

Article

Methods for Rapid Screening in Woody Plant Herbicide Development

William Stanley ^{1,*}, Shepard Zedaker ¹, John Seiler ¹ and Patrick Burch ²

¹ Department of Forest Resources and Environmental Conservation, 228 Cheatham Hall, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061, USA; E-Mails: willstanvt@gmail.com (W.S.); zedaker@vt.edu (S.Z.); jseiler@vt.edu (J.S.)

² Dow AgroSciences LLC, 3425 Elk Creek Drive, Christiansburg, VA 24073, USA; E-Mail: plburch@dow.com

* Author to whom correspondence should be addressed; E-Mail: willstanvt@gmail.com; Tel.: +1-540-661-6524.

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Abstract: Methods for woody plant herbicide screening were assayed with the goal of reducing resources and time required to conduct preliminary screenings for new products. Rapid screening methods tested included greenhouse seedling screening, germinal screening, and seed screening. Triclopyr and eight experimental herbicides from Dow AgroSciences (DAS 313, 402, 534, 548, 602, 729, 779, and 896) were tested on black locust, loblolly pine, red maple, sweetgum, and water oak. Screening results detected differences in herbicide and species in all experiments in much less time (days to weeks) than traditional field screenings and consumed significantly less resources (<500 mg acid equivalent per herbicide per screening). Using regression analysis, various rapid screening methods were linked into a system capable of rapidly and inexpensively assessing herbicide efficacy and spectrum of activity. Implementation of such a system could streamline early-stage herbicide development leading to field trials, potentially freeing resources for use in development of beneficial new herbicide products.

Keywords: rapid greenhouse screen; rapid seed screen; forestry; industrial vegetation management

1. Introduction

Since the early 1980s, there have been few herbicide products introduced specifically for control of woody plants. Barriers to development of new products are many and include large time and cost components of field trials as well as small expected markets relative to the cost of development. Many effective options (individual products and tank mixes) are currently available, but reasons for new product development could include management of resistance in weed populations, greater species selectivity, control of exotic invasive species, and effective control of vegetation using lower application rates.

Woody plants, being perennial and long-lived organisms, develop woody tissue and large root systems over time. Large areas (1/20 to 1/5 ha per treatment replication) for field test plots are needed, requiring large amounts of early-stage herbicide formulation for applications [1]. Following herbicide injury that does not completely kill the roots, woody plants are capable of re-growth [2]. Therefore, plants in field screenings must be observed in two growing seasons, commonly for one year and sometimes two years or more after treatment, to measure success of the herbicide application. These requirements may represent a significant time and monetary investment on the part of the herbicide developer and can discourage entries into smaller markets like forestry, right-of-way, and range and pasture.

Literature on standardized methods for herbicide field trials exists [3], but there is a lack of published information about how to conduct early-stage screening for herbicide activity on woody plants. The investment of woody plant field trials presents a hurdle for new compounds before entering the development process, potentially overlooking a promising product with a new mode of action or a new control for an exotic invasive like *Ailanthus altissima*.

Several authors have noted the need for improved woody plant herbicide screening procedures [1,4,5]. In response, methods for rapidly assessing herbicide activity in the early stages of product development have been developed over the past three decades. In the 1980s, Zedaker and Seiler were utilizing greenhouse space near their field trial locations to quickly grow seedlings in greenhouse pots and apply herbicide treatments in a controlled environment. Comparison of greenhouse and field results indicated that young, immature woody plants treated with 0.25 x to 0.5 x field rates can produce the same herbicide efficacy results in a shorter time period [1]. A subsequent study on the effect of herbicide solution on seed germination found that germination rates of seed soaked in herbicide solution were significantly different than water-soaked control treatments and paralleled known field trial results [6]. More recently, work by Blair *et al.* demonstrated the connection between greenhouse and field experiments by predicting results of triclopyr and imazapyr field treatments with greenhouse and seed screening techniques using regression modeling [4]. Focusing on greenhouse and seed techniques, this study evaluated an accelerated system for woody plant herbicide screening while providing efficacy data on a group of new herbicides from Dow AgroSciences and comparing their performance to a control standard, triclopyr. Our hypothesis was that results from shorter evaluations would correlate well with “standard” longer ones, thus allowing developers to make decisions quicker. This would allow woody plant screening with small amounts of chemical, with less cost, and possibly identify useful chemicals to continue in the screening process.

