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RESEARCH ARTICLE

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Efficacy of Dormant Season Herbicide Application on Control of Japanese Honeysuckle (*Lonicera japonica*) in Kentucky

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ABSTRACT: North America's remaining natural grassland communities provide habitat for native flora and fauna. We conducted a study to compare the efficacy of herbicides in control of the invasive Japanese honeysuckle (*Lonicera japonica* Thunb.) applied at times when most native plant species are dormant. Six herbicide mixtures (glyphosate, glyphosate + imazapyr, glyphosate + imazapic, imazapyr, triclopyr + diflufenzopyr, and metsulfuron methyl + diflufenzopyr) were applied once each in three seasons to assess the effect of application timing of each mixture on honeysuckle control. Herbicides were applied with a CO_2 pressurized sprayer at three sites in a randomized complete block design. Pretreatment sampling indicated that Japanese honeysuckle constituted over 70% of plant cover at the study sites. Post-treatment sampling was conducted 60 days, 180 days, 420 days, and 540 days after the final application. All mixtures in all application seasons decreased percent cover of honeysuckle with varying effectiveness. Results indicate that the glyphosate, imazapyr, and metsulfuron methyl + diflufenzopyr mixtures are particularly effective at controlling Japanese honeysuckle when applied at any time between October and April with suitable temperatures. Many native grasses and broadleaf forbs not found during pretreatment sampling also emerged post-treatment, benefiting from either application timing or indicating herbicide tolerance.

Index terms: grasslands, habitat restoration, herbicides, invasive species, *Lonicera japonica*

INTRODUCTION

Japanese honeysuckle (*Lonicera japonica* Thunb.) is recognized as one of the most aggressive nonnative invaders in the eastern United States due to its ability to crowd out or smother other species and monopolize resources (National Invasive Species Council 2004). It is a native of Southeast Asia, where it naturally occurs along roadsides and in sparsely vegetated forests below 1500 m in elevation (Zheng et al. 2004). Japanese honeysuckle's invasiveness is in part due to its ability to vigorously resprout after aboveground tissue (leaves and stems) has been removed following herbivory or cutting (Scheirenbeck 1994). It grows aggressively; each individual vine is capable of growing 10 m per year (Pelczar 1995). Japanese honeysuckle thrives in open areas, including natural grasslands. Although it is somewhat shade tolerant and does well at forest edges, it will often lurk in sunny openings or edges for years and exploit new openings in the canopy created by storms or other disturbances to colonize the forest interior (Yates et al. 2004).

Small natural grasslands such as glades and barrens are prone to disturbance and, therefore, invasion by Japanese honeysuckle, and are already in serious decline due to habitat loss and fragmentation caused by human activity (Noss et al. 1995). Less than 0.1% of the natural grasslands in the United States are intact, and most of these occur in small patches of just a few acres (Sampson and Knopf 1994). In Kentucky, natural grasslands provide habitat for over 60% of the rare plant communities monitored by the Kentucky State Nature Preserves Commission, Kentucky's natural heritage program (Taylor 1995). Japanese honeysuckle outcompetes most native species and also produces allelopathic compounds that alter soil chemistry and inhibit native plant growth (Skulman et al. 2004).

Although the Kentucky Exotic Pest Plant Council (KY-EPPC) recognizes nearly 200 invasive species as problems, ecological impacts vary in severity by species. KY-EPPC designates Japanese honeysuckle as a "severe threat," the category reserved for the species most ubiquitous and damaging to natural areas in the Commonwealth and defined as an "exotic plant species which possess characteristics of invasive species and spread easily into native plant communities and displaces native vegetation; includes species which are or could become widespread in Kentucky" (Kentucky EPPC 2013). The Southeast Exotic Pest Plant Council (SE-EPPC) has verified Japanese honeysuckle in 43 states and in 119 of Kentucky's 120 counties, although it is likely present in every county (EDDMapS 2014).

