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Research Article

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Effect of Method and Time of Management on European Buckthorn (*Rhamnus cathartica* L.) Growth and Development in Minnesota

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ABSTRACT

European buckthorn is an exotic problematic invasive woody species that has displaced native plant species in Minnesota woodlands. Buckthorn is also an overwintering host for oat crown rust and soybean aphids, which can cause significant crop yield losses. The overall goal of this study was to test multiple buckthorn control methods and examine the establishment of native plant species in colonized areas. Specific objectives were to 1) determine the effectiveness of buckthorn control methods when applied in different seasons, 2) monitor seedling recruitment and resprouting ability of buckthorn saplings following treatment, 3) monitor recruitment and survival of native plant species following treatment, and 4) characterize buckthorn carbohydrate fluctuations and considerations for timely and effective buckthorn management. Field experiments conducted for two years in two locations (Eagle Lake Regional Park and Battle Creek Regional Park, Minnesota, U.S.A), tested four buckthorn control treatments: 1) cutting only; 2) cutting+stump treatment with herbicide (triclopyr); 3) cutting+stump treatment with herbicide+burning, and 4) cutting+burning. Untreated controls were included in each experiment. Across management seasons, the cutting+stump treatment with herbicide resulted in higher seedling densities for buckthorn and other species the next season compared to cutting only without herbicide application. Spring management resulted in the lowest seedling density the next season for both buckthorn and other plant species, and spring control treatments that included herbicide and burning resulted in higher buckthorn and native species seedling densities than treatments without burning. Because seasonal total nonstructural carbohydrate levels in buckthorn crowns were highest in the fall season, we recommend applying systemic herbicides in the fall when carbohydrates are translocated for storage to facilitate herbicide translocation and efficacy. Our study shows that integrating multiple buckthorn control methods reduces buckthorn populations and increases native species diversity. For long-term control of buckthorn seedling establishment, follow-up treatments like applying foliar herbicide sprays can be used in addition to prescribed burning.

KEYWORDS: European buckthorn, invasive, control method, species diversity, species richness, total nonstructural carbohydrates

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INTRODUCTION

European buckthorn (*Rhamnus cathartica* L.) is an exotic invasive woody shrub associated with disturbed woodland habitats in Minnesota and throughout the upper Midwest [1,2]. Originally introduced to North America as an ornamental plant due to its hardiness and ability to tolerate a variety of soils and site conditions [2,3], buckthorn eventually dominated the understory of many woodland ecosystems preventing the recruitment and natural regeneration of native shrubs and herbs [1,4]. Buckthorn infestations have led to the loss of species diversity and displacement of wildlife-supporting native vegetation such as *Cornus* spp. (dogwood), *Corylus* spp. (hazel), and *Prunus* spp. (cherries) (Hennepin and Ramsey County parks management, personal communication). According to a Minnesota Department of Natural Resources management report [5], buckthorn can produce up to 5,000 seedlings m^{-2} and population densities of buckthorn in invaded habitats can exceed 14,000 saplings per acre. Archibold et al. [3] reported that the buried seed bank beneath mature seed-bearing buckthorn shrubs averaged 620 seeds m^{-2}. Buckthorn excludes understory species through shading and through allelopathic effects [1,6]. Buckthorn is also an overwintering host for the fungus (*Puccinia corotana*) that causes oat crown rust disease [7], and the soybean aphid (*Aphis glycines*) [8,9], both of which reduce grain yield and quality.

Since buckthorn is spread primarily by seed, a successful management strategy should be one that controls all stages of buckthorn populations, including seedlings, saplings, and seed-bearing plants. Because of resource constraints, most buckthorn removal programs have targeted removing mainly seed-bearing plants while saplings and seedlings still dominate the woodland understories. A variety of suppression methods, including mechanical and chemical approaches, were assessed by Archibold et al. [3], Boudreau and Willson [1], and Heidorn [4], but peer-reviewed publications on long-term sustainable control of buckthorn in the United States are still few. Several methods such as mowing, girdling, cutting, prescribed burns, and herbicide application suppress buckthorn but require frequent follow-up treatments, indiscriminately remove other woody species, and may cause severe soil disturbance [4,10]. Most published reports of effective buckthorn suppression employ herbicide treatment, either alone or combined with mechanical techniques such as burning or cutting. Applying triclopyr or glyphosate on saplings and mature buckthorn has shown some success when used in combination with either stem girdling or cutting [3,4] and when used with follow-up treatment to control seedlings [4,5].

Herbicides such as triclopyr have successfully controlled glossy buckthorn (*R. frangula*) and European buckthorn on several preserves across the United States, and of other invasive plants on The Nature Conservancy preserves in Hawaii [11]. Prescribed burns have also been effective in preventing buckthorn re-establishment via seedlings or saplings if used on an annual or biennial basis for five or more years despite the likelihood of killing desirable native species in some natural areas [4]. However, saplings greater than 2.5 cm in diameter are not effectively controlled by burning and later vigorously resprout [1]. Willson and Stubbendieck [12] reported that prescribed burning at the time of tiller elongation effectively controlled smooth brome (*Bromus inermis*) and encouraged the growth of native grasses. However, burning can also have detrimental effects on native plants, soil organisms, and seeds, and may also volatilize soil nutrients. Milberg and Lamont [13] reported that fire enhanced the invasion of exotic species and resulted in an overall reduction in the abundance of native species in extensively disturbed areas. While working on control of garlic mustard (*Alliaria petiolata* Bieb.), Schwartz and Heim [14] and Luken and Shea [15] reported that repeated prescribed burning was detrimental to native herbaceous species, reducing both density and richness.

Previous studies on other noxious weeds such as purple loosestrife (*Lythrum salicaria*) [16], leafy spurge (*Euphorbia esula*) [17], Russian olive (*Elaeagnus angustifolia* L.) [18], and parthenium weed [19] have reported that integration of two or more control methods is more effective compared to the use of single control methods. Burning a leafy spurge infestation reduced the litter layer stimulating the seeds of leafy spurge to germinate, followed by herbicide application, reducing the seed bank [11]. Tu et al. [11] also reported similar results for purple loosestrife and cogongrass (*Imperata cylindrica*). Caplan [20] described control of Russian olive stems greater than 10 cm in diameter with cutting and immediate application of a 50% solution of triclopyr ester to stump surfaces. The overall goal of this study was to test multiple buckthorn control methods and examine the establishment of native plant species in colonized areas. Specific objectives were to 1) determine the effectiveness of buckthorn control methods when applied in different seasons, 2) monitor seedling recruitment and resprouting ability of buckthorn saplings following treatment, 3) monitor recruitment and survival of native plant species following treatment, and 4) characterize buckthorn carbohydrate fluctuations and considerations for timely and effective buckthorn management. To minimize damage to native species and lower labor costs, Kline [21] suggested fall application of herbicides since buckthorn leaves remain green late in the season after native species have entered dormancy. However, no studies verified this suggestion, yet it is important for decisions related to the timing of herbicide application while conserving native species' diversity. Additionally, Harrington et al. [22] reported that buckthorn's average leaf longevity exceeds that of native species by 58 days, allowing control treatments to be applied later than the native species.