2. Experimental Design and Analysis

Facilities at the Reynolds Homestead Forest Resources Research Center (Reynolds) in Critz, Virginia, and Virginia Tech's Blacksburg campus were utilized to grow, treat, and over-winter plants in the rapid greenhouse seedling and germinal seedling screenings. Rapid seed screening was carried out in growth chambers located on Virginia Tech's Blacksburg campus.

2.1. Rapid Greenhouse Screening

Greenhouse screening was performed on 2 age groups of seedlings: first growing season and second growing season seedlings. Each experiment was a randomized complete block design (RCBD) and ran concurrently in the same greenhouse space using the methods described below. The first group of seedlings, hereafter referred to as the 1-0 group, was sprayed during their first growing season, approximately 4 months after planting. The other group of seedlings, the 2-0 group, was treated in their second growing season (approximately 11 months after planting) with the same herbicides and rates as the 1-0 group to allow the responses of the two seedling age groups to be compared. Visual observation revealed that the 2-0 group had more biomass than the 1-0 group and had one complete annual growth ring prior to treatment.

Seeds of four species, black locust (*Robinia pseudoacacia* L.), red maple (*Acer rubrum* L.), sweetgum (*Liquidambar styraciflua* L.), and water oak (*Quercus nigra* L.), were planted in greenhouse facilities at Reynolds during spring 2010 in D-16 containers (Stuewe and Sons, Corvallis, OR 97333) (16 cm³ volume) filled with a custom potting mix (Table 1). Bare-root loblolly pine (*Pinus taeda* L.) seedlings were purchased from a North Carolina Department of Forestry nursery and transplanted into D-16 containers with potting mix (Table 1) to guard against mortality caused by dampening off when starting this species from seed. Seedlings reached a height of approximately 51 cm, were topped for height consistency, and were allowed to flush out again prior to treatment.

Table 1. Potting mix recipe for greenhouse seedling and germinal screenings [†].

Growth Media		Plant Food	
Quantity	Product	Quantity (mL)	Product
2.0 bales (3.8 ft ³)	peat moss	2800	Osmocote Pro 8–9 month fertilizer (20-4-8)
3.5 bags (4 ft ³)	horticultural vermiculite	700	bone meal
1.5 bags (4 ft ³)	horticultural perlite	700	gypsum
		350	dolomite
		350	epsom salt

[†] Sufficient potting mix to fill 2450 16 cm³ cells.

Herbicide treatments were applied to seedlings in a spray booth (Model #SB 8, De Vries Manufacturing, 86956 State Highway 251, Hollandale, MN 56045, USA) fitted with an 8001E-HSS TeeJet spray tip (TeeJet Technologies Spraying Systems Co., P.O. Box 7900, Wheaton, IL 60187) mounted 30.5 cm above the seedling canopy and calibrated to deliver a volume of 187 L ha⁻¹. The randomized complete block was replicated three times with treatments applied to 10 seedling subsamples of each species, resulting in a sample size of 30 for each treatment. Experimental

herbicides supplied by Dow AgroSciences (DAS 313, 402, 534, 548, 602, 729, 779, and 896) (9330 Zionsville Road Indianapolis, IN 46268) were mixed in deionized water approximately 24 hours before application and foliar applied at 35, 70, 140, and 280 g ae ha⁻¹ in addition to the control standard, triclopyr as Garlon 4 Ultra, at 140, 280, and 560 g ae ha⁻¹ (Table 2). Agri-dex crop oil surfactant (Helena Chemical Company, 225 Schilling Blvd., Collierville, TN 38017) at 1.25% v/v was included in all treatments. Following treatment, seedlings were allowed to dry in the shade. They were then placed in the greenhouse under natural lighting, and sub-irrigated to prevent herbicide contamination from adjacent treatments.

Table 2. Rates of herbicide acid equivalent used in the current study.

