse (*Bonasa umbellus*), and redpolls (*Carduelis* spp.) consume speckled alder buds, flowers, and seeds (Cohen & Kost 2007), while alder stems furnish beaver with important dam and lodge construction materials (Hammerson 1994).

Native shrubs that coincide with speckled alder include silky dogwood (*Cornus amomum*), maleberry (*Lyonia ligustrina*), winterberry holly (*Ilex verticillata*), arrowwood viburnum (*Viburnum dentatum*), meadowsweet (*Spiraea latifolia*) and buttonbush (*Cephalanthus occidentalis*) (Richburg 1999; Cohen & Kost 2007). In the course of succession, alder wetlands may become swamp forests dominated by red maple (*Acer rubrum*), black ash (*Fraxinus nigra*), and poplar (*Populus tremuloides & P. balsamifera*) (Curtis 1959; Parker & Schneider 1974).

Interestingly, allelopathy may play a role in such replacement (Rice 1979). Jobidan & Thibault (1982) found water extracts of balsam poplar (*P. balsamifera*) to inhibit the height of alder seedlings, and to reduce *Frankia* nodulation and concomitant nitrogen fixation. Meanwhile, field assays of alder nitrogen fixation showed nodules in poplar stands to fix 83% less nitrogen than nodules distant

from poplar (Younger & Kapustka 1983). Phenolics and polyphenols were likely responsible for this inhibition. Thus, native plant successional dynamics indicate that phytochemicals have the capacity to disrupt the alder-*Frankia* symbiosis.

Frankia ia a filamentous, gram-positive actinomycete in the family Frankiaceae that is heterotrophic and aerobic (Baker & Schwintzer 1990). The bacteria, which was first described in the 1800s, is cosmopolitan and lives both freely in the soil as well as in symbiosis with actinorhizal plants. Nitrogen fixation rates in actinorhizal symbioses are similar to those of legumes and rhizobia, although actinorhizal symbioses occur mostly in temperate regions (Torrey 1978).

In actinorhizal relationships, the host plant provisions *Frankia* with carbon, receiving in exchange 70-100% of its requisite nitrogen (Myrold & Huss-Danell 2003). Vegetation may obtain nitrogen in three ways: ammonium uptake, nitrate uptake, or N_2 fixation. Of these, N_2 fixation is most expensive, in terms of ATP cost (Huss-Danell 1990). Although the *Frankia* symbiosis exacts a quantifiable toll, the benefit to the host plant outweighs the detriment, mainly because plants can't fix nitrogen on their own, whereas sunlight and CO_2 to make sugar for the bacteria is relatively abundant (Lundquist 2005). Inoculation with *Frankia* increases speckled alder biomass by 25-33% (Hendrickson et al. 1993).

Actinorhizal plants belong to eight families which, in addition to Betulaceae, include the Rosaceae, Myricaceae, Elaeagnaceae, Coriaceae, Datiscaceae, Rhamnaceae, and Casuarinaceae (Baker & Schwintzer 1990). Although actinorhizal vegetation exists on all continents except Antarctica, it is most diverse in North and South America (Benson & Dawson 2007).

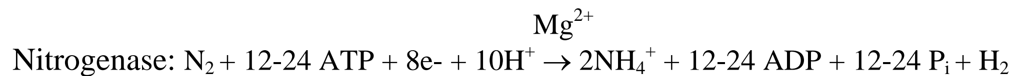
In concordance with the plants it colonizes, the *Frankia* genus is also varied, consisting of multiple strains (Torrey 1990). These fit into three major groups based on their ability to infect similar vegetation (Benson & Dawson 2007). Cluster 1 strains infect members of Betulaceae, Myricaceae, and Casuarinaceae; Cluster 2 strains form nodules on constituents of Rosaceae, Coriariaceae, Datiscaeae and the genus *Ceanothus* of Rhamnaceae; and Cluster 3 strains infect plants of Myricaceae, Elaeagnaceae, Rhamnaceae, and the genus *Gymnostoma* of Casuarinaceae (Benson & Dawson 2007). A single actinorhizal plant is commonly infected with multiple strains of *Frankia* (Benson & Schultz 1990).

Frankia dispersal from one plant or ecosystem to another occurs via either abiotic or biotic vectors (Paschke & Dawson 1993; Huss-Danell et al. 1997). Mass-wasting due to gravity, or movement of water and sediment during flooding are abiotic means of transport, while biotic vectors include earthworms, insects, rodents, and birds (Allen et al. 1992; Paschke & Dawson 1993; Huss-Danell et al. 1997; Yamanaka & Okabe 2006). Earthworms consume *Frankia*-infected soil and transfer it vertically through the substrate in their castings (Paschke 1993), while rodents on volcanic islands may unearth old *Frankia*-containing soil that has been buried by lava (Yamanaka & Okabe 2006). Additionally, infective *Frankia* has been seen to exist in the nests of five Illinois bird species including the American Robin, gray catbird, field sparrow, and common grackle. Since American robins build nests from soil, it makes sense that these structures would include *Frankia*. The presence of infective *Frankia* in nests made solely of grass and leaves,

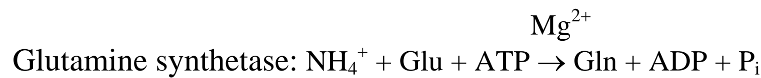
however, is more surprising, and suggests that birds feed their chicks prey that contain the bacteria, which subsequently becomes incorporated into nests (Paschke & Dawson 1993). Since many temperate bird species migrate long distances, it is possible they could transmit *Frankia* to far-off locales (Paschke & Dawson 1993).

Once present in soils, *Frankia* infects actinorhizal plants through hyphal contact with their root hairs, which subsequently deform and undergo cytokinesis in the process of nodulation (Baker & Schwintzer 1990). This is due to plant production of hormones such as auxins and cytokinins in response to *Frankia* (Wheeler et al. 1979). During nodulation, the plant also surrounds the bacteria with protective extracellular matrix (Berry & Sunell 1990). At maturity, nodules are essentially modified lateral roots with plant vasculature at the core, surrounded by tightly packed *Frankia* cells (Berry & Sunell 1990). In addition to extracellular matrix and other encapsulation material, *Frankia* vesicular cells, where nitrogen fixation takes place, are bounded by another membrane that keeps O_2 levels to a minimum. This is important because oxygen deactivates the enzyme nitrogenase, which is responsible for nitrogen fixation (Sylvia et al. 2005). Despite the necessity of protecting nitrogenase from oxygen, dinitrogen must still contact the enzyme for effective fixation, and lenticels at the nodule surface provide for this essential gas exchange (Berry & Sunell 1990). Plant-derived photosynthate for *Frankia*, meanwhile, enters nodules through the phloem, although the bacteria additionally obtain carbon by breaking down pectins and polysaccharides (Huss-Danell 1990).

In the process of nitrogen fixation, *Frankia* initially converts atmospheric N_2 into ammonium (Huss-Danell 1990). However, ammonium is toxic and must be assimilated into amino acids and amides for later transport in the plant. This conversion proceeds according to the nitrogenase-glutamine synthetase (GS) and glutamate synthase (GOGAT: glutamine oxo-glutarate aminotransferase) pathway, as follows:



Nitrogenase: $N_2 + 12\text{-}24\ ATP + 8e^- + 10H^+ \xrightarrow{Mg^{2+}} 2NH_4^+ + 12\text{-}24\ ADP + 12\text{-}24\ P_i + H_2$



Glutamine synthetase: $NH_4^+ + Glu + ATP \xrightarrow{Mg^{2+}} Gln + ADP + P_i$

Glutamate synthase: $Gln + \alpha\text{-ketoglutarate} + NAD(P)H + H^+ \rightarrow 2Glu + NAD(P)^+$

(From Benson & Schultz 1990; D. Benson, pers. comm.)

This process requires significant amounts of energy in the form of ATP, as well as NAD(P)H, which highlights the cost to the host plant of maintaining the symbiosis. To reduce one dinitrogen molecule to two ammonium (NH_4^+) molecules and then assimilate them to two glutamate molecules requires 14-26 ATP and 2 NAD(P)H (Benson & Schultz 1990). The products of assimilation, glutamine and glutamate, are important for synthesizing protein; one glutamate molecule additionally returns as a substrate for glutamine synthetase. In alder, the α-amino acid citrulline is the most common nitrogenous compound found in nodules, and is transmitted to the rest of the plant through the xylem (Huss-Danell 1990).

Wetlands, such as those inhabited by speckled alder and *Frankia*, are characterized by high light availability and copious moisture (Gopal & Goel

1993). Where nutrients are also abundant, wetland plants compete vigorously for access to these resources (Neori et al. 2000). In contrast to exploitative competition, in which plants directly struggle to obtain resources (Harper 1977), allelopathy is a form of interference competition in which plants suppress each other to thwart subsequent resource use (Gopal & Goel 1993). Neori et al. (2000) indicate that wetland plant genera possess multifunctional chemicals that, similar to emodin, both deter herbivores and pathogens as well as have allelopathic effects. Thus, I was interested in examining buckthorn's potential allelopathy in the context of native phytochemical interactions.

In order for allelopathy studies to be ecologically relevant, they should mimic natural processes as much as possible (Inderjit & Nilsen 2003). However, it is difficult to tease apart the effects of direct competition from those of allelopathy in the field (Dorning & Cipollini 2006). In addition to the aforementioned use of appropriate bioassay plants, procedures that increase the pertinence of allelopathy trials include the use of water (instead of acetone or alcohol) to extract active plant components, as well as incorporation of native substrate and soil microbes (Inderjit & Dakshini 1995; Inderjit & Duke 2003).

I executed four experiments to test either buckthorn allelopathic effects on alder and other native shrubs, or buckthorn chemical suppression of *Frankia*. Since I was interested in buckthorn chemical impacts on alder at a variety of alder life stages, I examined buckthorn extract effects on alder seed germination and seedling growth, as well as buckthorn mulch consequences for alder sapling growth.

Alder germination in buckthorn and native plant extracts

In this trial, I germinated alder seeds in petri plates with aqueous extracts of buckthorn and two native shrub species: arrowwood viburnum and silky dogwood, along with water as a control. I chose viburnum and dogwood because I found no indication in the literature that they were allelopathic. If buckthorn chemically inhibited alder, I expected that alder seeds would germinate less frequently in buckthorn extract versus other treatments.

Alder seedling growth in buckthorn and native plant extracts

Similar to the germination trial, I treated alder seedlings with buckthorn, viburnum, and dogwood extracts, or with water as a control. Due to putative buckthorn allelopathy, I expected that alder seedlings treated with invasive extract would demonstrate thinner stems, reduced height, and fewer leaves than alder seedlings treated with native extracts or water.

Growth of alder and other indigenous shrubs in buckthorn and native mulches

This experiment explored the impacts of buckthorn and native silky dogwood root and leaf mulches on indigenous alder, viburnum, and meadowsweet saplings. I expected bioassay plants to exhibit thinner stems, reduced height, and lower leaf production in buckthorn versus native mulch, due to the presumed effects of buckthorn allelochemicals, such as emodin.

Buckthorn effects on *Frankia* mycelial mat formation and growth

This trial examined the consequences of different concentrations of aqueous buckthorn extract on alder-infective *Frankia* mycelial mat morphology and growth. Due to phytochemical suppression, I hypothesized that bacterial growth responses would progressively diminish as concentrations of buckthorn extract increased.

Methods

Alder germination in buckthorn and native plant extracts

Bioassay species. In October 2007, I collected speckled alder seeds from three plants at each of three Western Massachusetts sites: Wentworth Farm and Plum Brook Conservation Areas in Amherst, and on the campus of Mount Holyoke College in South Hadley. I subsequently cold-stratified seeds at 2° C for seven months before the experiment began.

Site. This experiment took place between September 15th and October 20th 2008 in Talcott Greenhouse at Mount Holyoke College under a grow-light that created a 14-hour daily photoperiod. With this approach, I attempted to ameliorate the potential phenological difficulties posed by germinating temperate shrubs during New England autumn. I spatially organized treatment levels in a repeated array to control for varied light availability in the greenhouse.

Extracts. I collected whole-plant tissue from glossy buckthorn and native arrowwood and silky dogwood during the first week of September 2008. After air-drying the plant material on newspaper for one week, I cut it into 2 cm pieces and steeped it for 48 hours in species-specific 5 gallon plastic buckets at a

concentration of 50 g dried plant material per 1 L distilled water. Since I wanted to avoid using the high concentrations of plant tissue that have been critiqued in the allelopathy literature for lacking ecological relevance (Inderjit & Nilsen 2003), the concentrations used for this experiment were below those employed in some allelopathy trials (Gatti et al. 2004; Javaid et al. 2006; Modallal & Al-Charchafchi 2006). However, since it is difficult to determine the precise relationship between extract concentration and field conditions (Inderjit & Dakshini 1995), this decision was necessarily somewhat arbitrary.

After using nylon hardware cloth to remove coarse debris, I successively filtered extracts with Whatman 2.7 and 1.0 micron glass fiber paper and Zap-Cap 0.45 and 0.2 micron bottletop filters, subsequently storing the result in autoclaved bottles to maintain sterility. Distilled, autoclaved water served as a control.

To make one experimental unit, under a laminar flow hood I surface-disinfected 20 alder seeds in 5% bleach solution and placed them in a petri plate between two pieces of sterilized germination paper. I then added 8 mL of the appropriate extract (or water) and wrapped the plate in parafilm to prevent fluid loss and microbe infiltration. Four weeks into the experiment, I added an additional 2 mL of proper treatment liquid to each plate to prevent desiccation. I replicated each plant extract 25 times and replicated the control 50 times. By increasing water replication, I hoped to get a better sense of baseline alder germination.

Measured responses and statistical analysis. I assessed percent germination per plate at the trial's conclusion. Using JMP software, I performed an *a priori*

Student's t-test at α=0.05 for alder germination (arcsine transformed) in buckthorn extract or water. I also executed a one-way ANOVA (two-tailed) at α=0.05 for effects of extract and water on alder germination. For this analysis, I used a post-hoc Tukey test to detect differences among treatment levels.

Alder seedling growth in buckthorn and native plant extracts

Bioassay species. I started alder seedlings from seed collected and stratified as described for the germination trial. After one month of growth at a density of approximately one seedling per cm in trays with Fafard Germination Mix, I transplanted the seedlings into peat-filled potting flats with individual 5 x 5 cm compartments. To ensure the presence of alder's bacterial symbiont, I inoculated each seedling with 30 mL of *Frankia* solution. This was a water suspension of alder nodules gathered at Wentworth Farm and Mount Holyoke College, which I made according to a protocol developed at New England Wetland Plants (NEWP), a restoration nursery in Amherst, Massachusetts. I surface-treated the alder nodules for one minute with 9 mL Zeratol disinfectant in 1 L water to remove potentially confounding microbes. I then rinsed the nodules in water to prevent the Zeratol from killing *Frankia* at a later point in the process. After macerating the nodules with a mortar and pestle, I combined 150 g of the resulting paste with 5 L water, subsequently aerating the solution overnight with a Tetra air pump in a five-gallon plastic bucket.

Site. This trial took place from October 25th to January 31st 2008 in Talcott Greenhouse under a grow-light that created a 14-hour daily photoperiod. As for

the germination trial, I mixed treatment levels in a repeating array to prevent varying light availability from confounding the experimental results.

Extracts. In mid-October, I made buckthorn, arrowwood, and dogwood extracts as described for the germination trial but in this case used 75 g of dried plant tissue per 1 L water. This increased concentration of plant material was meant to correspond with older, and potentially hardier, bioassay plants, but still remained below concentrations used in several allelopathy trials (Gatti et al. 2004; Javaid et al. 2006; Modallal & Al-Charchafchi 2006). On October 25th, I applied 20 mL of the appropriate extract or water to each alder seedling and, after five weeks of regular watering, administered a second 20 mL of solution on November 29th. On December 2nd, the Plant, Soil, and Water Laboratory at the University of Georgia determined the amount of nitrate and ammonium in a sample of each extract type to allow for later differentiation of allelopathic and nutrient effects. There were 36 replicates per treatment level.

