POTENTIAL DETRIMENTAL EFFECTS OF USING GLYPHOSATE FOR HARDWOOD SEEDLING RELEASE

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Abstract—Chemical release treatments are commonly used in regeneration efforts to establish new hardwood stands. These applications target the competing vegetation surrounding the desired seedlings. Research findings have suggested that release treatments improve seedling survival and growth during the initial years after disturbance or planting. Glyphosate is one herbicide that has been used by land managers to accomplish this objective. This study suggests that glyphosate may actually inhibit height growth of natural oak (*Quercus* spp.) and yellow-poplar (*Liriodendron tulipifera*) reproduction. Seedlings treated using glyphosate conducted as radial sprays, treating the immediate 5-foot radius around the crop trees, reduced height growth compared to control treatments. Natural reproduction was measured post spray in the fall of 2014 and two growing seasons later in January and February of 2017. This paper presents the reduced growth response through treatment comparisons of glyphosate with untreated control. Hypotheses are presented to potentially explain the causes of reduced seedling growth following radial release using glyphosate.

INTRODUCTION

Foresters have great difficulty in successfully maintaining adequate oak (*Quercus* spp.) reproduction over time after a disturbance occurs within hardwood stands. Multiple silvicultural interventions are often required to enable oak to develop into competitive size classes. Even when an abundance of oak germinants occurs after a good seed crop, sheer numbers of small seedlings are not enough to ensure oak regeneration success to form the future stand (Beck 1991, Janzen and Hodges 1987, Lockhart and others 2000, Loftis 1983, Sander 1979, Schweitzer and Dey 2011, Smith 1986, Stringer 2005). Silvicultural practices including shelterwoods and clearcuts have been advocated to encourage oak regeneration. However, in most instances competing vegetation emerges after these disturbances that hinders or suppresses the preferred oak regeneration efforts.

Chemical applications have been prescribed to improve the growth and survival of oaks in hardwood stands. For example, seedling diameter and height growth can also be positively affected by chemical release. Hilt and Dale (1987) concluded that increased intensity of pre-commercial thinning resulted in greater diameter growth in stands 13, 17, and 21 years of age. A study by Thompson and Nix (1993) observed that early crop tree release within a 4-year-old clearcut using various herbicides significantly decreased herbaceous and woody plant competition. This reduction in competition resulted in increased seedling ground line diameter growth but did not improve height growth over untreated controls. Nix (2004) remeasured the released natural oak in the clearcut 10 years after the initial chemical release treatments and reported that four herbicide treatments significantly increased diameter growth of released oak seedlings. Various studies (Ezell and Catchot 1998, Ezell and others 2007, Hopper and others 1993, Self and others 2013) have also indicated that chemical release of planted hardwood seedlings improves early survival rates. The use of sulfometuron to control herbaceous weeds improved overall oak seedling survival by 20 percent or greater compared to untreated controls at the end of the growing season of the initial year (Ezell 2000, Ezell and others 2007). A survival rate of 80 to 90 percent is common for oak seedlings that receive chemical release during normal precipitation years (Grebner and others 2004). Likewise, post-emergent applications utilizing glyphosate improves height growth in hardwood species (Hopper and others 1993) in addition to oak seedling survival.

However, there may be some cause for concern with the type of herbicide used for hardwood release treatments. Avoidance of pesticide drift onto crop seedling foliage is paramount but there may be unforeseen effects that occur belowground. The possibility exists that glyphosate or a by-product could be absorbed belowground by the growing stock. Various studies

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(Kremer and others 2005, Neumann and others 2006, Tesfamariam and others 2009, Zobiole and others 2010) have reported that detrimental interaction in the root zone could allow glyphosate to restrict the growth of adjacent plants and seedlings.

This study examined the height growth response of preferred natural oak and yellow-poplar reproduction in response to multiple herbicide applications. We considered all reproduction together as well as separately by new germinants and resprout reproduction. All species were combined, regardless of class (white oak, red oak, and yellow-poplar) for all tests.