Rate	Experimental Herbicides	Triclopyr
	----- g ae ha ⁻¹ -----	
1 x	280	560
0.5 x	140	280
0.25 x	70	140
0.125 x	35	---

On July 19, 2010, four months after March planting, herbicide treatments were applied to the 1-0 seedlings. After treatment, seedlings entered a period of evaluation during which changes in height were measured and herbicide injury observed using the metric in Table 3.

Table 3. Injury rating scale for evaluation of greenhouse seedling and germinal screenings.

Rating	Plant Visible Injury Symptomology
1	No visible signs of injury
2	Slight Epinasty: <50% leaders/laterals bent
3	Severe Epinasty: >50% leaders/laterals bent
4	Necrosis: <50% leaf necrosis
5	Necrosis: >50% leaf necrosis
6	Dead (appearance)

Preliminary evaluations of 1-0 seedling treatments were carried out in the same growing season as herbicide application, at 2, 4, and 11 weeks after treatment (WAT). After the 11 week evaluation, heat was turned off in the greenhouse while naturally shortening fall daylengths and cooler temperatures were utilized to encourage bud set. Six weeks later, the 1-0 seedlings were placed in a cold room on site at 2 °C with no lighting for 1000 h (6 weeks) to satisfy dormancy requirements. This period of chilling mimicked the changing of the seasons for these species and put them into post-dormancy in only 6 weeks rather than the 3–4 months of dormancy that typically separates growing seasons. With chilling requirements satisfied, seedlings were now ready to begin growing again when placed into a spring-like environment with sufficient light and warmth.

Due to space constraints in the Reynolds greenhouse, the 1-0 seedlings were transported to a similar greenhouse on Virginia Tech's Blacksburg campus. Overnight temperatures in the Blacksburg greenhouse were kept above 13 °C, and artificial lighting provided 16 h photoperiods. In the

greenhouse, seedlings were allowed to re-flush for 8 weeks during January and February 2011 before herbicide efficacy was assessed 32 WAT, during the 1-0 seedlings' second growing season. This evaluation mimicked a 1 year after treatment (YAT) traditional field trial assessment, but the rapid screening process provided second growing season efficacy data in less than one year from seed to final evaluation.

Meanwhile, the 2-0 seedlings remained untreated for one cycle of growth and dormancy and were returned to the Reynolds greenhouse to re-flush before herbicide treatments were applied. After 6 weeks in the greenhouse during January and February 2011, the 2-0 seedlings had fully leafed out. Herbicides were applied on February 19, 2011, approximately 11 months after March planting, using the same rates and methods as with the 1-0 group. The 2-0 seedlings were evaluated once at 6 WAT due to greenhouse space constraints. This evaluation occurred in the second physiological growing season for the 2-0 seedlings, but during the same growing season that herbicide treatments were applied. Ideally, this group of seedlings would also have been kept through another dormancy cycle and evaluated in the next growing season after herbicide treatment.

2.2. Rapid Germinal Seedling Screening

The germinal (newly germinated seedling) screening (also RCBD) utilized the same greenhouse space at Reynolds and application methods as the greenhouse seedling screening. Differences between the experiments included planting method, time from planting to treatment (6 weeks), and size of seedlings (10–15 cm tall) at the time of treatment. The germinal screening was designed to be similar in method and duration to an early-stage herbaceous plant trial.

Seeds of four species, black locust, loblolly pine, sweetgum, and water oak, were planted in 52 cm × 40 cm × 6.35 cm greenhouse flats (trays) with holes for drainage. Flats were filled 5 cm deep with potting mix (Table 1) and seeds were planted approximately 5 cm apart in rows of 10, by species. Planting of species was staggered to account for differences in their rate of emergence and allow plants to reach a uniform height before herbicide application. This was important to the spray booth herbicide application to ensure that one plant would not overtop another, obstructing the overtopped plant from receiving the same amount of spray solution as adjacent plants. Germination rate of red maple seed was extremely low (<5%) and it was therefore not included in germinal screening.