Measured responses and statistical analysis. At the end of the trial on January 31st, I measured alder seedling basal diameter (mm), height (cm), and leaf number. Using JMP software, I performed *a priori* t-tests at $\alpha=0.05$ for alder growth in each of these response variables in buckthorn extract or water. I also executed one-way ANOVAs (two-tailed) at $\alpha=0.05$ for effects of buckthorn and native extract or water on alder seedlings for each response variable. I initially separated treatment means with Student's t-tests to detect as many potentially biologically meaningful signals as possible, later employing Tukey tests to perceive the strength of these signals while correcting for Type I error.

Growth of alder and other indigenous shrubs in buckthorn and native mulches

Bioassay species. With funding from Dr. Martha Hoopes, the Mount Holyoke College Department of Biological Sciences, and Harvard Forest, I purchased alder, meadowsweet, and arrowwood viburnum bioassay plants from New England Wetland Plants. NEWP growers started alder from local seed in the spring of 2006, and propagated arrowwood and meadowsweet from cuttings made in Amherst in June of 2007. These plants, similar in size, overwintered for one season in Shutesbury, Massachusetts prior to their inclusion in this trial. During experimental set-up, in July of 2008, I inoculated each alder plant with 150 mL of *Frankia* emulsion (as prepared above) to avoid potentially confounding effects of excluding alder's symbiont. I also wanted to ensure that alder plants possessed Frankia with the expectation that chemicals in buckthorn mulch could disrupt the mutualism, thereby affecting alder growth.

Site. From early July to late August 2008, this experiment took place at Harvard Forest in Petersham, Massachusetts, in a lath house. This slatted structure approximated natural conditions (i.e., temperature, light availability) more closely than would a greenhouse, but similarly excluded large herbivores. To supplement rainfall, I mist-irrigated plants in equal fashion across treatment groups. As for other experiments, I placed plants in a repeating treatment level array to control for differential light abundance in the lath house.

Mulches. I gathered root and leaf tissue from both glossy buckthorn and silky dogwood in September 2007 from Wentworth Farm and Plum Brook Conservation Areas and, in neighboring Shutesbury, at the corner of Buffam and North Valley Roads. I harvested material from three different plants at each site, subsequently air-drying the tissue for a month before storing it in sealed Ziploc bags through the winter. By collecting mulches in the fall, close to natural leaf abscission, I hoped to mimic ecological processes as much as possible.

On May 5th 2008, the Plant, Soil and Water Laboratory in Athens, Georgia analyzed the ratio of carbon to nitrogen present in the mulches. On July 6th, I applied mulches to bioassay vegetation in 12% concentrations of mulch to soil (dry weight), using Sungro Sunshine Mix #2 with no fertilizer to avoid deactivating the nitrogen-fixing capacity of *Frankia*. I mixed mulch with the top six cm of soil. In addition to buckthorn and dogwood root and leaf mulches, I also included a "no mulch" control, which was necessary for determining the directionality of mulch effects. I replicated each treatment 12 times.

Measured responses and statistical analysis. On July 7th, one day after mulch application, I measured bioassay plant height (cm), leaf number, and basal diameter (mm). With these data, I executed a one way ANOVA (two-tailed) at α=0.05 for each bioassay species to check the assumption that plants were of similar initial size across treatment groups. On August 26th, I assessed plants again, subsequently performing a two-way ANOVA (two-tailed) at α=0.05 for effects of mulch species, mulch organ, and the interaction between them on plant responses. In order to compare mulch effects with those of the control, I also

executed a one-way ANOVA (two-tailed) at α=0.05 for impacts of all mulch treatments (including the control) on bioassay plants. I used Tukey post-hoc tests to distinguish treatment means.

Buckthorn effects on *Frankia* growth and mycelial mat formation

Bioassay species and growth medium. I tested buckthorn effects on growth of alder-infective *Frankia* strain cpI1 cultured in *Frankia*-defined minimal medium (FDM). One liter of FDM consisted of 850 mL distilled water, 5 g succinate (carbon source), 0.5 g ammonium chloride (NH_4Cl), 50 mL 20X stock salt solution, 1 mL 1000X iron solution, and 100 mL 10X potassium phosphate buffer. Salt solution was comprised of 1 L distilled water mixed with 2 g $CaCL_2$ and 4 g $MgSO_4$, while iron solution combined 100 mL distilled water with 0.75 g Na_2EDTA, 0.56 g $FeSO_4$, and 0.02 g Na_2MoO_4. Phosphate buffer contained 68 g KH_2PO_4 (potassium phosphate monobasic) and 114.1 g K_2HPO_4 (potassium phosphate dibasic) per 1 L water, respectively. To create the final buffer mixture, my collaborator, Juliana Mastronunzio at the University of Connecticut, slowly added 500 mL of potassium phosphate dibasic to 1 L of potassium phosphate monobasic until a pH of 6.5 resulted.

Treatments. I made buckthorn extract as detailed for the alder seedling trial. From this stock solution, J. Mastronunzio and I created four concentrations: 0% (for positive and negative controls), 10%, 50%, and 70%. Using aseptic technique under a laminar flow hood, we pipetted 1 mL growth medium, 0.5 mL buffer, 0.1 mL *Frankia* CpI1, and quantities of autoclaved distilled water and extract appropriate to each concentration into glass test tubes. 0% tubes contained

no extract and 3.5 mL of water, 10% tubes contained 0.5 mL extract and 3.0 mL water, 50% tubes contained 2.5 mL extract and 1.0 mL water, and 70% tubes contained 3.5 mL extract and no water. We subsequently cultured all experimental tubes (except for the negative controls) for three days at 30° C in a Forma Scientific orbital shaker at 125 revolutions per minute. We stored negative control tubes, meanwhile, in a refrigerator at 2° C, a temperature known to retard *Frankia* growth (D. Benson, pers. comm.). In addition, we refrigerated calibration blanks we made of each extract concentration for use with a Klett Colorimeter. This device measures turbidity, a proxy for microbial abundance. We replicated each treatment five times.

Measured responses and statistical analysis. Although we planned to assess *Frankia* abundance with a Klett Colorimeter, our calibration blanks changed hue over the course of incubation such that they were no longer useful. Thus, we made qualitative observations of *Frankia* mycelial mat formation and quantified *Frankia* dry weight with a Mettler balance. This latter assessment took place after desiccation of tube contents for six hours at 60° C in a biological oven. To ensure that the dry weight appraisal sufficiently captured true differences, I compared positive and negative controls with a Student's t-test at α=0.05 with JMP software. I subsequently executed a one-way ANOVA (two-tailed) at α=0.05 for effects of buckthorn extract and controls on *Frankia* dry weight. For this analysis, post-hoc Tukey tests separated treatment means.

Results

Alder germination in buckthorn and native plant extracts

The *a priori* Student's t-test showed alder seeds to germinate significantly less frequently in buckthorn extract than in water (t_{73}=4.68, p=0.03; Figure 2). Although the overall treatment effect was significant when comparing all extract or water effects ($F_{3,121}$=6.67; p=0.0003), post-hoc Tukey tests did not distinguish between buckthorn extract or water means (Figure 3). Interestingly, even with the reduced capacity to detect differences in the combined analysis, alder germination in dogwood extract was significantly lower than germination in the control (Figure 3).

Alder seedling growth in buckthorn and native plant extracts

A priori Student's t-tests revealed buckthorn extract to reduce alder basal diameter (t_{70}=7.95, p=0.075; Figure 4), although not alder height (t_{70}=2.37, p=0.13) or leaf number (t_{70}=0.02, p=0.88). There was a significant effect of extract treatment on alder basal diameter, but not on other responses (Table 1). Post-hoc Student's t-tests showed smaller alder basal diameter in buckthorn extract versus viburnum extract (Figure 5), but more conservative Tukey tests indicated no differences in basal diameter among treatment means.

Laboratory analysis of plant-available nitrogen in the extracts (Table 2) revealed that buckthorn extract had approximately six times more ammonium than dogwood extract, and greater than 23 times more ammonium than viburnum extract. There was little difference among the extracts, however, in nitrate content.

Growth of alder and other indigenous shrubs in buckthorn and native mulches

At the beginning of the experiment, there was no statistical difference in alder or viburnum size among treatment levels (Table 3). Similarly, meadowsweet leaf number and basal diameter were initially comparable (Table 3). However, there was an unplanned height difference among meadowsweet bioassay plants (Table 3). Namely, meadowsweet in the buckthorn root mulch level was taller than meadowsweet in the dogwood root mulch level (Figure 6).

Although mulch species and mulch organ did not have a significant effect on alder growth responses, the interaction between these terms did impact alder basal diameter (Table 4). Specifically, alder grown in buckthorn root mulch had thinner stems than alder grown in buckthorn leaf mulch (Figure 7). The effects of all mulch treatments together also had a significant effect on alder basal diameter (Table 5), although here post-hoc Tukey tests did not distinguish buckthorn root mulch impacts from the control (Figure 8). Even with less power in the combined analysis, buckthorn root mulch looked to inhibit alder basal diameter more than did buckthorn leaf mulch (Figure 8). Perhaps the insignificant effect of mulch species on alder growth (Table 4) is due in part to this differential.

For meadowsweet, mulch species significantly affected this plant's height and leaf number (Table 4). Namely, meadowsweet grown in buckthorn mulch was taller and had more numerous leaves than meadowsweet grown in native mulch (Figure 9, Figure 10). Meadowsweet grown in any leaf mulch was also taller and had more leaves than meadowsweet grown in any root mulch (Table 4,

Figure 11, Figure 12). This was despite the initial bias toward greater height in the buckthorn root mulch level (Figure 6).

The interaction between mulch species and mulch organ affected meadowsweet leaf quantity (Table 4). Tukey analysis revealed meadowsweet to produce more leaves in buckthorn leaf mulch than in any of the other mulches (Figure 13). Comparing all mulch treatments indicated similarly high meadowsweet leaf production in buckthorn leaf mulch versus other levels (Table 5, Figure 14). Buckthorn leaf mulch thus contributed to the comparatively salutary effects of leaf mulch versus root mulch on meadowsweet height and leaf number (Figure 11, Figure 12).

For meadowsweet height, the impact of mulch was more complex. Although the effect of all mulch treatments was significant (Table 5), none of the mulch means were statistically distinct from that of the control (Figure 15). Additionally, meadowsweet grown in buckthorn leaf mulch was taller than meadowsweet grown in any native mulch. Meanwhile, meadowsweet grown in buckthorn root mulch was taller only than meadowsweet grown in native root mulch (Figure 15).

Distinct from alder and meadowsweet bioassay species, viburnum evidenced little impact of mulch treatment on its basal diameter, height, or leaf number (Table 4, Table 5).

Evaluation of carbon to nitrogen ratios of the mulches revealed leaf mulch to have more relative nitrogen than root mulch. Buckthorn leaf, in particular, contained the greatest relative amount of nitrogen (Table 6).

Buckthorn effects on *Frankia* growth and mycelial mat formation

Observation of *Frankia* mycelial mats showed intact mat morphology only in the positive control tubes. This assessment, however, was purely qualitative.

Evaluation of *Frankia* dry weight data indicated the presence of an outlier in the positive control level that was six times larger than the level mean. I imagined that this was due to measurement error and thus analyzed the data both with and without the outlier.

Including the outlier, *Frankia* dry weight did not differ between the positive and negative controls (t_7=1.70, p=0.23). This result did not bode well for further analysis with the outlier, since visual observation of the control units indicated more cloudiness (growth) in the positive versus negative controls. Additionally, control methodology was designed by D. Benson, a seasoned *Frankia* researcher.

Excluding the outlier, *Frankia* was significantly heavier (t_6=6.97, p=0.039) in the positive versus negative control levels (Figure 16). Therefore, I continued analysis without the outlier.

Buckthorn extract and control treatment significantly affected *Frankia* dry weight ($F_{4,18}$=10.17, p=0.002). Namely, *Frankia* grown in l0% buckthorn extract was heavier than the negative control but did not differ from the positive control, while *Frankia* grown in 50% and 70% buckthorn extracts was similiar in weight to the negative control but lighter than the positive control (Figure 17). Although the 70% extract level had a higher mean than the 50% extract level, there was no significant difference between them (Figure 17). Additionally, *Frankia* grown in

50% and 70% buckthorn extract was lighter than *Frankia* grown in 10% buckthorn extract.

Discussion

Alder germination in buckthorn and native plant extracts

Results indicate mild buckthorn chemical inhibition of alder germination but more pronounced suppression by native dogwood. This is surprising given that there is no reference in the literature to dogwood allelopathy, and suggests that chemical inhibition is indeed important in structuring competitive relationships among co-evolved native wetland plants, as Gopal & Goel (1993) and Neori et al. (2000) propose.

The novel weapons hypothesis is not supported by the results of this trial since native co-evolved dogwood suppressed alder germination more effectively than did buckthorn, contrary to the expectations of the theory.

If modest buckthorn allelopathic effects on alder germination combine with stronger dogwood effects, competitive relations between these two native shrubs may change over time. In other words, the addition of buckthorn's effects to dogwood's more pronounced allelopathy may tip the competitive balance between alder and dogwood, resulting in increased inhibition of alder.

It is intriguing that buckthorn chemical suppression of alder germination is modest, at best, in view of the invader's possession of allelopathic emodin. Work by Inoue et al. (1992), however, sheds light on this conundrum. Allelopathic *Polygonum sachalinense* exudes glycoside emodin into the soil, where the chemical degrades into aglycone emodin, which is the active form that suppresses

seedling growth. Thus, buckthorn aqueous extract may contain primarily the less active glycoside form of emodin, and further work exploring buckthorn allelopathy may be most effectively undertaken in a soil context that includes a functionally diverse representation of the native microbial community.

Alder seedling growth in buckthorn and native plant extracts

Buckthorn appeared to only mildly inhibit alder stem girth. Since this experiment involved soil, however, one might expect, according to the above reasoning, that substrate processes would degrade emodin into its active form, with correspondingly elevated allelopathic effects. However, the absence of soil microbes additional to *Frankia* may have prevented such degradation or, alternatively, buckthorn allelopathic effects were simply weak. Furthermore, since buckthorn extract contained a considerable amount of ammonium, this nutrient benefit may have worked in opposition to the effects of presumably suppressive chemicals.

Notably, dogwood extract did not inhibit alder seedling growth. This contrasted with its strongly suppressive effects on alder germination and indicates that allelopathic dogwood chemical impacts are alder life-stage specific.

Growth of alder and other indigenous shrubs in buckthorn and native mulches

Since meadowsweet bioassay plants in buckthorn root mulch exhibited greater initial height than those in dogwood leaf mulch, I needed to consider this bias. This unplanned difference made the allelopathy test, in effect, more conservative since plants in invasive buckthorn root mulch were bigger from the outset. However, this slant may have contributed to the overall greater height of

meadowsweet grown in buckthorn versus native mulch. Since a similar pattern emerged for meadowsweet leaf response, though, this apparently facilitative effect may be biologically accurate.

At the end of the experiment, alder basal diameter was significantly smaller in buckthorn root mulch versus buckthorn leaf mulch. Thus, if buckthorn has allelopathic effects on alder, this likely occurs via root exudation instead of leaf litter effects. This result points to the need for further investigation of belowground interactions in buckthorn invasion, particularly since the root mulch I used was nine months old at the time of application and was thus a very conservative proxy for active root exudation. Other invasive plants that demonstrate root-mediated allelopathy include aforementioned spotted knapweed (Weir et al. 2003), and common reed (Rudrappa et al. 2007), as well as Amur honeysuckle (*Lonicera maackii*) (Dorning & Cipollini 2006).

To examine root-mediated buckthorn chemical inhibition of alder in the future, I plan to condition soil by growing buckthorn in it for several months, then removing the plants. I will inoculate each pot with a quantity of soil from a local wetland margin to ensure the presence of the native microbial community, as well as apply *Frankia*. Subsequently, I will propagate alder seeds in this substrate and measure their germination rates and growth. The control treatment will consist of buckthorn-conditioned soil that is amended with activated charcoal, a substance that has been commonly employed in allelopathy trials to neutralize phytochemicals (Rudrappa et al. 2007). If alder seeds without activated charcoal germinate or grow less than the control seeds, this will support the hypothesis that

buckthorn allelopathically inhibits alder in a root-mediated fashion. Alternatively, it could mean that buckthorn root exudates suppress *Frankia* in a way that is meaningful for alder growth.