Previous studies suggested that management practices should be timed based on carbohydrate fluctuations [23,24,25]. The general pattern of carbohydrate use in deciduous temperate-zone woody plants [26] is similar to that observed in herbaceous perennial weeds such as purple loosestrife [25] and hemp dogbane (*Apocynum cannabinum*) [23]. The growth and survival of most plants heavily rely on the stored carbohydrate reserves [26]. Loescher et al. [27] reported that accumulation of carbohydrate reserves is very sensitive to late-season stresses and management. Therefore, decreased carbohydrate accumulation during the fall can profoundly affect the survival of a plant the following year. Understanding seasonal carbohydrate fluctuations and designing management strategies according to these seasonal changes would allow more effective implementation of management of these troublesome woody plant species.

In this study, we hypothesized that 1) integrated control strategies such as a combination of cut-stump herbicide treatment+prescribed burns provide more effective control of buckthorn than the application of single control methods such as cutting or burning alone, 2) the use of prescribed burns as a follow-up treatment increases seedling emergence of both buckthorn and other plant species from the seed bank, potentially enhancing native plant diversity, and 3) applying systemic herbicides late in the fall during basipetal carbohydrate translocation for storage lowers the survival rate of established buckthorn the following year. Field experiments and laboratory analyses were conducted to test these hypotheses.

MATERIALS AND METHODS

Buckthorn Management Study

Site description and experimental design

Field experiments were conducted at two study sites, Battle Creek Regional Park (Ramsey County) and Eagle Lake Regional Park (Hennepin County), MN, U.S.A in 2002 and 2003. The dominant tree species at both locations were northern red oak (*Quercus rubra)* and bur oak (*Quercus macrocarpa*) associated with scattered species such as European white birch (*Betula pendula*), American elm (*Ulmus americana*), and white ash (*Fraxinus americana*), and shrubs such as common prickly ash (*Zanthoxylum americanum*). The two parks were heavily infested with European buckthorn of multiple growth stages, ranging from seedlings to large shrubs up to trees with trunks 30.5 cm in diameter at breast height. The relative density of buckthorn saplings following the removal of large, seed-bearing buckthorns compared to other understory plant species was 75% of all understory species combined (data not shown). Relative density was expressed as a percentage of the total stems of the understory vegetation [22].

Preliminary study

Prior to conducting the detailed field management study, a preliminary study was undertaken during the spring of 2001 to evaluate the effectiveness of management practices such as cut-stump treatment using herbicide (triclopyr) and follow-up burning treatment on buckthorn survival at Battle Creek Regional Park. Park managers at Battle Creek Park had initiated a buckthorn control program in the previous year (2000) to control buckthorn and increase native species diversity. The initial buckthorn control program consisted of winter removal, by cutting all large and seed-bearing buckthorn trees and treating stumps with Garlon 3A, triethylamine salt formulation [active ingredient, triclopyr (44.4% amine salt) and inert ingredients (55.6%), WSSA 2002, Dow AgroSciences, Indianapolis, IN, 46268, U.S.A]. This was followed by a spring prescribed burn treatment in April 2001, only to a portion of the cut-stump treated area. To evaluate the effectiveness of the follow-up burning treatment on the survival of buckthorn seedlings and saplings, sixteen 1 m^2 quadrats were monitored in both burned and unburned areas at Battle Creek Regional Park. For each quadrat, data on buckthorn seedling population densities, number of live sapling stems, and average shoot height were recorded at three-week intervals in each quadrat starting June 6 to August 15, 2001.

Field study

After the preliminary study, management studies were initiated in 2002 and repeated in 2003 at both Eagle Lake and Battle Creek Regional Park, MN, and monitored the following season in 2003 and 2004, respectively. Four buckthorn control treatments were applied during four management seasons according to plant phenology, spring (bud break and leaf emergence), summer (leaf expansion), fall (leaf senescence), and winter (dormant stage). Each management season included nontreated checks and all or a subset of the four control treatments appropriate for that season: cutting only (C), cutting+stump-treatment with triclopyr amine (C+S), cutting+stump-treatment with triclopyr amine+burning (C+S+B), and cutting+burning (C+B). Triclopyr (Garlon 3A undiluted) was applied by spraying the surfaces of freshly cut stumps, making sure the cambium is uniformly wetted using a low-pressure spray bottle to avoid runoff. Follow-up burning treatments were conducted on April 29, 2003 only in treatments that were burned in 2002 at both Battle Creek and Eagle Lake Parks to evaluate the effect of follow-up treatment on buckthorn survival and native species diversity. The experiments were set up as a completely randomized design (CRD) with four replications. The experimental sites were fairly uniform, i.e., Battle Creek is gently sloping while Eagle Lake is fairly flat, and both have uniform soil and vegetation cover within the study area. The soil types at both study sites are Koronis-Kingsley complex, with 2 to 6% slope range. The soils are well drained, sandy loams with 2.5% organic matter in the upper 25 cm (Natural Resources Conservation Service, www.mn.nrcs.usda.gov). The experimental unit (quadrats) consisted of 1.5 by 2.0 m plots and included buckthorn sapling plants between sizes 1 to 4 cm in diameter. Ten buckthorn stems in each quadrat approximately 1 cm in diameter were tagged with white stakes and monitored throughout each management season and the following year. All buckthorn plants in the quadrat were subjected to the respective treatments as previously described, but data was collected for only the ten tagged plants.

Data collection

For each year and experimental locations, data on the number of resprouts, buckthorn seedling population density, and the density of other plant species were collected at three week-intervals in July (summer season) after applying respective buckthorn management treatments. Additionally, to assess the effect of buckthorn management treatments on species richness and species diversity, the number of other plant species other than buckthorn was collected during the summer of 2002 and 2003 in spring management plots. To monitor the response to long-term management, quadrats were left in place for follow-up data collection the following year.

Statistical Analysis

All data were analyzed by Analysis of Variance using Statistix Analytical Software for windows version 7.0 (P.O. Box 12185, Tallahassee FL 32317), and mean comparisons were made by Fisher's Protected LSD test at the 5% significance level. We compared all four management seasons (winter, spring, summer, and fall) for the two control treatments common to all: cutting only (C) and cutting + stump treatment (C+S) including nontreated checks. Within the spring management season, data was analyzed for all four control treatments: C+S+B, C+B, C+S, and C, including nontreated checks. When there were no significant location by treatment interactions, data for the locations were combined. Data were tested for homogeneity of variances and found to be homogenous and, therefore, were not transformed.

Characterization of Seasonal Carbohydrate Fluctuations

Qualitative and quantitative characterization of the predominant total nonstructural carbohydrates (TNC) in the crowns of European buckthorn were determined in the laboratory using conventional chemistry methods and high-performance liquid chromatography (HPLC) as described by Casterline et al. [28] and Chow and Landhausser [29].

