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The effects of pre- and post-emergent herbicides on non-target native plant species of the longleaf pine ecosystem

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KAESER, M. J. AND L. K. KIRKMAN (Joseph W. Jones Ecological Research Center, 3988 Jones Center Drive, Newton, GA, 39870). The effects of pre- and post-emergent herbicides on non-target native plant species of the longleaf pine ecosystem. J. Torrey Bot. Soc. 137: 420–430. 2010.—Native grasses and forbs are an important component of the longleaf pine (*Pinus palustris* Mill.) ecosystem; however, information about re-establishment of these species in a restoration context has become available only recently. Understanding the effects of herbicides on non-target native plants can advance the use of herbicides as an effective restoration tool in the control of competing vegetation and in maintenance of seed production fields. The objectives of this study were to determine the effects of several commonly used herbicides on non-target species of grasses, legumes, and composites native to the longleaf pine ecosystem of southwestern Georgia. We assessed the pre- and post-emergent properties of nine herbicides on ten species of grasses, legumes, and composites. For each species, we examined phytotoxic responses to two rates (low rate and high rate) of herbicide at three stages of plant growth: 0 (pre-emergent), 30, and 60 d post-emergent. Plants were visually rated for leaf damage 30 d after herbicide application to assess the phytotoxic effects of the herbicides. Plants were then harvested, dried, and weighed. Regardless of herbicide application rate or age of plant, legumes were extremely vulnerable to applications of aminopyralid, triclopyr, and hexazinone. Most pre-emergent grasses were vulnerable to triclopyr when applied at the high rate. Most 30-day-old grasses were killed when treated with the high rate of hexazinone. Our results indicate that several native species are more sensitive to herbicide application than expected based on the below maximum label rates used and the specificity implied on the herbicide labeling.

Key words: ground cover, herbicides, longleaf pine ecosystem, non-target plants, *Pinus palustris*, restoration.

The longleaf pine (*Pinus palustris* Mill.) ecosystem was once dominant in the southeastern United States prior to European settlement but has been drastically reduced to approximately 3% of its original extent (Hainds et al. 1999, Cox et al. 2004, Haywood 2009). High floristic diversity as it relates to the ground cover in this ecosystem has been recognized as some of the highest outside of the tropics, thus it is a regional conservation priority to protect what remains and begin to restore what has been lost (Peet and Allard 1993, Litt et al. 2001, Kirkman et al. 2004, Freeman and Jose 2009). Past longleaf pine restoration efforts have focused primarily on planting longleaf pine seedlings as part of the USDA's Conservation Reserve Program (CRP): Longleaf Pine Initiative (Holliday 2001). Increased landowner participation in CRP has raised awareness concerning the importance of using native ground cover species in combination with planting longleaf pine seedlings. Additionally, the newest component of CRP (CP36) is the requirement to establish native ground cover among planted longleaf pine trees, particularly native warm-season grasses.

The Poaceae (grasses), Fabaceae (legumes), and Asteraceae (composites) families are dominant in the ground cover of the longleaf pine ecosystem and are structurally and functionally important components of this ecosystem. Native grasses contribute to the diverse ground cover and the dominance of grasses plays a vital role in the reintroduction of prescribed fire necessary to sustain this ecosystem by serving as a fine fuel source to carry fire (Coffey and Kirkman 2006). The numerous species of legumes present in this ecosystem provide food and cover for many species of wildlife. In addition, they contribute a substantial percentage of nitrogen to the ecosystem through symbiotic nitrogen fixation

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(Hainds et al. 1999, Hendricks and Boring 1999, Cathey et al. 2010). As one of the most abundant and diverse families in the longleaf pine ecosystem, composites are a key contributor to the understory diversity (Drew et al. 1998, Coffey and Kirkman 2006).

When establishment or enhancement of native ground cover is a restoration objective, the degree of site preparation and use of herbicides depends on the initial conditions and land use history. The presence of undesirable weedy species, such as non-native species, hardwoods or common old field weeds, can interfere with the establishment and growth of native plant species. In particular, non-native invasive species such as bermudagrass (*Cynodon dactylon* (L.) Pers.), bahiagrass (*Paspalum notatum* Flüggé), and cogongrass (*Imperata cylindrica* (L.) P. Beauv.) can pose serious problems with establishment and growth of native ground cover, and it is therefore essential to control these grasses on sites prior to restoration planting efforts. Although herbicides have been widely used in southeastern pine plantations and forests as stand management tools, little is known about potential deleterious effects of herbicides on non-target, native ground cover species (Boyd et al. 1995, Litt et al. 2001, Freeman and Jose 2009). Information on the use of herbicides such as imazapyr, hexazinone, and sulfometuron methyl in longleaf pine restoration efforts has focused primarily on understory community responses rather than phytotoxic responses to individual species (Brockway et al. 1998, Miller et al. 1999, Brockway and Outcalt 2000, Litt et al. 2001). While a few studies document the effects of herbicide treatments on specific native ground cover species, such as wiregrass (*Aristida stricta* Michx.), the focus is typically on the response of mature, established ground cover plants to herbicides rather than response of seedlings or germinating seeds (Wilkins et al. 1993, Freeman and Jose 2009, Jose et al. 2010). As more landowners and practitioners initiate restoration efforts that include restoration of native ground cover species through programs such as CRP, a better understanding of herbicide impacts on common, native ground cover species of the longleaf pine ecosystem is necessary to develop appropriate tools and strategies for weed control during the establishment period of native species. In addition to control of undesirable species in restoration plantings, the use of herbicides as a management tool is particularly important in establishment and maintenance of native seed production fields to control aggressive species retained in the weedy seedbank.

