

## **Chapter 1:** **Introduction/Justification**

Vegetation management has been a concern for civilizations since the establishment of agriculture. Mechanical weed control, such as hand removal, cultivation, flooding, and burning, was the initial method of providing weed control and is still used today in certain situations. These mechanical methods are labor and time intensive, non selective, and may be counter productive by lengthening the time it takes for a crop to come to maturity (Saggers 1976). Salts, such as sodium chloride, and other inorganic compounds were the mainstay of chemical vegetation management until the turn of the 20<sup>th</sup> century. The development of organic compounds used for vegetation management has added significant benefits in the 20th century and into the 21<sup>st</sup> century. Organic compounds can provide true selectivity, a wide array of mechanisms of action, biodegradability, and a diverse selection of products available to agricultural, forestry, aquatic, industrial, public, and private landowners.

Forestry and rights-of-way vegetation managers have come to rely on herbicides as a means to maximize growth potential in timber stands, manage wildlife habitat, increase access and safety in rights-of-way, and manage invasive species in a cost effective manner with minimal environmental impact. Unfortunately, the introduction of new chemistries in these arenas is limited, as the last new product to be introduced for woody plant control was imazapyr in 1982. One significant barrier to the development of new chemistries is the cost and time involved in woody plant herbicide research and development. This barrier inhibits the introduction of chemistries that could possibly have less environmental impact, lower cost thresholds, or broader spectrums of control.

The agricultural industry continually invests in research and development in derivatives of existing chemistries and new market niches of existing chemistries. The economic factors and benefits of cheaper agricultural products drive these decisions to increase chemical alternatives. Traditionally, the market size of woody plant vegetation management has been approximately 1/10<sup>th</sup> that of the agricultural vegetation management market and this difference lends itself to the higher research and development efforts in agricultural markets by comparison to woody plant markets. Screening processes for herbaceous weed control in agriculture are often cheaper than woody plant screening due to the smaller amounts of herbicide active ingredient necessary to determine efficacy ratings. Time to evaluation is also shorter due to the annual nature of herbaceous weeds. It is not unreasonable to think that an efficient red maple (*Acer rubrum* L.) control product may have been developed in the agricultural sector but never researched and utilized in the woody plant market due to the damage it caused to an agricultural crop. A less cost and time intensive screening procedure for woody plant herbicides may have allowed this product to be screened and introduced into the woody plant market.

As stated, the forestry and rights-of-way industries could have new chemistries at their disposal if more cost and time efficient methods of screening woody plants for herbicide efficacy were available. The woody plant rapid greenhouse screening process developed by Drs. Seiler and Zedaker at Virginia Tech has shown promise in decreasing amounts of active ingredient necessary to perform woody plant screens from kilograms to milligrams. Time to evaluation can be decreased as well from up to 24 months to 12 to 16 months when starting from seed stock and decreased to 6 months with seedling stock. The actual time from application to final evaluation can be decreased from 12 months to as little as 5 months. These characteristics of rapid greenhouse screening could aid in the discovery and/or development of chemistries for woody

plant control. The use of rapid seed screening of woody plants in herbicide research has also shown possible benefits in determining initial activity on plant growth. This method could allow researchers to make a quick determination of initial herbicide efficacy and the need for further research in a time and cost efficient manner. If it can be shown that these two methods, rapid greenhouse screening and rapid seed screening, have strong statistical correlations with conventional field screening techniques, these two methods could become standard techniques for manufacturers conducting discovery and development research.

Foliar-applied herbicides are common in forest site preparation and right-of-way management. Leaf uptake is the primary route of entry for foliar-applied herbicides. The compound needs to translocate and move from the leaf surface to the site of action to kill or negatively impact living tissue. However, the ratio of intercepting surface area (i.e. leaf area) to biomass changes over time. This may alter efficacy in larger plants since more compound needs to enter though less leaf area per unit of biomass on a larger plant. Herbicide prescriptions are commonly made based on species composition and weed density. It is unclear; however, if a reduced relative absorbed dose due to a decrease in relative leaf area will result in the same amount of control of total biomass. It is also not known if the standard field rate could be applied to a lower uptake surface area (leaf area) and still provide satisfactory control of standing biomass. To understand the relationship between leaf area and biomass and its effect on herbicide efficacy, research should begin by collecting control (efficacy) data for a known leaf area to biomass ratio for selected age classes and/or growing conditions. These leaf area to biomass ratios used as covariates in a dose response curve could give an indication of the influence that this ratio has on herbicide efficacy. The ratios could also be used to test for significant differences in slopes of dose response curves between different age classes. This

initial research could lead to findings that a decreased rate (i.e. decreased cost of application) could be used to control a known biomass with a known leaf area for uptake.

## **Objectives**

The objectives of this research project were:

1. To determine if rapid greenhouse screens and rapid seed screens statistically correlate with traditional field screens by rate for woody species treated with imazapyr and triclopyr.
2. To determine effective rapid seed screening techniques and the rapid seed screens ability to provide information on potential species efficacy spectrums in a relatively short period of time.
3. To determine the effect that changes in leaf area / biomass ratios have on woody plants treated with imazapyr and triclopyr.

## **Chapter 2:** **Literature Review**

### *Section 1: Overview of Agricultural Pesticide Regulation and Development*

Pesticide development and manufacturing is regulated by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) of 1947 (amended in 1972, 1975, 1978, 1988, and 1996). The Federal Environmental Pesticide Control Act of 1972 granted the Environmental Protection Agency (EPA) administrative authority over the developing and manufacturing of all pesticides (Plater et al. 1998). FIFRA, and subsequent amendments, require that any pesticide chemical manufactured be registered with the EPA. Registration will be granted if certain criteria are met. These include determination of claims of efficacy by the manufacturer and if labeling data meet federal standards and documentation that there is no reasonable harm to humans or the environment (Plater et al. 1998). The amendments also moved the focus of the registration requirements from efficacy and labeling to human health and environmental safety (Plater et al. 1998). The EPA requires substantial information on biological activity, toxicology, environmental persistence, and physical and chemical characteristics for material safety data sheets. Collection of this information is the responsibility of the manufacturer and can cost anywhere from \$35 million to \$50 million and may take up to ten years to complete. Approximately 1 in 20,000 compounds entered into the manufacturers' testing process passes the EPA's standards and become available on the commercial market (CropLife America 1993). The time and cost involved in developing new chemistries can become prohibitive for manufacturers to invest in new discoveries, especially in the relatively small woody plant control market.

When a new pesticide compound is developed, the manufacturer synthesizes only minute quantities for testing. The initial testing is to provide data on biological activity, toxicological

activity and the potential ability to control pests (CropLife America, 1993). If the compound is deemed to be genetically harmful to animal cells, testing will be terminated or further tests are required for health considerations. This “primary” biological and toxicological screening provides the manufacturer information for further product development. The next step would be to design secondary biological screening using replicated operational trials to test the competency and characteristics of the pesticide compound under a broad range of realistic conditions (Croplife America 1993). This testing requires the manufacturer to obtain an experimental use permit (EUP) from the EPA (Bovey 2001). This typically includes applying the compound to different pest and crop species at varying rates by different application techniques under a broad range of environmental situations (Croplife America 1993). These operational trials, often performed by an outside source for the manufacturer (independent contractor, university, etc) near the end of the registration process, can also provide valuable information on environmental fate concerns such as persistence, dissipation, and impacts on non-target organisms.

These first few years of research, especially the primary screens described above, provide crucial information on whether or not to continue with the development process to make the product commercially available. As developmental research and screenings continue, operational and economic considerations must be evaluated and seen as beneficial for society and the manufacturer. Overall, in the process of discovery to product registration a chemical will undergo up to 142 tests to obtain EPA approval (Schulze 1993). Of these 142 tests, 18 of them relate to residual chemistry and efficacy. Of these 18 tests, only a few deal directly with efficacy in terms of a product performing up to acclaimed standards. The manufacturer will have more information on product efficacy than the EPA requires for testing and registration purposes. The

overall timeline for the developmental and registration process varies depending on product characteristics and potential uses. Total information collected by the manufacturer includes primary and secondary screenings, toxicology research, market research, patent reviews, pilot plant manufacturing data, and cost estimations. Once the EPA has granted a compound a product label, manufacturing and distribution of a new product will still depend on the time left on patent protection (up to 17 years) and the marketing considerations (Bovey 2001).

### Section 2: Herbicide Development Screening Techniques

All pesticides (fungicides, insecticides, herbicides, etc) must undergo primary and secondary screenings to determine their biological activity, toxicological ratings, and efficacy ratings (Bovey 2001). The screening process for herbicides, as with all pesticides, is an economically driven process occurring in stages to answer one question: should screening continue to the next stage or be terminated? Primary screenings are performed during the discovery stages of the compound while secondary screenings begin after the compound has reached the developmental stages and can continue even after product registration and further tests are desired on spectrum of control, tank mix options, etc. In the case of herbicides, screenings can be categorized into two main groups: the annual (herbaceous) plant screenings and perennial (herbaceous and woody) plant screenings. The remainder of this discussion on herbicide screening will deal with herbaceous and woody plant screens.

#### *Herbaceous Plant Herbicide Screening*

Herbaceous plant herbicide screenings are most common for the agricultural industry due to the prevalence of these types of weeds. These screenings typically take less time to perform than woody plant screens due to the rapid growth habits of the weed and crop species and the inherent physiological differences in herbaceous and woody plants. As with all pesticide

screens, the herbaceous plant herbicide screen is usually divided into two categories: a primary screen and a secondary screen (Saggers 1979).

A primary screen is conducted to determine biological activity (i.e. herbicidal activity) on plants of interest and toxicological effects on laboratory animals, as with any newly discovered pesticide (Bovey 2001). The overall design and extent of the primary screen is a function of the quantity of compound available (Saggers 1979). In the case of a primary screen and especially with an herbaceous screen, the quantity of active ingredient available is usually minimal (less than 2 grams) (Saggers 1979). In the case of herbicides, if no adverse effects are seen with animals in laboratory tests, screening continues for weed control options. Screening of a newly discovered compound for herbaceous weed control requires relatively small amounts (milligrams) of the active ingredient applied to small flats (~ 1 m<sup>2</sup>) containing several common weed and crop species (Zedaker and Seiler, 1988). This entire process, including growing the weed and crop species, application, and evaluation, is all performed in a greenhouse setting. The process of application and evaluation of control can be performed in less than 60 days (Zedaker and Seiler, 1988). Herbaceous screening can be easily established due to the small amounts of new compounds manufactured for testing. This type of screening is common for chemicals dedicated for the agriculture industries.

Herbaceous screening can incorporate secondary screens that occurs in a field setting. Field screens of newly discovered herbicides for herbaceous or woody species require the EPA to issue an experimental use permit (EUP). This type of screening can take in consideration the influence of environmental and operational constraints consistent with broad scale operations. This is not always necessary; however, as a majority of information needed to comply with EPA registration can be obtained from greenhouse studies. Field studies are more common after the



compound has been manufactured for an established market and more data is desired for other species control for different markets.

### *Conventional Woody Plant Herbicide Screening*

The defining characteristic of a woody species, compared to an herbaceous species, is the presence of secondary growth; more specifically, the development of woody tissue during the second and subsequent growing seasons. As stated earlier, herbaceous herbicide screening can take as little as 2 months to perform and requires minimal amounts of active ingredients to apply on greenhouse flats. There is no need for the development of secondary tissue and time for evaluation can be completed in the same growing season due to the annual nature of most herbaceous plants. Woody plants; however, are commonly much larger and well developed in field trials. The presence of these larger plants creates a need for larger amounts of active ingredient to perform initial screenings. Growth habits of woody plants create a requirement for longer process of herbicide screening due to the fact that it often takes one full growing season after application for the full extent of a product's efficacy to manifest. This increased time investment and the need for higher amounts of active ingredient for woody plant screens translate into a more costly process of herbicide screening for woody plants. As a consequence, most woody plant herbicides commercially available today came to the woody plant market after biological and toxicological testing was performed in the agricultural sector rather than being developed strictly for the woody plant control market.

Given that herbicides being considered for woody plant control have previously had biological and toxicological screens performed for the agricultural market, the terminology used to describe the screening process for woody plant, or forest, herbicides is different. A primary screening, or primary field evaluation, describes an evaluation of an experimental or untested

compound(s) on a single crop tree or weed species (Miller and Glover 1991). This screening is designed to remove compounds from consideration for a particular use and to determine rate spectrums or application timings of potentially successful compounds (Miller and Glover 1991). A secondary screening, or secondary field evaluation, refers to additional tests performed on compounds that have been shown to hold promise in the primary screens or from other sources (Miller and Glover 1991). Traditionally, the main difference between a forest herbicide primary screen and secondary screen is fewer treatments in the secondary screen than in the primary screen. Secondary screens can streamline treatment and control options and rate and efficacy ratings. These two types of screenings are performed with tightly calibrated and monitored research equipment and usually take 1 to 3 years to perform (Miller and Glover 1991).

There are two more screening types that occur in woody plant, or forest, herbicide testing. Once primary and secondary field evaluations have been performed, it is necessary to relate these findings to operational aspects and other specific factors. Operational field evaluations are performed using common operational ground or aerial equipment to determine the applicability of compounds in an operational or “real world” setting (Miller and Glover 1991). These screens are usually performed after a compound has been registered and is on the market. Operational field evaluations can confirm findings on rate, mix, and timing spectrums determined from the research findings. This type of screening connects those in research to industry, commercial applicators, and vendors as to the abilities of the compound (Miller and Glover 1991).

Field screening for specific factors such as herbicide - soil interaction characteristics, volatility, and preferred application method can also be performed (Miller and Glover 1991). This screening technique may also combine other silvicultural treatments such as fertilization

and burning to incorporate the compound(s) in question into common cultural treatments in order to determine compatibility and crop and weed response (Miller and Glover 1991).

### *Rapid Screening for Woody Plants*

The rapid screening process was developed in order to produce seedlings with secondary woody tissue in less than one year to be used in primary forest herbicide screening studies (Zedaker and Seiler 1988). It is a method to accelerate the conventional screening process of woody herbicide screening. This technique could benefit the woody plant management market by allowing introductions of compounds from the agricultural sector or new chemistries that have never been considered for the woody plant market due to cost considerations. The amount of chemical needed to perform these screens is also lessened, thereby further decreasing the cost of development and screening.

The rapid screening process can be started with either seeds or nursery stock. The timetable for a hypothetical herbicide screening can be seen in Table 2.1. Starting a trial from a seed source allows for a decrease in biological variance due to the controlled conditions in which the seedlings are grown. These seedlings can then be transplanted into field enclosures to allow for field plot trials with a decreased biological variance as well. These two applications can be of great use in woody plant herbicide screenings since herbicide efficacy is strongly correlated to the physiological condition, such as resulting from water stress of treated species (D'Anieri et al. 1987). Overall time to completion of the screening from seed source is 13 to 16 months after initiation; 9 to 12 months with seedling source. More importantly, time to first evaluation after herbicide application for both sources is only 6 weeks and time to second assessment is only 6 months.

*Table 2.1: Rapid Screening Timetable (Zedaker and Seiler 1988)*

<b>Starting from Nursery Stock</b>	
Month	Event
1	Begin Cycle 1
2-3	Spray
3-4	Harden Off, First Assessment
4-5	Chill
6-7	Begin Cycle 2, Break Dormancy
8-12	Final Assessment
<b>Starting from Seed</b>	
Month	Event
1	Plant Seed
3-4	Harden Off
4-5	Chill
6-7	Begin Cycle 2, Break Dormancy
7-9	Spray
8-11	Harden Off, First Assessment
9-12	Chill
11-14	Begin Cycle 3, Break Dormancy
13-16	Final Assessment

As previously stated, the process of rapid screening can begin with the stratification and planting of seeds or by purchasing seedlings from a nursery. The use of nursery stock can expedite the process; however, the inherent variance that exists with nursery stock may limit repeatability (Zedaker and Seiler 1988). Once the seeds have grown into seedlings, they, or the nursery stock seedlings directly, are then rotated between environmental control areas. These include greenhouses, shade or slat houses, and cold rooms. The rotation time depends on the natural season occurring at the time and the environmental conditions required to incite the growth cycle needed (Zedaker and Seiler 1988).

The environmental conditions and time periods needed to carry out the rapid screen process will vary from species to species. A major barrier to this process is determining a cycle

and time frame that is suitable for a wide array of seedlings and even different seed sources of the same species (Zedaker and Seiler 1988). The rapid screening studies performed to date have included mostly southern hardwood species and loblolly pine (*Pinus taeda* L.).

Herbicides are applied during a rapid screening process using a calibrated spray hood under tightly controlled conditions. This allows an application variance less than three percent and often less than one percent (Zedaker and Seiler 1988). Flat fan nozzles are accurately calibrated and ensure proper and accurate coverage of stems. The decrease in variance permits better precision than field applications where variances are rarely below three percent and are more often above five percent (Zedaker and Seiler 1988).

Evaluation of treated seedlings can occur in several stages. Initial observations could include visual estimates of percent mortality, percent foliar necrosis, and changes in height growth compared to a non-treated control during same growing season of application (commonly performed six weeks after treatment (WAT)). Other observations can be made in subsequent growing seasons relatively quickly due to the accelerated growth periods. These subsequent observations could include percent mortality, seedling height, root crown diameter, and dry weight expressed as percent control.

The rapid screening process has produced similar results to that of second year response data for conventional field studies (Zedaker and Seiler 1988). Published correlation values for rapid screening studies and conventional field studies for a few select species and chemistries are high (Table 2.2). The regressions predict the level of control in the field (Y or dependent variable) from rapid screens (X or independent variable). In this case, a two-year-old plantation was used. These values show success with species such as red maple but poor success on other species such as water tupelo (*Nyssa aquatica* L.). These values show the potential that the rapid

screening process holds for future screening studies and product development as well as the need for continued research and determination of correlation coefficients.

*Table 2.2: R-Squared Values for simple linear regression of seed screened and rapid screened seedlings for triclopyr and imazapyr. Regressions predict the level of control in the field (two-year old plantation saplings (Bunn et al. 1995)).*

Species	Triclopyr		Imazapyr	
	Seed	Seedling	Seed	Seedling
Loblolly pine	0.99	0.81	0.99	0.26
Red maple	0.90	0.68	0.98	0.91
Sweetgum ( <i>Liquidambar styraciflua</i> L.)	0.96	0.93	0.78	0.66
Water oak ( <i>Quercus nigra</i> L.)	0.60	NS	0.34	NS
Water Tupelo	NA	0.87	NA	0.47

### Seed Screening

The use of seed germination and tissue emergence efficacy data is common in research with pre-emergent herbicides. Preemergent products such as diuron, pendimethalin, and metolachlor have been screened for spectrum of control and rate recommendations. The majority of pre-emergent herbicides work by affecting the early root growth of the seed (Devine et al. 1993). This usually requires the presence of exposed growing tissue (root tip emergence from seed) for adsorption.