Sample preparation

Buckthorn crowns were collected monthly beginning March and ending December of both 2002 and 2003 in untreated areas adjacent to management study quadrats at Eagle Lake Regional Park (Hennepin County), Maple Grove, MN. A crown includes the woody, usually enlarged area in the transition region between the root and the stem, and in the buckthorn is an area where most resprouts occur. Five buckthorn saplings of similar crown size of 1 to 3 cm in diameter were randomly harvested within untreated areas and about 5 to 10 cm of the crown and stem was cut just above the soil surface using a clipper. Only the lower 5 cm of each crown were used for analysis. The sample sections were placed in plastic and immediately packed in ice until reaching the laboratory where they were stored in a -8 °C deep freezer at until analysis was done. The sampled plant saplings remained vegetative throughout the season. At the time of sample collection, various phenological characteristics such as leaf initiation, leaf expansion, leaf senescence, and dormancy were monitored. To determine carbohydrate stratification in the stem, crown, and roots for both vegetative and reproductive buckthorn growth phases, plant samples were taken during early spring in April 2004. This would establish carbohydrate demands for seed-bearing plants compared to the vegetative growth phase. Five buckthorn plants of each the vegetative and reproductive growth phases were harvested, and two samples were taken above and one below the soil surface, i.e., 10 cm above and 5 cm below the soil surface, and the entire length of sample stratified into 5 cm segments to determine whether carbohydrate levels vary along the plant axis in the stem, crown and the root. Subsamples were oven- and freeze-dried and analyzed for TNC or soluble sugars. Buckthorn crown samples for each sampling date were combined and oven-dried at 60 °C for 3 days and ground using a Wiley mill and collected through a 40-mesh screen before analysis.

Carbohydrate analysis

Buckthorn samples were analyzed for TNC including starch and the major soluble sugars (sucrose, glucose, fructose, fructans, raffinose, stachyose) using both conventional chemistry methods [29] and high-performance liquid chromatography (HPLC analysis) [28]. For soluble sugars, a subsample of 100 mg of plant material was placed into 50 ml centrifuge tube before adding 5 ml 80% ethanol to each sample, centrifuged at 2800 rpm for 20 min, the filtrate incubated in a water bath at 60 °C overnight, and stored in a -8 °C freezer before HPLC analysis. Starch levels were extracted from the insoluble residue remaining after soluble sugar extraction by gelatinization and use of α-amylase and amyloglucosidase enzymes [28,30]. Residual samples after soluble sugar extraction were stored overnight in a freezer, 20 ml of refrigerated nanopure water subsequently was added and centrifuged at 2800 rpm for 20 min before adding 5 ml of 0.1 M cold acetate buffer and 0.1 ml of heat stable amylase (Sigma A-3403, *Bacillus lichenformis*). Insoluble residue samples were heated at 90 °C in a water bath for 1 h and swirled every 20 min for starch gelatinization and hydrolysis to occur. The samples were cooled to 50 °C before adding 0.2 ml amyloglucosidase (Roche 102857, A*spergillus niger*) and then heated at 60 °C in a water bath for 3 h and swirled every 30 min. The samples were cooled at 50 °C before adding 28 ml 95% ethanol and refrigerated overnight. The sample was centrifuged at 2800 rpm for 15 minutes, and a 2 ml starch glucose aliquot was stored in a -8 °C freezer before HPLC analysis. The ethanol extract sample was used in starch determination in the HPLC by estimating the amount of glucose released by starch hydrolysis. Prior to running soluble and starch sugars on HPLC, samples were removed from the freezer, allowed to warm to room temperature, and then filtered. One ml of each sample was transferred into filter syringes before injection into the HPLC column (Agilent Technologies, Life Sciences and Chemical Analysis Group, 2850 Centerville Road, Wilmington, DE, 19808). The following were the HPLC specifications used and system used: Agilent 1100 with Quad pump, autosampler, column heater and Agilent refractive index detector. Chemstation A.08.03 software; Mobile phase: thermally stable Nanopure water, degassed; Column: BP-100 H+ 300mm X 7.8 mm Carbohydrate Column (cat# 802) with BP-100 H+50 mm X 4.6 mm guard column (cat# 802G) (Benson Polymeric Inc., 56 Glen Carran Cr, Sparks NV 89431, 775-356-5755); Column and detector temp: 35 C; Flow rate/run time: 0.4 ml/min for 45 minutes isocratic; Injection: 40 µl soluble sugars/100 µl starch analysis.

RESULTS AND DISCUSSION

Effect of Control Treatments and Management Season on Buckthorn Seedling Establishment and Sapling Resprouting Ability

Prior to conducting the full buckthorn management experiments, preliminary studies conducted in 2001 showed that burning as an additional management option to cutting and treating stumps of seed-bearing buckthorn shrubs with triclopyr reduced buckthorn sapling survival and plant height, but increased buckthorn seedling density (data not shown). However, the removal of seed-bearing buckthorn shrubs without additional management treatments enhanced buckthorn sapling and seedling densities due to the removal of the above canopy. Similarly, recent studies have shown that mowing of buckthorn in forests increases light in the understory, facilitating buckthorn growth and survival [31]. Our follow-up field experiments conducted in 2002 and 2003 showed higher buckthorn seedling population densities and a higher number of resprouts in 2002 than in 2003 regardless of location and method of control. This observation could have resulted from a drought experienced during the 2003 growing season (Table 1).

Table 1: Average monthly precipitation and air temperature for February through December 2002 and 2003

	Precipitation (cm)		Air temperature (°C)	
	2002	2003	2002	2003
February	0.91	1.37	-2.0	-9.1
March	3.51	3.66	-3.9	-0.4
April	8.20	6.10	7.6	9.1
May	7.19	5.44	12.5	14.3
June	21.08	11.84	21.7	20.1
July	13.18	5.23	25.0	23.2
August	21.08	2.84	21.6	24.1
September	9.88	5.59	18.6	16.9
October	11.18	1.57	5.4	10.6
November	0.23	1.80	1.0	0.1
December	0.53	1.57	-3.2	-3.9

Climate data was for the Twin Cities International Airport, Minneapolis-St. Paul, MN, the nearest station for both Eagle Lake and Battle Creek Regional Parks obtained from the Minnesota State Climatology Office (http://climate.umn.edu).