In contrast to potential buckthorn root mulch inhibition of alder basal diameter, buckthorn mulch facilitated meadowsweet height and leaf production. Since buckthorn leaf mulch had more relative nitrogen than other mulches, this effect was likely nutrient-mediated. The positive impact of all leaf mulch on meadowsweet height and leaf production was likely driven by beneficial buckthorn leaf mulch effects.

Since alder, meadowsweet, and viburnum plants exhibited species-specific responses to buckthorn mulches, there is apt to be a chemical and nutrient-mediated mechanism for buckthorn alteration of native plant community structure.

Buckthorn effects on *Frankia* mycelial mat formation and growth

Because *Frankia* did not form normal mycelial mats in buckthorn extract, this suggests that buckthorn chemicals suppress the bacteria. Since *Frankia* in 50% and 70% buckthorn extract levels weighed significantly less than the positive control, this also implies buckthorn inhibition of *Frankia* growth. Both of these results correspond well with emodin's known anti-bacterial activity (Izhaki 2002). Perhaps the quantity of active chemical in the 10% buckthorn extract concentration was not high enough to reduce *Frankia* growth.

Since buckthorn extract inhibits *Frankia* proliferation *in vitro*, the next step is to explore buckthorn disruption of *Frankia* in the context of its mutualism

with alder. This will involve conditioning soil with buckthorn, inoculating the substrate with *Frankia*, and growing alder from seed in the resulting soil. I will subsequently quantify alder growth and nodule formation and, if possible, will assess nitrogenase activity using acetylene-reduction. The control treatment will consist of alder seeds grown in buckthorn-conditioned (and *Frankia*-inoculated) soil that is additionally amended with activated charcoal. Both experimental and control soils will be inoculated with substrate from a local wetland to include other members of the native microbial community.

Since, as far as I know, there has been no report of an invasive plant disrupting an actinorhizal mutualism, this preliminary finding is exciting. Invasive plants, such as *Myrica faya* in Hawai'i, are known to increase soil nitrogen levels and thus change ecosystem-wide dynamics (Vitousek et al. 1987), but in this case, the putative disruption would probably result in decreased nutrient availability, with unknown consequences for indigenous wetland margin organisms. However, since native plants have been found to evolve resistance to invasive plant allelochemicals (Callaway et al. 2005), bacteria may do the same, especially since they exhibit considerably shorter generation times. Nevertheless, as Maron & Jefferies (1999) suggest, invasive plant-induced changes in nutrient dynamics have protracted effects on native ecosystems, resulting in long-term alteration of vegetative communities.

CHAPTER III

INDIGENOUS SPECKLED ALDER

FACILITATION OF GLOSSY BUCKTHORN

Introduction

Although the role of competitive interactions in organizing vegetative communities is clearly important (Wilson & Tilman 1991; Tilman 1994), and has been the focus of much ecology research, positive interactions are increasingly recognized as vital to the development of plant community structure and ecological succession (Callaway & Walker 1997; Stachowicz 2001; Chapin et al. 1994; Lockwood et al. 2007). According to the facilitation model of succession (Connell & Slatyer 1977), early colonizers promote the growth of later colonizers until a climax state is attained (Molles et al. 2005). Support for facilitation as a driver of natural succession comes from the work of Li & Wilson (1998), who found shrubs, which colonize old fields at high densities in Saskatchewan, to promote the growth of other shrubs, thus competitively excluding extant grasses. *Carmichaelia odorata*, an indigenous woody plant of New Zealand temperate rainforests, also differentially improves the growth of three native tree species, thus shaping their future relative abundance (Bellingham et al. 2001).

Mechanisms of plant facilitation include provision of physical support (i.e., trees sustaining vines and epiphytes), alteration of soil characteristics, and mycorrhizal resource transfer (Hunter & Aarssen 1998). Soil characteristics can be altered through leaf drop and nitrogen fixation, both of which increase nutrient content in the substrate (Hunter & Aarssen 1988; Richardson et al. 2000).

Mycorrhizal resource transfers take place when symbiotic fungi with minimal host specificity form hyphal webs connecting plants of different species, thus shifting nitrogen, phosphorus, and carbon among the vegetative constituents (Whittingham & Read 1982; Chiariello et al. 1982). These transfers may be direct, passing from plant to plant via fungal hyphae, or indirect, as plants leach nutrients into the soil, that mycorrhizae subsequently assimilate (Hunter & Aarssen 1988).

Although prevalent among co-evolved plants, facilitation also occurs at the native-invasive interface. For example, in California prairies, indigenous nitrogen-fixing bush lupine increases both root and stem biomass of the exotic grass *Bromus diandrus* (Maron & Connors 1996). Here, I examined the potential of native New England speckled alder to similarly promote glossy buckthorn.

<u>Buckthorn seedling growth in alder extract</u>

In this experiment, I tested alder facilitation of buckthorn by treating buckthorn seedlings with aqueous alder extract or water. Due to presumably high levels of nitrogen in alder tissue, at the experiment's end I expected alder-treated buckthorn seedlings to exhibit thicker stems, greater height, and more numerous leaves than water-treated buckthorn seedlings.

Methods

Buckthorn seedling growth in alder extract

Bioassay species. I started buckthorn seedlings from seeds gathered at Wentworth Farm, Plum Brook, and Plum Springs Conservation Areas in Amherst in September 2007. I stratified and propagated these as for alder seeds in the alder seedling trial, although without *Frankia*. In mid-October 2008, I transplanted the buckthorn seedlings into peat-filled potting flats with 5 x 5 cm separate compartments.

Site. This experiment took place for 14 weeks from October 22nd to January 29th 2008 in Talcott Greenhouse under a grow-light that created a 14-hour daily photoperiod. As for other experiments, I spatially mixed bioassay plants in a repeating array of treatment levels to control for differential light availability in the greenhouse.

Treatments. On October 22nd, I applied aqueous alder extract or sterilized, distilled water (as a control) to buckthorn seedlings. I made alder extract as detailed previously at a concentration of 75 g plant material per 1 L water. After five weeks of regular care, on November 26th, I administered a second 20 mL of appropriate solution to each seedling. There were 36 replicates per treatment group.

Measured responses and statistical analysis. At the conclusion of the trial on January 29th, I measured buckthorn height (cm), leaf number, and basal diameter (mm). For each response, I performed separate Student's t-tests at α=0.05 for alder extract and water effects on buckthorn growth.

Results

Buckthorn seedling growth in alder extract

Treatment with alder extract or water had a significant effect on buckthorn basal diameter, although not on buckthorn height or leaf number (Table 7). Specifically, buckthorn treated with alder extract had thicker stems than buckthorn treated with water (Figure 18).

Discussion

Buckthorn seedling growth in alder extract

Greater basal diameter of buckthorn seedlings treated with alder extract versus water suggests alder facilitation of buckthorn. However, since buckthorn height and leaf number responses were not affected by extract treatment, more work needs to be done to support this preliminary finding. Thus, in a future soil conditioning experiment, I will propagate alder in soil inoculated with the native microbial community (especially *Frankia*) for several months, successively removing the vegetation. Next, I will plant buckthorn seeds in the conditioned substrate, subsequently measuring their germination rate and growth. The control for this experiment will consist of buckthorn seeds propagated in soil conditioned by a variety of non-alder native wetland shrubs such as buttonbush, winterberry, and viburnum. If buckthorn seeds germinate with greater frequency and demonstrate increased growth in alder-conditioned versus other-native-shrub conditioned soil, this will furnish further evidence for alder facilitation of buckthorn.

If alder does facilitate buckthorn, this would lend further support for the importance of positive interactions in invasion success. Also, since a native legume has been seen to promote an invasive plant (Maron & Connors 1996) and invasive actinorhizal *Myrica faya* benefits exotic vegetation (Vitousek et al. 1987; Vitousek & Walker 1989), native actinorhizal facilitation of buckthorn represents a little-studied variation on the theme of nitrogen-fixation-mediated plant invasion.

Since speckled alder's *Frankia* mutualism exacts a carbon toll on the shrub (Vitousek 1982; Lundquist 2005), it may be expected to compete poorly with buckthorn, which putatively functions as a 'free-rider' – reaping the benefits of nitrogen-fixation without incurring the costs. Over time, this could lead to competitive exclusion of alder from wetland margin communities, particularly since buckthorn grows quickly, forming dense shade (Catling & Poresbski 1994), which alder tolerates poorly (Roy et al. 2007). In fact, alder is excluded in the course of natural succession in Alaska (Crocker & Major 1955). However, since buckthorn apparently benefits from alder, eliminating the native shrub would be to its detriment. Therefore, natural selection may favor buckthorn genotypes that are only moderately better competitors than alder, thus maintaining the invasive shrub's access to fixed nitrogen (Hunter & Aarssen 1988).

In terms of mycorrhizal nutrient transfer, I find it fascinating that both alder and buckthorn genera are ectomycorrhizal, and that Trappe (1962) reported the same species of symbiotic fungus, *Cenococcum graniforme*, to colonize them. Although this observation was made in Europe, it nonetheless suggests that

nitrogen from alder could be transmitted to buckthorn via shared hyphal connections. In light of ectomycorrhizal fungi's typical dearth of host-specificity (Finlay & Read 1986), this is an intriguing possibility.

CHAPTER IV

SYNTHESIS & MANAGEMENT IMPLICATIONS

Revisiting my hypothetical understanding of glossy buckthorn and speckled alder relations (Figure 1), I find mild support for allelopathic effects of buckthorn on alder and stronger indications of buckthorn suppression of *Frankia*. In coincidence with alder facilitation of buckthorn, powerful buckthorn allelopathy would result, over time, in small populations of alder and correspondingly greater buckthorn abundances. However, if alder populations declined sufficiently, their promotion of buckthorn growth would also become negligible, and the positive feedback would effectively halt.

Since current evidence suggests that buckthorn allelopathy is modest, however, I find it unlikely that this outcome will result, particularly since native plants can adapt to invasive allelochemicals (Callaway et al. 2005). Further exploration of the impacts of root exudates on alder should help elucidate the role allelopathy plays in buckthorn invasion of New England wetland margins. Testing buckthorn disruption of the alder-*Frankia* mutualism, and mycorrhizal transfer of nutrients between alder and buckthorn may shed additional light on belowground factors that drive buckthorn invasion.

In addition to allelopathy, the distinct life histories of alder and buckthorn suggest important roles for reproduction and resource competition in structuring their relative abundance. Since buckthorn appears to be more prolific than alder, its propagules may colonize gaps more successfully, thus exploiting extant resources. Buckthorn's rapid growth rate (Sanford et al. 2004; Knapp 2006) also

indicates that it may shade out alder. Furthermore, buckthorn's high genetic diversity and phenotypic plasticity (Hampe & Bairlein 2000) likely make it able to persist in a wider range of sites than alder, which could subsequently become buckthorn 'breeding grounds,' i.e., seed sources.

One of my concerns arising from this project is that alder promotion of buckthorn growth may lead to the invasive's competitive exclusion of rare native plants. One such threatened Massachusetts shrub is swamp birch (*Betula pumila*), a small member of the family Betulaceae that is confined to alkaline, mineral-rich wetlands, and persists at only four sites in the state (Natural Heritage & Endangered Species Program 1994). Unfortunately, drainage projects (Vickery 1985) and invasive vegetation, including buckthorn (Richburg et al. 2001), compromise the calcareous wetlands that provide habitat for swamp birch. Kampoosa Bog, in Stockbridge, Massachusetts, is one such mesic area where speckled alder, glossy buckthorn, and swamp birch spatially overlap (Coxe 1995; Richburg et al. 2001).

Swamp birch preferentially inhabits open areas (Jacobson et al. 1991), in which buckthorn also grows well (Knapp 2006). Buckthorn furthermore exhibits higher net photosynthetic assimilation rates per unit leaf area in sunny sites than do native plants (Knapp 2006). This is likely due to elevated levels of nitrogen in buckthorn leaves, which correspond to the invasive's ability to efficiently utilize soil nutrients (Knapp 2006).

Since conservation resources are limited, and it will likely be impossible to eliminate buckthorn from all of the New England wetland sites where it is

problematic, I suggest that its removal be prioritized where this will most benefit native vegetative diversity. Wetlands that contain buckthorn, alder and rare swamp birch, such as Kampoosa Bog, may be prime spots for such intervention.

BIBLIOGRAPHY

Allen, M.F., C.Crisafulli, C.F. Friese & S.L. Jeakins. 1992. Reformation of mycorrhizal symbioses on Mount St. Helens, 1980-1990: interactions of rodents and mycorrhizal fungi. *Mycological Research* **96**: 447-453.

Alpert, P. 2006. The advantages and disadvantages of being introduced. *Biological Invasions* **8**: 1523-1534.

Anderson, M.D., R.W. Ruess, D.D. Uliassi & J.S. Mitchell. 2004. Estimating N_2 fixation in two species of *Alnus* in interior Alaska using acetylene reduction and $^{15}N_2$ uptake. *Ecoscience* **11**: 102-112.

Bais, H.P., R. Vepachedu, S. Gilroy, R. M. Callaway & J.M. Vivanco. 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. *Science* **301**: 1377-1380.

Bais, H.P., T.S. Walker, F.R. Stermitz, R.A. Hufbauer and J.M. Vivanco. 2002. Enantiomeric dependent phytotoxic and antimicrobial activity of (-/+)-catechin: a rhizosecreted racemic mixture from *Centaurea maculosa* (spotted knapweed). *Plant Physiology* **128**: 1173-1179.

Baker, D.D. & C.R. Schwintzer. 1990. Introduction. Pages 3-16 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants.* Academic Press, San Diego.

Becerra, A., M.R. Zak, T.R. Horton & J. Micolini. 2005. Ectomycorrhizal and arbuscular mycorrhizal colonization of *Alnus acuminata* from Calilegua National Park (Argentina). *Mycorrhiza* **15**:S25-S31.

Bellingham, J.P., L.R. Walker & D.A. Wardle. 2001. Differential facilitation by a nitrogen-fixing shrub during primary succession influences relative performance of canopy tree species. *Journal of Ecology* **89**: 861-875.

Benson, D.R. & J.O. Dawson. 2007. Recent advances in the biogeography and genecology of symbiotic *Frankia* and its host plants. *Physiologia Plantarum* **130**: 318-330.

Benson, D.R. & N.A. Schultz. 1990. Physiology and biochemistry of *Frankia* in culture. Pages 107-128 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants.* Academic Press, San Diego.

Berry, A.M. & L.A. Sunell. 1990. The infection process and nodule development. Pages 61-82 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants.* Academic Press, San Diego.

Bertin, C., X. Yang & L.A. Weston. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant and Soil* **256**: 67-83.

Bischoff, J.M., P. Bukaveckas, M.J. Mitchell & T.M. Hurd. 2001. Nitrogen storage and cycling in a forested wetland: implications for watershed nitrogen processing. *Water, Air, and Soil Pollution* **128**: 97-114.

Blossey, B. & R. Notzold. 1995. Evolution of increased competitive ability in invasive, non-indigenous plants: a hypothesis. *Journal of Ecology* **83**: 887-889.

Bossdorf, O., A. Lipowski & D. Prati. 2008. Selection of preadapted populations allowed *Senecio inaequidens* to invade Central Europe. *Diversity and Distributions* **14**: 676-685.

Callaway, R.M. & L.R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. *Ecology* **78**: 1958-1965.

Callaway, R.M. & E.T. Aschehoug. 2000. Invasive plants versus their new and old neighbors: a mechanism for exotic invasion. *Science* **290**: 521-523.

Callaway, R.M. & W.M. Ridenour. 2004. Novel weapons: invasive success and the evolution of increased competitive ability. *Frontiers in Ecology and the Environment* **2**: 436-443.