Screening woody plant seeds for correlations with control data for established plants in field studies may provide information on continuing the developmental process for a new compound or insights on other controlled species (i.e. species spectrum) in a comparatively inexpensive and expedient manner. Seed screening methodologies such as presoaking woody plant seeds in a herbicide solution before germination have shown promise in using this process to differentiate between woody plant and herbaceous applications. In 1995, Bunn et al. showed significant reductions in germination rates of sweetgum seeds after a presoak with 1 % and 10 % v/v concentrations of triclopyr. Red maple showed a significant reduction in total germination

rates with a presoak of 10% v/v triclopyr compared to presoak in water. In the same study, presoaking woody seeds in clopyralid, known to be more effective on composite and legume broadleaf herbaceous species, failed to reduce sweetgum or red maple germination rates (Bunn et al. 1995). Seed screens were also correlated with field screens for the same species (Table 2.2).

The process of screening woody plant seeds can be performed in a greenhouse setting, growth chamber, or lab bench using Petri dishes and extremely minute amounts of chemical. This could allow for quick and inexpensive determination of initial control activity of a newly discovered compound on different growth stages and species.

### Section 3: Leaf Area / Aboveground Biomass Relationships

As a woody plant grows over time, physiological and morphological changes occur that affect relationships between certain physical aspects of the plant. For example, as a plant moves from seedling to sapling, the leaf area index / total biomass ratio changes (Norby et al. 2001). Seedlings undergo a period of rapid exponential growth. This rapid growth requires an increase in leaf area to support increased growth (biomass), which in turn supports the production of more leaf area, and so on (Norby et al. 2001). The aboveground biomass of a woody plant changes over time and in relation to specific leaf area (Norby et al 2001). More specifically, the ratio of leaf area (e.g. square centimeters) and above ground biomass (e.g. grams) decreases as the woody plant matures. It is unclear, to date, if this ratio has an effect on herbicide efficacy. It would be reasonable to assume that efficacy of a foliar applied herbicide may decrease on a stem with a lower leaf area and given stem biomass as compared to a stem with the same stem biomass but higher leaf area, due to a decrease in uptake surface area. No data to date has been found to support this hypothesis.

The dynamic allometric relationship between leaf area and biomass may have a direct or indirect influence on herbicide efficacy. Bovey (1998) examined the effect of hand defoliation of honey mesquite (*Prosopis galandulosa* Torr.) on herbicide efficacy with clopyralid and triclopyr. Greenhouse specimens were one stemmed species and field specimens were multistemmed. Hand defoliation at rates of 0%, 25%, and 50% of original foliage was performed prior to herbicide application. Defoliation did not reduce foliar-applied herbicide efficacy of clopyralid (140 g ai / ha), triclopyr (140 g ai / ha), or a 1:1 mixture of the two applied at the same rate (Bovey et al 1998). It is unclear if the application rates were too high to detect efficacy changes as a function of absorptive leaf area present. No mention was made of stem biomass and its affect on herbicide efficacy.

Past research has shown that the ratio between leaf areas and aboveground biomass changes over time (age) up to approximately full canopy (Madgwick et al. 1977, Landsberg 1986). Dose response curves have been established with multiple age classes on the same curve. If the relationship between age and leaf area / biomass ratios is strong, it may be possible to use the ratio as a covariate or indicator variable in these dose response curves. These combined dose response curves could show the effect that the change in ratios has on herbicide efficacy.

#### Section 4: Herbicide Classification by Mode of Action

Herbicides have been classified by several different methods over their brief history. These classifications include mode of action, chemical structure, and method of application. The most informative and logical method of herbicide classification is mode of action. By classifying by modes of action, weed scientists can group compounds that have an unknown mode of action into groups with similar symptomology. Each mode of action has distinctive symptoms of chemical affect and time to these symptoms. Environmental variables, such as



water stress, may also affect the mode of action and subsequent efficacy of a particular compound. It is necessary to understand or at least have an inclination as to the mode of action of an herbicide during screening trials. This will provide significant information on the time frame needed for an accurate screening and the proper application techniques.

This section of the literature review will focus on the mode of action, past research, and other pertinent information regarding imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-3-pyridinecarboxylic acid) and triclopyr ([[(3,5,6-trichloro-2-pyridinyl)oxy]acetic acid) since these two compounds are the focus of this study.

### Imazapyr

Imazapyr, commonly formulated as an isopropylamine salt, belongs to a chemical family named imidazolinones and is currently manufactured by BASF. Imidazolinones, along with sulfonlureas, make up the two major commercialized chemical families found to act on acetolactate synthase (Devine et al. 1993). The specific and only known mode of action for imidazolinones is inhibition of branched chain amino acid synthesis (Devine et al. 1993). This inhibition occurs in the chloroplast where 2 pyruvate molecules are joined by the enzyme acetolactate synthase (ALS) to form 2-acetolactate, the precursor for leucine and valine (Devine et al. 1993). Imidazolinones inhibit ALS and thus inhibit the production of leucine and valine, hence the name ALS inhibitors. Foliar absorption of imazapyr usually occurs rapidly (within 24 hours); however, this depends on species and leaf morphology (Ahrens 1994). Translocation readily occurs in both the xylem and phloem and physiological affects on plant growth can occur within hours after application (Ahrens 1994). Visual signs of growth inhibition; however, usually take one to two weeks to appear in herbaceous species and much longer for woody species. Meristematic regions of the plant become chlorotic followed by foliar chlorosis and

necrosis (Ahrens 1994). Woody species may not show signs of control until the following growing season.

Common commercial products include Arsenal ® Herbicide (amine) and Chopper ® Herbicide (both 2 lb ae / gal), Arsenal Herbicide Applicators Concentrate (AC) (amine) (4 lb a.e. / gal), Arsenal 0.5 Granule (0.5% ai), and Chopper RTU (0.255 lb a.e. / gal). Imazapyr is a common tool for industrial vegetation management and noncropland areas such as building sites, pipeline, highway and electric rights-of-way, utility and pumping stations, and non-irrigation ditch banks and is also heavily used in forestry site preparation and conifer release (Ahern 1994). Half-life values for field applications range from 25 to 140 days, depending on soil type (Bovey 2001). Plant metabolism occurs slowly or not at all (Bovey 2001). This in-plant persistence makes imazapyr an effective compound for control of perennial weeds and woody plants.

Imazapyr has a wide spectrum of control, including both herbaceous and woody species. Woody species of interest for this research are loblolly pine, yellow-poplar (*Liriodendron tulipifera* L.), and sweetgum. It has been reported that loblolly pine has high to moderate tolerance to imazapyr applications, hence imazapyr's wide spread use in intensive pine silviculture. Michael (1987) tested 2 rates of imazapyr (0.188 lb ai/ac and 0.375 lb ai/ac) on newly planted loblolly pine seedlings and established 1-year-old loblolly pine seedlings. The lower rate of imazapyr averaged a 52.3% survival rate across three sites and the higher rate averaged a 53.3% survival rate, both for the newly planted seedlings (Michael 1987). The 1-year-old established seedlings averaged a survival rate of 92.5% across two of the aforementioned sites with the lower rate and averaged a 93% survival rate for the same two sites with the higher rate (Michael 1987). Gnegy (1988) compared treatments of imazapyr alone and compared to tank mixes of imazapyr plus glyphosate, imazapyr plus metsulfuron methyl, and

glyphosate plus metsulfuron methyl. This study concluded that imazapyr alone at a 1 lb acid a.e. / ac rate had excellent woody species control and relatively similar pine response as compared to the other treatments and that the high rate of imazapyr tested (up to 1.5 lb a.e. / ac in this study) suggests a high degree of loblolly pine tolerance.

Miller (1990) compared crown volume reduction and rootstock reduction of sweetgum and yellow-poplar, and loblolly pine tolerance with a 0.5% solution of imazapyr (Arsenal ®) solution applied at three different times (May, July, and September of the same growing season). Yellow-poplar was effectively controlled at all three time periods. Crown volume reduction was 100% with May and July applications; 92% with the September application (Miller 1990). Rootstock reduction of yellow-poplar was 100% in May applications, 95% in July applications, and 90% in September applications (Miller 1990). Sweetgum crown volume reductions with imazapyr were 93% in the May treatment, 100% in the July treatment, and 97% in the September treatment (Miller 1990). Rootstock reductions of sweetgum with imazapyr were 50% in the May treatment, 100% in the July treatment, and 86% in the September treatment (Miller 1990). When applied directly over the top of the 2 – to 4 – year – old loblolly pines, the 0.5% Arsenal solution had a crown volume reduction of 37% with the May treatment, 33% with the July treatment, and 21% with the September treatment (Miller 1990). All three treatment timings resulted in no pine mortality (Miller 1990).

### Triclopyr

Triclopyr is commonly formulated as a butoxyethyl ester (oil soluble formulation) and as a triethylamine salt (water soluble formulation). For forestry and industrial purposes, DowAgrosciences manufactures and packages the two formulations of triclopyr as Garlon 4 ® (4 lb a.e. per gallon ester) and Garlon 3A® (3 lb a.e. per gallon amine). Triclopyr belongs to the

chemical family pyridinecarboxylic acids (Ahrens 1994). It is commonly referred to as an auxin-mimicking compound.

The natural auxin in plants, indole-3-acetic acid (IAA), occurs in all woody plants and is the primary growth hormone promoting and regulating plant growth. It is believed that auxins bind to specific receptors in the plasmalemma and are then transported across cell membranes (Devine et al. 1993). Auxin-type herbicides work by mimicking this IAA hormone receptor and binding to the protein responsible for IAA transport from the meristematic regions of the plant to other regions (Devine et al. 1993). This binding of the hormone to the herbicide structure prevents the transport of the IAA and progressively inhibits growth after a brief period of accelerated growth due to increased concentrations of the IAA hormone in meristematic regions. Symptomology of a woody plant treated with triclopyr includes stem and petiole elongation, leaf epinasty, leaf chlorosis and necrosis, stem and petiole swelling and thickening, and meristematic differentiation (Devine et al. 1993).

Studies have shown that the foliar applied oil-based triclopyr formulations have a faster penetration rate than the amine formulations (Bentson and Norris 1991). Deciduous broadleaf plants have higher absorption rates than evergreen broadleaf plants (Bentson and Norris 1991). However, the ester formulation can cause tissue damage on the leaf surface and prevents the absorption of the active ingredient located in the center of the droplet (Huang et al. 2000).

Triclopyr is translocated primarily through the symplast (including phloem) (Ahrens 1994). This characteristic provides insight on why triclopyr is used in forestry and other woody plant vegetation management industries to prevent resprouting after application. Foliar-applied triclopyr can translocate from the leaves of a woody plant to the root system, which contains

active meristematic tissue and the IAA hormone. Due to the mode of action of triclopyr, sprouting potential is negated or severely limited.

The oil soluble formulation of triclopyr, Garlon 4, is more commonly used for basal applications while the amine formulation, Garlon 3A, is used more commonly for foliar application. It is possible; however, to blend Garlon 4 with water to create an emulsion that can be applied through a foliar application. This is a common practice in forestry site preparation applications. Control data presented here will focus on the emulsion solution.

A study published in 1986 reported the results of forest access roadside spray trials comparing picloram and triclopyr alone and in mixes for control of common southeastern woody species (Schutzman et al. 1986). Results were reported as control efficacy 2 years after application (2 YAT). Triclopyr, formulated as Garlon 4, was tested at 2 rates, one at 32 oz /ac with 4 oz of Nalcotrol / ac (a drift control agent) and the second at 96 oz / ac with 2 oz Nalcotrol / ac. All treatments were applied at 25 GPA. The site where the 32 oz rate was tested was comprised primarily of privet (*Ligustrum vulgare* L.), sweetgum, sweet bay (*Magnolia virginiana* L.), willow (*Salix* L. spp), and red oak (*Quercus rubra* L.) before application. Evaluation of this plot 2 YAT showed less than 30% control of sweetgum, 30% to 50% control of privet, and excellent residual control of the remaining species (Schutzman et al. 1986). The 96 oz rate was tested on a site comprising of red oak, sweetgum, yellow-poplar, loblolly pine, sassafras (*Sassafras albidum* Nutt.), and sumac (*Rhus* spp L.) before application. Two-year evaluation showed less than 30% control on loblolly pine, 30 to 50% control of sassafras, and greater than 80% control after two years with the remaining species (Schutzman et al. 1986). Initial foliar brownout with all treatments of Garlon 4 was complete; however, residual efficacy was not sustained for the two year duration for most species (Schutzman et al. 1986). Lack of

absorption and subsequent translocation could have been an issue in this study as well due to gravel dust from the forest access road.

Miller (1990) examined triclopyr formulated as Garlon 4 and Garlon 3A as directed foliar sprays on common southeastern woody weeds in 2-year-old and 4-year-old loblolly pine plantations. Garlon 4 was applied at 2.5% v/v at three different timings, May, July, and September. Measurements of efficacy were measured through rootstock reduction and crown volume reduction. Both formulations of triclopyr tested provided greater than 80% crown volume reduction of sweetgum; however, they provided less than 40% rootstock reduction with the May application (Miller 1990). Rootstock reduction of sweetgum did increase to approximately 50% with the September application of both formulations (Miller 1990). This can be attributed to the translocation patterns to the root system common at that time of year. Yellow-poplar crown volume reduction averaged approximately 90% with all application times, with 100% reduction after the September application (Miller 1990). Rootstock reduction with triclopyr on yellow-poplar averaged approximately 90% across all timings (Miller 1990). Pine tolerance to triclopyr was relatively low in all application timings. Crown volume reduction of the loblolly pines averaged approximately 75%. Rootstock reductions were reported as 5% with Garlon 4 applied in May and 45% when applied in July (Miller 1990). No data was provided for the September application.

#### *Summary of usage of imazapyr and triclopyr in this study*

Imazapyr and triclopyr were chosen for this study for two reasons. First, each compound has its' own broad spectrum of control. These spectrums of control have some overlap in species controlled and also have differences in species which are tolerant to one or the other chemistry. For example, imazapyr is the most commonly used compound in intensive pine silviculture due

to pine species tolerance to the specific mode of action of imazapyr. This compound has also been shown to have little to no effectiveness in controlling legumes. However, triclopyr has been shown to be effective in controlling both pines and leguminous species. Secondly, each compound has its' own specific mode of action. As previously stated, imazapyr is a branched chain amino acid biosynthesis inhibitor while triclopyr is a compound that mimics the IAA compound naturally found in plants. These two different modes of action also produce two distinctly different symptomologies that are characteristic to each compound. These different modes of action also create two different times to visual symptomology as well. Differences in imazapyr and triclopyr provide an excellent model to use in an extensive herbicide screening study while using only two compounds.

### **Chapter 3:** **Methods and Materials**

The following null hypotheses were tested in this thesis:

1. There is no significant correlation between field screened efficacy and rapid screened efficacy of yellow-poplar, sweetgum, green ash (*Fraxinus pennsylvanica* Marsh.), and loblolly pine with imazapyr and triclopyr.
2. There is no significant correlation between seed screened efficacy and field screened efficacy of yellow-poplar, sweetgum, green ash, and loblolly pine with imazapyr and triclopyr.
3. Seed screening of woody plant herbicides can not establish significant treatment effect to determine initial activity and/or species spectrum in a relatively short period of time.
4. Changes in leaf area / stem biomass ratios in yellow-poplar, green ash, sweetgum, and loblolly pine do not significantly affect the efficacy (dose response) of triclopyr or imazapyr.

#### **Study Sites**

The study site for field and greenhouse was located at the Reynolds Homestead Forest Resources Research Center (RHFRRRC) in Critz, VA. Weather data collectors on site provided climatological summaries for the days of application.

Seed screening and leaf area biomass analysis were performed in facilities in Cheatham Hall and Price Hall on the Virginia Tech Campus in Blacksburg, VA.



### Section 1: Traditional Field Screening

The field application section of the study was a completely randomized design with three replications. The woody species used in the field enclosures were loblolly pine, yellow-poplar, green ash, and sweetgum. Plants were in rows incorporating up to ten individuals of each species per replication. Planting spacing in the field plantations was 3' X 2'. Two age classes were present in the field enclosures: three-year-old saplings (one physiological year in greenhouse conditions and two years in field) and two-year-old saplings (one physiological year in greenhouse conditions and one year in field). Field enclosures were located on the French soil series (USDA NRCS 2002). The saplings in both plantations were trimmed at a three foot level one to two months prior to application. This allowed the bicycle wheeled sprayer used for field applications to have enough clearance over the tops of saplings and still maintain spray pattern integrity. Spraying was not performed until the saplings had an opportunity to fully leaf out. This trimming also mimicked a site disturbance, such as harvesting. Trimming changed the leaf area to biomass ratios across age classes provided differentiation of control data across age and physiological stages.

Two herbicides were used: imazapyr, formulated as Arsenal AC (amine formulation at 4 lb a.e. / gal), and triclopyr, formulated as Garlon 4 (ester formulation at 4 lb a.e. / gal). Imazapyr applications were assigned a standard site preparation field application rate (x) of 24 fl oz per acre (0.75 lbs a.e. / ac or 0.8395 kg a.e. / ha). Triclopyr applications were assigned a standard site preparation field rate (x) of 4 qt per acre (4 lbs a.e. / ac or 4.48 kg a.e. / ha). A series of dilutions, 1x, .5x, .25x, .125x, and .0625x, were used to establish a total of five application rates for each herbicide (Table 3.1). All solutions contained a 0.25 % v/v rate of a non-ionic surfactant, CWC Surfactant 90. The fifth rate of .0625x was used for replications in the two-

year-old age class and not the three-year-old age class due to limitations in numbers of trees in the three-year-old field enclosure. All application rates are reported as ounces of formulated product per acre.

*Table 3.1: Summary of field application rates for imazapyr (Arsenal AC) and triclopyr (Garlon 4)*

Rate	Arsenal AC (oz / ac)	Garlon 4 (oz / ac)
1x	24	128
0.5x	12	64
0.25x	6	32
0.125x	3	16
0.0625x	1.5	8

The two-year-old plantation was treated on September 2, 2002 between 7:00 am and 1:00 pm. Temperatures ranged from 68° F at 7:00 am with a 100% relative humidity to 85° F at 1:00 pm with a 50% relative humidity. Wind speed ranged from 0 to 1 mph. The three-year-old plantation was treated on September 11, 2002 between 8:00 am and 2:00 pm. Temperatures ranged from 70° F at 8:00 am with a 70 % relative humidity to 90° F at 2:00 pm with a 44% relative humidity. Wind speed ranged from 0 to 5 mph. Applications were made using a bicycle wheeled sprayer equipped with a CO<sub>2</sub> tank attached to a shielded single nozzle spray boom. The CO<sub>2</sub> tank provided the constant and monitored pressure needed for an accurate application. The height of the boom was approximately four and a half feet and fitted with an 11003EVS Teejet ® nozzle. This 110-degree tip with a 03-size orifice delivered an even edge spray swath that covered the span of the sprayer. The application carrier rate for field plots was 20 gallons per acre. The spray system was flushed with water between treatments to prevent treatment contamination. Data collected included pre-application height, height 8 WAT, height 1 year after treatment (YAT), mortality 1 YAT and effect code 1 YAT. The effect code was established

to derive a rating system for individual seedlings that could differentiate between different stages of visual damage not captured by height and mortality data (Table 3.2).