Germinal treatments were applied on February 19, 2011, at Reynolds at the same time as 2-0 seedling applications. Each tray of germinals was placed in the spray booth with 2-0 seedlings and the same treatment (herbicide × rate) was applied to both experimental units simultaneously. Because the germinal screening shared greenhouse space at Reynolds with the 2-0 seedling experiment, germinals were limited to 12 treatments—3 herbicides × 4 rates. Based on preliminary data from the 1-0 seedlings, the most effective (DAS 729) and least effective (DAS 896) herbicides, and triclopyr as the control standard, were applied to the germinals. Following herbicide application, germinals were returned to the greenhouse, and watering from above was resumed after herbicide had been allowed to dry on leaf surfaces for several days. At 6 WAT, germinal treatments were evaluated by measuring height and herbicide injury (Table 3).

2.3. Rapid Seed Screening

Seed screening was carried out in growth chambers on Virginia Tech's Blacksburg campus following the procedures of Blair *et al.* 2006 [4]. After cold-moist stratification periods recommended by Young and Young [7], seeds were counted into groups of 20 and placed in Petri dishes containing dry filter paper. Each Petri dish represented one treatment. Applications were made as a pre-soak, consisting of a 24 h soak in 10 mL of herbicide solution to provide enough volume to saturate the filter paper and allow for absorption by the seeds. Herbicides DAS 402, DAS 729, and triclopyr were mixed in deionized water at concentrations equivalent to those used in rapid greenhouse seedling screening (Table 2). Three temporal replications (repeated in time) were completed, each including a deionized water control treatment per species. Excess liquid solution was drained from each Petri dish after the 24-h soak without removing the seeds and filter paper. Petri dishes were placed in a growth chamber at 25 °C and 80% relative humidity with 16 h photoperiods (16 h light, 8 h dark) to allow germination [8]. Seeds were measured at 4, 8, and 14 days after treatment (DAT). Length (mm) of live tissue (root and shoot combined) emerging from the seed was measured at each evaluation.

2.4. Data Analysis

Each of the four experiments utilized a randomized complete block design. Treatments were applied to sub-samples of 10 individuals for seedlings and 20 individuals for seeds. Treatments were replicated three times, resulting in an overall sample size in each experiment of 30 for seedlings and 60 for seeds. Using SAS JMP statistical software package, data were checked for normality and equal variance before analysis of variance was performed on each screening type separately to test for herbicide and rate main effects and interactions. Tukey's HSD test was used for pair-wise comparisons between treatment means. Percent mortality and percent germination responses were transformed before analysis using an arcsine data transformation procedure as recommended by Gomez and Gomez [9]. Seed data were not analyzed at 12 DAT because there were fewer than three replications at that time. In germinal and seedling screenings, an unbalanced design was created in which triclopyr did not have the same number of rates as the experimental herbicides. This was corrected using a data imputation method to estimate values for the triclopyr 0.125 x rate [10]. Regression analysis was run by species and herbicide using rates as data points on several combinations of data to test for correlation between screening results. Data combinations included: 1-0 seedling pre-dormancy (11 WAT) to post-dormancy (32 WAT); 1-0 to 2-0 seedling; germinal to 1-0 seedling; and seed to 1-0 seedling.

3. Results and Discussion

Analysis of variance detected differences in response means due to herbicide main effect during all four rapid screening experiments (Table 4). Significantly different response means caused by herbicide were recognized as early as 2 weeks after treatment in seedling screenings and as early as 4 days after treatment in seed screenings. Herbicides with early treatment differentiation often had the highest efficacy at the end of the trial.

Table 4. Summary table of ANOVA outputs from rapid screening trials [†].

Experiment	Time	Species	Response	Effect	p-value
1-0 Seedlings	2 WAT	All Species	Injury	Herbicide	<0.0001
	11 WAT	All Species	Height	Herbicide	<0.01
	11 WAT	All Species	Injury	Herbicide	<0.05
	32 WAT	All Species	Height	Herbicide	<0.0001
	32 WAT	All Species	Injury	Herbicide	<0.0001
	32 WAT	All Species	% Mortality	Herbicide	<0.01
2-0 Seedlings	6 WAT	All Species	Height	Herbicide	<0.0001
	6 WAT	All Species	Injury	Herbicide	<0.0001
Germinals	6 WAT	All Species	Height	Herbicide	<0.0001
	6 WAT	All Species	Injury	Herbicide	<0.0001
	6 WAT	All Species	% Mortality	Herbicide	<0.01
Seeds	4 DAT	Black Locust	% Germination	Herbicide	0.0261
	4 DAT	Black Locust	Tissue Length	Herbicide	0.0021
	8 DAT	Loblolly Pine	% Germination	Herbicide	<0.0001
	8 DAT	Loblolly Pine	Tissue Length	Herbicide	<0.0001
	14 DAT	Water Oak	% Germination	Herbicide	0.0034
	14 DAT	Water Oak	Tissue Length	Herbicide	<0.0001

