Methods for Rapid Screening in Woody Plant Herbicide Development

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ABSTRACT

Methods for woody plant herbicide screening were assayed with the goal of reducing resources and time required to conduct screenings for new products. Past studies have demonstrated reductions in required screening resources (time, amount of herbicide active ingredient, and size of seedlings) can be achieved relative to field exclosure screenings. Rapid screening methods including, greenhouse seedling screening, germinal screening, and seed screening were performed using triclopyr and 8 experimental herbicides supplied by Dow AgroSciences (DAS). Five woody species were included in screenings: black locust, loblolly pine, red maple, sweetgum, and water oak. Two groups of seedlings were used in greenhouse screening: 1-year-old (1-0) and 2-year-old (2-0). Seedling age was not calendar years, but was the number of greenhouse growing seasons seedlings experienced prior to herbicide treatment. Height and mortality responses showed that 1-0 seedlings were more susceptible to herbicide injury than 2-0 seedlings. Significant linear regression models were produced correlating 1-0 seedling pre-dormancy with post dormancy responses, shortening the length of that screening to 11 weeks from treatment to results. Species and herbicide specific models were produced correlating 2-0 seedling responses to 1-0 seedling data, germinal responses to 1-0 seedling data, and seed responses to 1-0 seedling data. 1-0 seedling pre to post dormancy predictions were more successful than other models. Results suggest that rapid screening methods have some usefulness in early stages of product development to determine herbicide activity and spectrum of efficacy to guide planning of larger scale field trials, resulting in savings of time and resources.

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Chapter 1: Introduction

Management of vegetation has been a time intensive activity since the establishment of agriculture over ten thousand years ago. Historically, the focus of vegetation management has been to increase crop yield by reducing competition between the crop and weed species for on-site resources. Until the 20^{th} century, the amount of food or fiber one farmer could produce was dependent on the number of people they employed in planting, weeding, and harvesting crops, but generally limited to feeding several families in the immediate area. With the advent of mechanized agriculture and pesticides, the potential yield per acre has increased many-fold.

Vegetation management techniques include chemical and mechanical methods. Mechanical methods and inorganic herbicides, including salts and ashes, have been in use for centuries to control undesired vegetation (Ware and Whitacre 2004). Reliable herbicide methods of vegetation control, such as synthetic organic herbicides, have evolved only in the last half-century. Synthetic organic herbicides, such as the phenoxy herbicides 2,4-D and 2,4,5-T, were discovered and found to have plant growth regulation properties during the 1940s (CAST 1975). In the late 1940s, forest land managers began aerially applying phenoxy herbicides to release conifers and found that they could provide crop trees with a competitive advantage and increase yields (McCormack 1990). Following the discovery of the effectiveness of phenoxy herbicides, chemical vegetation management expanded into a world-wide research effort yielding many other herbicides with different modes of action in the following decades (CAST 1975).

In 2007, herbicides accounted for the largest share of the world and domestic pesticide markets, 40% and 47% respectively (EPA 2011a). The United States applied 531 million pounds of herbicide active ingredient in 2007 across all market sectors (EPA 2011a). Herbicides are widely used by land managers solely or in combination with other cultural treatments to manage vegetation.

Industrial vegetation management (IVM), including herbicide use in forestland and rights-of-way management, represents a small portion of this market. Herbicide use in forestry includes site preparation treatments prior to stand establishment, release treatments to favor crop species after establishment, timber stand improvement (TSI) treatments, as well as invasive species management (Glover 1991). Rights of way managers employ herbicides to improve safety and access to their lands and to control vegetation so it does not interfere with functionality of industrial infrastructure (CAST 1975; Glover 1991). Electrical utilities spray beneath transmission lines to prevent interruption of service, highway managers spray roadside vegetation to increase visibility and maintain safe roadbed conditions, and railway managers spray their rights-of-way to control vegetation that might damage the ballast or limit line of sight (Gangstad 1982).

There is a limited research continuum from agricultural herbaceous weed control screening to IVM woody plant herbicide screening. This may be because the woody vegetation control sector represents a much smaller market volume than the agricultural herbicide market and new techniques for testing herbicides are not often funded. Woody plant screenings are also limited relative to agricultural screenings because they are time and resource intensive. Woody plants are capable of recovering after herbicide injury, further compounding the screening process. Generally, development of new herbicides and modes of action are targeted at crops with a high global market volume, such as maize, cereals, and rice (Rüegg et al. 2007). This attention is reasonable considering the large potential market for new compounds in these cropping systems, enabling manufacturers to recoup the large costs of registering a product.

However, many herbicides are discovered and not developed without having exhausted all potential applications because the cost of research, development, and registration of new herbicide products for IVM is high relative to the size of the expected market.

Herbicides that do not exhibit activity or control on common herbaceous weeds or that damage major crop species during agricultural primary screening may not screened for activity on woody species. It is not outside the realm of possibility that an effective herbicide for red maple (*Acer rubrum* L.) is in existence, but has never made it to woody plant screening (Zedaker and Seiler 1988). Woody plant screening is an intensive process and there is a need for better screening procedures to predict efficacy of promising new herbicide products on woody plants (Bovey 2001). This study seeks to demonstrate the feasibility of rapid herbicide screening for new herbicides for IVM applications.

Objectives

1. Reduce the resources (time, herbicide, and size of seedlings) required to conduct a greenhouse seedling screening.
2. For greenhouse seedling screening, determine if 1-year-old (1-0) seedling responses are capable of predicting 2-year-old (2-0) seedling responses and if 1-0 seedling pre-dormancy responses can predict post dormancy responses.
3. Determine if seed and germinal screenings can predict responses of greenhouse seedlings for the same species-herbicide combinations to further reduce the resources required to conduct screenings.

Chapter 2: Literature Review

Historical Summary of Herbicide Legislation

Pesticide legislation in the United States originated shortly after 1900 from the need to protect farmers from fraudulent pesticide products. The Federal Insecticide Act of 1910 was the first legislation aimed at the regulation of pesticides, but it did not include any language specific to herbicides (Ware and Whitacre 2004). This legislation was enacted to ensure the advertised efficacy of pesticide products on the market (Ware and Whitacre 2004). FIFRA, the Federal Insecticide, Fungicide, and Rodenticide Act of 1947 was the first pesticide legislation that specifically regulated the use of herbicides in the United States (Ware and Whitacre 2004). Since its creation in 1970, the U.S. Environmental Protection Agency (EPA) has been responsible for evaluating test data for registration of new herbicides prior to their labeling and subsequent release to the market (Ware and Whitacre 2004).

Herbicide Development Process

The development of a herbicide product from the discovery of a new herbicide chemistry to the registration of the product with the EPA is a time and capital intensive process. The EPA requires extensive testing of all new herbicides before they can be labeled and sold commercially. The development process for herbicides for agriculture and IVM follows a general pattern; however, the process of research and development for herbicides intended for the IVM market is slightly different (Bovey 2001; Glover 1991; Zedaker and Seiler 1988). Discussed below is a generic overview of the route that a new product might follow to registration. However, the stages of the development process described here are not necessarily linear and may differ widely by company and individual product. The development process for herbicides intended to control woody vegetation is more specific and a general outline will be discussed in subsequent sections, but should not be considered to be an absolute description of the process.

A new herbicide product begins in the discovery stage with a very small quantity of synthesized herbicide, usually several grams or less (Bovey 2001). Testing of the product is initiated with primary screening in laboratory or greenhouse facilities where the new herbicide is applied to test flats (trays filled with potting mix) containing as few as two or three herbaceous species to monitor for herbicide activity. If a herbicide exhibits activity on test species in primary screens, testing will continue on flats with increasing numbers of herbaceous crop and weed species. Testing of the new herbicide chemistry to fulfill EPA requirements may be conducted before, after, or concurrently with primary screening. The EPA requires approximately 140 tests encompassing the following (Bovey 2001; EPA 2011b):

- herbicide residue
- environmental fate
- toxicology
- re-entry protection
- spray drift
- wildlife and aquatic organisms
- plant protection
- non-target insects
- product performance
- product chemistry

Herbicides that have already been registered for use in other areas, agriculture for instance, will already have toxicology data and may only require additional testing specific to their intended use.

For herbicides that show promise in primary screening, secondary screening is usually the next step. This part of the herbicide development process involves testing in research plots under actual and simulated field conditions. Whereas primary screening during the discovery stage is usually conducted in-house by herbicide company researchers, secondary screening is a much more involved process and is often contracted out to research consultants, universities, and state or federal scientists (Bovey 2001; Glover 1991). Secondary screening involves collection of data to provide information on application methods, field rates, spectrum of control, crop tolerances, compatibility with other pesticides, half-life, biological longevity, and loss from runoff and leaching (Bovey 2001).

With data obtained from secondary screening, the decision may be made to continue to develop the product based on considerations such as remaining length of patent protection and support of the intended market (Bovey 2001). New products face an additional hurdle in that they must be shown to be more effective or selective than already tested, registered, and trusted products to justify the substantial cost of their registration.

At each step along the process from discovery to registration to production, before proceeding to the next step, the question is asked: do patent protection and intended market justify further testing of this product (Blair et al. 2004). Once the EPA grants a product label, manufacturing and distribution of the product will depend on the amount of time left on the patent (up to 17 years) as well as other market considerations (Bovey 2001).

In comparison with the agricultural herbicide market, the number of herbicides available to control woody vegetation is very small (Zedaker and Seiler 1988). The process from discovery to registration outlined above can cost in excess of 50 million dollars per product registered (Bovey 2001). Therefore, many herbicides that are registered for the control of woody vegetation have been developed for other markets with their usefulness for controlling woody species discovered during the development process or after registration for other uses (Bovey 2001).

Herbicide Screening Techniques

Herbaceous plant herbicide screening is commonly performed for herbicides intended for use in the agriculture industry. The short life span and rapid response of herbaceous species to herbicide treatment allows primary screening to be accomplished in less than 60 days, from application to final assessment (Zedaker and Seiler 1988).

Conventional field screening for herbicide efficacy in woody plants is a time and cost intensive process. Field screenings for IVM herbicides often begin after biological and toxicological screens have been performed on new herbicides intended for the agricultural market rather than coming directly to the IVM market (Blair et al. 2004). The resources required by these screenings are the result of the area needed to replicate treatments, the amount of newly formulated herbicide needed, and the physiology/phenology of woody plants. Per herbicide-rate-replication, field plots no less than 1/20 ha and as large as 1/5 ha are needed to ensure that a statistically adequate number of individuals are included in the screen (Zedaker and Seiler 1988). Newly formulated herbicides, still in the discovery stage, are often produced only several grams at a time. To apply herbicides across a spectrum of rates to several replications at typical field application rates may require several kilograms of newly discovered herbicide active ingredient, often very expensive to synthesize.

Woody species used in field screenings can be germinated and grown in greenhouse conditions and transplanted to field plots or germinated in the field. In either instance, these plants require an establishment period once planted in the field to develop similar hardiness to plants encountered in operational settings. When the establishment period is overlooked, screening results may not correlate well with operational results. Woody plants can be slow to respond to herbicide treatments, often not showing full effects of a product until the second growing season following application (Zedaker and Seiler 1988). Woody plants may also appear heavily damaged in the season of herbicide application and in the next growing season produce healthy, undamaged foliage or "grow-out" of the herbicide injury. Therefore, it is often necessary to record responses of treated plants from 2 growing seasons, requiring 15 to 26 months to complete a screening (Zedaker and Seiler 1988).

Given that herbicides being developed for IVM have already undergone screening for biological activity and toxicity testing, the terminology used to describe the stages of the screening process for these herbicides is different. Primary screening for woody plant herbicides describes field trials of experimental herbicides on crop and weed species to eliminate herbicides with little activity on species of interest (Glover 1991). During primary screening, application timing and rate spectrum may also be refined (Glover 1991). Products in primary screenings are always applied using tightly calibrated research equipment, while secondary screenings are generally applied using research equipment, but may also employ well calibrated operational equipment (Glover 1991). Secondary field evaluations usually contain fewer treatments than primary screens, focusing efforts on those treatments which exhibited promise during primary screening (Glover 1991).

Products with promising results in secondary screenings go on to operational evaluations, where they are applied using ground and aerial equipment to confirm rates and application timing from previous research trials (Glover 1991). This evaluation represents the primary cross-over from research and development to operations (Glover 1991). These evaluations usually involve few treatments and are installed at several locations across a range of soil and vegetation types (Glover 1991) to account for the variety of vegetation and soil combinations that might be encountered in operational settings.

Screening methods developed by Zedaker and Seiler (1988) for testing woody plant herbicide efficacy in greenhouse facilities have been shown to be less costly than and correlate well with conventional field screening techniques (Blair et al. 2006). The rapid screening process was developed to produce seedlings with secondary woody tissue in less than 1 year for use in primary screening for IVM herbicides (Zedaker and Seiler 1988). Rapid greenhouse screening has shown promise of decreasing the cost of testing new compounds for the IVM market and potentially increasing the number of labeled herbicides available to industrial vegetation managers.

The primary advantage of rapid greenhouse screening is a reduction in time and resources required to plant, establish, and measure results of a screen. Rapid greenhouse screening shortens the time necessary to produce sprayable seedlings, as defined by size and amount of woody tissue. This is begun by stratifying and planting seed or transplanting bare-root nursery stock and growing plants to desired size in greenhouse facilities. Nursery stock is not preferred due to the variability of the physiological condition of the plants and difficulty in repeating experiments with exactly the same stock (Zedaker and Seiler 1988).

Rapid screening trials require less space to conduct than traditional field screenings. Rapid screening is conducted on smaller plants (usually small seedlings) that can be grown in containers, saving space and increasing their portability. Containerized seedlings can be rotated through a variety of growth environments, reducing dependency on the seasons. Seedlings can be transferred from a greenhouse to a slat-house to a cold room as they set bud and enter dormancy. Their chilling requirements can be satisfied with six weeks of consistent cold temperatures (2°C). They can be subsequently returned to a greenhouse in spring-like conditions (16 hr photo-period, 13-32°C) and allowed to re-flush. This cycle shortens the time to pre-spray development of woody tissue or to post-spray second growing season evaluation. Overall time to final results from screening has been 13-16 months in past work (Blair et al. 2004; Zedaker and Seiler 1988).

Once seedlings have reached sprayable size, herbicide is applied using tightly calibrated research equipment. Variance in the application rate as low as 1% is possible and less than 3% is common with this type of application (Zedaker and Seiler 1988). The sprayhood makes use of a single flat fan nozzle that applies spray solution over the top of seedlings situated on an adjustable platform inside an enclosed spray booth. This type of application is much more accurate than typical field applications where variances in application rate are rarely below 3 percent and often above 5 percent (Zedaker and Seiler 1988). After herbicide application, seedlings are visually evaluated and measured, go through dormancy, and are returned to a greenhouse for final evaluation.

Rapid screening methods serve to benefit the IVM market by allowing testing of herbicides developed for agriculture on woody species at significantly lower cost in comparison to conventional field trials (Blair et al. 2004). Lessened costs and time commitments make woody plant herbicide screening less prohibitive and potentially increases the number of herbicides that companies in the IVM market might be able to screen in a given timeframe.

Forestry Herbicides

Herbicides used in forestry are usually agricultural and industrial herbicides that have been developed and labeled for use on forestland through special research efforts (Miller 1987). Many of the herbicides that are widely used today in forest management have been available since the early 1980s and before (Miller 1987). Silvicultural application of herbicides to control competing vegetation was first reported in the late 1940s with the use of phenoxy herbicides 2,4-D [2,4-dichlorophenoxy)acetic acid] and 2,4,5-T [2,4,5-trichlorophenoxy)acetic acid] (McCormack 1990; Vencill 2002). Many of these early applications were treatments to release crop trees, most often conifers, from competition (McCormack 1994). Use of herbicides for site preparation treatments began in the 1960s and 70s and included the use of materials with some residual soil activitiy, including picloram [4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid] (McCormack 1994; Vencill 2002). Following the loss of registration of 2,4,5-T and the declining use of 2,4-D in the 1970s, glyphosate [N-(phosphonomethyl)glycine] and triclopyr [[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid] became available to applicators, followed several years later by sulfometuron [methyl 2-[[[[(4,6-dimethyl-2-pyrimidinyl)amino]carbonyl]amino] sulfonyl]benzoate]and imazapyr [(±)-2-[4.5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-y1]-3-pyridinecarboxylic acid] (Vencill 2002). The latter two were especially significant for their silvicultural effectiveness at low rates of active ingredient per hectare relative to early herbicides (McCormack 1994).

Glyphosate is a broad spectrum foliar applied herbicide sold under the trade names Roundup® and Accord®, among many others (Vencill 2002). Glyphosate is absorbed through foliage and translocated symplastically in the target plant, but is rapidly adsorbed and inactivated by contact with soil (Vencill 2002). Several biochemical processes are disrupted by glyphosate, but inhibition of EPSP (5-enolpyruvylshikimate-3-phosphate) synthesis and resulting disruption of biosynthesis of aromatic amino acids is thought to be the most plant lethal action (Bovey 2001; Vencill 2002). Symptoms of herbicide injury caused by glyphosate include foliar chlorosis followed by necrosis (Bovey 2001).

Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], trade name Velpar®, is a soil and foliar applied triazine herbicide used to control many annual and perennial broadleaf and grass weeds as well as brush species (Vencill 2002). The herbicide is readily absorbed by roots and translocated upwards in the apoplast, but is less readily translocated out of leaves when foliar applied (Vencill 2002). Hexazinone is a photosynthetic inhibitor, killing plants by blocking the electron transport chain and disrupting the production of ATP and $NADPH_2$ (Vencill 2002). The disruption of plant energy production is lethal, but by-products of the blocked electron transport chain cause plant membranes to leak, bringing on tissue death through desiccation (Bovey 2001). Symptoms of plants injured by hexazinone include foliar chlorosis followed by necrosis (Vencill 2002).

Imazapyr, sold under the trade names Arsenal® and Chopper®, among others, is an imidazoline herbicide used to control annual and perennial broadleaf and grass weeds as well as deciduous trees in non-cropland areas (Vencill 2002). Imazapyr is soil active and can applied pre or post emergence, but maximum activity is obtained when foliar applied to actively growing weeds (Vencill 2002). Imazapyr is an ALS (acetolactate synthase) or AHAS (acetohydroxyacid synthase) inhibitor, killing plants by inhibiting an enzyme critical to branched chain amino acid synthesis (Vencill 2002). Growth is inhibited shortly after application and may be followed by foliar chlorosis and necrosis, however affected plants can be very slow to show symptoms of herbicide injury (Bovey 2001).

Metsulfuron [2-[[[[4-methoxy-6-methyl-1,3,5-triazin-2-y1)amino]carbonyl] amino]sulfonyl]benzoic acid] is a sulfonylurea foliar applied herbicide sold under the trade name Escort® (Vencill 2002). Metsulfuron is an ALS or AHAS inhibitor, killing plants by inhibiting branched chain amino acid synthesis (Vencill 2002). Growth of plants treated with metsulfuron is inhibited several hours after application, with foliar chlorosis and necrosis appearing gradually several weeks later (Vencill 2002).

Picloram is a plant growth regulator herbicide from the pyridinecarboxylic acid chemical family (Vencill 2002). It is readily absorbed by foliage and roots of target plants and translocated symplastically and apoplastically (Vencill 2002). Picloram is sold under the trade name Tordon® and can be used for the control of many annual and perennial broadleaf weeds and woody plantsm, while most grasses are resistant at labeled rates (Vencill 2002). Picloram is a plant growth regulator, mimicking the natural plant growth hormone auxin to control the growth of meristematic plant tissues (Bovey 2001). Symptoms of herbicide injury from picloram include epinasty or curling and twisting of leaves, stems, or roots followed by necrosis as the plant's vascular system becomes distorted and plugged (Bovey 2001). These symptoms are similar to other plant growth regulators, including triclopyr and the phenoxy herbicides 2,4-D and 2,4,5-T (Bovey 2001).

Sulfometuron is a sulfonylurea herbicide sold under the trade name Oust® that can be applied pre or post emergence (Vencill 2002). It has a broad spectrum of activity against annual and perennial grasses and broadleaf weeds (Vencill 2002). Sulfometuron is an ALS or AHAS inhibitor, killing plants by inhibiting branched chain amino acid synthesis (Vencill 2002). Plant growth is usually inhibited by sulfometuron several hours after application, but symptoms of injury, including foliar chlorosis and necrosis, may not appear for 2 to 3 weeks or more (Vencill 2002).

Triclopyr is an auxin type selective herbicide from the pyridinecarboxylic acid family (Vencill 2002). It is sold under the tradename Garlon® and can control annual broadleaf weeds as well as tree and brush species (Vencill 2002). Triclopyr is absorbed by roots and foliage and translocated to the site of action in the target plant where it acts as a synthetic auxin, inhibiting growth of plant tissues (Vencill 2002). Triclopyr causes epinasty, including leaf cupping and curling, followed by necrosis in affected tissues (Vencill 2002).

Chapter 3: Materials and Methods

Facilities

Facilities at the Reynolds Homestead Forest Resources Research Center in (RHFRRC) Critz, VA and Virginia Tech's Blacksburg Campus were utilized to grow, treat, and over-winter plants in the rapid greenhouse seedling and germinal screenings. Facilities at RHFRRC included: a heated glass greenhouse with a plastic double wall and shade cloth covering maintained at temperatures between 15°C and 43°C during the growing season, a plastic covered hoop-house maintained at minimum overnight temperature of 5°C, and two 4.9 m by 4.9 m walk-in coolers maintained at 2°C for overwintering plants. Facilities on the Virginia Tech Campus in Blacksburg included a heated glass greenhouse maintained at temperatures between 13°C and 26°C as well as growth chambers used to perform seed screening experiments.

Greenhouse Seedling Screening

The greenhouse seedling screening was performed summer 2010 – spring 2011 at RHFRRC. Five species were included in the screening: black locust (*Robinia Pseudoacacia* L.), loblolly pine (*Pinus taeda* L.), red maple (*Acer rubrum* L.), sweetgum (*Liquidambar styraciflua* L.), and water oak (*Quercus nigra* L.). Following recommended stratification periods (Table 3.1), hardwood seeds were germinated in D16-containers (16 cm^3) (Stuewe and Sons, Corvalis, OR) filled with potting mix (Table 3.2), using seed purchased from the Louisiana Seed Company. Within one week of planting, greenhouse trays were treated with Banrot® [etridiazole and thiophanate-methyl], a broad spectrum fungicide at labeled rates for containerized seedlings (Scotts-Sierra 2006).

Table 3.1. Published seed stratification periods (Schopmeyer 1989; Young and Young 1992).

Species	Published Time Period (days)	Published Temperature (°C)	Actual Time Period Stratified (days)
Black locust	n/a	n/a	30
Loblolly pine	30-60	3-5	transplanted
Red maple	n/a	n/a	0
Sweetgum	15-90	2-5	30
Water oak	30-60	0-5	90

Table 3.2. Potting mix recipe for greenhouse seedling and germinal screenings.†

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

†Sufficient potting mix to fill 2450 16 cm^3 cells.

Due to space constraints seedlings were germinated in different environments, black locust and sweetgum in the greenhouse and red maple and water oak in the hoop-house. Loblolly pine seedlings were purchased as 1-0 bare root stock from a North Carolina Department of Forestry nursery and transplanted with potting mix into D16-containers. Loblolly pine seedlings were not started from seed to guard against mortality from dampening off encountered in previous experiments. After the last expected frost date (May 1st), all seedlings were moved outdoors and allowed to reach a height of approximately 41 cm. They were topped for height consistency and allowed to flush out again prior to treatment.

Seedlings of adequate size and quality were split into two groups in an effort to further shorten the screening process. In past research, seedlings have been allowed to go through a dormancy period then to re-flush before receiving herbicide treatment in their second growing season (Blair et al. 2004). This ensures that they have developed woody tissue prior to treatment. In this project, one group of seedlings was sprayed during the first growing season – the 1-0 group. After herbicide application, the 1-0 seedlings were chilled and allowed to re-flush before final mortality evaluations. Concurrently, the unsprayed group of seedlings – the 2-0 group – were chilled and allowed to the reflush in the greenhouse before herbicide treatments were applied. The 2-0 seedlings had more woody tissue, greater starch reserves, and more closely resembled field-grown plants.

Herbicide treatments for the 1-0 seedlings were applied in a spray booth (De Vries Manufacturing – model #: SB8) located at RHFRRC on July 19, 2010. The experimental design was a randomized complete block replicated three times with treatments applied to 10 seedling subsamples of each species. Greenhouse trays containing 10 seedlings of each of the five species were arranged such that seedlings would receive equal coverage of spray solution without any significant leaf area overlap. Shorter seedlings were elevated to achieve a uniform canopy across the spray booth using extra D-16 containers. Herbicides supplied by Dow AgroSciences (DAS) were mixed in deionized water approximately 24 hours ahead of time and applied at rates specified in Table 3.3. Eight experimental herbicides were applied at 35, 70, 140, and 280 g ae ha^{-1}, triclopyr at 140, 280, and 560 g ae ha^{-1}, and DAS Agri-dex surfactant was mixed at 1.25% v/v in all treatments.

Table 3.3. Herbicide application rates used in this study.

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

The sprayhood was fitted with an 8001E-HSS TeeJet spray tip mounted 30.5 cm above the seedling canopy and calibrated to deliver a carrier rate of 187 L ha^{-1}. To monitor consistency of the application, each pass of the spray nozzle was observed and nozzle output calibrated before spraying each block. Herbicides were applied beginning with the lowest rate and proceeding in order to the highest (1x) rate. The nozzle system was flushed between herbicides with a 50% solution of acetone and deionized water, followed by a 1N NaOH solution, followed by deionized water, and a blast of CO_2 from the hood's pressure system. Finally, the walls of the spray booth were rinsed with water to remove any residual herbicide and rinse solution from the interior surfaces of the hood.

Sprayed plants were allowed to dry in the shade and moved into the greenhouse for protection from precipitation. They were placed in shallow metal trays which allowed them to be watered from below to prevent herbicide contamination from adjacent treatments. Plants remained in the greenhouse in bottom-watering trays exposed to shortening fall day-lengths until budset occurred, at which point they were placed in a cold room at 2°C with no lighting for 1000 hours (approximately 6 weeks) to satisfy dormancy requirements. During chilling, plants were watered every 2 weeks and treated twice with Zerotol® [hydrogen dioxide] algaecide/fungicide to control mold.

During budset development, evaluations were conducted to assess the level of herbicide injury and measure changes in plant height. These data were analyzed for correlation with final responses of height, herbicide injury, and mortality. Evaluations of 1-0 seedlings were conducted at 2, 4, 11, and 32 weeks after treatment (WAT) using the rating scale in Table 3.4 to visually evaluate herbicide injury. Following chilling at 2°C, 1-0 seedlings were moved to the Virginia Tech Department of Forest Resources and Environmental Conservation Greenhouse in Blacksburg, VA. The seedlings were brought into spring-like conditions (16 hr photoperiod and 13°C minimum overnight temperature) and allowed to re-flush for 6 weeks before final evaluation at 32 WAT. Time from treatment to measurement of final results was 32 weeks.

Table 3.4. Injury rating scale for evaluation of greenhouse seedling and germinal screenings.

Rating	Plant 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)

Shortening daylengths and outdoor exposure to overnight temperatures encouraged budset in the untreated 2-0 seedling group prior to placement in the cold room. Following 6 weeks of chilling at 2°C, seedlings were returned to the RHFRRC greenhouse under spring-like conditions (16-hr photoperiod and 15°C minimum overnight temperature) and allowed 6 weeks to re-flush. Herbicide treatments were applied to the 2-0 seedling group on February 19, 2011 with the same methodology used for the 1-0 seedlings. After application, the 2-0 seedlings were placed in bottom-watering trays in the greenhouse for 6 weeks before final evaluation of height, herbicide injury (Table 3.4), and mortality. Time from treatment to measurement of final results was 6 weeks.

Germinal Screening

The germinal screening experiment was carried out in the greenhouse at RHFRRC. Following completion of recommended stratification periods in Table 3.1, seeds of four species, black locust, loblolly pine, sweetgum, and water oak, were planted in 52 cm x 40 cm x 6.35 cm greenhouse flats with holes for drainage. Flats were filled 5 cm deep with potting mix (Table 3.2) and seeds were planted approximately 5 cm apart in rows of 10, by species. Planting of species was staggered to account for differences in the rate of emergence and allow plants to reach a uniform height before herbicide application (Table 3.5). Red maple was not included in germinal screening due to germination issues with seed at the time of planting.

Table 3.5. Germinal production timeline for this study.

Time (days)	Activity
0	Fill trays & plant water oak
1	Plant loblolly pine
7	Plant sweetgum
8	Plant black locust
9	Banrot® all trays
38	Spray
80	Evaluation

Germinal treatments were applied on February 19, 2011 at RHFRRC at the same time as 2-0 seedling applications. Based on 11 WAT evaluation data from 1-0 greenhouse seedlings, the most (DAS 729) and least effective (DAS 896) herbicides, and triclopyr were used in the germinal screening. Rates of herbicide acid equivalent, surfactant, and carrier rate used in germinal screenings were identical to 1-0 and 2-0 greenhouse seedling applications (Table 3.3). Each tray of germinals was placed in the spray booth with 2-0 greenhouse seedlings and the treatment applied to both experimental units simultaneously. Germinals were returned to the greenhouse and watering from above resumed several days after treatment. At 6 WAT, germinal heights and herbicide injury were evaluated using the same scale (Table 3.4) as the seedling screening. Time from treatment to measurement of final results was 6 weeks.

Seed Screening

The seed screening experiment was carried out in labs located on Virginia Tech's Blacksburg campus and followed procedures of Blair et al. 2004. Seeds of the five species used in the greenhouse screening, black locust, loblolly pine, red maple, sweetgum, and water oak were acquired from the Louisiana Seed Company. Seeds of each species were soaked overnight in water. Floating seeds were culled, the seed drained, placed in sealed bags, and placed in a cooler at 4°C for the required stratification times (Table 3.1).

Following stratification, seeds were counted into groups of 20 and placed in Petri dishes containing dry filter paper. Applications were made as a pre-soak treatment, consisting of a 24 hour soak in 10 mLs of herbicide solution to provide enough volume to saturate the filter paper and allow for absorption by the seeds. Due to the size of the Petri dishes (10 cm diameter) and the pre-soak application method, it was impractical to make application calculations based on area. Rates of herbicide acid equivalent from greenhouse seedling screenings were converted to a percent volume of the carrier rate (187 L ha^{-1}). Herbicides DAS 402, 729, and triclopyr were mixed in deionized water on a percent basis at rates (Table 3.3) equivalent to those used in the greenhouse seedling screening in addition to a deionized water control treatment. Three temporal replications were used in the seed screening study.

Excess liquid solution was drained from each Petri dish after the 24 hour soak without removing the seeds and filter paper (Blair et al. 2004). Petri dishes were placed in a growth chamber at 25ºC and 80% relative humidity with 16 hour photoperiods to allow germination. Seeds were measured at 4, 8, 12, and 14 days after treatment (DAT), except during the second temporal replication where seeds were measured only at 4,8, and 14 DAT. Length of live tissue emerging from the seed was measured at each evaluation. At the end of the observation period, mean tissue length per treatment and mean percent germination for each treatment were calculated (Blair et al. 2004). Time from application to measurement of final results was 14 days.

Data Analysis

Analysis of variance was performed on each screening type separately to test for herbicide and rate effects and Tukey's HSD test was used for pair-wise comparisons between treatment means. Seed data were not analyzed at 12 DAT because there were fewer than 3 replications at that time. An unbalanced design was created where triclopyr did not have the same number of rates as the experimental DAS herbicides. This was corrected using a data imputation method to estimate values for triclopyr 0.125x rate (Shaw and Mitchell-Olds 1993). Percent mortality and percent germination responses were transformed using an arcsine data transformation procedure as recommended by Gomez and Gomez (1984).

Regression analysis was run on 1-0 greenhouse seedling results from pre-dormancy (11 WAT) to determine if they correlate with post dormancy results (32 WAT). Regression analysis was also run between 1-0 and 2-0 seedling, 1-0 seedling and germinal, and 1-0 seedling and seed data to determine if very early screenings have the ability to predict seedling screenings.

Chapter 4: Results

1-0 Greenhouse Seedling Screening

1-0 seedlings were more sensitive to herbicide induced injury, height loss, and mortality than the 2-0 seedlings (Table 4.1). Paired t-test results indicate significant ($p<0.01$) differences between 1-0 and 2-0 final height, 26.3 and 45.3 cm, and percent mortality, 46.8% and 9.5%, respectively. Plant injury ratings were not significantly different, averaging 4.16 and 4.17, for the 1-0 and 2-0 groups, respectively.

Table 4.1. Comparison of 1-0 and 2-0 greenhouse seedling final evaluation results. †

Response	1-0 Seedling 32 WAT	2-0 Seedling 6 WAT
Height	26.3 cm***	45.3 cm
Injury	4.16	4.17
Mortality	46.8%***	9.5%

†Significant differences between seedling group means are ranked by t-values: <0.1*, <0.05**, <0.01***.

Seedlings died back from top, causing height to often decrease following treatment (Tables 4.2-4.4). In most species-herbicide combinations, height decreased through 11 weeks after treatment (pre-dormancy), then increased up to 32 weeks after treatment (WAT), post dormancy. For example, mean red maple seedling height decreased from 55.6 to 40.1 cm from 0 to 11 WAT and then increased to 48.3 cm at 32 WAT.

Table 4.2. Mean height response (cm) of 1-0 seedlings 2 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	37.1 a	41.2 a	48.0 a	47.1 ab	37.6 a	42.2 AB
DAS 402	40.5 a	39.6 a	46.5 a	45.5 ab	43.4 a	43.1 AB
DAS 534	39.2 a	39.0 a	48.8 a	46.5 ab	39.8 a	42.6 AB
DAS 548	38.0 a	39.6 a	44.5 a	44.2 ab	38.5 a	41.0 AB
DAS 602	40.4 a	41.4 a	49.1 a	46.9 ab	41.2 a	43.8 AB
DAS 729	39.1 a	39.1 a	45.1 a	42.2 b	39.1 a	40.9 AB
DAS 779	37.5 a	40.5 a	43.7 a	46.7 ab	35.6 a	40.8 B
DAS 896	38.7 a	42.5 a	50.5 a	49.1 a	44.0 a	45.0 A
Triclopyr	38.1 a	44.4 a	48.5 a	46.8 ab	41.2 a	43.8 AB
Overall Mean	38.7 B	40.8 B	47.2 A	46.1 A	40.0 B	42.6

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.3. Mean height response of 1-0 seedlings 11 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	12.3 a	27.1 a	31.9 bc	46.9 a	28.4 cd	29.3 A
DAS 402	1.4 b	0.0 c	49.6 a	24.2 d	43.9 ab	23.8 ABC
DAS 534	1.1 b	0.0 c	43.6 ab	13.6 e	30.2 bcd	17.7 BC
DAS 548	3.0 ab	1.2 c	27.2 c	37.4 abc	34.5 abcd	20.7 ABC
DAS 602	7.4 ab	1.4 c	49.4 a	35.0 bc	42.2 abc	27.1 AB
DAS 729	2.4 b	0.0 c	33.8 bc	5.2 e	36.3 abc	15.5 C
DAS 779	5.1 ab	17.0 b	33.9 bc	43.6 ab	20.0 d	23.9 ABC
DAS 896	2.3 b	9.3 bc	49.8 a	46.0 a	47.4 a	31.0 A
Triclopyr	4.6 ab	18.4 ab	41.4 abc	27.2 cd	27.2 cd	23.8 ABC
Overall Mean	4.4 C	8.3 C	40.1 A	31.0 B	34.4 B	23.6

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.4. Mean height response of 1-0 seedlings 32 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	10.5 a	28.0 a	40.0 bc	49.1 a	31.3 def	31.8 A
DAS 402	0.1 b	0.0 d	59.8 a	25.1 cd	47.2 abc	26.4 ABC
DAS 534	0.1 b	0.0 d	54.5 a	13.1 de	26.8 ef	18.9 BC
DAS 548	2.4 b	0.8 d	32.9 c	42.6 a	41.6 bcd	24.1 ABC
DAS 602	1.2 b	1.0 d	59.0 a	39.8 ab	54.8 ab	31.2 AB
DAS 729	0.8 b	0.0 d	39.3 bc	4.7 e	35.2 cde	16.0 C
DAS 779	3.7 b	13.7 bc	39.2 bc	50.7 a	18.5 f	25.2 ABC
DAS 896	1.9 b	8.7 cd	60.9 a	49.3 a	58.7 a	35.9 A
Triclopyr	5.5 ab	19.2 ab	49.1 ab	29.1 bc	33.0 de	27.2 ABC
Overall Mean	2.9 C	7.9 C	48.3 A	33.7 B	38.6 B	26.3

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Higher injury ratings corresponded with lower height measurements (Tables 4.2.-4.8). Injury ratings for overall species and herbicide means increased through 11 WAT and then decreased post dormancy at 32 WAT. For example, mean red maple injury ratings increased from 1.0 to 4.3 (scale of 1 to 6) from 0 to 11 WAT and then decreased following dormancy to 2.9. Comparison of 32 weeks after treatment (WAT) height, injury rating, and percent mortality suggest that black locust and loblolly pine were the most sensitive species, while red maple was the least sensitive species (Tables 4.4, 4.8, 4.9).

Table 4.5. Mean plant injury rating in 1-0 seedlings 2 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	5.0 d	2.4 e	3.8 ab	3.1 f	3.0 bcd	3.5 B
DAS 402	5.6 abc	4.1 abc	3.0 d	4.0 bc	2.6 de	3.9 AB
DAS 534	5.8 a	4.4 ab	3.8 ab	4.0 b	3.6 abc	4.3 A
DAS 548	5.7 ab	4.3 ab	4.2 a	3.8 bcd	3.0 cd	4.2 A
DAS 602	5.7 ab	4.0 bc	3.5 bc	3.6 cde	2.1 e	3.8 AB
DAS 729	5.9 a	4.6 a	4.2 a	4.4 a	2.0 e	4.2 A
DAS 779	5.4 c	3.0 d	3.9 ab	3.3 ef	3.7 a	3.8 AB
DAS 896	5.5 bc	3.7 c	3.1 cd	3.6 de	2.8 d	3.7 AB
Triclopyr	5.7 ab	2.8 de	4.0 a	3.7 bcd	3.6 ab	4.0 AB
Overall Mean	5.6 A	3.7 B	3.7 B	3.7 B	2.9 C	3.9

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.6. Mean plant injury rating in 1-0 seedlings 4 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	5.4 b	4.1 c	4.5 ab	4.0 d	4.2 ab	4.5 BC
DAS 402	5.8 a	5.8 ab	3.7 ef	4.6 bc	3.3 d	4.6 ABC
DAS 534	5.9 a	6.0 a	4.0 cde	5.0 ab	4.3 ab	5.0 A
DAS 548	5.8 a	5.7 ab	4.6 a	4.2 cd	3.9 bc	4.8 ABC
DAS 602	5.8 a	5.6 ab	3.9 def	4.3 cd	3.4 cd	4.6 ABC
DAS 729	5.8 a	5.9 a	4.4 abc	5.5 a	3.2 d	5.0 AB
DAS 779	5.6 ab	4.5 c	4.2 bcd	4.0 d	4.5 a	4.6 ABC
DAS 896	5.8 a	5.2 b	3.6 f	4.2 cd	3.3 cd	4.4 C
Triclopyr	5.7 ab	4.6 c	4.4 abc	4.6 bc	4.5 a	4.8 ABC
Overall Mean	5.7 A	5.3 B	4.2 D	4.5 C	3.9 E	4.7

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.7. Mean plant injury rating in 1-0 seedlings 11 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	5.6 a	4.9 c	4.7 ab	4.1 e	4.3 abc	4.7 AB
DAS 402	6.0 a	6.0 a	3.8 e	4.9 bc	3.6 d	4.9 AB
DAS 534	6.0 a	6.0 a	4.1 cde	5.3 b	4.4 ab	5.2 AB
DAS 548	5.9 a	6.0 a	4.8 a	4.3 e	4.0 abcd	5.0 AB
DAS 602	5.6 a	5.9 a	4.0 de	4.5 de	3.7 cd	4.7 AB
DAS 729	5.9 a	6.0 a	4.6 ab	5.8 a	3.8 bcd	5.2 A
DAS 779	5.9 a	5.1 bc	4.5 abc	4.0 e	4.7 a	4.8 AB
DAS 896	5.9 a	5.6 ab	3.9 e	4.2 e	3.9 bcd	4.7 B
Triclopyr	5.7 a	5.0 c	4.3 bcd	4.8 cd	4.5 ab	4.9 AB
Overall Mean	5.8 A	5.6 B	4.3 D	4.7 C	4.1 D	4.9

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.8. Mean plant injury rating in 1-0 seedlings 32 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	4.8 c	4.0 d	3.5 abc	2.5 e	4.0 ab	3.7 B
DAS 402	6.0 a	6.0 a	2.2 d	4.1 bc	3.0 bc	4.3 AB
DAS 534	6.0 a	6.0 a	2.8 bcd	4.8 ab	4.4 a	4.8 A
DAS 548	5.7 ab	5.9 a	3.6 ab	2.6 de	3.2 bc	4.2 AB
DAS 602	5.8 ab	6.0 a	2.2 d	2.6 de	2.7 c	3.9 B
DAS 729	5.8 ab	6.0 a	3.1 abcd	5.4 a	3.6 abc	4.8 A
DAS 779	5.6 ab	4.9 bc	3.7 a	2.3 e	4.4 a	4.2 AB
DAS 896	5.7 ab	5.4 ab	2.4 d	2.9 de	2.7 c	3.8 B
Triclopyr	5.2 bc	4.3 cd	2.6 cd	3.7 cd	3.4 abc	3.9 B
Overall Mean	5.6 A	5.4 A	2.9 C	3.4 B	3.5 B	4.2

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.9. Mean percent mortality in 1-0 seedlings 32 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	74.2 c	40.8 c	19.2 ab	10.0 d	20.8 ab	33.0 D
DAS 402	99.2 a	100.0 a	0.8 c	41.7 bc	8.3 b	50.0 ABC
DAS 534	99.2 a	100.0 a	5.0 bc	61.7 ab	29.8 ab	59.1 AB
DAS 548	90.0 abc	97.5 a	32.5 a	14.2 cd	13.3 ab	49.5 ABCD
DAS 602	94.2 ab	96.7 ab	1.7 c	17.5 cd	8.4 b	43.7 ABCD
DAS 729	95.8 ab	100.0 a	16.7 abc	85.0 a	15.8 ab	62.7 A
DAS 779	89.2 abc	59.2 c	15.8 bc	6.7 d	37.5 a	41.7 BCD
DAS 896	93.3 ab	79.2 b	2.5 bc	13.3 cd	5.8 b	38.8 ABCD
Triclopyr	82.3 bc	58.5 c	14.4 bc	31.3 cd	25.1 ab	42.3 CD
Overall Mean	90.8 A	81.3 A	12.1 C	31.3 B	18.3 C	46.8

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Response variables height and injury varied in their sensitivity to differences in treatments. Injury rating was generally the most sensitive to herbicide and rate, especially for early detection of treatment differentiation. Significant differences in injury rating due to herbicide were detected for all species as early as 2 WAT (Table 4.5). Height was less sensitive to differences in treatment means in response to herbicide and rate. At 2 WAT, there were significant differences in height due to herbicide only for sweetgum (Table 4.2). By 32 WAT, there were significant differences in height due to herbicide for all species, but especially water oak (Table 4.4). Differences in height and injury caused by herbicide were much more frequent and significant than differences caused by rates.

For consistency between species, figures for all herbicides were included in the results section. Although there may appear to be herbicide by rate interaction in some figures, it is not always statistically significant. Herbicides that had extremely high or low efficacy (<20% or >80% average mortality) prior to dormancy (11 WAT, Table 4.10) were not discussed in detail in the results text because their rates were too high or too low to produce good rate separation.

Table 4.10. Mean percent mortality in 1-0 seedlings 11 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	67.5 b	37.5 c	25.8 ab	5.0 c	19.2 ab	31.0 C
DAS 402	95.8 a	100.0 a	0.8 c	33.3 bc	3.3 b	46.7 ABC
DAS 534	96.7 a	100.0 a	4.2 c	53.3 b	22.5 ab	55.3 AB
DAS 548	88.3 ab	96.7 ab	33.3 a	15.0 c	12.5 ab	49.2 ABC
DAS 602	82.5 ab	95.0 ab	0.8 c	16.7 c	14.2 ab	41.8 ABC
DAS 729	92.5 a	100.0 a	16.7 abc	79.2 a	8.3 ab	59.3 A
DAS 779	85.8 ab	50.0 c	14.2 bc	4.2 c	30.8 a	37.0 BC
DAS 896	93.3 a	75.8 b	1.7 c	9.2 c	2.5 b	36.5 ABC
Triclopyr	80.9 ab	53.3 c	14.7 bc	30.7 bc	24.7 ab	40.9 BC
Overall Mean	87.0 A	78.7 A	12.5 C	27.4 B	15.3 C	44.2

†Means followed by the same lowercase letter are not significantly different within species at $\alpha=0.05$. Overall species and herbicide means followed by the same uppercase letter are not significantly different at $\alpha=0.05$.

Black Locust

Overall, black locust was highly susceptible to herbicide injury (Tables 4.2-4.10, Figures 4.1-4.27). The greatest decrease in mean height of black locust seedlings occurred between 4 and 11 WAT and heights of seedlings did not increase after dormancy (Figures 4.1-4.9). Significant differences in black locust seedling heights in response to herbicide occurred at 11 ($p<0.01$) and 32 ($p<0.0001$) WAT. Significant differences in height in response to rate also occurred at 32 ($p<0.1$) WAT. Height in response to rate for each herbicide appears to differ, however herbicide by rate interaction was not significant for plant height at any evaluation time. Based on height responses, DAS 402 and 534 treatments were most effective on black locust. Heights in these treatments were lower but not significantly different from all other herbicides at 11 WAT. This pattern continued post dormancy, but only DAS 313 treatment heights (10.5 cm) were significantly higher than DAS 402 (0.1 cm) and 534 (0.1 cm) (Table 4.4).

Injury ratings increased rapidly in response to all herbicides and remained high until final evaluation at 32 WAT (Figures 4.10-4.18). In some treatments, a decrease in injury rating was observed at final evaluation. A significant ($p<0.05$) herbicide by rate interaction occurred in injury at 2 WAT. Injury for rates of all herbicides was greater than 5.0 at 2 WAT, except for the lowest rate of DAS 313 (4.3). Significant differences in injury due to herbicide occurred at 4 ($p<0.0001$), 11 ($p<0.05$), and 32 ($p<0.0001$) WAT. At 11 WAT, significant differences in injury in response to herbicide were detected by ANOVA effects test, but were not captured in Tukey's HSD analysis (Table 4.7). Significant differences in injury due to rate occurred at 4 ($p<0.05$) and 32 ($p<0.05$) WAT. Black locust seedlings had the highest injury ratings in response to DAS 534 (5.8) and 729 (5.9) at 2 WAT (Table 4.5). However, injury ratings in these herbicides were not significantly higher than all other herbicides. Post dormancy, DAS 402 (6.0) and 534 (6.0) injury ratings were higher than, but not significantly different from all other herbicides (Table 4.8).

Height, injury, and percent mortality responses at 32 WAT suggest DAS 402 and 534 are the most effective herbicides on black locust seedlings, but responses were not significantly different from means of other herbicides, except for DAS 313 which generally had less of an effect (Tables 4.4, 4.8, and 4.9).

Most herbicides caused greater than 80% mortality in black locust seedlings by 11 WAT, with the exception of DAS 313 which only had 67.5% mortality (Table 4.10, Figures 4.19-4.27). Differences in injury rating in response to rates of DAS 313 were significant at 2 ($p<0.05$) WAT. Although not statistically significant, larger separation of injury ratings in response to rate occurred at 32 WAT when means were 3.7, 4.9, 5.4, and 5.3 for 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Heights of seedlings treated with DAS 313 were reduced by herbicide injury, but mean height did not go below 10.5 cm even at 32 WAT. Although not significant, good rate response was observed at 32 WAT when mean heights were 19.1, 10.2, 7.2, and 5.7 cm for 0.125x, 0.25x, 0.5x, and 1x rates, respectively.

