ASSESSING TOLERANCE OF LONGLEAF PINE UNDERSTORY HERBACEOUS PLANTS TO HERBICIDE APPLICATIONS IN A CONTAINER NURSERY

D. Paul Jackson, Scott A. Enebak, James West, and Drew Hinnant[1]

Abstract--Renewed efforts in longleaf pine (*Pinus palustris* Mill.) ecosystem restoration has increased interest in the commercial production of understory herbaceous species. Successful establishment of understory herbaceous species is enhanced when using quality nursery-grown plants that have a better chance of survival after outplanting. Nursery growing practices have not been clearly identified for propagating longleaf pine understory herbaceous species, especially for controlling weeds that can reduce plant growth in containers. To identify potential herbicides that could safely be applied postemergence to herbaceous plants in containers, trials were installed at the North Carolina Forest Service Nursery in Goldsboro, NC. Different container sets of wiregrass (*Aristida stricta* Michx.) and muhly grass [*Muhlenbergia expansa* (Poir.) Trin.] seedlings were treated at two growth stages with pendimethalin, oxyfluorfen, lactofen, or oxadiazon. One-year-old wiregrass was also treated 6 months after being outplanted at the nursery with the same treatments except that clopyralid was tested instead of oxadiazon. Oxyfluorfen killed all seedlings tested within 2 weeks of treatment with the exception of 7-week-old container-grown muhly grass. Lactofen and oxadiazon applications at 2 and 7 weeks post-sowing killed both grass species and caused moderate injury, respectively. Lactofen caused foliar damage, while pendimethalin and clopyralid did not affect outplanted wiregrass. Pendimethalin applied 7 weeks post-sowing did not injure muhly grass seedlings but did cause more severe injury when applied 2 weeks post-sowing. Results of the trials indicate that pendimethalin may be safe to use over-the-top of 7-week-old container-grown muhly grass and 1-year-old outplanted wiregrass as a preemergence application to targeted weeds.

INTRODUCTION

In the last several years, attention has focused on an ecosystem approach to longleaf pine (*Pinus palustris* Mill.) restoration rather than only on establishment and management of longleaf pine as a timber resource. Indicative of this interest, a Range-Wide Conservation Plan for Longleaf Pine was published in 2009 by the Regional Working Group for America's Longleaf (Lopez and others 2009). The 15-year goal of the conservation plan is to increase longleaf pine ecosystem acreage from 3.4 to 8 million acres. To achieve this goal, the plan calls for initiatives to: (1) maintain existing longleaf ecosystems in good condition, (2) improve areas classified as "longleaf forest types" that are missing key components of the understory necessary for ecosystem sustainability, and (3) restore longleaf pine ecosystems that are currently other forest types.

Of the six strategies presented in the conservation plan (Lopez and others 2009), one focused on understory regeneration and highlighted a key action that specifically states the need to

> "…develop the seed and plant production technologies, standards, and guidance needed to produce understory plant materials and identify species important in the ground-layer of the longleaf pine communities throughout the range with the goal to help development efforts for commercial production."

Developing sound and practical methods for growing understory plant species is necessary in order to make seedlings available to private landowners, industry, and government agencies for outplanting in longleaf pine ecosystem restoration projects. The longleaf pine ecosystem is second only to tropical rain forest systems in biological diversity with hundreds of endemic plant species found across its range from eastern Texas to southern Virginia (Jose and others 2006). Many of the plants serve as fuel to help carry low-intensity surface fires (Brockway and Lewis 1997) that help perpetuate pine canopy growth, maintain species richness in the ground-layer, and provide habitat for many indigenous animal species (Jose and others 2006).

Some longleaf pine understory herbaceous plant species are currently being grown commercially in southeastern nurseries. For instance, the

[1]Assistant Professor, Louisiana Tech University, Department of Agricultural Sciences, Ruston, LA 71270; Professor, Auburn University, School of Forestry and Wildlife Sciences, Auburn University, AL 36849; and Nursery/Tree Improvement Program Head and Nursery Technician, respectively, North Carolina Forest Service, Claridge Nursery, Goldsboro, NC 27530.