Reductions in invasive plant species populations have been shown to increase native biodiversity and productivity, a priority of natural areas managers (Price and Weltzin 2003). Previous research has investigated nonchemical control methods and found them to be ineffective or impractical due to the dense mats formed by the rhizomes of Japanese honeysuckle. Studies have found that only those methods that killed the belowground parts of the plant were effective in Japanese honeysuckle control (Stransky 1984). Removing the aboveground growth without subsequent chemical control does not kill the plant, and in many cases, stimulates even denser regrowth the following year. For instance, mowing alone is an ineffective control method, stimulating growth and encouraging formation of dense, albeit shorter, mats (Stransky 1984). While prescribed burns have proven effective in controlling many invasive species, fire only removes aboveground biomass while stimulating Japanese honeysuckle in much the same way as mowing (Barden and Matthews 1980; Munger 2002).

Herbicide use is one of the most practical strategies for controlling many invasive plants (Miller 2003). Herbicides are the predominant and most effective method for controlling Japanese honeysuckle invasions because they effectively target the persistent stolons and rhizomes in the soil organic layer (Prine and Starr 1971; Tu et al. 2001). In the southeastern United States, Japanese honeysuckle actively grows in the winter until temperatures drop below −1 °C, when most native species are dormant. Therefore, it may be possible to control Japanese honeysuckle during this dormant season and avoid damage to native broadleaf species while simultaneously releasing native species to germinate from the seedbank (Carter and Teramura 1988a). If this strategy proves successful, land managers could control Japanese honeysuckle populations while minimally affecting native species.

Chemical control of Japanese honeysuckle invasions has focused on foliar herbicide applications. However, foliar applications may only reduce the density of aboveground leaves and stolons temporarily without completely killing the plant's rhizome. In this case, new growth the following season can even exceed the original coverage prior to herbicide application (Prine and Starr 1971). Several studies found that two seasons of low-dose herbicide treatment are most effective for managing Japanese honeysuckle but still do not achieve adequate control over the long term (McLemore 1981; Miller 2003).

Among various herbicides tested for Japanese honeysuckle control, glyphosate has shown the most promise (Regehr and Frey 1988). Glyphosate phototoxicity differs seasonally as winter applications are generally less effective than those applied in other seasons due to lower photosynthetic activity by target species (Neal and Skroch 1985). However, honeysuckle leaves are physiologically active during all seasons in the Southeast (Carter and Teramura 1988b; Nyboer 1992). The imidazolinone herbicides, such as imazapyr and imazapic, have been used effectively to reduce competition from exotic species and promote establishment of native species, although imazapyr has been shown to limit recruitment of native seedlings (Masters et al. 1996; Ayeni et al. 1998; Beran et al. 2000; Washburn and Barnes 2000; Masters et al. 2001; Washburn et al. 2002). Studies indicate that imazapyr and metsulfuron methyl have potential for honeysuckle control (Edwards and Gonzalez 1986; Cain 1992; Yeiser 1999). While some research indicates that triclopyr is ineffective on Japanese honeysuckle, some agencies still recommend its use in honeysuckle control, suggesting further study is needed (Dreyer 1988; Miller 2003).

Due to the physiology of Japanese honeysuckle and the effectiveness of herbicides in its control, we hypothesized that herbicide mixtures applied to Japanese honeysuckle while the plant is actively growing, but while most native plants are dormant, can control honeysuckle while minimally affecting other species. In addition to application timing effects, we evaluated the efficacy of several different mixtures in the control of Japanese honeysuckle. Based on the physiology of Japanese honeysuckle, we expect the uptake and efficacy of herbicide application will not differ substantially regardless of application timing.

METHODS

We initiated a study to assess application-timing impacts on the efficacy of various herbicides on Japanese honeysuckle control. Each of six herbicide mixtures was applied once in three different seasons throughout the year to determine the effects of application timing on efficacy. The herbicide treatments in this study were 2.43 kg a.e./ha glyphosate, 1.21 kg a.e./ha glyphosate + 0.152 kg a.e./ha imazapyr, 0.152 kg a.e./ha glyphosate + 0.076 kg a.e./ha imazapic, 0.304 kg a.e./ha imazapyr, 0.202 kg a.e./ha triclopyr + 0.020 kg a.e./ha diflufenzopyr, and 0.243 kg a.i./ha metsulfuron methyl + 0.020 kg a.e./ha diflufenzopyr. These mixtures were chosen based on results of previous studies in the literature as well as recommendations from herbicide manufacturers. Herbicides were mixed with 0.383 L/ha methylated seed oil as an adjuvant.