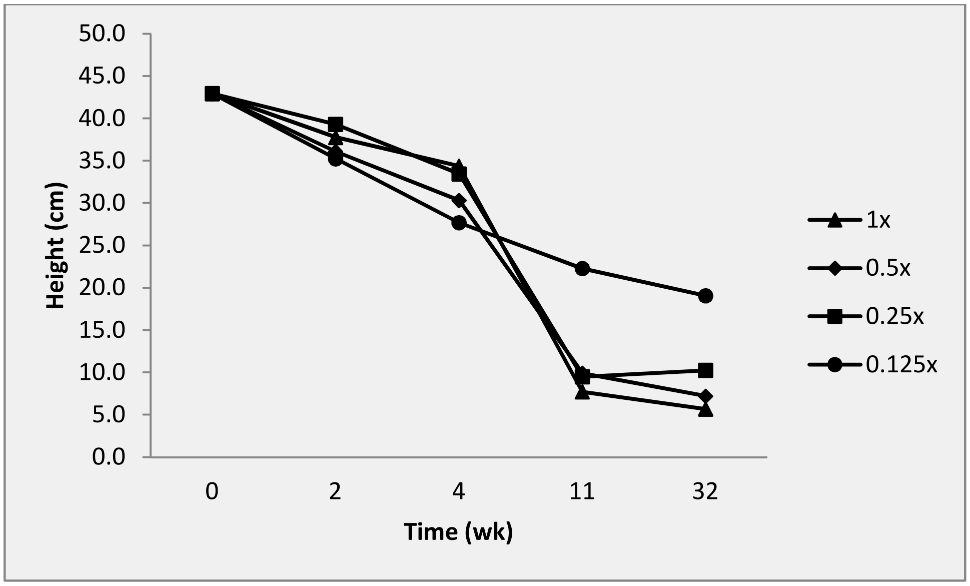

Figure 4.1. Mean height response of 1-0 black locust seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

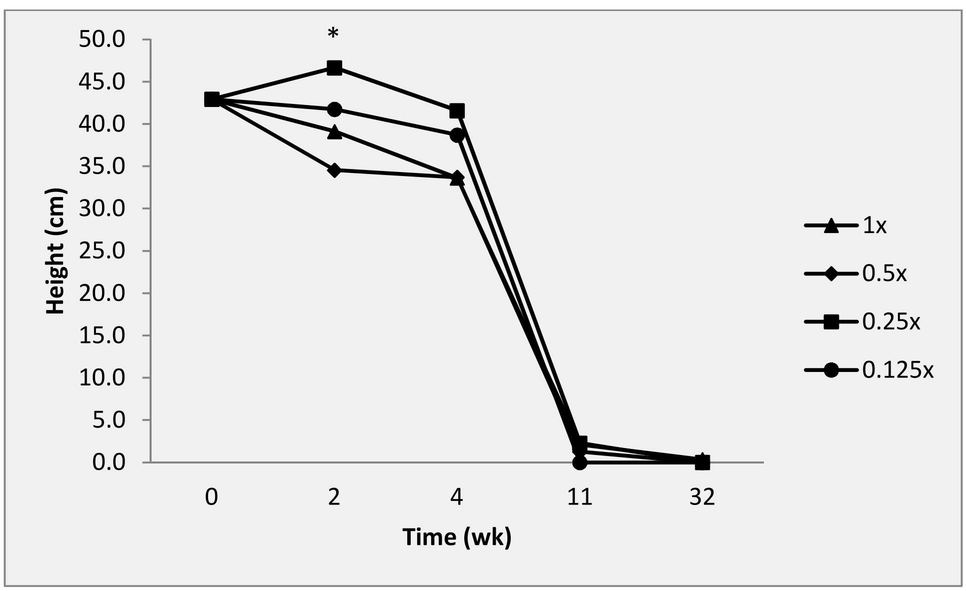

Figure 4.2. Mean height response of 1-0 black locust seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

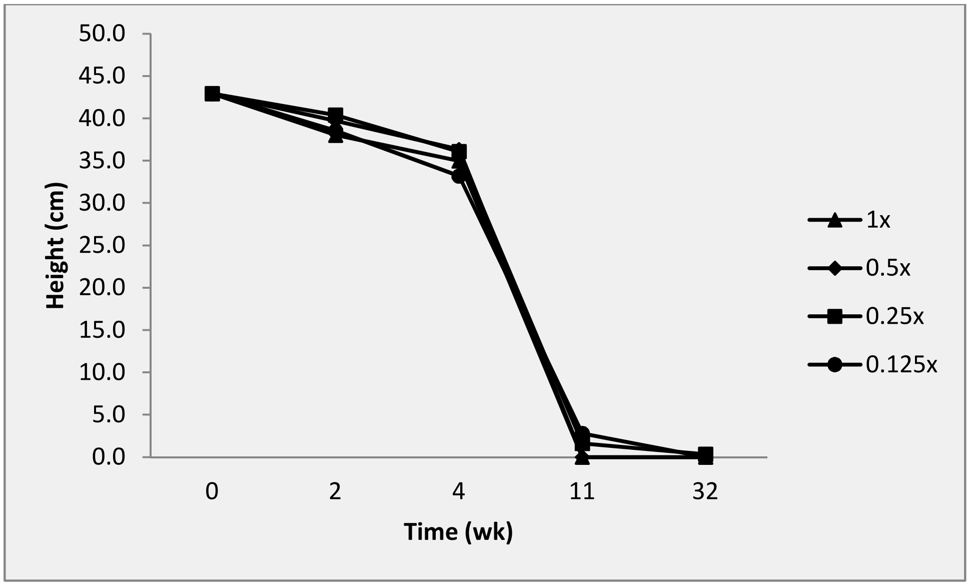

Figure 4.3. Mean height response of 1-0 black locust seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

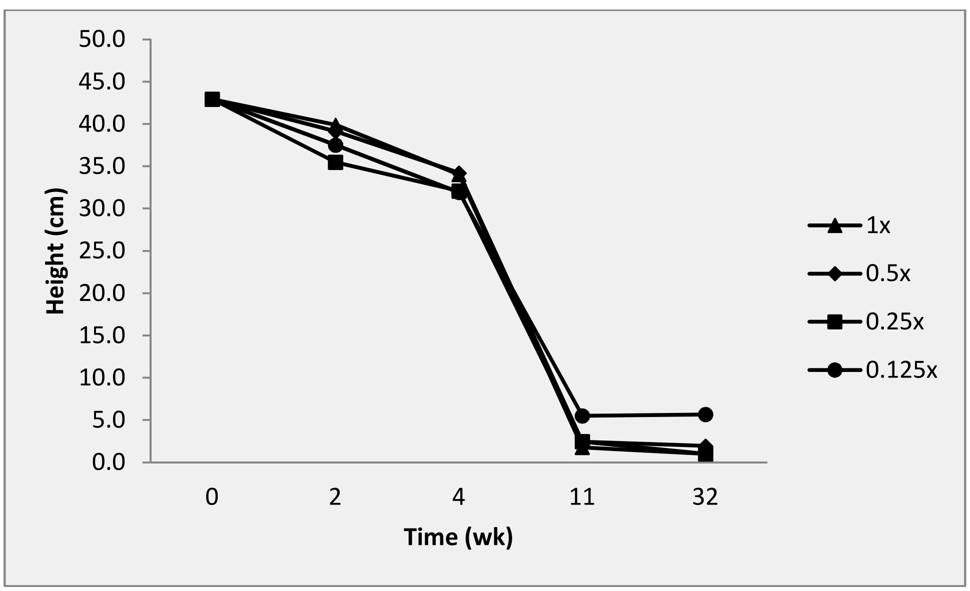

Figure 4.4. Mean height response of 1-0 black locust seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

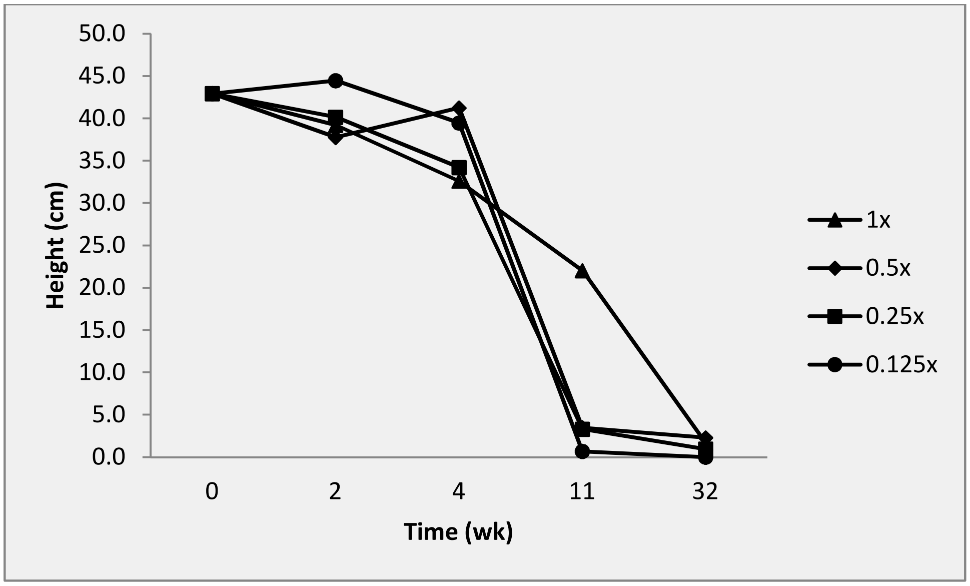

Figure 4.5. Mean height response of 1-0 black locust seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

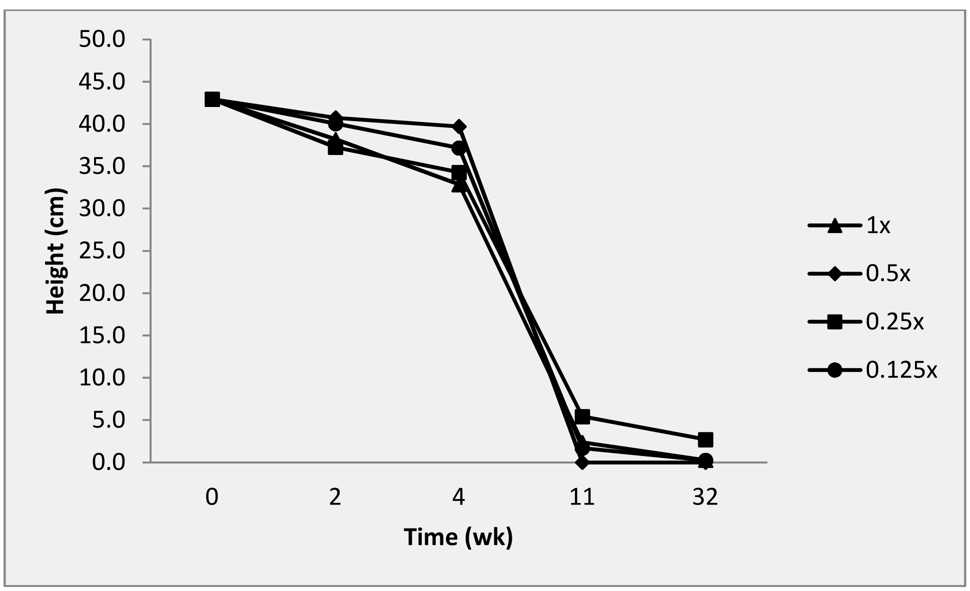

Figure 4.6. Mean height response of 1-0 black locust seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

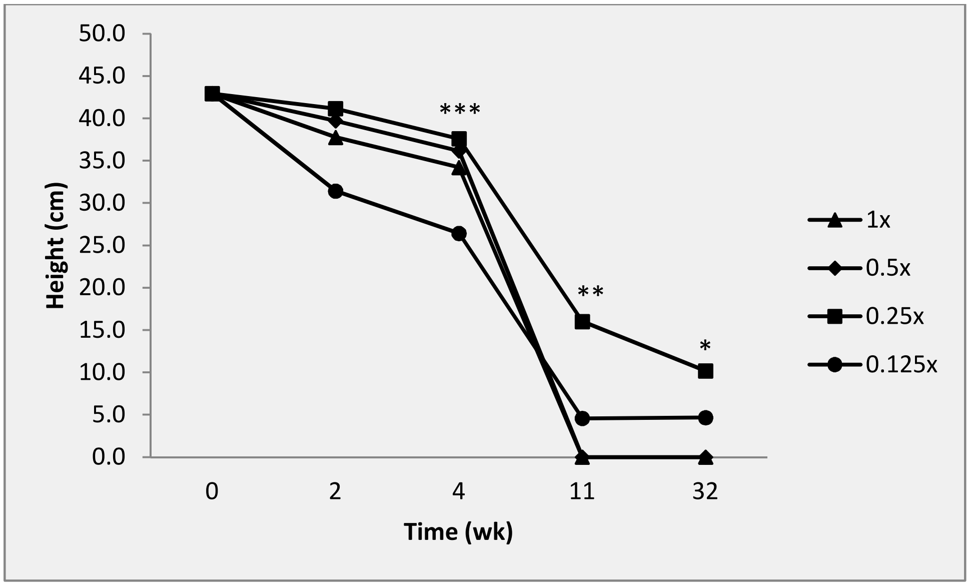

Figure 4.7. Mean height response of 1-0 black locust seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

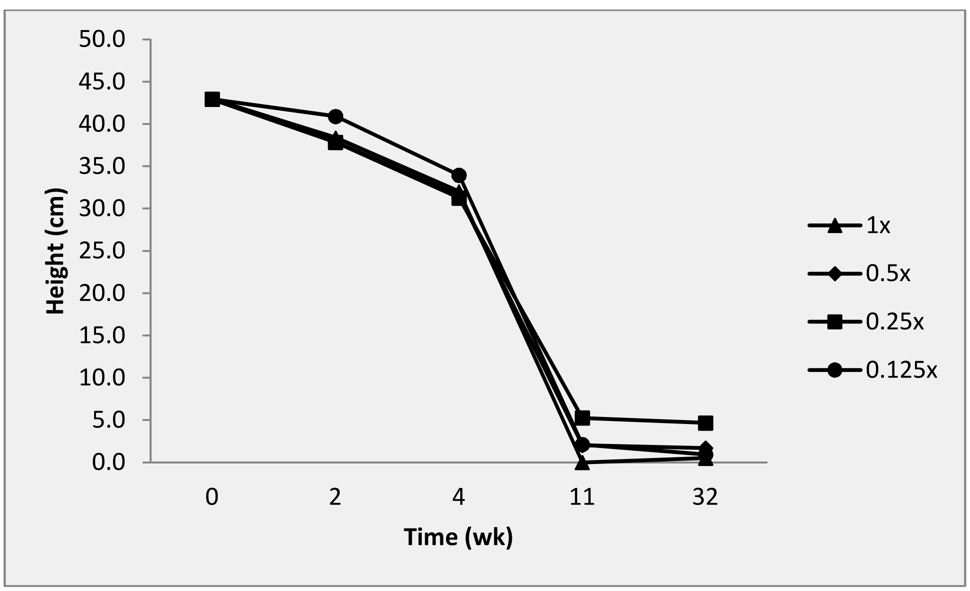

Figure 4.8. Mean height response of 1-0 black locust seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

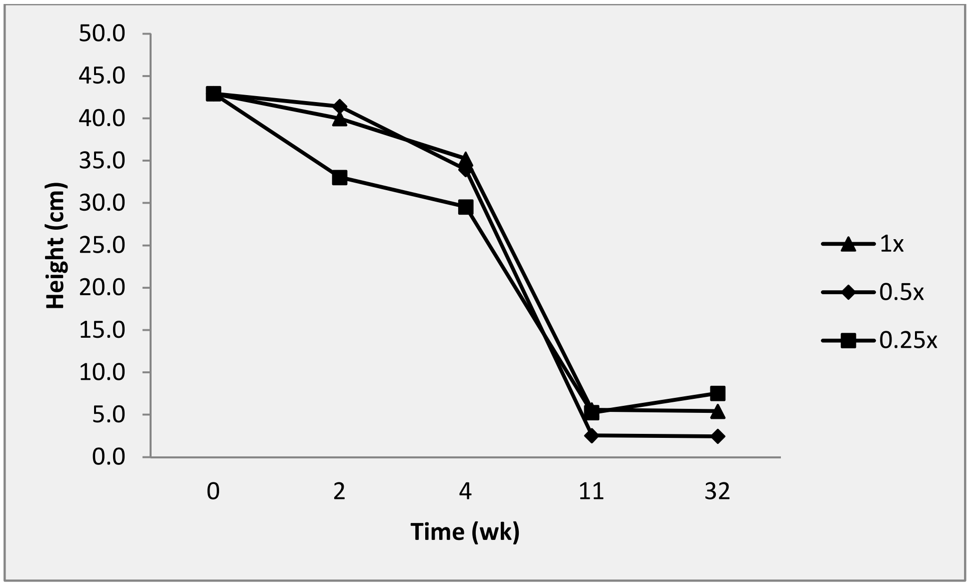

Figure 4.9. Mean height response of 1-0 black locust seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

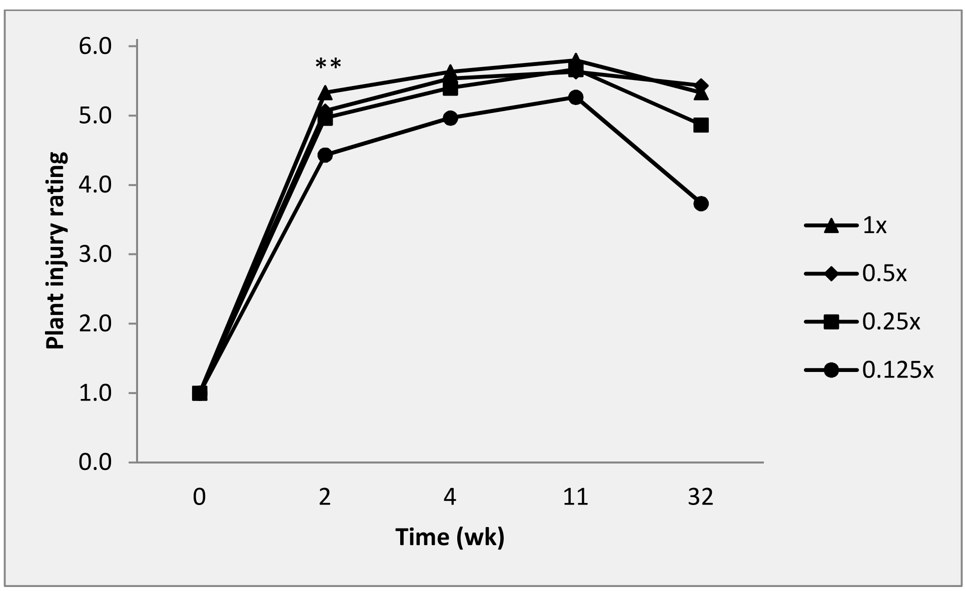

Figure 4.10. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

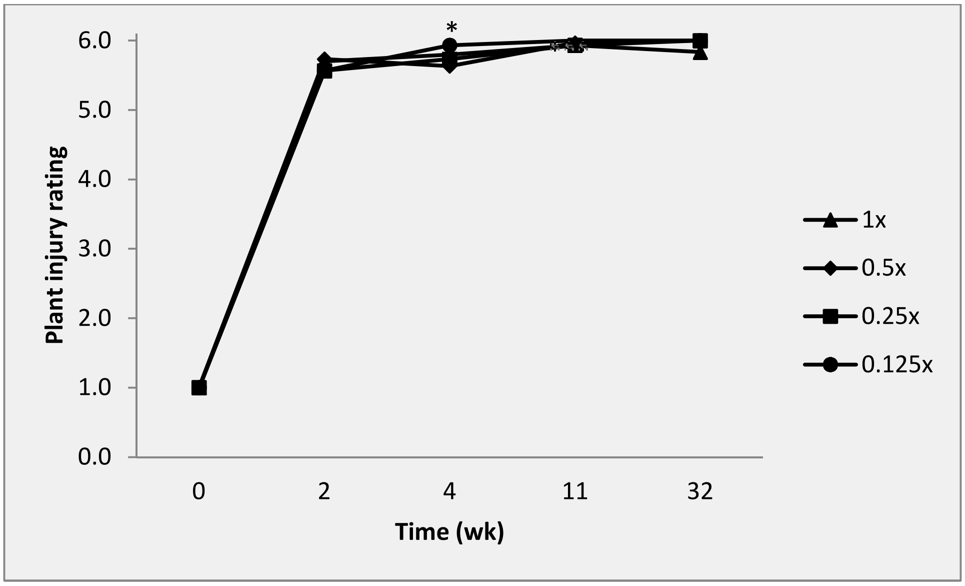

Figure 4.11. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

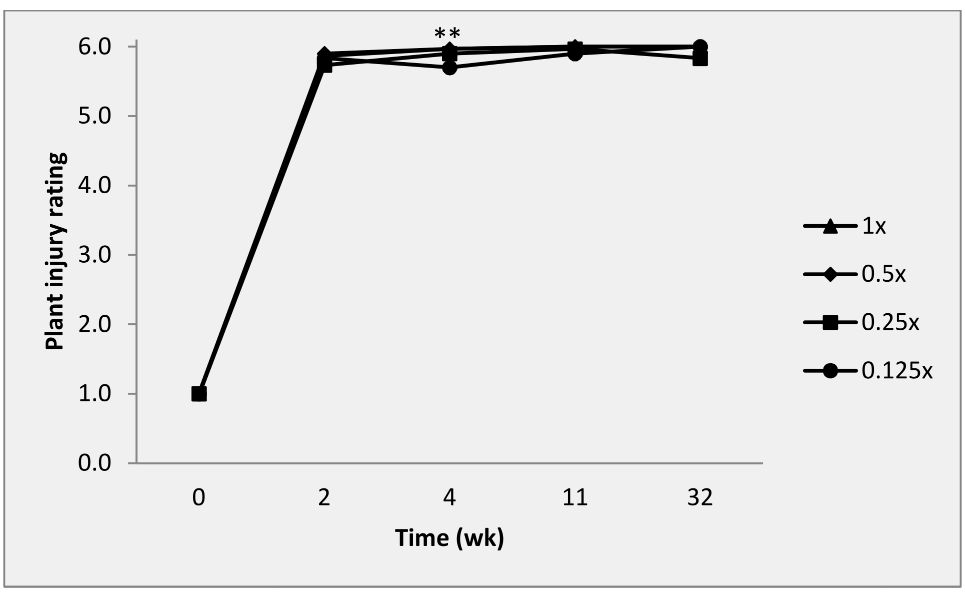

Figure 4.12. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

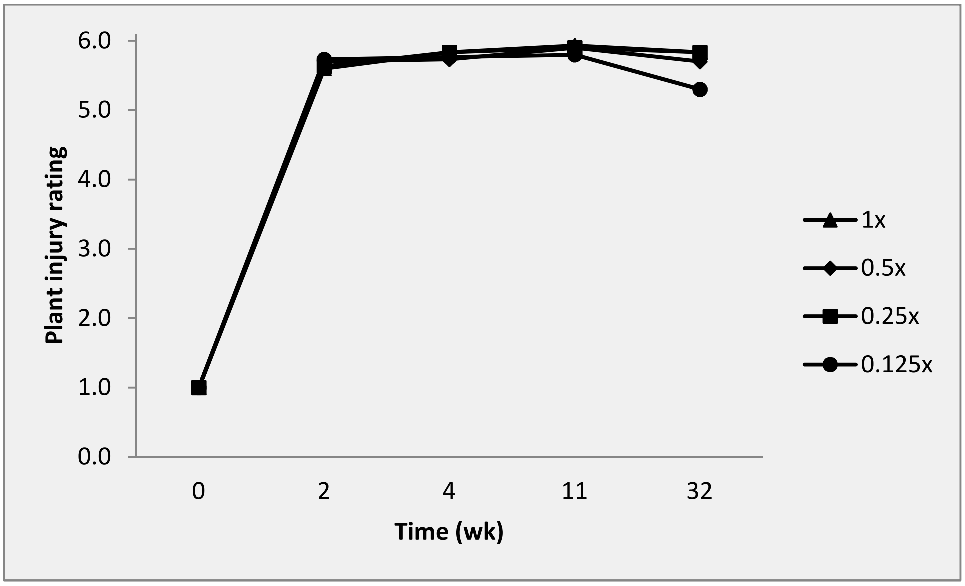

Figure 4.13. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

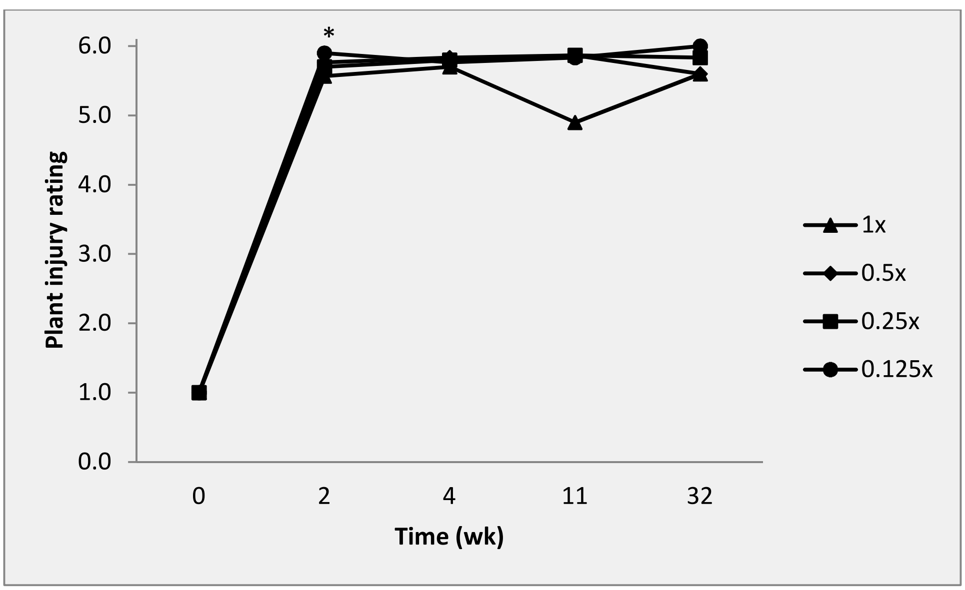

Figure 4.14. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

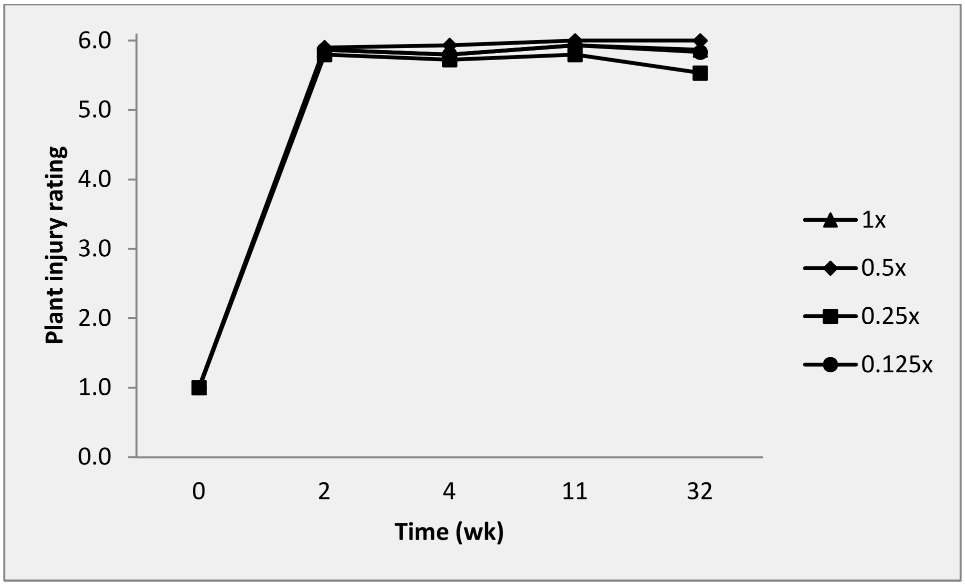

Figure 4.15. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

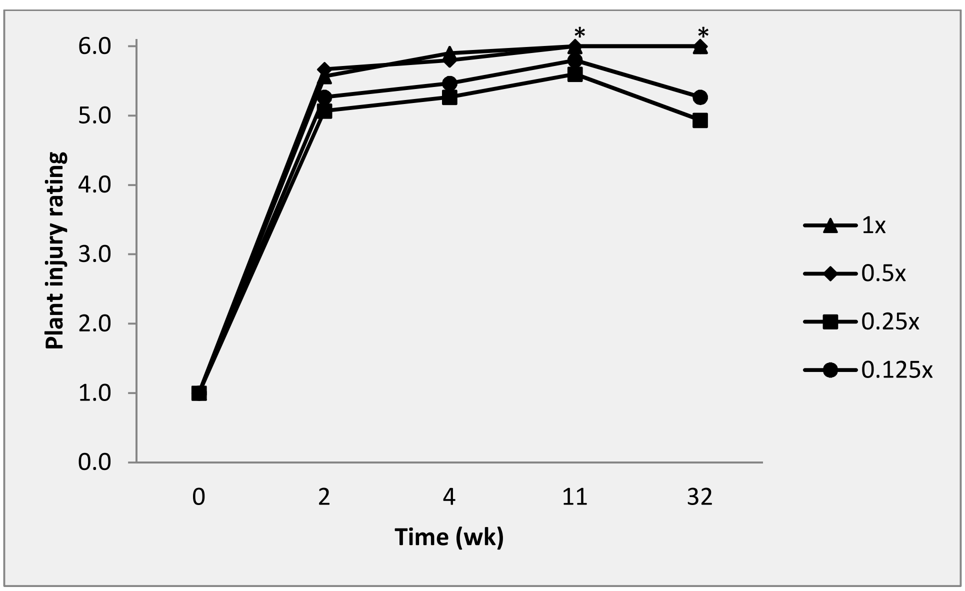

Figure 4.16. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

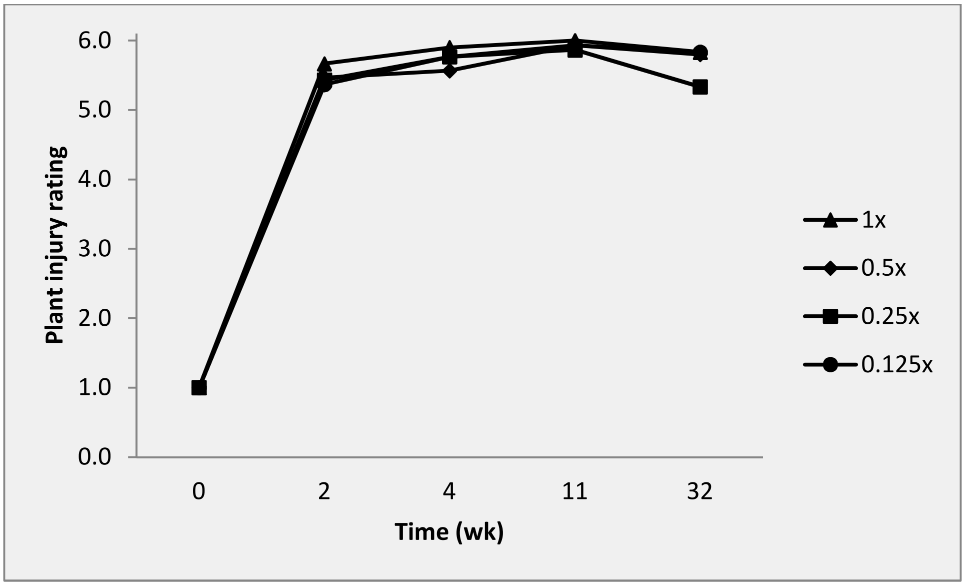

Figure 4.17. Mean plant injury rating of 1-0 black locust seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

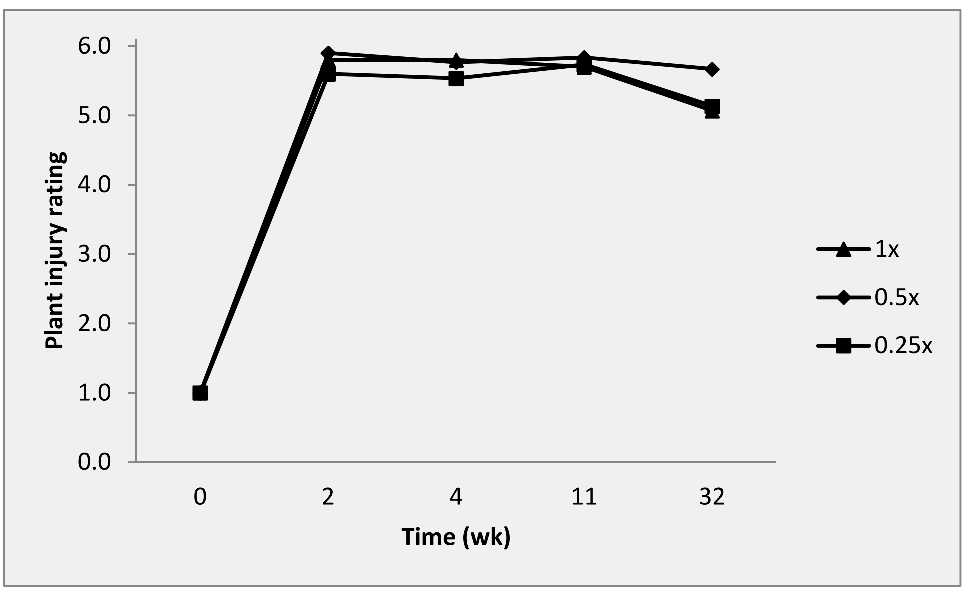

Figure 4.18. Mean plant injury rating of 1-0 black locust seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

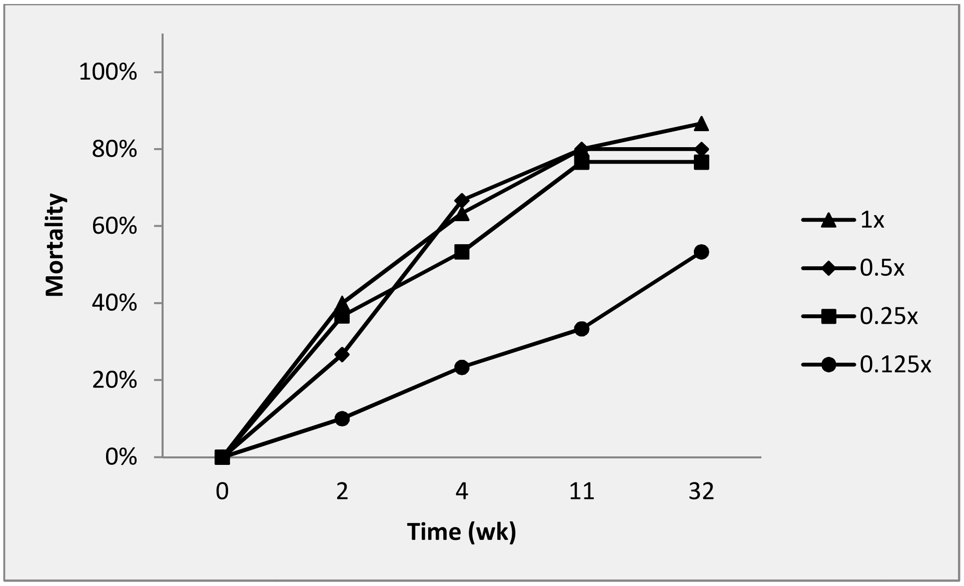

Figure 4.19. Mean percent mortality of 1-0 black locust seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

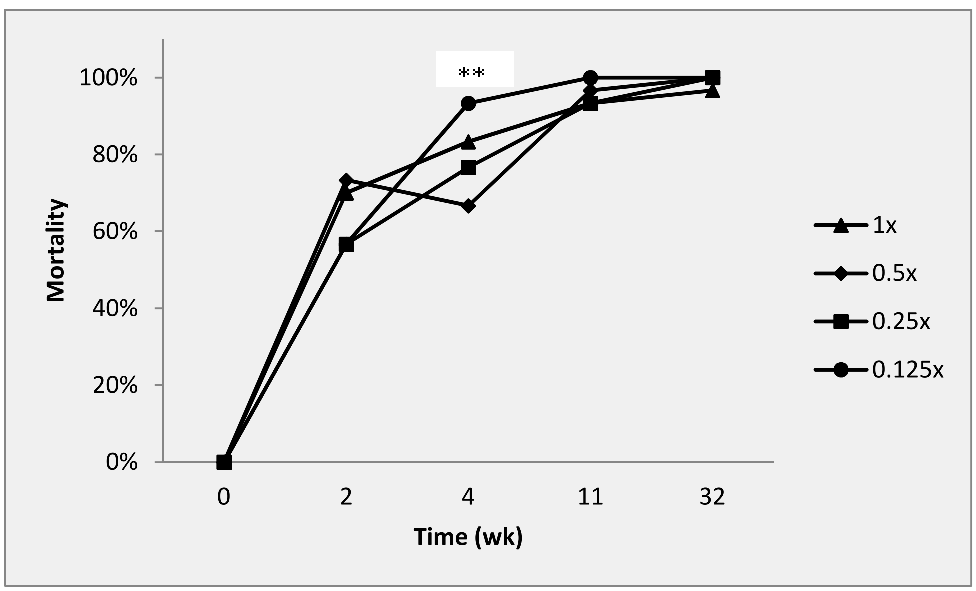

Figure 4.20. Mean percent mortality of 1-0 black locust seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

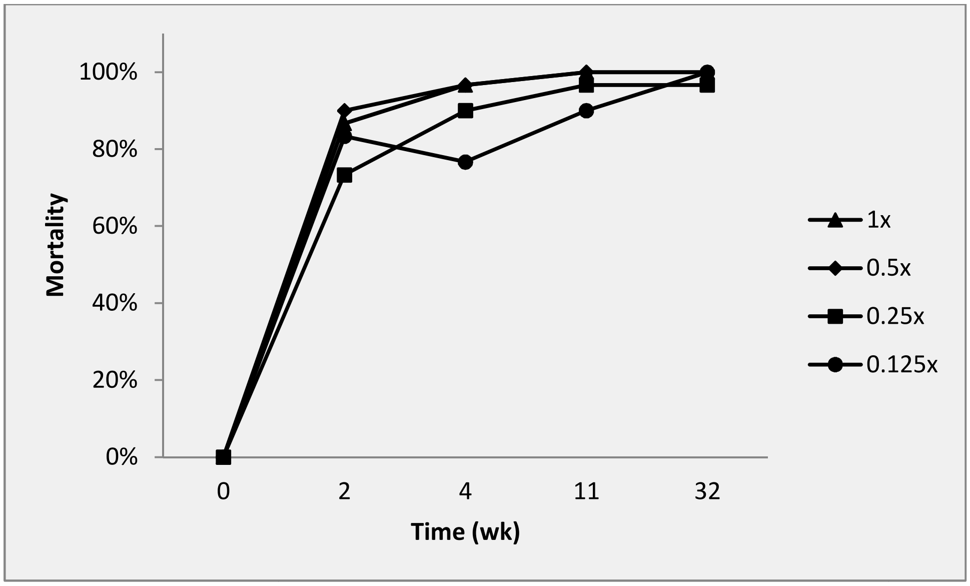

Figure 4.21. Mean percent mortality of 1-0 black locust seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

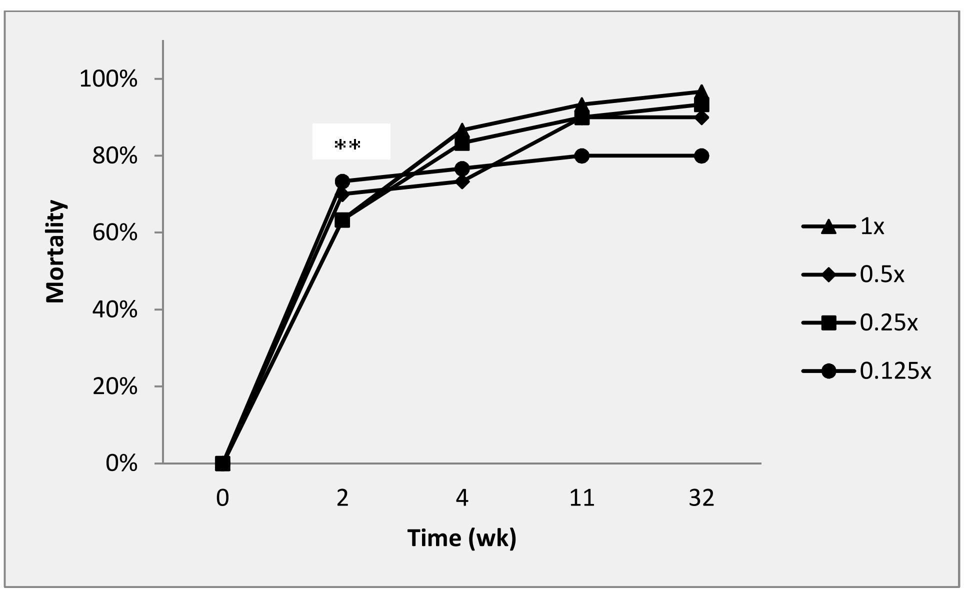

Figure 4.22. Mean percent mortality of 1-0 black locust seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

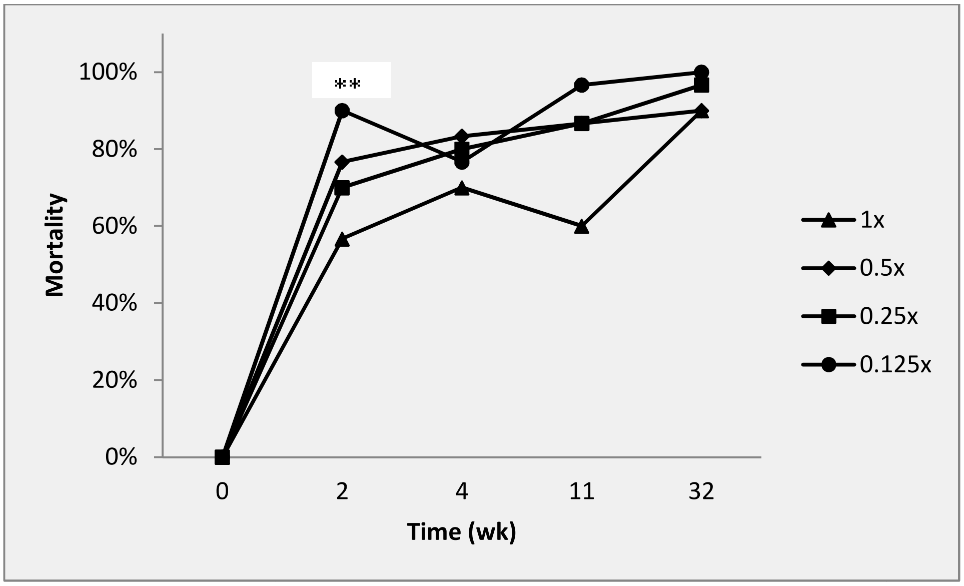

Figure 4.23. Mean percent mortality of 1-0 black locust seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

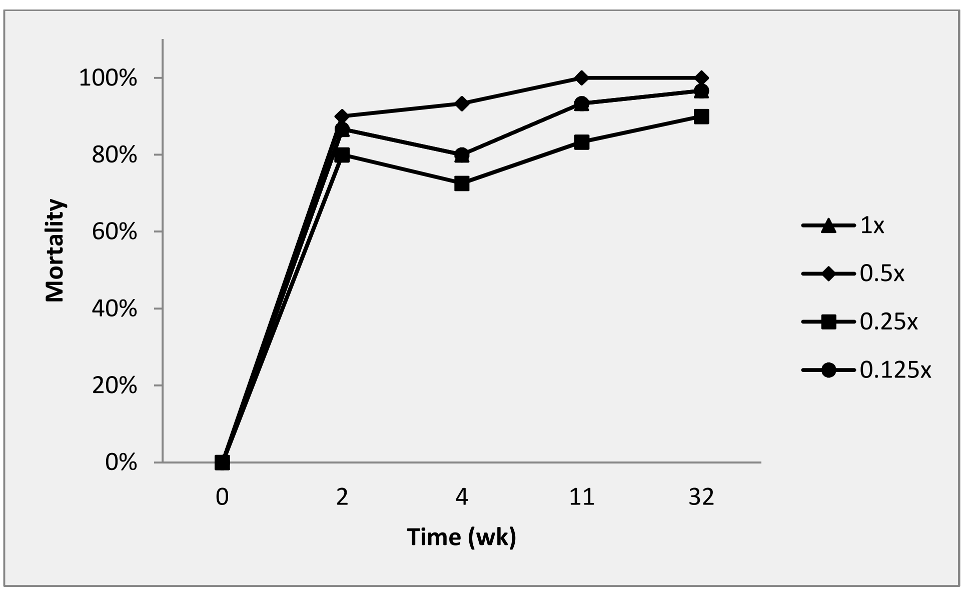

Figure 4.24. Mean percent mortality of 1-0 black locust seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

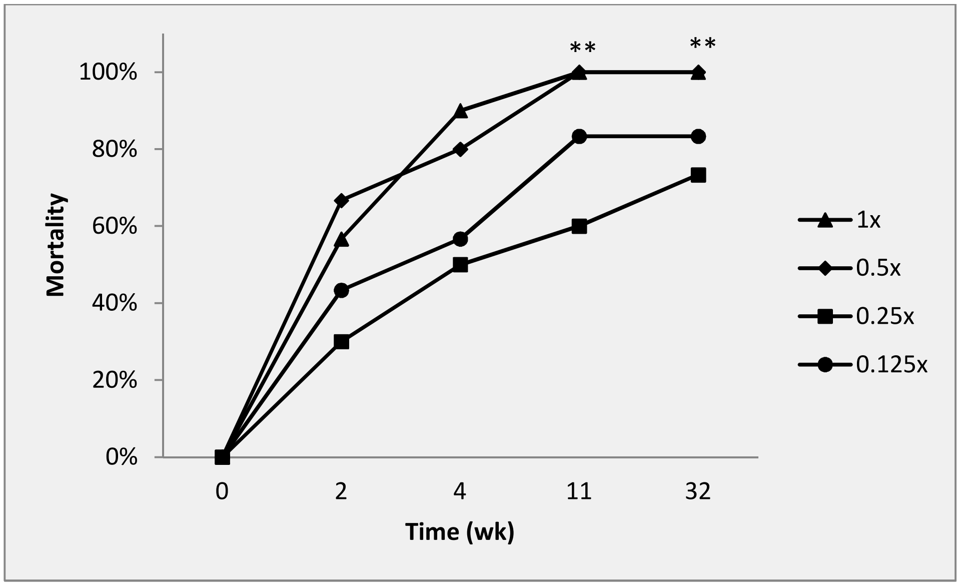

Figure 4.25. Mean percent mortality of 1-0 black locust seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

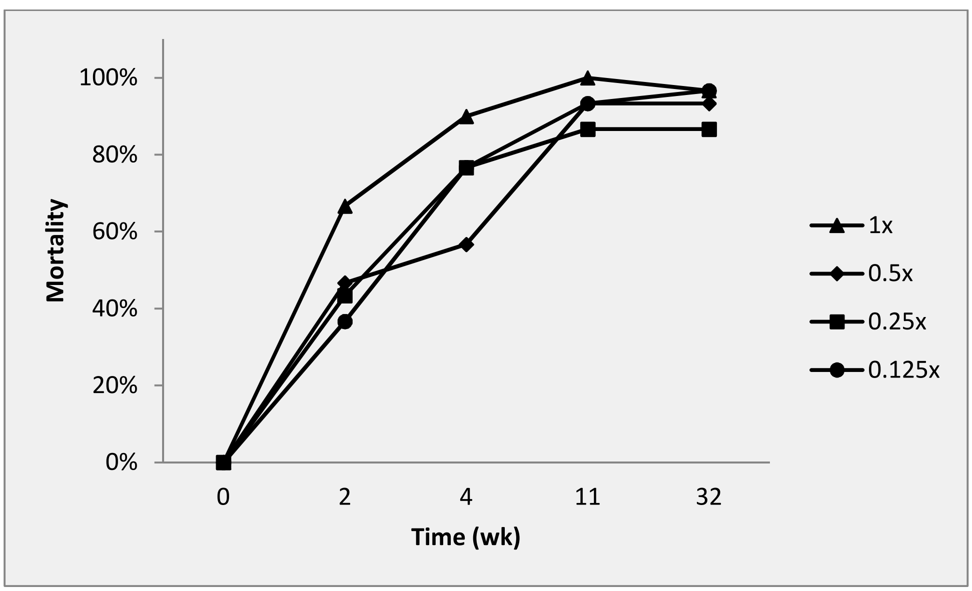

Figure 4.26. Mean percent mortality of 1-0 black locust seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

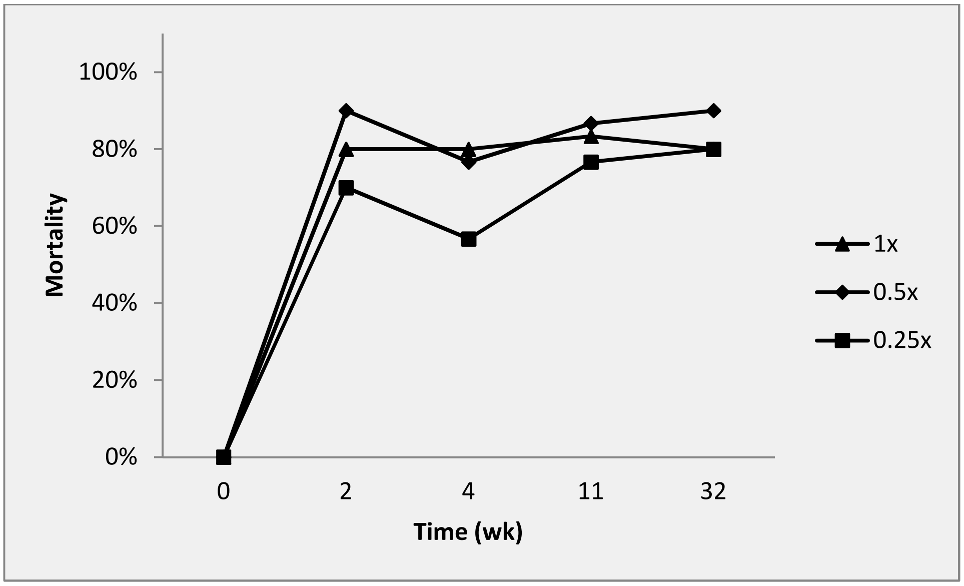

Figure 4.27. Mean percent mortality of 1-0 black locust seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Loblolly Pine

For many treatments (herbicide-rate combinations), mean height, injury, and mortality for loblolly pine seedlings reached a maximum response by 11 WAT and changed little after that (Figures 4.28-4.54). This pattern is particularly clear with DAS 402, 534, and 729.

Height decreased dramatically in response to most herbicides between 4 and 11 WAT. Significant ($p<0.05$) herbicide by rate interaction occurred for height of loblolly pine seedlings at 11 and 32 WAT. Decreases in height from 11 to 32 WAT were less severe in response to the lowest rates of DAS 896, 779, 548, 602, and triclopyr and all rates of DAS 313 (Figures 4.28-4.36). Heights in response to DAS 402, 534, and 729 were reduced to 0.0 cm by 11 WAT, but were not significantly lower than all other herbicides (Table 4.3). This pattern continued post dormancy (Table 4.4).

A significant ($p<0.01$) herbicide by rate interaction occurred at 4 and 11 WAT for injury rating. Injury rating increased rapidly from 0 to 11 WAT and remained high through 32 WAT in response to all rates of most herbicides, except for DAS 896, 548, and triclopyr (Tables 4.5-4.8 and Figures 4.37-4.45). Significant ($p<0.0001$) differences in injury due to herbicide occurred at 2 and 32 WAT. Significant differences in injury in response to rate occurred at 2 ($p<0.05$) and 32 ($p<0.01$) WAT. Injury in DAS 729 (4.6) treatments was significantly higher than most other herbicides at 2 WAT, except for DAS 402, 534, and 548 (Table 4.5). At 4 WAT, injury ratings in response to DAS 402, 534, 548, 602, and 729 ranged from 5.6 to 6.0 and were not significantly different from one another (Table 4.6). At 11 WAT, injury rating in these five herbicides increased, ranging from 5.9 to 6.0, and remained to 32 WAT (Tables 4.7-4.8). Height, injury, and mortality responses at 32 WAT suggest that DAS 402, 534, 548, 602, and 729 are the most effective herbicides on loblolly pine (Tables 4.4, 4.8, and 4.9).

Herbicides DAS 313 (37.5%), 779 (50.0%), 896 (75.8%), and triclopyr (53.3%) did not produce greater than 80% mortality by 11 WAT in loblolly pine (Table 4.10 and Figures 4.46-4.54). Significant differences in height or injury in response to rate did not occur in DAS 313 treatments (Figures 4.28 and 4.37). Differences in height or injury due to rates of DAS 779 were also not significant at any time (Figures 4.34 and 4.43).

Different rates of DAS 896 resulted in statistically significant differences in height at 11 ($p<0.001$) and 32 ($p<0.0001$) WAT (Figure 4.29). Height for the 0.125x rate at 11 WAT was 30.1 cm, while 0.25x, 0.5x, and 1x rates were 0.0, 0.0, and 6.7 cm, respectively. This gap between rates grew larger after dormancy at 32 WAT with a height difference of 29.4 cm between the 0.125x and 1x rates.

Significant differences in injury rating in response to rates of DAS 896 occurred at 4 (p<0.01), 11 (p<0.001), and 32 (p<0.01) WAT (Figure 4.44). Injury rating at 4 WAT for the 0.125x rate was 4.2 and the 0.25x, 0.5x, and 1x rates were 5.7, 5.8, and 5.4, respectively. Differences in injury in response to rates increased after dormancy to 3.8 for the 0.125x rate and 6.0, 6.0, and 5.6 for the 0.25x, 0.5x, and 1x rates, respectively.

Triclopyr rates also resulted in statistically significant differences in injury at 2 (p<0.01), 4 (p<0.05), and 11 (p<0.05) WAT (Figure 4.45). Injury ratings at 2 WAT were 2.0, 3.0, and 3.6 for the 0.25x, 0.5x, and 1x rates, respectively. Injury increased until 11 WAT to 4.1, 5.5, and 5.7 for the 0.25x, 0.5x, and 1x rates, respectively, and then decreased following dormancy at 32 WAT when differences in injury rating in response to rate were no longer significant.