When control treatments common to all management seasons (C+S and C) were compared for both 2003 and 2004, buckthorn seedling densities were higher when treatments were applied in the summer, fall, or winter compared to those applied in the spring (Table 2A and Table 2B). However, spring and summer treatments resulted in 70% more resprouts than when treatments were applied in either fall or winter. The higher number of resprouts could be because of high carbohydrate levels facilitating active plant growth, a trend observed in both 2003 and 2004 (discussed later). Interestingly, a higher buckthorn seedling emergence and resprouting ability was observed in July 2004 compared to July 2003. Fall and winter treatments resulted in fewer resprouts perhaps because at this time of the year, carbohydrates are being stored and thus cutting further reduced buckthorn survival the following season. Our carbohydrate study results (discussed later) showed that during the fall, carbohydrate reserves are getting translocated to the roots for winter storage, and thus, this would be the best time to apply systemic herbicides to facilitate herbicide translocation and efficacy. Our study shows that buckthorn control using cut-stump treatment with triclopyr prevented buckthorn sapling regrowth throughout the year regardless of application time. Cut-stump treatment with triclopyr during the fall or winter led to reduced buckthorn regrowth the following year due to management interfering with carbohydrate storage and consequently reducing buckthorn survival the following year. However, when control treatments common to all management seasons (C+S, C) were compared, cutting without herbicide application during the fall and winter completely controlled buckthorn regrowth but buckthorn sprouted after spring and summer management (Table 3). This could have been due to buckthorn plants having higher carbohydrate levels during spring and summer due to previous year storage and also the production of current photosynthates during leaf expansion. There was no regrowth during the fall, perhaps because carbohydrates are being stored, and the plant is not growing actively due to senescence. Also, since the plants were small (1 to 4 cm diameter under five years old), they could have had lower carbohydrate levels that could not support buckthorn survival the following year. When applying herbicides to control buckthorn, care must be taken to avoid potential negative nontarget effects on other species and drift [32]. Triclopyr is moderately persistent, with an average half-life of 10 to 46 days, depending on soil type, moisture, and temperature (WSSA 2002). Thompson et al. [33] reported that triclopyr may persist longer and may be susceptible to surface runoff. However, the authors also noted that at times triclopyr residues might be nonpersistent, dissipating through either rapid penetration or via photolytic degradation. In our study, there were minimal observable nontarget effects of burning on native plants since burning was conducted in the spring before native species emerged, but nontarget effects of burning were not evaluated in our study. When cutting seed-bearing buckthorn plants, it is better to cut during flowering or early fruit development when carbohydrate levels are at a minimum in late spring or summer due to the presence of reproductive sinks that sequester most of the assimilates. Cutting plants at this stage also interrupts fruit production, thus reducing seed input into the seed bank and seed dispersal by birds, both of which contribute to future buckthorn problems. Fall and winter cutting also controlled buckthorn sapling regrowth the following year, probably due to low carbohydrate levels in the young buckthorn saplings. Therefore, fall or winter cutting of saplings under five years old may reduce sapling regrowth and also minimize the use of herbicides. Our observations were contrary to previous reports that indicated that cutting alone without additional control methods results in vigorous resprouting [1]. Thus, there is need for further research to determine whether plant age and size affects the effectiveness of control methods.

Averaged across management seasons, all treatments that included cut-stump treatment using triclopyr (C+S) suppressed

Table 2A: Effect of 2002 control treatments and management season on buckthorn seedling density, number of resprouts per crown, and density of other plant species in July 2003

Treatments	Buckthorn seedling density[a] (plants ha^{-1})	Buckthorn resprouts per crown (no.)	Density of other species (plants ha^{-1})
Management seasons (Average across control treatment)			
Spring	1082	0.75	3226
Summer	1082	0.81	4212
Fall	1075	0.17	4212
Winter	1075	0.23	4212
LSD (0.05)	444	0.39	601
Control treatments (Average across management season)			
C + S	2016	0.00	6855
C	1344	0.89	2621
Nontreated	1075	0.57	2419
LSD (0.05)	384	0.34	1053

[a]all means are averaged across trial runs conducted at Eagle Lake and Battle Creek Park at P ≤ 0.05 comparing control treatments common to each management season: C+S - cutting + stump treatment with triclopyr amine, C - cutting only, and nontreated – checks

Table 2B: Effect of 2003 control treatments and management season on buckthorn seedling density, number of resprouts per crown, and density of other plant species in July 2004

Treatments	Buckthorn seedling density[a] (plants ha^{-1})	Buckthorn resprouts per crown (no.)	Density of other species (plants ha^{-1})
Management seasons (Average across control treatment)			
Spring	1613	1.71	5018
Summer	4032	1.69	8781
Fall	2688	0.17	8781
Winter	4032	0.18	8961
LSD (0.05)	710	0.99	1666
Control treatments (Average. across management season)			
C + S	4100	0.00	11156
C	3427	2.29	7796
Nontreated	1747	0.52	4704
LSD (0.05)	615	0.86	1442

[a]All means are averaged across trial runs conducted at Eagle Lake and Battle Creek Regional Parks at P ≤ 0.05 comparing control treatments common to each management season: C+S - cutting + stump treatment with triclopyr amine, C - cutting only, and nontreated – checks

buckthorn regrowth and killed the cut stump regardless of management season. Plants that were cut without the application of triclopyr (C) had 36%, and 76% more resprouts in 2003 and 2004, respectively, compared to nontreated plots (Tables 2A and 2B) probably indicating the effect of the drought in 2003 on resprouting ability. C+S-treated plots had 40% more buckthorn seedlings emerge than C-treated plots, probably because application of triclopyr to cut stumps prevented sapling regrowth, which removed buckthorn canopies, thus increasing light available to the soil surface. However, the cut-stump treatment application is labor intensive and mainly useful for smaller infestations despite its effectiveness throughout the growing season. To reduce labor costs, managers could also use foliar sprays on young, short plants, or basal bark treatments of triclopyr. Among spring control treatments, buckthorn plants that were cut without applying triclopyr or burning (C) consistently resulted in over 50% more resprouts than cutting followed by burning to suppress resprouting (C+B) indicating that burning suppressed buckthorn resprouting ability (Tables 4A and 4B).

Treatment combinations that included burning (C+S+B and C+B) resulted in higher buckthorn seedling densities than C+S and C treatments (Figure 1a; Figure 1b). However, there were no significant differences ($P \leq 0.05$) in buckthorn seedling population densities between the two burning treatments (C+S+B, C+B) for both years. For instance, treatments that included burning (C+S+B, C+B) resulted in over 70% more seedlings than treatments without burning (C+S, C, nontreated checks) (Tables 4A and 4B). This could be because burning removed surface litter in addition to canopy removal by cutting and increased soil temperatures and light availability to the soil surface, which facilitated seedling emergence. Results from previous surface litter studies showed that seedling emergence increased when litter cover was removed because of increased light transmittance and higher soil temperatures [34]. Burned plots had approximately 50% more seedlings than those that were not burned (Figure 1a; Figure 1b). Buckthorn seedling establishment did not vary among C+S, C treatments, and nontreated control checks in both years for all management seasons in the presence of surface litter. Treatments applied in summer and fall also had fewer buckthorn seedlings compared to those applied earlier in the spring (data not shown), probably because of shading by overstory canopies that limited seedling emergence. Buckthorn seedlings emerged primarily during June and July in Minnesota when soil conditions are more favorable, i.e., higher temperatures and high soil moisture (Table 1; http://climate.umn.edu). In our study, the spring 2003 follow-up prescribed burns, reduced buckthorn seedling density, and suppressed sapling regrowth but was not an effective method of controlling buckthorn since it facilitated further seedling emergence from the seed bank. Heidorn [4] suggested that periodic follow-up treatment is required to control buckthorn seedlings that are released by the removal of the overstory canopy by burning. However, Boudreau and Willson [1] reported that applying prescribed burns as saplings grow bigger than 2 cm in diameter is not effective at controlling sapling regrowth. Therefore, burning may be an effective means of reducing buckthorn populations long-term and may reduce the seed bank, but it is not clear whether repeated burns are the most effective means of continuing to reduce the buckthorn seed bank and also promote native species as increased fire frequencies required for buckthorn control may have negative