METHODS

Study Site

The study site was located on a private landholding in the Western Highland Rim-highly dissected plateau physiographic ecoregion of west-central Tennessee (Smalley 1984). The soils on the study site were Bodine gravelly silt loams (5-40 percent slopes). Site index values for white oak (Section *Quercus*) were moderate (value of 75 feet, base age 50). Most undisturbed forestland in that region was dominated (80 percent or greater) by oak species. The study site was covered by a mixed-mesophytic forest, with white oak (*Quercus alba*), southern red oak (*Q. falcata*), chestnut oak (*Q. montana*), black oak (*Q. velutina*), hickory (*Carya* spp.), blackgum (*Nyssa sylvatica*), red maple (*Acer rubrum*), sugar maple (*A. saccharum*), black cherry (*Prunus serotina*), and yellow-poplar (*Liriodendron tulipifera*) forming the majority of the overstory species composition. Midstory and understory canopy layers also contained flowering dogwood (*Cornus florida*), sourwood (*Oxydendrum aboreum*), sassafras (*Sassafras albidum*), eastern hophornbeam (*Ostrya virginiana*), elm (*Ulmus* spp.), and American beech (*Fagus grandifolia*). As indicated by residual stumps, one or more diameter-limit harvest probably occurred within the area (the most recent harvest likely occurred between 1990 and 1995).

Study Design

The study incorporated a randomized complete block sampling design. Three individual blocks were replicated on sites with uniform site productivity, and these blocks were clearcut in the early spring of 2014. Six individual treatment units, approximately 0.75 acres in size, were installed within each block:

1. Chemical seedling release treatments utilizing equivalent of 2 ounces per acre of sulfometuron methyl (SFM75® by Alligare LLC) only,
2. Alternating banded strip treatment utilizing foliar sprays of glyphosate (5 percent solution),
3. Radial spray release utilizing foliar sprays of glyphosate (5 percent solution),
4. Alternating banded strips plus release using sulfometuron methyl,
5. Radial sprays plus release using sulfometuron methyl, and
6. Untreated control.

The three units that received sulfometuron methyl treatments were applied in May-June of 2014. Glyphosate applications were conducted between July and August of 2014. Banded sprays were approximately 4 feet in width, with alternately treated (foliar sprayed) and untreated strips across the unit. Radial sprays treated vegetation in the surrounding area of approximately a 5-foot radius from the sample seedling. A stove pipe apparatus covered the seedling being released to protect foliage from incidental contact with herbicide solution. The banded and radial spray methods were also applied to two of the blocks that were previously sprayed with sulfometuron methyl. The final treatment unit (untreated control) did not receive any herbicide applications.

Approximately 150 naturally regenerated seedlings (approximately half oak species and half yellow-poplar) in each treatment unit in the three replicated blocks were measured in the fall of 2014 for overall height. Height measurements were taken after treatments with a standard retractable ruler to the nearest 0.5 inch. A total of 2,653 seedlings were measured on the site. Seedlings were measured a second time after two full growing seasons had elapsed in January and February of 2017. Only 1,559 seedlings were able to be relocated for measurement due to the robust response of warm-season grass vegetation. Height growth was the difference between the 2017 measurements and the 2014 measurements.

Statistical Analyses

The experimental design was a randomized complete block with sampling using height measurements taken from white oak, red oak (Section *Lobatae*), and yellow-poplar seedlings. Seedlings were classified as either new germinant or advance reproduction. One analysis looked at all seedlings combined to determine any differences between treatments. A second analysis separately evaluated differences among treatments for the new germinants and the advanced sprout reproduction. Treatment was considered a fixed variable. Random variables included blocks and seedlings. Statistical analyses were performed using linear mixed models (proc glimmix; SAS version 9.4, SAS Institute Inc., Cary, NC) with a normal distribution. The least squares means were separated using Tukey's significant difference test. The significance level was set at alpha = 0.05.