Due to the relatively large contiguous areas of dense Japanese honeysuckle required for this number of herbicide treatments, three sites were used: Hall's Prairie, a roadside natural area in Logan County, Kentucky, owned by the University of Kentucky; and two highway right-of-ways managed by the Kentucky Department of Transportation, one in Shelby County and the other in Powell County. The Logan and Shelby sites are in the Interior Plateau physiographic region, while the Powell County site is in the Western Allegheny Plateau (Woods et al. 2002). All three sites were open fields bordering major highways and were dominated by Japanese honeysuckle.

After deciduous tree leaf fall in October 2004, each site underwent visual vegetative sampling to estimate the percentage of live Japanese honeysuckle cover and the percent cover of all species combined other than Japanese honeysuckle. Initial Japanese honeysuckle coverage varied from 70% to 96% in all plots. Pretreatment data were used to determine how effective each herbicide was in reducing Japanese honeysuckle coverage by completion of the study and to determine the percent cover by other species (including both natives and nonnatives) because of herbicide applications.

All three study sites were situated on a <5° slope with no overstory cover. Pretreatment vegetation sampling showed that the Hall's Prairie site averaged 81.7% Japanese honeysuckle cover (SE = 9.1, n = 12). While the overall site was dominated by Japanese honeysuckle, the native tall goldenrod, *Solidago altissima* L., did dominate two plots. Pretreatment vegetation sampling showed that the Shelby County site averaged 95.8% Japanese honeysuckle cover (SE = 1.8, n = 12). While the site was dominated by Japanese honeysuckle, some plots contained scattered tall goldenrod, native purple milkweed (*Asclepias purpurascens* L.), and nonnative invasive crown vetch (*Securigera varia* L.). Pretreatment vegetation sampling showed that the Powell County site averaged 70.0% Japanese honeysuckle cover (SE = 8.94, n = 12). This site had several plots with 20%–25% cover of crown vetch scattered throughout the plots, as well as several blackberry bushes (*Rubus* spp.).

After pretreatment sampling, we located 19 plots at each site. Plots had no minimum distance between them; they were laid out immediately adjacent to one another in a grid pattern with shared boundaries. At each site, separate plots received one of the six herbicide treatments applied during one of three seasons (fall, winter, and spring) and one plot served as a control for all treatment periods (19 plots total). For example, glyphosate was applied to one treatment plot in fall, another plot in winter, and a third plot in spring. Each study plot was 3 m by 9 m, dictated by the width of the 3-m boom on the pressurized CO_2 sprayer and the distance it takes for the sprayer to empty a 2-L bottle of mixture at 241.3 kPa at a normal walking speed of approximately 4.5 km/hr. Each application was replicated three times, once at each site, per herbicide, per season.

On either 20 or 25 October 2004, after the first frost but before the first "killing frost" (temperature below −4 °C), each mixture was applied in three replications (once per each of the three sites). The order of mixture application was chosen randomly in a randomized complete block design. The same procedure was repeated on either 6 January or 6 February 2005, to assess winter applications. Winter application days were chosen based on climatological factors; herbicides begin to lose effectiveness at temperatures below 15 °C, and wind speeds exceeding 8 km/hr (5 mph), and most are not rainfast for at least 2 hr (Gover 2000). The final applications were conducted on either 10 or 11 April 2005, to assess spring herbicide application before many native species are actively growing or blooming and, therefore, less likely to experience non-target herbicide damage. Herbicides were applied on days near or exceeding 15 °C, with ground wind speeds at or under 8 km/hr, partly cloudy to sunny skies, and at least three days since measurable rain.