Table 3: Effect of 2002 and 2003 control methods common to all management seasons on buckthorn resprouting ability the season following treatment application, averaged across Eagle Lake and Battle Creek Parks, MN

	Resprouts per crown (no.)			
	C		C+S	
Management season	2003	2004	2003	2004
Spring	1.73	4.45	0.00	0.00
Summer	1.83	4.65	0.00	0.00
Fall	0.00	0.00	0.00	0.00
Winter	0.00	0.00	0.00	0.00
LSD (0.05)	0.11	0.13	0.00	0.00

[a]all means are averaged across trial runs conducted at Eagle Lake and Battle Creek Regional Parks at $P \leq 0.05$ comparing control treatments: C+S - cutting + stump treatment with triclopyr amine, C - cutting only, and nontreated – checks

Table 4A: Effect of 2002 spring control treatments on buckthorn seedling density, number of resprouts per crown, and density of other plant species in July 2003

Treatments	Buckthorn seedling density[a] (plants ha^{-1})	Buckthorn resprouts per crown (no.)	Density of other species (plants ha^{-1})
C+S+B	3226	0.00	6720
C+B	2957	0.55	3226
C + S	1075	0.00	2151
C	1075	1.73	2151
Nontreated	1075	0.53	2151
LSD (0.05)	370	0.11	709

Data was collected the season following treatment application at Eagle Lake Park, Maple Grove and Battle Creek Park, Maplewood, MN. [a]All means are averaged across trial runs conducted at Eagle Lake and Battle Creek Park at $P \leq 0.05$ comparing control treatments: C+S+B – cutting + stump treatment with triclopyr + burning, C+B – cutting + burning, C+S - cutting + stump treatment with triclopyr amine, C - cutting only, and nontreated – checks

Table 4B: Effect of 2003 spring control treatments on buckthorn seedling density, number of resprouts per crown, and density of other plant species in July 2004

Treatments	Buckthorn seedling density[a] (plants ha^{-1})	Buckthorn resprouts per crown (no.)	Density of other species (plants ha^{-1})
C+S+B	5376	0.00	20296
C+B	4301	1.78	13710
C + S	2688	0.00	5376
C	1075	4.63	5376
Nontreated	1075	0.53	5376
LSD (0.05)	428	0.10	2760

Data was collected the season following treatment application at Eagle Lake Park, Maple Grove and Battle Creek Park, Maplewood, MN. aAll means are averaged across trial runs conducted at Eagle Lake and Battle Creek Park at $P \leq 0.05$ comparing control treatments: C+S+B – cutting + stump treatment with triclopyr + burning, C+B – cutting + burning, C+S - cutting + stump treatment with triclopyr amine, C - cutting only, and nontreated – checks effects on native species overall. It is also important to note that burning initially increased species abundance and richness, but follow-up annual burning reduced species abundance. In a similar study, Willson and Stubbendieck [12] reported that timely prescribed burning of smooth brome increased native grass abundance. Also, because of earlier leaf emergence in buckthorn compared to most native species, Converse [10] recommended a late April or May burn in the upper Midwest to minimize injuring native species. However, increased fire frequencies have been suggested to promote invasion by exotic species [35], and repeated prescribed burning led to the loss of native species [13,15],. Although follow-up treatments are crucial in controlling buckthorn seedlings, the decrease in native species abundance by burning increases concerns that restoration will be difficult. Restoration may also be hindered by the lack of native seeds in the seed bank. Thus, planting of native species will likely be a necessary step in native species regeneration after buckthorn removal. More research should be conducted to ascertain the impacts of repeated annual spring burning as a buckthorn control method on desirable native species. This is because burning could also kill seeds, limit the chances of native plant regeneration after buckthorn removal, and, thereby, open niches for reinvasion by other invasive plants.

Effect of Control Treatments and Management Season on Seedling Establishment of Other Plant Species, Species Richness, and Diversity

Buckthorn was the predominant non-native plant species at both Battle Creek and Eagle Lake locations comprising 75% of all understory species before the application of control treatments. Understory species after buckthorn removal included both native species and weedy annual herbaceous plants such as common lambsquarters (*Chenopodium album*). Native species that emerged after burning included oak seedlings (*Quercus* spp.), white birch (*Betula* papyrifera), and cherries [pin cherry (*Prunus pennsylvanica*), wild cherry (*Prunus serotina*)] perhaps because of the existing high seed banks due to the current seed rain from existing trees.

A trend similar to buckthorn seedling establishment was observed for the establishment of other plant species for spring control treatments (Figure 2a; Figure 2b). Plots treated with prescribed burns resulted in higher seedling densities of other plant species (including herbaceous weeds and native species) than those that were not burned. Summer and fall treatments resulted in similar effects on the seedling population density of other species as that of buckthorn seedling establishment (data not shown). Seedling mortality occurred as fall temperatures decreased as indicated by fewer seedlings in November compared to early fall in September. When compared across control treatments similar to all management seasons (C+S and C), the population density of species other than buckthorn did not differ in July the season following treatment among summer, fall and winter management seasons but was 20 to 40% lower when treatments were applied in the spring in both 2003 and 2004 (Tables 2A and 2B). The lower establishment of other species in spring treated plots could have resulted from suppression by the 70% more buckthorn resprouts observed in the spring, compared to the fall or winter treatments. When averaged across management seasons, C+S-treated plots had, on average, 60% more seedlings of other species than nontreated plots, while C-treated plots had 10% more seedlings than nontreated plots (Tables 2A and 2B). The C+S-treated plots had over 30% more seedlings of other species than C-treated plots because cut-stump treatment with triclopyr prevented resprouting, which further suppressed canopy development allowing more light to reach the soil surface, increasing seedling emergence. Therefore, because C+S was effective throughout the growing season, it is the most suitable method to control buckthorn sapling regrowth and increase the establishment of native species. Across spring control treatments, C+S+B and C+B treated plots had more seedlings of other species than similar but unburned treatments (C+S and C). Thus, burning increased both buckthorn and native seedling emergence and establishment. Among spring treatments, C+S+B treatment resulted in over 45% higher abundance of seedlings of other plant species followed by C+B treatment, while treatments where litter was not removed by burning resulted in 40 to 70% fewer seedlings establishing compared to either C+S+B or C+B treatments (Table 4A and 4B). Therefore, the spring treatment including both herbicide application and burning (C+S+B), was the best management strategy for the spring buckthorn control program since it suppressed buckthorn