This study tests the sensitivity of common grasses, legumes, and composites native to the longleaf pine ecosystem to several conventionally used herbicides including aminopyralid, atrazine, butyric acid, fluazifop-p-butyl, hexazinone, imazapic, imazapyr, sulfometuron methyl, and triclopyr. Specific objectives of this study are 1) to assess the phytotoxicity responses of species of native grasses, legumes, and composites to pre- and post-emergent exposure to herbicides, and 2) to determine if the response varies with rate of herbicide application and age of plant.

Methods. Study Area. We conducted phytotoxicity experiments in a greenhouse at Ichauway, a 115 km^2 privately-owned property of the Joseph W. Jones Ecological Research Center, in Baker County, Georgia, USA (31° 13′ N, 84° 29′ W). This study was conducted in three separate trials, between February 2008 and June 2009.

Study Species and Herbicide Treatments. Plant species selected for this study represent three major families (Poaceae, Fabaceae, and Asteraceae) that are common to the frequently burned longleaf pine-wiregrass ecosystem and have important functional roles in this ecosystem. We examined ten ground cover species that included five grasses (*Andropogon virginicus* L., *Aristida stricta* Michx., *Saccharum alopecuroides* (L.) Nutt., *Sorghastrum secundum* (Elliot) Nash, and *Sporobolus junceus* (P. Beauv.) Kunth), three species of legumes (*Crotalaria rotundifolia* Walter ex J.F. Gmel., *Desmodium floridanum* Chapm., and *Lespedeza angustifolia* (Pursh) Elliot), and two composites (*Helianthus angustifolia* L. and *Rudbeckia hirta* L.). Species nomenclature is consistent with Wunderlin and Hansen (2003).

Nine herbicides were selected for testing including aminopyralid, atrazine, butyric acid, fluazifop-p-butyl, hexazinone, imazapic, imazapyr, sulfometuron methyl, and triclopyr (Table 1). We applied each herbicide at a low and high rate (below maximum label rates), with the application rates determined in consultation with a regional herbicide special-

Table 1. Herbicide chemical* and trade names, application rates (Rate 1 = low rate; Rate 2 = high rate), label specifications, and herbicide selectivity for each experiment.

Common Name	Product Name	Rate 1 kg ha^{-1}	Rate 2 kg ha^{-1}	Label Specifications	Herbicide Selectivity
Experiment I					
Aminopyralid	Milestone	0.27	0.47	pre- and post-emergent	broadleaves
Atrazine	Atrazine	1.60	3.30	pre- and post-emergent	broadleaves/grasses
Imazapic	Plateau	0.27	0.54	pre- and post-emergent	broadleaves/grasses
Experiment II					
Hexazinone	Velpar	1.60	3.30	post-emergent	broadleaves/grasses/woody
Imazapyr	Arsenal	0.27	0.54	pre- and post-emergent	broadleaves/grasses/woody
Sulfometuron methyl	Oust	0.14	0.27	pre- and post-emergent	broadleaves/grasses
Experiment III					
Butyric acid	2,4-DB	0.54	1.09	Pre-emergent	broadleaves
Fluazifop-p-butyl	Fusilade	0.81	1.63	post-emergent	grasses
Triclopyr	Garlon 3A	1.09	2.17	post-emergent	broadleaves

* The chemical names of the herbicides are as follows: aminopyralid (4-amino-3,6-dichloropyridine-2-carboxylic acid), atrazine (6-chloro-N2-ethyl-N4-isopropyl-1, 3, 5-triazine-2, 4-diamine), imazapic ((±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid), hexazinone (3-cyclohexyl-6-(dimethylamino)-1-methyl-1, 3, 5-triazine-2, 4 (1H, 3H)-dione), imazapyr ([2-(4-isopropyl-4-methyl-5-oxo-2-imidazolin-2yl)-nicotinic acid]), sulfometuron methyl (methyl2-[(4,6-dimethyl-pyrimidin-2-yl)carbamoylsulfamoyl]benzoate), butyric acid (4-2,4-dichlorophenoxy)butyric acid), fluazifop-p-butyl (butyl(R)-2-[4-(5-trifluoromethyl-2-pyridinyloxy)phenoxy]propionate), triclopyr (3, 5, 6-trichloro-2-pyridyloxyacetic acid)

ist (Mark Atwater, pers. comm., Weed Control Unlimited, Inc., Donalsonville, GA, January 2008). Rates are expressed as kilograms (kg) of active ingredient per hectare (ha). Control plants for each treatment were treated with deionized water only.

EXPERIMENTAL DESIGN. Seeds of each species were sown into forty cell (22 × 34.5 × 8.5 cm) or forty-five cell (22 × 34.5 × 10 cm) seedling flats that were filled with a mixture of potting soil, peat, sand, and perlite (ratio 8:2:2:1). Approximately 3–5 seeds were sown into each cell and covered with a thin layer of soil. We watered plants daily as needed and thinned to one plant per cell 2–3 weeks after planting. Plants were fertilized 6 weeks after flats were seeded with a N-P-K (20-20-20) fertilizer (Chem-Sol™, CNI Agriminerals LLC.) applied at a rate of 36 kg ha^{-1}.

To assess pre- and post-emergent properties of herbicides, we applied herbicides at three stages of plant growth: 0 (pre-emergent), 30 and 60 d post-emergent. Herbicides were applied at the same time to seedling flats of different ages (0-, 30-, and 60-day-old) with a calibrated sprayer designed to spray herbicide at precise rates on a small scale. The sprayer consisted of a pressurized CO_2 tank coupled to a tank containing the herbicide and was calibrated to deliver herbicide at a rate of 290 L ha^{-1}. The herbicide and CO_2 tanks were mounted on a push cart and spray rates were calibrated by timing the pace of walking and simultaneously pushing the cart a measured distance. For treatment application, we placed flats of plants on the ground and herbicide was delivered at the calibrated rate to the plants as we pushed the sprayer past the flats. Spray swath was 1.2 m with three nozzles attached to a boom delivering herbicide to the plants. We used a different tank for each herbicide, thoroughly flushing the tanks (containing herbicide) and spray lines with water between applications of each herbicide and application rate.