Following the spring 2005 applications, we visually estimated percent cover of plant species at each site in summer and fall of both 2005 and 2006. We sampled both Hall's Prairie and the Shelby County site on 2 June 2005, 1 September 2005, 16 June 2006, and 11 September 2006, while we sampled the Powell County site on 3 June 2005, 1 September 2005, 15 June 2006, and 12 September 2006. A 0.3-m^2 sampling frame quadrat was randomly placed along the center of the 9-m axis of each treatment plot five times to visually estimate the changes in honeysuckle coverage and coverage of species other than honeysuckle (Bonham 1989). Because plots were immediately adjacent, subsequent sampling was restricted to the center of each plot to account for potential drift—no sampling was conducted within 0.3 m of a plot border. Sampling was conducted at ground level with no minimum or maximum distance between quadrats. Cover was analyzed in two classes: live Japanese honeysuckle and total of all other species. Sampling was conducted by the same person to reduce bias and subjectivity. Total summed cover could not exceed 100%. Sampling was limited to the center one-third of each plot to reduce potential of interaction with the herbicide treatments on immediately adjacent plots that shared a border.

The effectiveness of each mixture for controlling Japanese honeysuckle was compared to the effectiveness of the other mixtures, as well as across the other fall, winter, and spring applications of the same mixture. A two-way repeated measures analysis of variance (with herbicide mixture and application timing as the factors) using PROC MIXED in SAS software version 9.1 for Windows (Little et al. 1996) and post-hoc least squares means comparison tests were used to determine any differences in herbicide treatments and application timing. The repeated measure was post-treatment sampling periods with site as a random effect. Data were arcsine square root transformed to meet the assumptions of an ANOVA (Zar 2010).

RESULTS

Across all herbicide treatments, the various application timings had no effect on Japanese honeysuckle control ($F = 1.91$, df = 2, $p = 0.15$). However, differences occurred in control of Japanese honeysuckle among different herbicide treatments ($F = 502.71$, df = 6, $p < 0.0001$).

At the final sample period, after the second full growing season following herbicide treatment application, the fall applications of all mixtures had <10% live Japanese honeysuckle cover (Figure 1). The metsulfuron methyl + diflufenzopyr treatments exhibited the lowest post-treatment cover of honeysuckle, but post-hoc analysis indicated that observed differences were only significant compared to triclopyr + diflufenzopyr. Winter applications of glyphosate, imazapyr, glyphosate + imazapyr, and metsulfuron methyl + diflufenzopyr treatment plots were all similar with <5% live Japanese honeysuckle, while the triclopyr + diflufenzopyr and glyphosate + imazapic plots exhibited significantly greater post-treatment cover of honeysuckle. Finally, spring applications yielded similar results to the winter applications, with no differences between the glyphosate, imazapyr, and metsulfuron methyl + diflufenzopyr plots once again, all with <5% live Japanese honeysuckle. Honeysuckle coverage in the glyphosate + imazapyr plot was no different from either of these highest-performing treatments nor the poorer performing glyphosate + imazapic. The triclopyr + diflufenzopyr and glyphosate + imazapic plots exhibited significantly greater honeysuckle cover than the glyphosate, imazapyr, and metsulfuron methyl + diflufenzopyr plots. Glyphosate + imazapyr did not differ significantly from glyphosate