Callaway, R.M., W.M. Ridenour, T.Laboski, T. Weir & J.M. Vivanco. 2005. Natural selection for resistance to the allelopathic effects of invasive plants. *Ecology* **93**: 576-583.

Callaway, R.M., D. Cipollini, K. Barto, G.C. Thelen, S.G. Hallett, D. Prati, K. Stinson & J. Klironomos. 2008. Novel weapons: Invasive plant suppresses fungal mutualists in America but not in its native Europe. *Ecology* **89**: 1043-1055.

Catling, P.M. & Z.S. Porebski. 1994. The history of invasion and current status of glossy buckthorn, *Rhamnus frangula*, in Southern Ontario. *Canadian Field Naturalist* **108**: 305-310.

Chapin, F.S., L.R. Walker, C.L. Fastie & L.C. Sharman. 1994. Mechanisms of primary succession following deglaciation at Glacier Bay, Alaska. *Ecological Monographs* **64**: 149-175.

Chiariello, N., J.C. Hickman & H.A. Mooney. 1982. Endomycorrhizal role for interspecific transfer of phosphorus in a community of annual plants. *Scoence* **217**: 941-943.

Cohen, J.G. & M.A. Kost. 2007. *Natural community abstract for northern shrub thicket.* Michigan Natural Features Inventory, Lansing, Michigan.

Connell, J.H. & R.O. Slatyer. 1977. Mechanisms of succession in natural communities and their role in community stability and organization. *The American Naturalist* **111**: 1119-1144.

Converse, C.K. 1985. *Element stewardship abstract for Rhamnus cathartica and Rhamnus frangula*. The Nature Conservancy, Arlington, Virginia.

Coxe, T. 1995. *Designation of the Kampoosa Bog Drainage Basin Area of Critical Environmental Concern.* Massachusetts Department of Environmental Management.

Crocker, R.L. & J. Major. 1955. Soil development in relation to vegetation and surface age at Glacier Bay, Alaska. *Journal of Ecology* **43**: 427-448.

Curtis, J.T. 1959. *The vegetation of Wisconsin: an ordination of plant communities.* University of Wisconsin Press, Madison.

D'Antonio, C.M. & P. Vitousek. 1992. Biological invasions by exotic grasses, the grass fire cycle, and global change. *Annual Review of Ecological Systems* **23**: 63-87.

Darwin, C. 1859. *The origin of species by means of natural selection*. J. Murray, London.

Davis, M.A., J.P. Grime & K. Thompson. 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* **88**: 528-534.

Dawson, J.O. 1990. Interactions among actinorhizal and associated plant species. Pages 299-316 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants.* Academic Press, San Diego.

Dorning, M. & D. Cipollini. 2006. Leaf and root extracts of the invasive shrub, *Lonicera maackii*, inhibit seed germination of three herbs with no autotoxic effects. *Plant Ecology* **184**: 287-296.

Elton, C.S. 1958. *The ecology of invasions by animals and plants*. Metheun, London.

Erichsen-Brown, C. 1989. *Medicinal and other uses of North American plants: a historical survey with special reference to the Eastern Indian tribes.* Courier-Dover Publications, Chelmsford, MA.

Fagan, M.E. & D.R. Peart. 2004. Impact of the invasive shrub glossy buckthorn (*Rhamnus frangula* L.) on juvenile recruitment by canopy trees. *Forest Ecology & Management* **194**: 95-107.

Fenner, M. & W.G. Lee. 2001. Lack of pre-dispersal seed predators in introduced Asteraceae in New Zealand. *New Zealand Journal of Ecology* **25**: 95-99.

Finlay, R.D. & D.J. Read. 1986. The structure and function of the vegetative mycelium of ectomycorrhizal plants. *New Phytologist* **103**: 143-165.

Forsyth, D.M., R.P. Duncan, M. Bomford & G. Moore. 2004. Climatic suitability, life-history traits, introduction effort, and the establishment and spread of introduced mammals in Australia. *Conservation Biology* **18**: 557-569.

Frappier, B., R.T. Eckert & T.D. Lee. 2004. Experimental removal of the non-indigenous shrub *Rhamnus frangula* (glossy buckthorn): effects on native herbs and woody seedlings. *Northeastern Naturalist* **11**: 333-342.

Gatti, A.B., S.C. Perez & M.I. Lima. 2004. Allelopathic activity of aqueous extracts of *Aristolochia esperanzae* in the germination and growth of *Lactuca sativa* and *Raphanus sativus*. *Acta Botanica Brasilica* **18**: 459-472.

Gopal, B. & U. Goel. 1993. Competition and allelopathy in aquatic plant communities. *Botanical Review* **59**: 155-210.

Glass, A.D.M. 1973. Influence of phenolics on ion uptake. *Plant Physiology* **51**: 1037-1041.

Gunton, J.A. 1921. A re-investigation of the proximate composition of *Rhamnus frangula*. *Doctoral dissertation, University of Illinois.*

Hammerson, G. 1994. Beaver (*Castor canadensis*): ecosystem alterations, management, and monitoring. *Natural Areas Journal* **14**: 44-57.

Hampe, A. 2004. Extensive hydrochory uncouples spatiotemporal patterns of seedfall and seedling recruitment in a 'bird-dispersed' riparian tree. *Ecology* **92**: 797-807.

Hampe, A. & F. Bairlein. 2000. Modified dispersal-related traits in disjunct populations of bird-dispersed *Frangula alnus* (Rhamnaceae): a result of its Quaternary distribution shifts? *Ecography* **23**: 603-613.

Harley, J.L. & E.L. Harley. 1987. a check-list of mycorrhiza in the British flora. *New Phytologist* **105**: 1-102.

Harrington, R.A., B.J. Brown, P.B. Reich & J.H. Fownes. 1989. Ecophysiology of exotic and native shrubs in southern Wisconsin II. Annual growth and carbon gain. *Oecologia* **80**: 368-373.

Haynes, R.J., K.C. Cameron, K.M. Goh & R.R. Sherlock. 1986. *Mineral nitrogen in the plant-soil system.* Academic Press, Orlando, Florida.

Hendrickson, O.Q., D. Burgess, P. Perinet, F. Tremblay & L. Chatatpaul. 1993. Effects of *Frankia* on field performance of *Alnus* clones and seedlings. *Plant and Soil* **150**: 295-302.

Heywood, V.H., R.K. Brummitt, A. Culham & O. Seberg. 2007. *Flowering plant families of the world*. Firefly Books, New York.

Hierro, J.L. & R.M. Callaway. 2003. Allelopathy and exotic plant invasion. *Plant & Soil* **256**: 29-39.

Howell, J.A. & W.H. Blackwell. 1977. The history of *Rhamnus frangula* in the Ohio flora. *Castanea* **42**: 11-115.

Huenneke, L.F. 1987. Demography of a clonal shrub, *Alnus incana* ssp. *rugosa* (Betulaceae). *American Midland Naturalist* **117**: 43-55.

Huenneke, L.F. & P.L. Marks. 1987. Stem dynamics of the shrub *Alnus incana* ssp. *rugosa*: transition matrix model. *Ecology* **68**: 1234-1242.

Hunter, A.F. & L.W. Aarssen. 1988. Plants helping plants. *BioScience* **38**: 34-40.

Hurd, T.M., K. Gokkaya, B.D. Kiernan & D.J. Raynal. 2005. Nitrogen sources in Adirondack wetlands dominated by nitrogen-fixing shrubs. *Wetlands* **25**: 192-199.

Hurd, T.M., D.J. Raynal & C.R. Schwintzer. 2001. Symbiotic N_2 fixation of *Alnus incana* ssp. *rugosa* in shrub wetlands of the Adirondack Mountains, New York, USA. *Oecologia* **126**: 94-103.

Hurd, T.M. & D.J. Raynal. 2004. Comparison of nitrogen solute concentrations within alder (*Alnus incana* ssp. *rugosa*) and non-alder dominated wetlands. *Hydrological Processes* **18**: 2681-2697.

Huss-Danell, K. 1990. The physiology of actinorhizal nodules. Pages 129-156 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of Frankia and actinorhizal plants.* Academic Press, San Diego.

Huss-Danell, K., D. Uliassi & I. Renberg. 1997. River and lake sediments as sources of infective *Frankia* (*Alnus*). *Plant and Soil* **197**: 35-39.

Inderjit & K.M.M. Dakshini. 1995. On laboratory assays in allelopathy. *Botanical Review* **61**: 28-44.

Inderjit & S. O. Duke. 2003. Ecophysiological aspects of allelopathy. *Planta* **217**: 529-539.

Inderjit & E.T. Nilsen. 2003. Bioassays and field studies for allelopathy in terrestrial plants: progress and problems. *Critical Reviews in Plant Science* **22**: 221-238.

Inderjit & H. Nishimura. 1999. Effect of the anthraquinones emodin and physcion on availability of selected soil inorganic ions. *Annals of Applied Biology* **135**: 425-429.

Inoue, M., H. Nishimura, H.H. Li & J. Mizutani. 1992. Allelochemicals from *Polygonum sachalinense* (Polygonaceae). *Journal of Chemical Ecology* **18**: 1833-1840.

Izhaki, I. 2002. Emodin – a secondary metabolite with multiple ecological functions in higher plants. *New Phytologist* **155**: 205-217.

Jacobson, G.L., H. Almquist-Jacobson & J.C. Winne. 1991. Conservation of rare plant habitat: insights from the recent history of vegetation and fire at Crystal Fen, northern Maine, USA. *Biological Conservation* **57**: 287-314.

Janiszewski, P., A. Gugolek & A. Lobanowska. 2006. Use of shoreline vegetation by the European beaver (*Castor fiber* L.). *Acta Scientiarum Polonorum* **5**: 63-70.

Javaid, A., S. Shafique & S. Shafique. 2006. Herbicidal potential of aqueous leaf extract of allelopathic trees against *Phalaris minor*. *Pakistan Journal of Weed Science Research* **12**: 339-346.

Jha, D.K., G.D. Sharma & R.R. Mishra. 1992. Mineral nutrition in the tripartite interaction between *Frankia*, *Alnus,* and *Glomus* at different soil phosphorus regimes. *New Phytologist* **123**: 307-311.

Jobidan, R. & J.R. Thibault. 1981. Allelopathic effects of balsam poplar on green alder germination. *Bulletin of the Torrey Botanical Club* **108**: 413-418.

Jutras, S., H. Hokka, J. Begin & A.P. Plamondon. 2006. Beneficial influence of plant neighbours on tree growth in drained forested peatlands: a case study. *Canadian Journal of Forest Research* **36**: 2341-2350.

Keane, R.M. & M.J. Crawley. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology & Evolution* **17**: 164-170.

Knapp, L. 2006. Photosynthesis, growth, and survival of native and invasive woody species: trade-offs and plasticity across open and understory environments. *Ph.D. thesis, Department of Organismal & Evolutionary Biology, University of Massachusetts, Amherst*.

Klironomos, J.N. 2002. Feedback with soil biota contributes to plant rarity and invasiveness in communities. *Nature* **417**: 67-70.

Lee, C.E. 2002. Evolutionary genetics of invasive species. *Trends in Ecology & Evolution* **17**: 386-391.

Le Van, T. 1984. Emodin as a fungal metabolite and the effects of emodin on the growth of some soil microorganisms. *Acta Agraria et Silvestria Seriea Agraria* **23**: 235-242.

Levin, H., R. Hazenfranz, J. Friedman & M. Perl. 1988. Partial purification and some properties of antibacterial compounds from *Aloe vera*. *Phytotherapy Research* **1**: 1-3.

Levine, J.M., M. Vila, C.M. D'Antonio, J.S. Dukes, K. Grigulis & S. Lavorel. 2003. Mechanisms underlying the impacts of exotic plant invasions. *Proceedings of the Royal Society of London* **270**: 775-781.

Li, X. & S.D. Wilson. 1998. Facilitation among woody plants establishing in an old field. *Ecology* **79**: 2694-2705.

Lockwood, J.L., M.F. Hoopes & M.P. Marchetti. 2007. *Invasion ecology*. Blackwell Publishing, Malden, Massachusetts.

Lonsdale, W.M. 1999. Global patterns of plant invasions and the concept of invasibility. *Ecology* **80**: 1522-1536.

Lundquist, P.O. 2005. Carbon cost of nitrogenase activity in *Frankia-Alnus incana* root nodules. *Plant and Soil* **273**: 235-244.

Mack, R.N. 2003. Phylogenetic constraint, absent life forms, and preadapted alien plants: a prescription for biological invasions. *International Journal of Plant Sciences* **164**: S185-S196.

Marchetti, M.P., P.B. Moyle & R. Levine. 2004. Alien fishes in California watersheds: characteristics of successful and failed invaders. *Ecological Applications* **14**: 587-596.

Maron, J.L. & P.G. Connors. 1996. A native nitrogen-fixing shrub facilitates weed invasion. *Oecologia* **105**: 302-312.

Maron, J.L. & R.L. Jefferies. 1999. Bush lupine mortality, altered resource availability, and alternative vegetation states. *Ecology* **80**: 443-454.

Maron, J.L. & M. Vila. 2001. When do herbivores affect plant invasion? Evidence for the natural enemies and biotic resistance hypotheses. *Oikos* **95**: 361-373.

McDermott, E.L. & A.M. Berry. 2003. Mycorrhizal fungi. *Native Plants Journal* **4**: 141-142.

Medan, D. 1994. Reproductive biology of *Frangula alnus* (Rhamnaceae) in southern Spain. *Plant Systematics and Evolution* **193**: 173-186.

Mitchell, C.E. & A.G. Power. 2003. Release of invasive plants from fungal and viral pathogens. *Nature* **421**: 625-627.

Mitchell, C.E., A.A. Agrawal, J.D. Bever, G.S. Gilbert, R.A. Hufbauer, J.N. Klironomos, J.L. Maron, W.F. Morris, I.M. Parker, A.G. Power, E.W. Seabloom, M.E. Torchin & D.P. Vasquez. 2006. Biotic interactions and plant invasions. *Ecology Letters* **9**: 726-740.

Modallal, N.M. & F.M.R. Al-Charchafchi. 2006. Allelopathic effect of *Artemesia harba alba* on germination and seedling growth of *Anabasis setifera*. *Pakistan Journal of Biological Sciences* **9**: 1795-1798.

Molles, M.C. 2005. *Ecology: concepts and applications*. McGraw-Hill, Boston.

Myrold, D.D. & K. Huss-Danell. 1994. Population dynamics of *Alnus*-infective *Frankia* in a forest soil with and without host trees. *Soil Biology & Biochemistry* **26**: 533-540.

Natural Heritage & Endangered Species Program. 1994. *Threatened species of Massachusetts: swamp birch*. Westborough, Massachusetts.

Neori, A., K.R. Reddy, H. Ciscova-Koncalova & M. Agami. 2000. Bioactive chemicals and biological-biochemical activities and their functions in rhizospheres of wetland plants. *Botanical Review* **66**: 350-377.

Nesom, G. 2003. *Plant guide: speckled alder Alnus incana L. Moench ssp. rugosa (Du Roi) Clausen.* United States Department of Agriculture Natural Resources Conservation Service, Washington, D.C.

Parker, G.R. & G. Schneider. 1974. Structure and edaphic factors of an alder swamp in northern Michigan. *Canadian Journal of Forest Research* **4**: 499-508.

Paschke, M.W. 1993. Distribution and dispersal of *Frankia*. *Ph.D. thesis, Department of Biology, University of Illinois, Urbana-Champaign.*

Paschke, M.W. & J.O. Dawson. 1993. Avian dispersal of *Frankia*. *Canadian Journal of Botany* **71**: 1128-1131.

Pimentel, D., R. Zuniga & D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* **52**: 273-288.

Possessky, S.L., C.E. Williams and W.J. Moriarity. 2000. Glossy buckthorn (*Rhamnus frangula* L.): a threat to riparian plant communities of the northern Allegheny Plateau. *Natural Areas Journal* **20**: 290-292.

Post, T.W., E. McClosky and K. Klick. 1989. Two-year study of fire effects on *Rhamnus frangula* L. *Natural Areas Journal* **9**: 175-176.