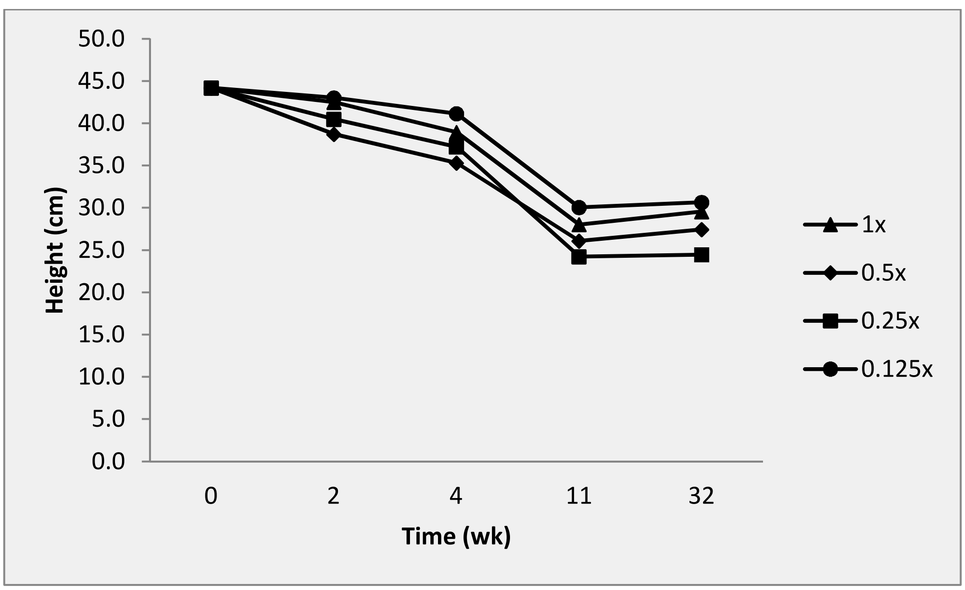

Figure 4.28. Mean height response of 1-0 loblolly pine seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

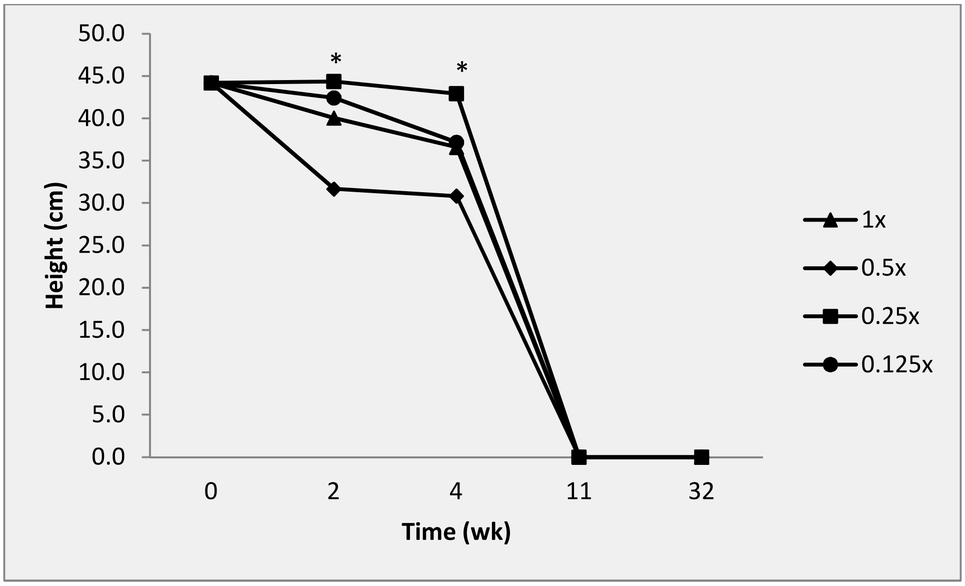

Figure 4.29. Mean height response of 1-0 loblolly pine seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

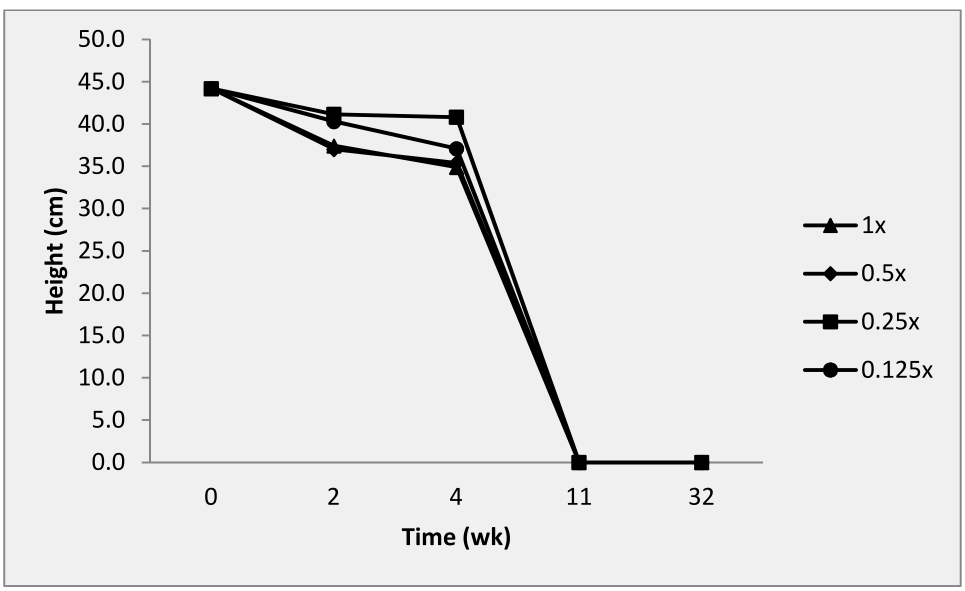

Figure 4.30. Mean height response of 1-0 loblolly pine seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

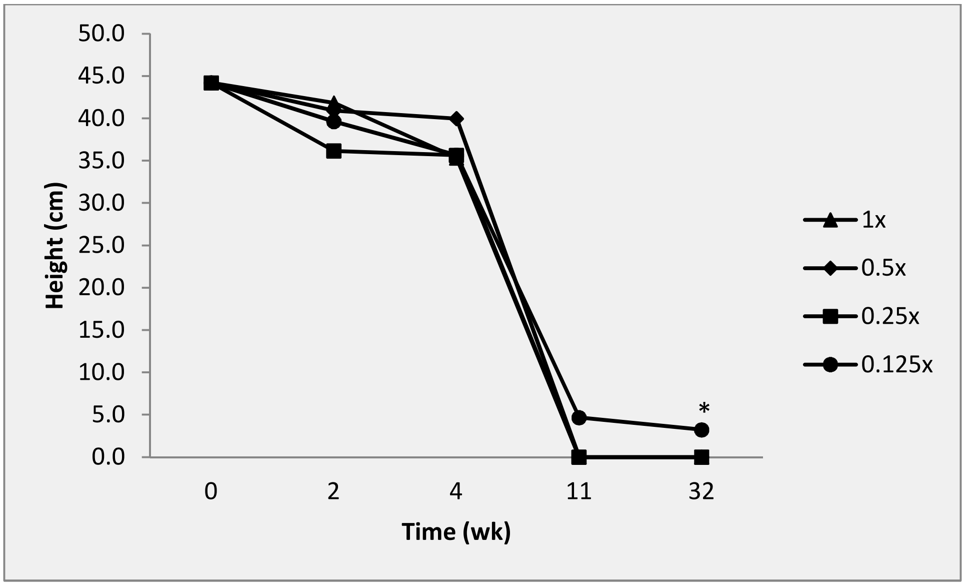

Figure 4.31. Mean height response of 1-0 loblolly pine seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

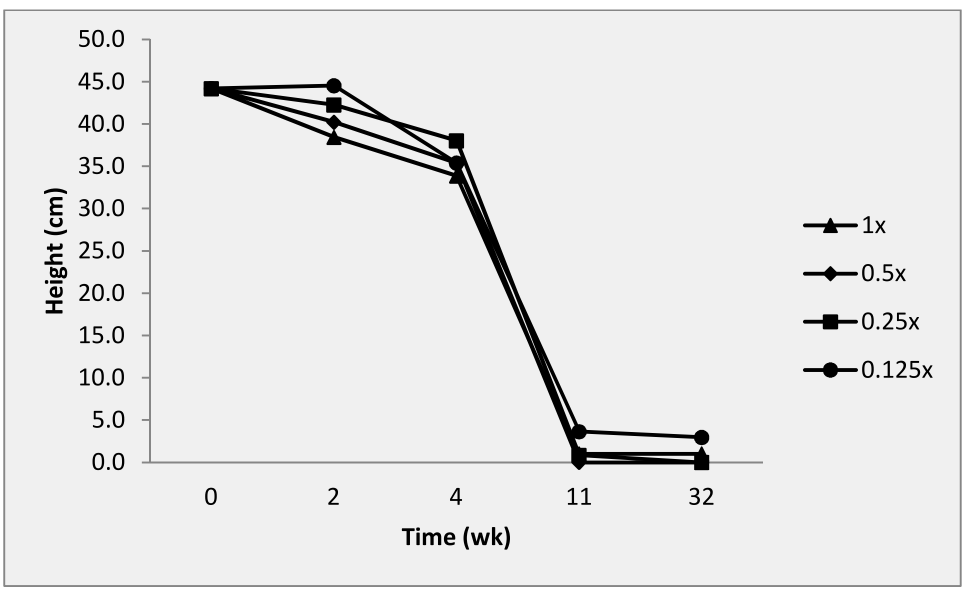

Figure 4.32. Mean height response of 1-0 loblolly pine seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

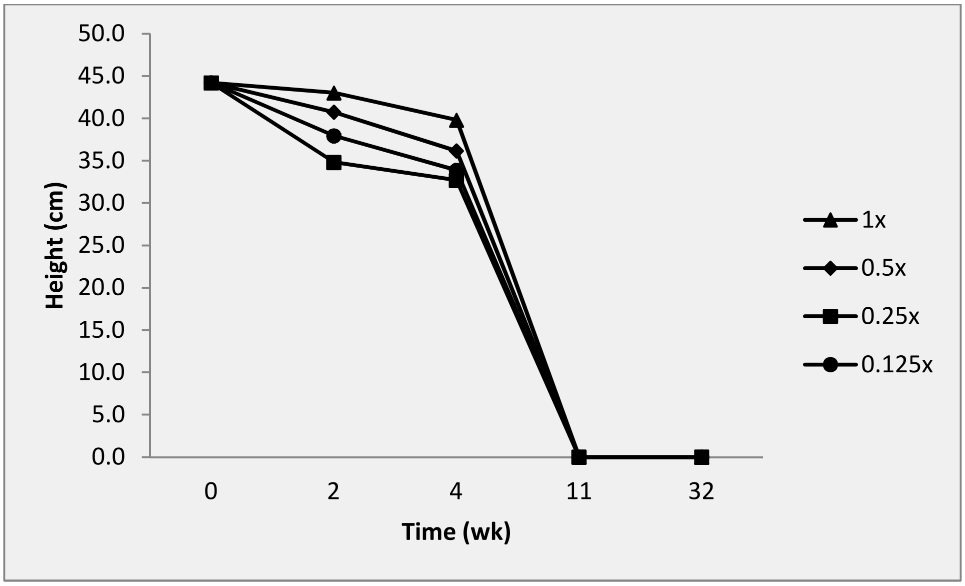

Figure 4.33. Mean height response of 1-0 loblolly pine seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

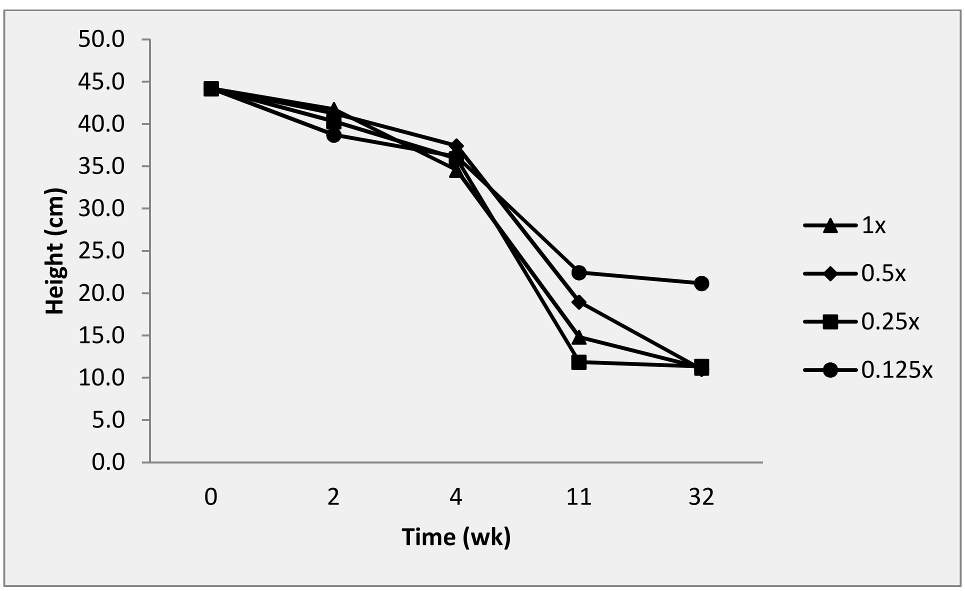

Figure 4.34. Mean height response of 1-0 loblolly pine seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

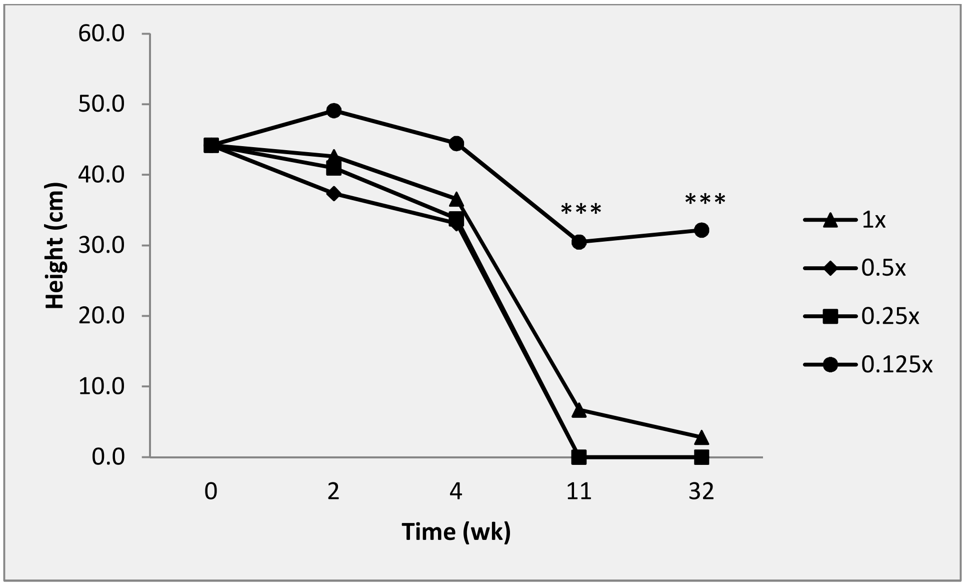

Figure 4.35. Mean height response of 1-0 loblolly pine seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

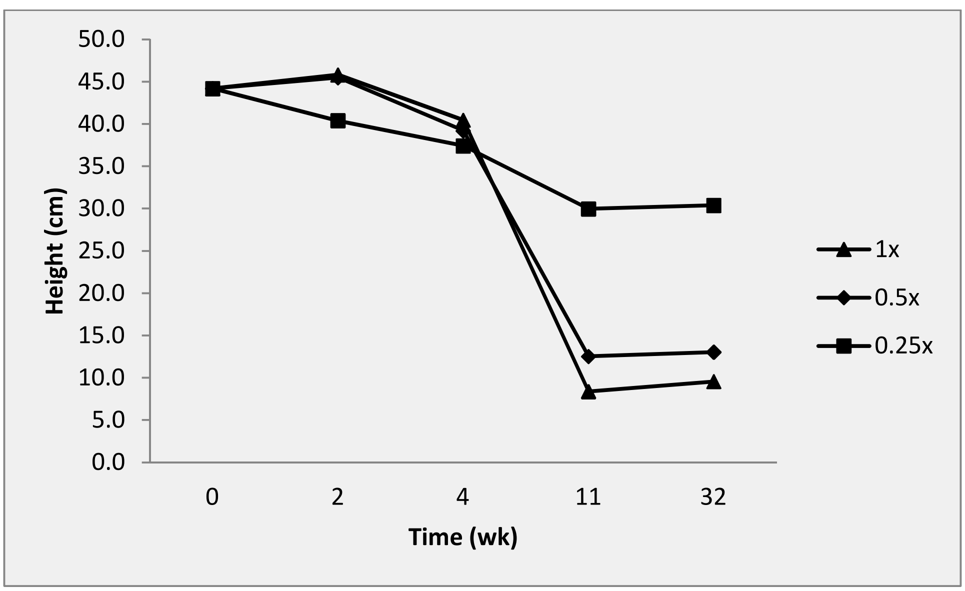

Figure 4.36. Mean height response of 1-0 loblolly pine seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

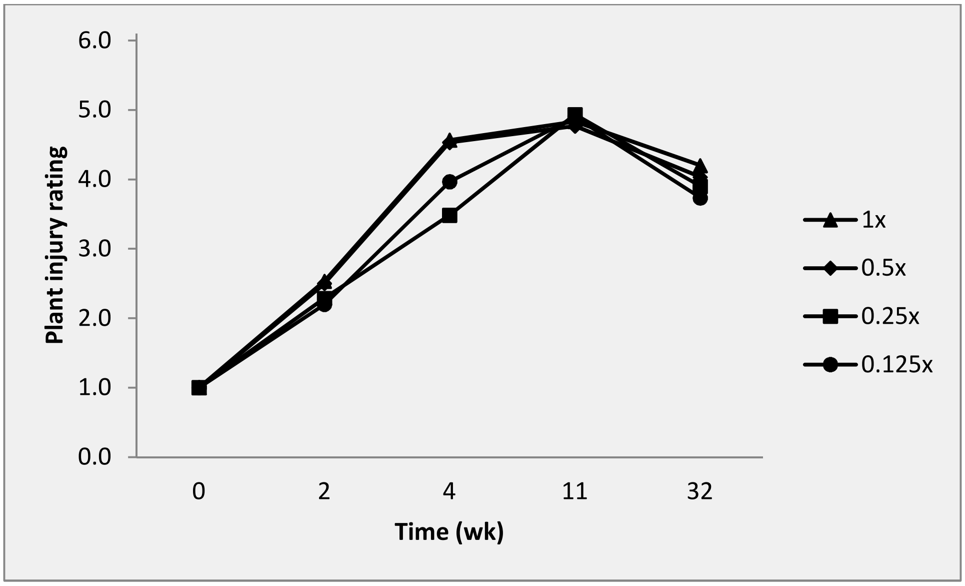

Figure 4.37. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

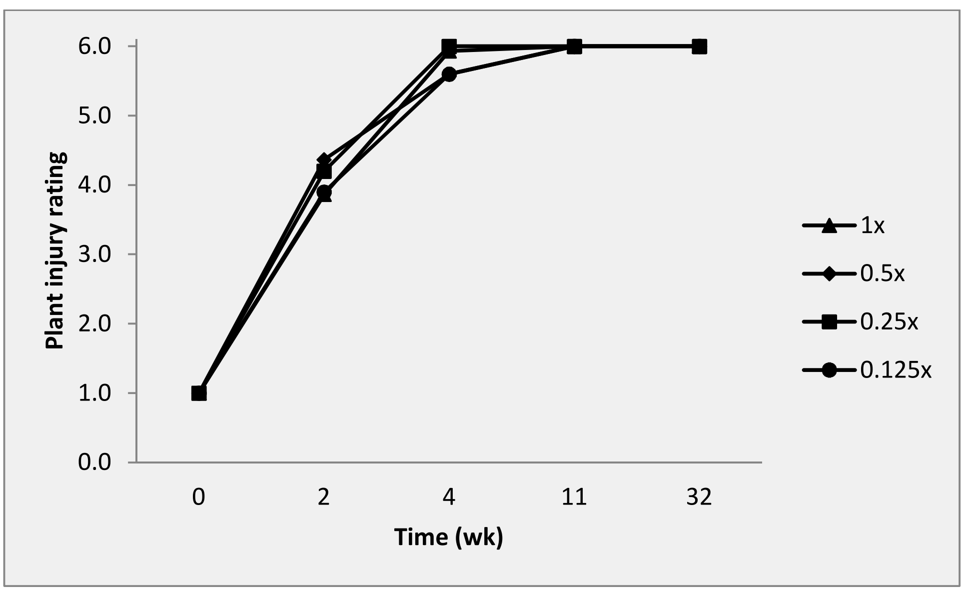

Figure 4.38. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

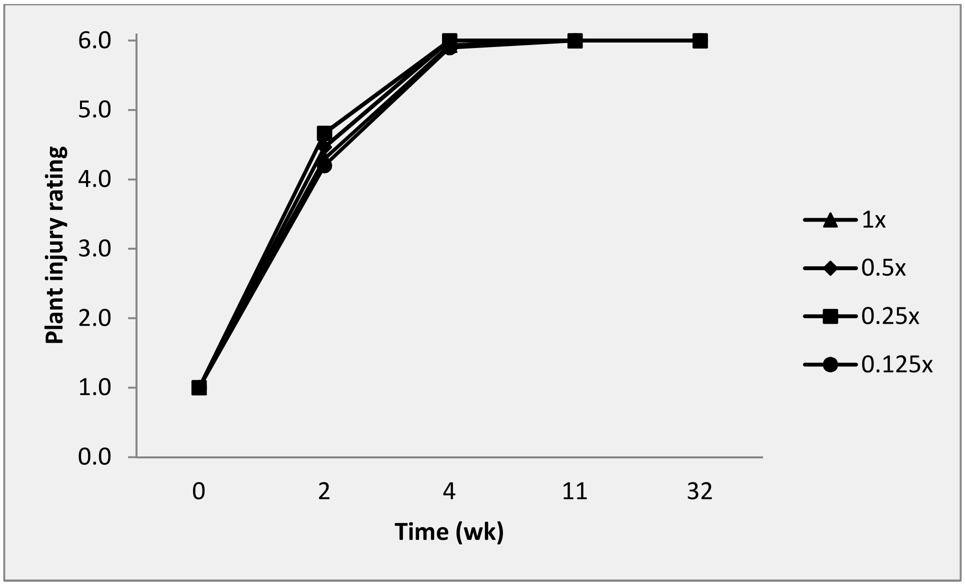

Figure 4.39. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

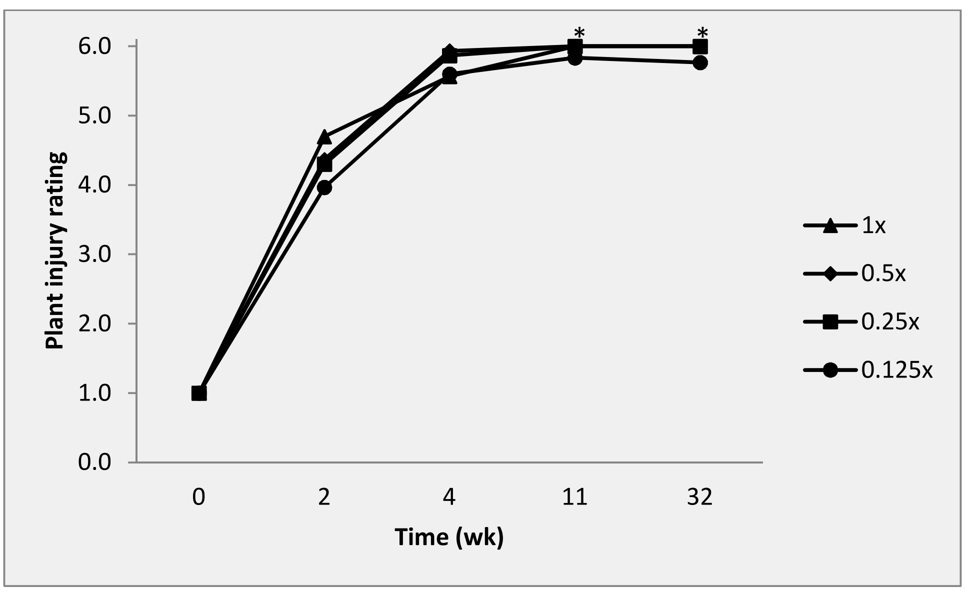

Figure 4.40. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

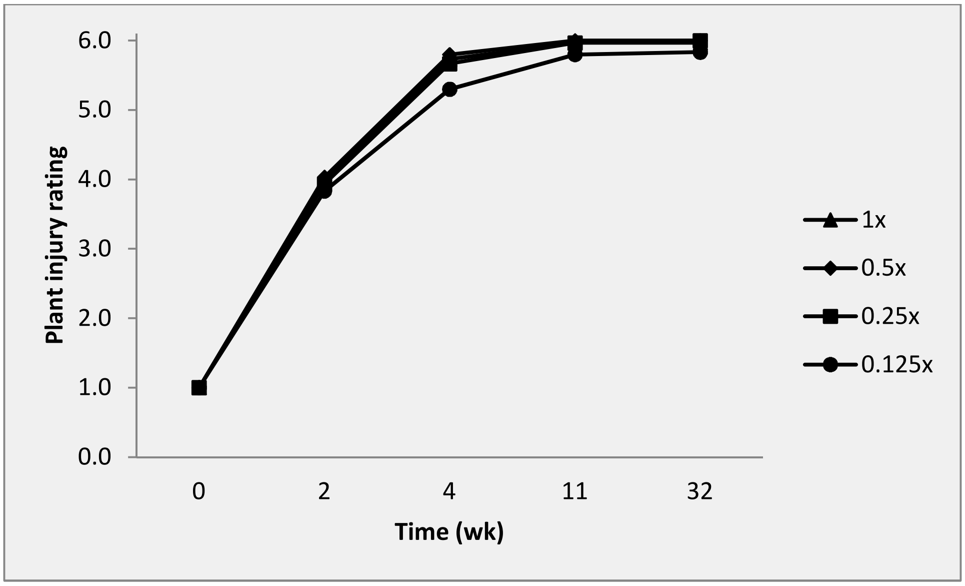

Figure 4.41. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

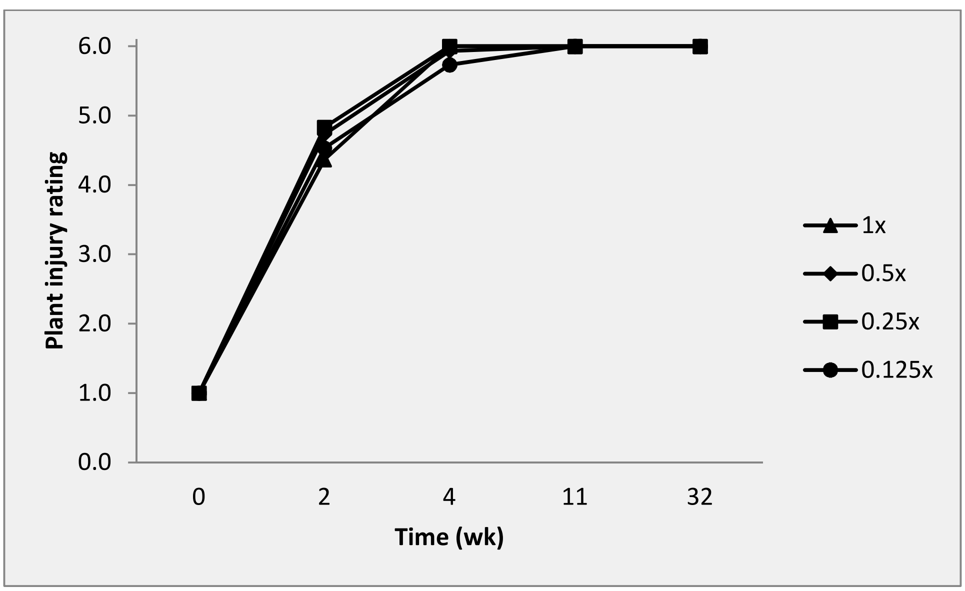

Figure 4.42. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

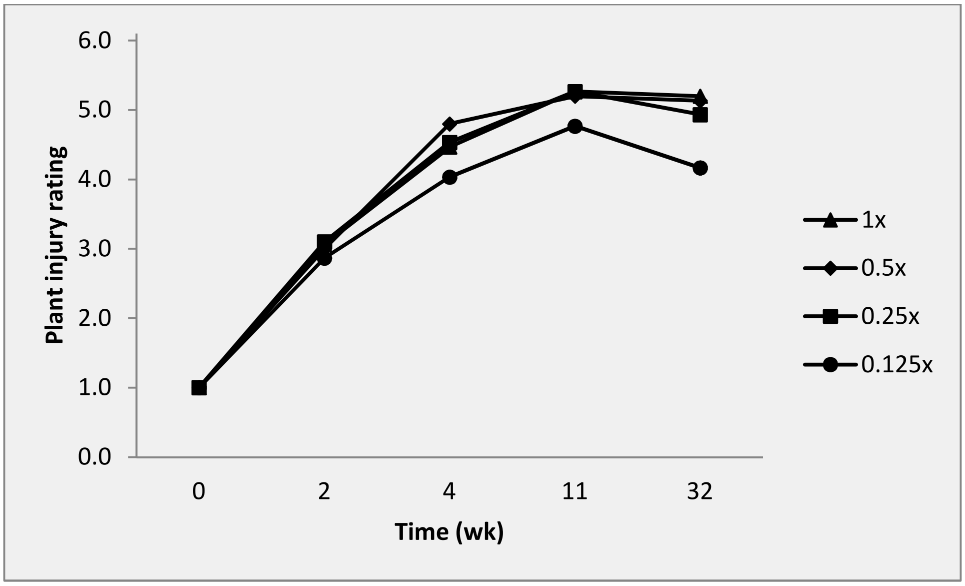

Figure 4.43. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

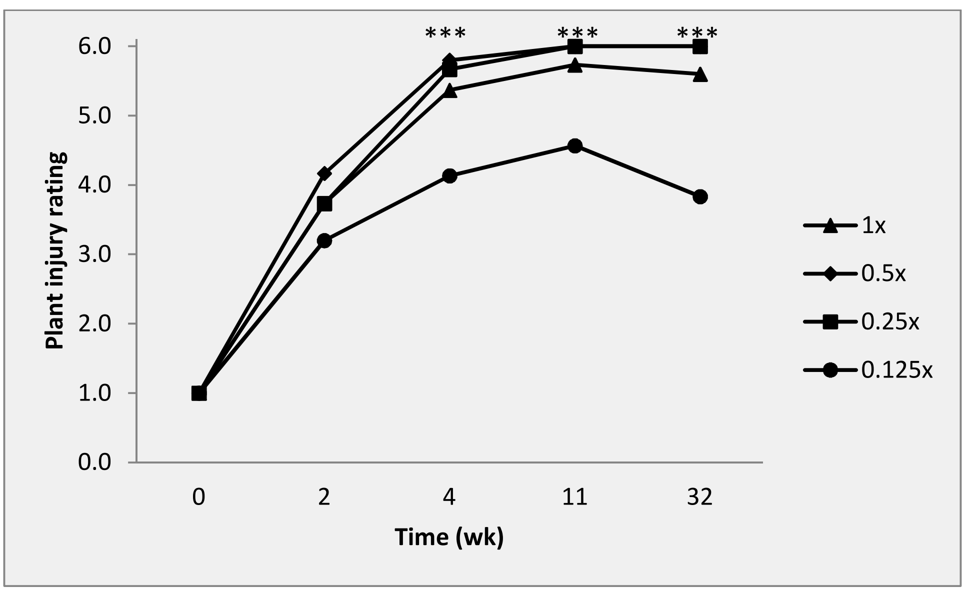

Figure 4.44. Mean plant injury rating of 1-0 loblolly pine seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

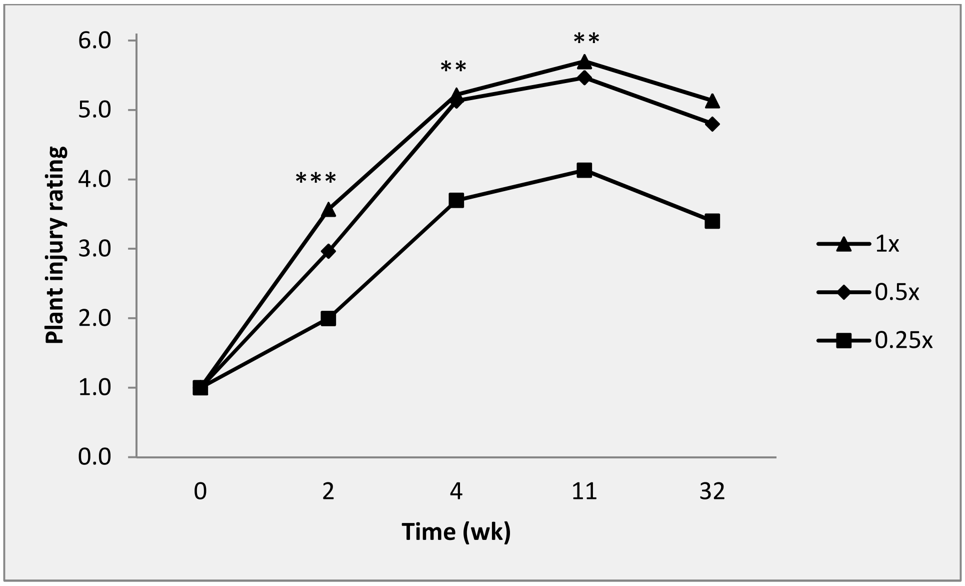

Figure 4.45. Mean plant injury rating of 1-0 loblolly pine seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

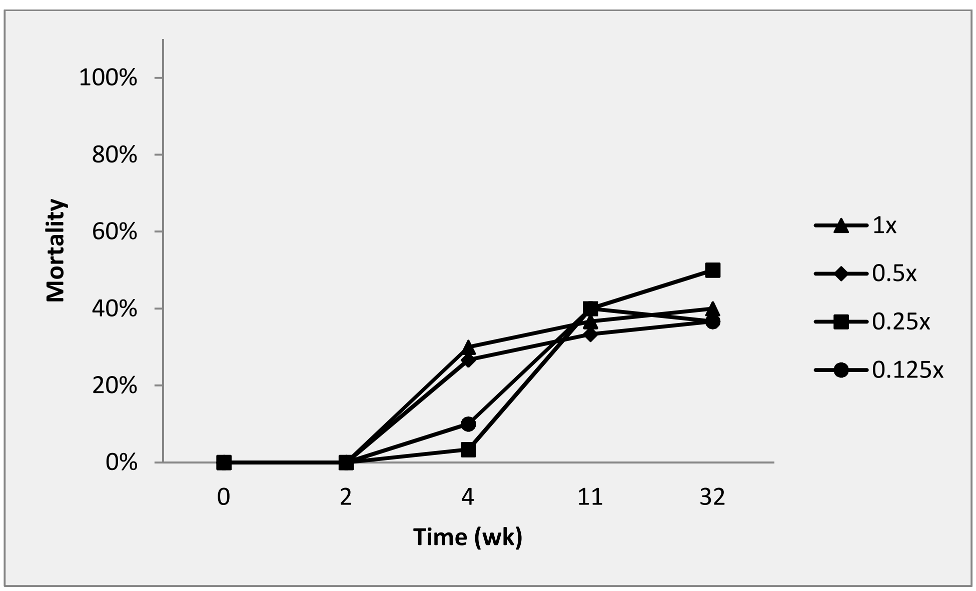

Figure 4.46. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

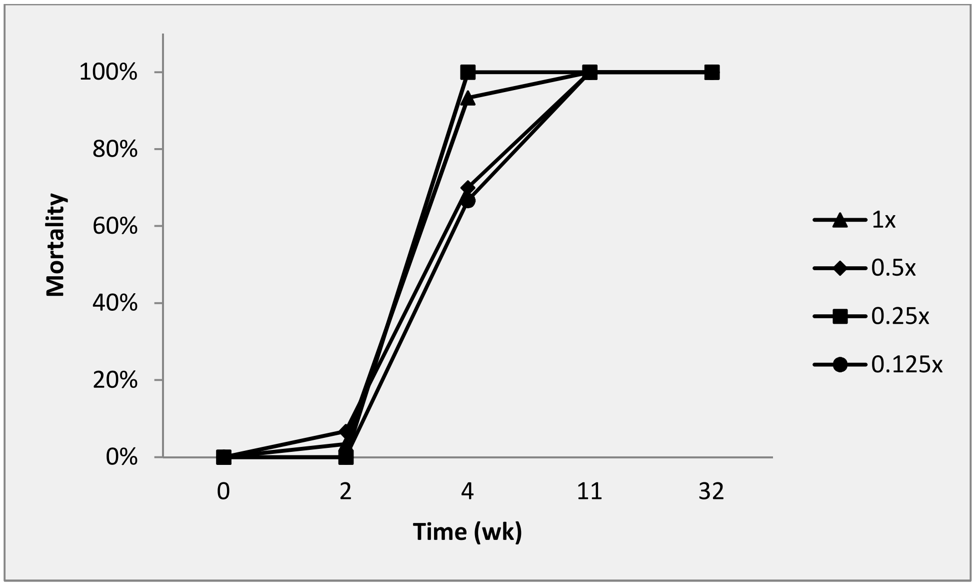

Figure 4.47. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

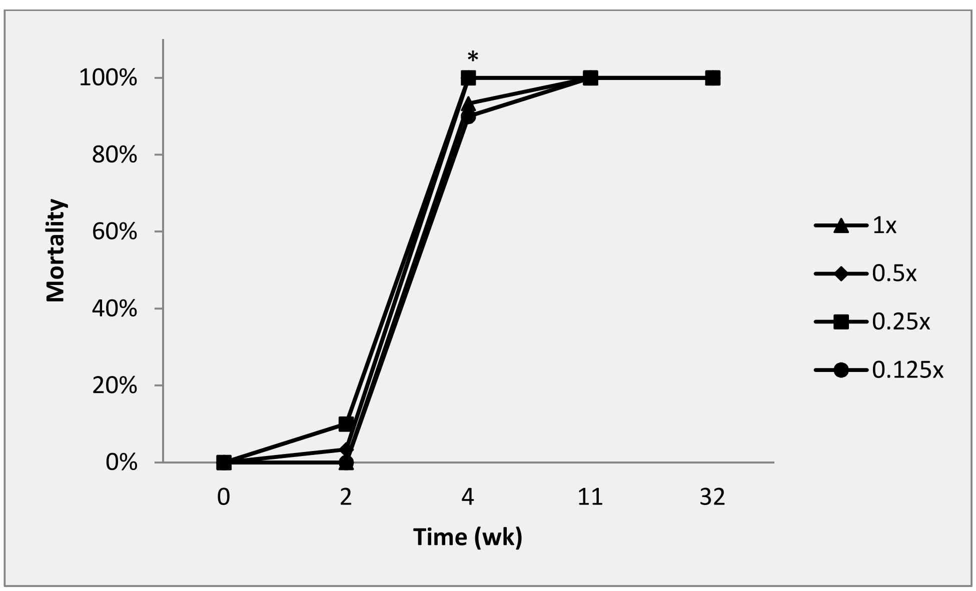

Figure 4.48. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

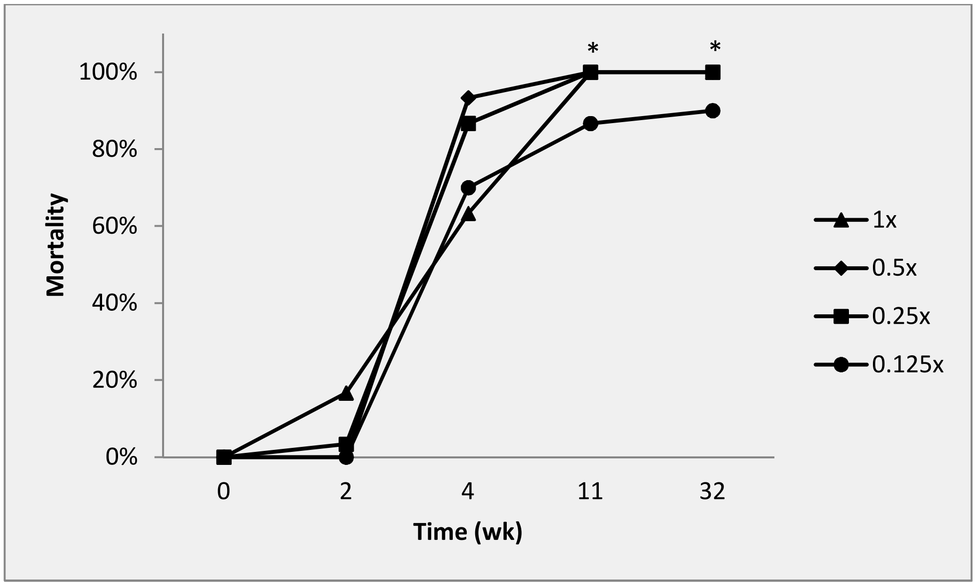

Figure 4.49. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

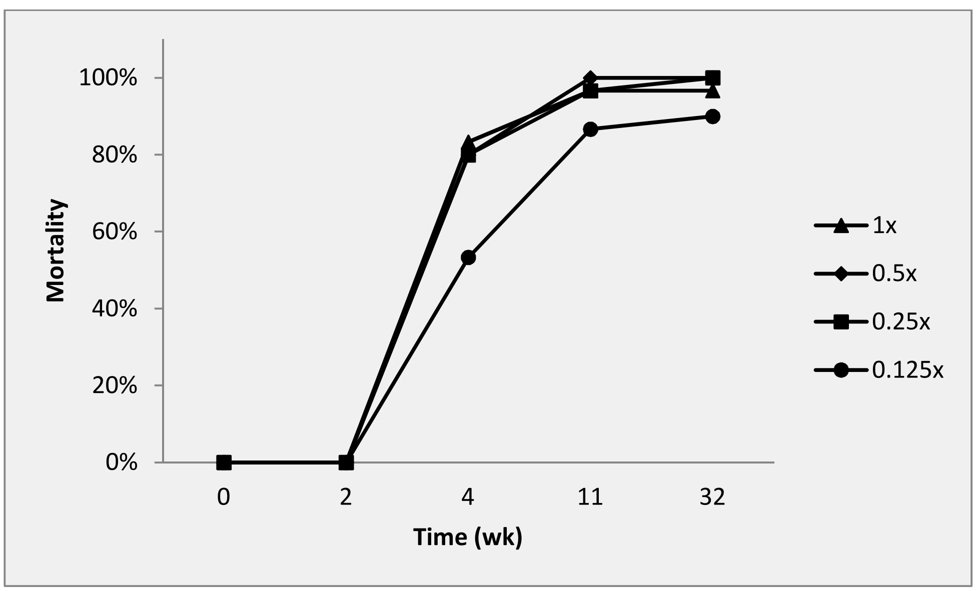

Figure 4.50. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

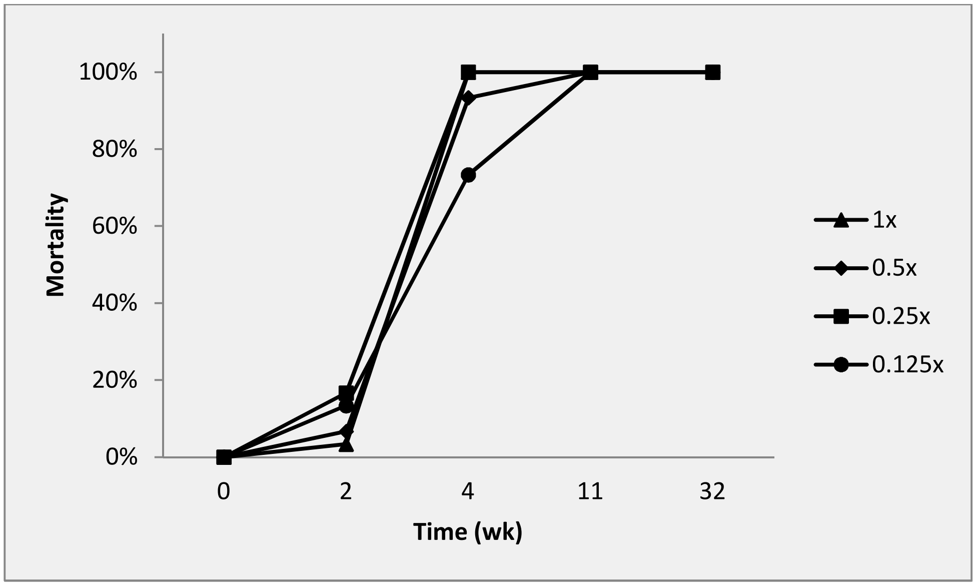

Figure 4.51. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

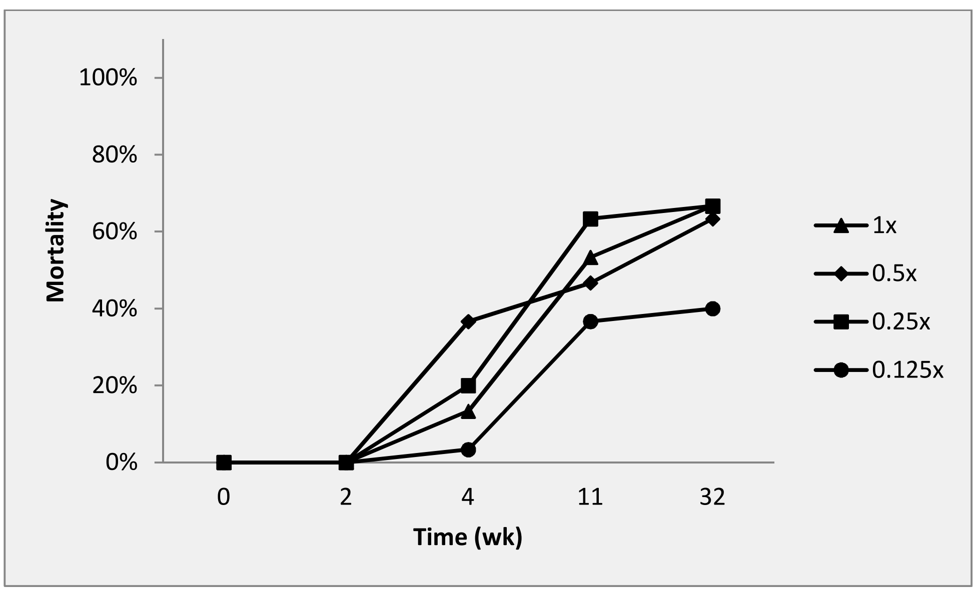

Figure 4.52. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

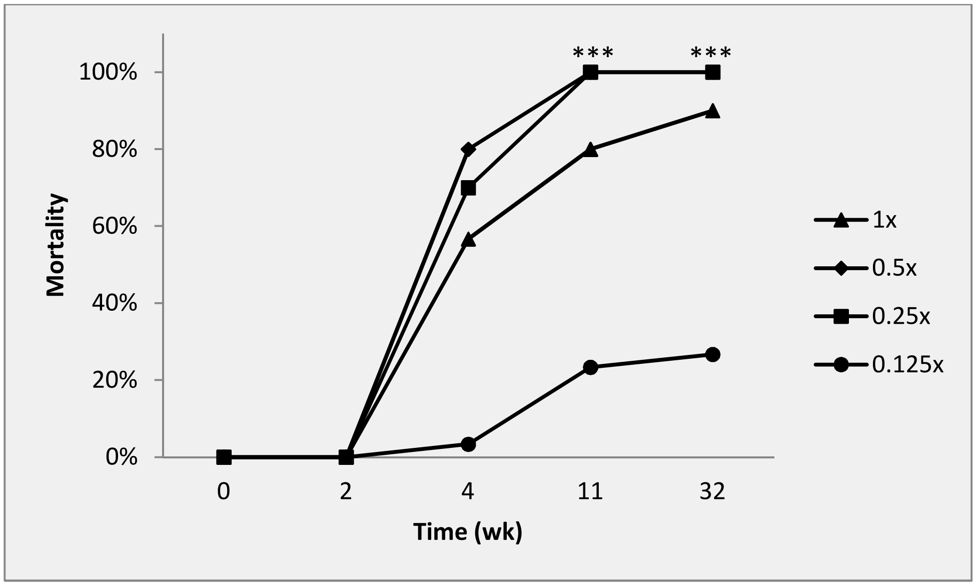

Figure 4.53. Mean percent mortality of 1-0 loblolly pine seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

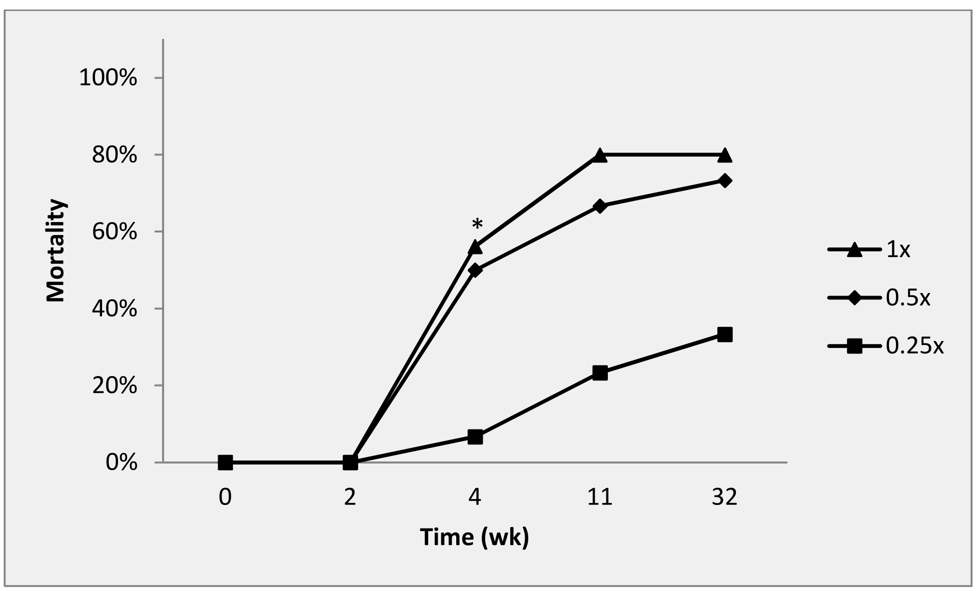

Figure 4.54. Mean percent mortality of 1-0 loblolly pine seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Red Maple

Red maple height decreased little in response to herbicide until 11 WAT (Figures 4.55-4.63). Following dormancy, mean heights in all treatments increased through final evaluation at 32 WAT. Significant differences in height in response to herbicide occurred at 4 ($p<0.1$), 11 ($p<0.0001$), and 32 ($p<0.0001$) WAT. Significant differences in height in response to rate occurred at 11 ($p<0.001$) and 32 ($p<0.0001$) WAT. Although plant heights appear to respond differently to rates of each herbicide, no herbicide by rate interactions were detected. At 11 WAT, DAS 548 treatments had the lowest height (27.2 cm) but not significantly lower than DAS 313 (31.9 cm), 729 (33.8 cm), 779 (33.9 cm), and triclopyr (41.4 cm) (Table 4.3). Post dormancy, DAS 548 treatment heights remained lowest (32.9 cm) but were not significantly lower than DAS 313 (40.0 cm), 729 (39.3 cm), and 779 (39.2 cm) (Table 4.4).