*Table 3.2: Effect Code Rating System for Treated Seedlings and Saplings*

Code	Hardwood Species Injury Rating
0	No visible sign of injury
1	Stem damage with effective leaf out
2	Stem damage with marginal leaf out
3	Stem damage with no leaf out but bud activity present (swelling, green, regardless of size)
4	Stem damage with no leaf out and no bud activity but green stem present (at any height)
5	Dead
Code	Loblolly Pine Injury Rating
0	No visible sign of injury
1	Needle burn with no tip damage
2	Needle burn with tip damage but reflushing
3	Needle burn with tip damage and not reflushing
4	Main terminal killed, deformed growth, severe damage, survival unlikely
5	Dead

*Section 2: Rapid Greenhouse Screen Herbicide Application and Seedling Handling*

The rapid greenhouse screening was performed twice, the first in late 2002 / early 2003 (late fall / winter) and the second in mid-spring / late summer 2003. Four species, loblolly pine, sweetgum, green ash, and yellow-poplar, were used to mirror the field applications. Seedlings for the first rapid greenhouse screen (RGS1) were purchased from Vernon Barnes and Sons Nursery in McMinnville, TN, shipped to the RHFRRRC as dormant seedlings in early 2002, transplanted into D40-pots (Stuewe and Sons, Corvallis, OR), which had a volume of 40 cubic centimeters, and allowed to completely flush out, trimmed for height consistency, and allowed to completely flush out again. There were 3 replications per treatment, including the non-treated control, with 10 individual seedlings per replication for loblolly pine, sweetgum, and yellow-poplar and 8 individual seedlings per replication for the green ash screen.

Herbicide treatments for the first rapid screen were conducted in a spray booth located at the Reynolds Homestead on September 27 and 30, 2002. The treatments were applied in a completely randomized experimental design. The seedlings were placed in seedling racks and spatially arranged in the spray booth so as to provide equal coverage of spray solution with no leaf area overlap. Uniform seedling height was obtained by stacking racks so as to further reduce spray variance. The spray booth was fitted with an 11001VS spray tip and the seedling rack was adjusted to provide a twenty-four inch spray swath. The spray system was periodically calibrated during application to ensure consistency and a constant 20 GPA application rate. Spray solutions were mixed in 16 ounce bottles prior to application. The spray system was flushed between treatments with 50 % acetone / 50 % distilled water solution to prevent contamination.

Chemical rates and spray volume for the first rapid greenhouse screening were consistent with the field applications (Table 3.1). Pre-application measurements of the first rapid screen were a sample of forty randomly selected individual seedlings of each species to obtain a mean pre-application height by species.

After application, the seedlings were placed in the greenhouse to utilize both natural light and fluorescent lights for 16 hour photoperiods. The seedlings were watered from below so that the herbicide was not washed from the leaves before it had a chance to effectively penetrate the leaf surface and translocate. The seedlings remained in the greenhouse for eight weeks under the 16-hour photoperiods to allow for an evaluation at 8WAT. The 8WAT evaluation measured heights of all individual seedlings in the screen. Due to the time of year that the 8 WAT evaluation took place (late November), the lights were turned off in the greenhouse to utilize the naturally occurring photoperiods to induce dormancy. Temperature in the greenhouse was

maintained at ambient levels (60-75° F (15 – 24° C)). At approximately two weeks after the 8 WAT evaluation, the seedlings were placed in a controlled growth room on site. The growth room mimicked a dormancy period with shorter photo periods (8 hours of fluorescent light and 16 hours of dark) and a lower temperature of 39.2° F (4° C). Light levels in the growth chamber for this and subsequent screens were 35  $\mu\text{E} / \text{m}^2 / \text{sec}$ . The seedlings were then cooled for a total 1500 hours (approximately 9 weeks). The 1500 hour time period allowed enough time for the seedlings to set winter buds, undergo dormancy, and begin dormancy release. After 1500 hours, the seedlings were returned to the greenhouse to promote and initiate bud break and grown with a 16-hour photoperiod until the control seedlings had fully developed a new flush of foliage.

Final evaluation of the first rapidly screened seedlings was performed at approximately 6 weeks after returning to the greenhouse. Mortality, effect code, and height were measured on all individual seedlings. The second rapid greenhouse screen (RGS2) utilized the same experimental design as the first rapid greenhouse screen with 3 replications per treatment, including the non-treated control, with 10 individuals per replication for all four species. Initial analysis of the first rapid greenhouse screen showed a dose response for all species with high degrees of mortality for all rates tested (except the control). Therefore, the second rapid greenhouse screen incorporated a more diverse rate spectrum in order to capture a more conclusive dose response curve. Table 3.3 shows the rates tested for the second rapid screen. Application rates are reported in ounces of formulated product per acre.

*Table 3.3: Rapid Greenhouse Screen 2 Application Rates*

Rate	Arsenal AC (oz / ac)	Garlon 4 (oz / ac)
1x	24	128
0.25x	6	32
0.0625x	1.5	8
0.015625x	0.375	2
.00390625x	0.09375	0.5

The seedlings used for the second rapid screen were lifted from nursery beds at the end of their first growing season in 2002 at Vernon Barnes and Sons Nursery in McMinnville, TN. The seedlings were received as dormant seedlings at the RHFRRRC in December of 2002 and transplanted into D40-pots in January 2003. They were then moved to the greenhouse to allow for the second year flush. There were no pre-application measurements taken for the second rapid greenhouse screen. The herbicide application was treated with the same methodologies as the first rapid screen on May 1<sup>st</sup> and 2<sup>nd</sup>, 2003. The seedlings were then placed in the greenhouse after application to be watered from below for 8 weeks. At 8 WAT, percent necrosis and effect code were measured for every individual seedling. The seedlings were then placed in the growth room for dormancy induction, chilling, and initial dormancy release. Due to the time of year and natural photoperiods occurring, the seedlings were placed in the growth room with 8 hour photoperiods and the temperature gradually reduced to 39.2° F (4° C) over 1 week in an effort to expedite dormancy induction and the entire rapid screening process. Once 39.2° F was obtained, the seedlings were chilled for 1500 hours. After 1500 hours, the sweetgum seedlings were not as progressed in the dormancy induction process as the other seedlings, based on the number and size of winter buds observed on the sweetgum seedlings as compared to the other species. All seedlings were therefore kept in the cooler for an additional 500 hours, for a total of 2000 hours,

to ensure that dormancy had been achieved and winter buds were set. At 2000 hours, Hurricane Isabelle hit the area and the research center lost power. It was felt that it would be best if the seedlings were moved to the slat house to allow for longer photoperiods until the power was restored and the greenhouse ventilation system was operational. The seedlings were moved into the greenhouse 1 week after being moved to the slat house. The greenhouse supplied the seedlings with 16 hour photoperiods to break dormancy and remained there until the control plants reached effective leaf out (approximately 6 weeks). The final evaluation measured height, effect code, and mortality.

Section 3: Rapid Seed Screening

The seed screening was performed twice in an effort to clarify effective seed screening techniques. Seeds were acquired for four species, loblolly pine, sweetgum, yellow-poplar, and green ash, from the Louisiana Seed Company. Seeds were weighed out by species by replication, placed in a Ziploc® bag filled with enough distilled water to cover the seeds, and placed in a cooler at 39.2° F (4° C) for 24 hours. The bags were then drained and placed back in the cooler at the same temperature for the required stratification times. Table 3.4 shows the published stratification requirements.

*Table 3.4: Published Stratification Periods (Young and Young, 1992)*

Species	Published Time Period (days)	Actual Time Period Stratified (days)	Published Temperature (degrees Celsius)
Loblolly pine	30-60	45	3-5
Green Ash	60-150	60	0-5
Sweetgum	15-90	45	2-5
Yellow-poplar	140-168	140	None cited

Once removed from the cooler after stratification, the seeds were counted into groups of 20 and placed in Petri dishes containing dry filter paper. Treatments for the first screening included the ten rates (5 imazapyr and 5 triclopyr) used in the second rapid greenhouse screen (Table 3.3), surfactant alone, and a distilled water control for a total of 12 treatments. All herbicide treatments in the first seed screen included surfactant at 0.25% v/v. Three replications were performed with twenty seeds per species per Petri dish as one replication. Due to the size of the Petri dishes, it was impractical to make applications based on area. Therefore, applications were made on a percent volume concentration based on the standard field rate and delivery rate (20 GPA) used with the field and greenhouse applications. Table 3.5 shows field application rates in a percent volume notation. Herbicide treatments were mixed accordingly. Application rates are reported in formulated product ounces per acre.

*Table 3.5: Tested Rates for Screening Trials*

Rate	Imazapyr		Triclopyr	
	oz / ac	% Conc.	Oz / ac	% Conc.
1x	24	0.9375	128 oz	5.000
0.25x	6	0.23475	32 oz	1.25
0.0625x	1.5	0.05859	8 oz	0.3125
0.015625x	0.375	0.01465	2 oz	0.078125
0.00390625x	0.03975	0.00366	0.5 oz	0.01955

Applications were made as a pre-soak treatment. Presoaking in the first screen consisted of a 24 hour soaking in 15 mL of the assigned solution. 15 mLs of solution provided enough volume to saturate the filter paper and provide an even level of solution to allow the seeds to absorb herbicide solution. The excess liquid solution was then drained from each Petri after the 24 hour soak while keeping the seeds in the same Petri with the same filter paper, which undoubtedly had residual herbicide solution in it. The dishes were then placed in a growth chamber at 77° F (25°



C) with 12 hour photoperiods to allow germination. Seeds were evaluated at least every 4 days (every other day was the ideal observation period) for up to 20 days. This allowed for a continuum of measurements and re-application of distilled water if the dish became dry. It was desirable to maintain the same amount of moisture in the Petri dish for the entire 20-day period. The evaluation scheme also allowed for collecting data for at what time period differentiation of treatments was exhibited quantifying an evaluation protocol (i.e. at what time a post treatment evaluation could be performed in an actual manufacturing screening).

Live tissue emerging from the seed was measured in length at each interval. At the end of the evaluation period, percent germination was calculated for each treatment along with mean tissue length per treatment at each evaluation period.

The second seed screen incorporated slightly different methodologies than the first screen due to a high degree of what appeared to be non-herbicide seed mortality in the first screen. Seeds were treated with Banrot® (a.i. = etridiazole and triphosphate-methyl) (Scotts – Sierra Crop Protection, 2001) for 24 hours after the stratification period was over. The seeds were then drained and separated into groups of 20 into Petri dishes. All herbicide treatments remained the same as the first screen except that surfactant at .25% v/v was removed from each mix as was the surfactant alone treatment. Seeds in the Petri's were treated with 10 mL of herbicide solution as the presoaking treatment. Humidity levels in the growth chamber fluctuated in the first rapid seed screen and the second rapid seed screen attempted to maintain a high level of constant humidity in an effort to prevent the seeds from drying out. Measurements and duration of screening was the same as the first screen.

#### Section 4: Data Analysis of Field, Rapid Greenhouse, and Rapid Seed Screenings

##### *Field versus rapid greenhouse screens*

Due to planting inconsistencies across the two age classes, available greenhouse species for comparison, and natural mortality, correlations between field screens and the first rapid greenhouse screen were determined for two-year-old loblolly pine, yellow-poplar, and sweetgum using five rates of each compound while correlations were estimated for three-year-old loblolly pine, sweetgum, and green ash using four rates of each compound and untreated controls. Correlations between the field trials and the second rapid screen were made between the same species with three rates and an untreated control versus the two-year-old plantation and two rates and an untreated control versus the three-year-old plantation due to a change in the rate titrations for the second rapid screen.

All parameters measured at each time interval for both rapid greenhouse screens were correlated with all parameters measured at all time intervals with the field screens. This was done in an attempt to find the best parameter for each screen that best predicted the response in the field. Simple linear regressions were run for each species and chemical individually with the field screen parameters as the response variable and the rapid greenhouse field screen parameters as regressors using Statistical Analysis Software (SAS<sup>®</sup>). For example, mean height difference of loblolly treated with triclopyr in the field screen was used as a response variable against all parameters measured for rapid greenhouse screened loblolly treated with triclopyr at all time intervals in both rapid greenhouse screens. This was performed on an individual regressor parameter basis with a p value set a  $p < 0.1$ .

SAS provided scatter and residual plots that were used to determine if regressor variable transformation was necessary. Regressions were run on individual species and chemicals either

with no data transformation or utilizing standard data transformations until a model was created with a maximum coefficient of determination ( $R^2$ ) while minimizing model error. This means that the  $R^2$  reported may not necessarily be the highest one possible but efforts were made to minimize error and create a strong model.

#### *Field versus rapid seed screens*

Two parameters measured in the seed screen, percent control of seed tissue growth and percent control of germination, were correlated with three parameters measured from the field screen in the 2-year-old plantation. These dependent variables were percent control of height 1 YAT, percent mortality 1 YAT, and effect code 1 YAT. The regressions were run for the three species, yellow-poplar, sweetgum, and loblolly pine, using proc glm in SAS®. As with the rapid greenhouse screens, the p value for significance of a model was  $< 0.1$ . Regressions were run for every data set (observation interval) to determine at what time future seed screens should be measured and to determine if a pattern, if any, existed across species and chemistries. ANOVAs were also performed at every time (observation) interval to determine when significant treatment effect is first observed on a chemical by species basis.

#### *Section 5: Establishment of Leaf Area – Biomass Ratios and Their Effect on Herbicide Efficacy*

##### *Leaf Area – Biomass Ratios*

Leaf area to biomass ratios were established for the two age classes in the field enclosures and for the second rapid greenhouse screen. Ratios were not established for the first rapid greenhouse screen due to the lack of plant material available.

Ten individuals for the three species evaluated (loblolly pine, green ash, and sweetgum for age three and loblolly pine, yellow-poplar, and sweetgum for age two) in each age class for the field enclosures were randomly selected for a destructive harvest. One leaf was harvested

from three height locations (top, middle, and bottom) and four directional locations (the enclosure walls) for a total of twelve leaves from each sapling. The twelve leaves were placed in a labeled Ziploc® bag and put into a cooler for transport back to the lab. The remaining leaves were harvested from each sapling and placed in a labeled paper bag, dried at 140° F (60° C) at the lab, and weighed to determine dry leaf weight. The stem of the sapling was harvested at ground level, cut into manageable sections, and placed in a labeled paper bag, oven dried at 60° Celsius, and weighed.

Leaf area ( $\text{cm}^2$ ) was determined on the twelve leaf sub-samples using a Li-Cor Model 3100 Area Meter (Li-Cor, Inc, Lincoln, NE). The leaf area meter was calibrated between each species to ensure accuracy. Loblolly samples were scanned three times for each sapling and then averaged due to the higher variance in the leaf samples. The samples were then oven dried at 140° F (60° C) and weighed to determine dry weight (g). These measurements were used to determine the specific leaf area (leaf area ( $\text{cm}^2$ ) to leaf biomass (g)) for each sub-sample.

The remaining leaves from each sapling were oven dried at 140° F (60° C) and weighed to determine dry weight (g). The specific leaf area of each stem was then used to extrapolate a total leaf area for each stem based on weight of each leaf sample (twelve individual leaves versus remaining leaves). The corresponding stem samples were oven dried and weighed to determine dry weight (g). These weights were added to the total leaf dry weight for each stem to establish a total leaf area ( $\text{cm}^2$ ) to total above ground biomass (g) ratio to use as a covariate or indicator variable in dose response curves.

Rapidly grown greenhouse seedlings were sampled in the spring of 2003 to establish the same ratios as described above. The seedlings came from the same sample that was used for the second rapid screen. These seedlings were destructively sampled and all leaves were scanned for

leaf area measurements and then oven dried. Stems were cut at ground level and oven dried. These ratios were used with the two sets of field sapling leaf area / biomass ratios in the data analysis.

### *Hand Defoliation Screening*

A variation of the previously described leaf area to biomass ratio experiments was the hand defoliation trial. This provided a simply designed experiment to test the hypothesis that changes in the leaf area to biomass ratios do not affect herbicide efficacy. Two of the species (yellow-poplar and sweetgum) examined in the greenhouse screens were used. These seedlings were lifted from nursery beds at the end of their first growing season at Vernon Barnes and Sons Nursery in McMinnville, TN. The seedlings were received at Reynolds Homestead in December of 2002 and transplanted into D40-pots in January 2003. They were then moved to the greenhouse to allow for the second year flush. Once the flush was complete, a hand defoliation regime was established. The experimental design was a two-way factorial (defoliation and herbicide being treatments) with 4 defoliation classes, 3 herbicide application rates per chemical (imazapyr and triclopyr), 10 individual seedlings per replication and 3 replications of each defoliation class and herbicide rate. Application rates for each chemical were 1x, .0625x, and 0.00390625x. Defoliation rates were 0, 25, 50, and 75%. This was achieved by removing individual leaves to the point of the desired defoliation class. For example, the 25% defoliation class was achieved by removing 1 out of every 4 leaves and the 75% defoliation class was achieved by removing 3 out of 4 leaves.

Herbicide treatments were applied in the same manner in the hood sprayer as the rapid greenhouse screens. Applications were performed on June 9<sup>th</sup> and 10<sup>th</sup>, 2003. Seedlings were treated in the same manner after application as the second rapid screen. Hurricane Isabelle hit

the area during the middle of the dormancy induction process and power was lost to the controlled growth room for 4 days. Measures were taken to minimize the stress that this placed on the seedlings, such as opening both doors in the growth room, which was located outside, to allow natural light to enter the room and allow airflow. However, there was still a chance that the seedlings would still be greatly affected by the loss of power as they were in the middle of the chilling process with short photoperiods.

Data collected included necrosis at 8 WAT, height at 1 physiological year after treatment, and mortality 1 physiological year after treatment.