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North Carolina Forest Service's Claridge Nursery, Goldsboro, NC, produces species such as wiregrass (*Aristida stricta* Michx.), muhly grass [*Muhlenbergia expansa* (Poir.) Trin.], little bluestem [*Schizachyrium scoparium* (Michx.) Nash], and Indian grass [*Sorghastrum nutans* (L.) Nash]. The cultural practices required for growing these plant species have not been clearly identified. Nursery managers are currently using a "figure it out as you go" approach for applying herbicides to understory herbaceous plants at various growth stages. Controlling weeds during production is important to prevent plants from being stunted or smothered due to increased competition for nutrients and water. At the Claridge Nursery, some of the more troublesome weeds include annual sedge (*Cyperus compressus* L.), false pimpernel [*Lindernia dubia* (L.) Pennell], Florida pusley (*Richardia scabra* L.), prostrate spurge [*Chamaesyce maculata* (L.) Small] and smooth pigweed (*Amaranthus hybridus* L.). The weeds can be a problem in the container production area as well as in nearby nursery beds that contain outplanted 1-year-old wiregrass seedlings from which seeds are harvested each year. The objective of the following trials was to determine the level of tolerance for container-grown wiregrass and muhly grass seedlings at various growth stages and outplanted 1-year-old wiregrass seedlings to a range of herbicides that target troublesome weeds encountered at the Claridge Nursery.

METHODS

Container-Grown Seedlings

The trials took place at the North Carolina Forest Service's Claridge Nursery. Wiregrass and muhly grass seeds were collected at Holly Shelter Gamelands Nature Reserve in Pender County, NC and sown on April 30, 2012 into Forestry Tray (FT-135) containers placed on T-rail benches in full sun. Each container consisted of 135 cavities (581/m^2) with a volume of 113 cm^3 and depth of 13 cm. Container cavities were filled with growing media that consisted of 85 percent peat moss, 10 percent perlite and 5 percent vermiculite.

Wiregrass and muhly grass seedlings were challenged with four postemergence herbicide treatments: lactofen (Cobra®) at 1.16 L/ha [0.28 kg active ingredient (a.i.)/ha]; oxyfluorfen (Goal® 2XL) at 2.33 L/ha (0.56 kg a.i./ha); pendimethalin (Pendulum® AquaCap™) at 2.48 L/ha (1.12 kg a.i./ha); and oxadiazon (Ronstar Flo®) at 2.92 L/ha (1.12 kg a.i./ha). A fifth treatment received no herbicide (non-treated control). All herbicide treatments were applied using a CO_2 hand-sprayer calibrated at 205 L/ha. Herbicide applications occurred at two different growth stages to both plant species: at 2 weeks post-sowing when seedlings were about 1 to 2 inches in height and at 7 weeks post-sowing when seedlings were about 4 to 6 inches in height. There were five replications (reps) of each treatment for wiregrass seedlings, with a total of 25 containers and 3,375 seedlings compromised at each application period. There were three reps of each treatment for muhly grass seedlings, with a total of 15 containers and 2,025 seedlings compromised at each application period. A rep was considered one container of seedlings or one experimental unit, and containers were arranged in a randomized complete block design.

Wiregrass and muhly grass tolerance to the herbicide treatments was evaluated by rating the amount of plant injury using a scale from 1 to 10 (1 = no injury; 10= dead plant or no green foliage). Ratings of 2 through 9 reflected a progressive increase in the amount of either chlorotic or brown foliage. Injury ratings were recorded at 8, 13, 16, and 25 days after treatment (DAT) for seedlings treated 2 weeks post-sowing and at 9, 23, 33, 44, and 51 DAT for seedlings treated 7 weeks post-sowing. Foliage fresh weights were also recorded at 51 DAT for seedlings treated 7 weeks post-sowing by clipping and weighing the foliage (dead or alive) from each container (experimental unit). Foliage fresh weights per seedling were calculated by dividing the total amount of foliage from a container of seedlings by the number of seedlings sampled from that container.