Injury ratings reached maximum levels for all treatments at 11 WAT and decreased following dormancy (Figures 4.64-4.72). Significant herbicide by rate interaction occurred for injury rating at 4 ($p<0.01$) and 32 ($p<0.1$) WAT. Injury ratings were tightly grouped in response to rates of most herbicides, but there were significant differences in response to rates of DAS 313, 779, 548, and triclopyr (Figures 4.64-4.72). Significant ($p<0.0001$) differences in injury in response to herbicide occurred at 2 and 11 WAT. At 2 WAT, DAS 548 (4.2), the most effective herbicide on red maple seedlings, had one of the highest injury ratings (Table 4.5). DAS 548 treatments continued to have the highest or second highest injury from 2 until 32 WAT, but injury was not significantly higher than other herbicides at any time (Tables 4.5-4.8). In contrast, injury of seedlings treated with other herbicides changed rank over time.

Only herbicides DAS 313 (25.8%) and 548 (33.3%) produced greater than 20% mortality at 11 WAT (Table 4.10 and Figures 4.73-4.81). Significant differences in height and injury rating in response to rates of DAS 313 occurred at 4, 11, and 32 WAT (Figures 4.55 and 4.64). Differences in height in response to rate were most significant ($p<0.01$) at 11 WAT: 52.2, 36.9, 26.8, and 11.7 cm for 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Differences were still significant ($p<0.05$) at 32 WAT but the rate response collapsed slightly to: 60.0, 46.2, 29.8, and 24.1 cm for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Differences in injury ratings in response to rate were most significant ($p<0.05$) at 4 WAT where injury ratings were 4.0, 4.2, 4.9, and 5.0 for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Statistical significance of differences in injury rating decreased ($p<0.1$) by 32 WAT, but numerical differences between injury ratings for rates of DAS 313 increased to 2.3, 3.0, 4.1, and 4.6 for the 0.125x, 0.25x, 0.5x, and 1x rates of DAS 313, respectively.

Significant differences in height and injury in response to rates of DAS 548 occurred at 32 WAT (Figures 4.58 and 4.67). Significant ($p<0.05$) differences in height in response to rate occurred at 32 WAT when means were 54.4, 26.8, 20.8, and 29.7 cm for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Significant ($p<0.1$) differences in injury in response to rate occurred at 32 WAT when ratings were 2.4, 3.8, 4.2, and 3.8 for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively.

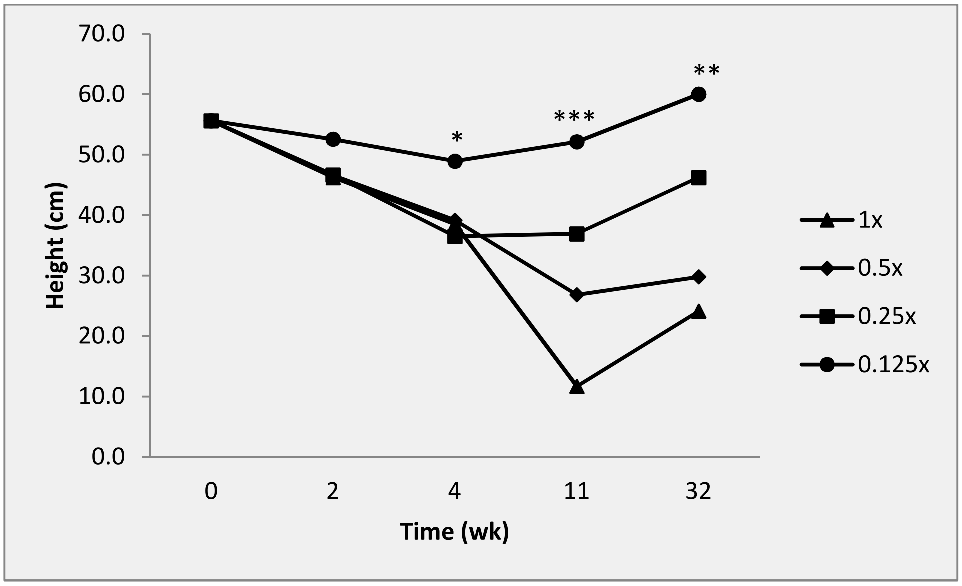

Figure 4.55. Mean height response of 1-0 red maple seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

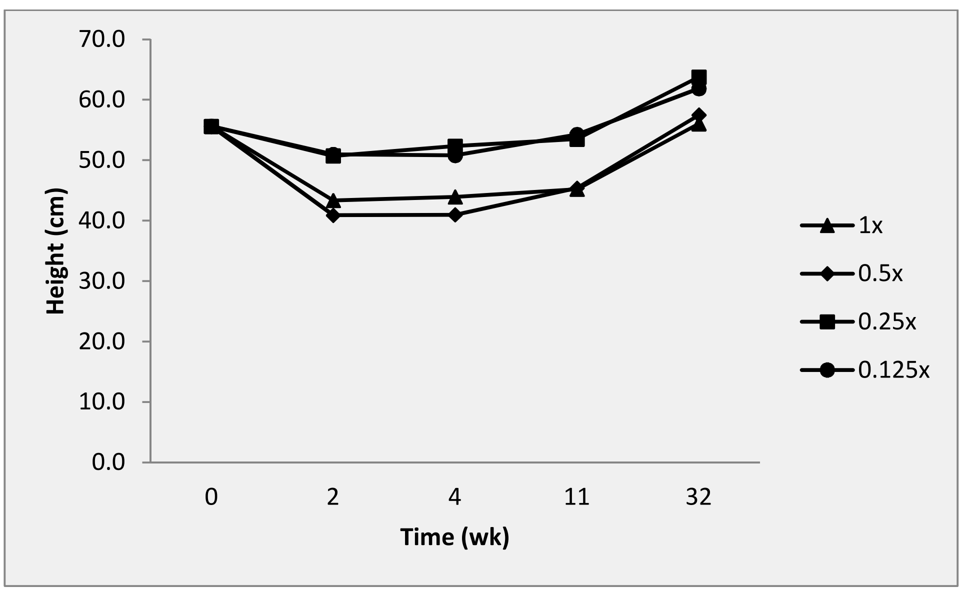

Figure 4.56. Mean height response of 1-0 red maple seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

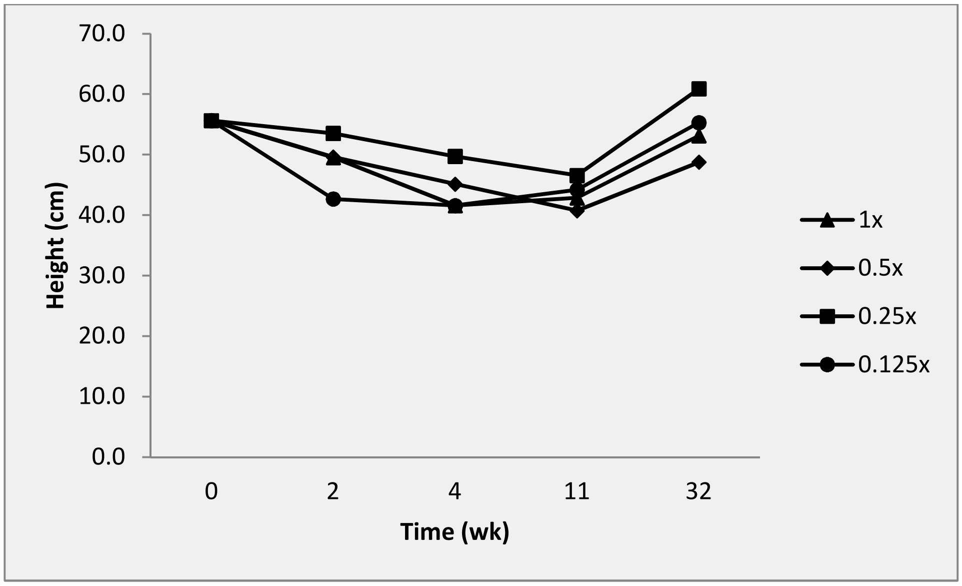

Figure 4.57. Mean height response of 1-0 red maple seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

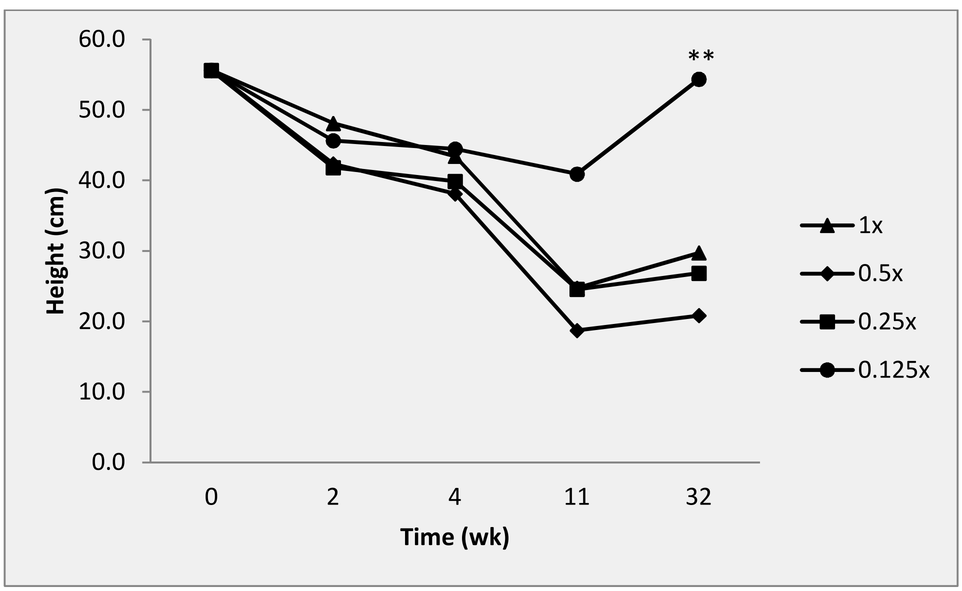

Figure 4.58. Mean height response of 1-0 red maple seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

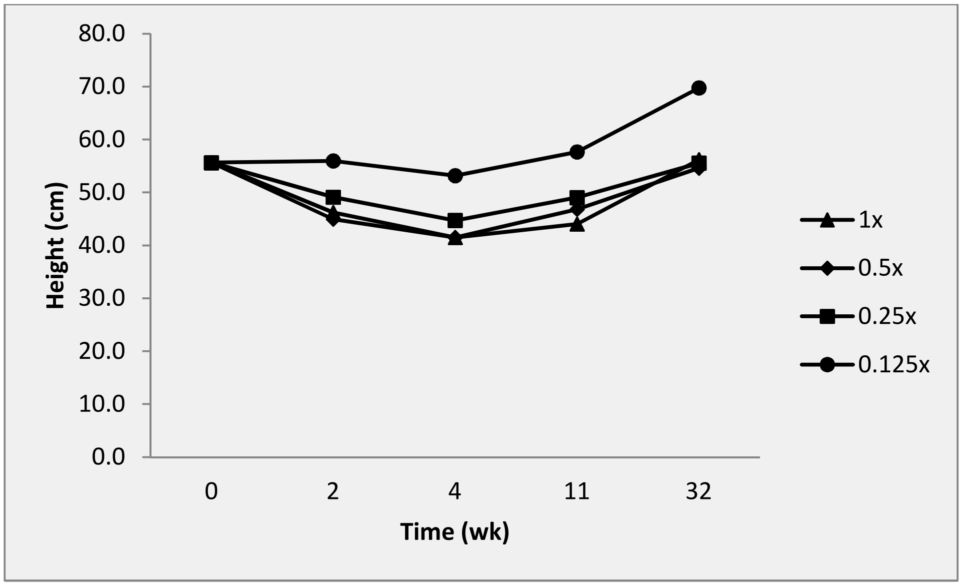

Figure 4.59. Mean height response of 1-0 red maple seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

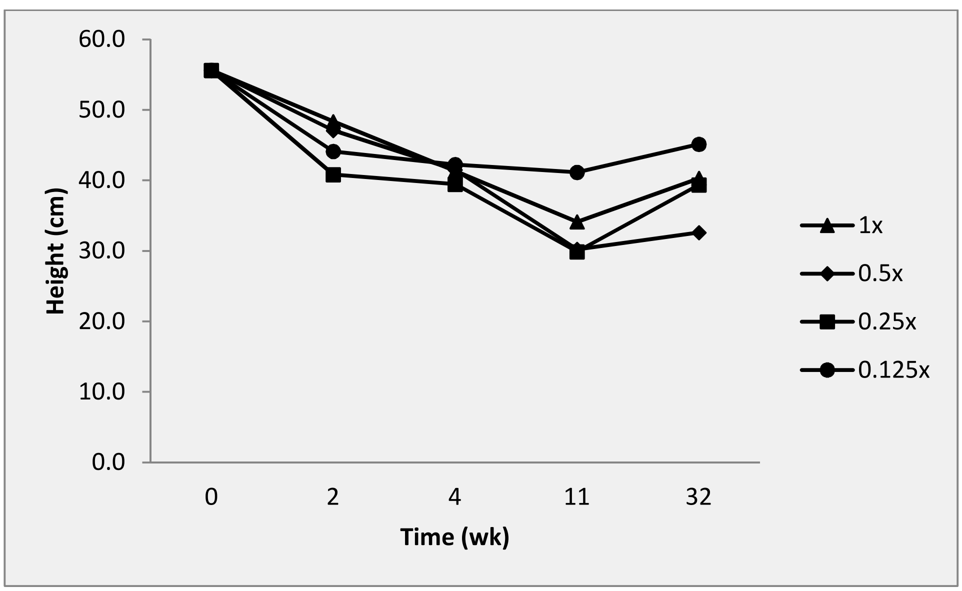

Figure 4.60. Mean height response of 1-0 red maple seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

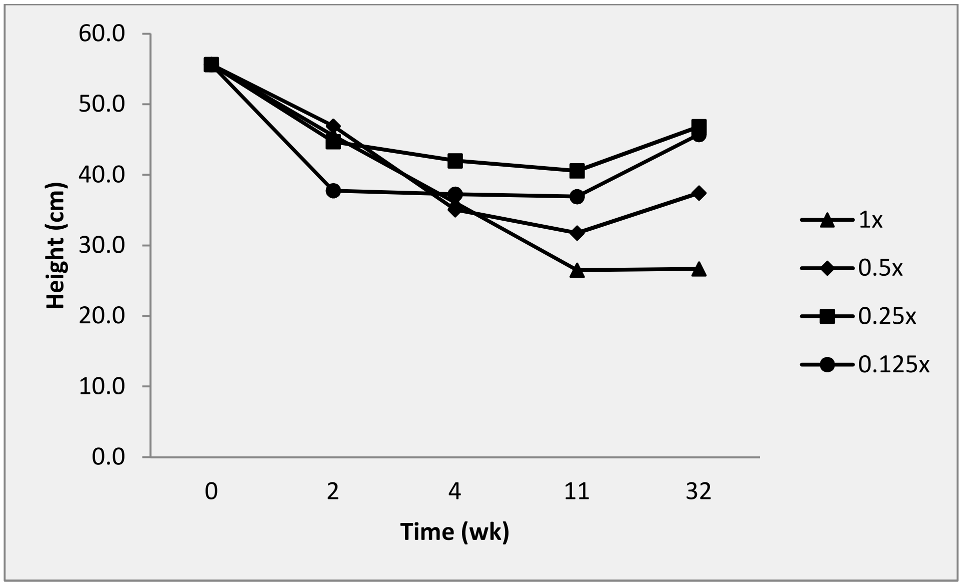

Figure 4.61. Mean height response of 1-0 red maple seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

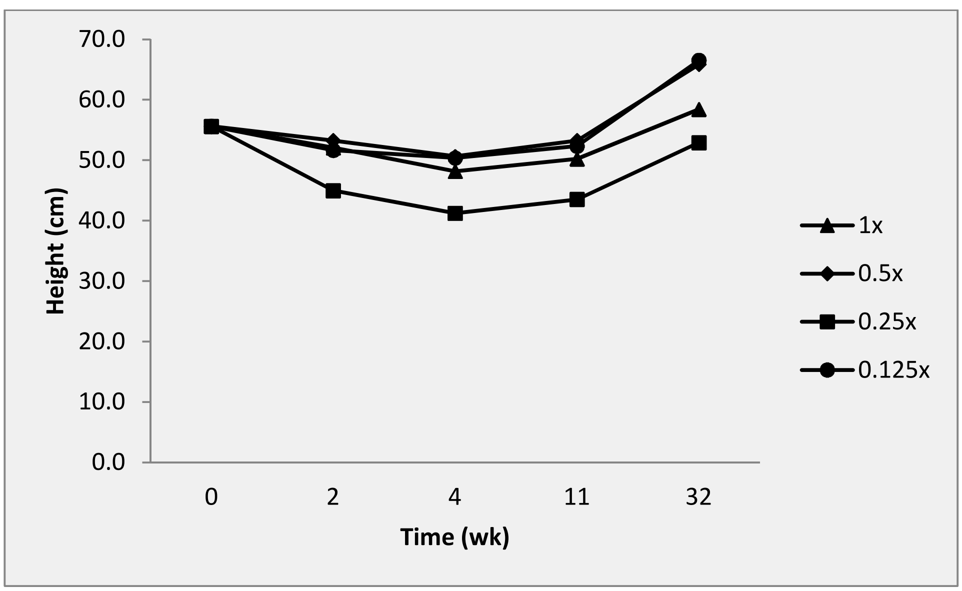

Figure 4.62. Mean height response of 1-0 red maple seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

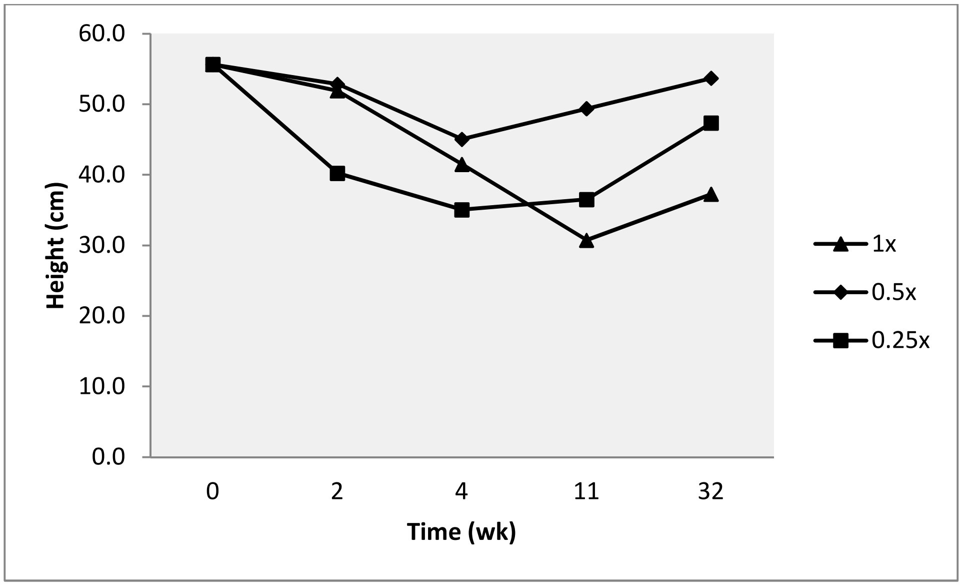

Figure 4.63. Mean height response of 1-0 red maple seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

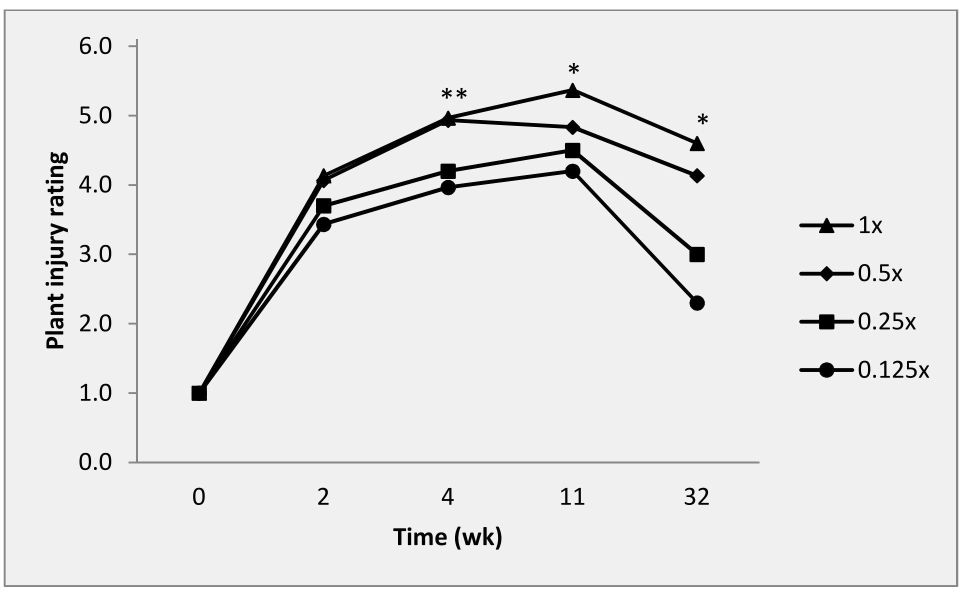

Figure 4.64. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

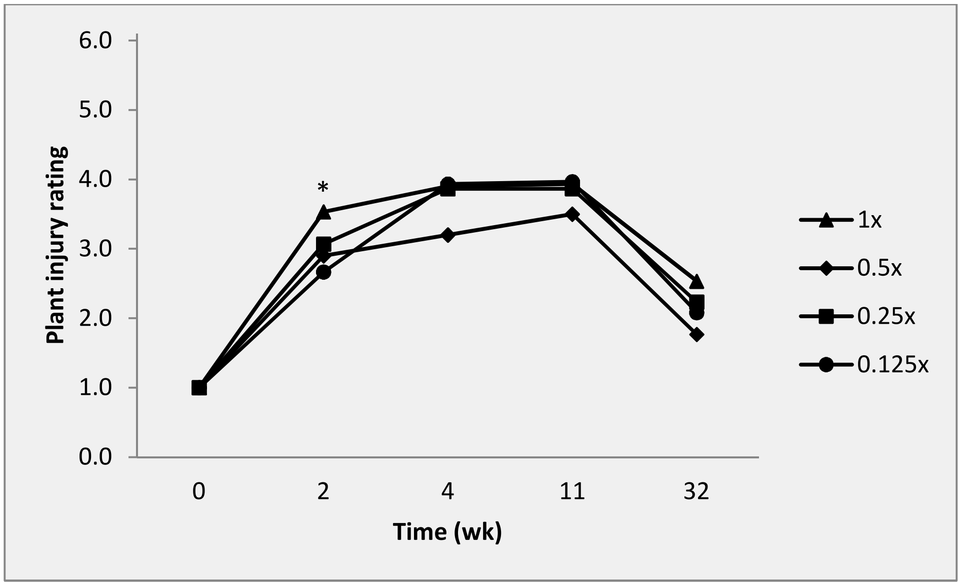

Figure 4.65. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

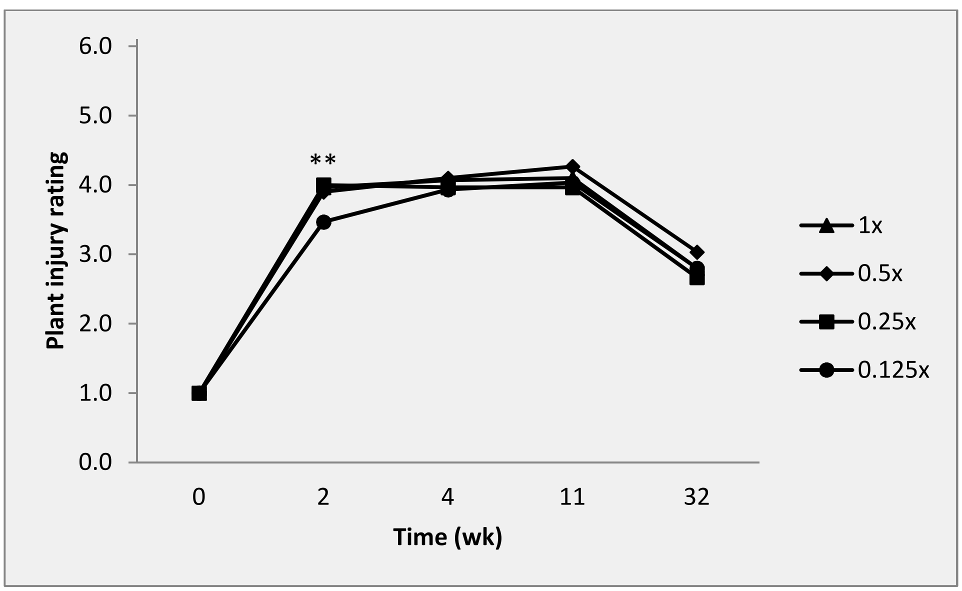

Figure 4.66. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

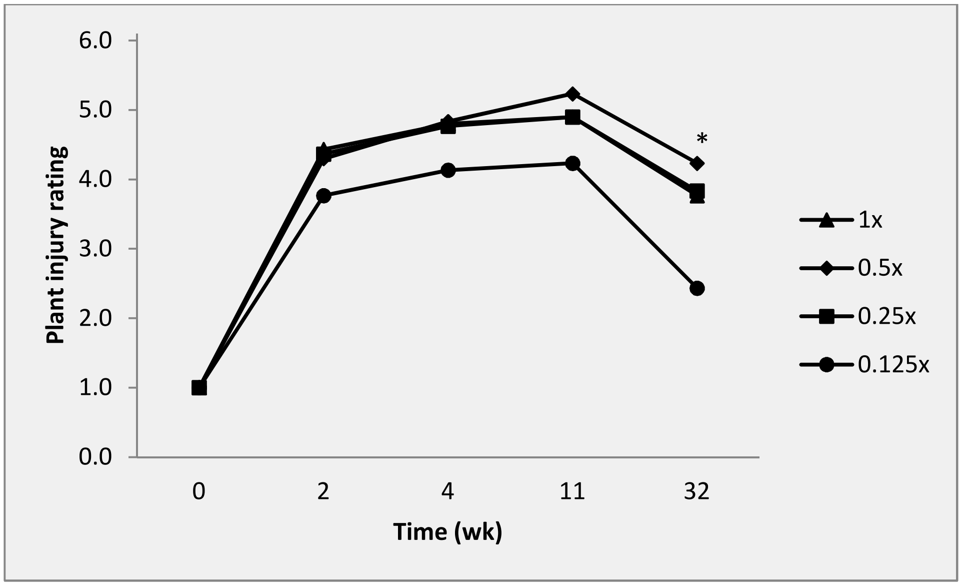

Figure 4.67. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

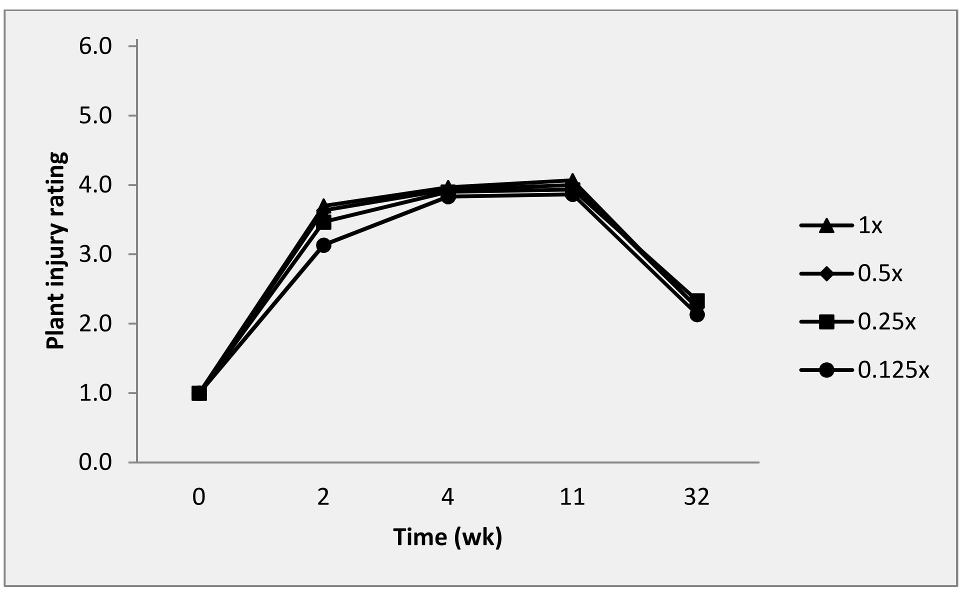

Figure 4.68. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

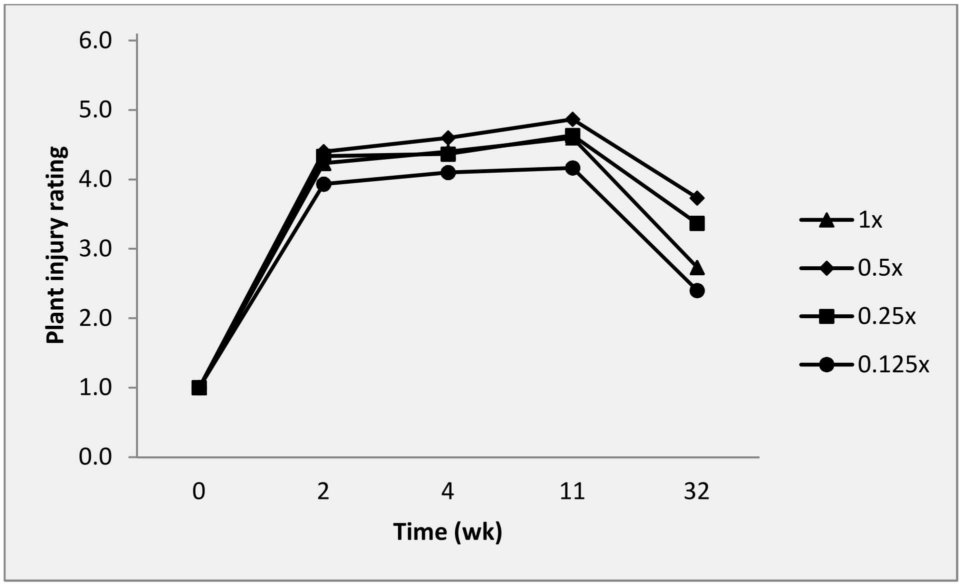

Figure 4.69. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

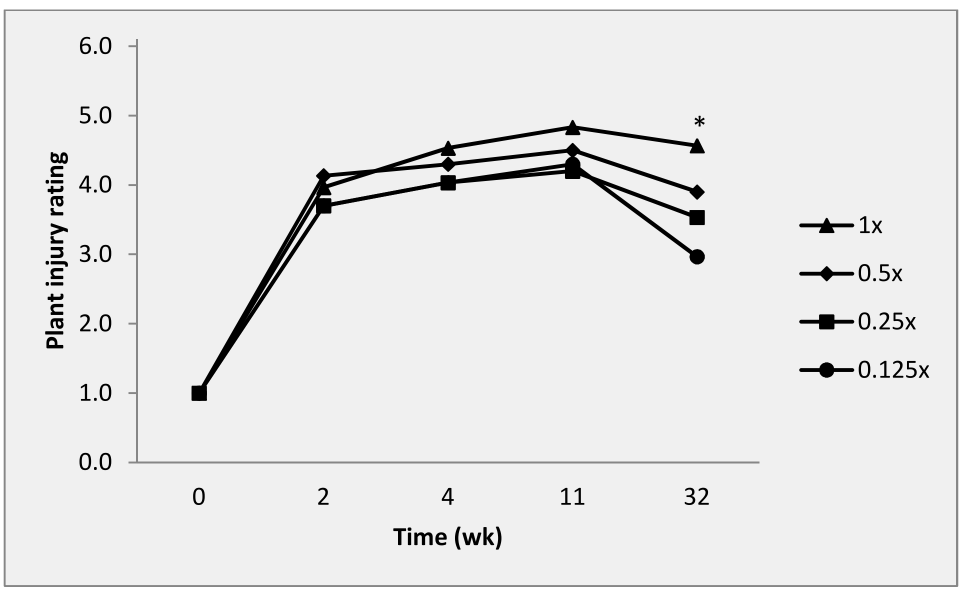

Figure 4.70. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

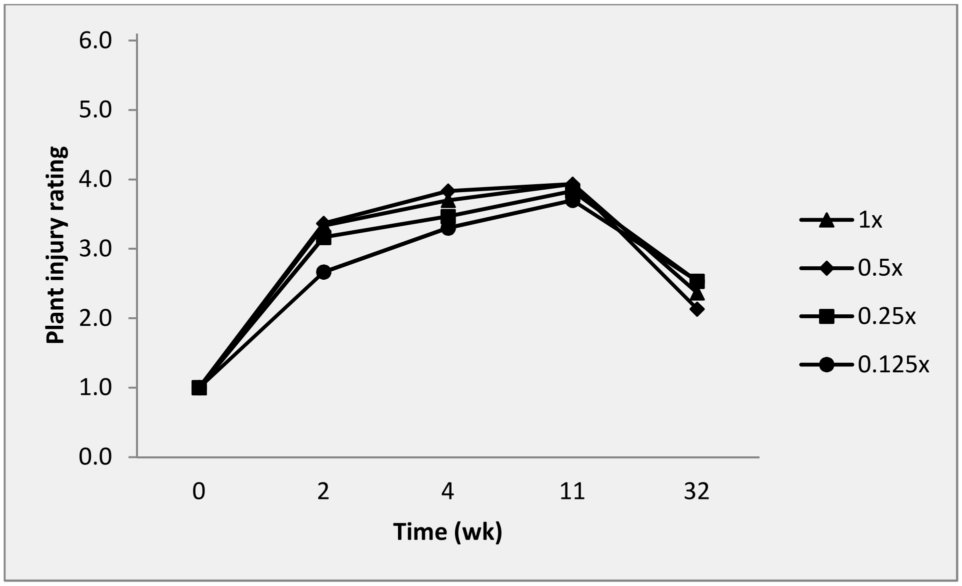

Figure 4.71. Mean plant injury rating of 1-0 red maple seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

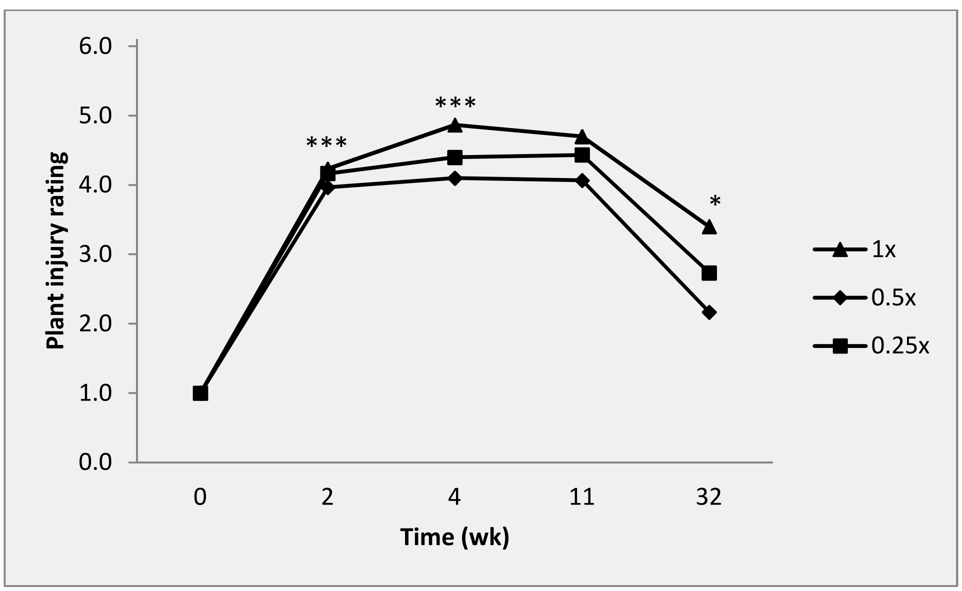

Figure 4.72. Mean plant injury rating of 1-0 red maple seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

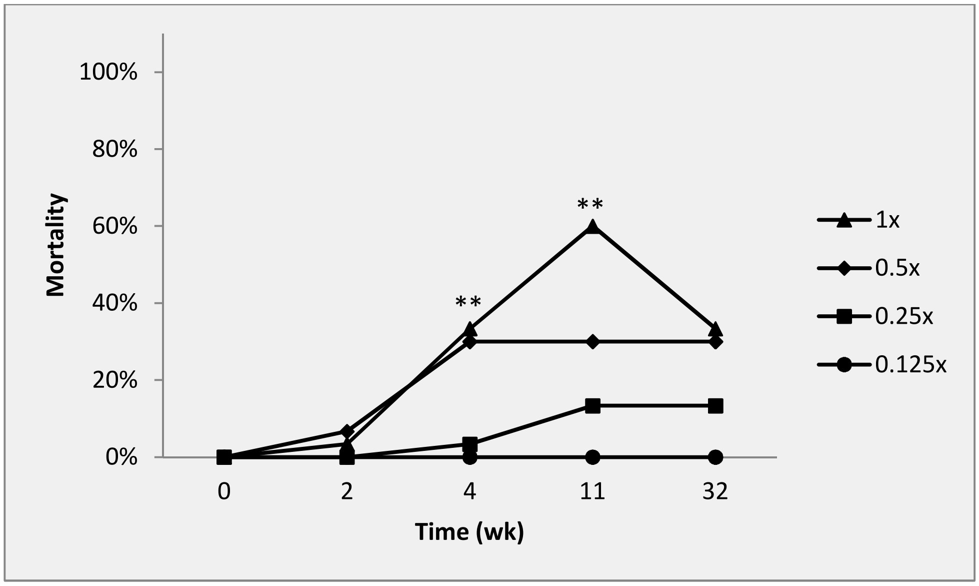

Figure 4.73. Mean percent mortality of 1-0 red maple seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

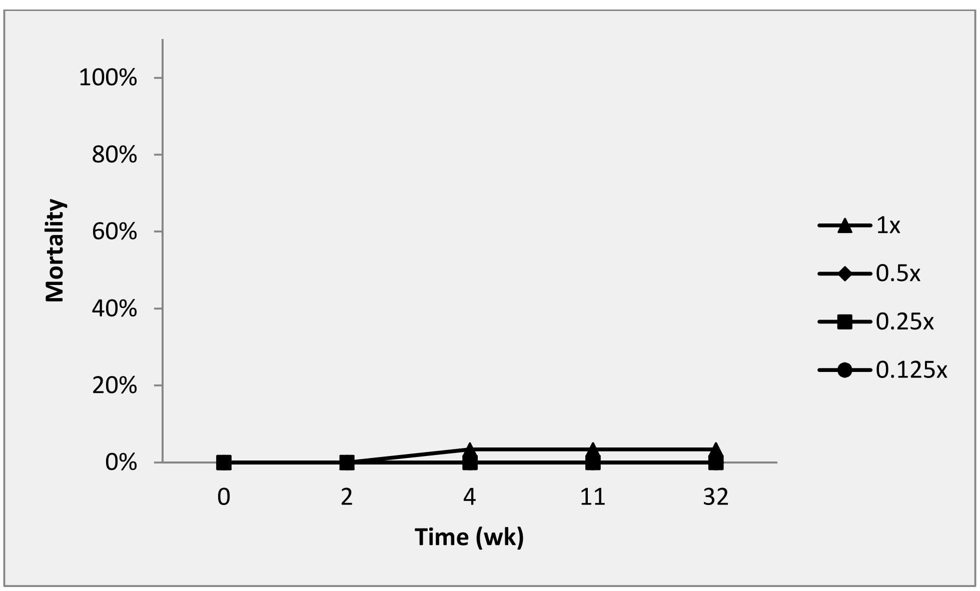

Figure 4.74. Mean percent mortality of 1-0 red maple seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

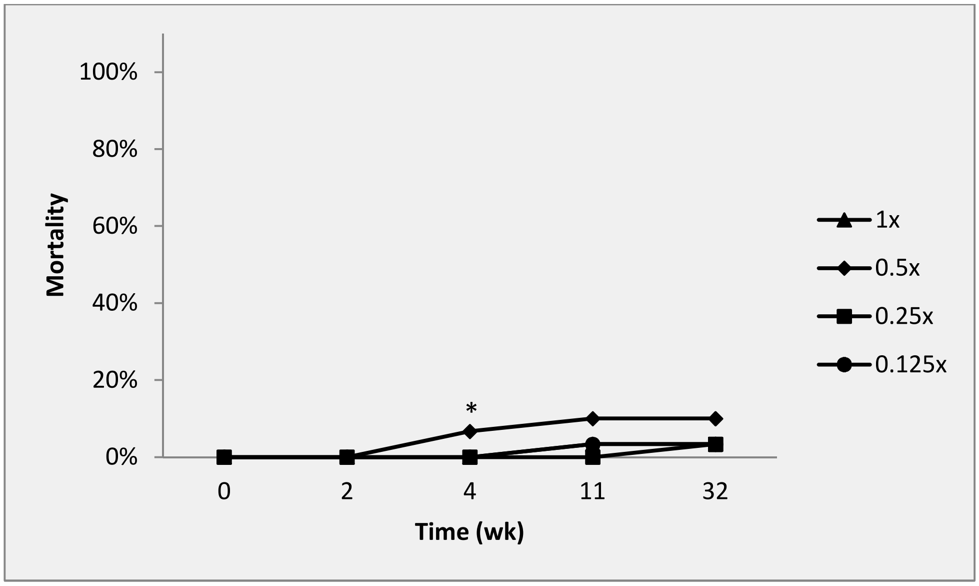

Figure 4.75. Mean percent mortality of 1-0 red maple seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

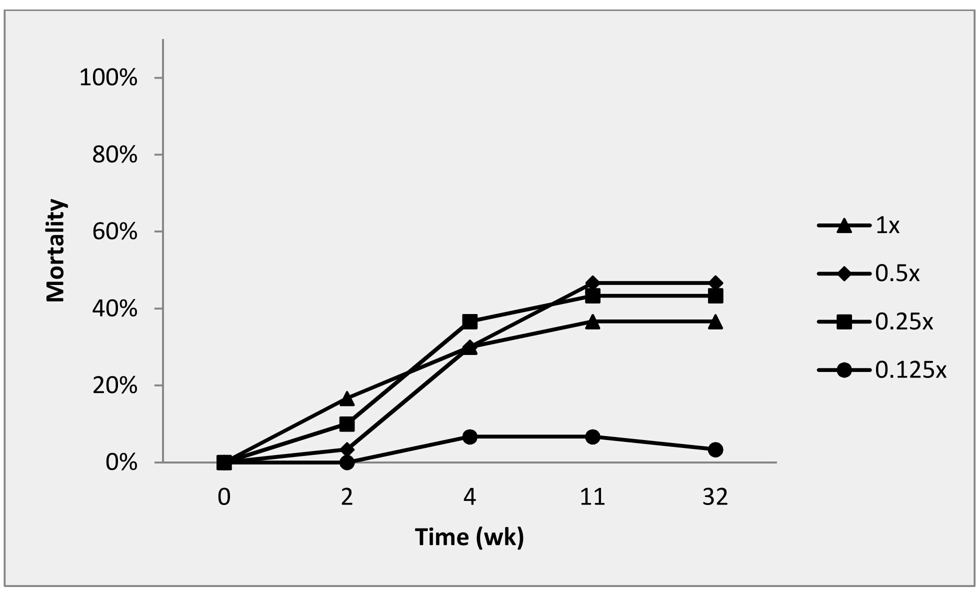

Figure 4.76. Mean percent mortality of 1-0 red maple seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

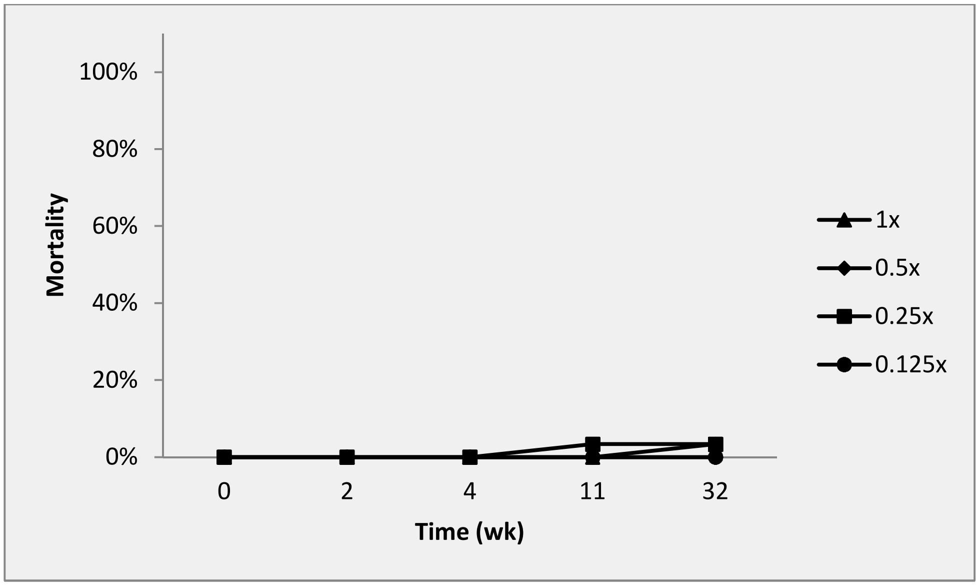

Figure 4.77. Mean percent mortality of 1-0 red maple seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

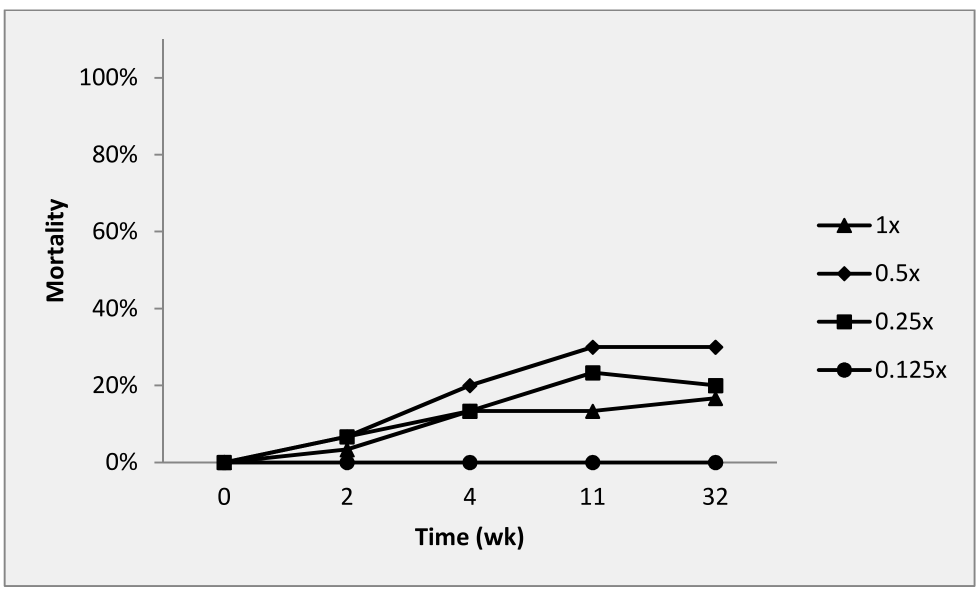

Figure 4.78. Mean percent mortality of 1-0 red maple seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

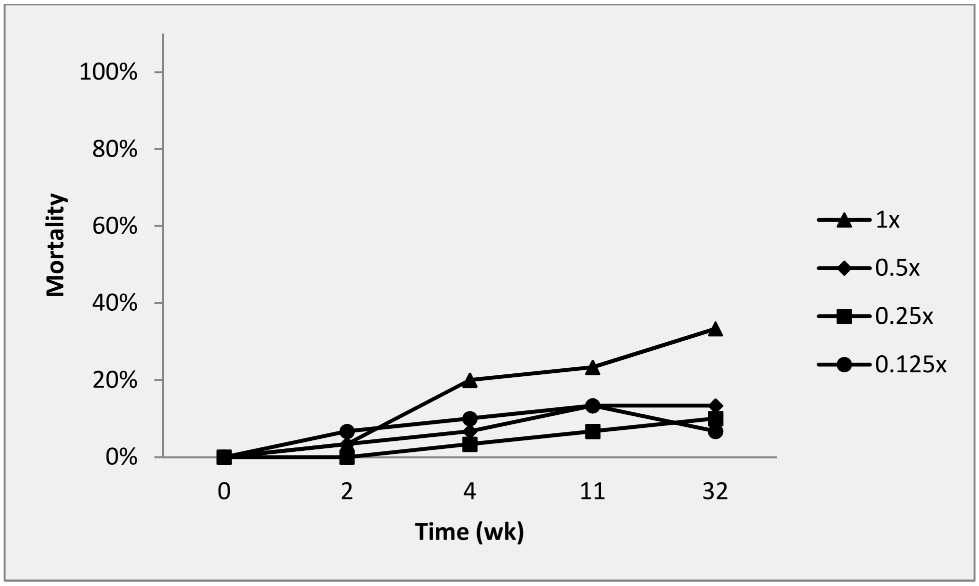

Figure 4.79. Mean percent mortality of 1-0 red maple seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

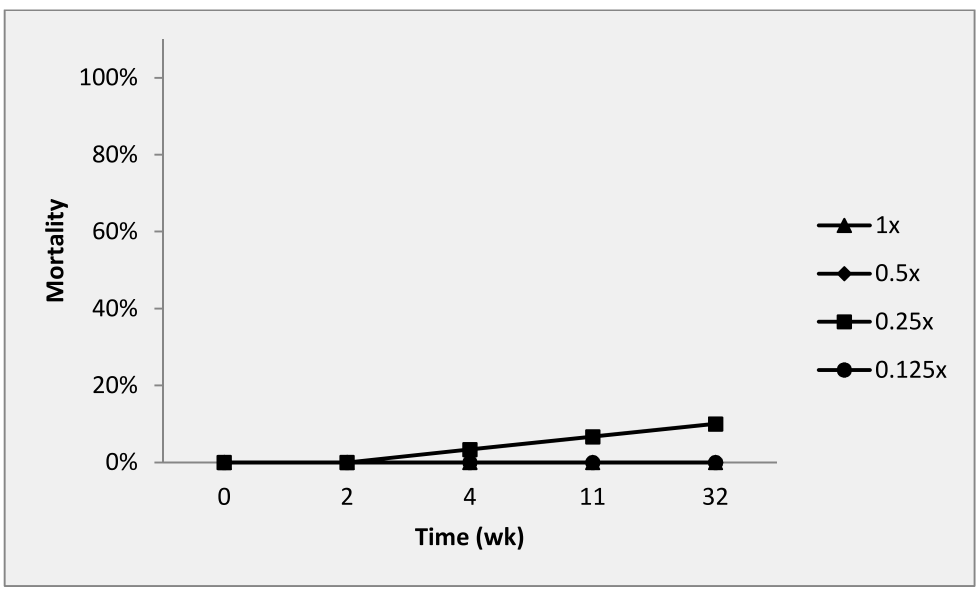

Figure 4.80. Mean percent mortality of 1-0 red maple seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

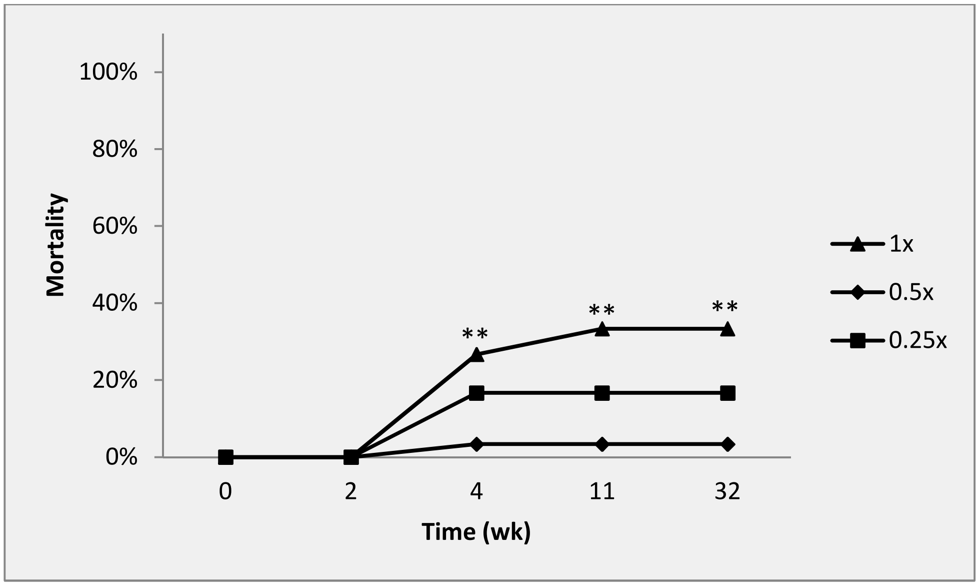

Figure 4.81. Mean percent mortality of 1-0 red maple seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Sweetgum

Height decreased by nearly 40 cm in sweetgum seedlings at 32 WAT with DAS 534 and 729 (Figures 4.82-4.90). In other herbicide treatments, such as DAS 313, 779, and 896, an increase in height of approximately 5 cm was observed from 0 to 32 WAT. A significant ($p<0.1$) herbicide by rate interaction occurred for height at 2 WAT. Height responses due to rate were tightly grouped for most herbicides at 2 WAT, but significant differences in height due to rate occurred for seedlings treated with DAS 313 and 402 (Figures 4.82 and 4.83). Significant ($p<0.0001$) differences in height in response to herbicide occurred at 4, 11, and 32 WAT and significant ($p<0.0001$) differences in height due to rate occurred at 11 and 32 WAT. Heights in DAS 729 treatments (42.2 cm) were lowest at 2 WAT, but were not significantly lower than most other herbicides (Table 4.2). At 11 and 32 WAT, DAS 729 heights (5.2 and 4.7 cm, correspondingly) continued to be the lowest of all herbicides, but were not significantly lower than DAS 534 heights (13.6 and 13.1 cm, respectively) (Tables 4.3-4.4).