#### Section 6: Data Analysis for Leaf Area - Biomass Ratios and Hand Defoliation

##### *Leaf Area – Biomass Ratios*

Data was analyzed using percent control of height efficacy data for two-year-old and one-year-old yellow-poplar, sweetgum, and loblolly pine screens for both imazapyr and triclopyr. The goal of the analysis was to compare the slopes of the linearized dose response curves by age for the same chemical. Linearization of the dose response curves was performed using proc GLM in SAS while comparison of these linear dose response curves was performed using proc REG in SAS. The proc REG analysis allowed for a test of interaction between the herbicide dose variable and age class (i.e. leaf area - biomass ratio). The leaf area - biomass ratio was substituted for age as the covariate qualitative variable. That is, since age is a variable describing the status of the seedlings, the leaf area – biomass ratio could replace this variable as a qualitative variable and perform the same function. An indicator variable was created in the code to indicate the qualitative variable age. The indicator variable, x2, was equal to 1 if the ratio equal to  $a$ , or that of the two-year-old species, and equal to 0 otherwise. The full model tested was  $y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \beta_3x_1x_2 + \varepsilon$ , where  $\beta_3$  is the interaction term between the

variables dose and ratio. The interaction term is the difference in slopes between the two lines and thus the hypothesis tested is  $\beta_3 = 0$  versus  $\beta_3 \neq 0$ . Significance at the  $p < 0.05$  level for the t value for this term indicated that the hypothesis was true, there was no difference in slope, and there is no interaction between dose and leaf area – biomass ratios.

### *Hand Defoliation*

The data was analyzed as a two way factorial design to test for interactions between application rate and reduced leaf area, or defoliation rate. Two responses measured in RGS2, height and survival, along with the two treatment variables, herbicide rate and defoliation rate, were analyzed with proc glm in SAS® where the model was ‘response = herbicide defoliation herbicide\*defoliation’. A t value for the interaction term that was significant at the  $p < 0.05$  level indicated that an interaction exists between the two treatment variables.

## **Chapter 4:** **Results**

### **Section 1: Prediction of Field Response using Rapid Greenhouse Screens**

Results for the prediction of field response using the rapid greenhouse screening process are presented in two parts. The first section deals with the prediction of field response of the three-year-old plantation and the second section deals with the prediction of field response of the two-year-old plantation. These two sections provide results of the models using the field parameters as dependent (y) variables and the rapid greenhouse screens as the independent (x) variables and only report up to the three best models (i.e. models with the highest  $R^2$  while minimizing error) per species / chemical combination. In the figures presented in the following sections, R1 refers to rapid greenhouse screen 1 while R2 refers to rapid greenhouse screen 2.

Initial analysis included the use of scatter plots of the raw data to determine if any response pattern existed. These scatter plots showed that the majority of the rapid screen versus field responses held either a linear or log-linear pattern. This simple analysis allowed for the determination of what, if any, data transformation needed to be performed; the most common of which performed here was the log y transformation of the field (dependent) variable. This transformation was performed with every regression analysis along with the linear form for two models for every parameter set tested per chemical per species. When scatter plots showed that the log y transformation was not adequate along with the linear model, other transformations were performed on an individual model basis.

### **Predicting Three-Year-Old Species Efficacy**

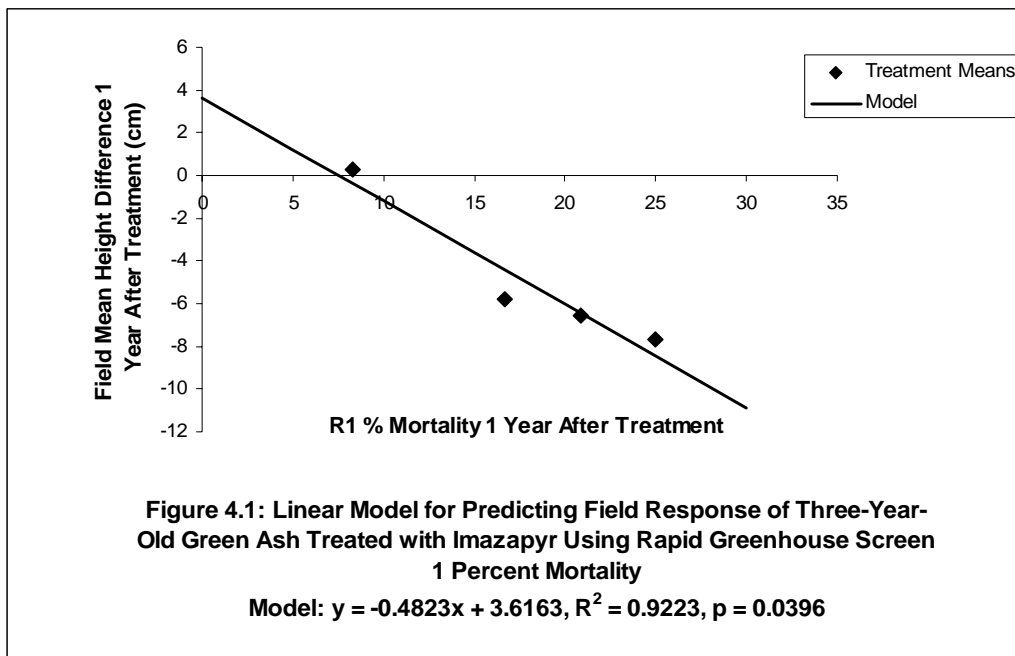
Prediction of three-year-old response involved the use of 30 models per chemical per species (3 dependent variables X 5 independent variables X 2 models). Dependent variables for the three-year-old plantation included mean height difference from pre application height 1



YAT, effect code 1 YAT, and mortality 1 YAT. Independent variables included rapid greenhouse screen 1 (RGS1) % control of height 1 YAT, RGS1 % mortality 1 YAT, and RGS1 effect code 1 YAT. No regression model could be generated to predict three-year-old response using rapid greenhouse screen 2 (RGS2) or rapid seed screen (RSS) due to the minimal points available. There were only 2 rates that overlapped between the field and the RGS2 and RSS due to the difference in rate titrations in the rapid screens and the use of only 4 rates in the three-year-old plantation with no control treatment.

*Green Ash*

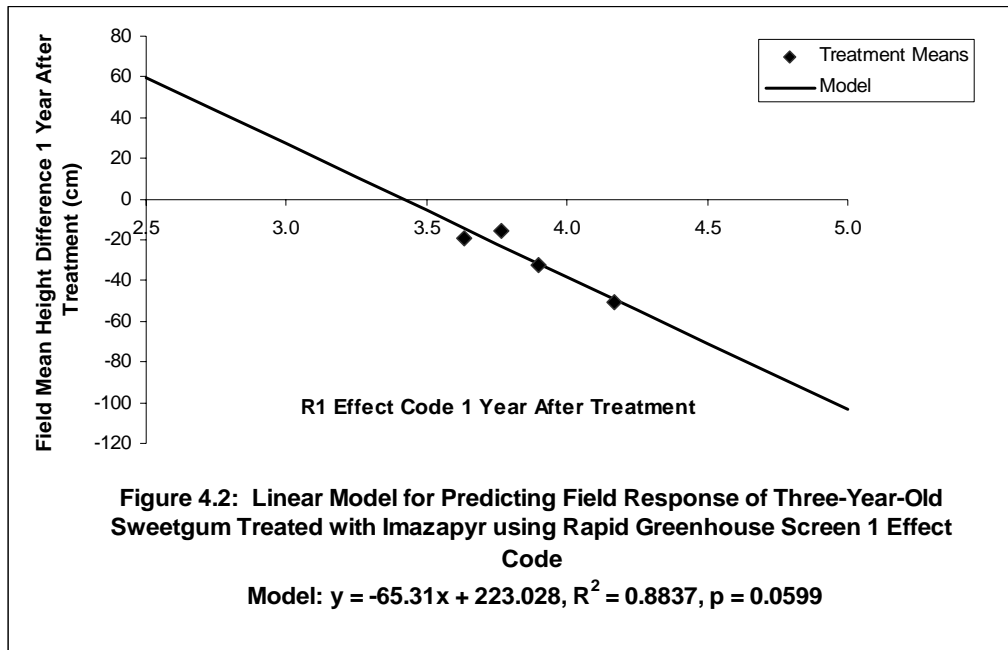
There was only one significant model produced that could adequately predict field efficacy of imazapyr treated on three-year-old green ash. This model used mean height difference as compared to pre application heights as the dependent variable and RGS1 % mortality 1 YAT as the independent variable (Figure 4.1). The model produces a negative slope due to the fact that as imazapyr rates increase, the mean difference in height from pre-application to 1 YAT becomes increasingly negative.

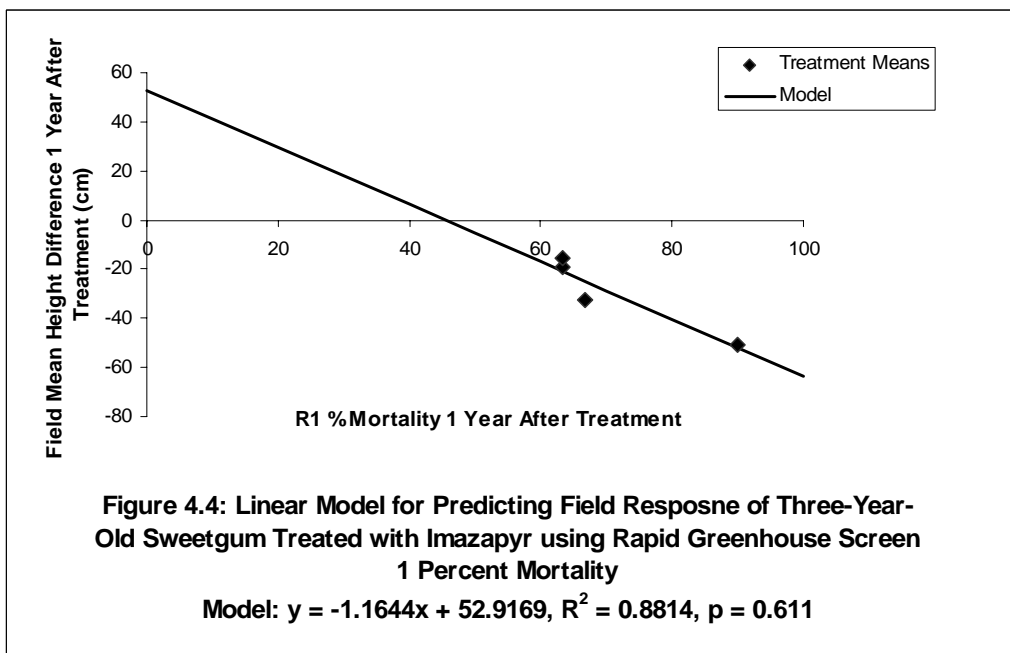
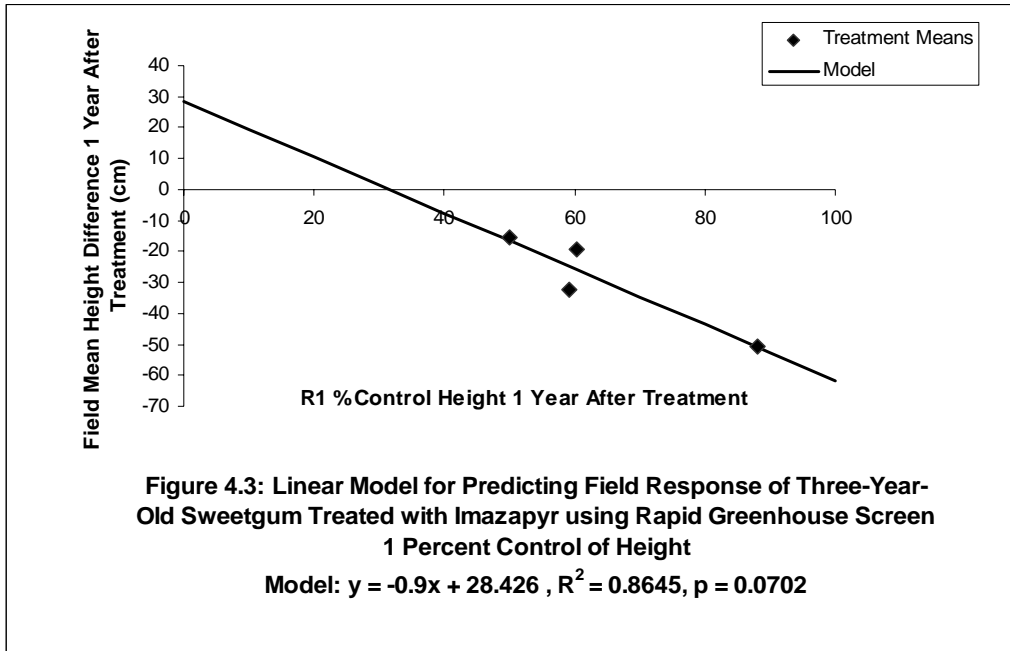


There were no significant models produced at the  $p < 0.1$  level that could adequately predict field response of three-year-old green ash treated with triclopyr.

### *Sweetgum*

Mean height difference 1 YAT was the best dependent (i.e. predicted) efficacy variable for three-year-old sweetgum treated with imazapyr. Linear equations using this variable paired with RGS1 effect code 1 YAT, RGS1 % control of height 1 YAT, and RGS1 % mortality 1 YAT, had  $R^2$  values ranging from 0.86 to 0.88, and were significant at the  $p < 0.1$  level (Figures 4.2, 4.3, 4.4). The models for predicting mean difference in sweetgum height treated with imazapyr 1 YAT using these three independent variables all have negative slopes due the decrease in height growth as rates of imazapyr increases. The three models are effective in utilizing a full range of independent data (0 – 5 for effect code and 0 - 100% for height and mortality).





There were no significant models for the prediction of field response of three-year-old sweetgum treated with triclopyr using rapid greenhouse screen 1 data. These data sets never presented a linear, log-linear, or any other type of linearizable form to base a statistically significant model on.

### *Loblolly Pine*

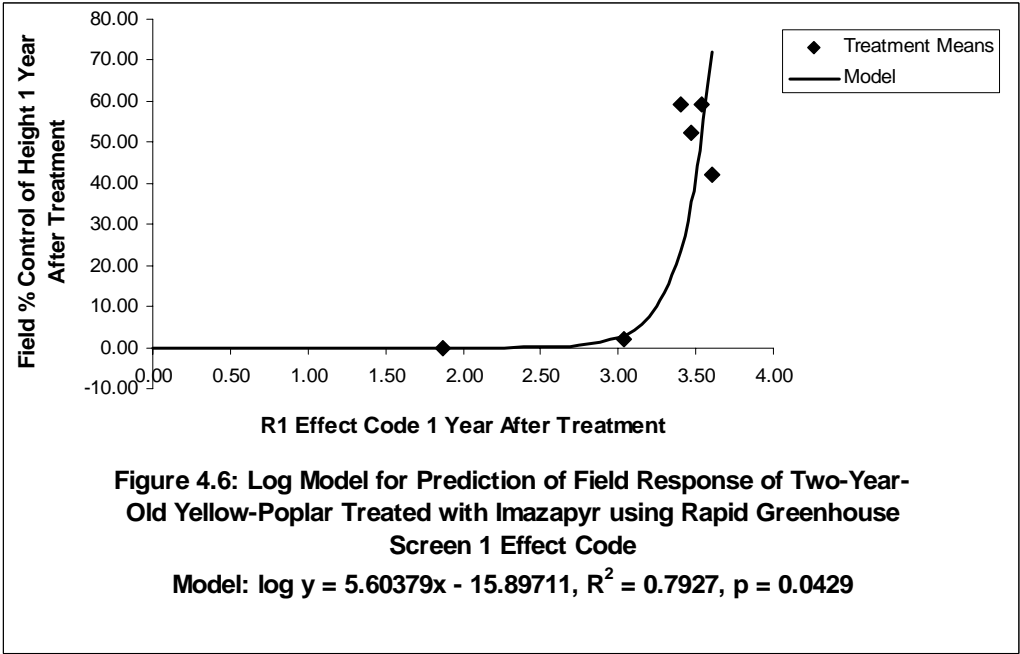
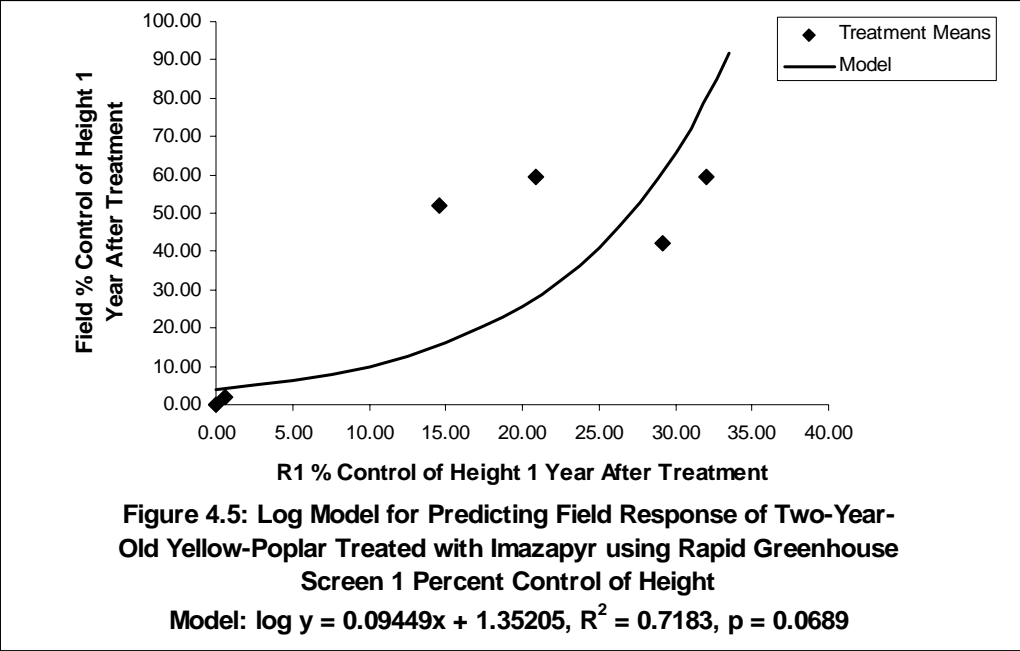
There were no significant ( $p < 0.1$ ) models produced that could predict field response of three-year-old loblolly pine treated with either imazapyr or triclopyr using the RGS1 data as the independent variable.