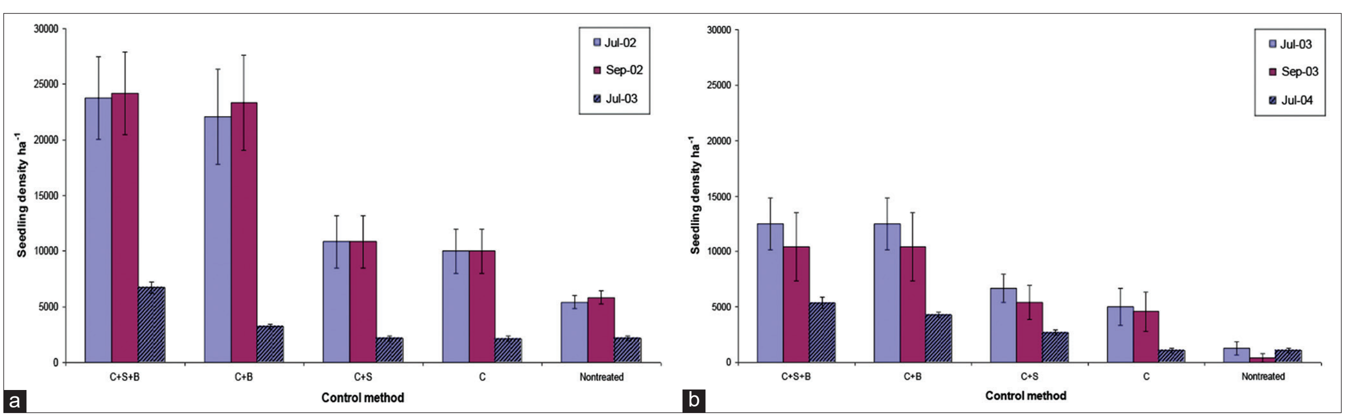

Figure 1: Effect of spring treatments on buckthorn seedling establishment averaged across Battle Creek and Eagle Lake Reginal parks, MN in 2002 (Figure 1a) and 2003 (Figure 1b)

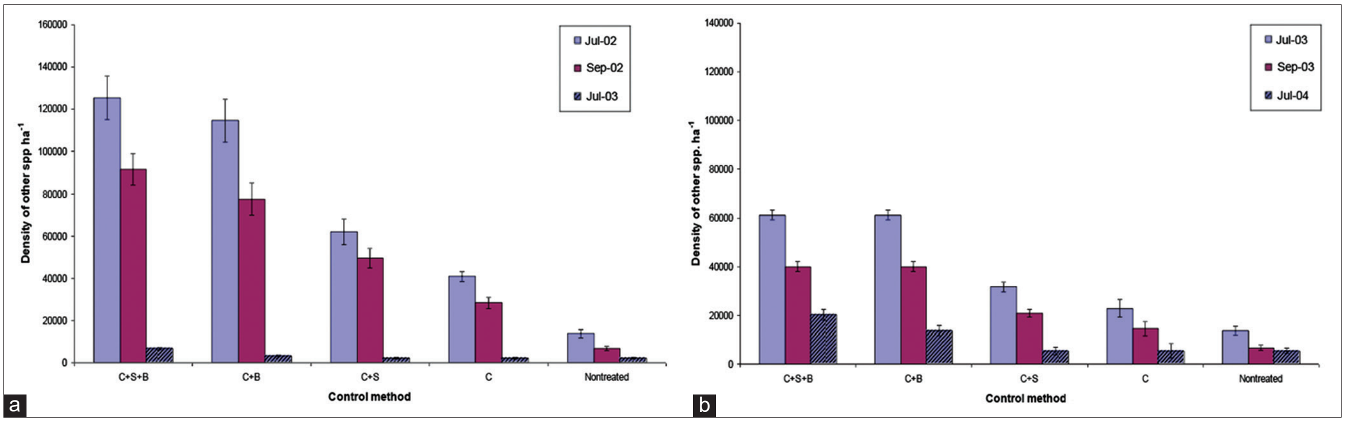

Figure 2: Effect of 2002 treatments on establishment of other species averaged across Battle Creek and Eagle Lake Reginal parks, MN in 2002 (Figure 2a) and 2003 (Figure 2b)

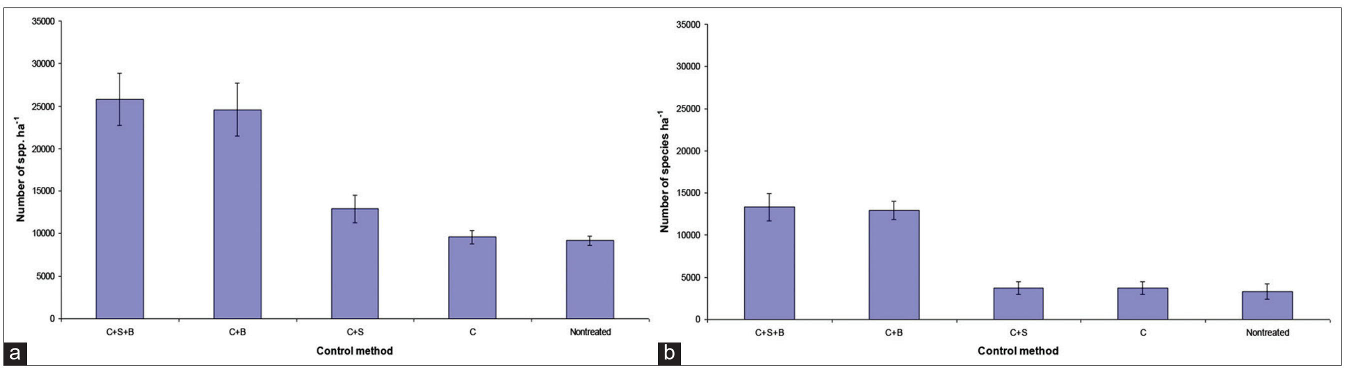

Figure 3: Effect of 2002 spring control treatments on species richness in July 2002 at Battle Creek Reginal park, Maplewood MN (Figure 3a). Effect of 2003 spring control treatments on species richness in July 2003 at Battle Creek Reginal Park, Maplewood, MN (Figure 3b)

regrowth and increased plant species abundance and diversity. These findings support our hypothesis that integrating more than one control technique increases species diversity despite the fact that buckthorn seedling emergence also increased due to a more open canopy. This, therefore, indicates that follow-up management options should be carefully planned for long-term control of buckthorn seedling establishment.

To evaluate species richness and diversity in each control treatment, individual plant counts were taken during July 2002 and 2003 in only spring treated plots, which unlike other management seasons, included all four control treatment options (C+S+B, C+B, C+S, C). Species richness, including native and herbaceous weedy species, differed across years and control treatments (Figure 3a; Figure 3b, $P \leq 0.05$). As observed for species abundance or density, species richness was higher for treatments that included burning (C+S+B, C+B) but was similar and low for the remaining treatments, C+S, C, and the nontreated checks. Treatments that included burning as an additional control option had 1 to 3 more species present compared with treatments that did not include controlled burns. Comparing the two treatments that included burning (C+S+B and C+B), a combination of cut-stump treatment and burning (C+S+B) resulted in a 25% higher species diversity than cutting and burning without herbicide (C+B) probably because the competitive canopy was further suppressed by cut-stump treatment using triclopyr which prevented resprouting, and litter was removed by burning, thus leading to higher seedling emergence in C+S+B treatments. Species diversity was low in the C+S, C, and the untreated control plots probably because of the presence of litter that may have reduced light availability and soil temperatures reducing seedling emergence as observed in previous studies [36,37].