This study was conducted as three separate experiments due to space constraints in the greenhouse (Table 1). We evaluated the response of all ten species to four herbicide treatments (three herbicides and control) in each experiment. A total of 720 flats were used for each experiment; 4 herbicides (including control) × 10 species × 3 stages of plant growth × 2 herbicide application rates with each treatment combination replicated 3 times (3 flats). Herbicides used in Experiment I included aminopyralid, atrazine, and imazapic. Experiment II herbicides included hexazinone, imazapyr, and sulfometuron methyl and

Table 2. Phytotoxicity rating scale used to score the effects of foliar damage due to herbicide exposure. Adapted version of the European Weed Research Council rating scale (Dear et al. 2006).

Score	Description of plant tolerance to herbicide
1	No effect of herbicide on plants
2	Very slight effects; stunting and chlorosis of foliage just visible
3	Slight effects; stunting and chlorosis more obvious but effects reversible
4	Substantial chlorosis and stunting; effects likely reversible
5	Strong chlorosis, stunting, and thinning
6	Increasing severity of damage; recovery doubtful
7	Heavy injury
8	Plants nearly dead
9	Plants dead

plants were treated with butyric acid, fluazifop-p-butyl, and triclopyr in Experiment III.

Seedlings in each flat were visually rated for foliage damage 30 days following herbicide application using a modified version of the European Weed Research Committee rating scale (Dear et al. 2006). The phytotoxicity scale ranged from 1 (no effect) to 9 (complete destruction/plants dead) (Table 2). Plants in each cell were scored and the mean phytotoxicity rating score was then calculated for each flat for statistical analysis. The aboveground portions of 15 systematically selected seedlings were harvested from each flat after the 30 day phytotoxicity rating. Plant material was then dried at 70°C for a minimum of 48 hours and weighed to obtain dry weights.

Statistical Analyses. Analysis of variance (ANOVA) was used to examine differences in biomass and phytotoxicity due to herbicide treatment for each species separately (Proc ANOVA, SAS 2004). The dependent factor was mean biomass or phytotoxicity score by flat for each species and plant age and the independent factor was herbicide treatment (herbicide and application rate). Mean biomass and phytotoxicity scores for each species due to herbicide treatment were compared to the untreated control of that species using Duncan's multiple range tests.

Results. Experiment I. In general, seedlings of most species were sensitive to both low and high application rates of aminopyralid and atrazine, the notable exception being *Sporobolus junceus* (Table 3). Regardless of age, all legume species were vulnerable to applications of aminopyralid (phytotoxicity scores ranging from 7 to 9) with plants exhibiting irreversible foliage damage such as severe injury and plant death. Compared to control plants, biomass of all legumes treated with applications of aminopyralid was lower, except for 30-day-old *Crotalaria rotundifolia* at the low rate (Table 4). Biomass of all pre-emergent grasses (except *Sporobolus junceus*) was reduced relative to untreated control plants when treated with applications of aminopyralid. Phytotoxic symptoms included strong chlorosis and stunting, severe injury, and plant death at phytotoxicity scores of 5 to 9 (Table 3). Additionally, mean aboveground biomass of all pre-emergent grasses (except *Saccharum alopecuroides*) treated with atrazine was less than the untreated controls (Table 4). The 0- and 30-day-old *Rudbeckia hirta* seedlings were killed when treated with aminopyralid and atrazine, while only the high application rates of both herbicides killed 60-day-old seedlings (Table 4).

Experiment II. Mean aboveground biomass of pre-emergent and 30-day-old legumes was less than untreated control plants in response to treatments with hexazinone, imazapyr, or sulfometuron methyl (Table 5). Treated seedlings exhibited phytotoxic symptoms ranging from substantial chlorosis and stunting to plant death with phytotoxicity scores of 4 to 9 (Table 3). All pre-emergent legumes treated with the high rate of hexazinone had lower biomass than plants treated with imazapyr or sulfometuron methyl or the control plants. In addition, biomass of all post-emergent (30- and 60-day-old) legume seedlings treated with both application rates of hexazinone was less than untreated controls (Table 5). Phytotoxic symptoms ranged from severe damage to plant death at phytotoxicity scores of 6 to 9 (Table 3). All 30-day-old grass seedlings (except *Sorghastrum secundum*) were killed when treated with the high application rate of hexazinone, and most 60-day-old grass seed-

Table 3. Summary of phytotoxic effects of herbicides on seedlings by species, plant age (days), experiment, herbicide, and application rate (R1 = low rate; R2 = high rate). The symbols represent phytotoxicity score ranges (H = score range 7–9; M = score range 4–6; L = score range 1–3) with "H" (bold) representing the most severe damage to seedlings.