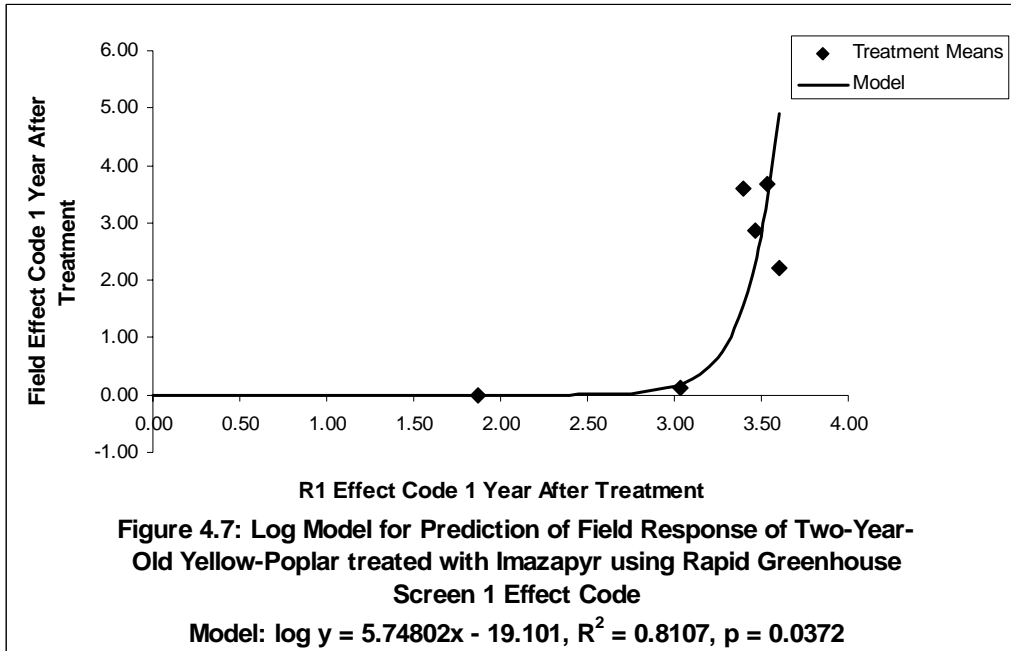
### *Predicting Two-Year-Old Species Efficacy*

There was a higher number of overlapping rates between the field screened two-year-old plantation and both rapid greenhouse screens. This allowed for a higher number of significant models to be produced as compared to the prediction of three-year-old species response. Due to the fact that the second rapid greenhouse screen (RGS2) could be used to predict field response of the two-year-old plantation, results presented here will be separated by rapid greenhouse screen trial (RGS1 and RGS2) as well as species and chemical. The presence of a non-treated check treatment in the two-year-old plantation also allowed for height measurements to be transformed as a percent control of height.

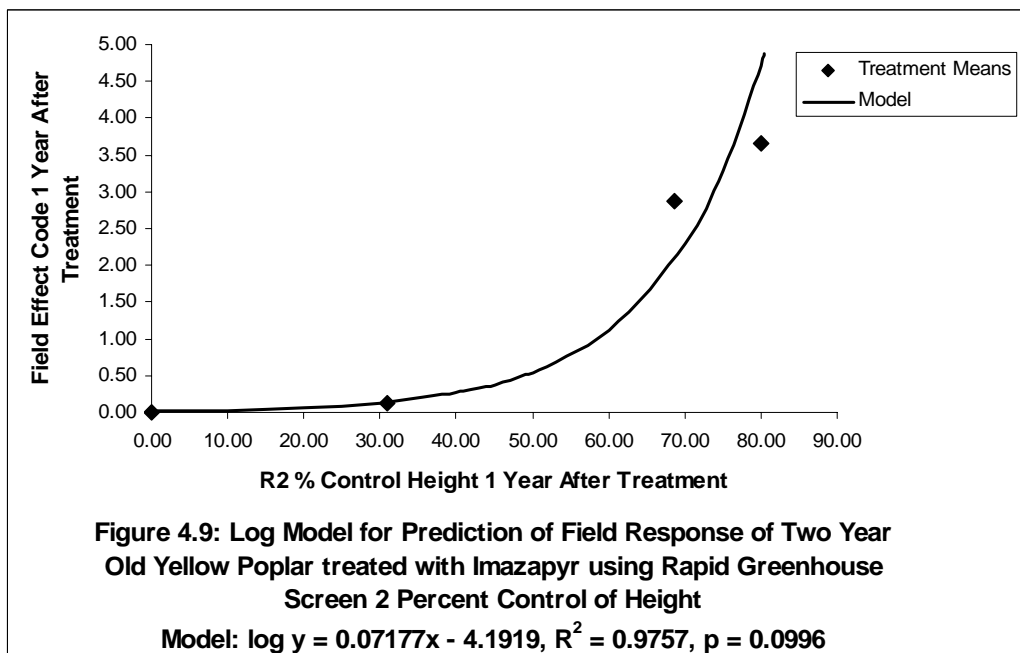
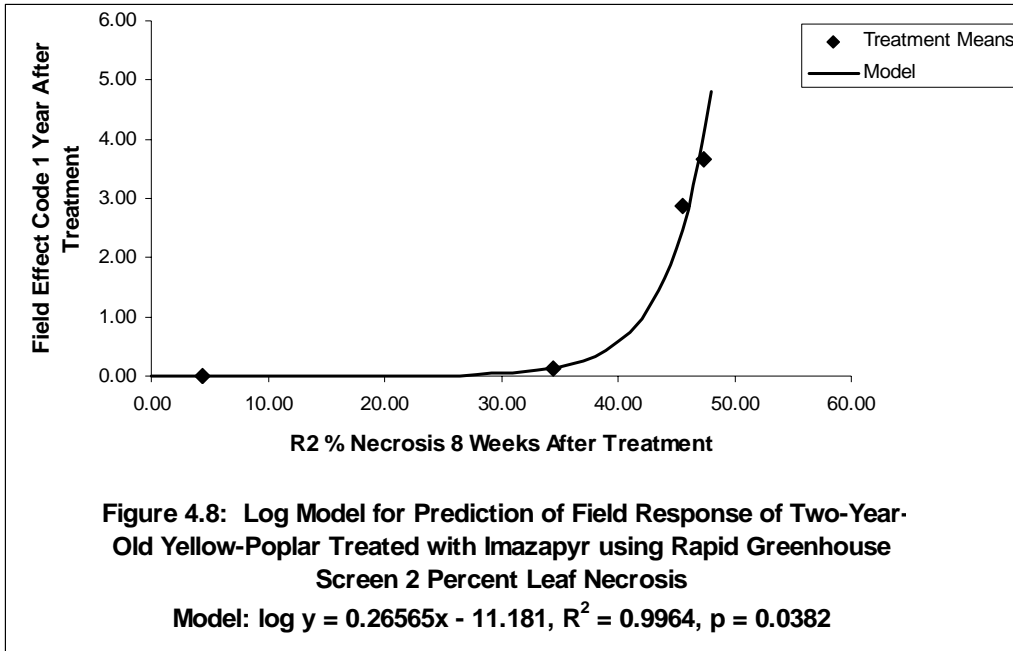
### *Yellow-poplar*

RGS1 consistently predicted the log response of two-year-old yellow-poplar treated with imazapyr. The three best regression models used both percent control of height 1 YAT and effect code 1 YAT for both the independent and dependent variables and had  $R^2$  values ranging from 0.7183 to 0.8107 (Figures 4.5, 4.6, and 4.7). Using RGS1 percent control of height 1 YAT provided a model that could predict a full range of percent control of height (0 – 100 %) of field screened trees (Figure 4.5). Using RGS1 effect code to predict either percent control of height or effect code, both 1 YAT, appears to be effective only at the  $x > 2.5$  levels (Figure 4.6, Figure 4.7).



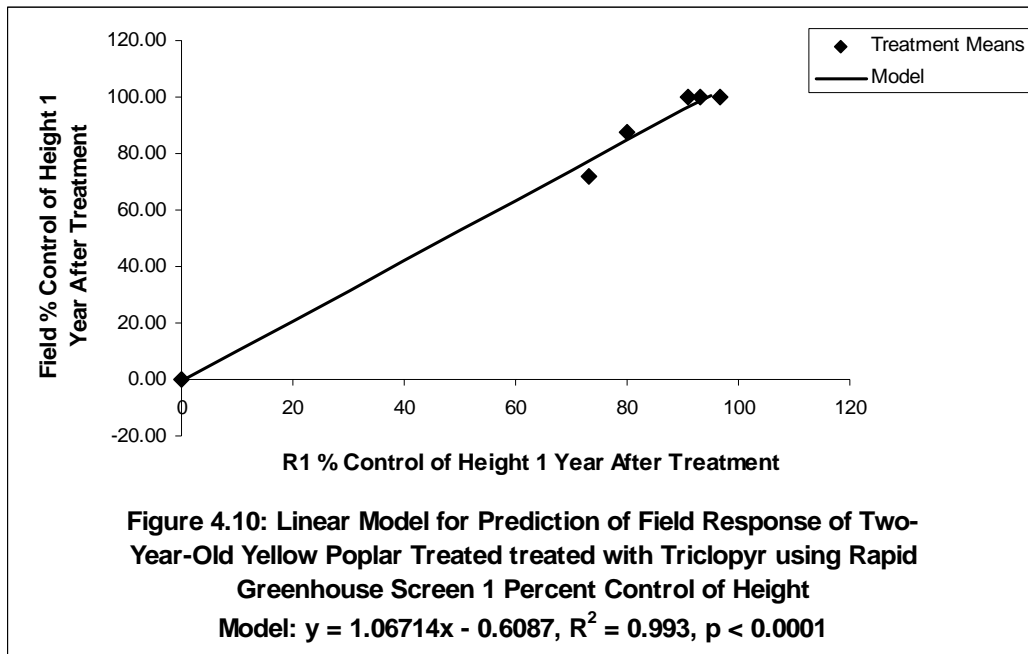


The dependent variable, log effect code, provided the strongest prediction of field response of two-year-old yellow-poplar treated with imazapyr using the RGS2 data. Field effect code of yellow-poplar was predicted using the rapid greenhouse screen 2 percent leaf necrosis measured at 8 WAT. This appears only to be effective when the percent necrosis level is less than 50 % and greater than 30 % (Figure 4.8). The independent variable percent control of height 1 YAT used a more dispersed range of values of percent control of height to predict field effect code 1 YAT (Figure 4.9). Both models show the log trend of responses for one-year-old seedlings versus two-year-old saplings and have  $R^2$  values greater than 0.97.

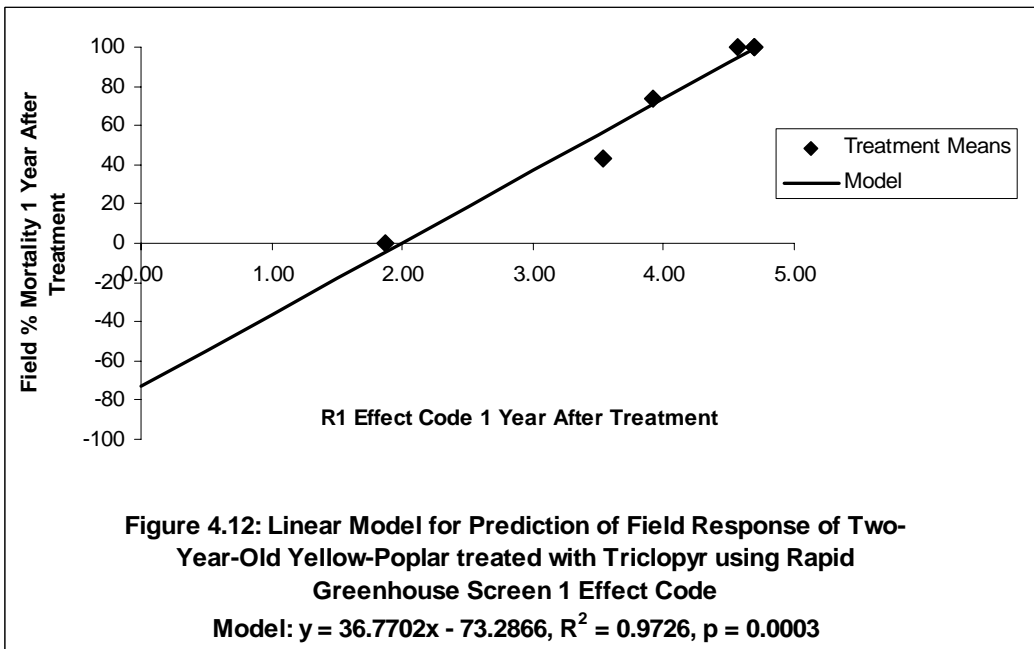
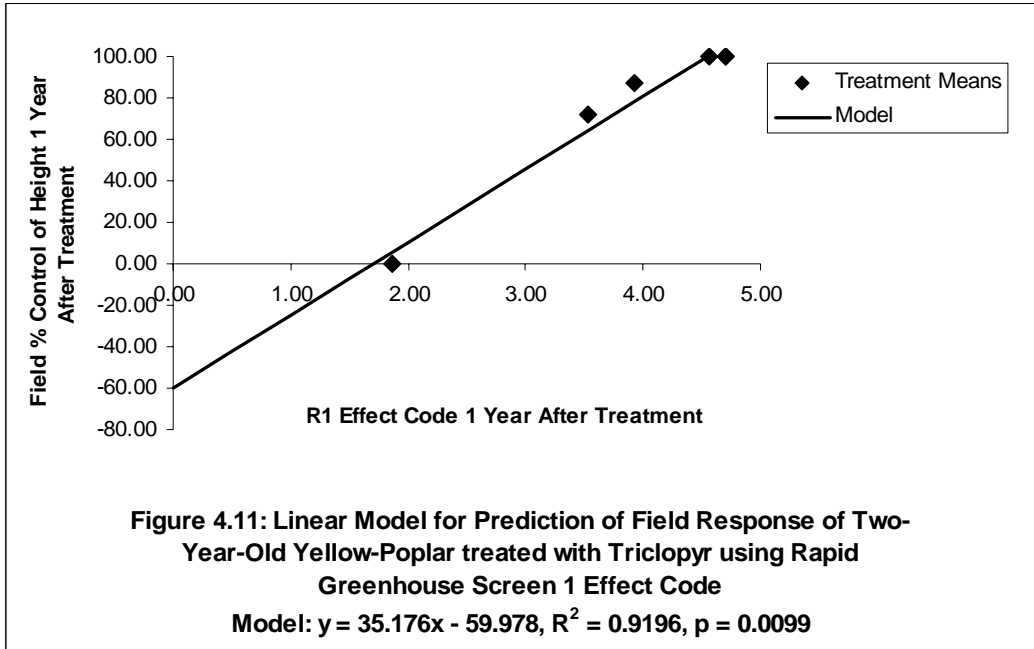


RGS1 effectively predicted field response of two-year-old yellow-poplar treated with triclopyr using percent control of height and effect code, both 1 year after treatment, as independent variables. These models used field percent control of height and field percent mortality as dependent variables and had  $R^2$  values ranging from 0.9196 to 0.993 (Figures 4.10,

4.11, and 4.12). The linear model incorporating percent control of height as independent and dependent variable has the ability to predict control through the full range of percent control of height values (0 – 100 %) (Figure 4.10). Using effect code 1 YAT as the independent variable predicts that at the  $x < 1.5$  level, there is a loss in percent control of height and percent mortality in field response; thus the negative response of the y (dependent) variable (Figures 4.11 and 4.12).





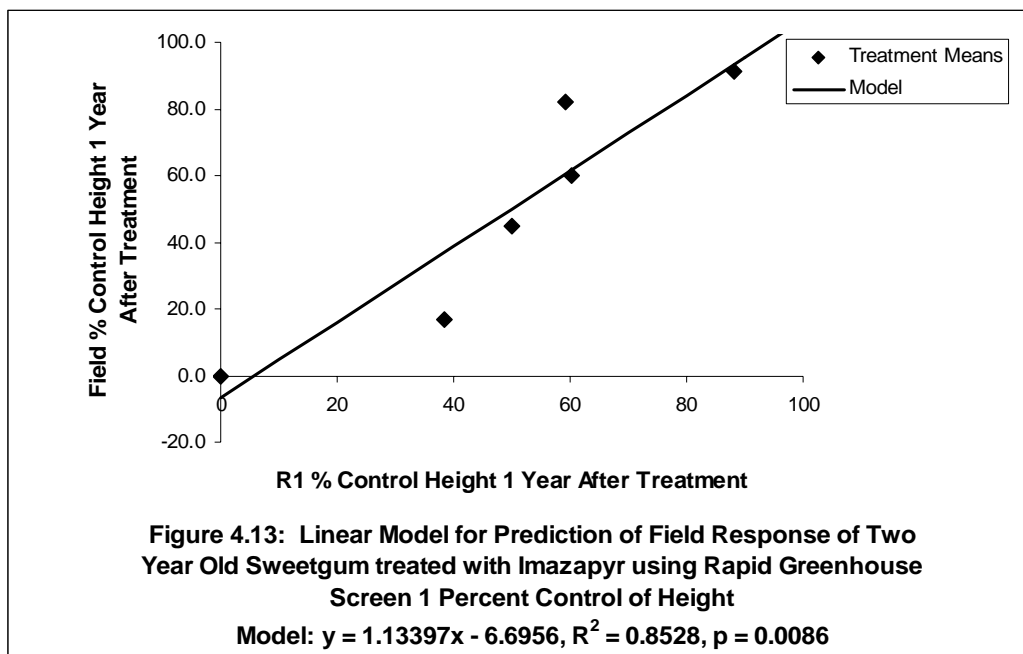


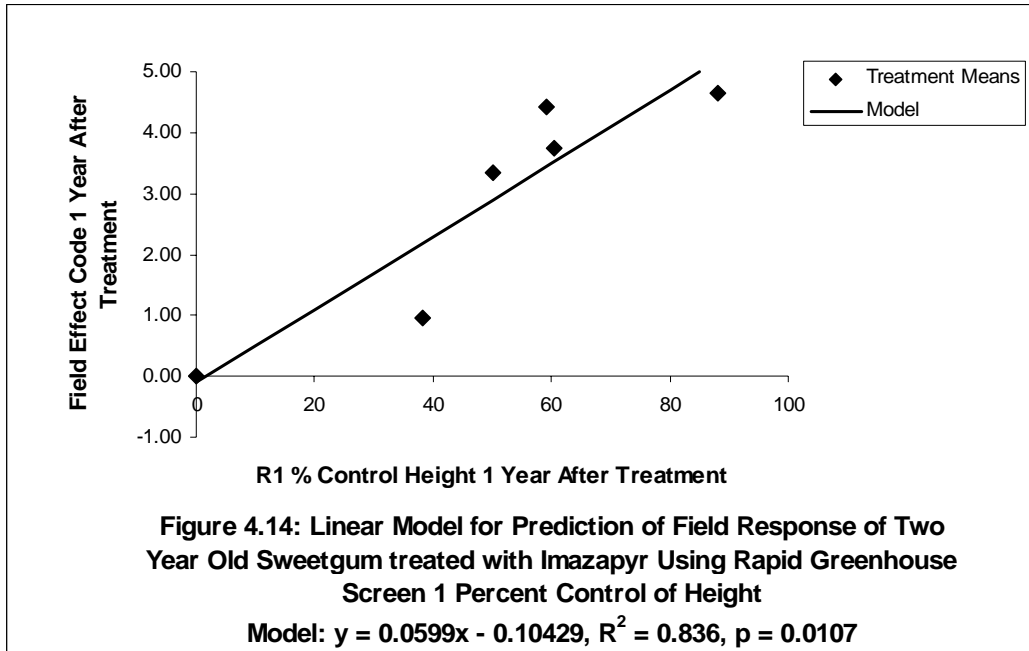
There were only two significant models produced using RGS2 data that could predict field response of two-year-old yellow-poplar treated with triclopyr. These two models used percent leaf necrosis 8 WAT as the independent variables and predicted the log response of percent control of height and percent mortality, both 1 YAT. These two models had high  $R^2$

values; however, only seem to be effective to predict field response once percent leaf necrosis was greater than 80% to predict percent control of height (Appendix Figure 1) and greater than 90% to predict field mortality (Appendix Figure 2). These models are considered to be *extremely* inadequate for discussion purposes and are, for the most part, considered to be invalid and of no use. Models are presented in the appendix, and not in the results, for comparison purposes only and should not be considered to have scientific merit.