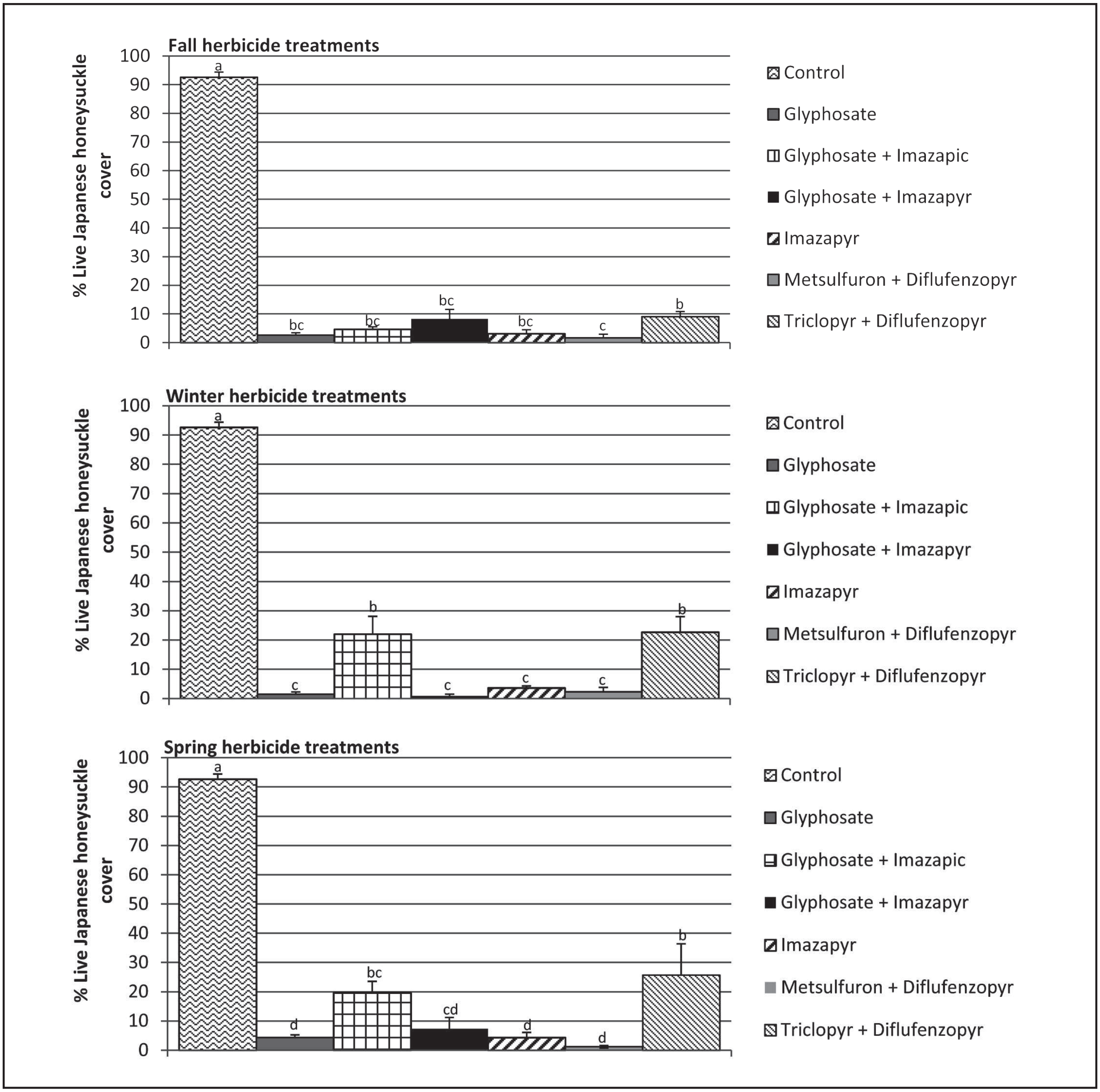

Figure 1. Percent cover of Japanese honeysuckle two growing seasons after herbicide applications. Means within the same treatment block with the same letter are not different (*P* = 0.05) and graphs are nontransformed data. Rates: 2.43 kg a.e./ha glyphosate, 0.152 kg a.e./ha glyphosate + 0.076 kg a.e./ha imazapic, 1.21 kg a.e./ha glyphosate + 0.152 kg a.e./ha imazapyr, 0.304 kg a.e./ha imazapyr, 0.243 kg a.i./ha metsulfuron methyl + 0.020 kg a.e./ha diflufenzopyr, 0.202 kg a.e./ha triclopyr + 0.020 kg a.e./ha diflufenzopyr.