		Experiment I						Experiment II						Experiment III					
		Atrazine		Aminopyralid		Imazapic		Imazapyr		Sulfometuron methyl		Hexazinone		Butyric acid		Fluazifop -p-butyl		Triclopyr	
Species	Age	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
Grasses																			
Andropogon	0	**H**	**H**	M	**H**	L	**H**	**H**	**H**	L	M	**H**	**H**	L	L	L	M	M	M
virginicus	30	**H**	**H**	L	M	L	L	**H**	**H**	L	L	**H**	**H**	L	L	L	M	**H**	**H**
	60	M	M	L	L	L	L	M	M	L	L	M	**H**	L	L	L	M	L	L
Aristida	0	L	L	**H**	**H**	M	M	M	M	**H**	M	M	**H**	L	L	L	L	M	M
stricta	30	M	M	M	M	M	M	**H**	**H**	**H**	**H**	**H**	**H**	M	M	M	**H**	M	**H**
	60	L	M	M	M	M	M	M	M	L	M	**H**	**H**	M	M	M	**H**	M	M
Saccharum	0	M	M	M	M	**H**	**H**	M	**H**	M	M	L	M	L	L	L	L	L	L
alopecuroides	30	M	M	M	M	M	**H**	**H**	**H**	**H**	M	**H**	**H**	L	L	M	**H**	M	M
	60	M	M	L	L	M	M	M	M	M	M	**H**	**H**	L	L	M	**H**	L	L
Sorghastrum	0	L	L	M	M	M	M	M	M	M	M	M	M	L	L	L	L	M	M
secundum	30	M	M	M	M	M	M	**H**	**H**	**H**	**H**	M	**H**	L	L	M	**H**	L	M
	60	M	M	M	M	M	M	M	M	M	M	L	**H**	L	L	L	M	L	L
Sporobolus	0	**H**	**H**	L	L	M	**H**	M	M	M	M	L	M	L	L	L	L	L	L
junceus	30	M	**H**	L	L	M	M	**H**	**H**	M	M	**H**	**H**	L	L	M	**H**	L	L
	60	**H**	**H**	L	L	L	M	M	L	M	M	M	**H**	L	L	L	M	L	L
Legumes																			
Desmodium	0	M	M	**H**	**H**	L	M	M	M	M	M	M	**H**	L	L	L	L	M	M
floridanum	30	**H**	**H**	**H**	**H**	L	M	**H**	**H**	M	**H**	**H**	**H**	M	L	L	L	**H**	**H**
	60	M	M	**H**	**H**	M	M	L	M	M	L	M	M	L	L	L	L	**H**	**H**
Crotalaria	0	L	L	**H**	**H**	L	L	M	M	M	M	M	M	L	L	L	L	M	M
rotundifolia	30	**H**	**H**	**H**	**H**	M	M	**H**	**H**	M	**H**	**H**	**H**	L	L	L	L	M	**H**
	60	**H**	**H**	**H**	**H**	M	**H**	M	M	M	M	M	**H**	M	L	L	L	M	M
Lespedeza	0	**H**	**H**	**H**	**H**	M	M	M	M	M	M	M	M	L	L	L	L	M	M
angustifolia	30	**H**	**H**	**H**	**H**	M	M	M	M	M	M	**H**	**H**	M	M	L	L	**H**	**H**
	60	L	L	**H**	**H**	M	M	L	L	M	L	M	**H**	L	L	L	L	**H**	**H**
Composites																			
Helianthus	0	**H**	**H**	**H**	**H**	M	M	M	M	M	M	M	M	L	L	L	L	M	M
angustifolia	30	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	**H**	L	L	L	L	M	**H**
	60	L	**H**	M	M	M	M	**H**	**H**	**H**	**H**	**H**	**H**	L	L	L	L	M	M
Rudbeckia	0	**H**	**H**	**H**	**H**	M	M	M	M	M	M	**H**	**H**	L	L	L	L	M	M
hirta	30	**H**	**H**	**H**	**H**	M	M	**H**	M	**H**	**H**	**H**	**H**	L	L	L	L	M	**H**
	60	**H**	**H**	**H**	**H**	M	M	M	M	M	M	**H**	**H**	L	L	L	L	M	M

Table 4. Mean (± SE) aboveground biomass (mg per seedling) of seedlings following treatment with herbicides in Experiment I by species, plant age, herbicide, and application rate (Rate 1 = low rate and Rate 2 = high rate). Asterisks (*) denote significant differences of treatments relative to the untreated control at $\alpha \leq 0.05$ (ANOVA).