Injury rating reached highest levels at 11 WAT in most treatments and decreased following dormancy, except for the 1x rates of DAS 534 and 729 (Figures 4.91-4.99). The highest rates of these herbicides behaved differently than others even though a significant herbicide by rate interaction did not occur. There were significant ($p<0.001$) differences in injury in response to herbicide and rate at 2, 4, 11, and 32 WAT. Injury rating of DAS 729 treatments was higher than other herbicides at 2 WAT (4.4) and remained highest through 32 WAT (5.4) (Tables 4.5-4.8). DAS 534 also produced high injury at 32 WAT (4.8) in sweetgum which was not significantly lower than DAS 729.

At 11 WAT, DAS 402 (33.3%), 534 (53.3%), 729 (79.2%), and triclopyr (30.7%) produced greater than 20% and less than 80% mortality in sweetgum seedlings (Table 4.10 and Figures 4.100-4.108). Significant differences in height in response to rate occurred in DAS 402 treatments at 2 ($p<0.1$), 11 ($p<0.05$), and 32 ($p<0.1$) WAT (Figure 4.83). Heights were: 45.6, 53.0, 39.5, and 43.9 cm at 2 WAT, 31.6, 35.6, 17.9, and 11.6 cm at 11 WAT, and 30.5, 39.2, 20.4, and 10.5 cm at 32 WAT for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. The difference in heights due to rate continued to increase numerically up to 32 WAT, but was most significant at 11 WAT. Injury rating in DAS 402 treatments reached maximum levels at 11 WAT where differences in injury due to rate were significant ($p<0.05$) (Figure 4.92). Differences in injury rating due to rate increased numerically from 11 to 32 WAT, but were no longer significant.

Significant differences in height in response to rate occurred in DAS 534 treatments at 11 ($p<0.05$) and 32 ($p<0.01$) WAT (Figure 4.84). Treatment means at 11 WAT were 22.1, 18.4, 8.0, and 5.8 cm for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. At 32 WAT difference in heights due to rate had increased; means were: 25.7, 18.8, 7.0, and 0.7 cm for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Differences in injury in response to rate were significant ($p<0.1$) at 11 and 32 WAT (Figure 4.93). Means at 11 WAT were 4.9, 5.1, 5.6, and 5.6 for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. Differences in injury due to rate increased numerically from 11 to 32 WAT to 3.7, 4.3, 5.5, and 5.9 for the 0.125x, 0.25x, 0.5x, and 1x rates.

No significant differences in height or injury in response to rate occurred in DAS 729 (Figures 4.87 and 4.96) or triclopyr (Figures 4.90 and 4.99) treatments.

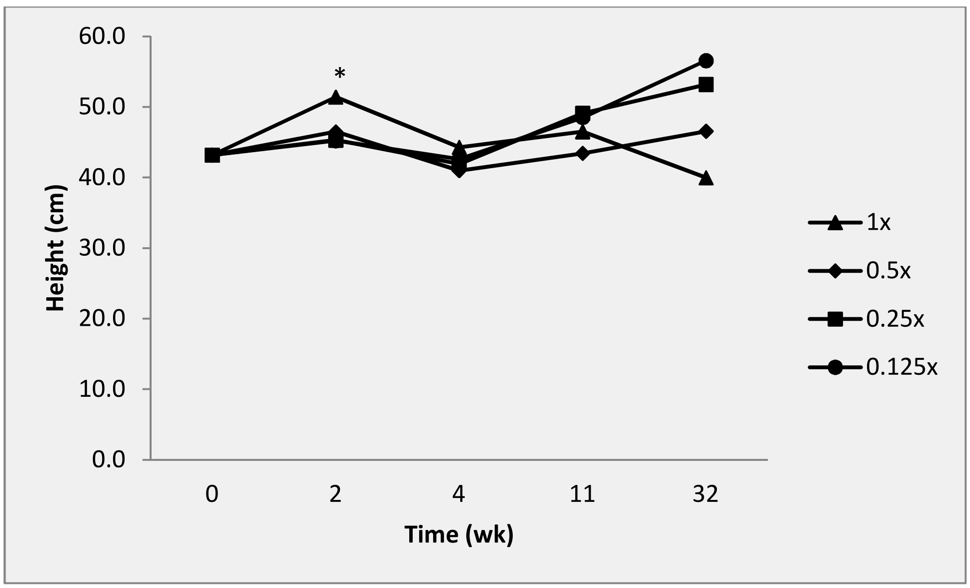

Figure 4.82. Mean height response of 1-0 sweetgum seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

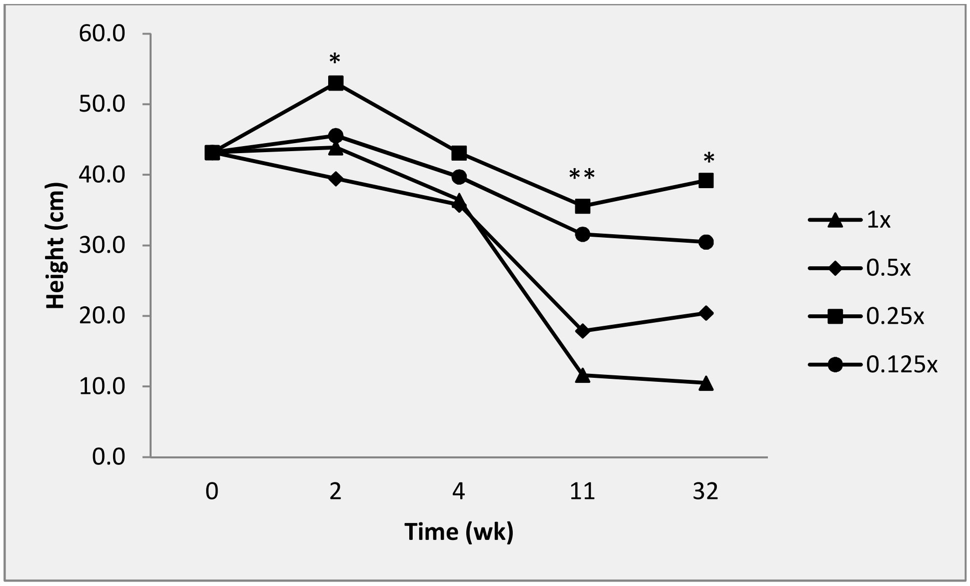

Figure 4.83. Mean height response of 1-0 sweetgum seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

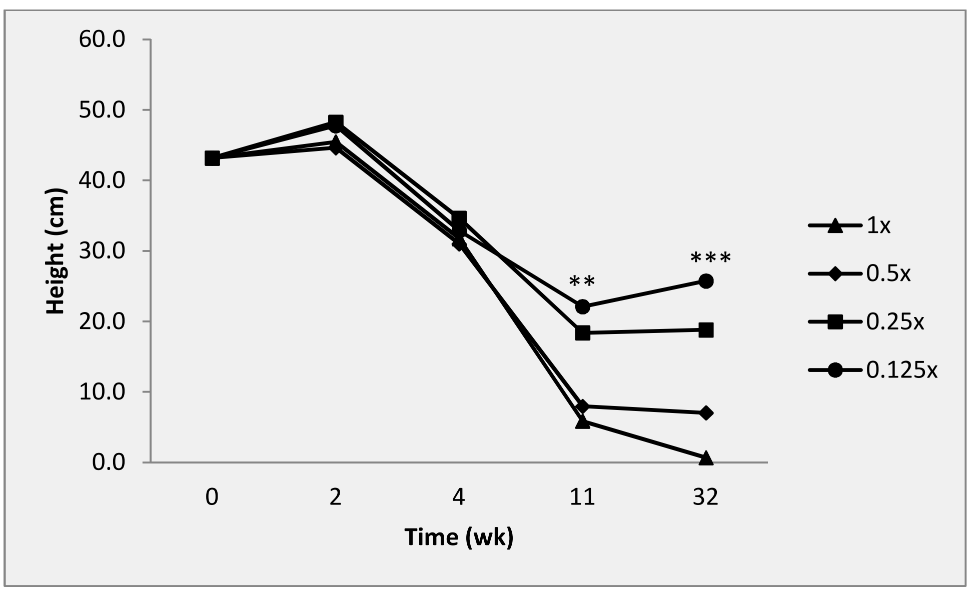

Figure 4.84. Mean height response of 1-0 sweetgum seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

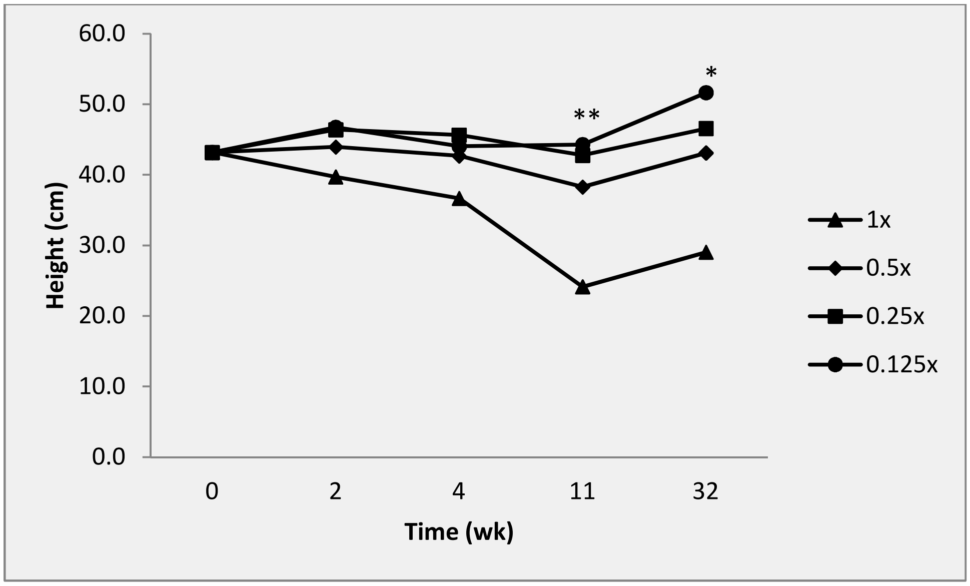

Figure 4.85. Mean height response of 1-0 sweetgum seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

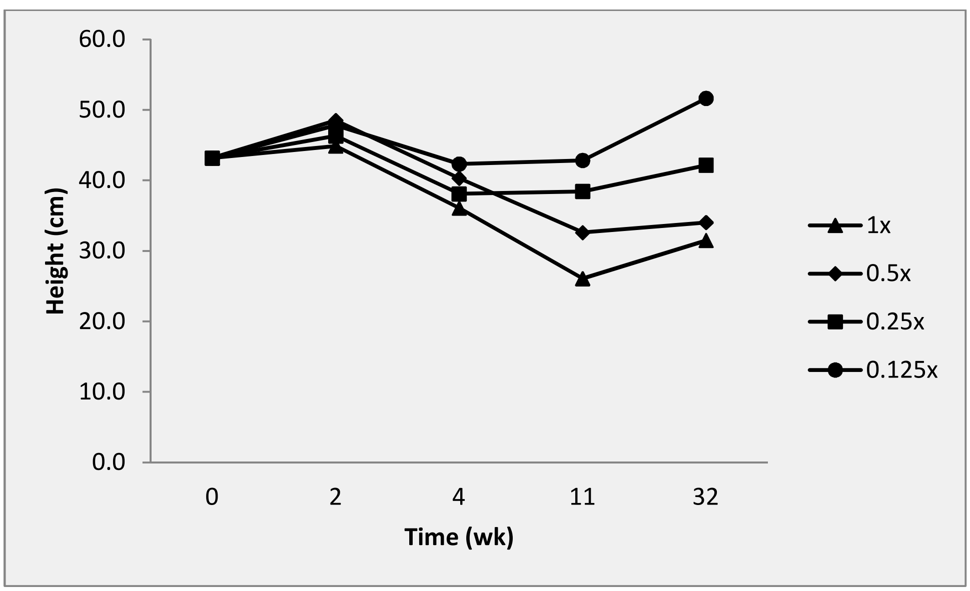

Figure 4.86. Mean height response of 1-0 sweetgum seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

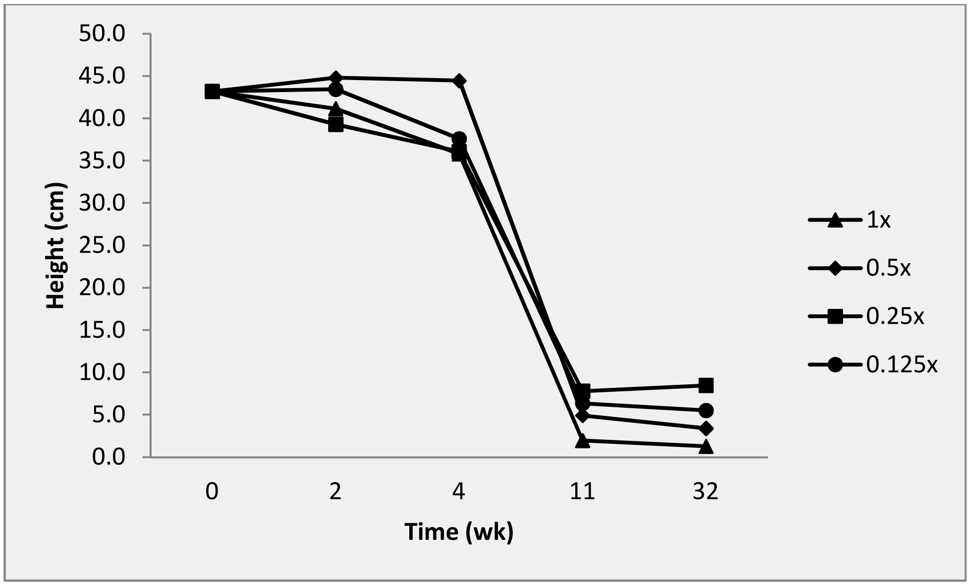

Figure 4.87. Mean height response of 1-0 sweetgum seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

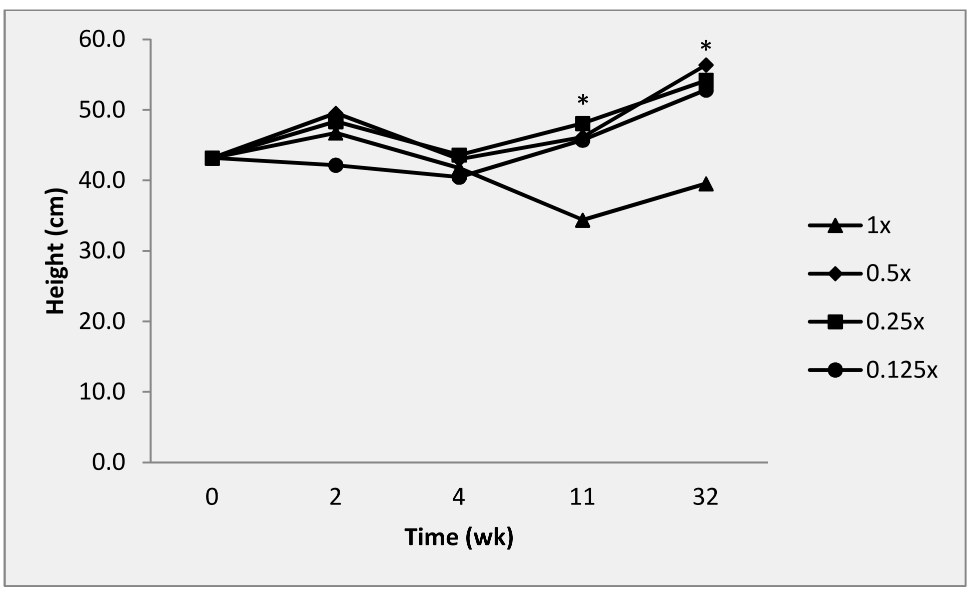

Figure 4.88. Mean height response of 1-0 sweetgum seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

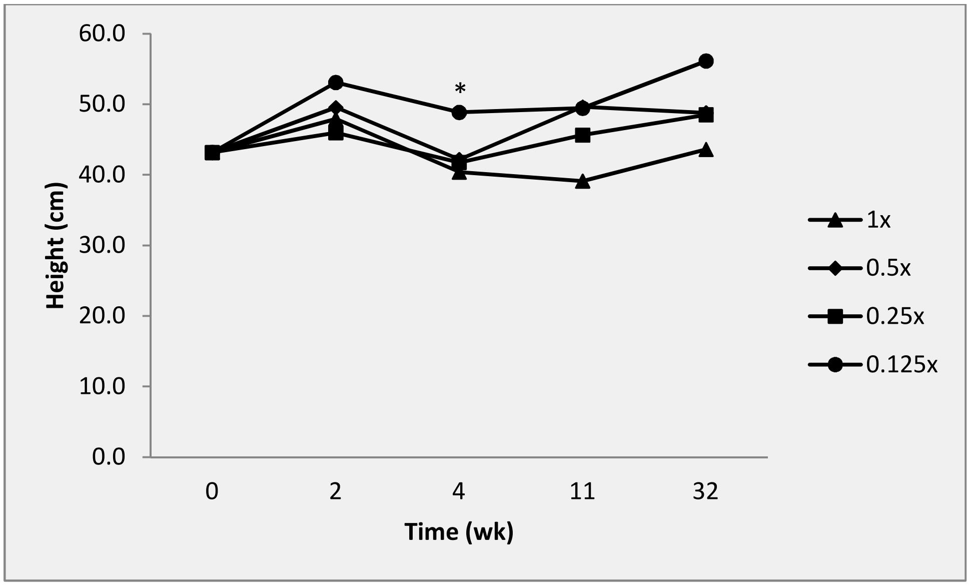

Figure 4.89. Mean height response of 1-0 sweetgum seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

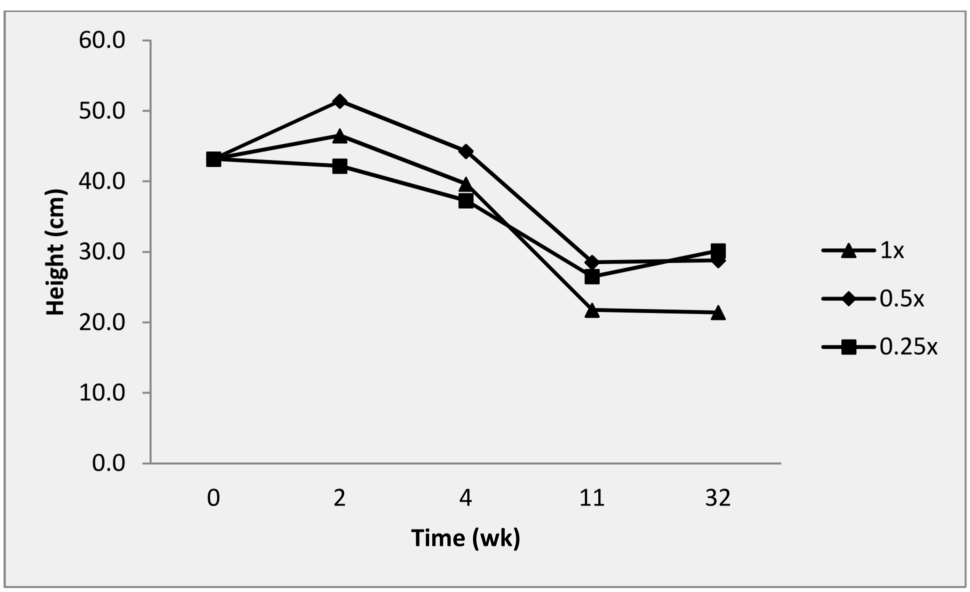

Figure 4.90. Mean height response of 1-0 sweetgum seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

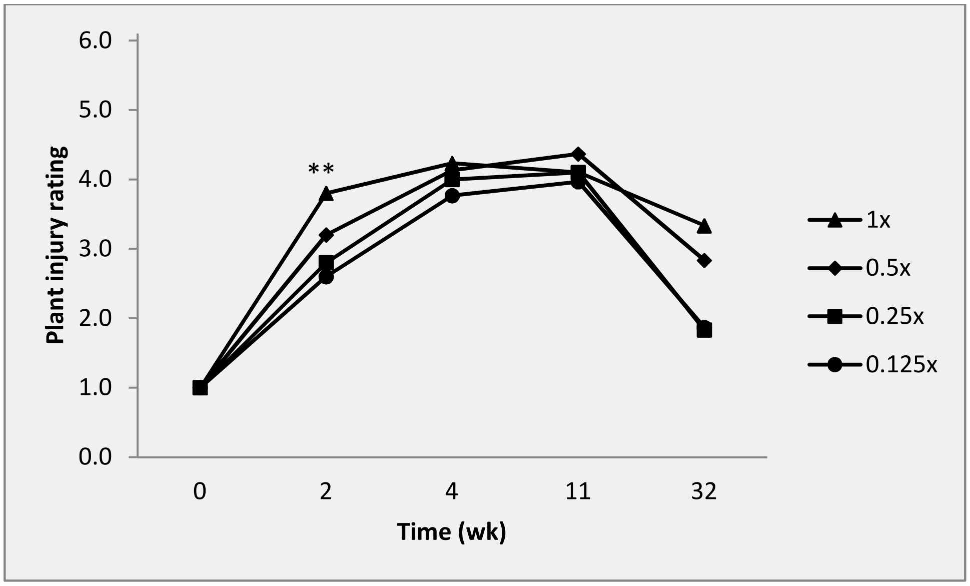

Figure 4.91. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

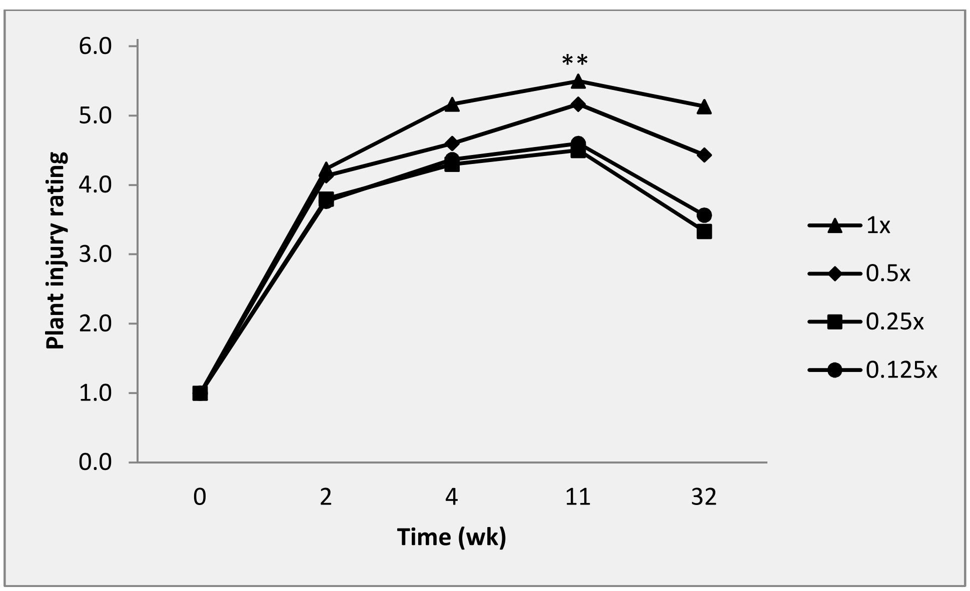

Figure 4.92. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

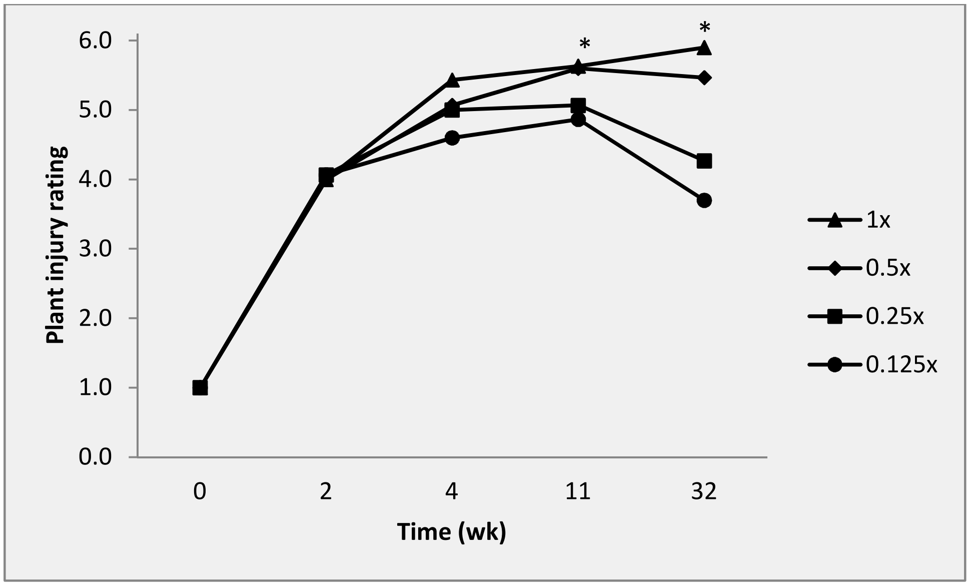

Figure 4.93. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

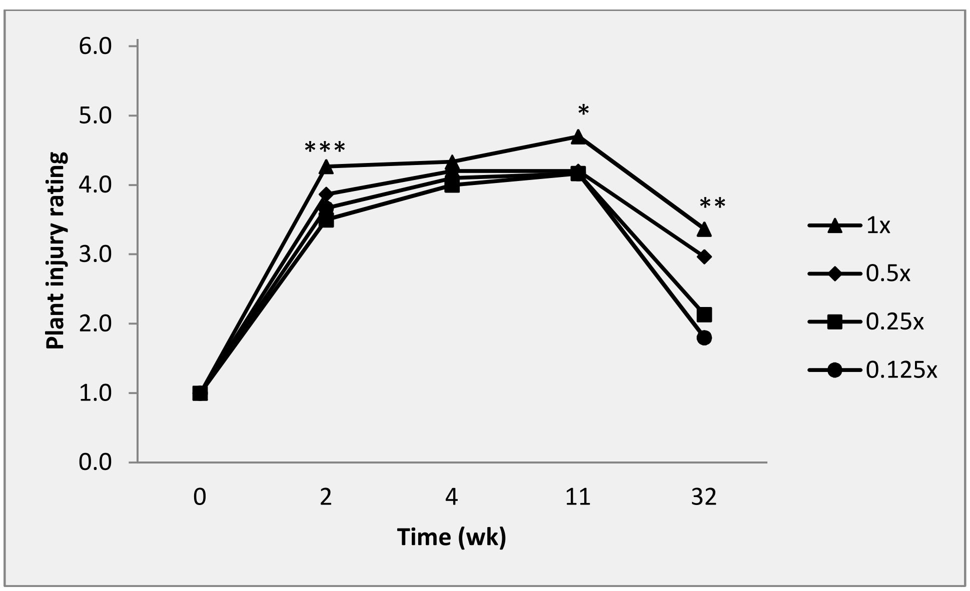

Figure 4.94. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

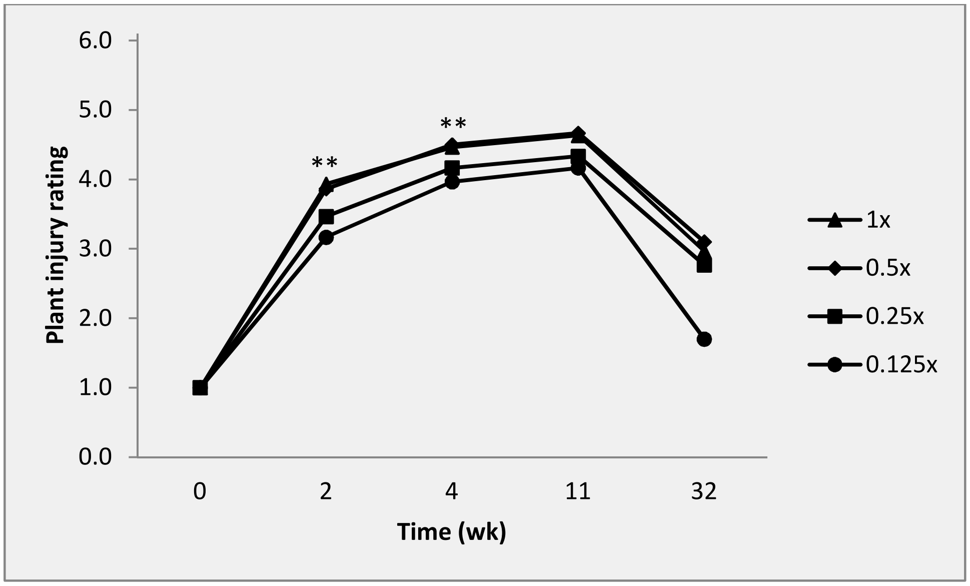

Figure 4.95. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

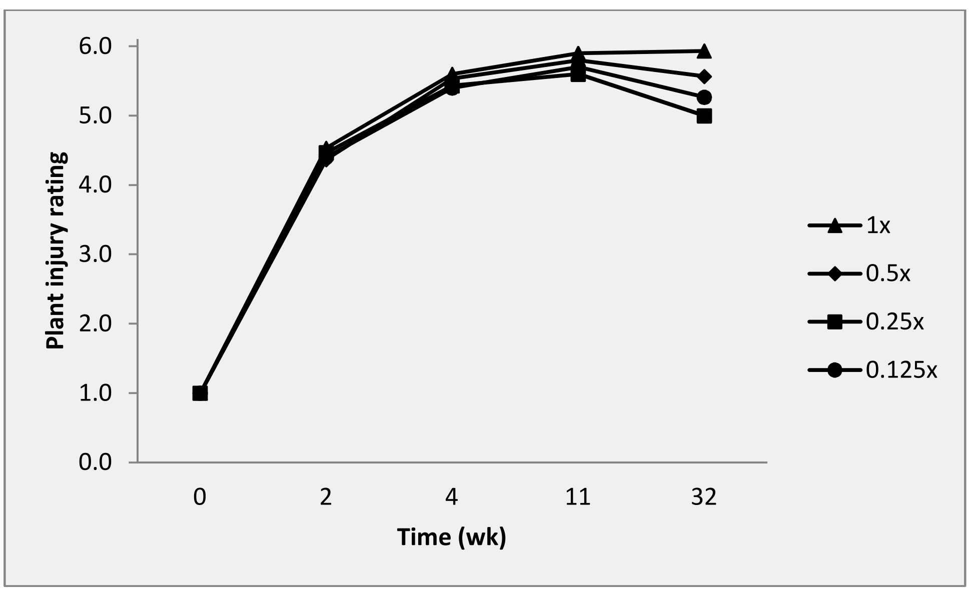

Figure 4.96. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

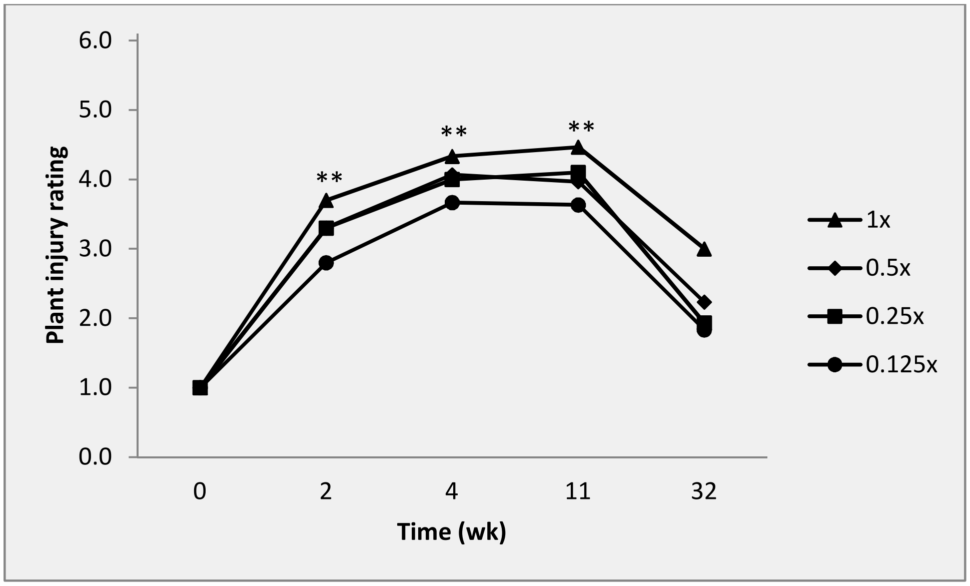

Figure 4.97. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

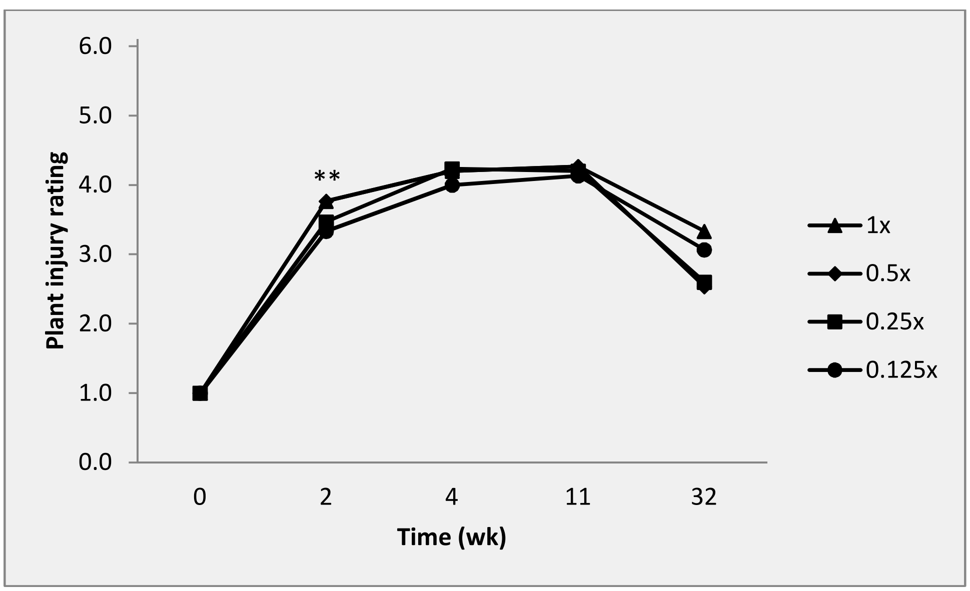

Figure 4.98. Mean plant injury rating of 1-0 sweetgum seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

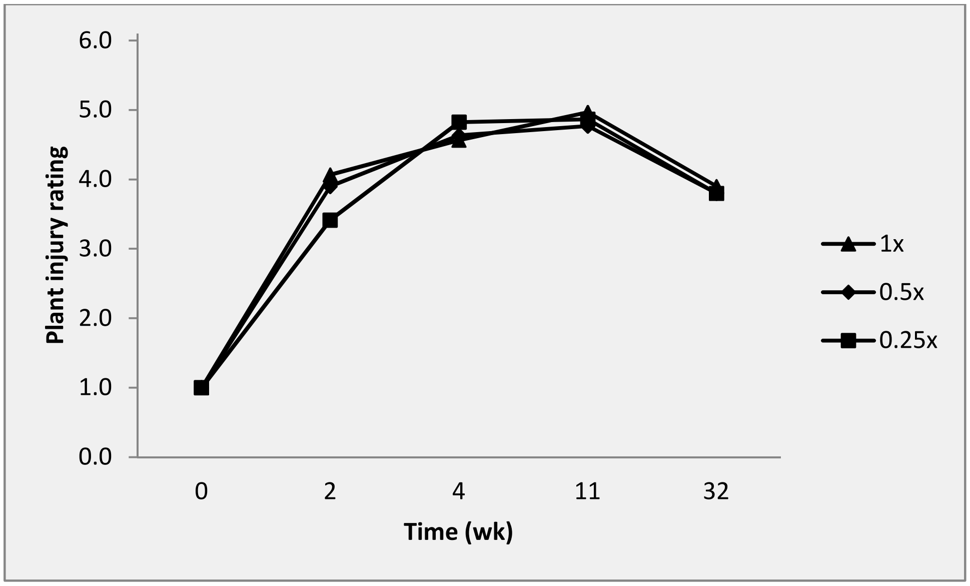

Figure 4.99. Mean plant injury rating of 1-0 sweetgum seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

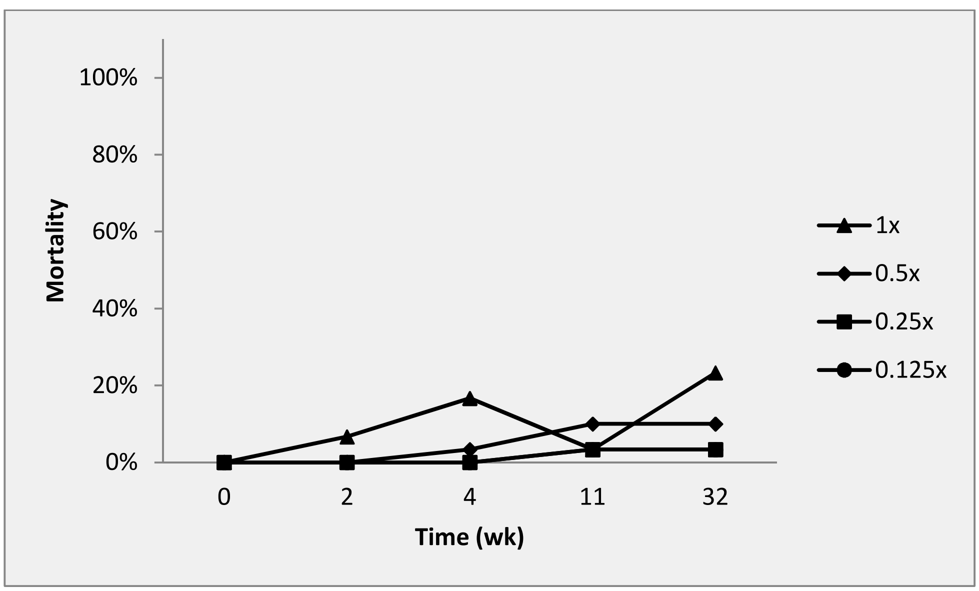

Figure 4.100. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

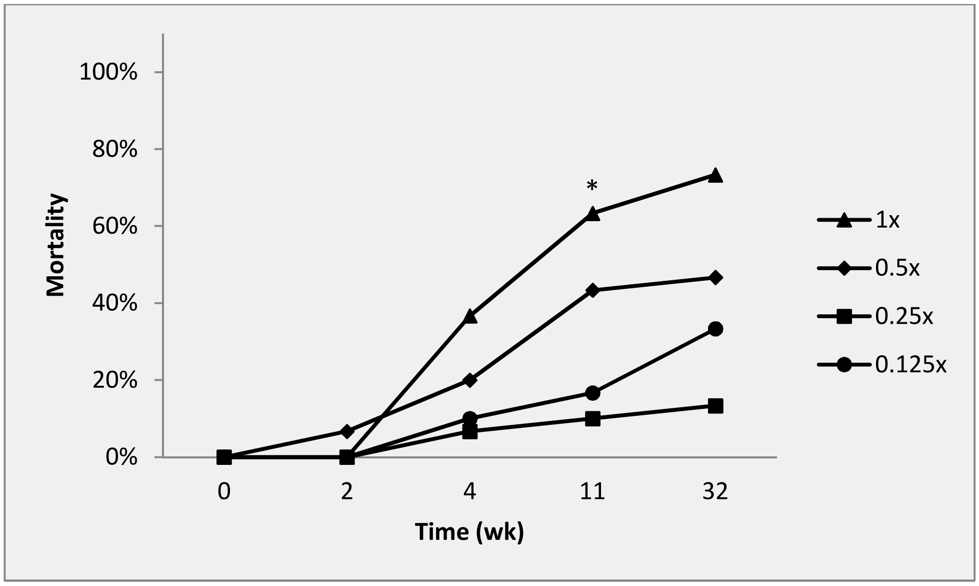

Figure 4.101. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

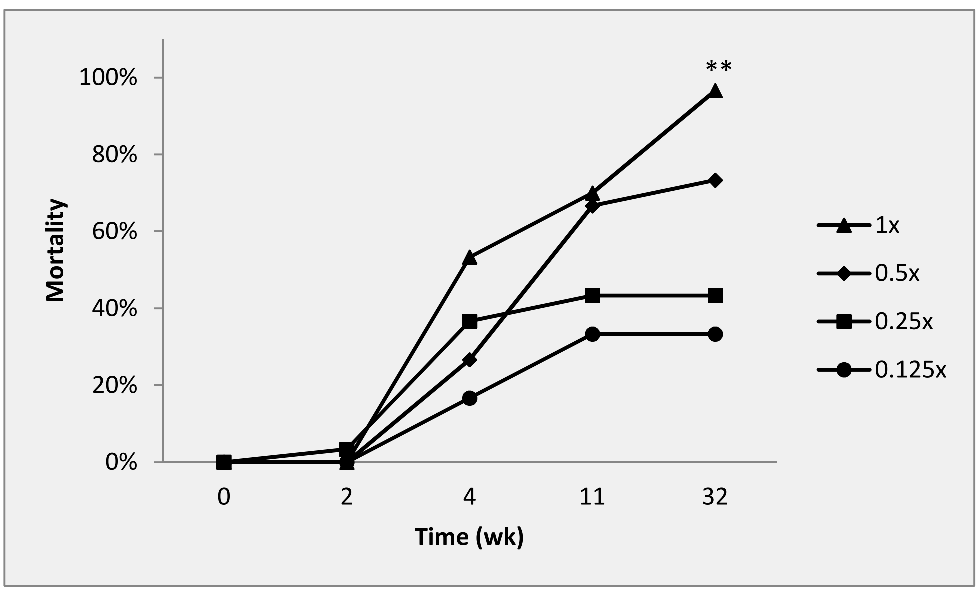

Figure 4.102. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

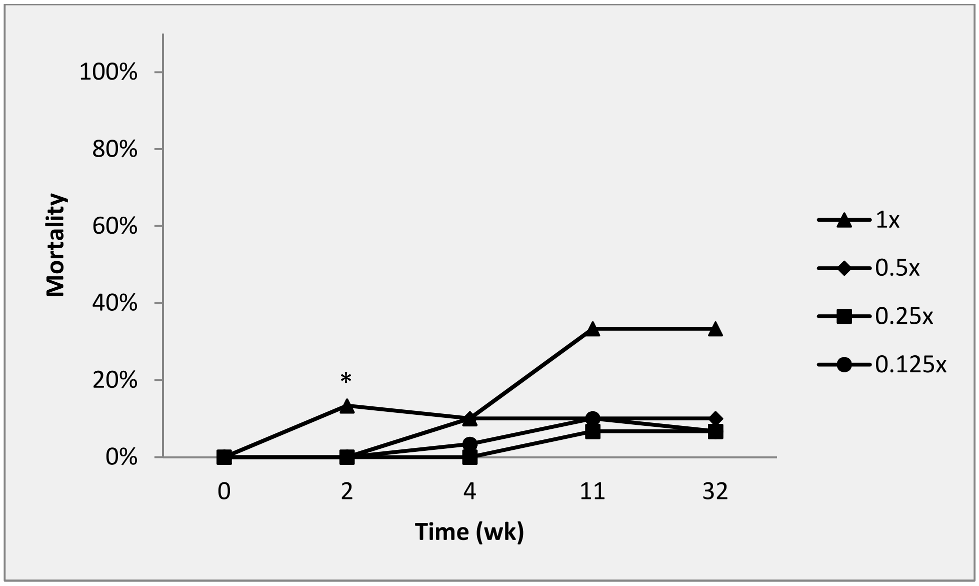

Figure 4.103. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

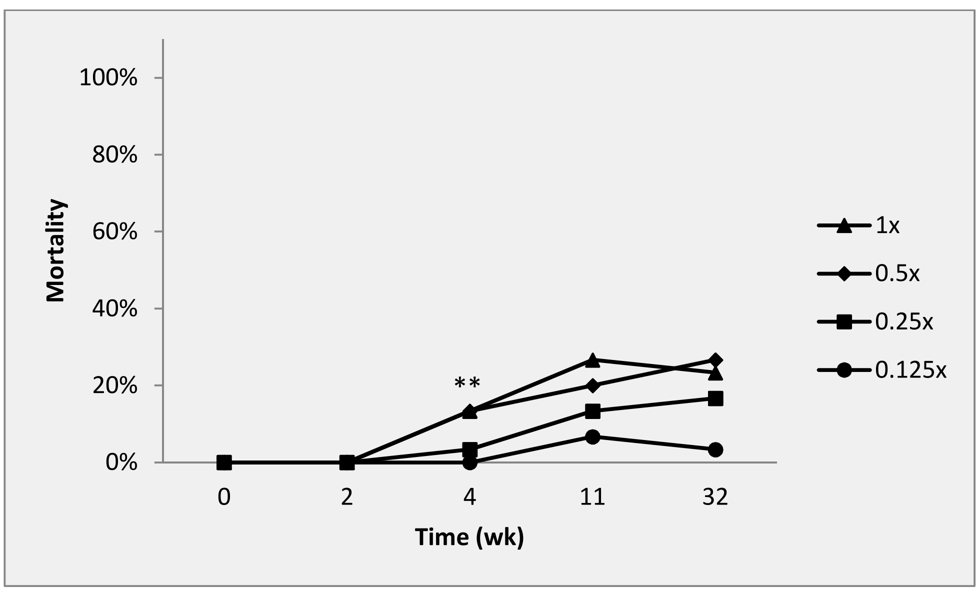

Figure 4.104. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

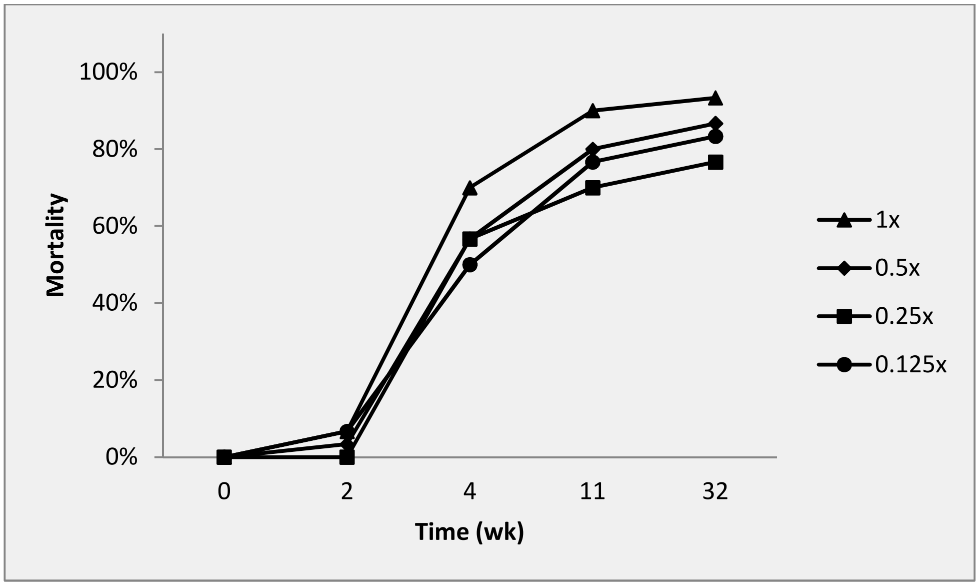

Figure 4.105. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

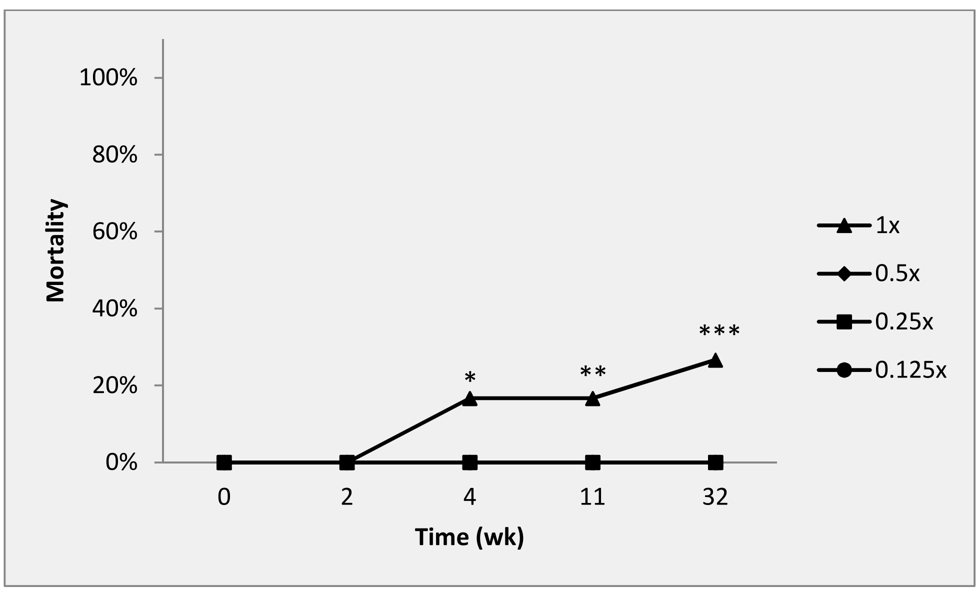

Figure 4.106. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

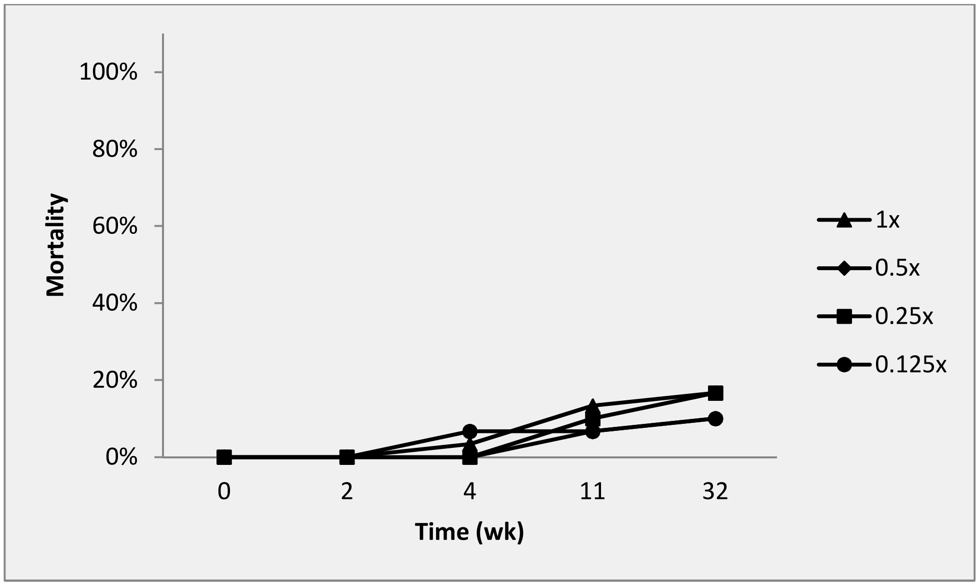

Figure 4.107. Mean percent mortality of 1-0 sweetgum seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

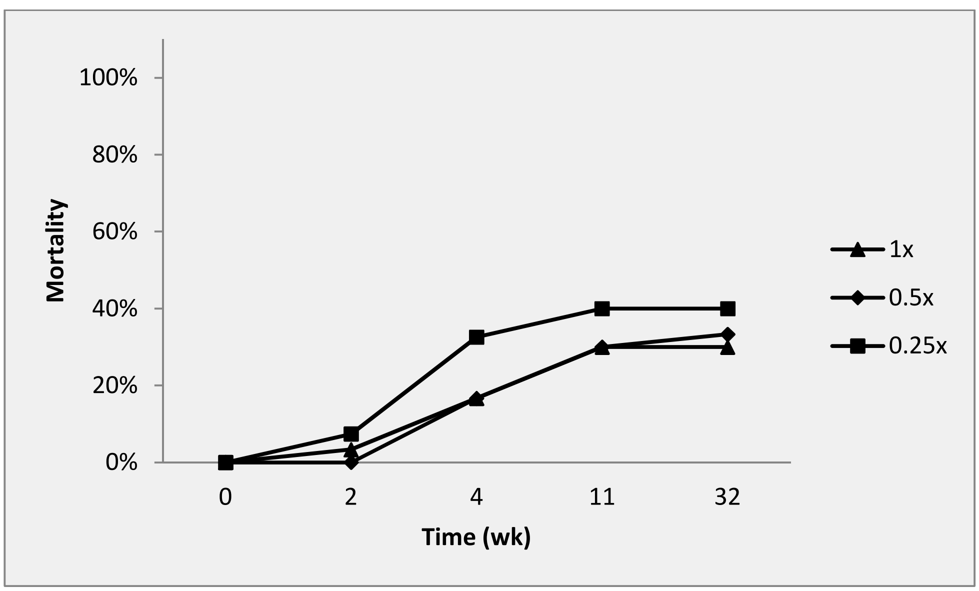

Figure 4.108. Mean percent mortality of 1-0 sweetgum seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Water Oak

In response to DAS 779 and triclopyr, water oak height decreased from 40.6 cm to 18.5 and 33 cm, respectively, from 0 to 32 WAT (Figures 4.109-4.117). In other herbicides, such as DAS 896, an increase in height of 18 cm occurred from 0 to 32 WAT. However, no significant herbicide by rate interaction occurred. Significant differences in height in response to herbicides occurred at 4 ($p<0.01$), 11 ($p<0.0001$), and 32 ($p<0.0001$) WAT. Significant differences in height in response to rate occurred at 11 ($p<0.05$) and 32 ($p<0.001$) WAT. At 2 (35.6 cm), 11 (20.0 cm), and 32 (18.5 cm) WAT DAS 779 resulted in the lowest height responses, but DAS 779 heights were not significantly different from other herbicides (Tables 4.2-4.4).