Outplanted Seedlings

Wiregrass seedlings grown during the 2011 growing season were outplanted the first week of December 2011 in nursery beds at the Claridge Nursery. The seedlings are maintained as a seed source for successive crops grown each growing season at the nursery. These 1-year-old seedlings were treated on May 16, 2012 with the same herbicide treatments as described for the container-grown wiregrass and muhly grass trials with one exception: instead of oxadiazon, the herbicide clopyralid (Stinger®) was tested at 0.29 L/ha (0.1 kg a.i./ha). There were five reps of each treatment with 10 seedlings in each rep (plot) set up in a randomized complete block design. Each plot represented an experimental unit. A total of 250 wiregrass seedlings were compromised in the trial. Herbicide tolerance was recorded using the same injury rating scale as previously described for container-grown seedlings at 8, 13, 16, 25, and 33 DAT.

Data Analysis

Data were analyzed using a generalized linear model (GLM) in SAS (9.3 ed., SAS Institute, Cary, NC). Means of each dependent variable for each experimental unit were analyzed using analysis of variance (ANOVA) and mean separation tests were performed using Duncan's Multiple Range Test. Duncan groupings and least significant differences (LSD) are only reported for wiregrass and muhly grass data collected the final evaluation day for the 7-week post-sowing trial. Injury rating data in the 2-week post-sowing trial lacked variation, and thus, those statistics are not being reported.

RESULTS AND DISCUSSION

Container-Grown Seedlings

Wiregrass--All seedlings treated with oxadiazon, lactofen, and oxyfluorfen were dead at or before13 DAT when the herbicides were applied 2 weeks post-sowing (table 1). Pendimethalin applications resulted in severe seedling damage when applied at 2 weeks post-sowing, and the damage was more gradual compared to the other herbicide treatments.

Wiregrass seedlings treated at 7 weeks post-sowing were killed by oxyfluorfen 9 DAT (table 1). Oxadiazon and lactofen applications caused seedling injury by 9 DAT, but the herbicidal effects began to slightly improve by 44 DAT. Similar to the 2 weeks post-sowing treatments, foliar injury caused by pendimathalin applications at 7 weeks post-sowing was delayed, but the injury was less severe (table 1). All four herbicide applications resulted in less foliage fresh weight per seedling compared to the non-treated control with oxyflourfen having significantly less foliage than all treatments at 7 weeks post-sowing (fig. 1).

Muhly grass--Herbicide injury ratings for muhly grass followed a similar trend to those reported for wiregrass seedlings when treated 2 weeks post-sowing (table 2). When treated 7 weeks post-sowing, pendimethalin applications did not injure muhly grass seedlings (table 2). Treatments of oxadiazon and lactofen at the older stage of growth resulted in half the level of damage as seedlings treated 2 weeks post-sowing, and seedlings recovered from the damage to a level "2" rating by 44 DAT. Similarly, oxyfluorfen caused severe damage to seedlings by 8 DAT, but the plants recovered to a rating of "4" by 51 DAT (table 2). Foliage fresh weights were similar among all treatments, with seedlings treated with oxadiazon and pendimethalin having numerically more foliar biomass than non-treated control seedlings (fig. 2).

Outplanted Wiregrass

Applications of oxyfluorfen killed the 1-year-old outplanted wiregrass seedlings by 13 DAT (table 3). Lactofen applications resulted in moderate seedling injury, while clopyralid injury was rated as a "2" from 8 to 25 DAT before being upgraded to a "1" by 33 DAT (table 3). Along with clopyralid, pendimethalin applications did not affect wirergrass.

DISCUSSION

Applying pendimethalin, oxadiazon, lactofen, and oxyfluorfen at the rates used in these trials 2 weeks post-sowing may cause severe damage and death to both wiregrass and muhly grass seedlings. Two-week old seedlings are more susceptible to herbicidal injury because they have not developed much beyond the germinant (embryonic) phase of growth (about 1 to 2 inches tall). Another study reported that treatments of atrazine at 6.35 L/ha 30 days after wiregrass seedling emergence caused severe

Table 1--Mean injury ratings (1 = no injury; 10 = dead) for container-grown wiregrass seedlings on certain days after treatment (DAT) with herbicides at 2 and 7 weeks post-sowing. Means with the same letter within the "51 DAT" column in the 7-week post-sowing trial are not significantly different based on Duncan's Multiple Range Test. Due to a lack of variation, statistics for the 2-week post-sowing trial are not being reported