			Atrazine		Aminopyralid		Imazapic	
Species	Age	Control	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
Grasses								
Andropogon virginicus	0	5.0 ± 0.2	0.3 ± 0.2*	0.8 ± 0.3*	0.7 ± 0.0*	0.6 ± 0.1*	2.8 ± 0.3*	2.2 ± 0.3*
	30	166.7 ± 8.1	43.2 ± 3.3*	38.8 ± 0.5*	143.7 ± 8.2	102.5 ± 4.3*	182.7 ± 17.1	154.9 ± 10.6
	60	227.1 ± 20.2	96.0 ± 14.9*	92.1 ± 17.8*	156.7 ± 24.3	188.8 ± 29.5	292.4 ± 40.4	276.6 ± 20.7
Aristida stricta	0	2.2 ± 0.1	1.5 ± 0.1*	1.3 ± 0.1*	1.1 ± 0.0*	0.9 ± 0.3*	1.5 ± 0.5*	0.7 ± 0.0*
	30	40.9 ± 7.3	18.3 ± 1.8	30.3 ± 8.4	29.4 ± 9.2	30.7 ± 2.2	21.1 ± 2.8	23.5 ± 3.0
	60	81.4 ± 9.5	50.0 ± 10.2	72.1 ± 11.8	58.4 ± 2.9	109.6 ± 7.6	53.4 ± 17.5	61.4 ± 20.5
Saccharum alopecuroides	0	6.3 ± 0.5	4.6 ± 0.2	4.0 ± 1.6	2.9 ± 0.4*	2.8 ± 0.6*	2.6 ± 0.9*	1.6 ± 0.2*
	30	103.6 ± 22.1	206.0 ± 13.2*	210.0 ± 38.5*	142.2 ± 7.9	143.5 ± 7.7	81.7 ± 5.7	78.5 ± 14.1
	60	276.3 ± 39.3	255.8 ± 24.0	152.1 ± 43.0*	437.0 ± 29.3*	309.0 ± 30.2	326.2 ± 25.7	210.2 ± 11.6
Sorghastrum secundum	0	10.0 ± 0.7	1.9 ± 0.2*	2.2 ± 0.4*	1.3 ± 0.3*	0.8 ± 0.1*	11.4 ± 2.9	13.6 ± 4.4
	30	107.1 ± 7.1	68.9 ± 7.5*	67.9 ± 15.6*	133.9 ± 8.5	103.6 ± 14.6	79.2 ± 6.7	80.3 ± 2.5
	60	253.7 ± 24.6	137.1 ± 12.6*	152.2 ± 16.2*	139.4 ± 6.3*	143.8 ± 24.1*	91.2 ± 18.3*	119.5 ± 5.1*
Sporobolus junceus	0	3.3 ± 0.3	1.3 ± 0.1*	1.3 ± 0.3 *	3.2 ± 0.3	2.5 ± 0.1	1.6 ± 0.1*	1.9 ± 0.7*
	30	36.0 ± 3.1	7.9 ± 1.1*	7.5 ± 1.1*	24.5 ± 1.6*	40.8 ± 2.2	13.4 ± 1.9*	7.9 ± 0.8*
	60	102.8 ± 4.6	47.2 ± 4.6*	34.8 ± 9.0*	90.2 ± 5.4	110.4 ± 2.7	56.5 ± 13.9*	51.9 ± 8.1*
Legumes								
Desmodium floridanum	0	15.5 ± 2.5	8.7 ± 2.8	5.9 ± 0.5*	1.2 ± 1.2*	0.9 ± 0.9*	13.8 ± 2.0	7.0 ± 0.2*
	30	41.1 ± 9.4	23.7 ± 4.9	12.9 ± 2.4*	0.0 ± 0.0*	0.0 ± 0.0*	44.5 ± 3.3	36.6 ± 1.5
	60	175.2 ± 11.1	81.1 ± 21.7	84.7 ± 7.9	0.0 ± 0.0*	0.0 ± 0.0*	100.8 ± 20.4	71.4 ± 12.9
Crotalaria rotundifolia	0	3.8 ± 0.3	1.8 ± 0.4*	1.6 ± 0.6*	1.4 ± 0.9*	0.3 ± 0.3*	2.3 ± 0.3	2.3 ± 0.3
	30	161.0 ± 19.7	57.2 ± 28.7*	13.3 ± 13.3*	93.3 ± 52.1	21.7 ± 21.7*	165.3 ± 14.5	162.8 ± 18.6
	60	210.4 ± 18.5	21.7 ± 21.7	0.0 ± 0.0*	40.0 ± 20.8	0.0 ± 0.0*	115.3 ± 10.5	75.7 ± 5.0
Lespedeza angustifolia	0	10.0 ± 1.3	0.1 ± 0.1*	0.0 ± 0.0*	1.1 ± 0.6*	2.3 ± 1.2*	4.8 ± 0.6*	7.0 ± 1.3
	30	36.4 ± 3.6	14.7 ± 0.8*	16.2 ± 2.0*	5.7 ± 3.6*	5.1 ± 2.6*	16.1 ± 3.1*	22.8 ± 3.3*
	60	125.6 ± 6.5	81.5 ± 3.3	79.4 ± 2.6	42.8 ± 13.0*	22.2 ± 11.3*	82.2 ± 2.6	85.8 ± 7.8
Composites								
Helianthus angustifolia	0	12.7 ± 0.8	2.3 ± 1.1*	3.5 ± 1.4*	1.1 ± 0.2*	2.2 ± 0.3*	2.0 ± 0.3*	3.0 ± 0.2*
	30	45.1 ± 8.4	0.0 ± 0.0*	0.0 ± 0.0*	28.6 ± 3.2	35.9 ± 17.7	42.3 ± 11.8	48.2 ± 13.0
	60	187.3 ± 20.2	167.9 ± 43.2	70.7 ± 13.7*	128.2 ± 26.8	79.6 ± 6.4*	113.3 ± 14.3	112.8 ± 12.0
Rudbeckia hirta	0	9.2 ± 0.9	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	4.1 ± 0.4	3.4 ± 0.5
	30	62.1 ± 5.9	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	0.0 ± 0.0*	37.7 ± 6.1*	20.2 ± 4.5*
	60	141.6 ± 9.3	20.0 ± 20.0*	0.0 ± 0.0*	25.6 ± 25.6*	0.0 ± 0.0*	45.7 ± 1.9*	58.4 ± 3.0*

Table 5. Mean (± SE) aboveground biomass (mg $seedling^{-1}$) of seedlings following treatment with herbicides in Experiment II by species, plant age, herbicide, and application rate (Rate 1 = low rate and Rate 2 = high rate). Asterisks (*) denote significant differences of treatments relative to the untreated control at $\alpha \leq 0.05$ (ANOVA).