RESULTS

Differences existed among the treatments when all seedlings were combined for the analysis ($F_{5,1553} = 14.02$, $p < 0.0001$). Tukey mean separation tests found a significant difference between the sulfometuron methyl only treatment and the radial treatment compared to all other treatments in regards to the change in height growth. These treatments also differed from one another. The radial release treatment had the lowest mean value of 17.3 inches (table 1).

The type III test indicated potential differences ($F_{5,504} = 2.3$, $p = 0.0481$) in height growth among treatments for new germinant reproduction. The sulfometuron methyl only treatments had the largest height growth for new germinant reproduction, 30.8 inches, approximately 6.5 inches greater than the control germinant reproduction. The radial treatment had the least growth increase with an estimate of only 22.6 inches. This value was also lower than the control estimate (table 1).

There were also differences in height change among treatments for sprout reproduction ($F_{5,1043} = 13.21$, $p < 0.0001$). The sulfometuron methyl treatment had the largest increase in height growth with an estimate of 28.1 inches. The sprouts in the radial treatments had the poorest height change response with an estimate of 15.6 inches (table 1).

DISCUSSION

Seedlings in the radial spray treatment using glyphosate experienced minimal increases in height. Radial application yielded the lowest mean height growth (table 1) of all treatments. The same trend was observed when reproduction was analyzed by size class as advanced sprout reproduction or new germinants (table 1), which suggests that the glyphosate herbicide when applied as a radial spray application had a detrimental effect.

While our treatments applied glyphosate within inches of the covered crop tree seedlings, extreme care was taken to avoid contact with the crop tree foliage during applications. Because seedling foliage was protected, it seems likely that the active ingredient or a by-product could have been absorbed belowground. Glyphosate eventually reaches the soil through either direct contact or by release from dead plant matter (Neumann and others 2006). Once in the soil, glyphosate may be absorbed onto soil particles, experience degradation by soil microorganisms, or leach through pores or root canals into deeper soil horizons. Tesfamariam and others (2009) also proposed that there may be some possibility of toxicity to non-target plants due to rhizosphere transfer of glyphosate. Neumann and others (2006) observed the exudation of glyphosate from treated plant roots into the adjacent soil. Kremer and others (2005) reported that exudation of glyphosate can restrict growth of adjacent plants and seedlings. Negative effects to non-target plants may include heightened sensitivity to plant diseases connected with low magnesium and iron availability in soil, increased nematode infections, inhibition of root growth, and reduced nitrogen fixation (King and others 2001). Glyphosate has been witnessed to alter nitrogen metabolism by directly affecting mycorrihizae or indirectly by causing an effect on plant physiology (Zobiole and others 2010). Glyphosate applications can reduce nodulation due to reductions of symbiotic bacteria in glyphosate resistant soybeans (Zobiole and others 2010, Zobiole and others 2012). This factor leads to a loss of energy and fixed nitrogen that could inhibit plant growth and production.

Table 1—Least squares mean estimates for change in height (inches) for combined oak/yellow-poplar seedlings, sprouts, and new germinant reproduction among treatments for the herbicide seedling release study on the Western Highland Rim of Tennessee

	Combined			Germinants			Sprouts		
Treatment	*n*	lsmean	SE	*n*	lsmean	SE	*n*	lsmean	SE
SFM75 only	271	28.9 A	0.98	88	30.8 A	1.74	183	28.1 A	1.18
Banded	265	24.4 B	0.99	116	25.6 AB	1.52	149	23.4 AB	1.30
Control	294	23.7 B	0.94	87	24.3 AB	1.75	207	23.5 AB	1.10
Radial + SFM75	220	23.0 B	1.09	56	27.3 AB	2.19	164	21.6 B	1.24
Banded + SFM75	262	21.9 B	1.00	104	26.7 AB	1.60	158	18.8 BC	1.26
Radial	247	17.3 C	1.03	59	22.6 B	2.13	188	15.6 C	1.16

n = number of seedlings, lsmean = least squares means, SE = standard error.