Mean plant injury ratings peaked at 11 WAT and decreased following dormancy (Figures 4.118-4.126). Significant ($p<0.0001$) differences in injury rating due to herbicides occurred at 2, 4, 11, and 32 WAT. In response to rates, significant differences in injury occurred at 2 ($p<0.01$), 4 ($p<0.01$), 11 ($p<0.05$), and 32 ($p<0.01$) WAT. Significant differences in injury due to herbicide occurred in as little as 2 weeks (Table 4.5). DAS 779 had the highest injury rating at 2 (3.7) and 11 (4.7) WAT (Tables 4.5 and 4.7), but differences were not always statistically significant from other herbicides. DAS 779 also had the highest mortality at 32 WAT, 37.5%, although not significantly higher than DAS 313, 534, 548, 729, or triclopyr (Table 4.9).

DAS 534 (22.5%), 779 (30.8%), and triclopyr (24.7%) produced mortality results greater than 20% at 11 WAT in water oak seedlings (Table 4.10 and Figures 4.127-4.135). Significant ($p<0.1$) differences in injury rating in response to rates of DAS 779 occurred at 2 WAT (Figure 4.124). Injury ratings were 3.0, 3.5, 4.1, and 4.1 for the 0.125x, 0.25x, 0.5x, and 1x rates, respectively. No significant differences in height or injury in occurred in response to rates of DAS 534 (Figures 4.111 and 4.120) or triclopyr (Figures 4.117 and 4.126).

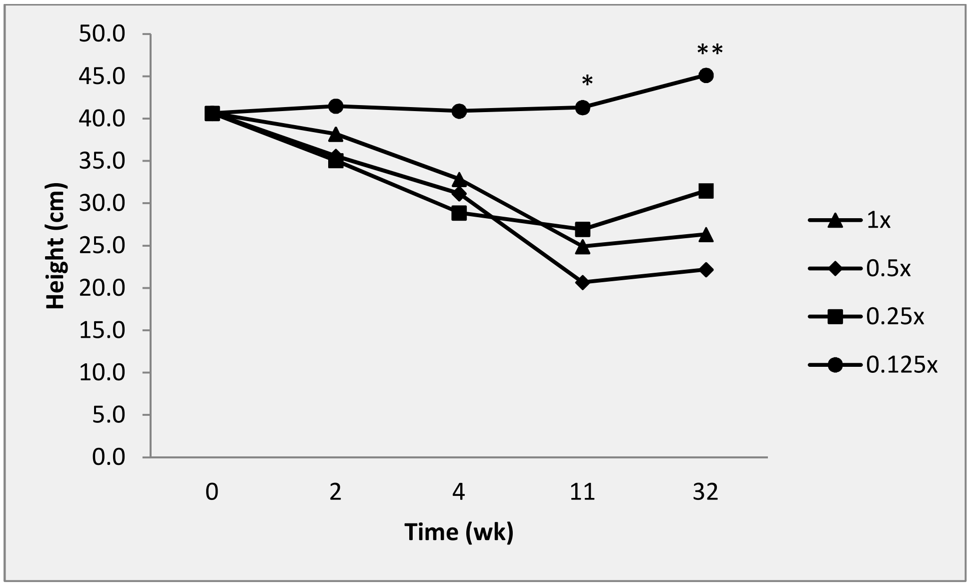

Figure 4.109. Mean height response of 1-0 water oak seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

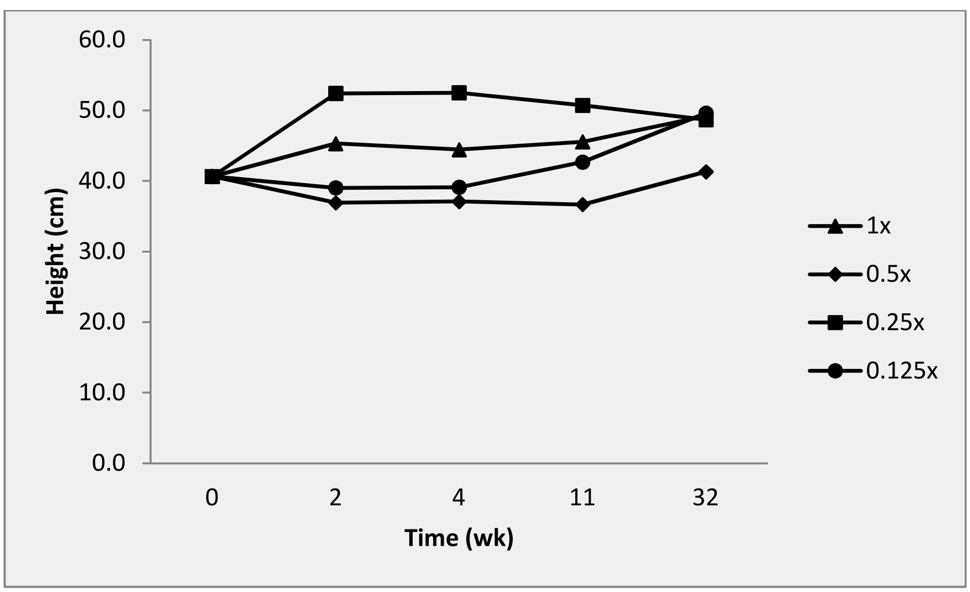

Figure 4.110. Mean height response of 1-0 water oak seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

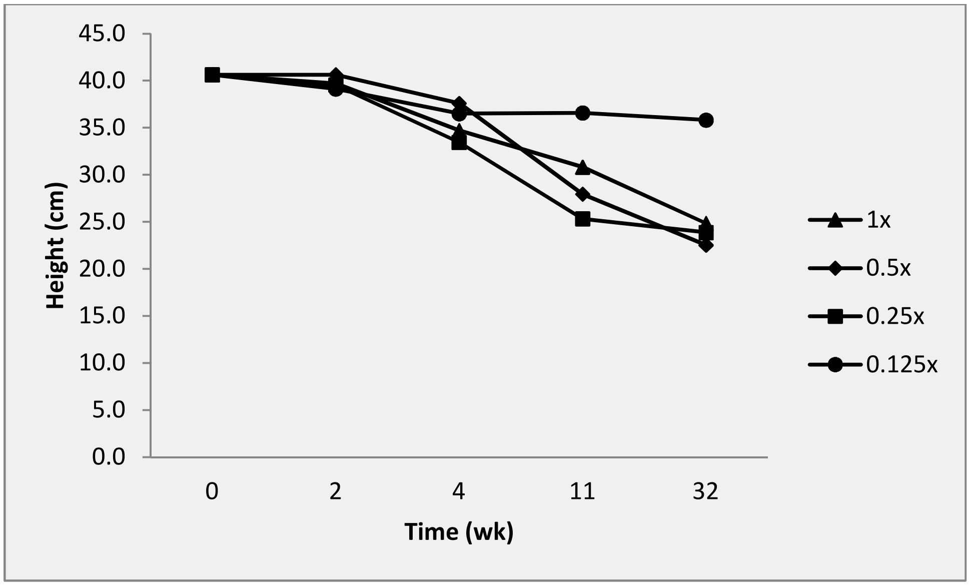

Figure 4.111. Mean height response of 1-0 water oak seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

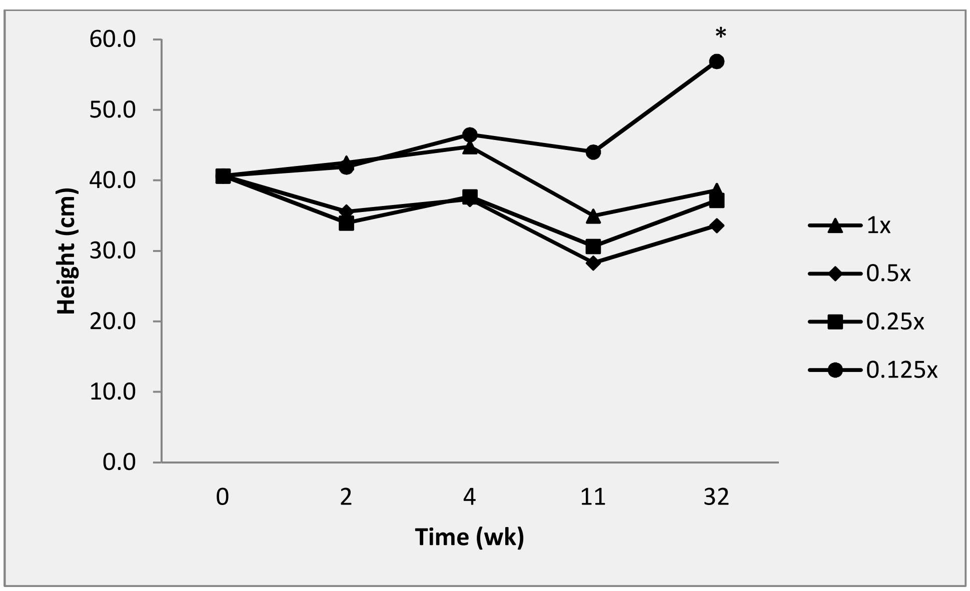

Figure 4.112. Mean height response of 1-0 water oak seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

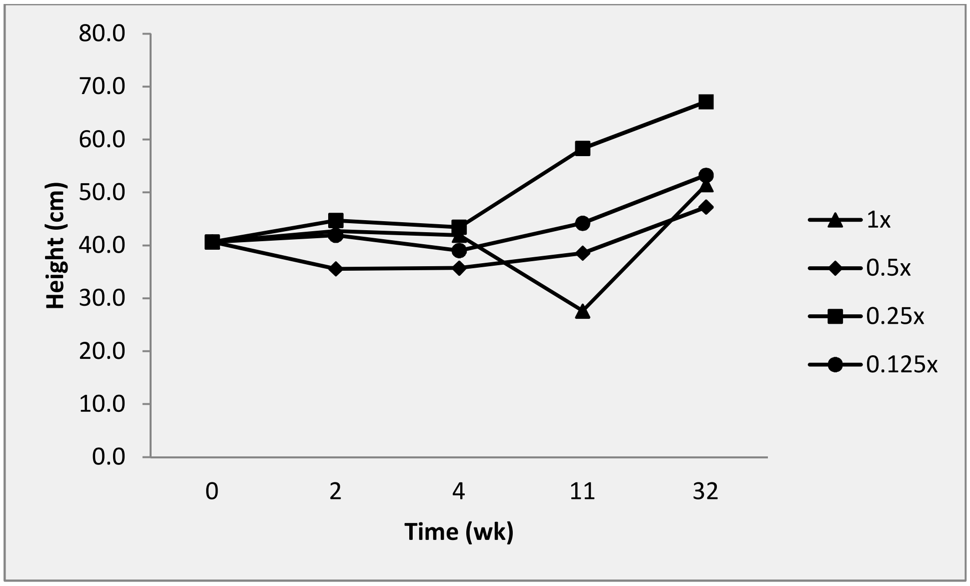

Figure 4.113. Mean height response of 1-0 water oak seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

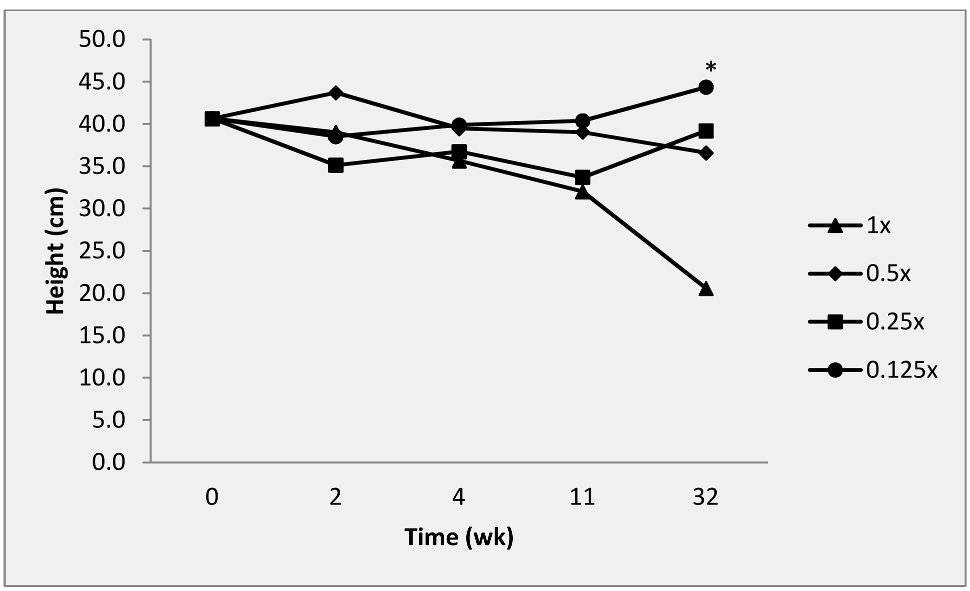

Figure 4.114. Mean height response of 1-0 water oak seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

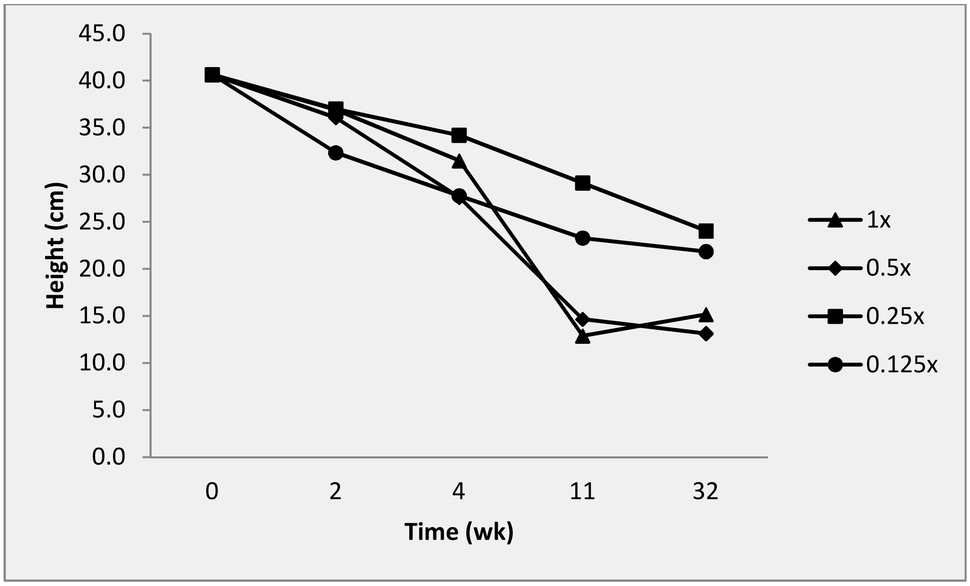

Figure 4.115. Mean height response of 1-0 water oak seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

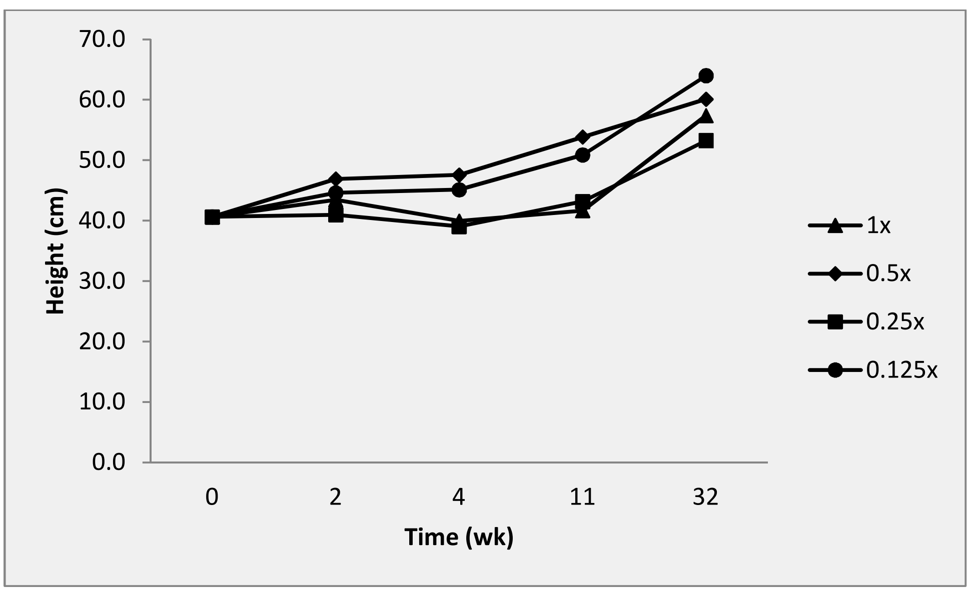

Figure 4.116. Mean height response of 1-0 water oak seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

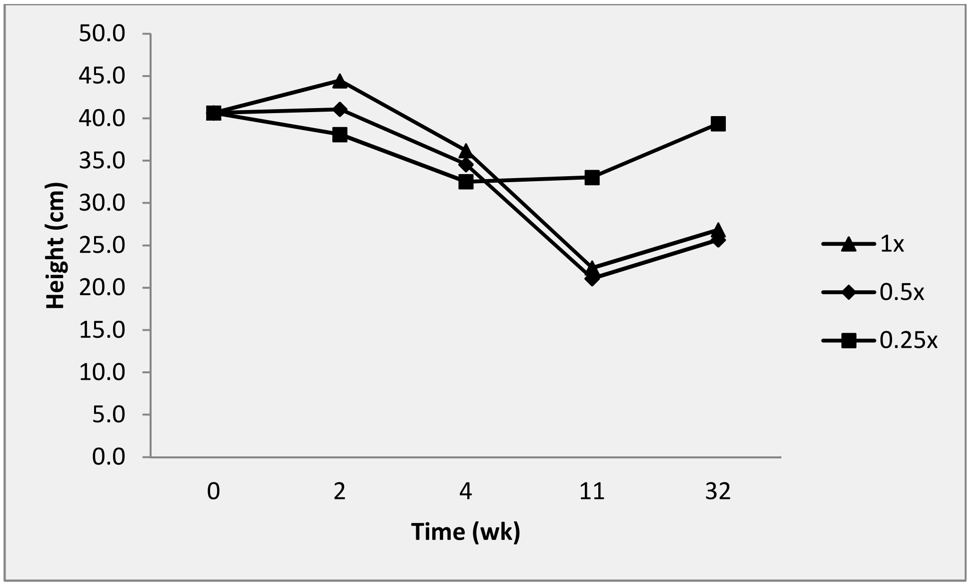

Figure 4.117. Mean height response of 1-0 water oak seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

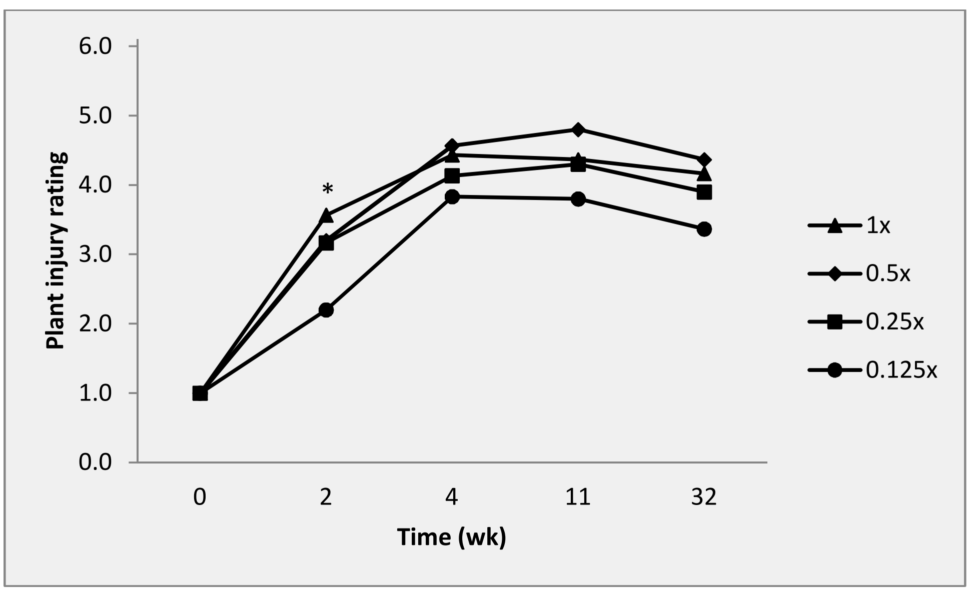

Figure 4.118. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

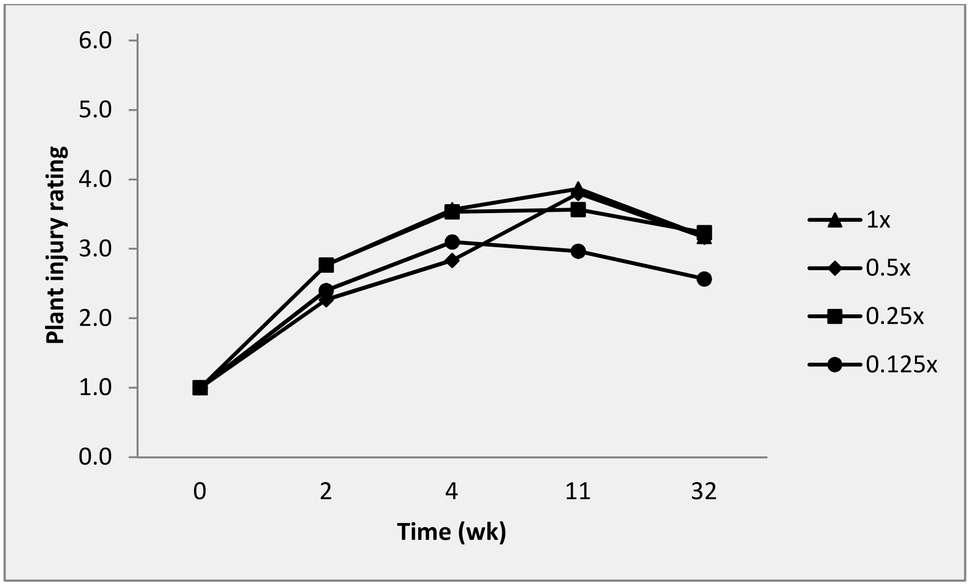

Figure 4.119. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

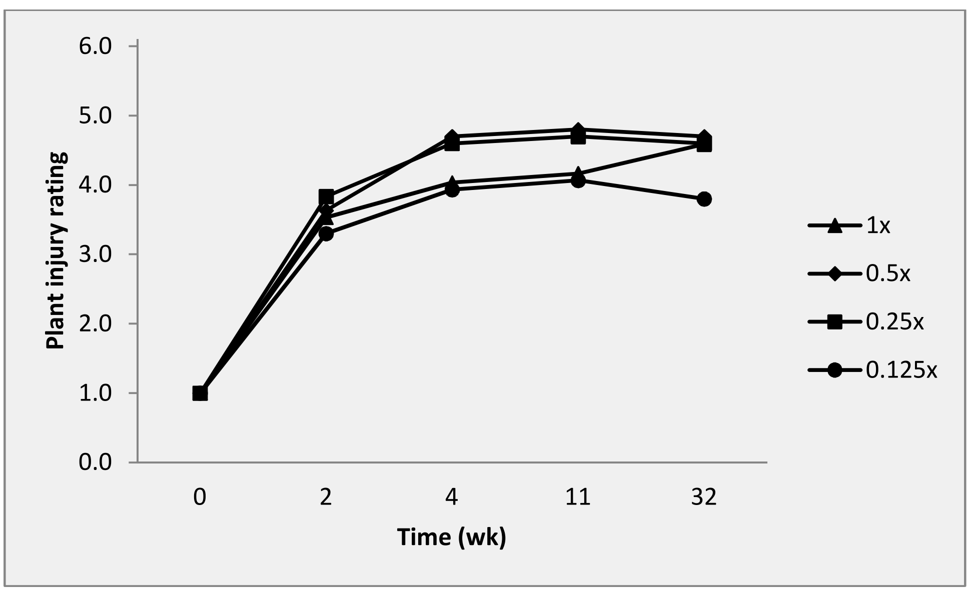

Figure 4.120. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

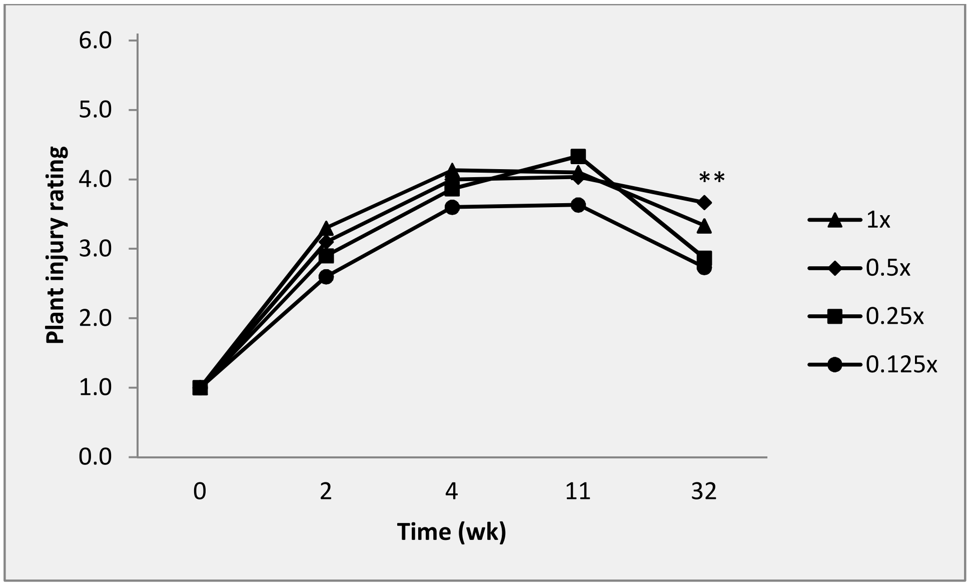

Figure 4.121. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

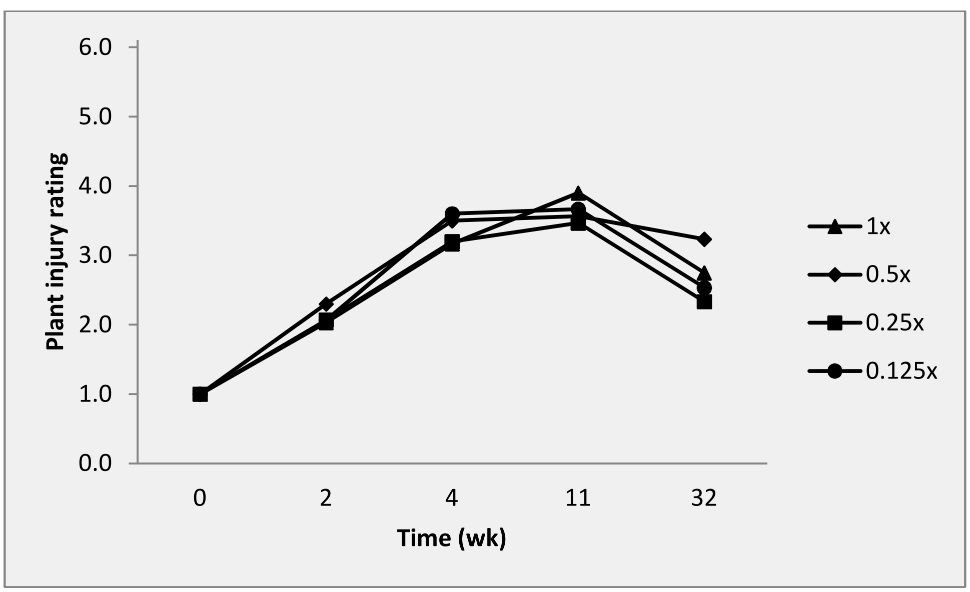

Figure 4.122. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

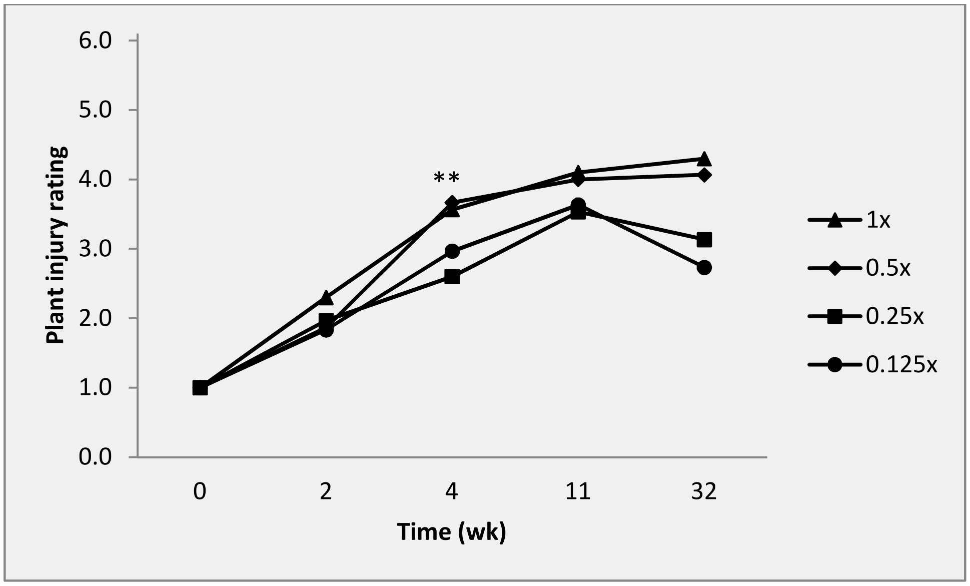

Figure 4.123. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

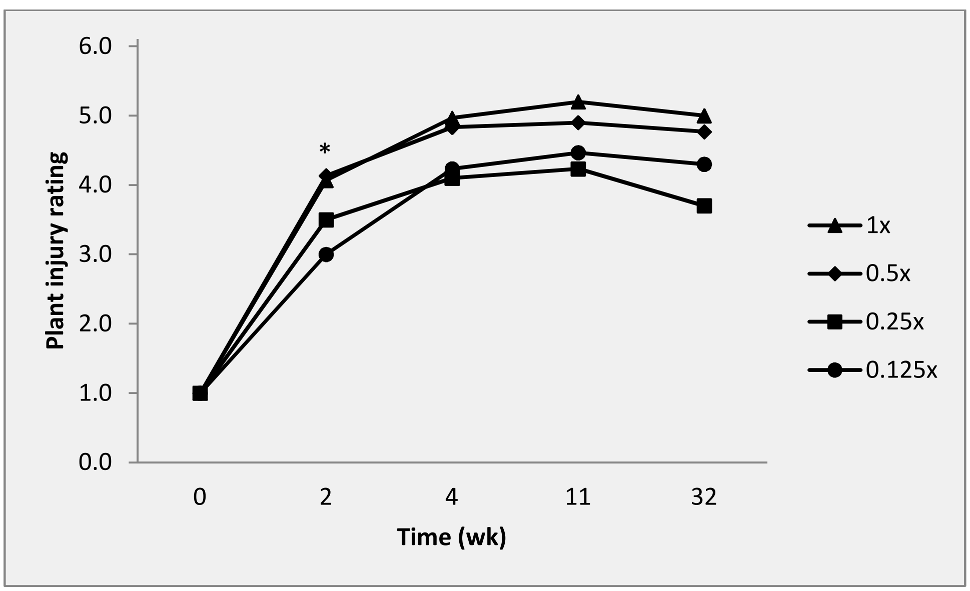

Figure 4.124. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

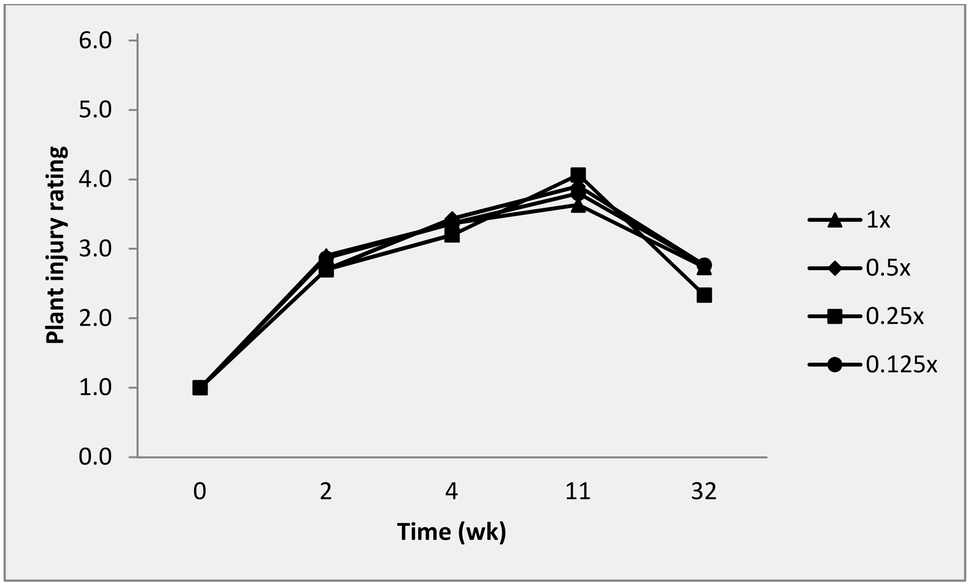

Figure 4.125. Mean plant injury rating of 1-0 water oak seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

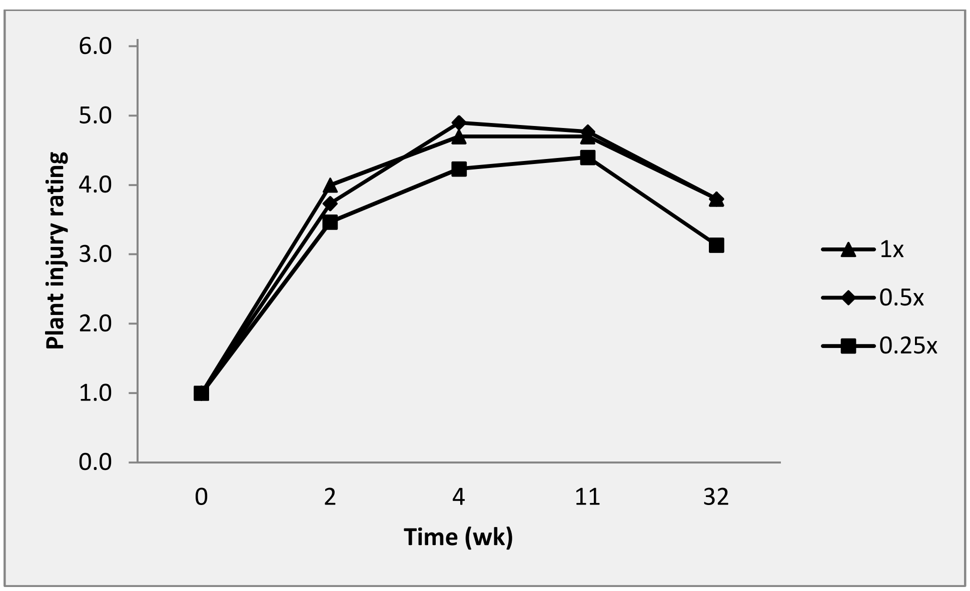

Figure 4.126. Mean plant injury rating of 1-0 water oak seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

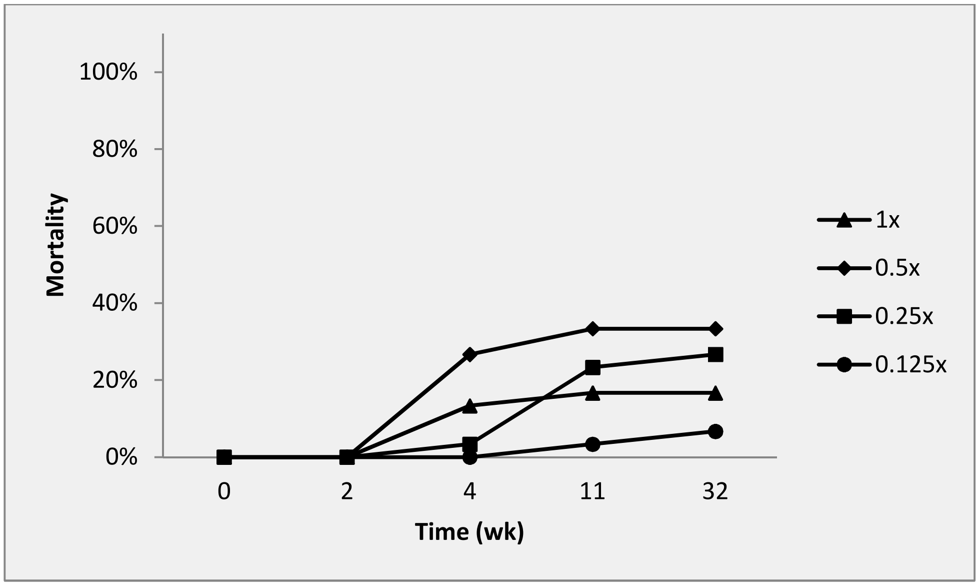

Figure 4.127. Mean percent mortality of 1-0 water oak seedlings treated with DAS 313 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

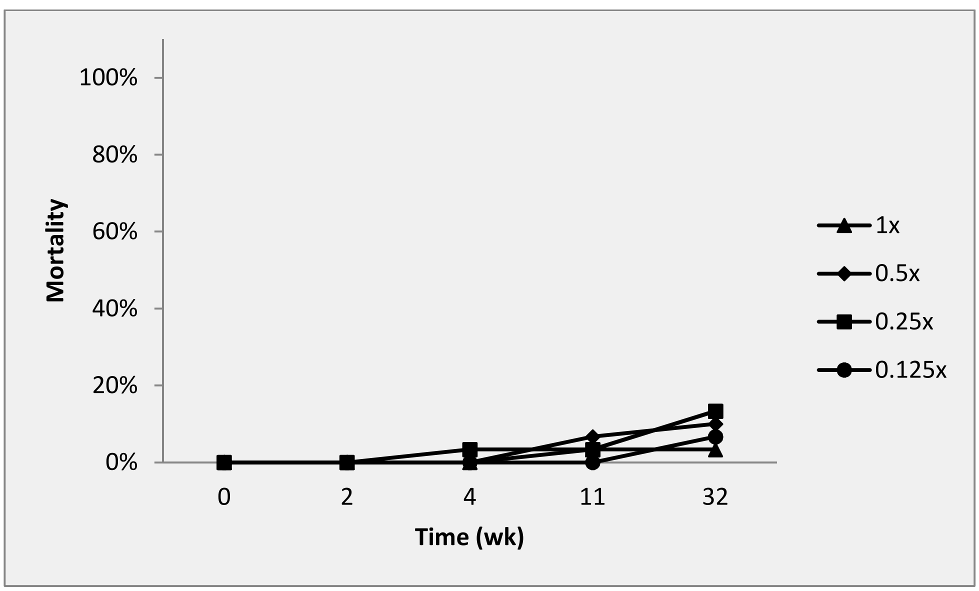

Figure 4.128. Mean percent mortality of 1-0 water oak seedlings treated with DAS 402 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

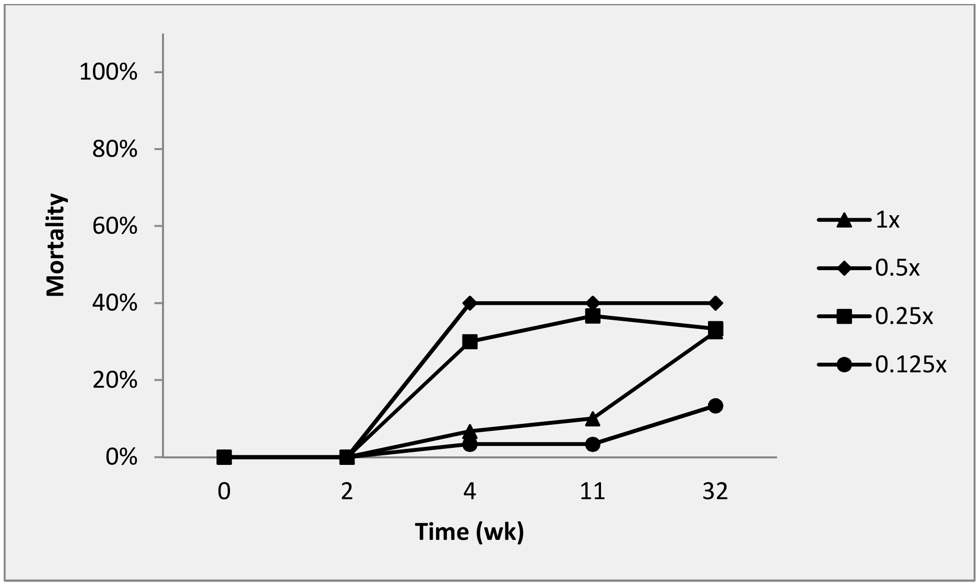

Figure 4.129. Mean percent mortality of 1-0 water oak seedlings treated with DAS 534 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

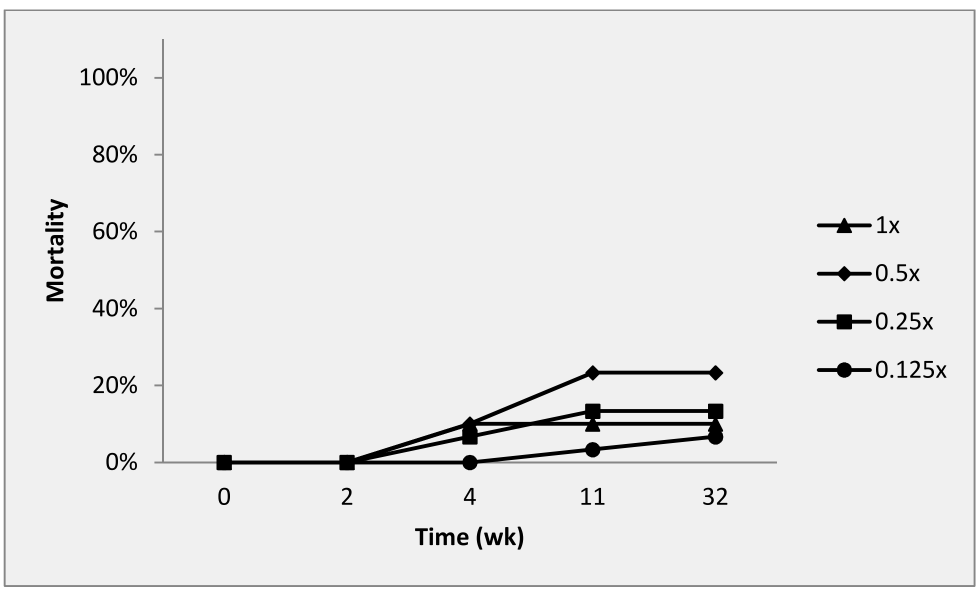

Figure 4.130. Mean percent mortality of 1-0 water oak seedlings treated with DAS 548 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

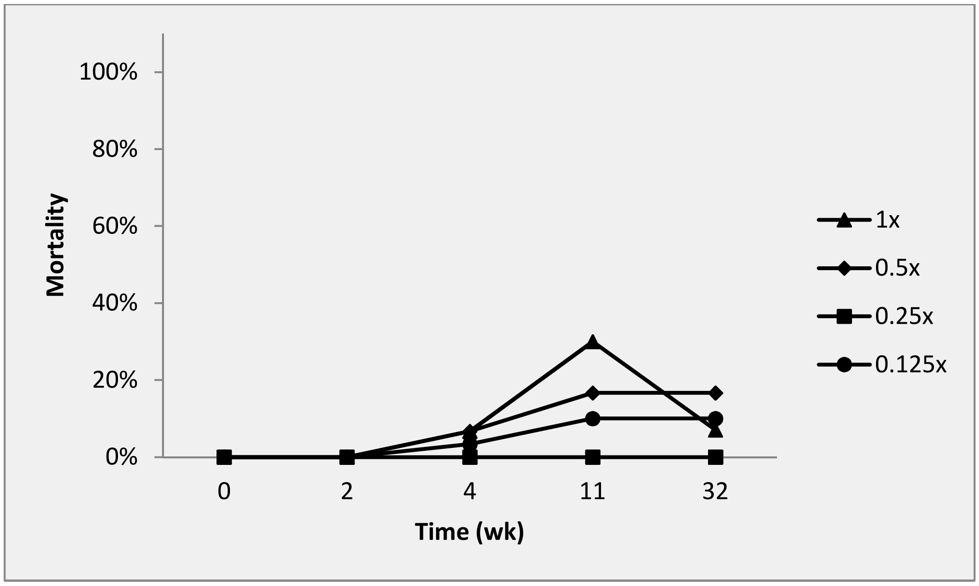

Figure 4.131. Mean percent mortality of 1-0 water oak seedlings treated with DAS 602 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

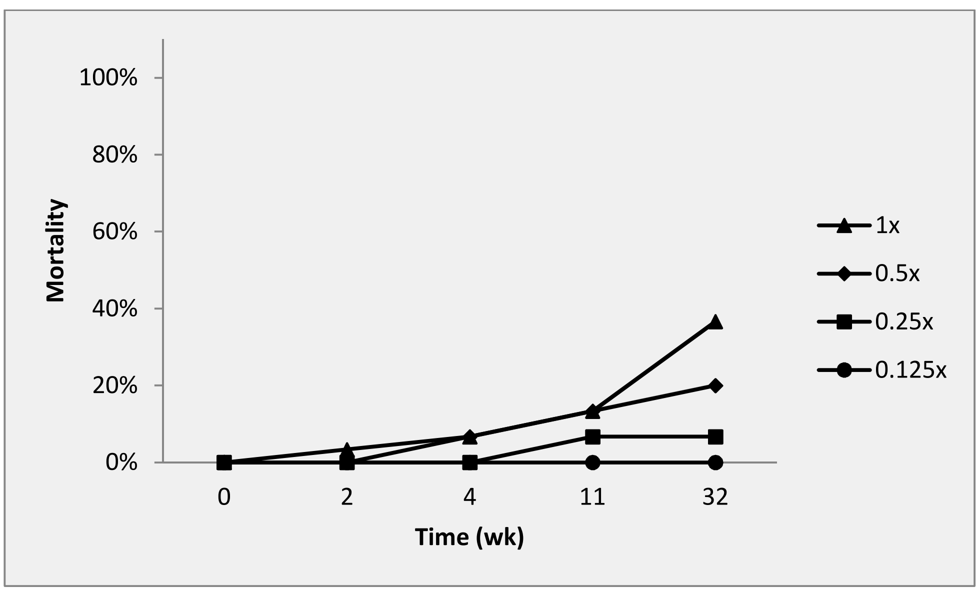

Figure 4.132. Mean percent mortality of 1-0 water oak seedlings treated with DAS 729 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

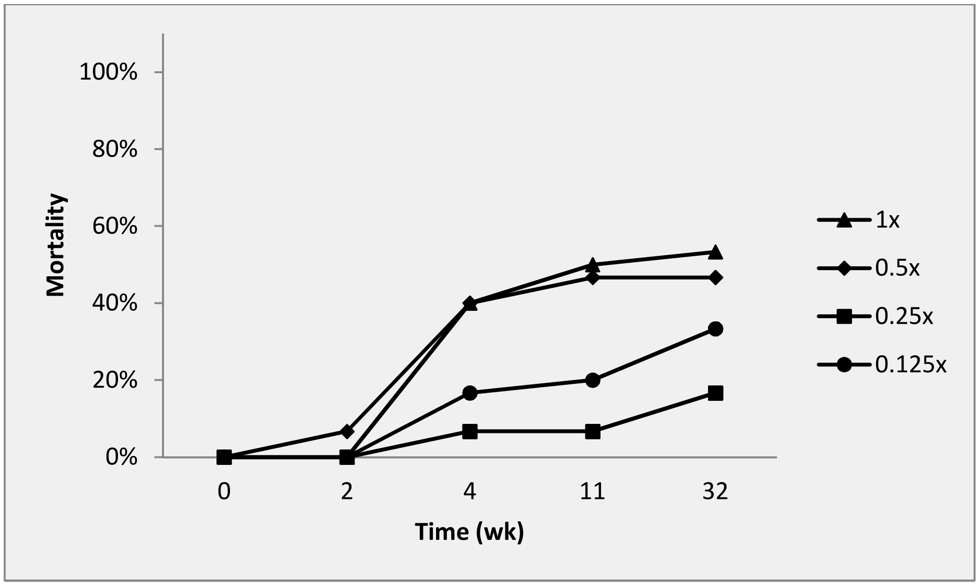

Figure 4.133. Mean percent mortality of 1-0 water oak seedlings treated with DAS 779 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

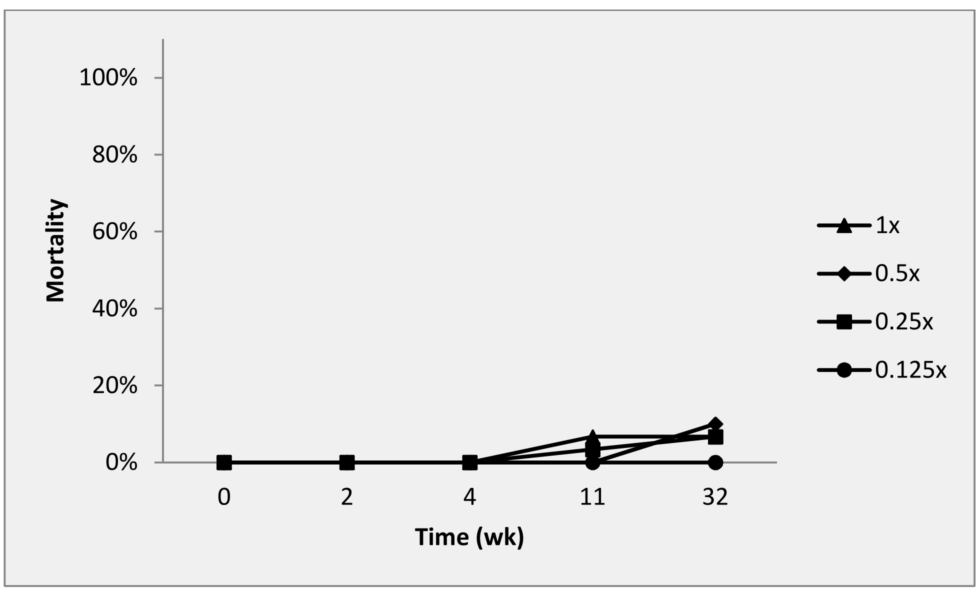

Figure 4.134. Mean percent mortality of 1-0 water oak seedlings treated with DAS 896 in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

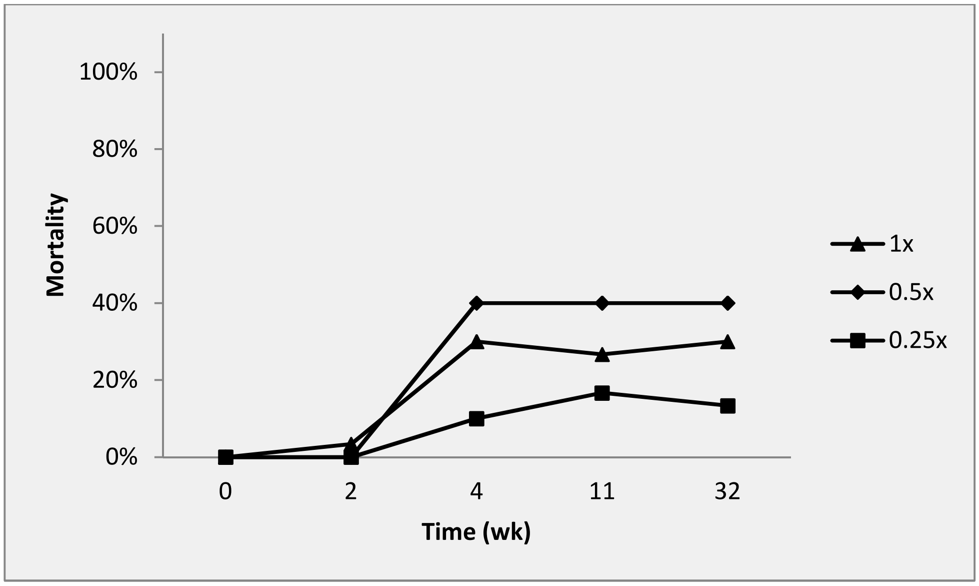

Figure 4.135. Mean percent mortality of 1-0 water oak seedlings treated with triclopyr in greenhouse seedling screening. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

2-0 Greenhouse Seedling Screening

Paired t-test results indicate that 2-0 seedlings were less sensitive to herbicide damage than 1-0 seedlings (Table 4.1). Height, injury, and percent mortality results indicate that black locust was the most sensitive species in the 2-0 group (27.9 cm, 5.0, and 30.9%) at 6 WAT (Tables 4.11-4.13). Height and mortality responses at 6 WAT were not significantly different for red maple, sweetgum, and water oak (52.8, 50.8, and 53.1 cm and 3.6%, 2.0%, and 0.2%, correspondingly). They were least susceptible to herbicide damage. Loblolly pine (4.3) was slightly less susceptible to herbicide injury than black locust (5.0) and water oak had the lowest herbicide injury rating (3.5) at 6 WAT (Table 4.12). Significant ($p<0.0001$) differences in height and injury in response to herbicide occurred in all species. There were significant differences in height and injury in response to rate, but the magnitude differed by species. No significant herbicide by rate interactions occurred for any species or response variable combination in the 2-0 seedling group.