Prati, D. and O. Bossdorf. 2004. Allelopathic inhibition of germination by *Alliaria petiolata* (Brassicaceae). *American Journal of Botany* **91**: 285-288.

Radford, I.J. and R.D. Cousens. 2000. Invasiveness and comparative life-history traits of exotic and indigenous *Senecio* species in Australia. *Oecologia* **125**: 531-542.

Reinartz, J.A. 1997. Controlling glossy buckthorn with with winter herbicide treatments of cut stumps. *Natural Areas Journal* **17**: 38-41.

Reinartz, J.A. and J. Kline. 1988. Glossy buckthorn (*Rhamnus frangula*), a threat to the vegetation of Cedarburg Bog. *University of Wisconsin-Milwaukee Field Station Bulletin* **18**: 1-48.

Reinhart, K.O. & R.M. Callaway. 2004. Soil biota facilitate exotic *Acer* invasions in Europe and North America. *Ecological Applications* **14**: 1737-1745.

Rejmanek, M. & D.M. Richardson. 1996. What attributes make some plant species more invasive? *Ecology* **77**: 1655-1661.

Rice, E.L. 1979. Allelopathy: an update. *Botanical Review* **45**: 15-111.

Richardson, D. M., N. Allsop, C.M. D'Antonio, S.J. Milton & M. Rejmanek. 2000. Plant invasions: the role of mutualisms. *Biological Reviews* **75**: 65-93.

Richburg, J.A. 1999. Water chemistry, *Phragmites* invasion, and changing plant communities at Kampoosa Bog, Stockbridge, Massachusetts. *M.S. thesis, Department of Forestry, University of Massachusetts, Amherst*.

Richburg, J.A., W.A. Patterson & F. Lowenstein. 2001. Effects of road salt and *Phragmites australis* invasion on the vegetation of a western Massachusetts calcareous lake-basin fen. *Wetlands* **21**: 247-255.

Roy, S., D.P. Khasa & C.W. Greer. 2007. Combining alders, frankiae, and mycorrhizae for the revegetation and remediation of contaminated ecosystems. *Canadian Journal of Botany* **85**: 237-251.

Rudrappa, T., J. Bonsall & H.P. Bais. 2007. Root-secreted allelochemical in the noxious weed *Phragmites australis* deploys a reactive oxygen species response and microtubule assembly disruption to execute rhizotoxicity. *Journal of Chemical Ecology* **33**: 1898-1918.

Sacerdote, A.B. 2008. Developmental and ecological impacts of European buckthorn invasion on amphibians. *Proceedings of the Society of Wetlands Scientists 'Capitalizing on Wetlands' Conference.* May 26-30, Washington, D.C.

Sanford, N.L., R.A. Harrington & J.H. Fownes. 2003. Survival and growth of native and alien seedlings in open and understory environments. *Forest Ecology and Management* **183**: 377-385.

Schinazi, R.F., C.K. Chu, J.R. Babu, B.J. Oswald, V. Saalmann, D.L. Cannon, B.F.H. Eriksson & M. Nasr. 1990. Anthraquinones as a new class of antiviral agents against human immunodeficiency virus. *Antiviral Research* **13**: 265-272.

Serdar, B., K. Coskuncelebi, S. Terzioglu & A. Hampe. 2007. Anatomical notes on Turkish *Frangula alnus* Mill. (Rhamnaceae). *Plant Biosystems* **141**: 69-74.

Shea, K. & P. Chesson. 2002. Community ecology as a framework for biological invasions. *TRENDS in Ecology and Evolution* **17**: 170-176.

Singh, U.P., K.P. Singh, S.P. Singh & V.B. Ram. 1992. Effect of emodin isolated from *Rhamnus triquetra* on spore germination of some fungi. *Fitopatology Brasileira* **17**: 420-422.

Stachowicz, J.J. 2001. Mutualism, facilitation, and the structure of ecological communities. *BioScience* **51**: 235-246.

Stinson, K.A., S.A. Campbell, J.R. Powell, B.E. Wolfe, R.M. Callaway, G.C. Thelen, S.C. Hallett, D. Prati & J.N. Klironomos. 2006. Invasive plant suppresses the growth of native tree seedlings by disrupting belowground mutualisms. *PLoS Biology* **4**: 727-731.

Sydiskis, R.J., D.G. Owen, J.L. Lohr, K.H.A. Rosler & R.N. Blomster. 1991. Inactivation of enveloped viruses by anthraquinones extracted from plants. *Antimicrobial Agents and Chemotherapy* **35**: 2463-2466.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel & D.A. Zuberer. 2005. *Principles and applications of soil microbiology.* Prentice Hall, Upper Saddle River, New Jersey.

Taft, J.B. & M.K. Solecki. 1990. Vascular flora of the wetland and prairie communities of Gavin Bog and Prairie Nature Preserve, Lake County Illinois. *Rhodora* **92**: 142-165.

Tilman, D. 1994. Competition and biodiversity in spatially structured habitats. *Ecology* **75**: 2-16.

Tilman, D. 2004. Niche tradeoffs, neutrality, and community structure: a stochastic thery of resource competition, invasion, and community assembly. *Proceedings of the National Academy of Sciences* **101**: 10854-10861.

Tilton, D.L. & J.M. Bernard. 1975. Primary productivity and biomass distribution in an alder shrub ecosystem. *American Midland Naturalist* **94**: 251-256.

Torrey, J.G. 1978. Nitrogen-fixation by actinomycete-nodulated angiosperms. *Bioscience* **28**: 586-592.

Torrey, J.G. 1990. Cross-inoculation groups within *Frankia* and host-endosymbiont associations. Pages 83-106 in C.R. Schwintzer and J.D. Tjepkema, editors. *The biology of* Frankia *and actinorhizal plants.* Academic Press, San Diego.

Trappe, J.M. 1962. Fungus associates of ectotrophic mycorrhizae. *Botanical Review* **28**: 538-606.

Upper Thames River Conservation Authority. 2007. *Buckthorn control methods*. Upper Thames River Conservation Authority, London, Ontario.

Vickery, B. 1985. *Element stewardship abstract for* Betula pumila. The Nature Conservancy, Arlington, Virginia.

Vitousek, P.M. 1982. Nitrogen cycling and nutrient use efficiency. *The American Naturalist* **119**: 553-572.

Vitousek, P.M. & L.R. Walker. 1989. Biological invasion by *Myrica faya* in Hawai'i: plant demography, nitrogen fixation, and ecosystem effects. *Ecological Monographs* **59**: 247-265.

Vitousek, P.M., L.R. Walker, L.D. Whiteaker, D. Mueller-Dombois & P.A. Matson. 1987. Biological invasion by *Myrica faya* alters ecosystem development in Hawai'i. *Science* **238**: 802-804.

Vitousek, P.M., C.M. D'Antonio, L.L. Loope & R. Westbrooks. 1996. Biological invasions as global environmental change. *American Scientist* **84**: 468-478.

Vivanco, J.M., H.P. Bais, F.R. Stermitz, G.C. Thelen & R.M. Callaway. 2004. Biogeographical variation in community response to root allelochemistry: novel weapons and exotic invasion. *Ecology Letters* **7**: 285-292.

Vivrette, N.J. & C.H. Muller. 1977. Mechanism of invasion and dominance of coastal grassland by *Mesembryanthemum crystallinum*. *Ecological Monographs* **47**: 301-318.

Webster, C.R., M.A. Jenkins & S. Jose. 2006. Woody invaders and the challenges they pose to forest ecosystems in the eastern United States. *Journal of Forestry* **104**: 366-374.

Weir, T.L., H.P. Bais & J.M. Vivanco. 2003. Intraspecific and interspecific interactions mediated by a phytotoxin, (-)-catechin, secreted by the roots of *Centaurea maculosa* (spotted knapweed). *Journal of Chemical Ecology* **29**: 2397-2412.

Wheeler, C.T., I.E. Henson & M.E. McLaughlin. 1979. Hormones in plants bearing actinomycete nodules. *Botanical Gazette* **140**: S52-S57.

Whittingham, J. & D.J. Read. 1982. Nutrient transfers between plants with mycorrhizal interconnections. *New Phytologist* **90**: 277-284.

Wiens, J.J. & C.H. Graham. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annual Review of Ecology, Evolution, and Systematics* **36**: 519-539.

Wilcove, D.S., D. Rothsein, J. Dubrow, A. Philips & E. Loso. 1998. Quantifying threats to imperiled species in the United States. *Bioscience* **48**: 607-615.

Williamson, M. & A. Fitter. 1996. The varying success of invaders. *Ecology* **77**: 1661-1666.

Wilson, S.D. & D. Tilman. 1991. Components of plant competition along an experimental gradient of nitrogen availability. *Ecology* **72**: 1050-1065.

Wyman, D. 1971. *Shrubs and vines for American gardens*. MacMillan, New York.

Yamanaka, T. & H. Okabe. 2006. Distribution of *Frankia*, ectomycorrhizal fungi, and bacteria in soil after the eruption of Miyake-Jima (Izu Islands, Japan) in 2000. *Journal of Forest Restoration* **11**: 21-26.

Younger, P.D. & L.A. Kapustka. 1983. Nitrogenase activity by *Alnus incana* ssp. *rugosa* in the northern hardwood forest. *American Journal of Botany* **70**: 30-39.

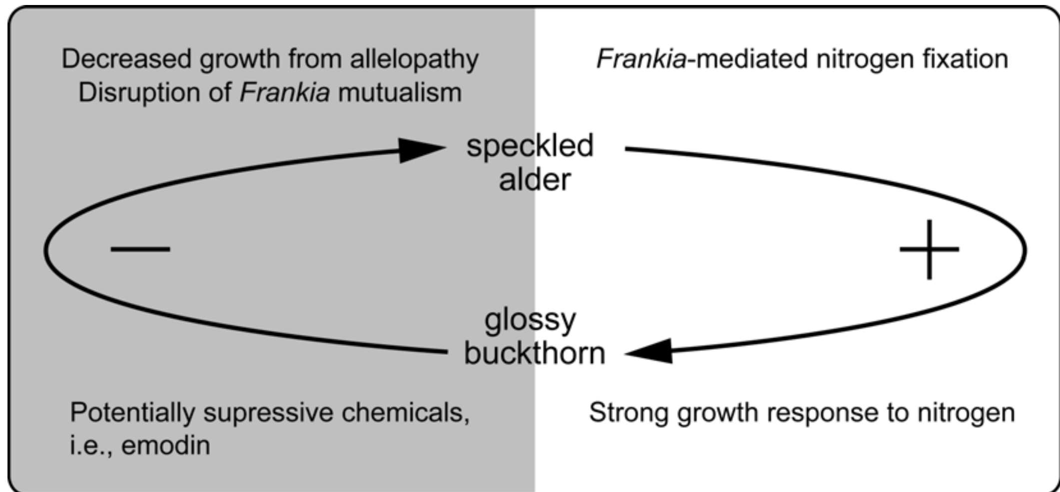


Figure 1: Hypothetical relationship between glossy buckthorn and speckled alder

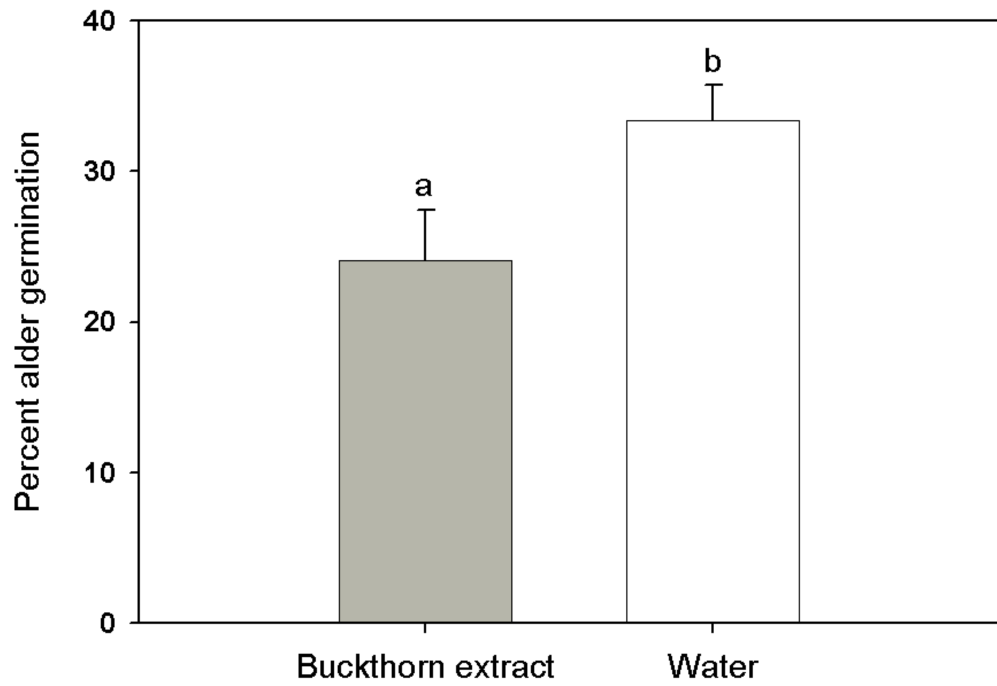


Figure 2: Percent alder seeds germinated (per petri plate) in buckthorn extract or water. Each bar represents the mean (+ 1 SE). Means with different letters are statistically distinct as determined by a Student's t-test.

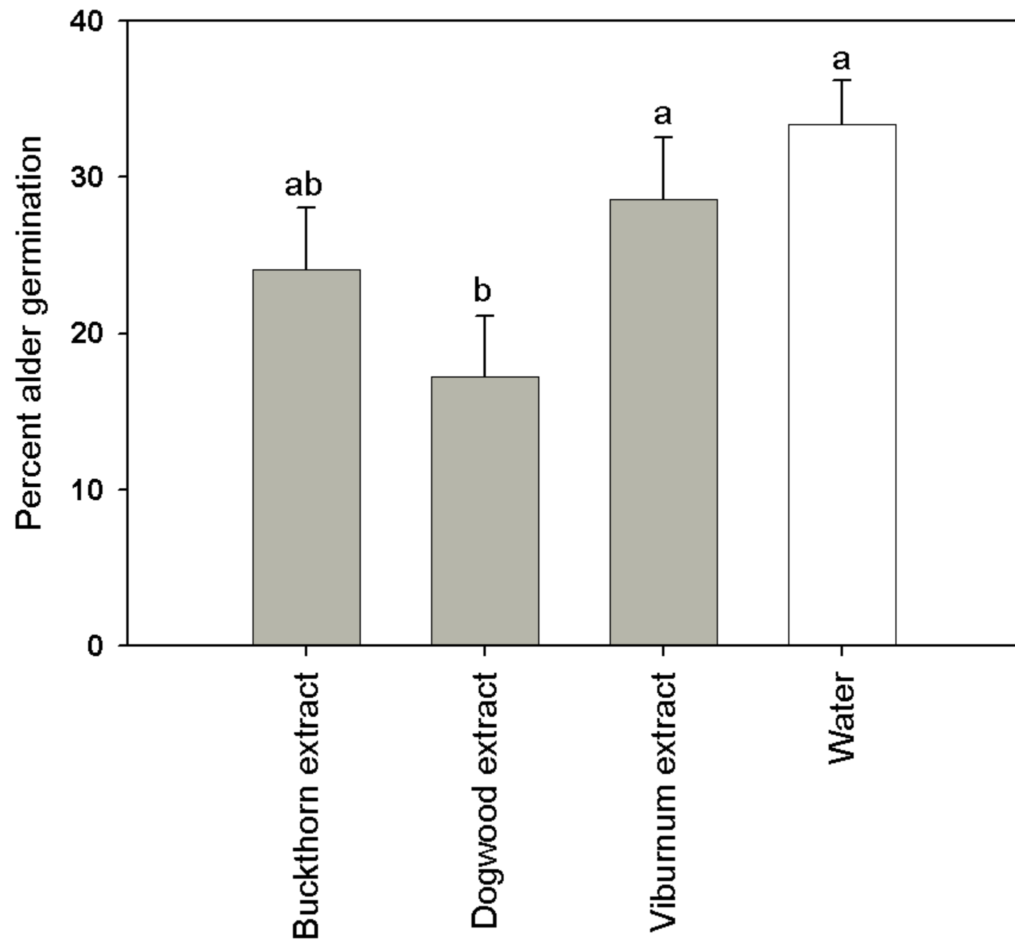


Figure 3: Percent alder seeds germinated in buckthorn and native extracts or water. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical distinctions among treatments were determined by Tukey HSD post-hoc analysis.