[†] Abbreviations: 1-0, 2-0, first growing season and second growing season seedling groups, respectively; WAT, weeks after treatment; DAT, days after treatment.

Past research has shown that predicting field efficacy from greenhouse-grown plants is possible and repeatable [1,4,11]. However, comparison to field efficacy can be affected by several factors, including timing and method of application, physiological condition of the plant, and rate of herbicide active ingredient relative to plant size [12]. In this study, the same herbicides and rates were applied to the germinal, 1-0, and 2-0 seedling groups, but overall mortality at the end of the screenings varied widely: 56.0%, 46.8%, and 9.5%, respectively. The authors expected plants of different sizes and life stages to respond differently to the same herbicide rates, but the identical rates provide a basis for comparison of results from the various screenings. The wide range of the response means from germinal and two seedling experiments makes comparing the relative ranking of herbicide efficacy (best, middle, worst) more useful than comparison of responses such as mortality or change in height. Because rapid screening is an early part of the development process, the most important question when testing on a group of new herbicides is: Which ones warrant more research and development efforts?

Rankings of herbicide efficacy can be assigned based on any appropriate response variable and used to objectively compare responses of plants from different life stages. Although these rankings are not statistically derived, regression models were used to establish a relationship between the screening datasets (Table 5). Regression models of the form $Y = b_0 + b_1X$, where Y is the responses from the longer screening and X is the response from the shorter screening, were used to check for correlation. The greatest number of statistically significant models predicted post-dormancy results from pre-dormancy data for 1-0 seedlings. Table 5 highlights the best significant models ($R^2 > 0.80$, $p < 0.1$) connecting the screening types, while including 1-0 pre-dormancy to post-dormancy models where no other significant models were available.

Table 5. Summary table for best significant linear regression models predicting greenhouse seedling responses using rapid screening data [†].

Species	Herbicide	Dependent	Independent	<i>p</i>	<i>R</i> ²
Black locust	DAS 313	2-0% mortality 6 WAT	1-0% mortality 32 WAT	0.064	0.88
		1-0 height 32 WAT	Seed % germination 14 DAT	0.097	0.82
	DAS 402	1-0 injury 32 WAT	Seed % germination 14 DAT	0.097	0.82
		1-0% mortality 32 WAT	Seed % germination 14 DAT	0.097	0.82
	DAS 729	1-0 injury 32 WAT	1-0 injury 11 WAT	0.003	0.99
Loblolly pine	DAS 779	2-0 injury 6 WAT	1-0 injury 32 WAT	0.068	0.87
		1-0 height 32 WAT	Germinal height 6 WAT	0.034	0.93
		1-0 injury 32 WAT	Germinal injury 6 WAT	0.046	0.91
	Triclopyr	1-0% mortality 32 WAT	Germinal % mortality 6 WAT	0.084	0.84
		1-0 height 32 WAT	Seed % germination 14 DAT	0.070	0.86
		1-0 injury 32 WAT	Seed % germination 14 DAT	0.058	0.89
Red maple	DAS 534	2-0 height 6 WAT	1-0 height 32 WAT	0.013	0.97
	DAS 548	1-0 height 32 WAT	1-0 height 11 WAT	0.004	0.99
	DAS 779	2-0 height 6 WAT	1-0 height 32 WAT	0.004	0.99
		2-0 injury 6 WAT	1-0 injury 32 WAT	0.022	0.96
	Triclopyr	1-0 injury 32 WAT	1-0 injury 11 WAT	0.008	0.99
Sweetgum	DAS 313	2-0 height 6 WAT	1-0 height 32 WAT	0.022	0.96
	DAS 534	1-0 height 32 WAT	1-0 height 11 WAT	0.010	0.98
	DAS 548	2-0% mortality 6 WAT	1-0% mortality 32 WAT	0.007	0.99
	DAS 602	2-0 injury 6 WAT	1-0 injury 32 WAT	0.034	0.93
	DAS 729	1-0 injury 32 WAT	1-0 injury 11 WAT	0.003	0.99
Water oak	DAS 402	1-0% mortality 32 WAT	Seed % germination 4 DAT	0.077	0.85
		1-0% mortality 32 WAT	Seed % germination 14 DAT	0.057	0.89
	DAS 729	2-0 injury 6 WAT	1-0 injury 32 WAT	0.043	0.92
	Triclopyr	2-0 height 6 WAT	1-0 height 32 WAT	0.021	0.96
		1-0 injury 32 WAT	Germinal injury 6 WAT	0.094	0.82