			Imazapyr		Sulfometuron methyl		Hexazinone	
Species	Age	Control	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
Grasses								
Andropogon virginicus	0	2.3 ± 0.1	0.4 ±0.2*	0.4 ± 0.2*	2.3 ± 0.2	1.1 ± 0.2*	0.5 ± 0.1*	0.6 ± 0.4*
	30	39.0 ± 2.4	12.9 ± 12.9*	21.0 ± 6.0	31.5 ± 5.5	48.1 ± 7.1	13.3 ± 6.7*	0.0 ± 0.0*
	60	144.6 ± 22.9	169.5 ± 30.3	153.5 ± 24.1	66.6 ± 20.6	250.7 ± 8.3	104.0 ± 17.6	90.8 ± 70.7
Aristida stricta	0	2.4 ± 0.4	1.0 ± 0.3*	0.4 ± 0.1*	0.8 ± 0.3 *	0.4 ± 0.0*	1.1 ± 0.1*	0.2 ± 0.1*
	30	16.2 ± 2.3	5.6 ± 0.1*	6.0 ± 2.2*	7.7 ± 0.4*	7.9 ± 3.2*	7.1 ± 0.8*	0.0 ± 0.0*
	60	67.7 ± 5.7	50.0 ± 2.3	30.0 ± 8.1*	27.4 ± 0.8*	11.6 ± 5.3*	26.2 ± 8.7*	12.7 ± 12.7*
Saccharum alopecuroides	0	6.0 ± 2.1	1.2 ± 0.2	1.0 ± 0.0	1.1 ± 0.1	0.9 ± 0.2	2.0 ± 0.4	0.8 ± 0.2
	30	92.1 ± 12.9	10.6 ± 6.4*	10.4 ± 10.4*	27.2 ± 2.8*	60.6 ± 14.0	23.3 ± 23.3*	0.0 ± 0.0*
	60	199.3 ± 17.2	316.2 ± 21.5	406.6 ± 128.0*	60.2 ± 18.0	253.9 ± 25.2	130.0 ± 75.1	0.0 ± 0.0*
Sorghastrum secundum	0	3.0 ± 0.7	1.1 ± 0.1*	0.9 ± 0.1*	1.0 ± 0.2*	1.1 ± 0.3*	0.5 ± 0.3*	2.1 ± 0.9
	30	34.5 ± 2.4	41.4 ± 4.3	25.3 ± 12.4	27.7 ± 2.4	28.5 ± 7.3	32.3 ± 3.2	12.9 ± 12.9
	60	120.2 ± 26.0	226.7 ± 12.2	136.1 ± 16.1	64.4 ± 9.3	74.8 ± 1.8	184.1 ± 45.3	149.1 ± 29.3
Sporobolus junceus	0	2.1 ± 0.2	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.4 ± 0.1	6.7 ± 5.7	1.8 ± 0.2
	30	28.4 ± 2.3	15.0 ± 1.6*	11.9 ± 3.5*	5.4 ± 0.5*	6.6 ± 1.9*	14.4 ± 4.5*	0.0 ± 0.0*
	60	102.2 ± 8.1	50.9 ± 1.2*	102.7 ± 2.1	35.6 ± 5.8*	72.7 ± 3.3	47.7 ± 8.9*	82.7 ± 7.1
Legumes								
Desmodium floridanum	0	15.8 ± 0.9	5.9 ± 0.7*	4.1 ± 0.4*	3.9 ± 0.4*	5.3 ± 0.4*	8.4 ± 2.7*	1.2 ± 1.2*
	30	91.2 ± 12.1	59.2 ± 2.4*	35.7 ± 4.4*	50.0 ± 0.7*	47.8 ± 0.3*	0.0 ± 0.0*	0.0 ± 0.0*
	60	112.4 ± 7.6	178.7 ± 19.2*	169.8 ± 16.9*	69.6 ± 5.4*	208.0 ± 7.1*	44.7 ± 8.2*	63.5 ± 9.3*
Crotalaria rotundifolia	0	13.2 ± 1.3	2.0 ± 0.1*	1.9 ± 0.2*	2.1 ± 0.1*	2.4 ± 0.2*	3.5 ± 1.4*	1.4 ± 0.9*
	30	150.7 ± 14.5	57.3 ± 7.0*	45.1 ± 1.4*	63.4 ± 9.6*	87.2 ± 4.5*	0.5 ± 0.5*	0.0 ± 0.0*
	60	217.3 ± 10.4	185.6 ± 7.1	151.0 ± 25.9*	250.5 ± 11.4	224.6 ± 7.6	135.1 ± 15.6*	89.2 ± 1.5*
Lespedeza angustifolia	0	9.0 ± 0.5	3.6 ± 1.1*	2.5 ± 0.2*	5.3 ± 2.2*	3.5 ± 0.4*	0.2 ± 0.2*	0.0 ± 0.0*
	30	62.7 ± 3.2	21.0 ± 2.3*	13.2 ± 2.4*	13.6 ± 2.6*	18.9 ± 3.6*	0.0 ± 0.0*	0.0 ± 0.0*
	60	92.1 ± 2.1	129.6 ± 8.0*	118.4 ± 1.4*	61.5 ± 8.7*	137.3 ± 0.4*	41.8 ± 1.8*	46.6 ± 5.1*
Composites								
Helianthus angustifolia	0	8.1 ± 0.8	1.5 ± 0.1*	4.3 ± 3.6	0.7 ± 0.3*	1.0 ± 0.1*	1.1 ± 0.6*	0.9 ± 0.3*
	30	71.2 ± 5.0	1.6 ± 0.3*	1.9 ± 1.1*	0.5 ± 0.2*	1.5 ± 0.4*	1.2 ± 1.0*	0.2 ± 0.2*
	60	114.3 ± 5.8	92.2 ± 26.4	43.9 ± 30.6*	21.2 ± 9.1*	0.7 ± 0.2*	53.7 ± 36.1	17.6 ± 16.2*
Rudbeckia hirta	0	5.9 ± 0.3	0.8 ± 0.1*	0.7 ± 0.1*	0.8 ± 0.3*	0.5 ± 0.1*	0.0 ± 0.0*	0.0 ± 0.0*
	30	79.7 ± 4.3	18.4 ± 4.3*	17.0 ± 3.2*	20.3 ± 2.7*	39.2 ± 1.5*	0.0 ± 0.0*	0.0 ± 0.0*
	60	171.4 ± 10.3	133.2 ± 17.4	112.0 ± 17.2*	137.7 ± 3.4	96.4 ± 18.8*	0.0 ± 0.0*	24.5 ± 18.2*

lings including *Aristida stricta* and *Saccharum alopecuroides* were severely injured or killed when treated with hexazinone at the high rate (Table 3). Only the 30-day-old composites treated with hexazinone, imazapyr, or sulfometuron methyl had lower biomass values than the untreated controls (Table 5) and showed extreme phytotoxic response including severe injury and plant death with phytotoxicity scores of 7 to 9 (Table 3).