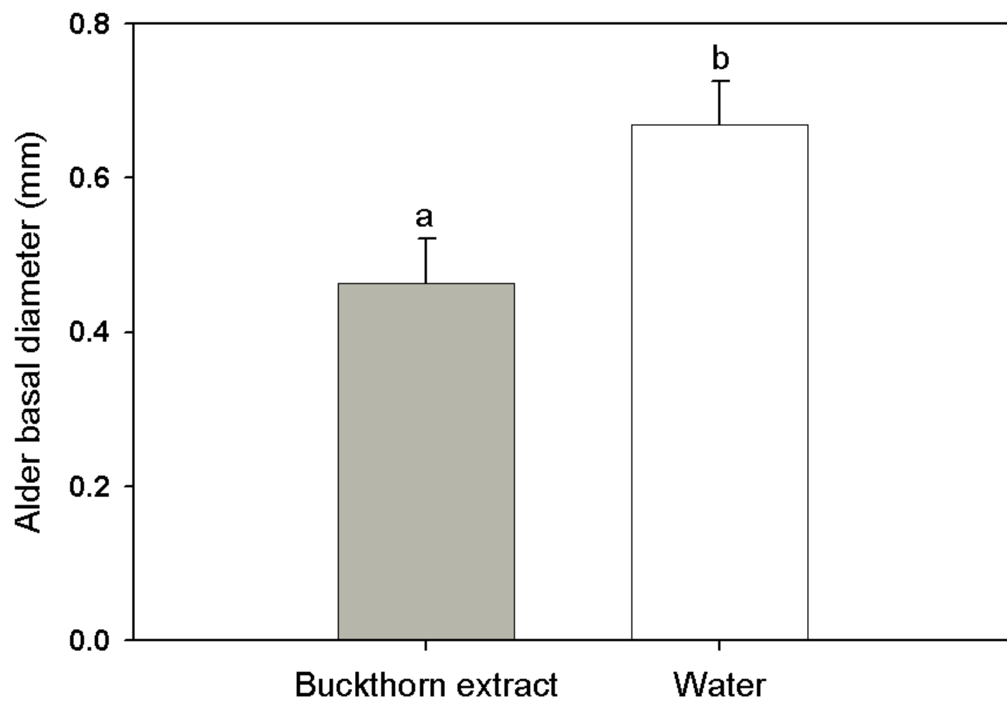


Figure 4: Alder seedling basal diameter in buckthorn extract or water. Each bar represents the mean (+ 1 SE). Differences in treatment means were determined by a Student's t-test.

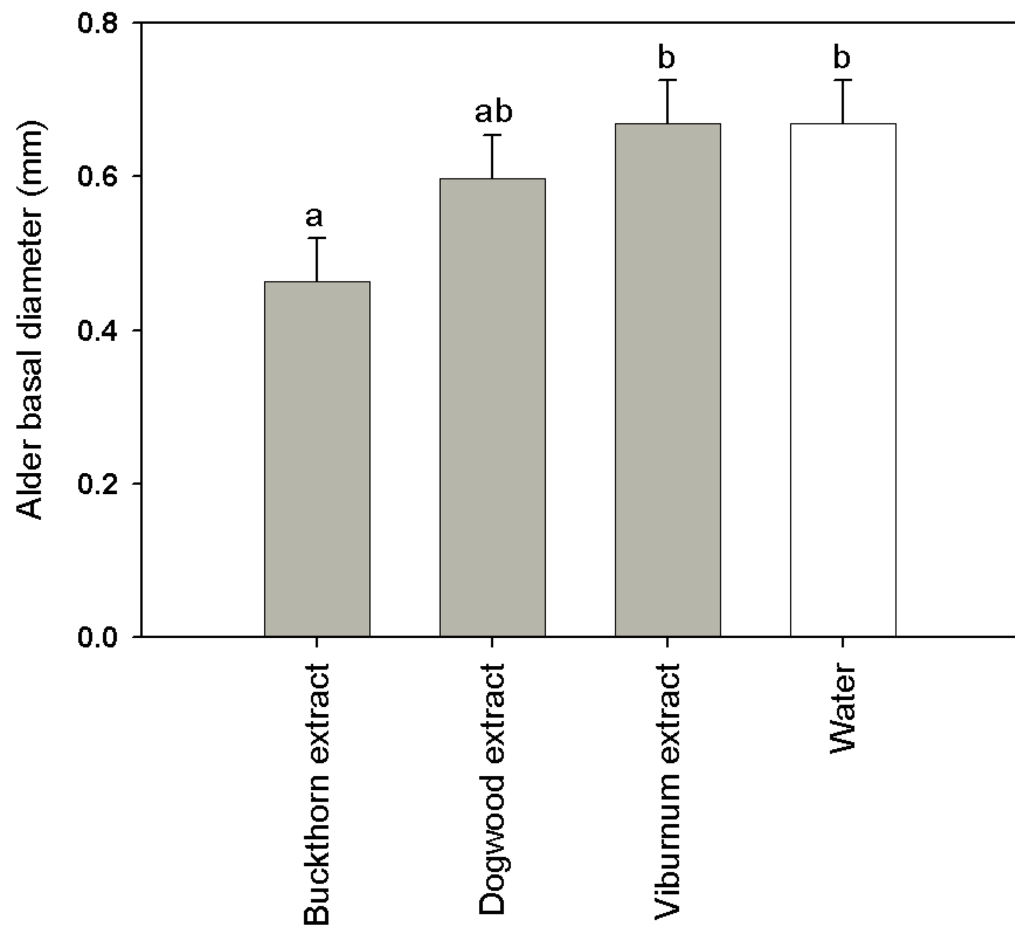


Figure 5: Alder seedling basal diameter in buckthorn and native extracts or water. Each bar represents the mean (+ 1 SE). Statistical differences among treatments were determined by Student's t-tests.

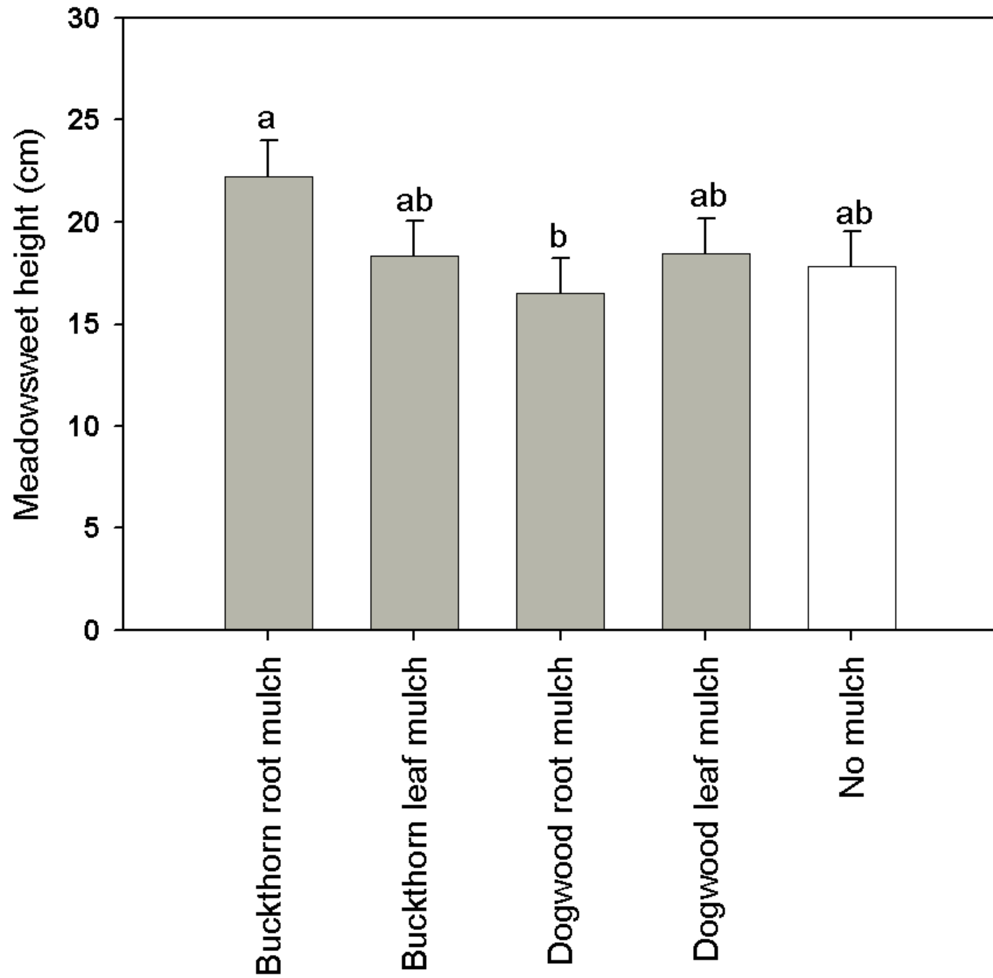


Figure 6: Initial meadowsweet height in all mulch treatments. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

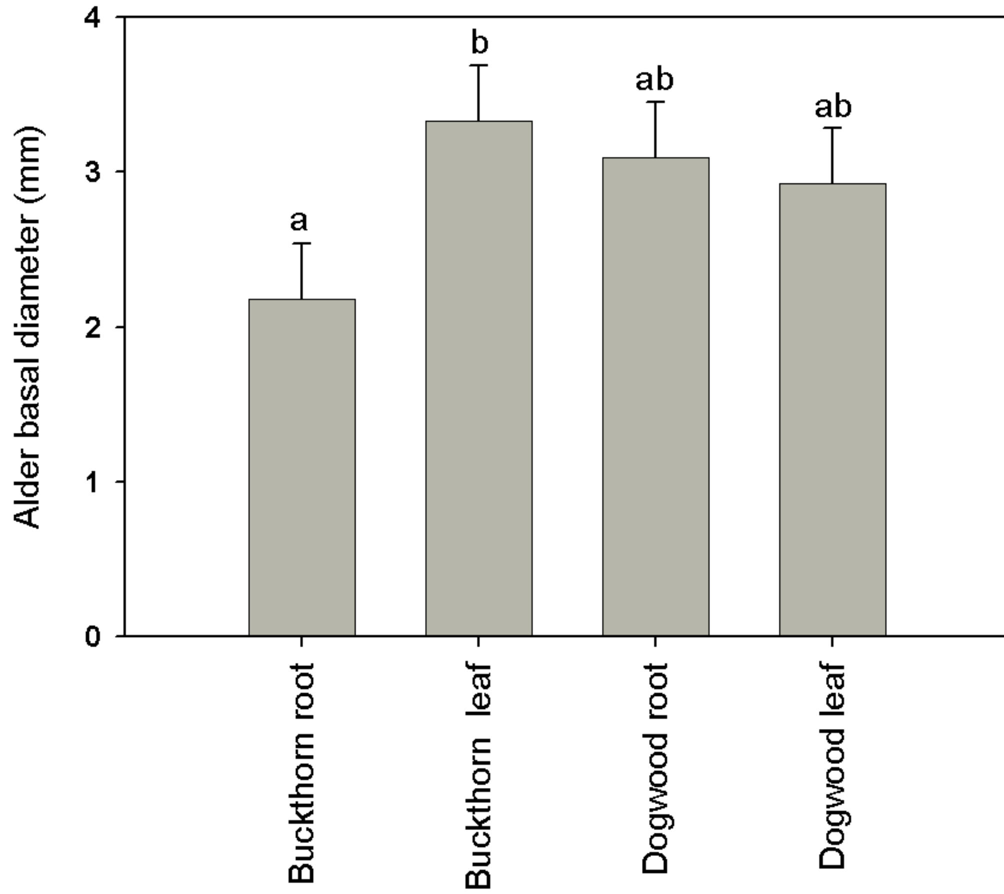


Figure 7: Alder basal diameter in buckthorn and native root or leaf mulch. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

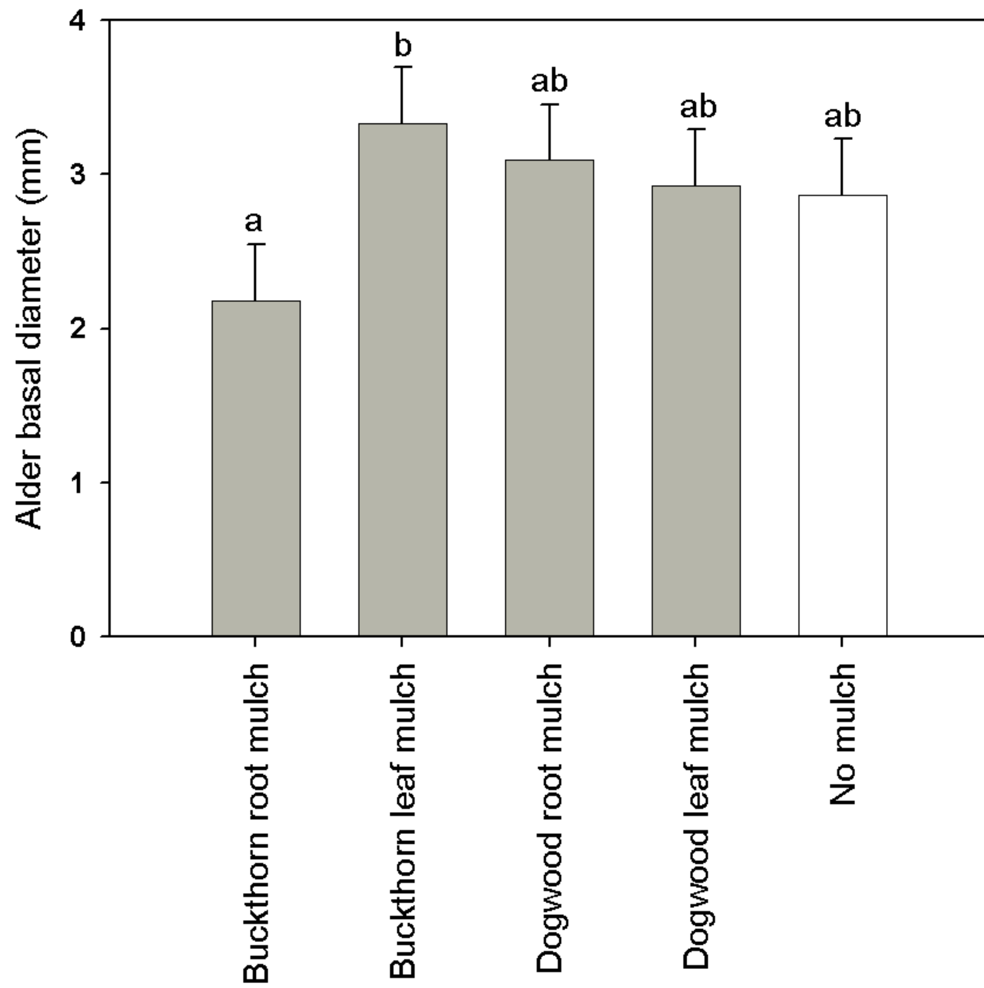


Figure 8: Alder basal diameter in all mulch treatments. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

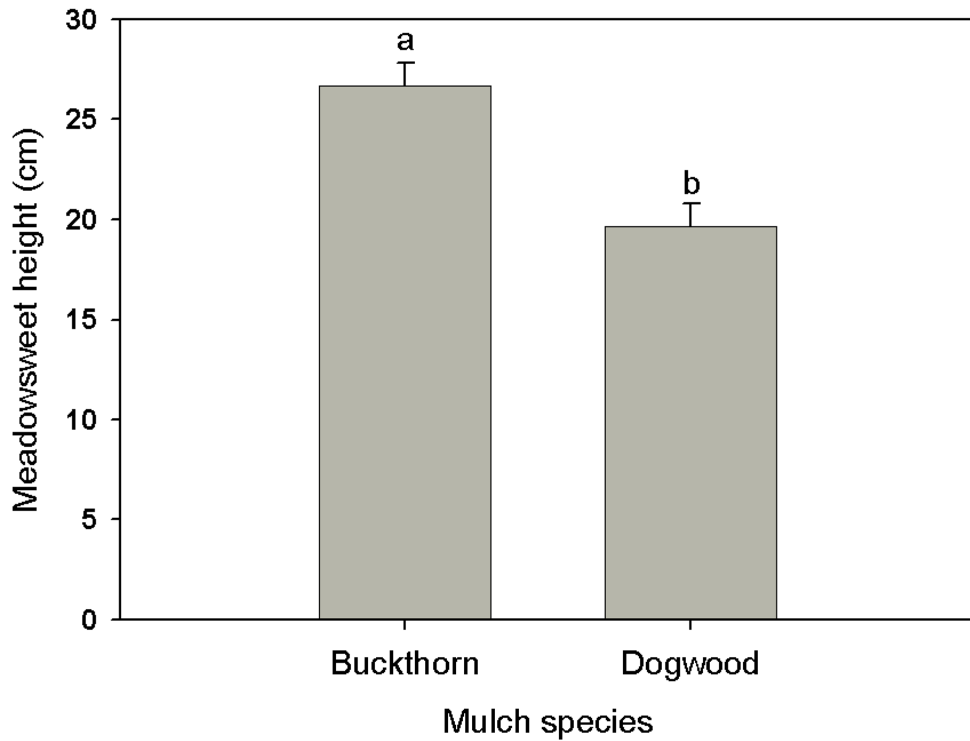


Figure 9: Meadowsweet height in buckthorn or native mulch. Each bar represents the mean (+ 1 SE). Statistical differences between treatments were determined by a Student's t-test.