	-------------2 weeks post-sowing--------------				---------------------7 weeks post-sowing---------------------				
Herbicide	8 DAT	13 DAT	16 DAT	25 DAT	9 DAT	23 DAT	33 DAT	44 DAT	51 DAT
Non-treated	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0 d
Pendimethalin	2.4	5.4	7.6	9.0	1.0	5.8	5.8	5.8	6.2 b
Oxadiazon	9.0	10.0	10.0	10.0	7.8	7.8	7.8	6.0	5.4 bc
Lactofen	9.4	10.0	10.0	10.0	7.2	7.4	7.4	6.0	5.2 c
Oxyfluorfen	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0 a
LSD[a]									0.86

[a]LSD = least significant difference.

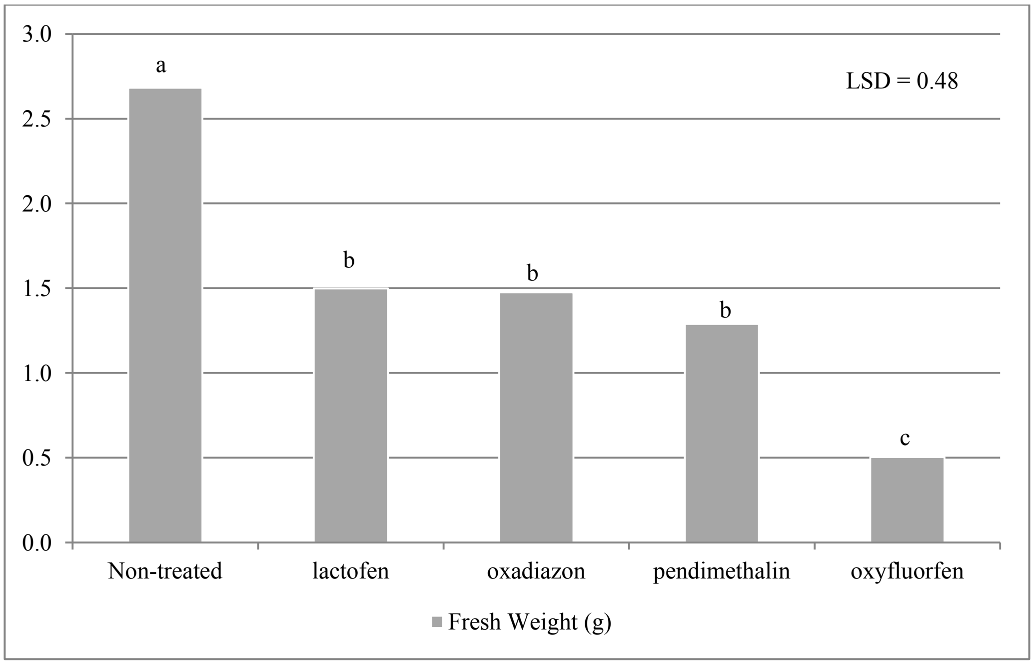

Figure 1--Mean seedling foliage fresh weights (live and dead) for container-grown wiregrass seedlings 51 days after treatment with herbicides at 7 weeks post-sowing. Means with the same letter are not significantly different based on Duncan's Multiple Range Test.

Table 2--Mean injury ratings (1 = no injury; 10 = dead) for container-grown muhly grass seedlings on certain days after treatment (DAT) with herbicides at 2 and 7 weeks post-sowing. Means with the same letter within the "51 DAT" column in the 7-week post-sowing trial are not significantly different based on Duncan's Multiple Range Test. Due to a lack of variation, statistics for the 2-week post-sowing trial are not being reported

	--------------2 weeks post-sowing------------				--------------------7 weeks post-sowing--------------------				
Herbicide	8 DAT	13 DAT	16 DAT	25 DAT	9 DAT	23 DAT	33 DAT	44 DAT	51 DAT
Non-treated	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0 b
Pendimethalin	3.0	7.0	9.0	9.0	1.0	1.0	1.0	1.0	1.0 b
Oxadiazon	9.0	10.0	10.0	10.0	5.0	2.3	1.7	2.0	2.0 b
Lactofen	9.7	10.0	10.0	10.0	5.7	5.0	4.3	2.0	2.0 b
Oxyfluorfen	10.0	10.0	10.0	10.0	8.0	8.0	7.7	6.0	4.3 a
LSD[a]									0.97

[a]LSD = least significant difference.