Experiment III. Regardless of age, biomass of legumes treated with triclopyr was usually less (Table 6) than untreated control plants, with phytotoxicity scores ranging from 5 to 9 and symptoms ranging from substantial chlorosis and stunting to plant death (Table 3). Additionally, biomass of all pre-emergent legumes treated with butyric acid was significantly lower than control plants (Table 6). Although biomass of pre-emergent legumes treated with butyric acid was substantially less than the untreated control, plants exhibited only slight or no phytotoxic symptoms with phytotoxicity scores of 1 to 2 (Table 3). Pre-emergent and 30-day-old composites had significantly lower biomass values relative to control plants when treated with the high application rate of triclopyr (Table 6), causing foliar damage such as substantial chlorosis and stunting, severe injury, and near death with phytotoxicity scores of 4 to 8 (Table 3). Biomass of all pre-emergent grasses (except *Saccharum alopecuroides*) treated with triclopyr was significantly lower than biomass of control plants (Table 6).

All 30- and 60-day-old legumes and composites treated with applications of fluazifop-p-butyl grew just as well as or better than untreated control plants with the exception of 30-day-old *Helianthus angustifolia* at the low rate (Table 6). In general, most species of grasses were least sensitive to the low application rate of butyric acid compared to seedlings treated with fluazifop-p-butyl or triclopyr.

Discussion. This study demonstrates that several native ground cover species were more sensitive to herbicide application than expected based on the below maximum label rates used and use implied by the herbicide labeling (Table 1). For example, most pre-emergent grasses were severely damaged by applications of aminopyralid, a herbicide labeled for control of broadleaf weeds (Table 3). Additionally, triclopyr, an herbicide with post-emergent properties labeled to control broadleaf weeds, was injurious to most pre-emergent grass species (Tables 3 and 6). All pre-emergent legumes were sensitive to butyric acid, an herbicide labeled to control broadleaf weeds growing among leguminous crops.

Hexazinone is one herbicide in particular that has been broadly studied in relation to longleaf pine ecosystem restoration efforts. Longleaf pine restoration studies that have used hexazinone as a site preparation tool or to control undesirable woody species in the understory have reported results regarding effects of this herbicide on mature, established ground cover plants and often found this herbicide to have only minimum toxic effects (Wilkins et al. 1993, Brockway et al. 1998, Brockway and Outcalt 2000, Provencher et al. 2001, Freeman and Jose 2009, Jose et al. 2010). Although our study was limited to young seedlings (plant age of 60 d or less), this herbicide caused severe phytotoxic damage (heavy injury or plant death) to nearly all species tested in this study (Table 3). Heavy injury and plant death of all of our pre-emergent legumes and composites treated with hexazinone was an unexpected result considering that this herbicide is labeled as a post-emergent herbicide. In particular, our study showed that biomass of *Aristida stricta* (wiregrass - historically a functionally important understory component of the longleaf pine ecosystem) seedlings of all ages treated with hexazinone was significantly lower compared to untreated control plants (Table 5). This sensitivity to hexazinone may provide an explanation for the puzzling findings of Outcalt et al. (1999) who observed decreased survival of planted *Aristida stricta* seedlings on sites pre-treated with hexazinone.

While most herbicides tested in this study were harmful to seedlings, some herbicides such as fluazifop-p-butyl, imazapic, and butyric acid, performed appropriately based on their label specifications and certain species were relatively tolerant of these herbicides. The 30- and 60-day-old legumes and composites grew just as well as or better than control plants when treated with fluazifop-p-butyl, a post-emergent herbicide used to control grasses (Table 6). Use of fluazifop-p-butyl could be advantageous where native broadleaf ground cover species are planted in a production setting and it is necessary to control problem-

Table 6. Mean (± SE) aboveground biomass (mg seedling^{-1}) of seedlings following treatment with herbicides in Experiment III by species, plant age, herbicide, and application rate (Rate 1 = low rate and Rate 2 = high rate). Asterisks (*) denote significant differences of treatments relative to the untreated control at $\alpha \leq 0.05$ (ANOVA).