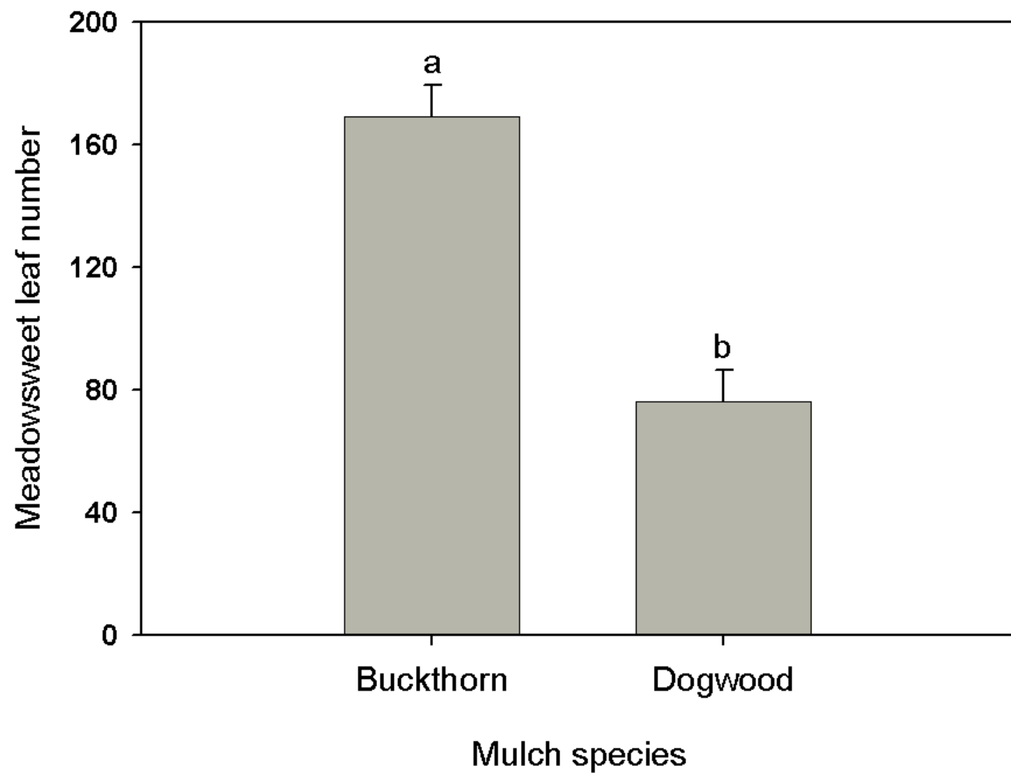


Figure 10: Meadowsweet leaf production in buckthorn or native mulch. Each bar represents the mean (+ 1 SE). Statistical differences between treatments were determined by a Student's t-test.

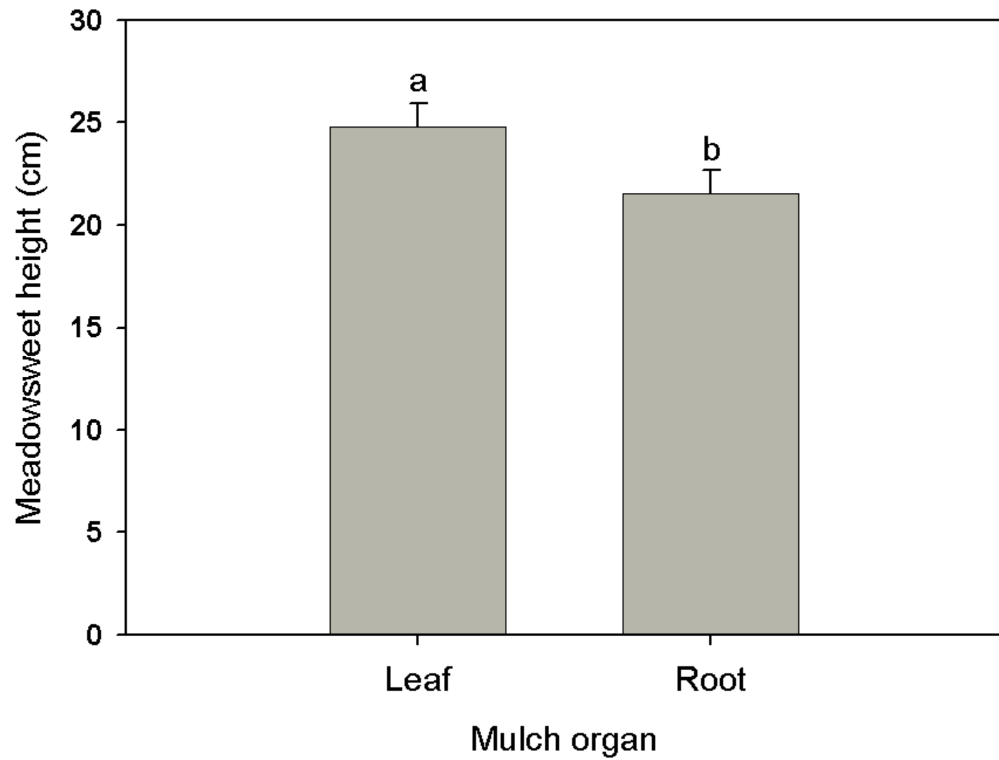


Figure 11: Meadowsweet height in leaf or root mulch. Each bar represents the mean (+ 1 SE). Statistical distinctions between treatments were determined by a Student's t-test.

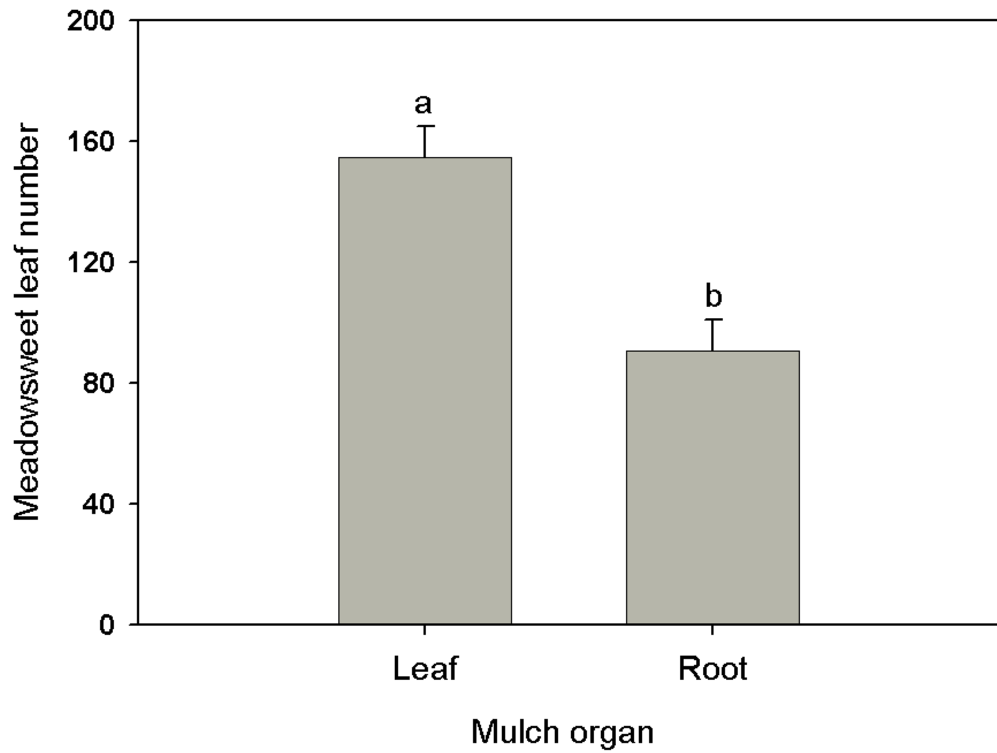


Figure 12: Meadowsweet leaf production in leaf or root mulch. Each bar represents the mean (+ 1 SE). Statistical distinctions between treatments were determined by a Student's t-test.

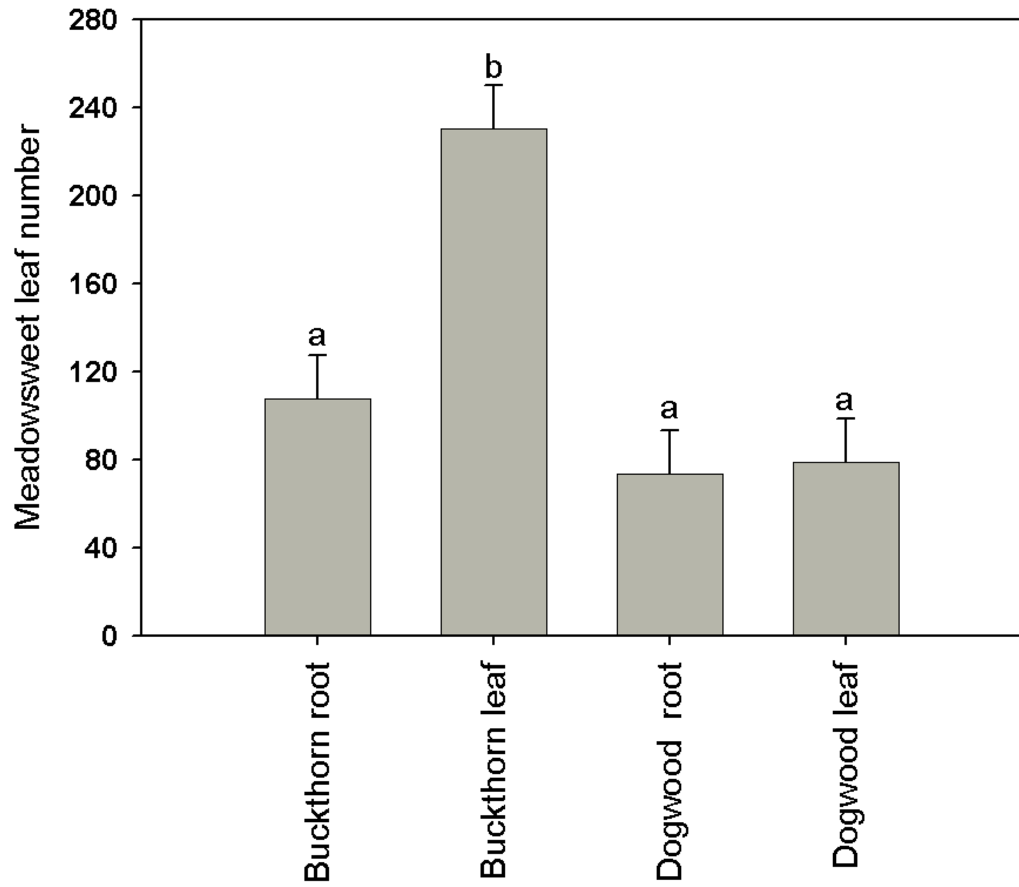


Figure 13: Meadowsweet leaf production in buckthorn and native root and leaf mulch. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

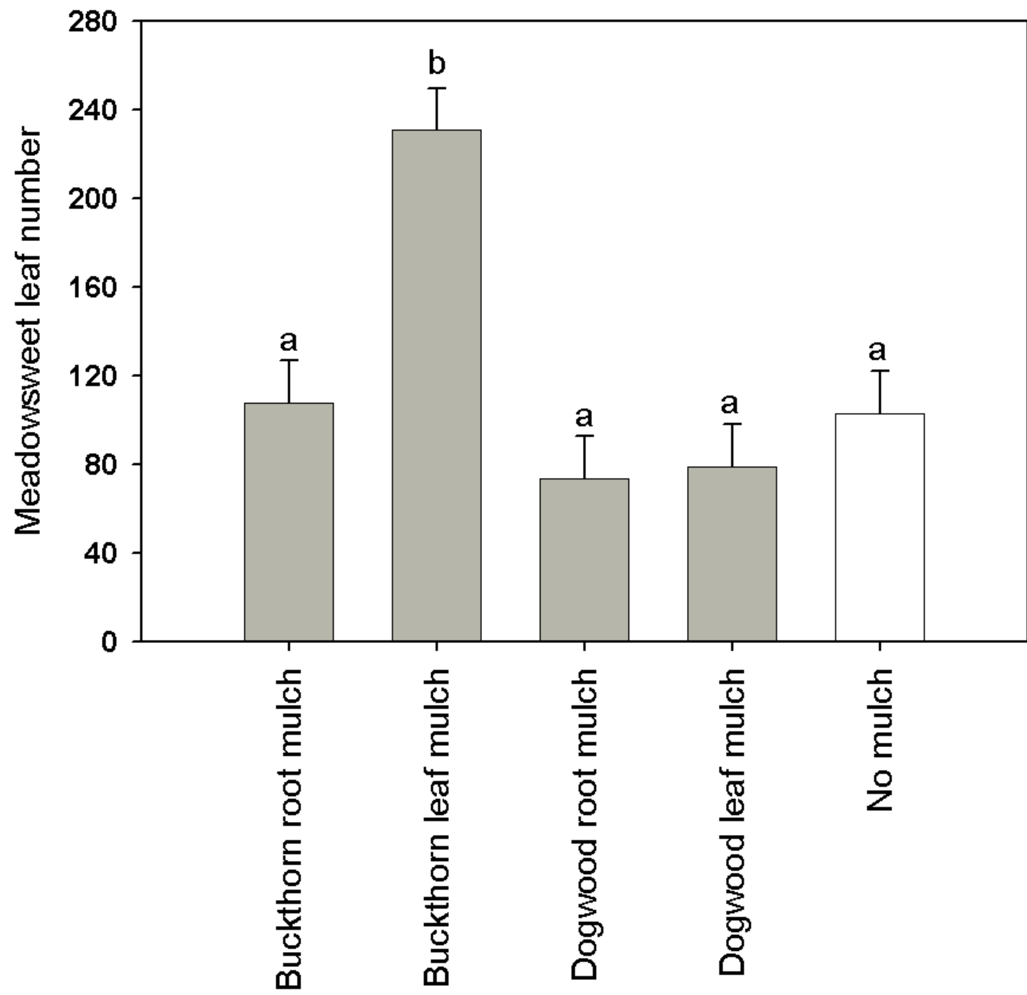


Figure 14: Meadowsweet leaf production in all mulch treatments. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical distinctions among treatments were determined by Tukey post-hoc analysis.