Table 4.11. Mean height response (cm) of 2-0 seedlings 6 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	47.6 a	46.0 ab	50.6 bcd	55.1 abc	54.6 ab	50.8 ab
DAS 402	25.1 bcd	40.8 ab	60.2 ab	49.5 bcde	55.7 ab	46.3 abcd
DAS 534	15.4 d	26.7 c	54.1 abcd	48.0 cde	53.1 ab	39.5 d
DAS 548	22.4 cd	40.5 ab	50.2 bcd	47.7 de	53.6 ab	42.9 cd
DAS 602	28.8 bc	41.8 ab	55.9 abc	53.8 abcd	56.8 ab	47.4 abc
DAS 729	23.5 cd	37.5 bc	53.3 abcd	45.7 e	48.9 bc	41.8 cd
DAS 779	29.3 bc	47.5 ab	43.2 d	57.3 a	44.3 c	44.3 bcd
DAS 896	36.5 ab	46.4 ab	61.9 a	56.8 ab	59.4 a	52.2 a
Triclopyr	22.4 cd	51.6 a	45.4 cd	42.8 e	51.1 abc	42.7 cd
Overall Mean	27.9 C	42.1 B	52.8 A	50.8 A	53.1 A	45.3

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.12. Mean plant injury rating of 2-0 seedlings 6 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	4.4 c	4.2 bc	4.5 a	3.9 bcd	3.9 a	4.2 ab
DAS 402	5.0 ab	4.5 abc	3.5 de	4.3 abc	3.2 cd	4.1 abc
DAS 534	5.4 a	5.2 a	3.9 bcd	4.5 a	3.3 bcd	4.4 a
DAS 548	5.1 ab	4.3 bc	4.3 ab	4.1 abcd	3.4 abcd	4.2 ab
DAS 602	4.8 bc	4.4 bc	3.7 cde	3.8 d	3.2 cd	4.0 bc
DAS 729	5.1 ab	4.6 ab	4.1 abc	4.4 a	3.5 abcd	4.3 ab
DAS 779	4.9 ab	4.0 bc	4.4 ab	3.8 d	3.7 abc	4.2 abc
DAS 896	4.8 bc	3.9 c	3.3 e	3.8 cd	3.1 d	3.8 c
Triclopyr	5.3 ab	3.8 c	4.5 a	4.4 ab	3.8 ab	4.4 ab
Overall Mean	5.0 A	4.3 B	4.0 C	4.1 C	3.5 D	4.2

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.13. Mean percent mortality of 2-0 seedlings 6 weeks after treatment.†

Chemistry	Black Locust	Loblolly Pine	Red Maple	Sweetgum	Water Oak	Overall Mean
DAS 313	4.2 d	8.3 ab	2.5 a	0.0 b	0.0 a	3.0 b
DAS 402	30.8 abcd	10.8 ab	0.0 a	0.0 b	0.0 a	8.3 ab
DAS 534	53.7 a	33.0 a	1.7 a	1.0 ab	0.0 a	17.9 a
DAS 548	38.3 abc	11.7 ab	5.8 a	2.1 ab	0.0 a	11.6 ab
DAS 602	24.2 bcd	12.5 ab	0.0 a	0.0 b	0.0 a	7.3 b
DAS 729	36.7 abc	16.7 ab	0.8 a	6.3 ab	0.0 a	12.1 ab
DAS 779	29.0 abcd	0.0 b	10.8 a	0.0 b	1.7 a	8.3 ab
DAS 896	15.1 cd	5.0 ab	0.0 a	0.9 ab	0.0 a	4.2 b
Triclopyr	45.8 ab	1.7 b	10.3 a	8.1 a	0.0 a	13.2 ab
Overall Mean	30.9 A	11.1 B	3.6 C	2.0 C	0.2 C	9.5

†Means followed by the same lowercase letter are not significantly different within species at $\alpha=0.05$. Overall species and herbicide means followed by the same uppercase letter are not significantly different at $\alpha=0.05$.

Height and injury averaged across species suggest that DAS 534 and 729 are among the top 3 most active herbicides in 1-0 seedlings at 32 WAT and in 2-0 seedlings at 6 WAT (Tables 4.4, 4.8, 4.9, and 4.13). Height and injury responses suggest that DAS 896 is among the 3 least active herbicides in both groups. Ranking of injury responses in triclopyr treatments was very different between the two seedling groups. Triclopyr treatments had the second highest injury (4.4) at 6 WAT in 2-0 seedlings, but the third lowest injury (3.9) at 32 WAT in 1-0 seedlings.

Black Locust

At 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.001$, height; $p<0.05$, injury). Based on height and injury rating, DAS 534 was the most effective treatment (Tables 4.11-4.12). However, height in DAS 534 treatments (15.4 cm) was not significantly different from heights in DAS 402 (25.1 cm), 548 (22.4 cm), 729 (23.5 cm), and triclopyr (22.4 cm). Also, injury in DAS 534 treatments (5.4) was not significantly different from injury in DAS 402 (5.0), 548 (5.1), 729 (5.1), 779 (4.9), and triclopyr (5.3).

Loblolly Pine

At 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.01$, height; $p<0.001$, injury). Based on height and injury, DAS 534 was the most effective treatment (Tables 4.11-4.12). However, height in DAS 534 (26.7 cm) treatments was not significantly different from height in DAS 729 (37.5 cm). Injury averaged across rates of DAS 534 (5.2) was not significantly different from injury in DAS 402 (4.5) and 729 (4.6) treatments.

Red Maple

In red maple at 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.05$, height; $p<0.001$, injury). Based on height responses, DAS 779 (43.2 cm) was the most effective treatment, but heights in DAS 313 (50.6 cm), 534 (54.1 cm), 548 (50.2 cm), 729 (53.3 cm), and triclopyr (45.4 cm) treatments were not significantly different (Table 4.11). Injury rating suggests that triclopyr was the most effective treatment, but triclopyr injury (4.5) was not significantly higher than DAS 313 (4.5), 548 (4.3), 729 (4.1), and 779 (4.4) injury ratings (Table 4.12).

Sweetgum

At 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.0001$, height; $p<0.001$, injury). Based on height responses, triclopyr (42.8 cm) was the most effective treatment, but was not significantly different from DAS 402 (49.5 cm), 534 (48.0 cm), 548 (47.7 cm), and 729 (45.7 cm) treatments (Table 4.11). Injury rating suggests DAS 534 (4.5) was the most effective treatment, but injury in DAS 402

(4.3), 548 (4.1), 729 (4.4), and triclopyr (4.4) treatments was not significantly different (Table 4.12).

Water Oak

In water oak at 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.01$). Height responses suggest DAS 779 was the most effective treatment, but DAS 779 height (44.3 cm) was not significantly lower than DAS 729 (48.9 cm) and triclopyr (51.1 cm) treatment heights (Tables 4.11). Based on injury rating, DAS 313 (3.9) was the most effective treatment, but was not significantly higher than DAS 548 (3.4), 729 (3.5), 779 (3.7), and triclopyr (3.8) treatments (Table 4.12).

Germinal Screening

Germinal seedlings were very susceptible to herbicide injury (Table 4.14-4.16). Average mortality at final evaluation was 56.0% compared to 1-0 seedlings with 46.8% and 2-0 seedlings with 9.5% (Tables 4.9, 4.13, and 4.16) Mean height was lowest for sweetgum and loblolly pine, while water oak and black locust were taller and less affected by herbicide (Table 4.14). Sweetgum had the highest mean injury rating at 5.6, but was not significantly different from black locust at 5.2 (Table 4.15). Black locust (71.3%), loblolly pine (66.1%), and sweetgum (82.8%) mortality was not significantly different (Table 4.16). Water oak was much less sensitive than other species with only 3.6% mortality. Significant ($p<0.01$) differences in response to herbicide occurred in height and injury rating in all species.

Table 4.14. Mean height response (cm) of germinals 6 weeks after treatment.†

Chemistry	Black Locust	Lobolly Pine	Sweetgum	Water Oak	Overall Mean
DAS729	0.0 b	0.0 b	0.0 b	11.1 b	2.8 B
DAS896	0.6 b	3.0 a	3.0 a	22.1 a	7.2 A
Triclopyr	23.0 a	4.0 a	0.3 b	9.4 b	9.1 A
Overall Mean	7.8 B	2.3 C	1.1 C	14.2 A	6.4

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.15. Mean injury rating of germinals 6 weeks after treatment.†

Chemistry	Black Locust	Lobolly Pine	Sweetgum	Water Oak	Overall Mean
DAS729	6.0 a	6.0 a	6.0 a	3.2 a	5.3 A
DAS896	5.9 a	4.6 b	5.0 b	2.3 b	4.4 B
Triclopyr	3.8 b	4.1 b	5.9 a	3.7 a	4.4 B
Overall Mean	5.2 AB	4.9 B	5.6 A	3.0 C	4.7

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.16. Mean percent mortality of germinals 6 weeks after treatment.†

Chemistry	Black Locust	Lobolly Pine	Sweetgum	Water Oak	Overall Mean
DAS729	100.0 a	99.1 a	99.2 a	1.9 b	75.0 A
DAS896	95.8 a	55.0 b	53.2 b	0.0 b	51.0 B
Triclopyr	17.9 b	44.3 b	96.1 a	8.9 a	41.8 B
Overall Mean	71.3 A	66.1 A	82.8 A	3.6 B	56.0

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Height and injury responses suggest that herbicides in the germinal screening were generally ranked in a similar order to herbicides in the 1-0 seedling responses. Averaged across species, germinal responses to DAS 729 treatments are significantly more effective than DAS 896 and triclopyr treatments. Similarly, in 1-0 seedlings at 32 WAT, DAS 729 efficacy was ranked higher than DAS 896 and triclopyr, even though responses were not always significantly different.

Black Locust

Significant ($p<0.0001$) differences in height in response to herbicide occurred at 6 WAT. Significant herbicide by rate interaction ($p<0.05$) occurred for injury rating. Injury ratings averaged across rates for DAS 729 (6.0) and 896 (5.9) were very high, while triclopyr rate response ranged from 2.9 to 4.7 (Table 4.15). Height and injury responses suggest DAS 729 was the most effective treatment, but DAS 729 height (0.0 cm) and injury (6.0) were not significantly different from DAS 896 height (0.6 cm) and injury (5.9) (Tables 4.14-4.15). DAS 729 and 896 treatments were significantly more effective than triclopyr (23.0 cm, 3.8) on black locust germinals.

Loblolly Pine

At 6 WAT, significant differences in height and injury occurred in response to herbicide ($p<0.0001$) and rate ($p<0.05$). Based on these height and injury responses, DAS 729 (0.0 cm, 6.0) was the most effective treatment on loblolly pine, significantly different from DAS 896 and triclopyr (Tables 4.14-4.15).

Sweetgum

Statistically significant differences in height and injury due to herbicide ($p<0.0001$) occurred at 6 WAT. Height and injury responses suggest DAS 729 was the most effective treatment, but height (0.0 cm) and injury (6.0) in DAS 729 treatments were not significantly different from triclopyr (0.3 cm, 5.9) treatments (Tables 4.14-4.15). Height and injury in DAS 729 and triclopyr treatments were significantly more effective than DAS 896 treatments (3.0 cm, 5.0).

Water Oak

At 6 WAT, statistically significant differences in height and injury occurred in response to herbicide ($p<0.0001$). Based on these height and injury responses, triclopyr was the most effective treatment, but height (9.4 cm) and injury (3.7) in triclopyr treatments were not significantly different from DAS 729 (11.1 cm, 3.2) treatments (Tables 4.14-4.15). Height and injury in DAS 729 and triclopyr treatments were significantly different from DAS 896 treatments (22.1 cm, 2.3).

Seed Screening

Successful germination of seeds was observed in control treatments in the seed screening for all species, except red maple. Germination tests indicated that red maple seed had become dormant. Therefore, stratification was performed prior to the beginning of the seed screening. However, no germination resulted in red maple petri dishes. Sweetgum seeds germinated successfully in control petri dishes (90.0% at 14 DAT), but no germination was observed in any herbicide treated dishes. In paired t-test comparisons, percent germination averaged over time for black locust ($p<0.01$), loblolly pine ($p<0.01$), and water oak ($p<0.1$) was significantly lower for herbicide treated petri dishes than controls (Table 4.17). Overall, mean percent germination in treated petri dishes was 44.6% and 60.6% in controls.

Table 4.17. Mean percent germination of seeds in control and treated petri dishes.†

	Black Locust		Loblolly Pine		Water Oak		Overall Mean	
Time	Treated	Control	Treated	Control	Treated	Control	Treated	Control
4	49.6**	65.0	27.4	45.0	8.8	21.7	28.6***	43.9
8	55.0*	66.7	61.8**	83.3	25.1	40.0	47.3**	63.3
14	56.8***	78.3	75.0	93.3	41.5	51.7	57.8**	74.4
Overall Mean	53.8***	70.0	54.7***	73.9	25.1*	37.8	44.6***	60.6

†Significant differences between control and treated means are ranked by t-values: <0.1*, <0.05**, <0.01***.

Percent germination of seeds of all species increased steadily over time (Tables 4.18-4.20). Water oak had the lowest germination rate in herbicide treated dishes (41.5%) and loblolly pine had the highest (75.0%) at 14 DAT. Black locust appeared to have the fastest rate of germination, 49.6% in 4 days, but only increased to 56.8% germination through day 14. Black locust and loblolly pine had significantly higher germination rates than water oak. Overall mean germination across species at 14 DAT was 57.8%. Across species, changes in percent germination in response to herbicide appeared at 8 DAT and stayed consistent through time. Germination in DAS 402 treatments was significantly lower than DAS 729 (which had highest germination); differences were 17.5% and 12.8% at 8 and 14 DAT, respectively.

Table 4.18. Mean percent germination of seeds 4 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	60.8 a	11.7 a	7.9 a	26.8 A
DAS 729	51.3 ab	37.5 a	11.7 a	33.5 A
Triclopyr	36.7 b	32.9 a	6.7 a	25.4 A
Overall Mean	49.6 A	27.4 B	8.8 C	28.6

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.19. Mean percent germination of seeds 8 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	62.5 a	32.5 b	21.7 a	38.9 B
DAS 729	55.8 a	81.7 a	31.7 a	56.4 A
Triclopyr	46.7 a	71.3 a	22.1 a	46.7 AB
Overall Mean	55.0 A	61.8 A	25.1 B	47.3

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.20. Mean percent germination of seeds 14 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	64.6 a	51.7 b	42.5 ab	52.9 B
DAS 729	57.9 a	90.8 a	48.3 a	65.7 A
Triclopyr	47.9 a	82.5 a	33.8 b	54.7 AB
Overall Mean	56.8 B	75.0 A	41.5 C	57.8

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Paired t-tests revealed that tissue length averaged across time in herbicide treated dishes was significantly ($p<0.05$) lower than controls for black locust, loblolly pine, and water oak (Table 4.21). Mean herbicide treated tissue length was 1.4 mm and mean control tissue length was 12.0 mm.

Table 4.21. Mean tissue length (mm) of seeds in control and treated petri dishes.†

	Black Locust		Loblolly Pine		Water Oak		Overall Mean	
Time	Treated	Control	Treated	Control	Treated	Control	Treated	Control
4	1.5**	17	0.4*	0.9	0.2	1.5	0.7**	6.5
8	2.2**	20.0	1.9**	8.6	0.5	6.4	1.5**	11.7
14	1.5**	19.8	3.3**	19.6	1.1	14.5	2.0***	18.0
Overall Mean	1.7***	18.9	1.9***	9.7	0.6**	7.5	1.4***	12.0

†Significant differences between control and treated means are ranked by t-values: <0.1*, <0.05**, <0.01***.

Tissue length increased over time for all species, except black locust (Tables 4.22-4.24). Black locust seeds germinated very rapidly with some individuals developing into plants with clearly differentiated root, stem, and leaf tissues during the experiment. Black locust had the highest tissue length at 4 DAT, 1.5 mm, which increased to 2.2 mm at 8 DAT, but decreased to 1.5 mm at 14 DAT. Loblolly pine and water oak tissue length increased steadily through time. As tissue length increased over time, herbicide treatment effects became significant in loblolly pine and water oak (8 DAT and 14 DAT). However, black locust herbicide effects occurred at 4 and 8 DAT and then disappeared at 14 DAT. Tissue length effects due to herbicides averaged across species developed at 8 DAT. Although the effect of herbicides on tissue length changed from 8 to 14 DAT, the ranking of herbicides across species did not. DAS 402 treatments continued to have the shortest tissue length and DAS 729 the longest.

Table 4.22. Mean tissue length (mm) of seeds 4 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	1.9 a	0.1 a	0.1 a	0.7 A
DAS 729	1.8 a	0.6 a	0.2 a	0.9 A
Triclopyr	0.8 b	0.4 a	0.1 a	0.4 A
Overall Mean	1.5 A	0.4 B	0.2 B	0.7

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.23. Mean tissue length (mm) of seeds 8 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	2.1 ab	0.5 b	0.4 b	1.0 B
DAS 729	2.8 a	2.9 a	0.7 a	2.1 A
Triclopyr	1.6 b	2.4 a	0.3 b	1.4 AB
Overall Mean	2.2 A	1.9 A	0.5 B	1.5

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Table 4.24. Mean tissue length (mm) of seeds 14 days after treatment.†

Chemistry	Black Locust	Loblolly Pine	Water Oak	Overall Mean
DAS 402	1.3 a	0.7 b	0.9 b	1.0 B
DAS 729	2.0 a	4.5 a	1.9 a	2.8 A
Triclopyr	1.2 a	4.6 a	0.7 b	2.2 A
Overall Mean	1.5 B	3.3 A	1.1 B	2.0

†Means followed by the same lowercase letter are not significantly different within species at α=0.05. Overall species and herbicide means followed by the same uppercase letter are not significantly different at α=0.05.

Percent germination and tissue length at 14 DAT in seed screening did not produce similar ranking order to1-0 seedling responses (Tables 4.4, 4.8, 4.20, and, 4.24). Treatments with DAS 729, one of the most effective herbicides in 1-0 seedlings at 32 WAT, exhibited the highest percent germination and tissue length in the seed screen. Treatments with DAS 402, lower efficacy than DAS 729 in 1-0 seedlings, had the greatest control of percent germination and tissue length in the seed screening at 14 DAT.

Black Locust

There were significant differences in tissue length ($p<0.01$) and percent germination ($p<0.05$) in response to herbicide at 4 DAT in black locust seeds (Tables 4.18-4.20 and 4.22-4.24). At 8 DAT, significant ($p<0.05$) herbicide by rate interaction occurred for tissue length. For some herbicides, there were large rate responses, especially percent germination in triclopyr treatments. However, rate effects were significant only for tissue length at 8 DAT in DAS 402 treatments (Figures 4.136-4.141). Based on percent germination (36.7%) and tissue length (0.8 mm), triclopyr was the most effective herbicide at 4 DAT, but by 14 DAT differences in tissue length and percent germination in response to herbicide and rate were no longer statistically significant.

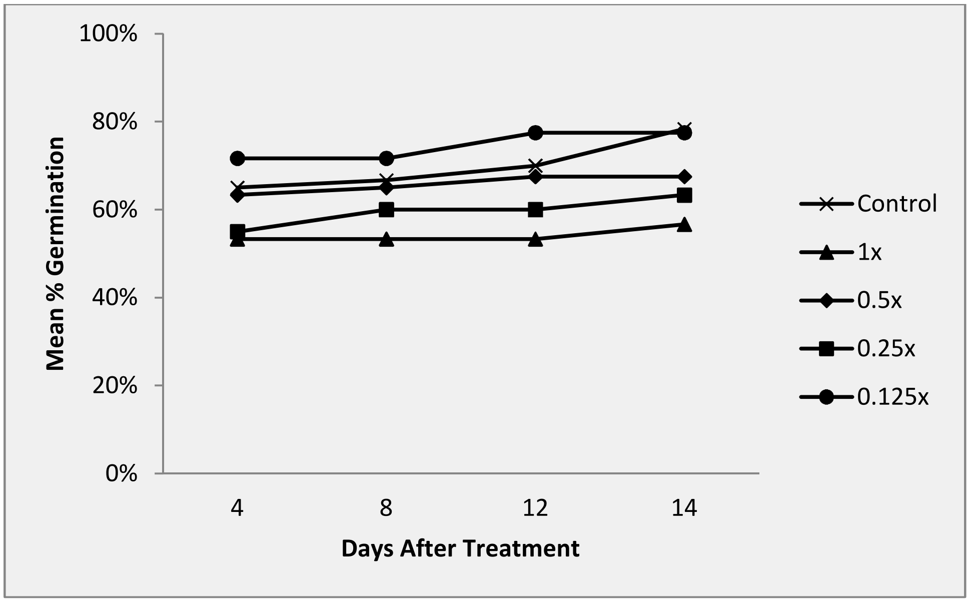

Figure 4.136. Mean percent germination of black locust seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

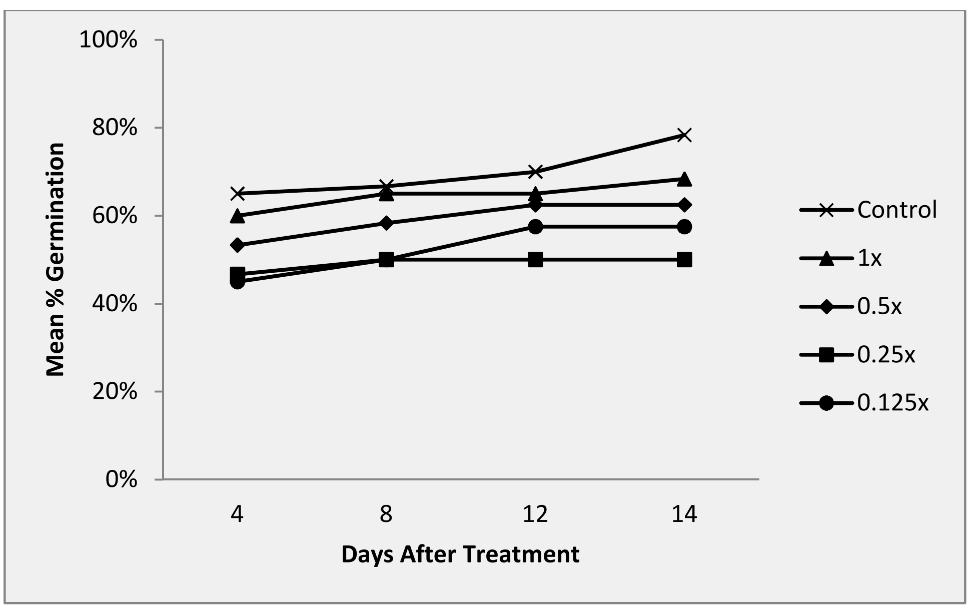

Figure 4.137. Mean percent germination of black locust seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

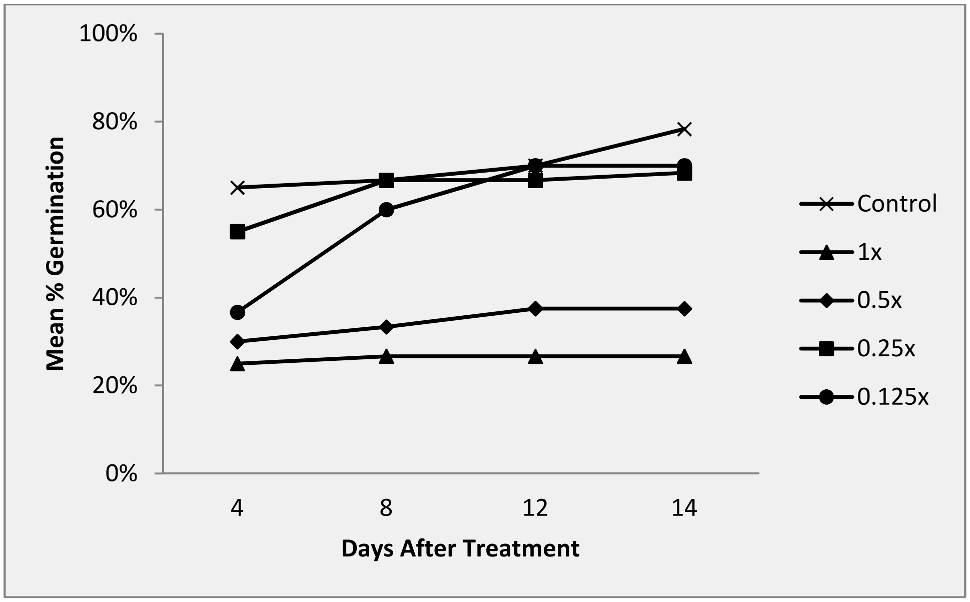

Figure 4.138. Mean percent germination of black locust seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

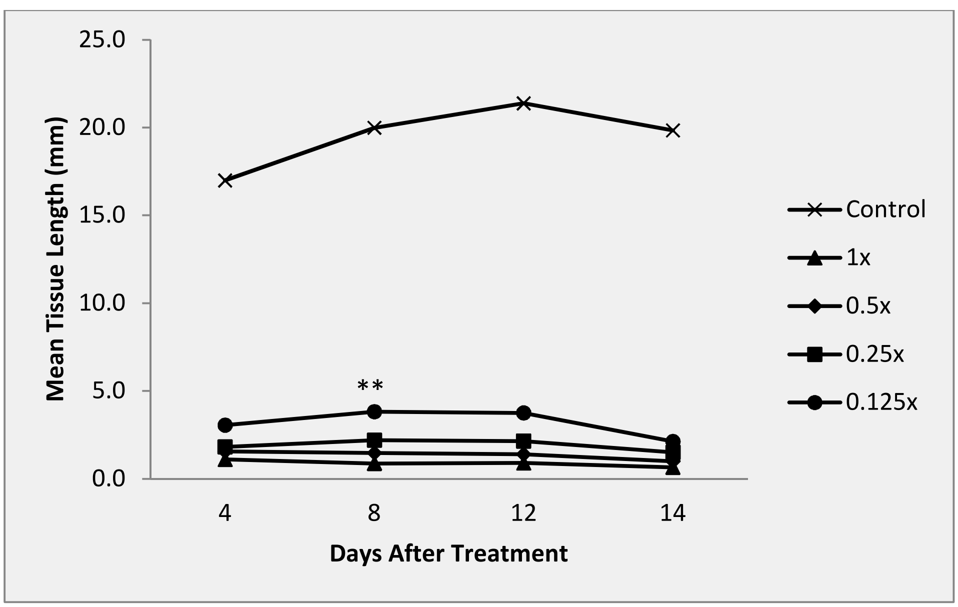

Figure 4.139. Mean tissue length of black locust seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

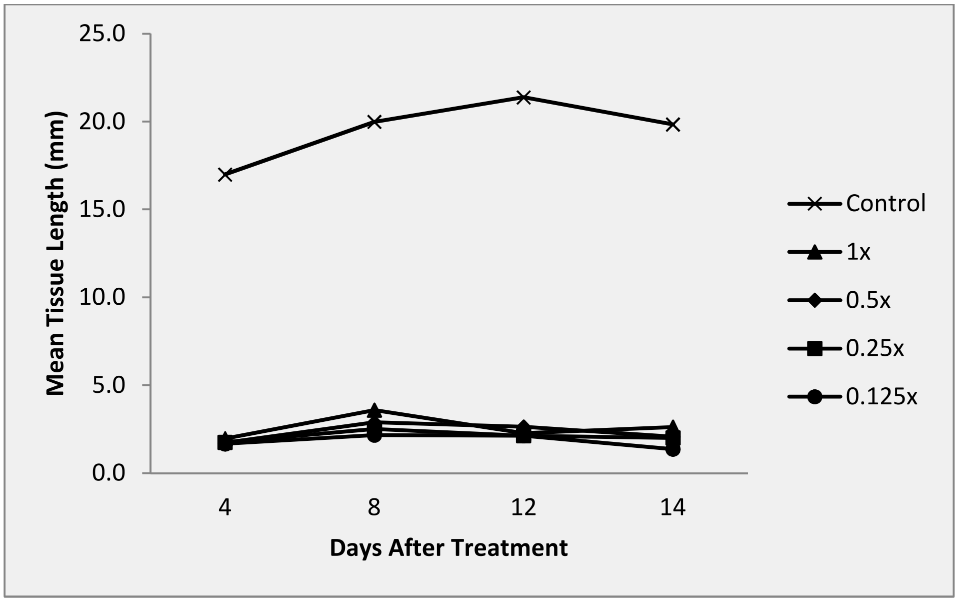

Figure 4.140. Mean tissue length of black locust seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

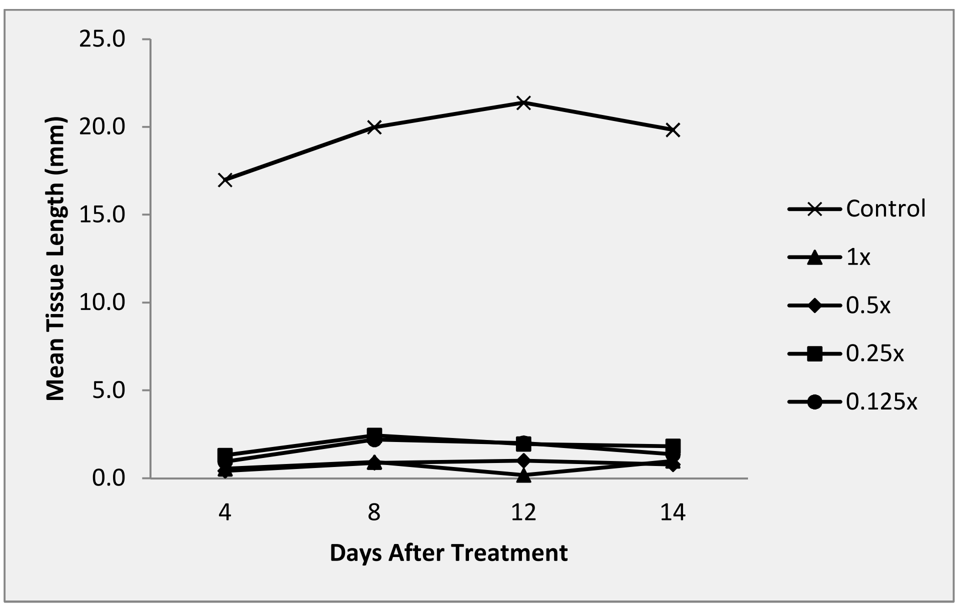

Figure 4.141. Mean tissue length of black locust seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Loblolly Pine

Significant differences in percent germination occurred in response to herbicide at 4 ($p<0.05$), 8 ($p<0.0001$), and 14 ($p<0.0001$) DAT. There were significant ($p<0.05$) differences in percent germination in response to rates of triclopyr at 8 DAT (Figure 4.142-4.147). Significant differences in tissue length occurred in response to herbicide at 4 ($p<0.1$) and 8 ($p<0.0001$) DAT and significant ($p<0.1$) herbicide by rate interaction occurred for tissue length at 14 DAT. There were significant ($p<0.05$) differences in tissue length in response to rates of DAS 402 and triclopyr at 14 DAT (Figures 4.145 and 4.147). The difference between tissue length between the high and low rates of triclopyr was 3.7 mm, while the difference between DAS 402 treatments was only 0.87 mm. Based on percent germination (8 DAT, 32.5%; 14 DAT, 51.7%) and tissue length (8 DAT, 0.5 mm; 14 DAT, 0.7 mm), DAS 402 was the most effective herbicide on loblolly pine seeds at 8 and 14 DAT (Tables 4.18-4.20 and 4.22-4.24).

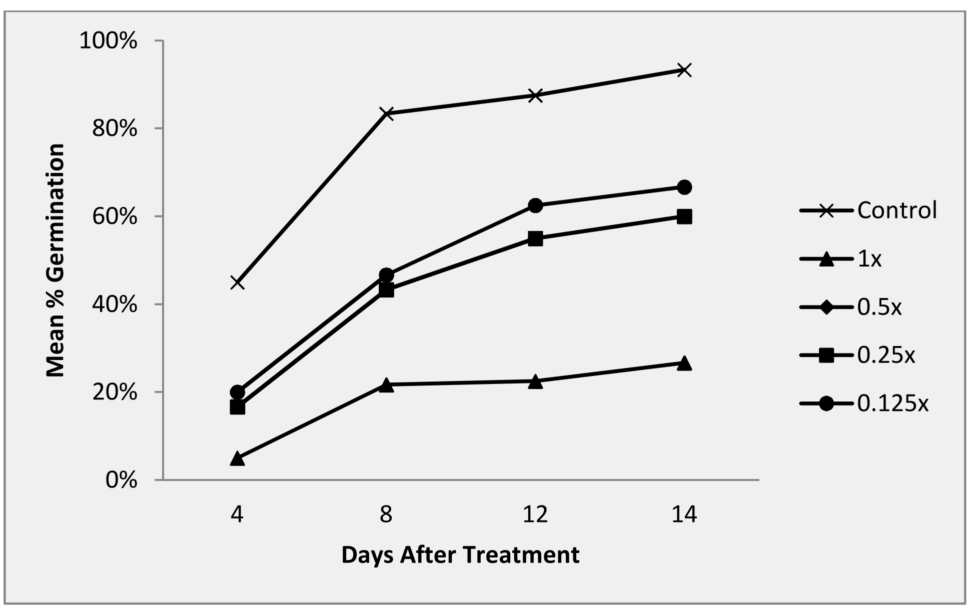

Figure 4.142. Mean percent germination of loblolly pine seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

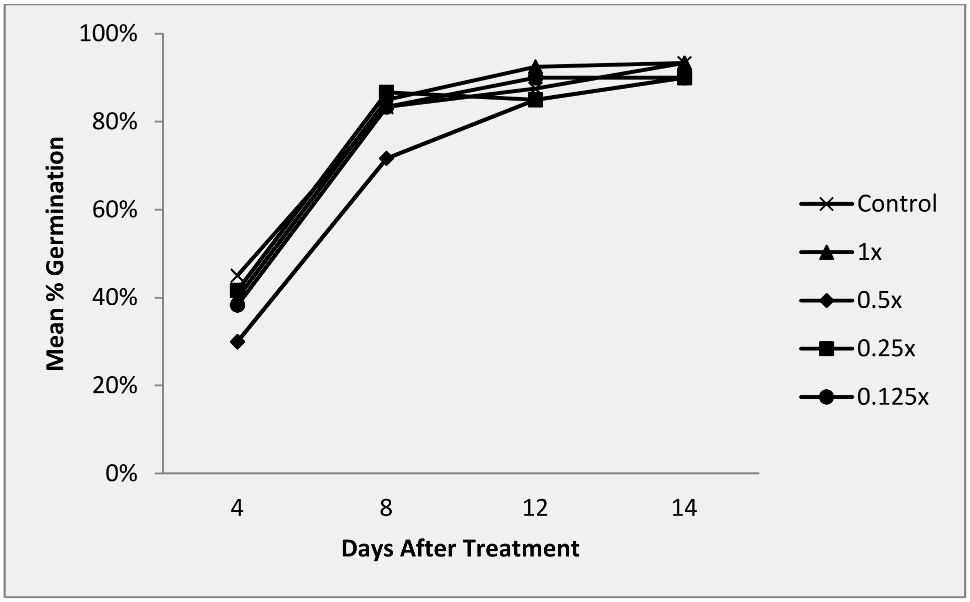

Figure 4.143. Mean percent germination of loblolly pine seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

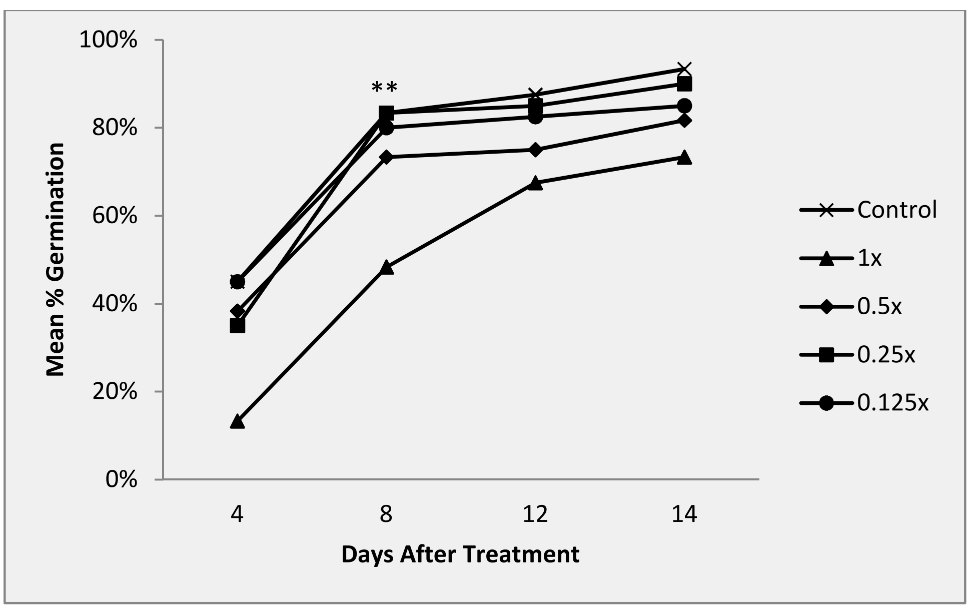

Figure 4.144. Mean percent germination of loblolly pine seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

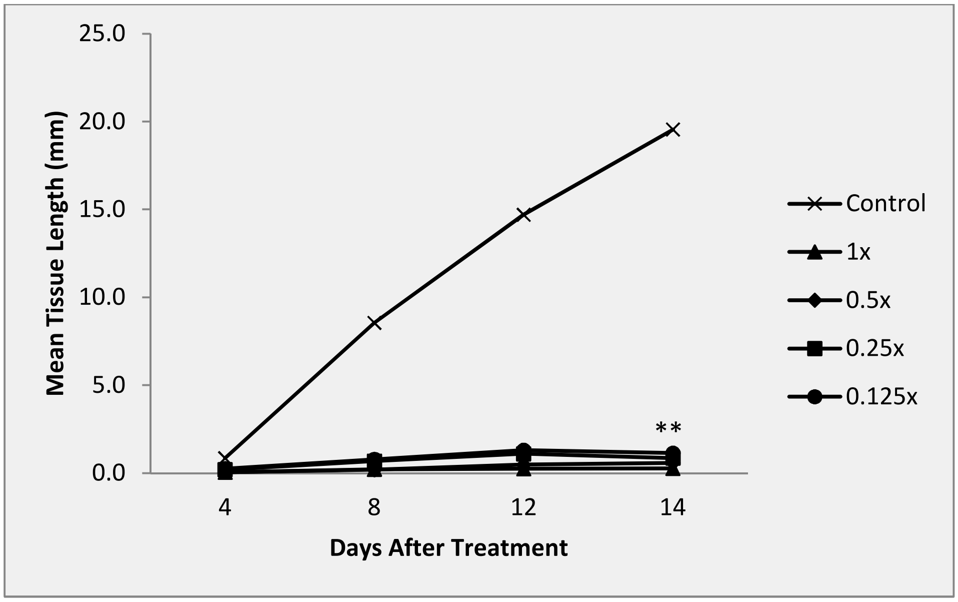

Figure 4.145. Mean tissue length of loblolly pine seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

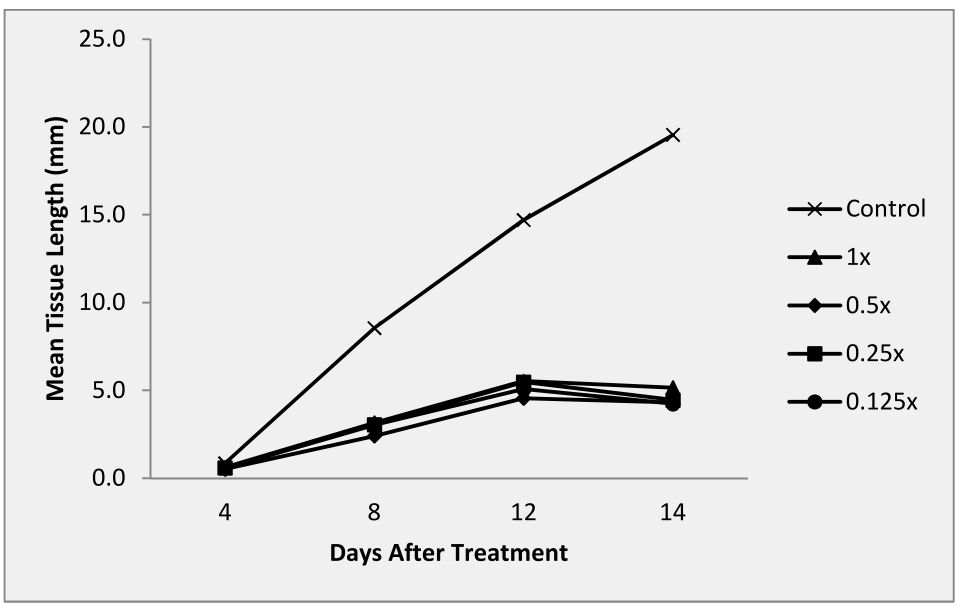

Figure 4.146. Mean tissue length of loblolly pine seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

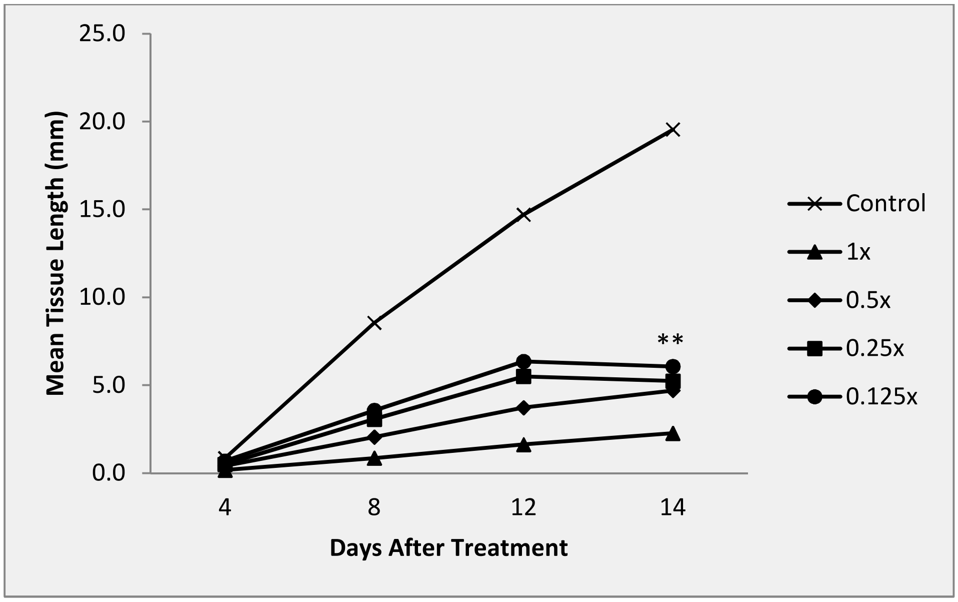

Figure 4.147. Mean tissue length of loblolly pine seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Sweetgum

No significant differences in sweetgum percent germination or tissue length occurred in response to herbicide. Successful germination and elongation of tissue was observed in sweetgum control treatments: mean germination was 90.0% and tissue length 20.4 mm by 14 DAT in control treatments. However, complete control of seed germination (0%) and tissue length (0 mm) was observed in herbicide treatments (Figures 4.148-4.149).