[†] Abbreviations: 1-0, 2-0, first growing season and second growing season seedling groups, respectively; WAT, weeks after treatment; DAT, days after treatment.

Regression analysis of pre-dormancy and post-dormancy 1-0 seedling responses produced numerous significant linear regression models (Table 5). Because woody plants can resprout following herbicide injury, it is quite possible that the pre- and post-dormancy responses would not be well correlated. The similarity in response to treatments described by the models indicate that the effects of treatments did not change greatly after dormancy, effectively shortening the length of the seedling screening to 11 weeks (time of pre-dormancy measurements) from application to results.

Species data were aggregated and herbicides were objectively assigned a numeric rank based on their injury rating and height responses within each screening (Table 6). Injury rating was chosen because it was the most sensitive to differences in response means caused by herbicide main effect, while height responses were used to make distinctions in rankings when injury ratings were equal. Results for some herbicides were very stable across screening types, including DAS 534, DAS 548, and DAS 779. The most effective herbicides were DAS 534 and DAS 729. This ranking was stable

across germinal, 1-0, and 2-0 seedling screenings with one notable exception: DAS 729 was the least effective chemistry in the seed screening. In this instance, if seed screening was the first test used by a researcher to differentiate chemistries and no other information was available about DAS 729, it would not make it to the next round of testing, *i.e.*, germinal screening. This anomaly is important, but future testing may detect which groups of chemistries or species might perform more reliably than others.

Table 6. Overview of herbicide rankings[‡] across screening types.

Efficacy	Herbicide	Decision Points				
		Seed 14 DAT	Germinal 6 WAT	1-0 Seedling 11 WAT	1-0 Seedling 32 WAT	2-0 Seedling 6 WAT
High	DAS729	3	1	1	1	2
	DAS534	†	†	2	2	1
	DAS548	†	†	3	4	4
	Triclopyr	2	3	4	7	3
Intermediate	DAS402	1	†	5	3	7
	DAS779	†	†	6	5	6
	DAS313	†	†	7	9	5
	DAS602	†	†	8	6	8
Low	DAS896	†	2	9	8	9

† A subset of 3 chemistries was tested in seed and germinal screenings. Symbol denotes herbicide was not tested; ‡ rankings: 1 to 9, where 1 is most effective and 9 is least effective.

Triclopyr ranking was relatively consistent (ranked #2–4) except in post-dormancy (32 WAT) 1-0 seedlings (#7). The 32 WAT evaluation of 1-0 seedlings is the only evaluation of herbicide efficacy that took place in the growing season after application. Results from this evaluation could be different because the seedlings had a chance to recover from herbicide injury before evaluation. If 2-0 seedlings were kept through a dormancy cycle, seedlings in triclopyr treatments might have recovered from initial injury as well. Rankings from seed screenings were not consistent with greenhouse trials, but germinal, 1-0, and 2-0 rankings of herbicide efficacy were relatively stable overall. Experimental herbicides from Dow AgroSciences were often more effective at lower rates than triclopyr in rapid screenings, but no other published results exist to confirm these findings.