			Butyric acid		Fluazifop-p-butyl		Triclopyr	
Species	Age	Control	Rate 1	Rate 2	Rate 1	Rate 2	Rate 1	Rate 2
Grasses								
Andropogon virginicus	0	7.7 ± 0.2	2.6 ± 0.5*	5.0 ± 0.3*	4.6 ± 0.4*	0.8 ± 0.1*	3.9 ± 0.9*	1.2 ± 0.6*
	30	51.9 ± 5.7	62.6 ± 16.3	14.0 ± 3.5*	15.0 ± 3.7*	22.3 ± 1.6*	47.6 ± 2.5	30.5 ± 3.0
	60	99.2 ± 8.4	104.3 ± 8.3	67.1 ± 6.4*	71.4 ± 2.2*	104.9 ± 1.3	77.8 ± 10.3	58.5 ± 0.7*
Aristida stricta	0	2.5 ± 0.1	1.7 ± 0.1*	1.5 ± 0.1*	0.6 ± 0.1*	0.9 ± 0.6*	0.6 ± 0.1*	0.6 ± 0.2*
	30	21.6 ± 2.6	18.7 ± 4.5	30.7 ± 26.1	5.2 ± 0.7	7.9 ± 1.0	9.7 ± 1.7	5.2 ± 1.8
	60	53.9 ± 7.4	26.4 ± 9.1*	15.8 ± 1.7*	14.0 ± 0.6*	25.4 ± 4.0*	18.9 ± 2.6*	14.9 ± 2.3*
Saccharum alopecuroides	0	3.0 ± 1.1	3.6 ± 0.9	4.4 ± 2.8	1.4 ± 0.8	1.8 ± 1.6	0.3 ± 0.3	0.6 ± 0.2
	30	196.2 ± 20.3	202.2 ± 42.0	95.0 ± 17.5*	23.0 ± 7.9*	10.1 ± 3.4*	57.9 ± 18.6*	45.6 ± 25.1*
	60	213.3 ± 27.1	241.5 ± 61.7	203.0 ± 78.5	250.9 ± 94.0	154.6 ± 39.2	230.0 ± 23.6	179.1 ± 18.0
Sorghastrum secundum	0	10.4 ± 0.8	13.4 ± 2.7	11.8 ± 1.3	7.4 ± 1.8	4.5 ± 0.8*	5.0 ± 1.1*	2.2 ± 0.6*
	30	101.1 ± 8.9	114.3 ± 72.8	80.8 ± 7.8	29.9 ± 4.9	38.6 ± 2.3	128.4 ± 18.7	37.3 ± 5.5
	60	187.8 ± 13.0	227.0 ± 32.1	154.8 ± 10.4	169.1 ± 6.4	160.4 ± 20.1	175.5 ± 21.8	112.8 ± 39.9*
Sporobolus junceus	0	5.2 ± 0.3	2.7 ± 0.5*	1.6 ± 0.2*	1.2 ± 0.6*	0.0 ± 0.0*	1.7 ± 0.2*	0.7 ± 0.2*
	30	74.0 ± 3.3	92.0 ± 6.2	38.8 ± 5.8*	11.1 ± 1.4*	11.1 ± 1.3*	56.0 ± 2.5*	47.2 ± 5.0*
	60	83.5 ± 3.3	119.8 ± 8.4*	103.4 ± 2.7	76.4 ± 7.3	72.3 ± 5.5	102.1 ± 15.5	79.0 ± 4.1
Legumes								
Desmodium floridanum	0	42.6 ± 1.8	26.7 ± 1.5*	23.8 ± 3.2*	27.4 ± 1.0*	40.0 ± 1.8	3.9 ± 0.9*	4.3 ± 2.2*
	30	54.1 ± 7.7	52.9 ± 7.0	67.8 ± 8.1	121.6 ± 13.1*	171.9 ± 8.6*	24.6 ± 12.9	35.0 ± 18.2
	60	115.4 ± 10.9	109.0 ± 24.9	85.2 ± 13.1	211.7 ± 14.9*	235.9 ± 34.4*	50.9 ± 31.3	0.0 ± 0.0*
Crotalaria rotundifolia	0	43.3 ± 1.4	29.4 ± 0.7*	27.5 ± 1.1*	33.8 ± 1.1 *	27.7 ± 1.1*	6.6 ± 2.5*	11.0 ± 7.2*
	30	80.1 ± 11.6	104.9 ± 2.9*	100.2 ± 7.8*	177.7 ± 7.7*	192.1 ± 14.9*	57.1 ± 4.6*	29.5 ± 10.8*
	60	356.7 ± 19.2	241.3 ± 68.9*	265.3 ± 33.0	319.9 ± 36.1	280.7 ± 19.2	100.0 ± 20.5*	169.2 ± 11.6*
Lespedeza angustifolia	0	12.5 ± 1.0	9.2 ± 0.5*	7.7 ± 1.0*	6.1 ± 0.7*	8.5 ± 0.9*	1.8 ± 0.7*	1.0 ± 0.5*
	30	44.3 ± 5.6	31.7 ± 8.7	12.6 ± 2.3*	65.8 ± 3.2*	70.7 ± 8.6*	0.0 ± 0.0*	0.0 ± 0.0*
	60	122.5 ± 11.6	74.1 ± 7.6*	70.5 ± 16.2*	150.5 ± 5.6	109.2 ± 12.8	49.6 ± 12.6*	0.0 ± 0.0*
Composites								
Helianthus angustifolia	0	24.0 ± 2.6	17.0 ± 3.8	13.3 ± 2.1*	19.1 ± 1.6	21.2 ± 4.3	1.4 ± 0.4*	3.2 ± 0.5*
	30	62.1 ± 4.7	32.5 ± 6.2*	45.8 ± 0.9*	34.9 ± 2.6*	72.0 ± 3.7	36.3 ± 5.6*	32.3 ± 7.5*
	60	165.0 ± 9.0	101.2 ± 8.7*	123.8 ± 16.9*	154.9 ± 20.9	131.0 ± 7.1	62.8 ± 11.1*	110.6 ± 8.4*
Rudbeckia hirta	0	17.4 ± 1.4	11.1 ± 1.6*	9.2 ± 1.8*	6.6 ± 1.4*	11.4 ± 2.4*	1.8 ± 0.6*	0.7 ± 0.4*
	30	102.3 ± 12.3	51.3 ± 3.6*	50.6 ± 4.5*	124.8 ± 16.7	94.5 ± 14.8	73.4 ± 29.6	36.7 ± 4.2*
	60	168.9 ± 17.5	110.3 ± 21.8	120.1 ± 6.0	177.2 ± 18.2	168.3 ± 19.6	114.5 ± 12.1	132.5 ± 18.1

atic, undesirable grass species such as bermudagrass, bahiagrass, and cogongrass using herbicides. *Andropogon virginicus* and *Sorghastrum secundum*, species used in restoration plantings, appeared to be somewhat tolerant of applications of imazapic, an herbicide labeled to control grasses and broadleaves. Additionally, most grasses showed tolerance to butyric acid, an herbicide used to control broadleaves growing among leguminous plants. The tolerance of certain grass species (*Andropogon*, *Sorghastrum*, and *Aristida*) to these herbicides shows particular promise for programs such as the CRP-CP36 in the state of Georgia because landowners and practitioners often seek advice regarding the use of herbicides to control undesirable species while simultaneously promoting desirable native species.

Understanding the sensitivity of common non-target native ground cover species to several different herbicides can advance the use of herbicides as effective tools, leading to more restoration successes in the longleaf pine-wiregrass ecosystem. This study clearly demonstrates that herbicide use for native ecosystem restoration cannot necessarily be generalized from that of agricultural or silvicultural use. Additionally, results from this study should be interpreted with caution because we are uncertain about the effects of these herbicides on young seedlings in a field setting and how mature plants will respond to these herbicides and application rates. Furthermore, selection of appropriate herbicides is largely dependent on restoration objectives including the species attempting to establish and age of the plants.

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