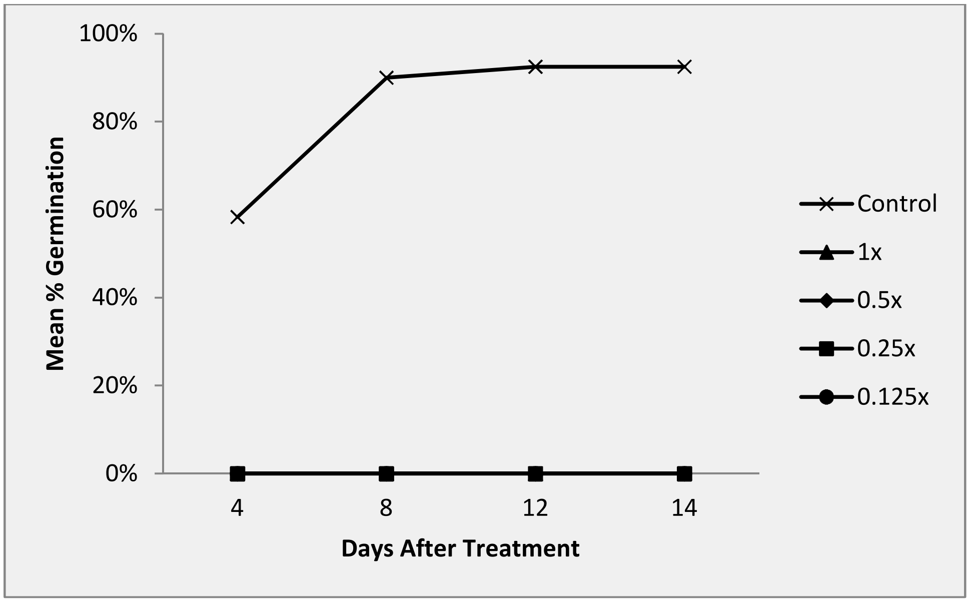

Figure 4.148. Mean percent germination of sweetgum seeds for all herbicides. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

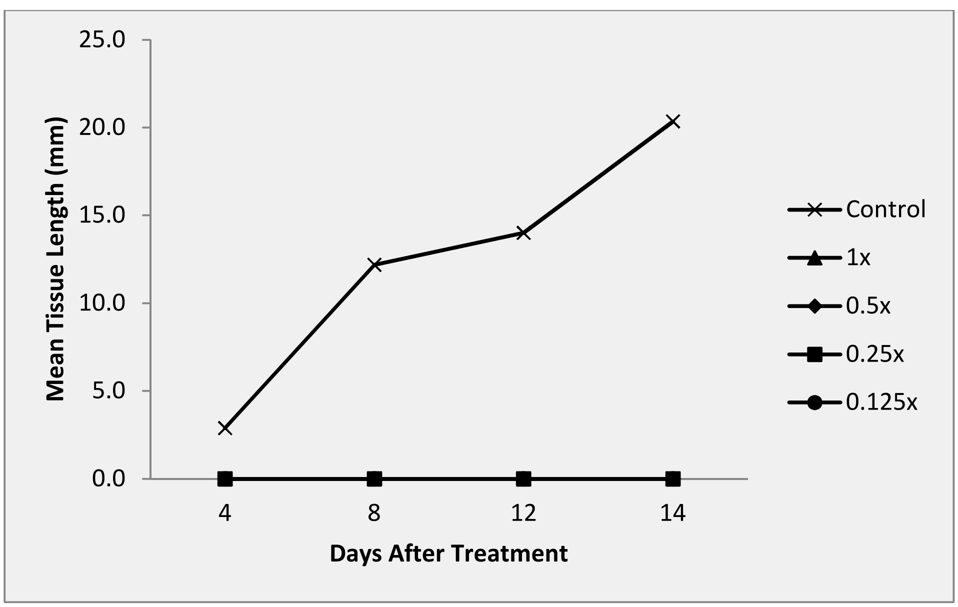

Figure 4.149. Mean tissue length of sweetgum seeds for all herbicides. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Water Oak

Significant differences in percent germination in response to herbicide occurred at 14 (p<0.01) DAT for water oak seeds. Tissue length was significantly changed by herbicide at 8 (p<0.01) and 14 (p<0.0001) DAT. Triclopyr appeared to be the most effective treatment in water oak seeds at 14 DAT (33.8%, 0.7 mm), but responses were not significantly different from DAS 402 (42.5%, 0.9 mm) (Tables 4.20 and 4.24, Figures 4.150-4.155). A significant difference in percent germination for rates of DAS 729 was detected by the rates ANOVA, but not the two-way ANOVA main effects test.

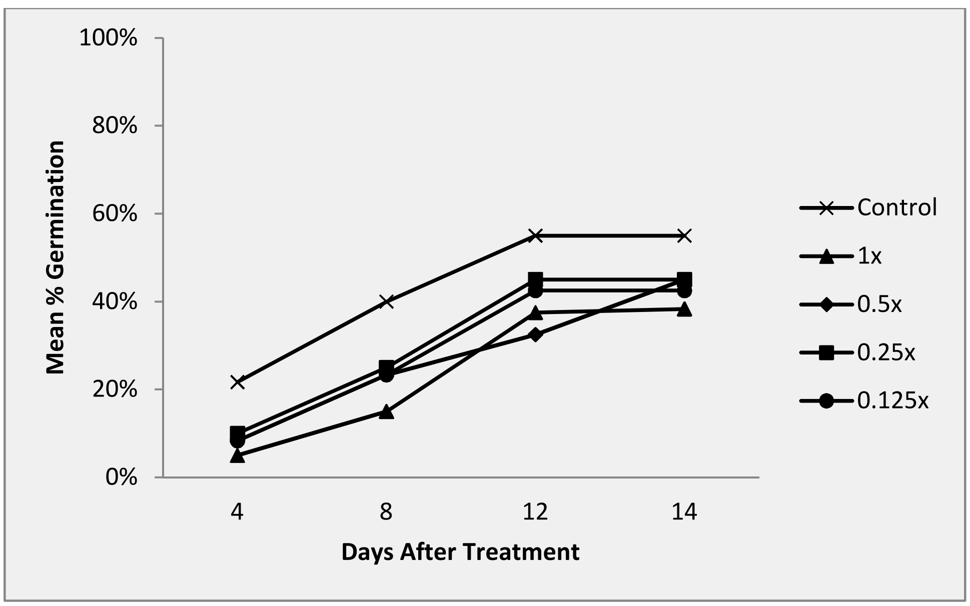

Figure 4.150. Mean percent germination of water oak seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

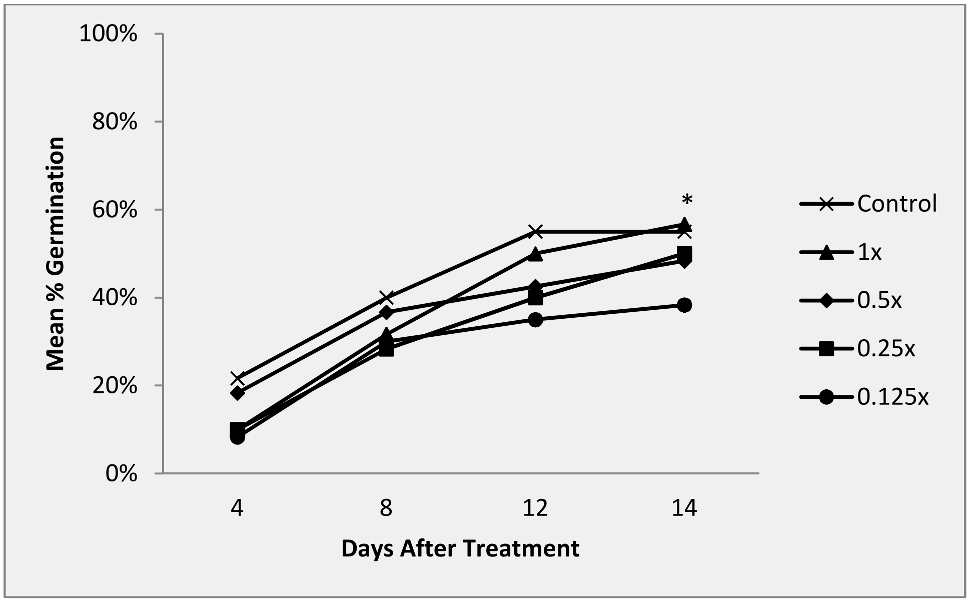

Figure 4.151. Mean percent germination of water oak seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

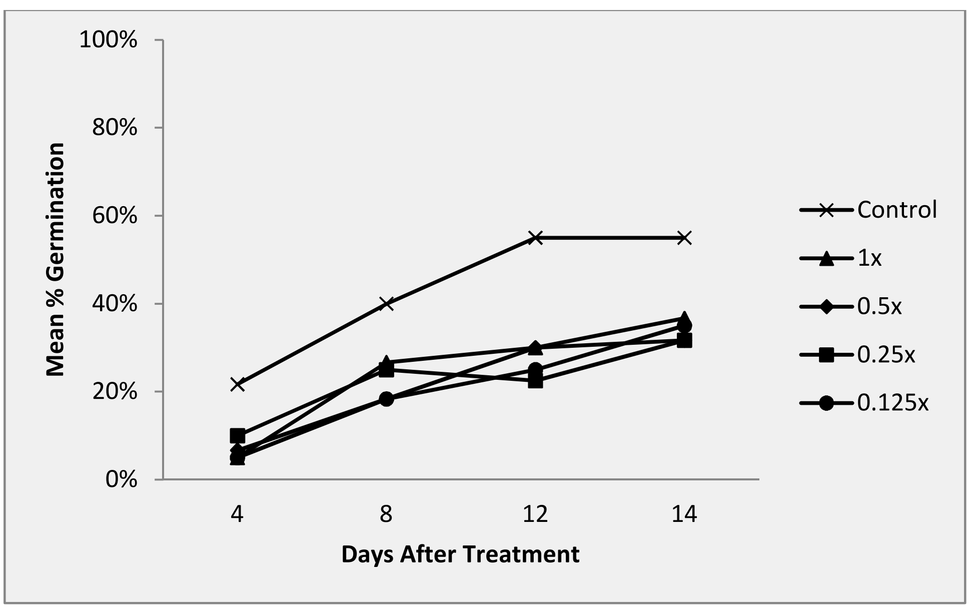

Figure 4.152. Mean percent germination of water oak seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

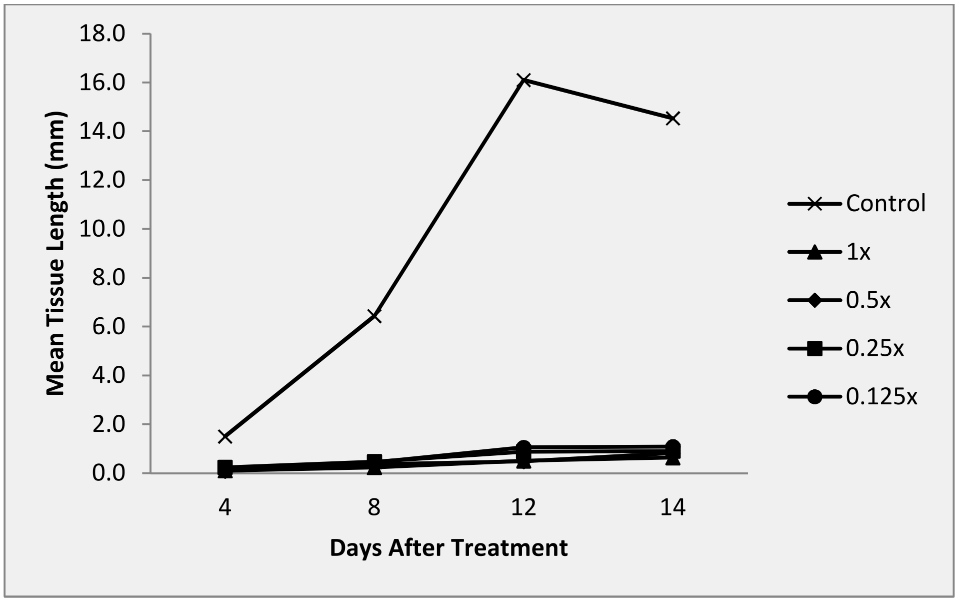

Figure 4.153. Mean tissue length of water oak seeds after treatment with DAS 402. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

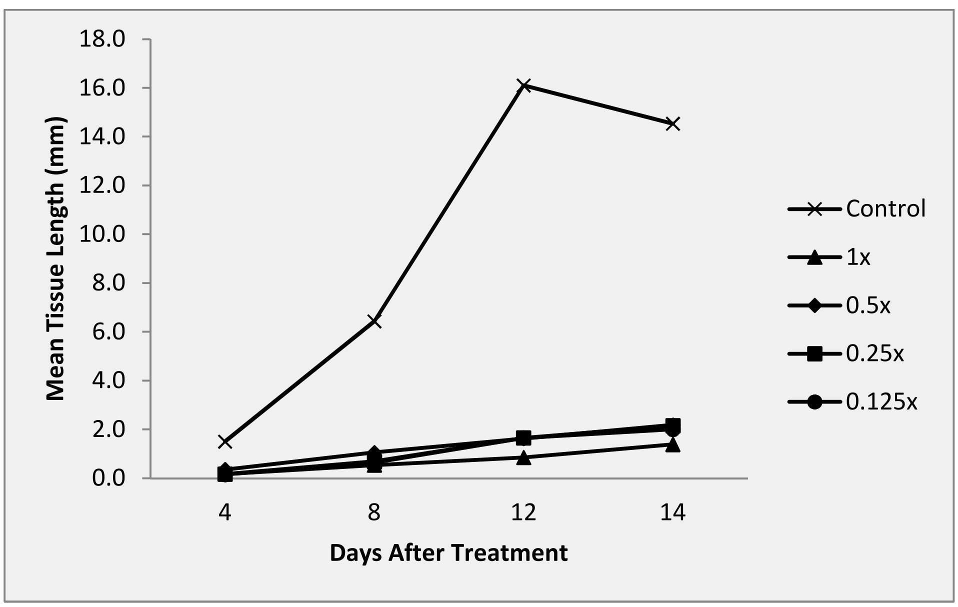

Figure 4.154. Mean tissue length of water oak seeds after treatment with DAS 729. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

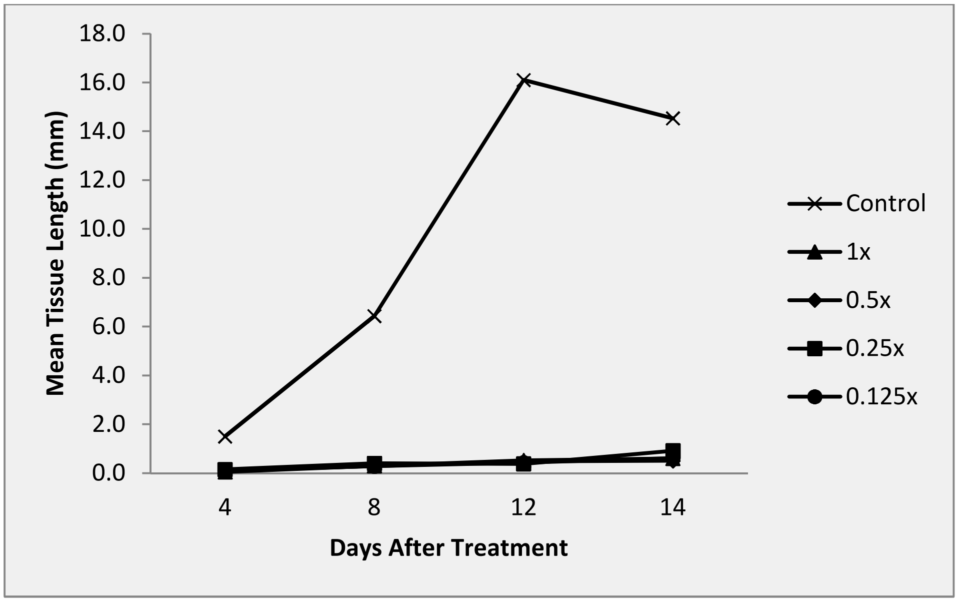

Figure 4.155. Mean tissue length of water oak seeds after treatment with triclopyr. Significant differences between rates are ranked by p-values: <0.1*, <0.05**, <0.01***.

Prediction of 1-0 Seedling Post Dormancy Responses Using Pre-dormancy Responses

Fifty-four significant (p<0.1) regression models (out of 90 possible) predicted post dormancy responses (32 weeks after treatment) using pre dormancy (11 week) responses for 1-0 seedlings (Tables 4.25-4.26). The greatest number of significant (p<0.1) models predicted post dormancy height; mean R^2 across all species and herbicides was 0.81. Injury had similar prediction capabilities for post dormancy responses, but average R^2 (0.71) was not as high as height. Across species, triclopyr produced strong correlations between pre-dormancy and post dormancy height (R^2 = 0.91) and injury (R^2 = 0.95). Height models in DAS 548 (R^2 = 0.98) treatments also had strong relationships, as did models predicting injury in DAS 729 (R^2 = 0.92) treatments. Herbicides with good predictions of both post dormancy height and injury were not consistent. Exceptions to this were triclopyr (height R^2 = 0.91; injury R^2 = 0.95) and DAS 779 (height R^2 = 0.87; injury R^2 = 0.87). By species, black locust and water oak relationships were least consistent, while loblolly pine post dormancy responses were the most predictable.

Loblolly pine treated with triclopyr produced very strong models predicting post dormancy responses (R^2 = height, 1.00; injury, 0.99). Red maple and sweetgum post dormancy height correlations were consistent, but injury ratings were not.

Table 4.25. Summary table of regression models predicting 1-0 seedling height post dormancy (32 WAT) using pre-dormancy (11 WAT) height. ††

	Black Locust		Loblolly Pine		Red Maple		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 313	**0.033**	**0.93**	**0.024**	**0.95**	**0.027**	**0.95**	0.405	0.35	**0.008**	**0.98**	0.099	0.83
DAS 402	0.554	0.20	†	†	**0.050**	**0.90**	**0.017**	**0.97**	0.235	0.58	0.214	0.66
DAS 534	0.751	0.06	†	†	**0.002**	**1.00**	**0.010**	**0.98**	**0.094**	**0.82**	0.214	0.71
DAS 548	**0.019**	**0.96**	**<0.001**	**1.00**	**0.004**	**0.99**	**0.011**	**0.98**	**0.028**	**0.94**	0.016	0.98
DAS 602	0.623	0.14	**0.038**	**0.93**	**0.082**	**0.84**	**0.048**	**0.91**	0.176	0.68	0.193	0.70
DAS 729	**0.067**	**0.87**	†	†	0.175	0.68	**0.034**	**0.93**	0.250	0.56	0.131	0.76
DAS 779	**0.017**	**0.97**	0.240	0.58	**0.027**	**0.95**	**0.031**	**0.94**	**0.043**	**0.92**	0.071	0.87
DAS 896	**0.050**	**0.90**	**0.009**	**0.98**	**0.059**	**0.89**	0.198	0.64	0.276	0.52	0.118	0.79
Triclopyr	0.128	0.76	**0.001**	**1.00**	**0.050**	**0.90**	**0.040**	**0.92**	**0.004**	**0.99**	0.045	0.91
Overall Mean	0.249	0.64	0.062	0.91	0.053	0.90	0.088	0.85	0.124	0.78	0.120	0.81

†Pre and post dormancy heights were 0 cm for all rates of this chemistry.
††Statistically significant models are bolded.

Table 4.26. Summary table of regression models predicting 1-0 seedling injury rating post dormancy (32 WAT) using pre-dormancy (11 WAT) injury rating. ††

	Black Locust		Loblolly Pine		Red Maple		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 313	**0.083**	**0.84**	0.393	0.37	**0.036**	**0.93**	0.523	0.23	**0.034**	**0.93**	0.214	0.66
DAS 402	0.478	0.27	†	†	0.223	0.60	**0.002**	**1.00**	**0.082**	**0.84**	0.196	0.68
DAS 534	1.000	0.00	†	†	**0.023**	**0.95**	**0.010**	**0.98**	0.282	0.51	0.329	0.61
DAS 548	**0.034**	**0.93**	**<0.001**	**1.00**	**0.008**	**0.98**	0.226	0.60	0.750	0.06	0.255	0.72
DAS 602	0.482	0.27	**0.018**	**0.97**	0.684	0.10	0.108	0.80	0.851	0.02	0.428	0.43
DAS 729	**0.003**	**0.99**	†	†	**0.099**	**0.81**	**0.003**	**0.99**	**0.068**	**0.87**	0.043	0.92
DAS 779	**0.017**	**0.97**	**0.046**	**0.91**	0.124	0.77	0.136	0.75	**0.029**	**0.94**	0.070	0.87
DAS 896	0.167	0.69	**<0.001**	**1.00**	0.248	0.57	0.924	0.01	0.248	0.56	0.397	0.57
Triclopyr	**0.009**	**0.98**	**0.007**	**0.99**	**0.008**	**0.99**	**0.068**	**0.87**	**0.025**	**0.95**	0.023	0.95
Overall Mean	0.252	0.66	0.116	0.87	0.161	0.74	0.222	0.69	0.263	0.63	0.214	0.71

†Pre and post dormancy injury ratings were "6" for all rates of this chemistry.
††Statistically significant models are bolded.

Prediction of 2-0 Seedling Responses Using 1-0 Seedling Responses

Models correlating responses of seedlings from different age groups were much weaker than models predicting responses within the same age group. Only sixteen regression models were statistically significant ($p<0.1$) when trying to predict 2-0 seedling responses using 1-0 seedling responses (Tables 4.27-4.29). Mean r-squared values across species and herbicide were similar for height, injury, and mortality: 0.38, 0.37, and 0.33, respectively. No overall patterns in species or herbicide were present within 2-0 regression models. Even loblolly pine treated with triclopyr did not produce consistent results. Sweetgum appears to be the most predictable species in the 2-0 group, r-squared for height, injury, and mortality were 0.60, 0.51, and 0.47, respectively. Significant ($p<0.1$) models predicted 2-0 height, injury, and mortality of seedlings treated with DAS 779 better than most herbicides; mean R^2 across species were 0.51, 0.60, and 0.75, respectively. DAS 779 was also consistently predicted in 1-0 pre to post dormancy analysis. However, injury was best predicted for seedlings treated with DAS 548 (mean R^2 = 0.75).

Table 4.27. Summary table of regression models predicting 2-0 seedling height using 1-0 seedling height. ††

	Black Locust		Loblolly Pine		Red Maple		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 313	0.690	0.10	0.460	0.29	0.551	0.20	**0.022**	**0.96**	0.469	0.28	0.438	0.37
DAS 402	0.771	0.05	†	†	0.445	0.31	**0.084**	**0.84**	0.195	0.65	0.374	0.37
DAS 534	0.488	0.26	†	†	**0.013**	**0.97**	0.387	0.38	0.588	0.17	0.369	0.36
DAS 548	0.346	0.43	0.261	0.55	0.859	0.02	0.112	0.79	0.401	0.36	0.396	0.43
DAS 602	0.727	0.07	0.661	0.11	0.555	0.20	0.365	0.40	0.104	0.80	0.482	0.32
DAS 729	0.950	0.00	†	†	0.597	0.16	0.181	0.67	0.454	0.30	0.545	0.23
DAS 779	0.312	0.47	0.494	0.26	**0.004**	**0.99**	0.457	0.29	0.260	0.55	0.306	0.51
DAS 896	0.445	0.31	0.195	0.65	0.714	0.08	0.259	0.55	0.612	0.15	0.445	0.35
Triclopyr	0.604	0.16	0.738	0.07	0.248	0.57	0.279	0.52	**0.021**	**0.96**	0.378	0.45
Overall Mean	0.592	0.21	0.468	0.21	0.443	0.39	0.238	0.60	0.345	0.47	0.414	0.38

†1-0 seedling height at 32 WAT was 0 cm for all rates of this chemistry.
††Statistically significant models are bolded.

Table 4.28. Summary table of regression models predicting 2-0 seedling injury rating using 1-0 seedling injury rating. ††

	Black Locust		Loblolly Pine		Red Maple		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 313	0.959	0.00	0.625	0.14	0.550	0.20	0.151	0.72	0.604	0.16	0.578	0.24
DAS 402	0.691	0.10	†	†	0.725	0.08	0.263	0.54	0.649	0.12	0.582	0.17
DAS 534	0.735	0.07	†	†	0.686	0.10	0.987	0.00	0.962	0.00	0.843	0.03
DAS 548	0.178	0.68	**0.092**	**0.83**	0.316	0.47	**0.072**	**0.86**	**0.044**	**0.91**	0.140	0.75
DAS 602	0.984	0.00	0.348	0.43	0.118	0.78	**0.034**	**0.93**	0.943	0.00	0.485	0.43
DAS 729	0.541	0.21	†	†	0.317	0.47	0.216	0.61	**0.043**	**0.92**	0.279	0.44
DAS 779	0.272	0.53	**0.068**	**0.87**	**0.022**	**0.96**	0.196	0.65	0.975	0.00	0.307	0.60
DAS 896	0.142	0.74	0.565	0.19	0.272	0.53	0.968	0.00	0.918	0.01	0.573	0.29
Triclopyr	0.907	0.01	0.775	0.05	0.103	0.80	0.457	0.30	**0.068**	**0.87**	0.462	0.41
Overall Mean	0.601	0.26	0.412	0.28	0.345	0.49	0.371	0.51	0.579	0.33	0.465	0.37

†1-0 seedling injury rating at 32 WAT was "6" for all rates of this chemistry.
††Statistically significant models are bolded.

Table 4.29. Summary table of regression models predicting 2-0 seedling mortality using 1-0 seedling mortality. ††

	Black Locust		Loblolly Pine		Red Maple		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 313	**0.064**	**0.88**	0.194	0.65	0.460	0.29	†	†	†	†	0.239	0.61
DAS 402	0.560	0.19	†	†	†	†	†	†	†	†	0.560	0.10
DAS 534	0.498	0.25	†	†	0.423	0.33	0.556	0.20	†	†	0.492	0.20
DAS 548	0.261	0.55	0.321	0.46	0.699	0.09	**0.007**	**0.99**	†	†	0.322	0.52
DAS 602	0.816	0.03	0.759	0.06	†	†	†	†	†	†	0.788	0.05
DAS 729	0.646	0.13	†	†	0.844	0.02	0.265	0.54	†	†	0.585	0.17
DAS 779	0.103	0.80	†	†	**0.039**	**0.92**	†	†	0.302	0.49	0.148	0.74
DAS 896	0.633	0.13	0.499	0.25	†	†	0.423	0.33	†	†	0.518	0.24
Triclopyr	0.615	0.15	0.284	0.51	0.845	0.02	0.468	0.28	†	†	0.553	0.24
Overall Mean	0.466	0.35	0.411	0.24	0.551	0.28	0.344	0.47	0.302	0.49	0.445	0.33

†For all rates of this chemistry, 1-0 seedling mortality was 100% at 32 WAT or 2-0 seedling mortality was 0% at 6 WAT.
††Statistically significant models are bolded.

Prediction of 1-0 Seedling Responses Using Germinal Responses

Models using germinal data to predict 1-0 seedling responses were inconsistent (Tables 4.30-4.32). High overall mean herbicide efficacy in germinal treatments (average mortality 56% at 6 WAT) and high mortality in specific 1-0 seedling species-herbicide combinations (100% in all rates at 32 WAT) contributed to a low number of significant models predicting 1-0 seedling responses (Tables 4.9 and 4.16). Seedlings treated with triclopyr created the only significant models. Models ($p<0.1$) predicting height ($R^2 = 0.93$), injury ($R^2 = 0.91$), and mortality ($R^2 = 0.84$) for 1-0 loblolly pine had the best R^2 values. Water oak treated with triclopyr produced the only other significant ($p<0.1$) model; R^2 was 0.82. Models could not be produced that predicted 1-0 black locust or loblolly pine seedling response to DAS 729 because 100% mortality at all rates of herbicide during the seedling screening.

Table 4.30. Summary table of regression models predicting 1-0 seedling height at 32 weeks after treatment using germinal height at 6 weeks after treatment. ††

	Black Locust		Loblolly Pine		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 729	†	†	†	†	0.816	0.03	0.571	0.18	0.694	0.07
DAS 896	0.943	0.00	0.315	0.47	0.356	0.41	0.768	0.05	0.596	0.24
Triclopyr	0.948	0.00	**0.034**	**0.93**	0.240	0.58	0.125	0.77	0.337	0.57
Overall Mean	0.945	0.00	0.175	0.70	0.471	0.34	0.488	0.33	0.512	0.31

†1-0 seedling or germinal height at final evaluation was 0 cm for all rates of this chemistry.
††Statistically significant models are bolded.

Table 4.31. Summary table of regression models predicting 1-0 seedling injury rating at 32 weeks after treatment using germinal injury rating at 6 weeks after treatment. ††

	Black Locust		Loblolly Pine		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 729	†	†	†	†	0.709	0.08	0.622	0.14	0.666	0.08
DAS 896	0.694	0.09	0.163	0.70	0.849	0.02	0.359	0.41	0.516	0.31
Triclopyr	0.924	0.01	**0.046**	**0.91**	0.234	0.59	**0.094**	**0.82**	0.324	0.58
Overall Mean	0.809	0.03	0.104	0.81	0.597	0.23	0.358	0.46	0.469	0.34

†1-0 seedling or germinal injury rating at final evaluation was "6" for all rates of this chemistry.
††Statistically significant models are bolded.

Table 4.32. Summary table of regression models predicting 1-0 seedling mortality at 32 weeks after treatment using germinal mortality at 6 weeks after treatment. ††

	Black Locust		Loblolly Pine		Sweetgum		Water Oak		Overall Mean	
Chemistry	P	R^2	P	R^2	P	R^2	P	R^2	P	R^2
DAS 729	†	†	†	†	0.889	0.01	0.804	0.04	0.846	0.02
DAS 896	0.877	0.02	0.365	0.40	0.459	0.29	†	†	0.567	0.18
Triclopyr	0.601	0.16	**0.084**	**0.84**	0.923	0.01	0.172	0.69	0.445	0.42
Overall Mean	0.739	0.06	0.224	0.62	0.757	0.10	0.488	0.24	0.575	0.22

†1-0 seedling or germinal mortality at final evaluation was either 0% or 100% for all rates of this chemistry.
††Statistically significant models are bolded.

Prediction of 1-0 Seedling Responses Using Seed Screening Responses

Seed screening models were also largely inconsistent. All significant models predicting 1-0 seedling responses using percent germination of seed are presented below (Table 4.33). Tissue length produced no significant models. The strength of predictions created using seed screening responses varied by species and herbicide. DAS 402 produced the greatest number of significant models (5) predicting 1-0 seedling responses, while DAS 729 produced none. Height, injury, and mortality of 1-0 black locust seedlings treated with DAS 402 were predicted using 14 DAT percent germination. These models have the same p-value and R^2 because 1-0 black locust seedlings had nearly 100% mortality in response to all rates of DAS 402 at 32 WAT. There were two significant (p<0.1) models that predicted 1-0 loblolly pine seedling height and injury using percent germination at 14 DAT. Percent mortality at 32 WAT for 1-0 water oak seedlings treated with DAS 402 could be predicted using 4 DAT percent germination.

Table 4.33. Summary of significant regression models predicting 1-0 seedling responses at 32 weeks after treatment using seed screening responses.

Species	Chemistry	Predictor (X)	Response (Y)	P	R^2
Black locust	DAS 402	% Germination 14 DAT	Height (cm) 32 WAT	0.097	0.82
Black locust	DAS 402	% Germination 14 DAT	Injury 32 WAT	0.097	0.82
Black locust	DAS 402	% Germination 14 DAT	% Mortality 32 WAT	0.097	0.82
Loblolly pine	Triclopyr	% Germination 14 DAT	Height (cm) 32 WAT	0.070	0.86
Loblolly pine	Triclopyr	% Germination 14 DAT	Injury 32 WAT	0.058	0.89
Water oak	DAS 402	% Germination 4 DAT	% Mortality 32 WAT	0.077	0.85
Water oak	DAS 402	% Germination 14 DAT	% Mortality 32 WAT	0.057	0.89

Chapter 5: Discussion

All rapid screening experiments were able to detect differences in response means due to species and herbicide at specific times. The ability to quickly differentiate herbicides based on their activity is critical to herbicide researchers. Although quick evaluation of herbicide activity is accomplished with rapid screening, 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 (Miller and Edwards 1996).

Past research has shown that predicting field efficacy from greenhouse grown plants is possible and repeatable (Blair et al. 2006; Bunn et al. 1996; Zedaker and Seiler 1988). In a study testing large numbers of herbaceous and woody plants for responses to herbicides, there was found to be less than a two-fold overall variation between greenhouse and field efficacy (Fletcher et al. 1990). The Fletcher study went on to conclude that taxonomic grouping of the plants in the study had a greater effect on results than did environment (field vs. greenhouse).

1-0 Greenhouse Seedling Screening

Changes in height from pre to post dormancy varied by species and treatment, but overall species means increased from 11 to 32 WAT. This indicates seedlings grew through herbicide injury or in some cases of severe injury, resprouted from dormant buds lower on the stem (Coble et al. 1969). Resprouting makes predictions of post dormancy efficacy difficult given that seedlings heavily injured before dormancy can resprout and regrow post dormancy.

Visual injury rating detected differences between herbicides very early in the experiment (2 weeks after treatment). Foliage began to show signs of herbicide injury (epinasty, curling or twisting of plant tissues, followed by necrosis) before plant height became reduced by death of leaf and stem tissues in the top of the crown and certainly before a plant would appear to be dead. Seedlings that had 100% crown necrosis were given an injury rating of “6” (on a scale of 1 to 6) and appeared to be dead based on visual assessment, but mortality could not be confirmed until evaluation in the growing season after application.

Injury rating was the most subjective measure of herbicide efficacy. Due to the size of the experiment, crews of 6 to 10 evaluators were employed to evaluate all seedlings within a reasonable timeframe, i.e. 1-2 days. This inherently increased variability of the results. To control for variation among workers conducting visual assessments of injury rating, evaluators were tested on a check tray during each evaluation workday. Data from check tray testing indicate a coefficient of variation equal to 22% of the mean (averaged across all species and evaluation times). Evaluation of injury for black locust and sweetgum in check trays was most consistent (CV 14% and 15% respectively) and varied most on water oak (CV 34%).

Height measurements had a coefficient of variation equal to 25% of the mean. Check tray height measurements of some species were more variable than others; loblolly pine had the most consistent check tray height measurements (CV 10%), while black locust check tray height measurements were least consistent (CV 51%). Other species had CV’s of approximately 20%.

Higher injury ratings generally corresponded with lower height measurements. Injury ratings in some species and treatment combinations increased through 11 WAT and decreased after dormancy (Tables 4.5-4.8). This indicates that seedling growth following dormancy produced stem and leaf tissue without symptoms of herbicide injury (Coble et al. 1969). For example, mean red maple injury ratings increased from 1.0 to 4.3 from 0 to 11 WAT and then decreased following dormancy to 2.9 (Tables 4.5-4.8).

Height and injury were more frequently different due to herbicides than due to rates. This may have been the result of the rate spectrum of some herbicides being too high for the size of the seedlings in the 1-0 screening. For example, mortality at 32 WAT for loblolly pine seedlings treated with DAS 402, 534, and 729 was 100.0% even at the lowest rate (Table 4.9). Adjustment of rate spectrum to be specific to each species and herbicide may produce more detailed screening results (Blair et al. 2004). However, some preliminary results are required with the herbicides and species in question to direct refinements in rate.

Models predicting post dormancy height and injury for black locust seedlings were inconsistent. Even when herbicide injury resulted in 100% crown necrosis, black locust seedlings were observed to resprout. This is consistent with Sterrett and Chappell (1967) which showed that black locust will produce stump sprouts and root suckers when the plant's crown is removed. Resprouting potential of locust may have affected post dormancy response correlations.

In contrast, predictions of loblolly pine post dormancy efficacy were very reliable. Triclopyr models, especially, predicted post dormancy height and injury consistently for 1-0 loblolly pine seedlings; mean R^2 was 0.99 (Tables 4.25-4.26). Most pines, including loblolly, cannot resprout from their root systems following severe damage to their crown (Little and Somes 1960). Once pines appeared to be dead aboveground (injury rating "6"), they did not recover.

Previous research also shows that triclopyr produces strong linear and log(y) transformed models predicting field response of 2 year old loblolly pine using 2-0 greenhouse seedlings (Blair et al. 2006). One year after treatment (YAT), percent mortality in 2 year old loblolly pine field trials was predicted with 1 YAT percent mortality of 2-0 greenhouse seedlings (p=0.02, R^2=0.97) (Blair et al. 2006). Log transformed injury of 2 year old loblolly pine field trials at 1 YAT was also predicted using 1 YAT injury rating of 2-0 loblolly pine greenhouse seedlings (p=0.01, R^2=0.99) (Blair et al. 2006). The logarithmic best fit line indicates responses of greenhouse seedlings increase exponentially in comparison to 2 year old saplings as herbicide dosage increases, suggesting a relationship between plant size and herbicide response.

Several published sources, including the product label, indicate that loblolly pine can be damaged by triclopyr (DowAgrosciences 2008). At 1 YAT in a field study, three year old loblolly pine treated with a broadcast application of triclopyr formulated as Garlon 4 at 4481 g ae ha^{-1} exhibited 100% mortality (Miller and Edwards 1996). Similarly, in an earlier study triclopyr (also as Garlon 4) applied as a broadcast spray at 2241 g ae ha^{-1} caused 67% mortality 1 YAT in loblolly pine (Minogue et al. 1985). However, at rates used in the current study (Table 5.1), 1-0 loblolly pine seedling mortality was only 58.5% at 32 weeks after treatment (Table 4.9). Although the highest rate of triclopyr used in this experiment was 8 times lower than one standard site preparation rate (4 lbs ae ac^{-1}) (Table 5.1), smaller plants are expected to be more susceptible to the same amount of herbicide active ingredient. Past studies on rapid screening have shown that rates 0.5x to 0.25x standard field rates produced results in greenhouse seedlings similar to two year old field trials (Zedaker and Seiler 1988). Additionally, herbicides can be more effective when applied to greenhouse grown plants because conditions for absorption and translocation may be ideal in young, well hydrated plants (Beckie et al. 2000).

Table 5.1. Comparison of rates used in the current study with previous research (Blair et al. 2006).

	Current Study		Blair et al. 2006	
Rate	Numbered DAS Compounds	Triclopyr (Garlon 4 Ultra)	Imazapyr (Arsenal AC)	Triclopyr (Garlon 4)
	g ae ha^{-1}			
1x	280	560	841	4481†
0.5x	140	280	421	2241
0.25x	70	140	210	1120
0.125x	35	70	105	560

†4 lbs ae ac^{-1} (4481 g ae ha^{-1}) is a standard site preparation rate.

Control of red maple seedlings by triclopyr (1-0 seedling mortality = 14.4% at 32 WAT) was low compared to published field efficacy where mortality was 85% 1 YAT in response to triclopyr broadcast applied at 4481 g ae ha^{-1} (Miller and Edwards 1996). The strength of 1-0 red maple post dormancy predictions of height and injury was very good; red maple had more significant relationships than any other species.

Sweetgum seedling mortality (31.3%) in triclopyr treatments was consistent with results of some field trials. May and July application of triclopyr (Garlon 4) as directed sprays at 2% v/v produced 33% and 36% mortality, respectively, in sweetgum 1 YAT (Miller 1990). However, broadcast applications of triclopyr (Garlon 4) produced 56% mortality in sweetgum 1 YAT (Miller and Edwards 1996). Previous research has shown that 1 YAT log transformed injury rating of 2 year old field grown sweetgum saplings treated with triclopyr can be predicted using 2-0 greenhouse sweetgum injury ($p=0.04$, $R^2=0.81$) (Blair et al. 2006). In this experiment, pre and post dormancy responses of sweetgum seedlings could be correlated using linear regression models, but the average model strength was not as strong as other species. Sweetgum was able to consistently come out of dormancy producing stem and leaf tissue without symptoms of herbicide injury (from 11 WAT to 32 WAT sweetgum overall mean injury decreased from 4.7

to 3.4). Post dormancy recovery may have been a factor decreasing predictability of injury results.

Water oak 1-0 seedling mortality in this experiment (25.1%) was less than results of published field trials. One year after treatment, triclopyr (Garlon 4) broadcast applied at 4481 g ae ha^{-1} caused 55% mortality in water oak (Miller and Edwards 1996). In July directed spray application at 2% v/v, triclopyr (Garlon 4) caused 77% mortality (Miller 1990). Lower mortality observed in this study may be due to the lower rate of herbicide applied to plants in greenhouse trials. Differences in growth rates within species caused some water oak seedlings in greenhouse screening to have tops of new, succulent growth at the time of spray while other seedlings had tops with more mature leaves. In most plants, there is a relationship between thickness of leaf cuticle and the absorption of foliar applied herbicides (Hull et al. 1975). The Hull study showed that leaf cuticle thickness develops over time in honey mesquite and new growth absorbed herbicide solution more readily than mature leaves with their thicker, waxier cuticle. Differences in herbicide absorption by individual water oak seedlings may have increased variation in results and contributed to low average R^2 values for models predicting water oak post dormancy height and injury.

Overall, triclopyr treatment post dormancy responses could be consistently predicted, especially injury ratings; mean R^2 was 0.95 and all models were significant ($p<0.1$) (Table 4.26). Models predicting height in triclopyr treatments also had high mean R^2 values (0.91), expect for black locust which did not produce any significant height models (Table 4.25).

Regression models produced from this study make it apparent that overwintering 1-0 seedlings may not be necessary to measure results that are consistent with those of overwintered seedlings. The length of 1-0 greenhouse seedling screen without overwintering was 11 weeks from treatment to pre-dormancy evaluation. Removing overwintering from the screening process represents a time savings of five months.

2-0 Greenhouse Seedling Screening

The same herbicides and rates were applied to the 1-0 and 2-0 seedlings groups, but 2-0 seedlings (9.5% mortality) were much less susceptible to herbicide injury than 1-0 seedlings (46.8% mortality). At rates applied in this study, 2-0 seedlings were also much less susceptible than plants in field trials (Miller 1990; Miller and Edwards 1996).

Differences between 1-0 and 2-0 seedling responses may be explained by the relationship between leaf area to biomass ratios and herbicide efficacy. Past research indicates that the leaf area to biomass ratio decreases as seedlings age (Blair et al. 2006). A lower leaf area to biomass ratio requires more foliar applied herbicide active ingredient to reach the site of action in the plant through a given leaf surface area to maintain herbicide efficacy as plant size increases (Blair et al. 2004).   In other words, lower leaf area to biomass ratio in the 2-0 seedlings would require more herbicide to be absorbed by the plant in order to have efficacy similar to the 1-0 seedlings.

The relationship correlating 1-0 and 2-0 seedling results is much weaker than the relationship between 1-0 seedling pre to post dormancy responses, 16 significant models compared to 54 (Tables 4.25-4.29). Autocorrelation between 1-0 pre to post dormancy responses is certainly a factor in the difference in the number of significant models. Models predicting 2-0 mortality were very weak, especially for water oak. Inconsistencies in model predictions across species and herbicide may indicate that models are species and herbicide specific.

Measurement intervals for the 2-0 group (6 WAT) were different from the 1-0 group (2, 4, 11, and 32 WAT). Responses from 1-0 seedlings indicated that height and injury changed over time, especially from 4 to 11 WAT (Tables 4.2-4.8). Although injury symptoms caused by the herbicides in this study developed rapidly, some plants recovered from initial herbicide injury. Therefore, six weeks may not have been a long enough time period to form conclusions about the effects on the 2-0 seedlings.

Germinal Screening

Consistent with the relationship between plant size and herbicide efficacy described above, germinal seedlings were more susceptible to herbicide injury than 1-0 and 2-0 seedlings. Size of germinal seedlings varied widely by species and this seemed to have an impact on the susceptibility of individual plants to herbicide injury; smaller plants were more easily damaged than large ones. Loblolly pine and sweetgum germinals were very small (3 – 6 cm height) and still had their juvenile leaves on the spray date. Black locust, however, germinated and grew very rapidly, reaching an average height of 13.5 cm in control trays and greater than 20 cm at 6 WAT in triclopyr treated trays. This was unexpected considering that black locust is known to be susceptible to most herbicides, including triclopyr. Hormesis, a phenomenon observed in toxicology where a non-lethal dose of a plant toxic substance causes a stimulation in growth, may explain the difference in height between black locust herbicide and control trays (Calabrese and Baldwin 2003). Water oak germinals, planted several weeks in advance of the other species, were also well established with mature foliage by the spray date. Water oak also exhibited a hormetic effect on height. Mean height in control trays was 8.3 cm while mean height in herbicide treated trays was 14.2 cm; mean height specifically in DAS 896 treated trays was 22.1 cm at 6 WAT.

Models predicting efficacy in 1-0 seedlings from germinal responses were not very successful (Tables 4.30-4.32). Loblolly pine and water oak treated with triclopyr produced the only significant ($p<0.1$) models predicting responses of 1-0 seedlings at 32 WAT. However, mortality observed in germinal control trays, black locust (35%), loblolly pine (40%), and sweetgum (100%), further reduces reliability of predictions made from germinal herbicide treatments.

The goal was to produce uniformly sized germinal seedlings before treatment. Because the species were started from seed together as ready-to-spray trays (4 species x 10 individuals per tray), timing of plantings was critical and will require more refinement in future screenings (Table 3.5). In seedling screenings, species were planted individually in cells (pots) and kept separately in the greenhouse to be on different watering schedules based on their rate of germination and speed of growth. The use of individual cells or pots instead of trays would allow germinals to be kept in trays of pure species on different watering regimes from planting until sorting prior to application. Cells would also probably have better drainage for improved control of watering levels. This system should help prevent overwatering of some species while under-watering others, which, coupled with timing of plantings, may have been an issue with the development of sweetgum and loblolly germinals.

Seed Screening

Significant differences in percent germination and tissue length between control and treated Petri dishes were observed, indicating that seed screening is a good test for general herbicidal activity (Tables 4.17 and 4.21). Few significant ($p<0.1$) models of seed responses were produced that predicted 1-0 seedling efficacy, but differences were detected between herbicides used in seed treatments and this is consistent with past research (Blair et al. 2006; Bunn et al. 1996).

Control of sweetgum germination (0%) and tissue length (0 mm) with triclopyr observed in this study is expected based on previous seed screening work (Blair et al. 2004; Bunn et al. 1995). The mode of action of triclopyr is similar to IAA (endogenous auxin) and other synthetic auxin-type herbicides and may disrupt seed development by inhibiting cell division and growth (Vencill 2002). In a laboratory study testing the effect of herbicide concentrations on the forest floor seed bank, triclopyr caused the greatest reductions in germination relative to control out of four other herbicides (Morash and Freedman 1988). However, in the field, seed response to herbicide residues from operational applications was not significantly different in treated versus untreated areas (Morash and Freedman 1988).

Loblolly pine germinated successfully and exhibited response to rates of triclopyr, similar to past research (Blair et al. 2004). In this experiment, models were produced predicting height and injury at 32 WAT of 1-0 loblolly pine treated with triclopyr using 14 DAT seed percent germination (Table 4.33). No research has been found predicting greenhouse seedling results from seed screening, but loblolly pine seed screenings have previously been shown to parallel triclopyr efficacy in field screenings (Bunn et al. 1995). In another study, significant models were produced predicting percent control of height ($p=0.08$, $R^2=0.85$) and injury rating ($p=0.05$, $R^2=0.90$) at 1 YAT in 2 year old loblolly pine saplings using percent control of germination in loblolly pine seeds at 10 days after treatment (Blair et al. 2006).

Time until differentiation of treatments was species specific as the species in this experiment are known to have different rates of emergence. Black locust seeds emerged rapidly, but tissue length was highly variable with some seeds elongating into fully developed plants and others not germinating at all. Differences in tissue length and percent germination across species in response to herbicide appeared by 8 DAT. Treatment differentiations disappeared for black locust after 8 days as plant tissue became infected with an unknown fungal pathogen likely due to the high humidity levels (80% relative humidity) that were maintained in the seed germination chamber. It was not clear whether the decrease in tissue length in black locust treatments was related to any herbicidal activity, although black locust treated dishes had significantly lower tissue length and germination rates than controls. No other species experienced this fungal infection. The decline in tissue length indicates that percent germination may be a better response for ranking herbicides in seed screening because it was more stable over time – once treatment differentiations and rankings appeared they did not go away.

Seed screening was able to determine herbicide activity relative to control and treatment differentiation in a very short period of time (4 days for some species). Responses were not useful for differentiating rates or ranking herbicides relative to seedling efficacy, but some species-herbicide combinations produced significant regression models that were able to predict herbicide efficacy in 1-0 seedlings.

The screening methods tested in this study offer several "decision points" to researchers using these methods to screen new herbicides. These decision points are times in the development process where the researcher must pose and answer the question: should new herbicide "X" continue to be developed based on the results of screening? Each successive screening offers the opportunity to filter out herbicides that do not have properties that are a fit for the desired market. Early stage screenings, like seed and germinal screenings, offer fast, inexpensive information on new herbicides. However, if the results of these screenings are not consistent with greenhouse seedling and field trials, they could cause promising new compounds to be disregarded. To provide an overview of the results of herbicide trials, herbicides were objectively ranked based on their injury rating and height responses and assigned a numeric rank within each screening (Table 5.2).

Table 5.2. Overview of herbicide rankings†† across screening types.

		Decision Points				
Efficacy	Herbicide	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.
†† Ranking: 1 to 9 where 1 is most effective and 9 is least effective.

Results for some herbicides were very stable across screening types, including DAS 534, 548, and 779 (Table 5.2). The most effective herbicides were DAS 534 and 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. If seed screening were the first test used by a researcher to differentiate chemistries and no other information were available about DAS 729, it would not make it to the next round of testing, i.e. seedling screening.

Triclopyr ranking was relatively consistent (ranked #2-#4) except for 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. If 2-0 seedlings were kept through a dormancy cycle, seedlings in triclopyr treatments may 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 compounds from Dow AgroSciences were often more effective at lower rates than triclopyr in rapid screenings, but no published field results exist to confirm these findings.

Preliminary agricultural screening techniques are moving to genomics and in vitro testing to identify new herbicide modes of action and sites of herbicide activity in weed species (Hess et al. 2001; Weller et al. 2001). But, greenhouse and growth chamber trials are still used in agricultural screening for new herbicides. In one study examining resistance in seed populations of weed species, soil-less screening methods including agar and petri dishes with filter paper were used as precursors to greenhouse pot screenings, reinforcing the usefulness of advance predictor tests in screening large numbers of herbicides and species (Beckie et al. 2000). However, published material on specific methods currently used for correlating greenhouse screening with operational trials for new woody and herbaceous herbicides has been difficult to locate. This may be related to the proprietary nature of screening processes used by chemical companies. Although screening methods for woody vegetation management are well behind the agriculture sector, rapid screening methods presented here continue to show promise for quickly providing information on new herbicides that may be useful for guiding product development.

Chapter 6: Conclusions and Future Applications

Woody plant herbicide screening is a time and resource intensive process due to the physiology of woody plants. The ability to rapidly rank and predict herbicide efficacy and spectrum of species activity is critical to efficient research and development efforts. Results of advance studies may be used to predict and guide larger scale research and operational trials in the process from discovery to registration of new herbicide products.

Recognition of herbicides with high efficacy as early as 2 weeks after treatment in seedling screenings and as early as 4 days after treatment in seed screenings is important. Significant results of this research are shortening the greenhouse seedling screening to 11 weeks by eliminating overwintering and demonstrating that species and herbicide specific predictions of results can be made using germinal and seed screening techniques.

Future research is needed to tie the screening types in this experiment to field enclosure screenings. This work would include fewer herbicides, but would include several rapid screening methods: seed, germinal, 1-0 and 2-0 greenhouse seedlings, as well as 2 year old field exclosure screenings. This would allow for species and herbicide specific response patterns to be captured across all rapid screening types and compared to field results. Herbicides, especially standards, should be analogous for comparison purposes. Fewer treatments would allow for a greater number of evaluations in the weeks after treatment to fine tune the ideal evaluation time for various species-herbicide combinations. Results of this research may be useful to future rapid screening and woody plant herbicide researchers with a potential outcome of larger numbers of herbicides available for vegetation management.

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