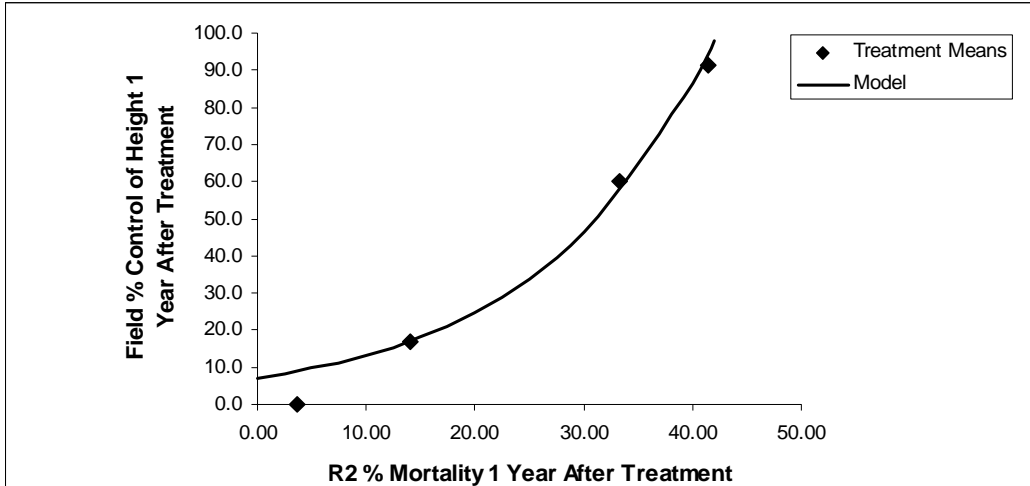
### *Sweetgum*

RGS1 could only produce two significant models to predict field response of two-year-old sweetgum treated with imazapyr. These models used percent control of height as the independent variables and had  $R^2$  values of 0.8528 and 0.836 to predict percent control of height and effect code, both 1 YAT, respectively (Figure 4.13 and 4.14). The linear models were significant and provided prediction of field response through the entire range of percent height control (Figure 4.13) and effect code (Figure 4.14).



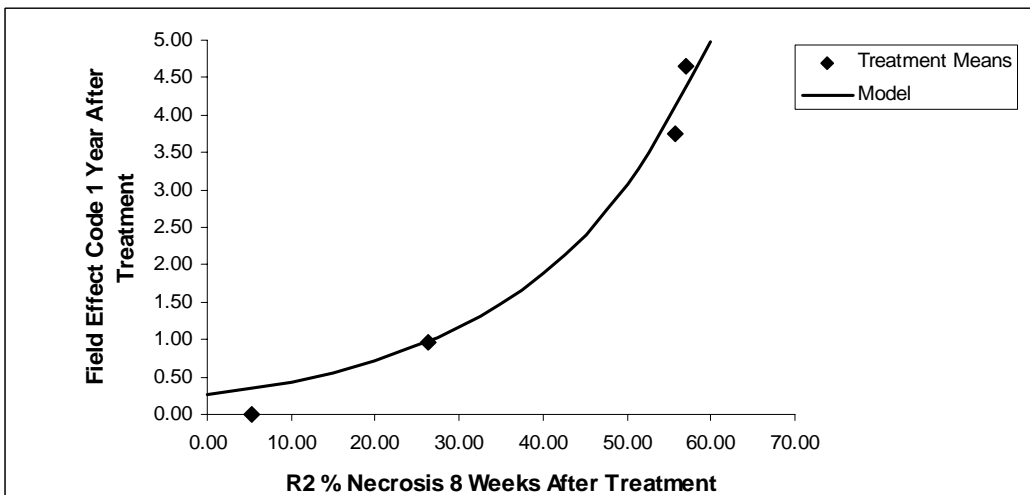


RGS2 used three different parameters to predict percent control of height and effect code, both 1 YAT. These three models all had  $R^2$  values greater than 0.98 and were significant at the  $p < 0.1$  level (Figures 4.15, 4.16, and 4.17). All three models used the log response of the dependent variables to predict field efficacy. Using RGS2 effect code to predict field response effect code appears only to be effective once the greenhouse screen effect code reaches 3.5 (Figure 4.17). Unlike the two-year-old yellow-poplar / rapid greenhouse screen 2 / triclopyr model reported above, Figure 4.17 appears to be of more importance and adequacy due to its use of more data points to create a log relationship and is therefore included in this results section.



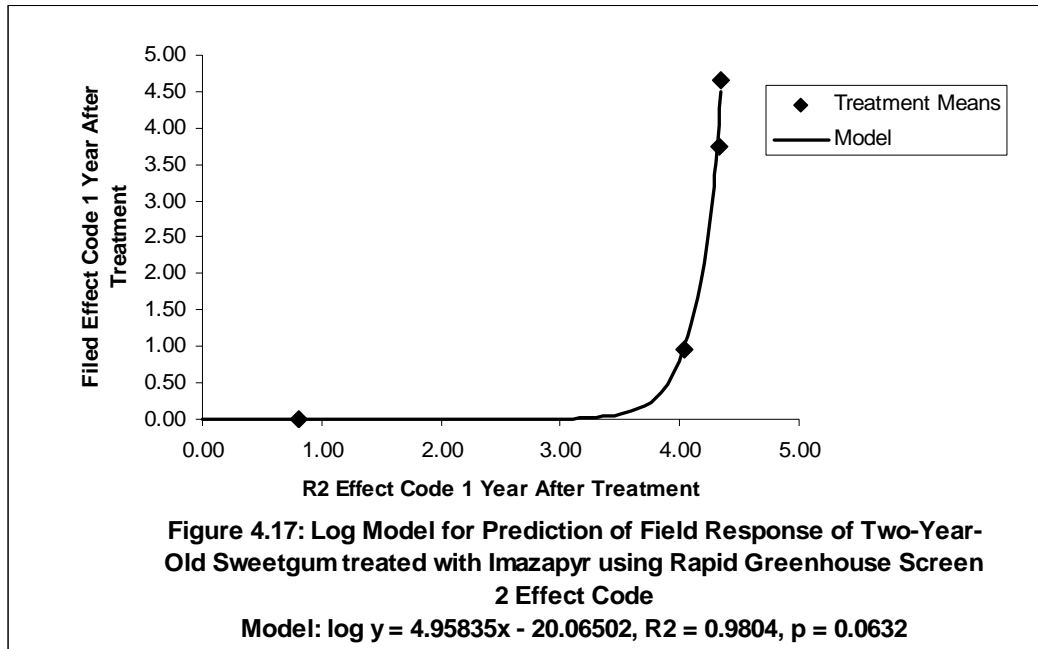
**Figure 4.15: Log Model for Predicting Field Response of Two Year Old Sweetgum treated with Imazapyr using Rapid Greenhouse Screen 2 Percent Mortality**

**Model:  $\log y = 0.0623x + 1.96684$ ,  $R^2 = 0.982$ ,  $p = 0.0857$**

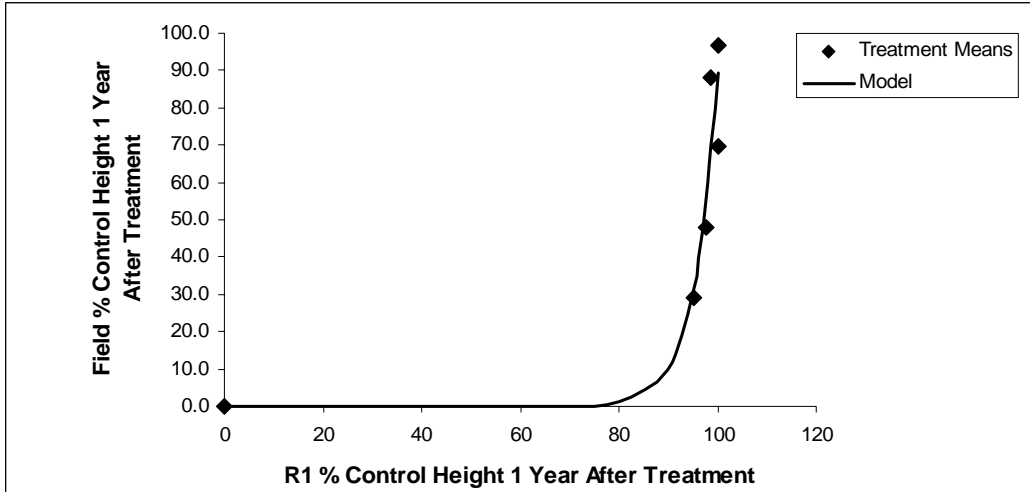


**Figure 4.16: Log Model for Prediction of Field Response of Two Year Old Sweetgum treated with Imazapyr using Rapid Greenhouse Screen 2 Percent Leaf Necrosis**

**Model:  $\log y = 0.04866x - 1.31296$ ,  $R^2 = 0.9916$ ,  $p = 0.0586$**

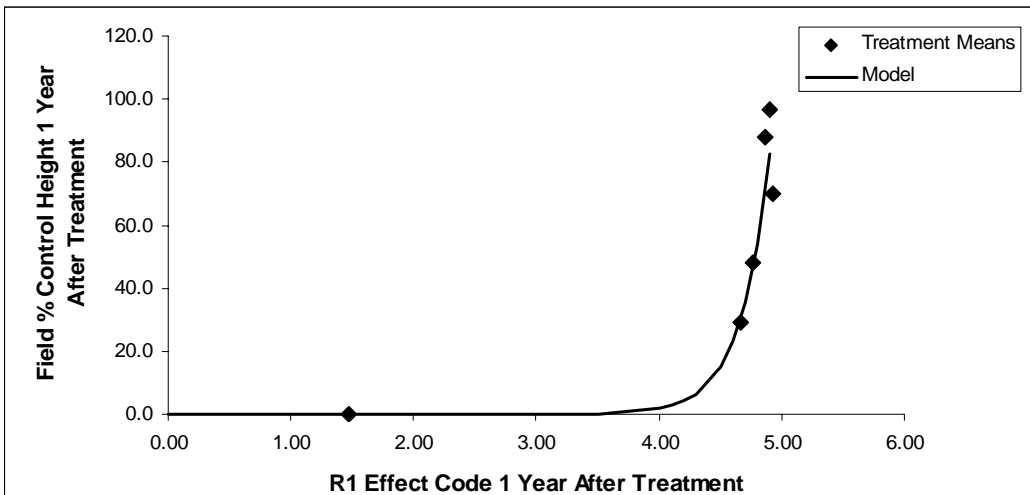


Prediction of field response of two-year-old sweetgum treated with triclopyr using RGS1 data was accomplished by using the log y transformation of percent control of height and effect code, both 1 YAT (Figure 4.18, 4.19, and 4.20). These models incorporated the RGS1 parameters percent control of height and effect code to create three models with  $R^2$  values ranging from 0.8122 to 0.8428. These log models can only predict field response once the rapid screened seedlings have a percent control of height greater than 80% (Figure 4.18) and an effect code greater than 3.75 (Figures 4.19 and 4.20). Also, using RGS1 percent control of height to predict percent control of height in the field can only predict control of field height up to 90% once RGS1 percent control reaches 100% (Figure 4.18). This limitation in prediction of the high values of response also occurs using the RGS1 effect code as the independent variable (Figures 4.1.9b and 4.1.9c). As with Figure 4.17, these models are included here and are considered to have more statistical importance than Appendix Figure 1 and Figure 2 due to the inclusion of more data points, comparatively.



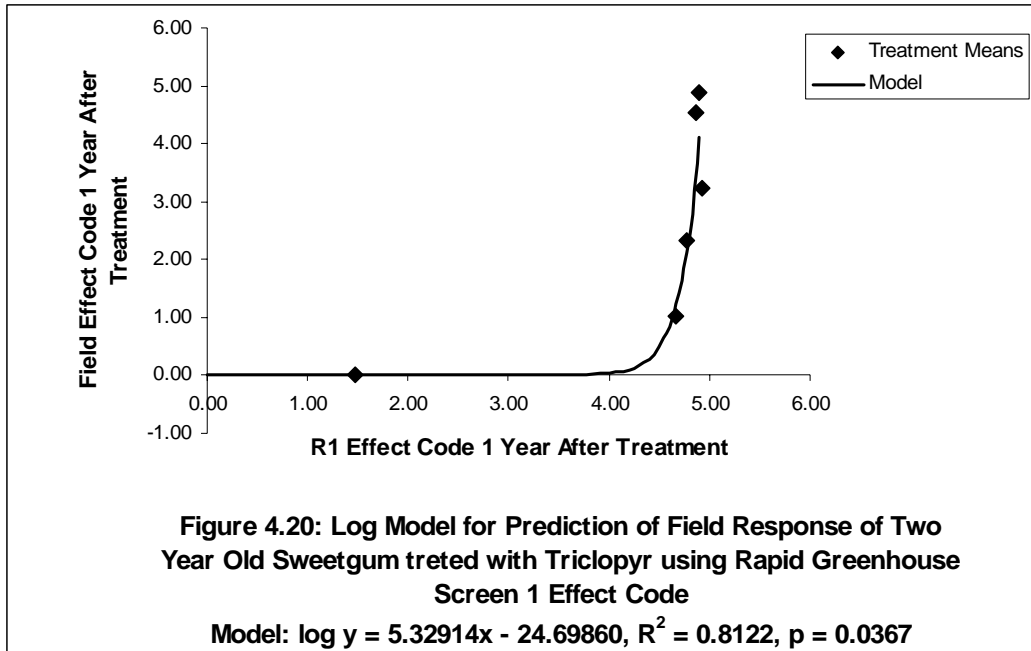
**Figure 4.18: Log Model for Prediction of Field Response of Two Year Old Sweetgum treated with Triclopyr using Rapid Greenhouse Screen 1 Percent Control of Height**

**Model:  $\log y = 0.22041x - 17.54734$ ,  $R^2 = 0.8257$ ,  $p = 0.0327$**



**Figure 4.19: Log Model for Prediction of Field Response of Two Year Old Sweetgum treated with Triclopyr using Rapid Greenhouse Screen 1 Effect Code**

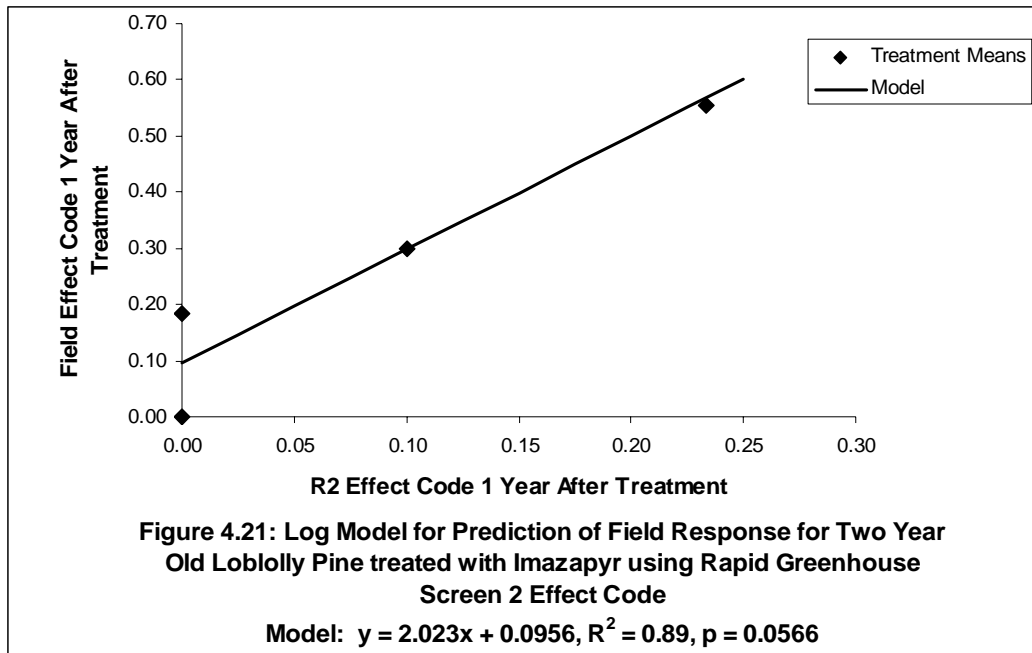
**Model:  $\log y = 4.24131x - 16.37105$ ,  $R^2 = 0.8428$ ,  $p = 0.0278$**



There were no significant models produced that utilized the RGS2 data set that could predict either a linear or log response of two-year-old sweetgum treated with triclopyr.