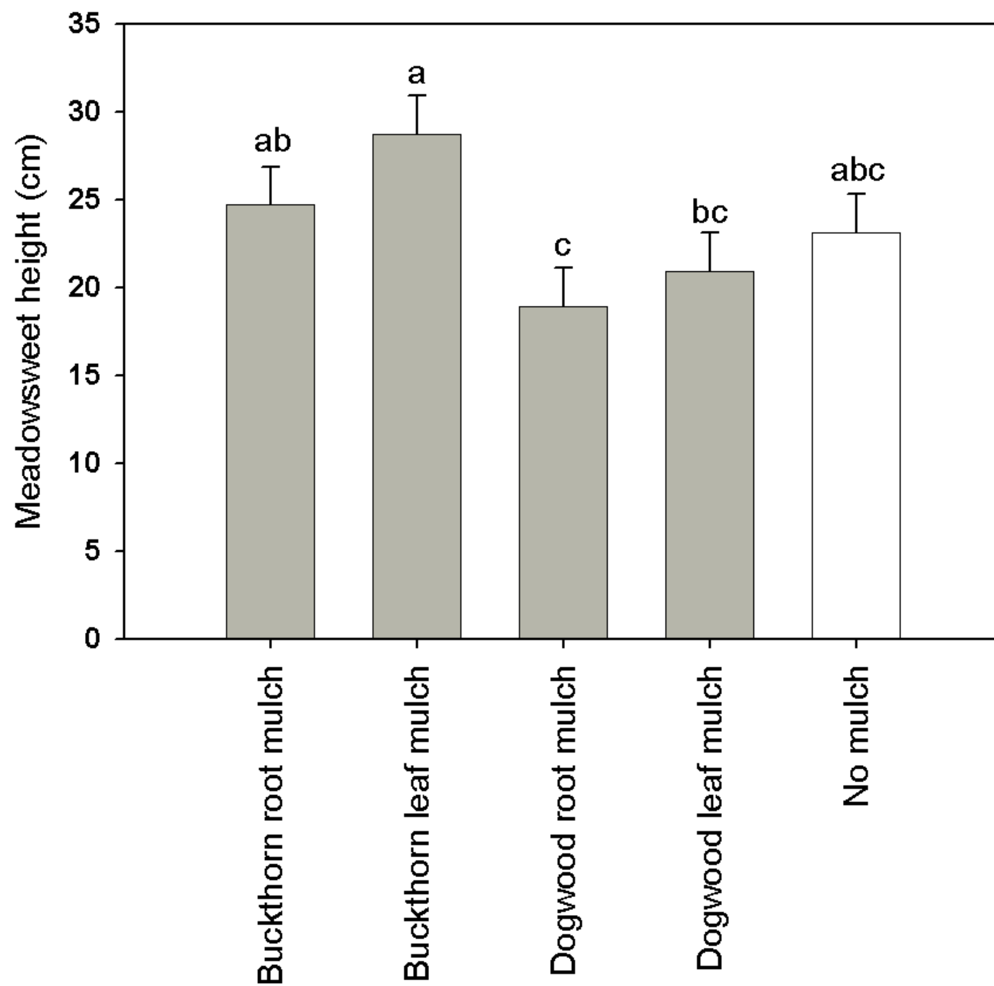


Figure 15: Meadowsweet height in all mulch treatments. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

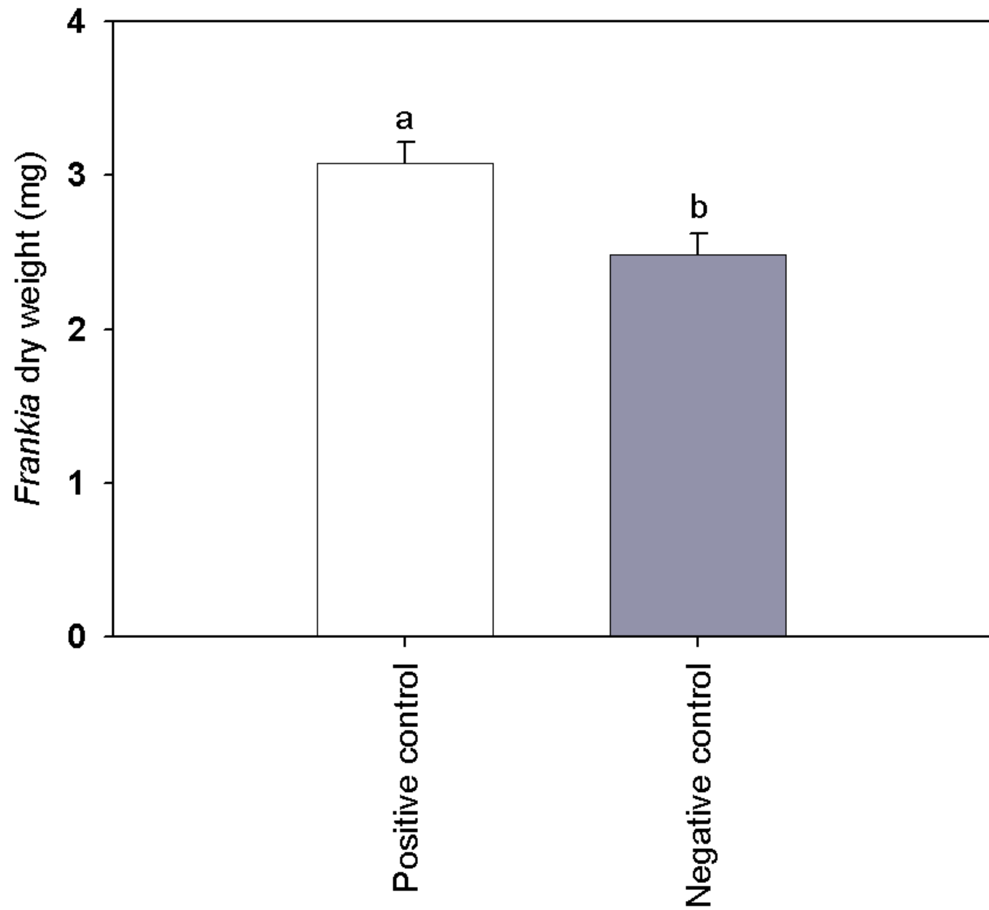


Figure 16: *Frankia* dry weight in positive and negative controls. This analysis excluded an outlier in the positive control group. Each bar represents the mean (+ 1 SE). Statistical distinctions between the controls were determined by a Student’s t-test.

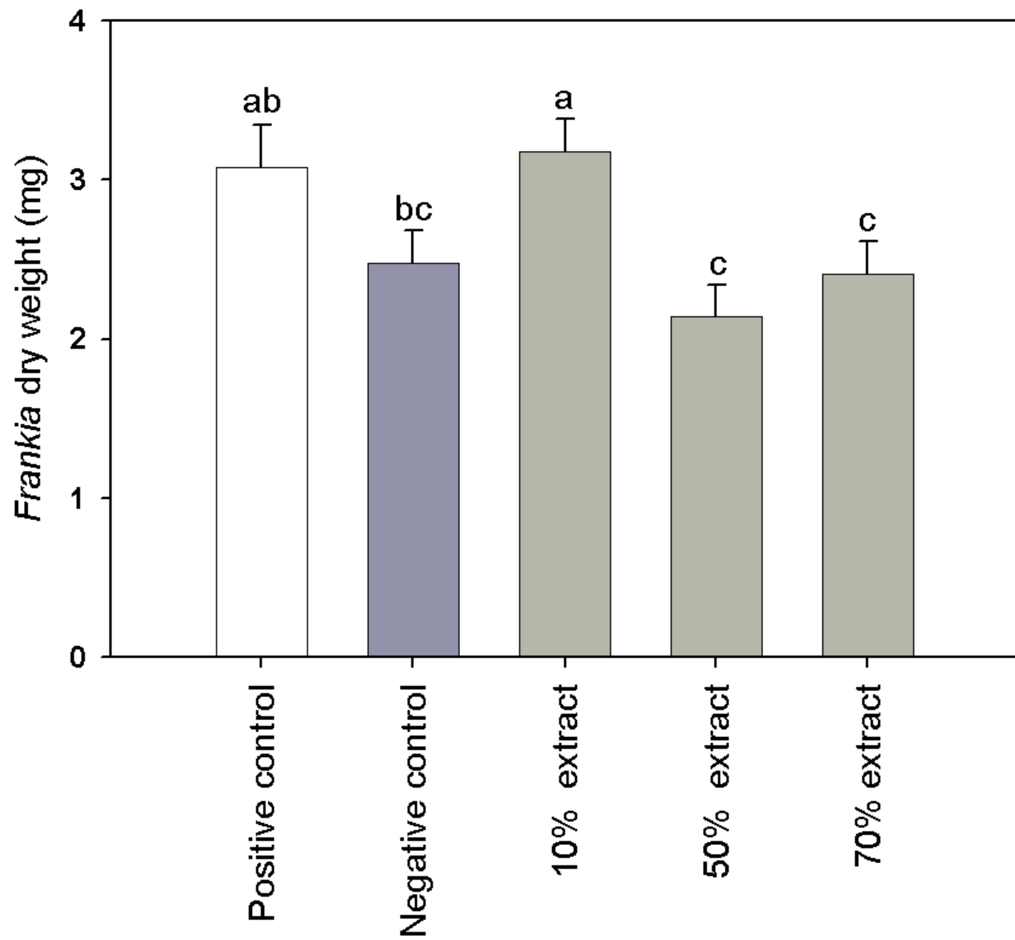


Figure 17: *Frankia* dry weight in buckthorn extract concentrations and controls. This analysis excluded an outlier in the positive control group. Each bar represents the mean (+ ½ Tukey confidence interval). Statistical differences among treatments were determined by Tukey post-hoc analysis.

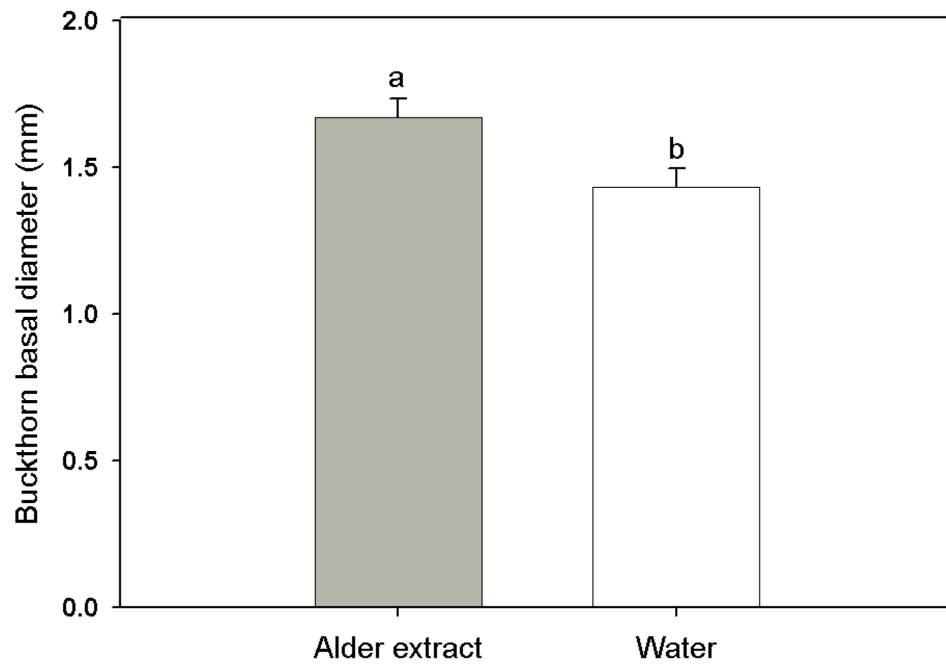


Figure 18: Buckthorn seedling basal diameter in alder extract or water. Each bar represents the mean (+ 1 SE). Statistical differences between treatments were determined by a Student’s t-test.

Table 1: ANOVA for impacts of buckthorn and native extracts or water on alder seedling growth. For this trial, the model effect has 3 df, with 140 df in the error term.

	Alder basal diameter (mm)		Alder height (cm)		Alder leaf number	
Term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Extract or water effect	2.95	0.03*	1.71	0.16	1.35	0.26

Table 2: Ammonium and nitrate content (in ppm) of buckthorn and native plant extracts

Extract type	Ammonium (NH_4)	Nitrate (NO_3^-)
Buckthorn	6.50	<0.12
Viburnum	<0.28	<0.12
Dogwood	1.10	0.19

Table 3: ANOVA for initial size of indigenous in all mulch treatments (including control). For this analysis, the model effects have 4 df, with 55 df in the error terms.

	Alder basal diameter (mm)		Alder height (cm)		Alder leaf number	
Term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Treatment group allocation	1.32	0.27	1.07	0.38	0.86	0.49
	Meadowsweet basal diameter (mm)		Meadowsweet height (cm)		Meadowsweet leaf number	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Treatment group allocation	1.20	0.32	3.04	0.02	0.97	0.63
	Viburnum basal diameter (mm)		Viburnum height (cm)		Viburnum leaf number	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Treatment group allocation	0.24	0.92	0.99	0.42	0.97	0.43

Table 4: ANOVA for effects of buckthorn and native root and leaf mulch on indigenous shrub growth. For this experiment, all model effects have 1 df, with 44 df in the error terms.

Term	Alder basal diameter (mm)		Alder height (cm)		Alder leaf number	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch species (buckthorn or dogwood)	0.89	0.35	0.01	0.99	0.49	0.49
Mulch organ (leaf or root)	3.42	0.07	1.07	0.31	0.09	0.76
Mulch species x mulch organ	6.11	0.02*	0.98	0.33	0.29	0.59
	Meadowsweet basal diameter (mm)		**Meadowsweet height (cm)**		**Meadowsweet leaf number**	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch species (buckthorn or dogwood)	2.52	0.12	18.76	<.0001*	40.13	<.0001*
Mulch organ (leaf or root)	1.37	0.25	4.08	0.0495*	18.98	<.0001*
Mulch species x mulch organ	0.01	0.93	0.22	0.64	15.91	0.0002*
	Viburnum basal diameter (mm)		**Viburnum height (cm)**		**Viburnum leaf number**	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch species (buckthorn or dogwood)	0.28	0.60	0.01	0.93	1.05	0.31
Mulch organ (leaf or root)	2.31	0.14	0.001	0.98	0.01	0.91
Mulch species x mulch organ	0.09	0.77	0.12	0.73	0.12	0.73

Table 5: ANOVA for effects of all mulch treatments (including control) on indigenous shrub growth. For this analysis, the model effects have 4 df, with 55 df in the error terms.

	Alder basal diameter (mm)		Alder height (cm)		Alder leaf number	
Term	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch or no mulch effect	2.81	0.03*	0.94	0.45	2.44	0.06
	Meadowsweet basal diameter (mm)		**Meadowsweet height (cm)**		**Meadowsweet leaf number**	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch or no mulch effect	1.36	0.26	6.34	0.0003*	22.34	<.0001
	Viburnum basal diameter (mm)		**Viburnum height (cm)**		**Viburnum leaf number**	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
Mulch or no mulch effect	0.55	0.70	1.60	0.19	0.51	0.73

Table 6: Carbon to nitrogen ratios of buckthorn and native leaf and root mulches

Mulch type	C:N
Buckthorn leaf	19:1
Buckthorn root	66:1
Dogwood leaf	33:1
Dogwood root	55:1

Table 7: Student's t-tests for impacts of alder extract or water on buckthorn seedling growth. For these trials, the model effect has 1 df, with 70 df in the error term.

	Buckthorn basal diameter (mm)		Buckthorn height (cm)		Buckthorn leaf number	
Term	t-stat	p-value	t-stat	p-value	t-stat	p-value
Extract or water effect	7.00	0.01*	0.001	0.99	3.23	0.08