Banded = Alternating banded strip treatment utilizing foliar sprays of glyphosate (5 percent solution), Banded + SFM75 = Alternating banded strips plus release using sulfometuron methyl, Control = no treatment, SFM75 only = 2 ounces per acre of sulfometuron methyl only, Radial = Radial spray release utilizing foliar sprays of glyphosate (5 percent solution), Radial + SFM75 = Radial sprays plus release using sulfometuron methyl.

Means in a column followed by the same letter are similar (Tukey's adjusted $p > 0.05$).

Glyphosate is stored within plant metabolic sites including root and shoot meristems after translocation within the plant. Enhanced growth rate areas including nodules, root tips, and shoot apices are important sinks for glyphosate storage. A small transference of herbicide may occur at these belowground locations. A metabolite of glyphosate named amino-methylphosphonic acid (AMPA) forms from glyphosate after degradation by microorganisms. AMPA is a recognized phototoxin that has been suggested to cause glyphosate-induced injuries in glyphosate-resistant plants (Reddy and others 2004). The authors also observed that AMPA affected chlorophyll biosynthesis and caused plant growth reduction. AMPA has also been observed to reduce the amino acids glycine, serine, and glutamate in treated mouse ear cress (*Arabidopsis thaliana*) plants (Serra and others 2013). Thus, a possibility exists on the study site in treatment units receiving glyphosate applications that measured seedlings may have had AMPA transferred through the rhizosphere. The uptake of some limited amount of AMPA could have stunted growth without causing mortality to the affected seedlings.

Though most of these research findings are associated with genetically modified soybeans and other plants, similar effects could have occurred within the oak and yellow-poplar seedlings within the radial treatments. Our data supports the likelihood that glyphosate applied around crop seedlings may somewhat inhibit growth. This may especially be true of the radial treatments as greater amounts (greater volume used to adequately wet at least 70 percent of foliage) of herbicide were applied to live vegetation that completely surrounded the favored seedlings. The banded spray treatments also used glyphosate; however, not all of the sample seedlings were located on the edge of the untreated bands. Thus, the herbicide may not have transferred into the crop seedling due to the buffer distance between the root stock and any movement of the herbicide directly down the soil profile. The sampled seedlings that were on the edge of the treated strip would be potentially exposed to only half (one side) the area compared to the entire area around radial sprays. The radial treatments applied after using sulfometuron methyl, only treated the live woody stems that survived as the herbaceous forbs had already been controlled during the initial spray. The reduced amount of glyphosate applied during the second spray, in conjunction with reduced belowground root contact points also meant less uptake of the active ingredient by crop trees. Thus, the lack of or reduced amount of exposure may have had little to no effect on the sample seedlings.

CONCLUSIONS

Herbicides are often applied at various stages of early development to alleviate high mortality and enhance early regeneration growth. However, in our study the use of glyphosate appears to have had negative impacts on seedling growth and development. A notable detrimental response was observed in seedling height growth when glyphosate only was applied in a radial fashion around the immediate area of the crop seedlings. This treatment was significantly lower than all other treatments including the untreated check. It is suspected that some level of either the active ingredient or a secondary by-product was transferred into the plant within the rhizosphere. It is believed that the transference was enough to suppress height growth but not sufficient to induce mortality of released seedlings. The negative height growth impact was also noted in the advanced resprout reproduction but not in the new germinant reproduction. The larger root stock of the advanced reproduction, which likely had greater contact points with adjacent root stock of treated plants, is likely the notable factor that resulted in this difference. This possible observation being more evident in the resprout reproduction would support the theory of transference of active ingredient through root stock. Overall, our study further suggests that the greater application rate (more active ingredient used to cover 70 percent or more of live foliage) of glyphosate when applied as a radial spray around the crop seedlings reduced height growth to levels lower than all other treatments including the untreated control. Better results could likely be expected if directed sprays targeted only woody stems around the plant as opposed to all vegetation. This would minimize the amount that is available for potential uptake within the crop tree. Targeting woody plants would potentially provide more available growing space that may improve the crop tree's persistence into a greater canopy position as the stand develops over time.

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