Species and herbicides used in this project were chosen to serve as a test for the rapid screening process. Early detection of efficacy in germinal and seedling experiments may have been aided by the rapid expression of herbicide symptomology that was observed. The plant injury rating scale used in this experiment is well suited to plant growth regulator-type (PGR) herbicides with rapid activity, like triclopyr. Determining an appropriate injury rating scale would be an important part of a research project with a new group of herbicides from a different chemical family. Although information on experimental herbicides used in this project was limited at the time of publication, we do know that DAS 534 is a picolinic acid [13], while DAS 402 and 896 are close structural analogs. Symptomology observed in all herbicide treatments was consistent and included curling and cupping of leaves and stems followed by necrosis so we are confident our injury rating scale was appropriate.

Loblolly pine rankings appeared to be more stable across screening types than rankings for other individual species (Table 7). Herbicides DAS 402, DAS 534, and DAS 729 were consistently ranked most effective (#1–3) by seedling screenings. Consistency between seed and seedling screenings was much improved for loblolly pine *versus* other individual species as well as all species pooled; DAS 402 was ranked #1 in seed, #1 in 1-0 seedling screening, and #3 in 2-0 seedling screening. Germinal screening efficacy ranking also corresponded with 1-0 seedling rankings. The consistency in efficacy rankings for loblolly pine suggests that this method is effective in predicting the response for a species of special concern for forestry. As loblolly pine is a major plantation crop in the southeastern United States, herbicide treatments that can be broadcast over top of loblolly pine to control hardwood competition are in demand. Hardwood competition in pine plantations is not limited to, but can include, species such as black locust, red maple, sweetgum, and water oak. These species are also often found on road banks, fence lines, and utility rights of way. The adoption of advanced genetics in southern pine silviculture has increased the need for herbicides that control seedling pines as well.

Table 7. Overview of herbicide rankings[‡] across screening types for loblolly pine.

Efficacy	Herbicide	Decision Points				
		Seed 14 DAT	Germinal 6 WAT	1-0 Seedling 11 WAT	1-0 Seedling 32 WAT	2-0 Seedling 6 WAT
High	DAS402	1	†	1	1	3
	DAS534	†	†	2	2	1
	DAS729	3	1	3	3	2
	DAS602	†	†	5	4	4
Intermediate	DAS548	†	†	4	5	5
	DAS896	†	2	6	6	8
	DAS779	†	†	7	7	7
	DAS313	†	†	9	9	6
Low	Triclopyr	2	3	8	8	9

† A subset of 3 chemistries was tested in seed and germinal screenings; symbol denotes herbicide was not tested; ‡ rankings: 1 to 9, where 1 is most effective and 9 is least effective.

4. Conclusions

This research showed that the resources and time required for early-stage herbicide screening can be greatly reduced. Greenhouse seedling screenings were carried out in 300 ft² of greenhouse space using less than 500 mg herbicide acid equivalent per herbicide in each round of screening (Table 8). Responses from 1-0 seedlings' second physiological growing season were measured in 32 weeks with preliminary evaluations taking place at 11 WAT. In comparison, field trials often require 12 to 15 months before second growing season results can be measured.

Field trials are necessary to verify results from greenhouse work, but researchers have the capability to build a base of information about new chemistries quickly and inexpensively using rapid screening. Results of this research may be useful to herbicide researchers and developers, with the potential for a greater number of products to become available for woody plant control due to a reduction in the cost of development.

Table 8. Resources required for rapid screening: amount of herbicide acid equivalent used (mg) and time (from herbicide application to end of trial).

Screening Type	Experimental Herbicides	Triclopyr	Time
Traditional Field Sampling *	-	188,521 mg	1 year
1-0 Seedling	450 mg	900 mg	32 weeks
2-0 Seedling	450 mg	900 mg	6 weeks
Germinal	†	†	6 weeks
Seed	500 mg	1,000 mg	14 days

* Data from previous field screening work; † Germinal treatments were applied with 2-0 seedling treatments, so no additional herbicide was required for these treatments.

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Author Contributions

Will Stanley coordinated this research project for his M.S. thesis under advisement of the other authors. Experimental design was a collaborative effort based on the years of experience shared by Shep Zedaker and John Seiler (co-advisors), and Pat Burch, our industry committee member with Dow AgroSciences. William Stanley was responsible for fieldwork, data analysis, and writing, while the other authors reviewed and edited the work.

Conflicts of Interest

The authors declare no conflict of interest.

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