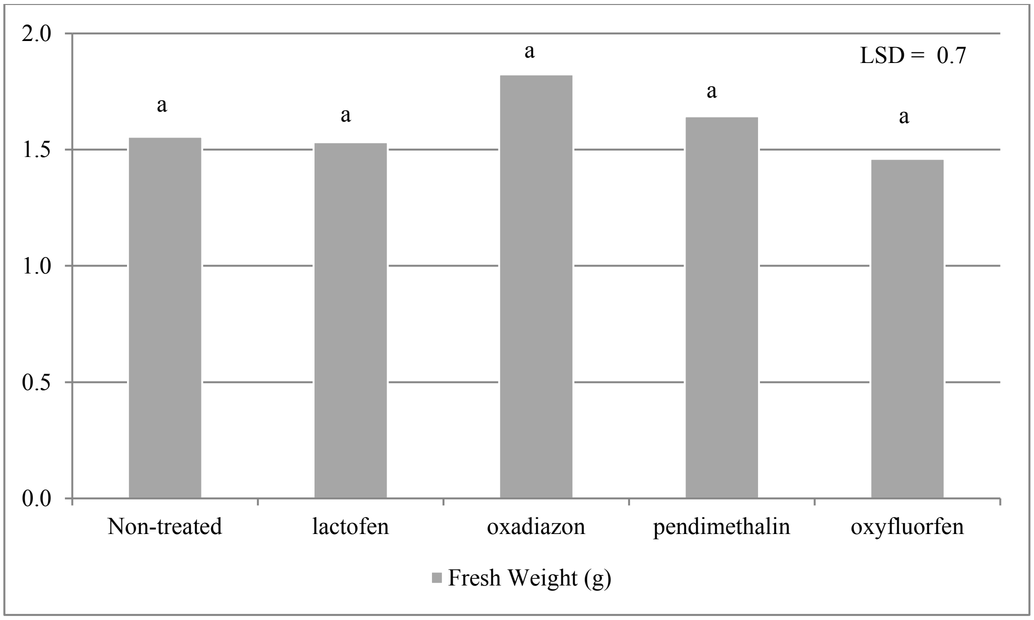

Figure 2--Mean seedling foliage fresh weights (live and dead) for container-grown muhly seedlings 51 days after treatment with herbicides at 7 weeks post-sowing. Means with the same letter are not significantly different based on Duncan's Multiple Range Test.

Table 3--Mean injury ratings (1 = no injury; 10 = dead) for 1-year-old outplanted wiregrass seedlings on certain days after treatment (DAT) with herbicides. Herbicides were applied 6 months after outplanting. Means with the same letter within the "33 DAT" column are not significantly different based on Duncan's Multiple Range Test

Herbicide	8 DAT	13 DAT	16 DAT	25 DAT	33 DAT
Non-treated	1.0	1.0	1.0	1.0	1.0 c
Pendimethalin	1.0	1.0	1.0	1.0	1.0 c
Clopyralid	1.6	1.6	1.6	1.6	1.0 c
Lactofen	3.6	5.8	5.8	5.8	3.6 b
Oxyfluorfen	9.2	10.0	10.0	10.0	10.0 a
LSD[a]					0.32

[a]LSD = least significant difference.

foliar damage and even seedling death, while applications 60 days after emergence caused either no injury or slight injury with the capacity to recover (Kaeser and Kirkman 2010). In that study, wiregrass seedlings also exhibited severe damage to death when treated with aminopyralid (Milestone®), imazapic (Plateau®), imazapyr (Arsenal®), hexazinone (Velpar®), butyric acid (2,4 DB), fluazifop-p-butyl (Fusilade®), and triclopyr (Garlon® 3A) at either 30 or 60 days after emergence (Kaeser and Kirkman 2010).