+ imazapic, but did have less honeysuckle cover than the triclopyr + diflufenzopyr. It is worth noting both glyphosate and imazapyr performed better individually in fall and spring applications than when combined. It is unclear whether this outcome was due to interactions between the chemicals or simply because the label rate for both was lower in the combined mixture.

The triclopyr + diflufenzopyr treatments were consistently among the worst performing at each sampling period regardless of application timing, although the observed difference in the fall application was slight. Although the fall application of glyphosate + imazapic was similar to some of the better performing mixtures, the winter and spring applications were no different from the least effective triclopyr + diflufenzopyr. The metsulfuron methyl + diflufenzopyr treatments consistently exhibited no differences with the best performing treatments regardless of application timing along with glyphosate, imazapyr, and glyphosate + imazapyr.

Across all herbicide treatments, percent cover of plant species other than Japanese honeysuckle differed among herbicide treatments during each sampling period ($F = 6.39$, df = 2, $p = 0.002$). There was also a difference in percent cover of other species in each plot among the different herbicide treatments ($F = 79.51$, df = 6, $p < 0.0001$).

During the first growing season sampling period in summer 2005, the observed coverage by other species in the fall herbicide treatment plots varied from <6% with the glyphosate + imazapyr application to between 21%–22% with the glyphosate and the imazapyr treatments and >40% with the glyphosate + imazapic and triclopyr + diflufenzopyr applications. The winter and spring applications were more similar, with the highest percentage of coverage by other species in the triclopyr + diflufenzopyr plots at <22% with spring treatments and <16% winter treatments at the high end and <5% coverage with imazapyr, glyphosate + imazapic, or metsulfuron methyl + diflufenzopyr at the low end with both applications.

By the end of the second growing season in fall 2006 all treatment plots were >74% covered with species other than Japanese honeysuckle regardless of herbicide treatment or application timing, with glyphosate + imazapic, imazapyr, and triclopyr + diflufenzopyr treatments consistently showing the lowest cover by other species and glyphosate, metsulfuron methyl + diflufenzopyr, and glyphosate + imazapyr consistently the highest (Figure 2). In the fall treatments plots, glyphosate + imazapic plots exhibited the lowest coverage of other species, but did not differ in cover from the glyphosate + imazapyr, imazapyr, or triclopyr + diflufenzopyr treatments. Metsulfuron methyl + diflufenzopyr plots had the greatest coverage by other species and displayed significantly greater cover than glyphosate + imazapic and imazapyr. Cover on glyphosate plots was significantly greater than that on glyphosate + imazapic plots. Cover on glyphosate, glyphosate + imazapyr, imazapyr, and triclopyr + diflufenzopyr plots did not differ significantly. There was greater separation in the winter treatment plots, with glyphosate + imazapic, imazapyr, and triclopyr + diflufenzopyr treatment plots showing significantly lower cover of other species than metsulfuron methyl + diflufenzopyr, glyphosate, and glyphosate + imazapyr. In the spring treatment plots, metsulfuron methyl + diflufenzopyr exhibited greater cover of other species than glyphosate + imazapic, imazapyr, and triclopyr + diflufenzopyr. No other differences between treatments were observed.

DISCUSSION

This study demonstrated that applying one of several herbicide mixtures during the dormant season can control Japanese honeysuckle for two growing seasons while allowing revegetation by other species. The glyphosate, imazapyr, glyphosate + imazapyr, and metsulfuron methyl + diflufenzopyr consistently controlled Japanese honeysuckle in all of the application seasons, with <10% of each plot covered by live Japanese honeysuckle in the final sampling period regardless of application timing. While this study indicates that application of glyphosate, imazapyr, or metusulfuron + diflufenzopyr performed better than the other mixtures when applied in the dormant season, all of the herbicide treatments reduced Japanese honeysuckle cover two years after treatment relative to control levels. Post-hoc analysis indicated the triclopyr + diflufenzopyr treatments were consistently among the worst performing regardless of application timing, while the glyphosate + imazapic fall application was the only application timing of that mixture that held Japanese honeysuckle cover under 10% and was not one of the poorest performing treatments.