#### *Loblolly Pine*

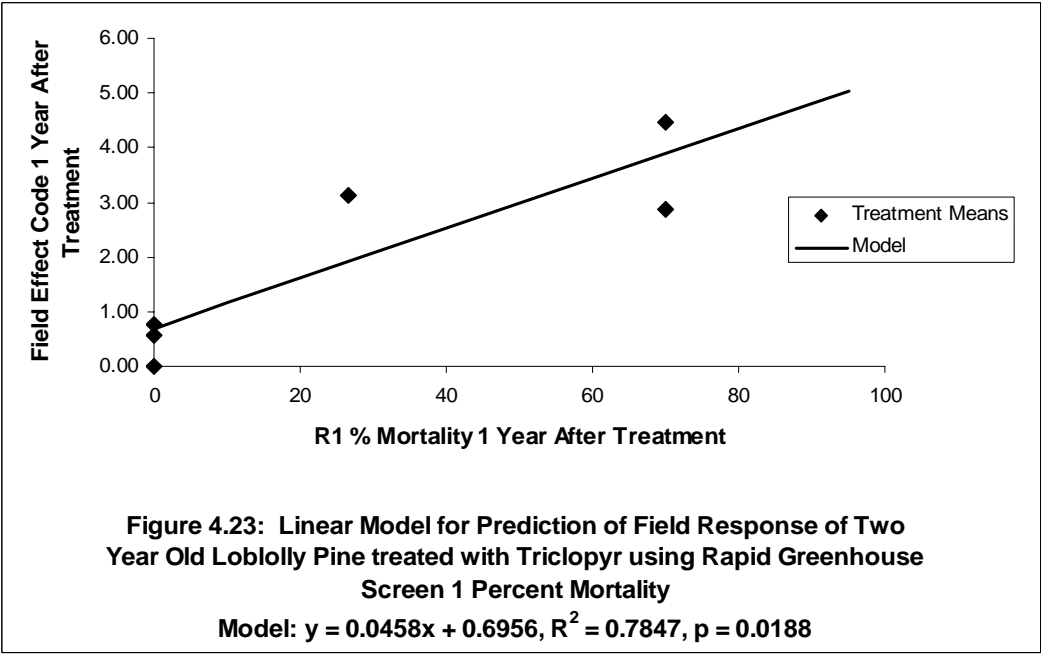
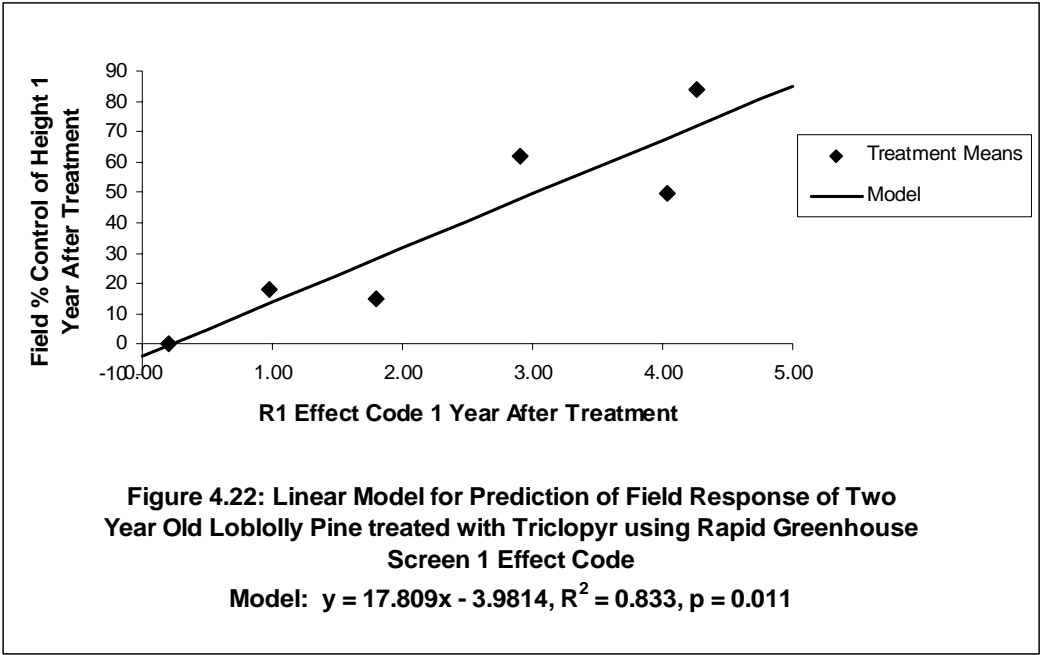
Parameters measured with RGS1 were unable to produce significant models to predict field response of any parameter measured in the field screens for loblolly pine treated with imazapyr at the  $p < 0.1$  level. The only significant model produced that could predict field response of two-year-old loblolly pine treated with imazapyr was effect code measured from RGS2 (Figure 4.21). It should be noted that the model is made possible by using the effect code on a species tolerant to imazapyr. This creates a linear model that predicts little to no response (less than 1 for the field response and less than 0.5 for the RGS response).

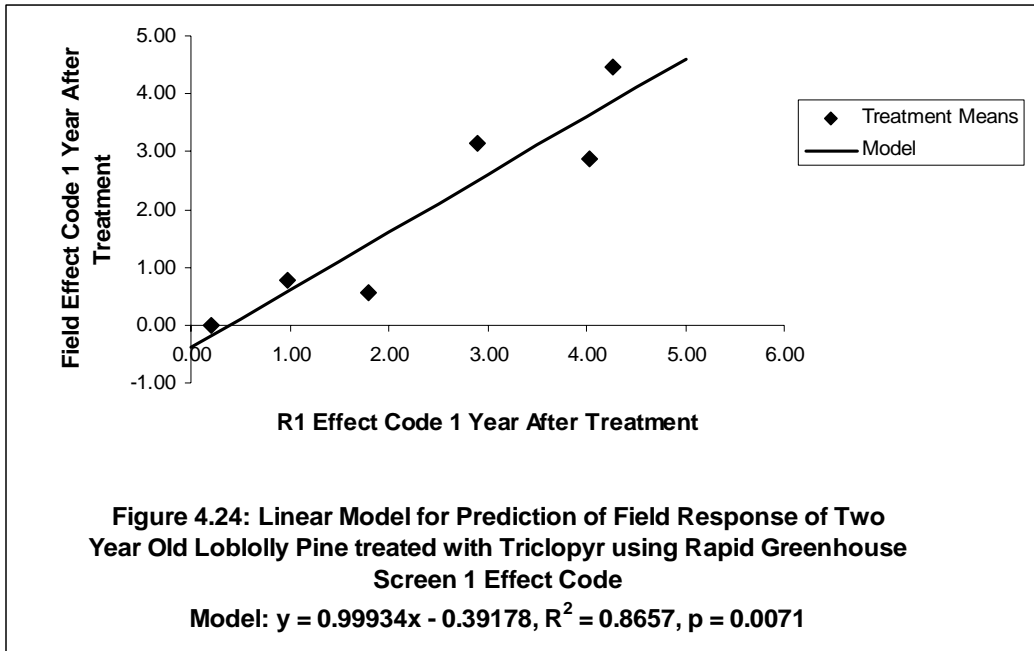


There were a higher number of significant models produced predicting the field response of two-year-old loblolly pine treated with triclopyr as compared to the imazapyr screen. This is due to the susceptibility of loblolly pine to triclopyr and the resulting treatment response differentiation.

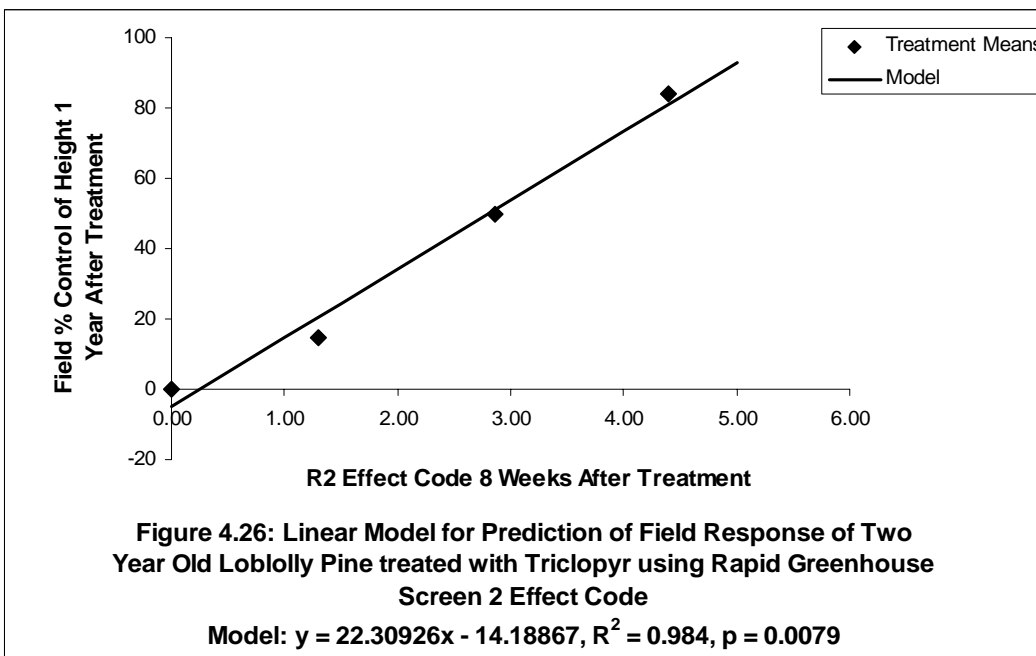
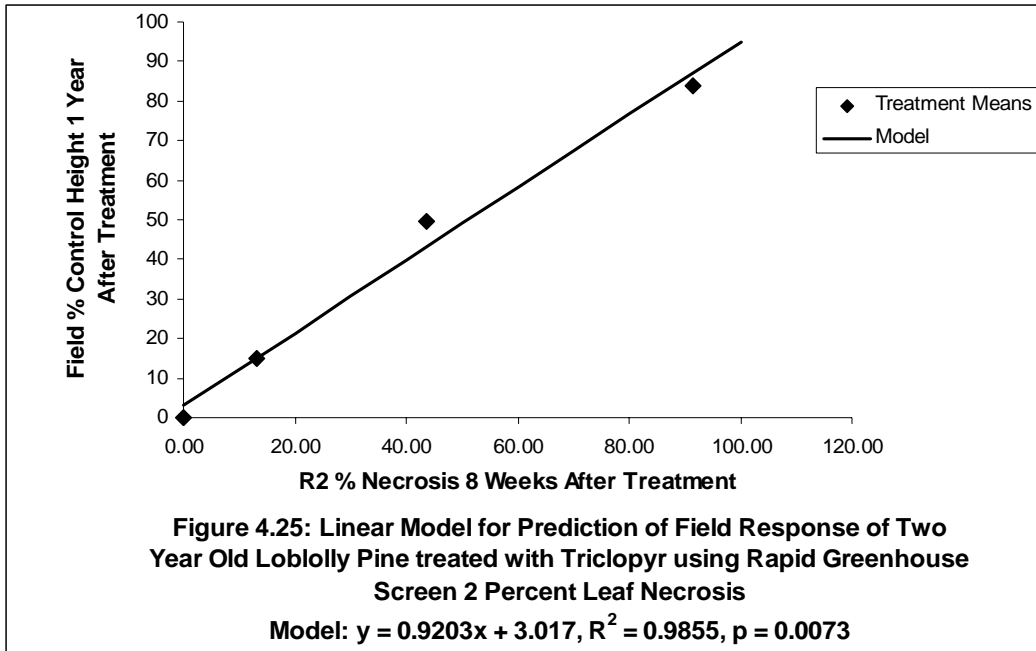
Field effect code 1 YAT and percent control of height 1 YAT could be predicted using RGS1 data measuring seedling effect code and percent mortality, both 1 YAT. These three linear models had  $R^2$  values ranging from 0.7847 to 0.8657 (Figures 4.22, 4.23, and 4.24). All three models have a positive slope, showing the positive relationship increasing rates of triclopyr have on effect code, percent control of height, and percent mortality for loblolly pine treated with triclopyr.

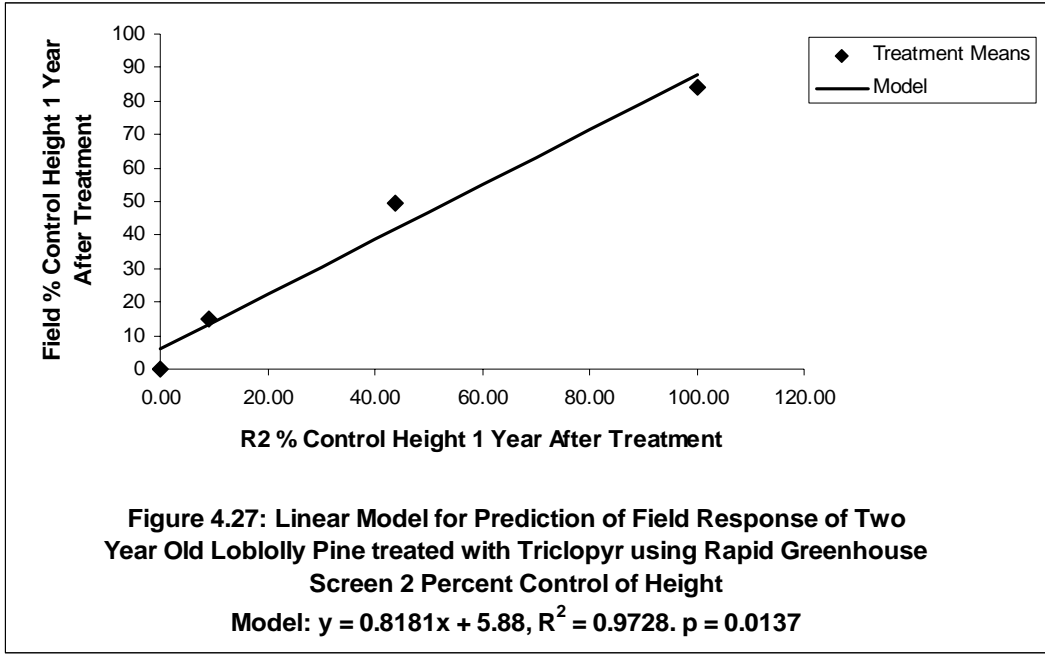






RGS2 data was extremely effective in predicting field response of two-year-old loblolly pine treated with triclopyr. Results presented here are a synopsis of the total results for prediction of field response of two-year-old loblolly pine. Percent control of field height was predicted using parameters measured at both 8 WAT and 1 YAT. These linear models had  $R^2$  values ranging from 0.9728 to 0.9855 and can predict at least up to 90 % control of height in field response (Figures 4.25, 4.26, and 4.27).





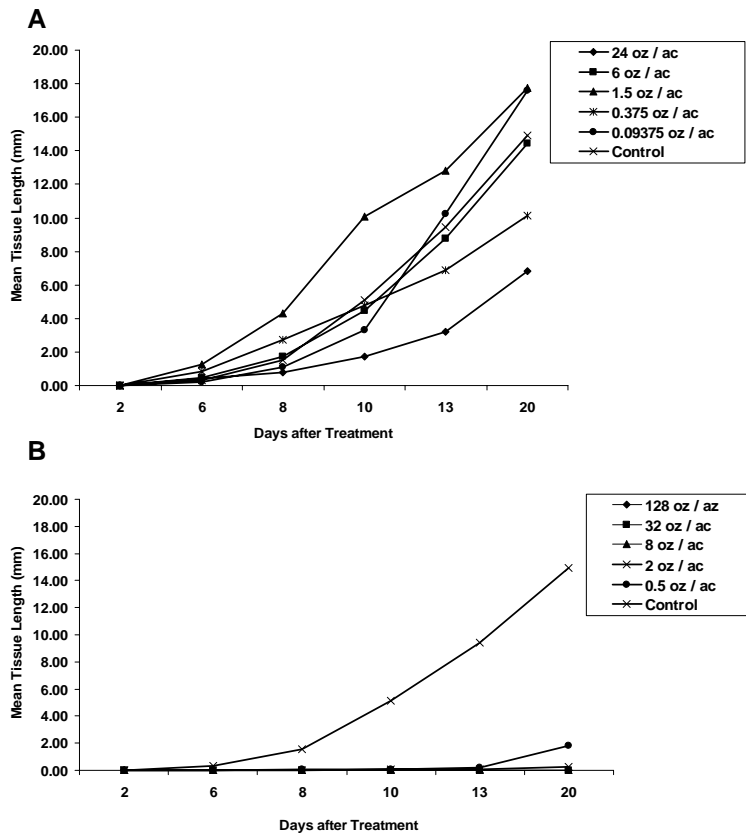
## Section 2: Rapid Seed Screening

Results presented in this section will be presented in two parts. The first part will provide a summary of the overall response of the four species tested in the seed screen with imazapyr and triclopyr. This will give some insight as to the potential for seed screening to allow for testing of initial herbicide activity. Legends in figures for the first part refer to application rates of the respective chemical and are in formulated product ounces per acre units. The second part will provide a summary of regression models for prediction of response of two-year-old saplings treated with imazapyr and triclopyr using seed data as the independent variable. These regression models will also summarize the effective time of observation in the tested seed screens to allow the statistically strongest regression models.

### Overall Response of Rapid Seed Screen

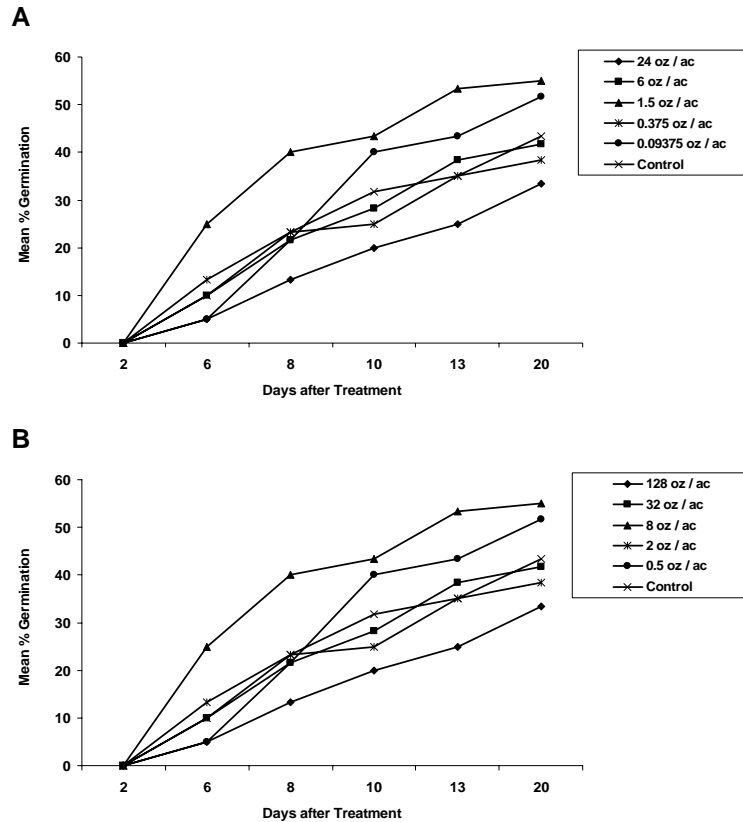
#### *Green Ash*

The green ash imazapyr seed screen exhibited a growth stimulation response on mean tissue length with the 1.5 and 0.09375 oz / ac rates as compared to the control (Figure 4.28 A). It is unclear why this response was not exhibited with the 0.375 oz / ac rate. All rates tested allowed germination and growth of seed tissue and showed adequate rate differentiation throughout the screen. Mean percent germination was observed earlier in the screen as compared to mean tissue length. The growth stimulation response observed with mean seed tissue is also exhibited with mean percent germination with the same two rates, 1.5 and 0.09375 oz / ac, as compared to the control (Figure 4.29 A).



**Figure 4.28: Mean Tissue Length for Green Ash Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

Seed germination and tissue growth of green ash seeds were severely inhibited in the triclopyr seed screen. Mean tissue length was never greater than 1.8 mm throughout the entire seed screen and three of the five treatments, 128, 32, and 8 oz / ac, never germinated and grew (Figure 4.28 B). Only two rates, 2 and 0.54 oz / ac, had any seed tissue emerge and these were minimal as compared to the control. Germination rates were low as well, never exceeding approximately 10% (Figure 4.29 B). Only the lowest two rates, 2 and 0.5 oz / ac, had any germination activity.