Oxyfluorfen applications quickly killed the container-grown wiregrass at 2 and 7 weeks post-sowing, muhly grass at 2 weeks post-sowing, and the 1-year-old outplanted wiregrass. Oxyfluorfen has preemergence and postemergence activity on weeds with postemergence applications being the most successful on juvenile plants not exceeding the 2-leaf stage and that are < 4 inches in height. Container seedling applications of oxyfluorfen occurred when plants were < 6 inches in height and more susceptible to herbicide injury. The killing of the 1-year-old outplanted wiregrass confirms that using 32 ounces per acre of the herbicide may be excessive. Lowering the rate of oxyfluorfen in future trials may result in an increased tolerance for wiregrass seedlings.

Muhly grass treated 7 weeks post-sowing were able to tolerate lactofen, oxadiazon, and oxyfluorfen but only after incurring foliar injury by 9 DAT from which they recovered later in the growing season. The ability of muhly grass to tolerate applications of the herbicides better than wiregrass at 7 weeks post-sowing may be attributed to differences in genetics between the two species. This fact highlights the need to develop specific herbicide regimes that can be implemented in nurseries for growing the different species required for longleaf pine ecosystem restoration.

Pendimethalin applications 7 weeks after sowing moderately injured wiregrass and did not affect muhly grass or outplanted wiregrass. Pendimethalin is a preemergence herbicide that interferes with cell division and elongation of emerging shoots and roots during the germination phase and has been known to cause non-target plant injury when applied postemergence to cotton (*Gossypium hirsutum* L.) (Miller and Carter 1980) and loblolly pine (*Pinus taeda* L.) (South and Hill 2009). The increased tolerance of muhly grass to pendimethalin at 7 weeks post-sowing compared to at 2 weeks post-sowing may be explained by the plants having time to develop a more extensive root system and surpass the stage of susceptibility to the pendimethalin. Due to their size, outplanted wiregrass seedlings were not affected by pendimethalin, and at this point it is unclear how early pendimethalin can be safely used over-the-top of container-grown wiregrass during propagation.

Outplanted wiregrass was also injured by lactofen applications but recovered slightly. Thus, these herbicides are not suited for use on either grass species at any growth stage with the rates used in these trials. Oxadiazon and lactofen have preemergence activity during the germination phase and on tender juvenile tissues. This may explain (like with pendimethalin) why seedlings treated 2 weeks post-sowing were killed, and seedlings treated 7 weeks post-sowing were initially affected but then recovered as they grew older and more developed.

Clopyralid performed well over-the-top of outplanted wiregrass. This herbicide is used to control broadleaf weeds in areas where grasses are being grown for seed harvesting, which is the purpose of the outplanted wiregrass at the Claridge Nursery. Clopyralid can be used over-the-top of conifer seedlings in some southern states to control sicklepod [*Senna obtusifolia* (L.) Irwin and Barneby] in bareroot nursery beds (South 2000). Extending its use over-the-top of the outplanted wiregrass would be beneficial in reducing broadleaf weed competition and hopefully increasing annual seed harvests for sowing future wiregrass crops at the nursery.

CONCLUSIONS

These results indicate that, of the applications of oxyfluorfen, lactofen, oxadiazon, and pendimethalin at 2 and 7 weeks post-sowing over-the-top of container-grown wiregrass and muhly grass seedlings, pendimethalin applied at 2.48 L/ha was the only herbicide that did not injure muhly grass seedlings at 7 weeks post-sowing. For outplanted 1-year-old wiregrass seedlings, pendimethalin at 2.48 L/ha and clopyralid at 0.29 L/ha performed well and showed promise as an option to combat weeds that may compete with and reduce seed yields from the wiregrass beds. These results give nursery managers a start on developing an herbicide program against troublesome weeds in the herbaceous understory plant nursery.

Future trials could test some of these same herbicides over-the-top of wiregrass, muhly grass, and other herbaceous understory species such as little bluestem or Indian grass but at lower rates and different growth stages. Other herbicides should be identified and tested that target problematic weeds. For instance, halosulfuron (Sedgehammer®) has activity on sedges and oxyfluorfen (Goal Tender®) is one that may offer more flexibility when applied over younger herbaceous seedlings.

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