Effect of Follow-up Burning Treatment on Establishment of Buckthorn and Other Plant Species

Follow-up burning treatment was only conducted in April 2003 and only in plots previously burned in spring 2002 at both Battle Creek and Eagle Lake Parks, and data were collected in July 2003. This treatment reduced buckthorn seedling density in July 2003 by 33% compared to the population present in July 2002 (Figure 4a). The fire killed buckthorn seedlings present after the 2002 treatment, though more emerged from the seed bank after burning by summer 2003. Burning appeared to be a self-defeating contradiction as a follow-up control method because although it killed buckthorn seedlings, it also seemed to facilitate buckthorn seedling emergence from the seed bank. This paradox surrounding prescribed burning may suggest that an alternative control approach should be developed in order to effectively control buckthorn while facilitating the growth of native species.

However, burning may eventually deplete the buckthorn seed bank, especially when used in combination with additional control techniques such as foliar sprays to control emerging seedlings. Burning also reduced the resprouting ability of the surviving saplings from 2 to 1 resprouts per crown in the C+B plots (Figure 4b). Follow-up spring burns decreased species abundance from 48,000 seedlings ha^{-1} in July 2002 to 20,000 seedlings ha^{-1} in July 2003 (Figure 4c), and the number of buckthorn seedlings was reduced from 16,000 seedlings ha^{-1} in July 2002 to 10,000 seedlings ha^{-1} in July 2003 at Battle Creek Park (Figure 4a). This contradicts the findings observed by Moriarty et al. [5], who reported that buckthorn removal using triclopyr was followed by controlled burns, species diversity increased from 6,000 species to 16,000 species ha^{-1} while the number of buckthorn seedlings was reduced from 3 seedlings in 1996 to 0 m^{-2} in 1998. However, unlike ours was a two-year study, the Moriarty et al. [5] study was conducted across a four-year period, and factors such as differences in location, climate, and causes in mortality could have led to observed differences. In previous studies, by Boudreau and Willson [1], reported that burning did not eliminate buckthorn saplings but suppressed resprouting and killed buckthorn seedlings. In plots where no follow-up treatments were applied, the number of buckthorn saplings continued to increase throughout the experiments.

Characterization of Buckthorn Crown Carbohydrates and Implications for Buckthorn Management

Total nonstructural carbohydrates fluctuated throughout the year with the lowest TNC in buckthorn crowns at leaf expansion and highest at bud break (leaf initiation) and leaf senescence of both years (Figure 5a; Figure 5b). The TNC followed a similar trend across the growing season for both 2002 and 2003 (Figure 5a; Figure 5b), although 2002 had 15% higher TNC levels than 2003. This difference could have resulted from drier weather conditions experienced in the 2003 growing season. Latt et al. [38] did correlations between carbohydrate levels and weather variables while working on tropical tree species and reported that weather variables seem to interact with reserve carbohydrates. For example, TNC levels were highest early in the spring in April and during the fall in October, while the lowest levels occurred during late spring and early summer during June to meet carbohydrate demands for early growth and leaf expansion. Several investigators have reported seasonal changes in carbohydrate composition in both woody and herbaceous plant tissues [24,25,26]. Kozlowski [26] reported that carbohydrate reserves decrease during spring growth to a minimum in early summer, and then after vegetative growth has slowed or stopped, increase to a peak in the fall. A similar trend has been observed in herbaceous perennial weeds such as purple loosestrife [25] and hemp dogbane [23]. In our study, a comparison of TNC among buckthorn growth stages showed that buckthorn crowns at vegetative (non-seed bearing) stage had 25% more sucrose and starch than crowns at reproductive (seed-bearing) stage (Figure 6a; Figure 6b). This could be because plants in the vegetative phase produced and stored more assimilates compared to the reproductive stage that used up

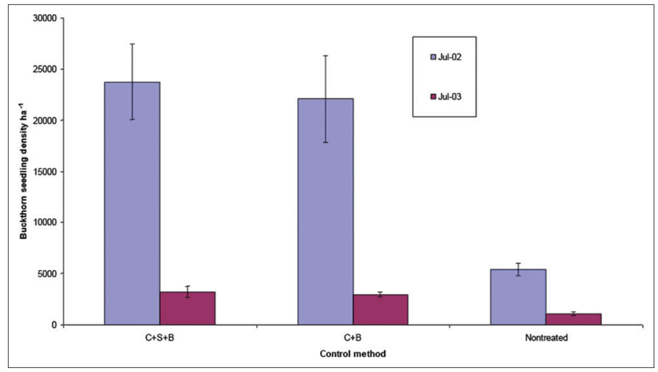

Figure 4a: Effect of 2003 two-year sequential burning on buckthorn seedling density, Battle Creek Regional Park, Maplewood, MN

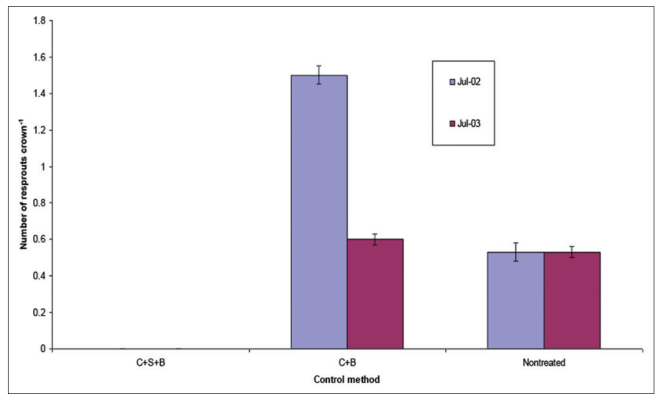

Figure 4b: Effect of 2003 two-year sequential burning on buckthorn resprouting ability, Battle Creek Regional Park, Maplewood, MN