These results are somewhat consistent with previous studies on herbicide efficacy on Japanese honeysuckle control, but there are some differences. Regehr and Frey (1988) indicated that October applications of glyphosate at concentrations of 0.75% and 1.5% were equally effective in killing honeysuckle with a 99% reduction in coverage by April and with very little resprouting at the completion of their 30-month study. However, while their December applications of glyphosate were less effective (68% mortality at the 0.75% rate and 86% mortality at 1.5%), our winter glyphosate application was actually the most effective. Cain (1992) reduced Japanese honeysuckle coverage from 45% to 31% in one season with a 0.585 mL/ha application of imazapyr, much poorer results than in our study. Previous growing season studies have shown that metsulfuron methyl controls Japanese honeysuckle effectively, which is supported by our findings, but no dormant season studies were found in the literature. For example, Edwards and Gonzalez (1986) found that a May application of 0.28 kg/ha resulted in 99% mortality of Japanese honeysuckle while Yeiser (1999) stated a June application of 0.105 kg/ha appeared promising in reducing honeysuckle while releasing oak seedlings in a logged hardwood bottom. Our revegetation results support those of Yeiser (1999), who also found that metsulfuron methyl did not inhibit revegetation. Our findings also support previous research that has questioned the effectiveness of triclopyr on Japanese honeysuckle control (Dreyer 1988; Miller 2003). There are no published studies of the effectiveness of diflufenzopyr on Japanese honeysuckle control.

In addition to the relative efficacy on Japanese honeysuckle control, our results suggest that revegetation by species other than Japanese honeysuckle is possible following a dormant season herbicide treatment**.** Percent cover of species other than Japanese honeysuckle increased dramatically in all treatment plots; species other than Japanese honeysuckle were well established with all herbicide mixtures. Establishment of other species quickly after herbicide treatment is important, as bare ground tends to encourage the establishment of other invasive species (Adkins and Barnes 2013). Previous research also shows that eradication of undesirable vegetation is necessary for establishment