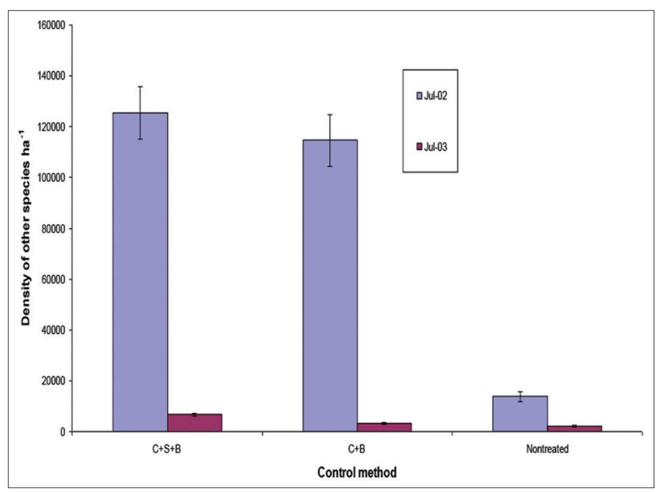

Figure 4C: Effect of 2003 two-year sequential burning on density of other plant species, Battle Creek Regional Park, Maplewood, MN

most of the photoassimilates for other developmental processes such as flowering and fruit production. Our tissue-specific analysis of TNC for buckthorn stem, crown, and root samples for both vegetative and reproductive growth phases showed higher levels of TNC in roots than in crowns or stems with 70, 29, and 16 mg g^{-1}, respectively in seed-bearing plants and, 80, 36, and 20 mg g^{-1}, respectively in vegetative plants. Among the two buckthorn growth stages, buckthorn roots at vegetative

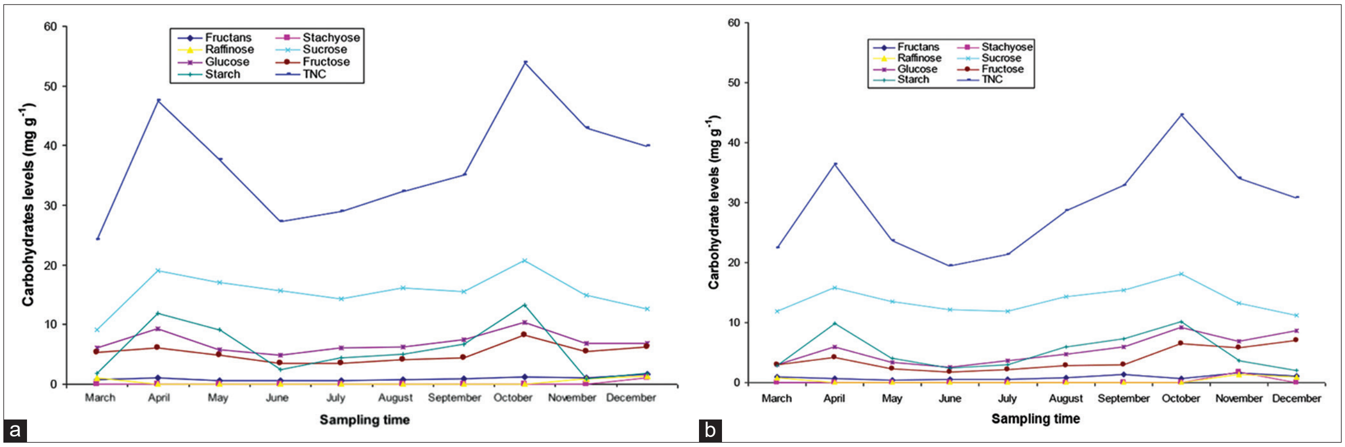

Figure 5: Sesonal fluctuations of total nonstructural carbohydrate levels in buckthron crowns at vegetative stage, Eagle Lake Regional Park, MN, 2002 (Figure 5a) and 2003 (Figure 5b)

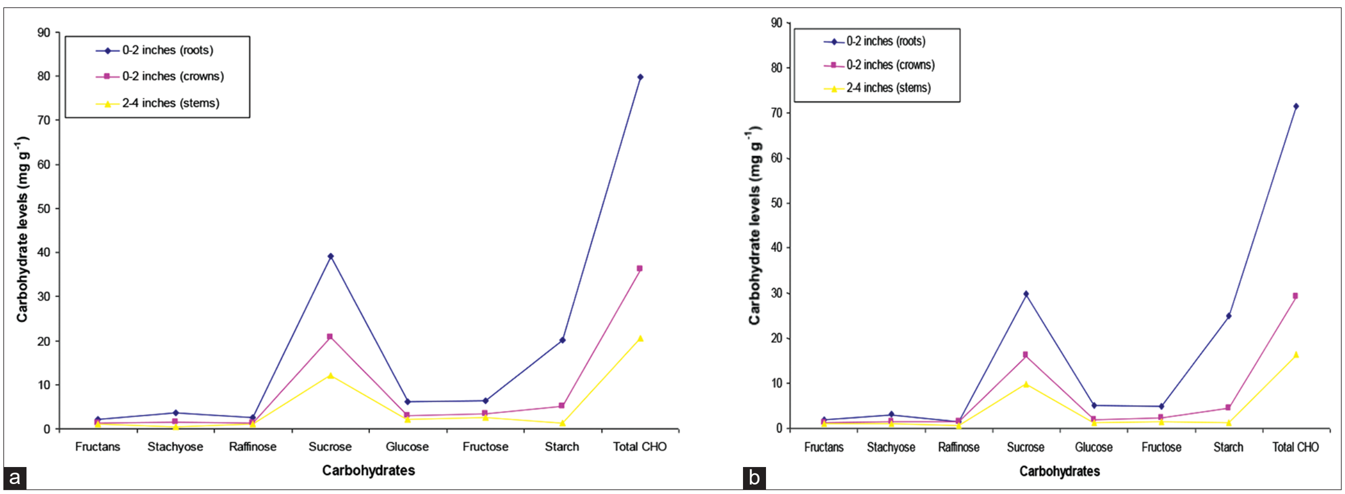

Figure 6: Carbohydrate stratification in buckthron roots, crowns and stems at vegetative (Figure 6a) and reproductive (Figure 6b) growth stages. All samples were collected in spring 2004 at Eagle Lake Regional Park, MN

stage had more sucrose (40 mg g^{-1}) than roots of reproductive plants, which had 30 mg g^{-1}. Studies on reserve carbohydrates in woody species have indicated that carbohydrate levels in plant tissues may be reduced by the presence of reproductive sinks such as flowers and fruits [26,27]. Therefore, the presence of reproductive sinks in seed-bearing buckthorn may have reduced carbohydrate levels in buckthorn crowns compared to vegetative growth. Carbohydrate fluctuations in buckthorn crowns during the growing season have management implications. As previously stated, the ideal time to apply herbicide is in the fall, to facilitate herbicide translocation and efficacy.

CONCLUSION

Our findings indicate that an integrated weed management approach that includes a combination of control strategies such as cutting+stump herbicide treatment, followed by an additional prescribed burn (C+S+B), was more effective at reducing established buckthorn populations and increasing species diversity than the use of single methods such as cutting alone. Therefore, to optimize resources, reduce buckthorn infestation, and increase native species' diversity, an integrated approach combining knowledge of the biology and ecology of buckthorn is a more ecologically and economically feasible management approach. Additionally, understanding the buckthorn invasion process, including factors that favor spread and persistence such as allelopathy [6,39] will help in designing appropriate management technologies. When timing herbicide treatments in integrated weed management programs, fall applications are most suitable to facilitate herbicide efficacy and reduce buckthorn populations in subsequent years.

COMPETING INTERESTS

The authors have declared that there are no competing interests.

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