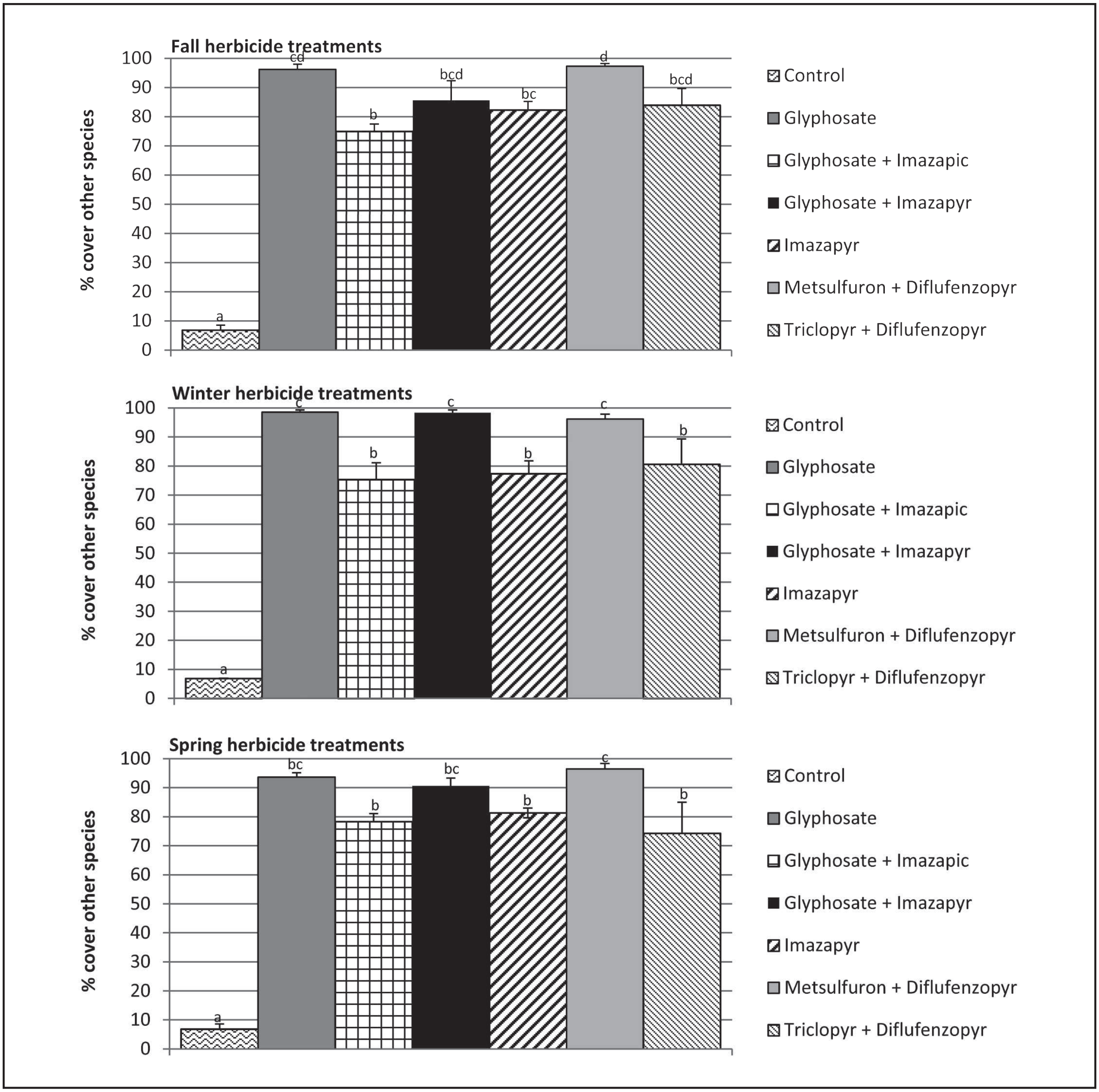

Figure 2. Percent cover of other species two growing seasons after herbicide applications. Means within the same treatment block with the same letter are not different (*P* = 0.05) and graphs are nontransformed data. Rates: 2.43 kg a.e./ha glyphosate, 0.152 kg a.e./ha glyphosate + 0.076 kg a.e./ha imazapic, 1.21 kg a.e./ha glyphosate + 0.152 kg a.e./ha imazapyr, 0.304 kg a.e./ha imazapyr, 0.243 kg a.i./ha metsulfuron methyl + 0.020 kg a.e./ha diflufenzopyr, 0.202 kg a.e./ha triclopyr + 0.020 kg a.e./ha diflufenzopyr.

of native plant communities (Wilson and Gerry 1995; Masters et al. 1996; Barnes 2004). Species present in this study other than Japanese honeysuckle varied by site and treatment and included both nonnative invasive species and native grassland and woody species, suggesting that restoration of native species is highly site specific and dependent on species availability in the seedbank and adjacent or nearby areas.

Retreatment of sites after periodic assessments or monitoring is recommended to maintain acceptable control levels. Given the growth characteristics of Japanese honeysuckle, it may be assumed that if left unchecked a 5% cover of Japanese honeysuckle in any plot will eventually return to pretreatment levels. It is important to note that the highest-performing herbicides in this study—glyphosate, imazapyr, and metsulfuron methyl—are all systemic enzyme inhibitors. If herbicide resistance becomes a concern, then occasional rotation between an herbicide with a different mode of action should be considered even if it offers less control, such as triclopyr. Overall, these findings suggest dormant season herbicide applications are an effective means for reducing Japanese honeysuckle cover in natural areas while promoting revegetation by other species. Managers of natural areas with significant grassland attributes should consider chemically treating Japanese honeysuckle infestations between October and April on days with appropriate weather conditions.

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The late Thomas G. Barnes, Ph.D., was extension professor and wildlife specialist at the University of Kentucky. His research focused on the restoration, rehabilitation, and recreation of native grasslands using herbicides. He was the author of seven books on the natural history of Kentucky, including Kentucky, Naturally: The Kentucky Heritage Land Conservation Fund at Work. He passed away during the completion of this project.

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