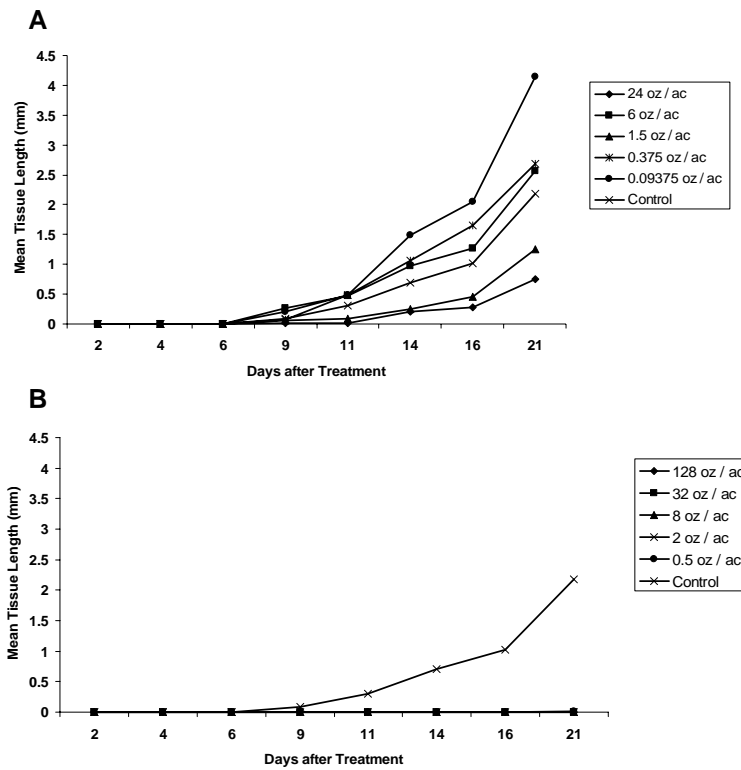
**Figure 4.29: Mean Percent Germination of Green Ash Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

*Yellow-poplar*

All yellow-poplar seeds in the imazapyr screen had germination and tissue growth regardless of treatment. There was the presence of a tissue growth stimulation response with the 6, 0.375, and 0.09375 oz / ac treatments as compared to the control (Figure 4.30 A).

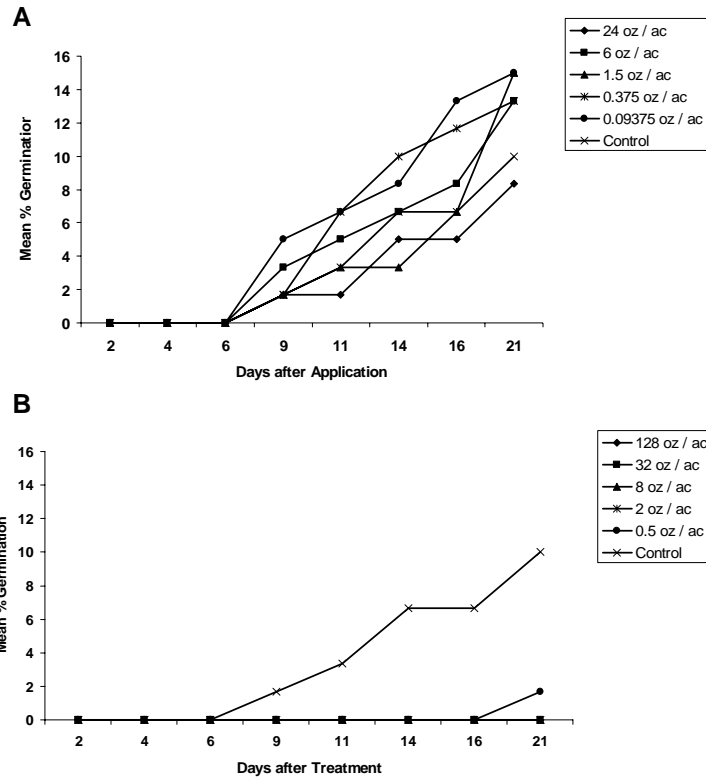
Germination rates are also stimulated in yellow-poplar seeds treated with imazapyr. The only rate not to germinate as well as the control is the highest rate of imazapyr, 24 oz /ac (Figure 4.31 A). These germination rates are very low and indicate a poor germination success rate due to non-herbicidal effects.

Triclopyr effectively inhibited all germination and subsequent growth of yellow-poplar seeds. There was a small response of seed tissue growth with the lowest rate of triclopyr, 0.5 oz / ac (Figure 4.30 B) and with percent germination (Figure 4.31 B). This germination response at the 21 DAT interval is only 1.67%, which is equivalent to only 1 seed germinating over all replications for that treatment.



**Figure 4.30: Mean Tissue Length for Yellow-Poplar Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

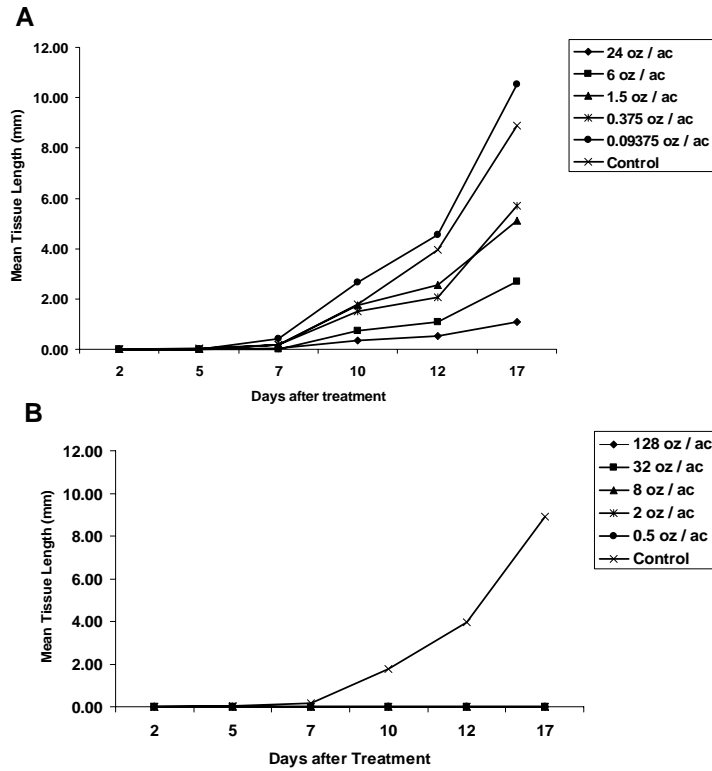




**Figure 4.31: Mean Percent Germination of Yellow-Poplar Seed Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

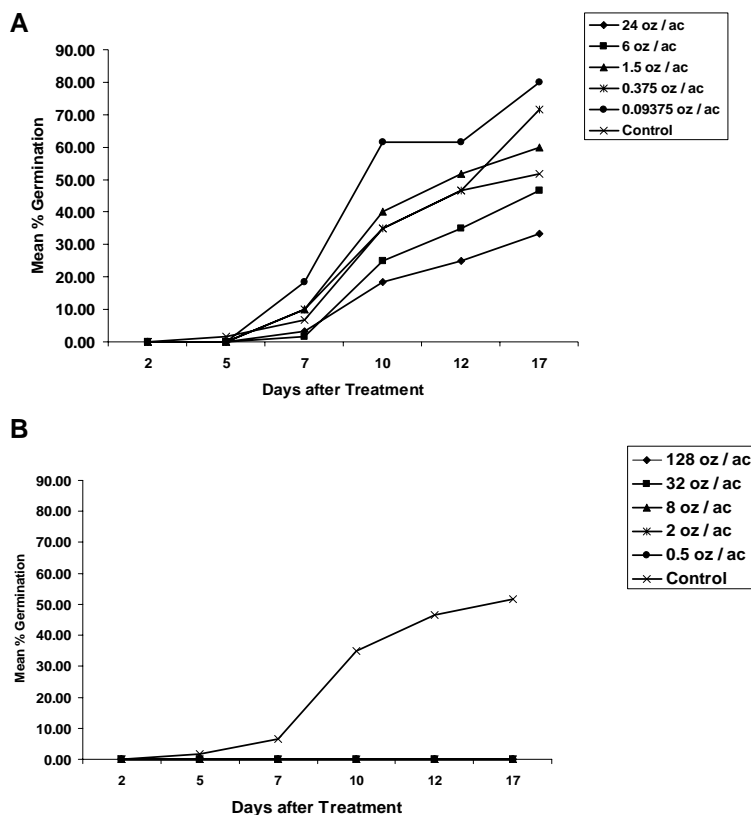
*Sweetgum*

Sweetgum seeds, as with the green ash and yellow-poplar seeds, exhibited a growth stimulation effect at the low rates of imazapyr. The lowest rate of imazapyr tested, 0.09375 oz / ac, had a higher mean tissue length than the control (Figure 4.32 A). The other four rates tested all resulted in consistent treatment differentiation and control of tissue length at the 17 DAT interval as rates increased. Mean percent germination exhibited a stimulation effect as the lowest three rates tested, 0.09375, 0.375, and 1.5 oz / ac, had higher mean germination percentages as compared to the control (Figure 4.33 A). Seed screens on sweetgum seeds using imazapyr appear to be effective in determining initial herbicide activity and rate differentiation.



**Figure 4.32: Mean Tissue Length of Sweetgum Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

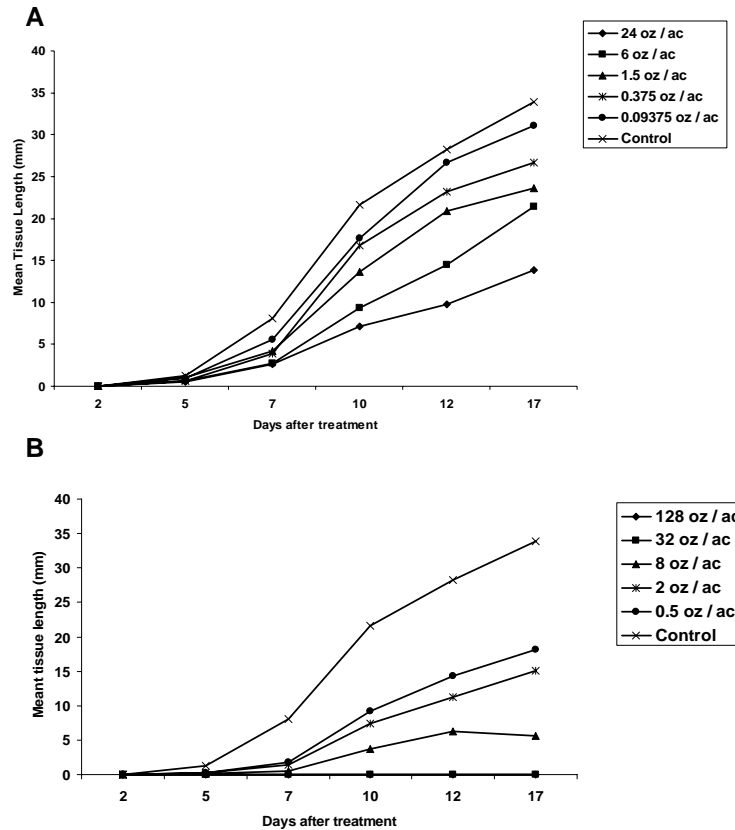
Sweetgum seed germination and subsequent growth was completely inhibited during the triclopyr screens (Figures 4. 32 B and 4.33 B).



**Figure 4.33: Mean Percent Germination of Sweetgum Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

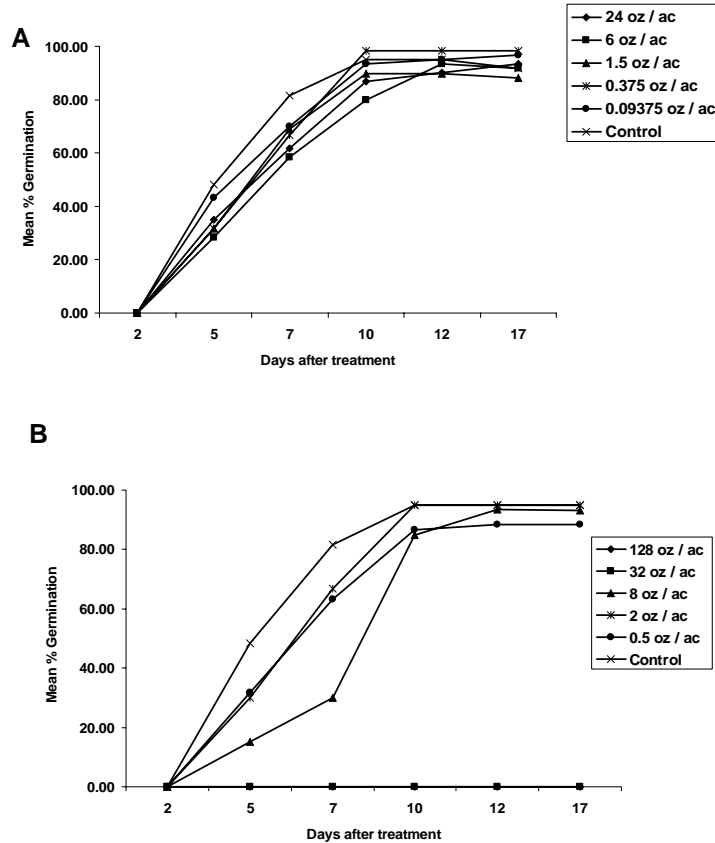
*Loblolly Pine*

Loblolly pine seeds treated with imazapyr exhibited a consistent growth response in line with treatment rates (Figure 4.34 A). As rates of imazapyr increased from 0 to 24 oz / ac, mean tissue response decreased. There was no complete inhibition of growth or germination as loblolly pine is tolerant to imazapyr. Loblolly pine seeds all had high and consistent success rates of germination across treatments (Figure 4.35 A).



**Figure 4.34: Mean Tissue Length of Loblolly Pine Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

Loblolly pine was the only species tested that exhibited an effective response to the triclopyr screen. No one treatment exhibited as high mean tissue length response as the control treatment; however, the three lowest rates, 0.5, 2, and 8 oz / ac, did allow for tissue growth (Figure 4.34 A). There was no tissue response for the two highest rates, 128 and 32 oz / ac. Mean germination rates for the lower three rates were close to that of the control treatment by the end of the seed screen (Figure 4.35 B).



**Figure 4.35: Mean Percent Germination of Loblolly Pine Seeds Treated with Imazapyr (A) and Triclopyr (B) Over Entire Length of Rapid Seed Screen**

An important statistic for the rapid seed screen is the time to first significant treatment effect. This may allow for quick determination of initial herbicide activity and possible species control spectrum to provide information on the total screening process required for future research. For example, time to significant treatment effect was determined to be as little as 5 days after treatment for loblolly pine (Table 4.1) Loblolly pine consistently provided quicker time to significant treatment effect, regardless of chemical, than the other three species. Loblolly pine was followed by green ash, sweetgum, and then yellow-poplar. Yellow-poplar seed never exhibited significant treatment effect of imazapyr throughout the entire length of the rapid seed

screen. There was no difference in time to significant treatment effect within species regardless of chemical for loblolly pine, green ash, and sweetgum.

*Table 4.1: Days to Significant Treatment Effect for Rapid Seed Screens (DAT = Days After Treatment)*

Species	Chemical	Response	DAT
Loblolly Pine	Imazapyr	Tissue	5
		Germination	5
	Triclopyr	Tissue	5
		Germination	5
Green Ash	Imazapyr	Tissue	6
		Germination	6
	Triclopyr	Tissue	6
		Germination	6
Sweetgum	Imazapyr	Tissue	7
		Germination	7
	Triclopyr	Tissue	7
		Germination	7
Yellow-Poplar	Triclopyr	Germination	14
		Tissue	16
	Imazapyr	Tissue	N/A
		Germination	N/A

*Prediction of Field Response of Two-year-old Saplings Using Rapid Seed Screen Data*

Rapid seed screen data for three species, yellow-poplar, sweetgum, and loblolly pine, were correlated with the response data for the same species in the two-year-old plantation. Simple linear regressions were performed for every observation period in an attempt to use either percent control of seed tissue or percent control of germination to predict percent control of field height 1 YAT, percent mortality 1 YAT, or effect code 1 YAT. These results will show the ability of the rapid seed screen to predict field response as well as the effective time of observation of a rapid seed screen with the species in question.

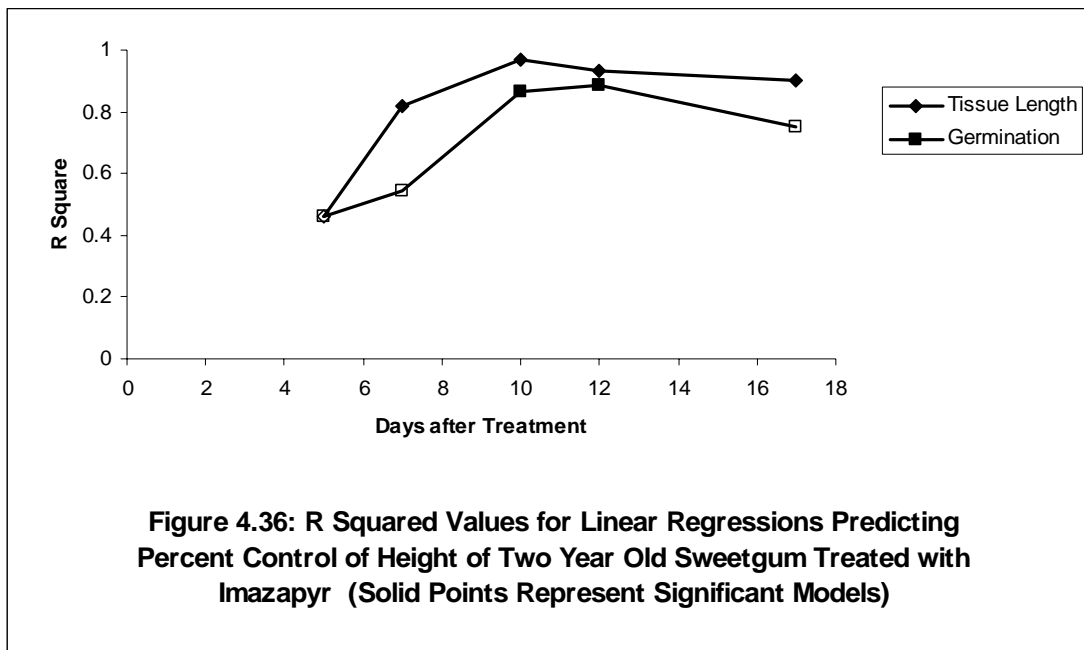
*Yellow-poplar*

No significant models were produced that could predict any of the field responses measured of two-year-old yellow-poplar saplings treated with either imazapyr or triclopyr using

the rapid seed screen data. The imazapyr seed screen did allow for seed tissue growth or seed germination (Figures 4.30 A and 4.31 A, respectively); however, the germination rates and subsequent growth was very low compared to field response. There was no statistically significant treatment differentiation (Figures 4.30 B and 4.31 B) with the triclopyr seed screen to use as independent data for regression modeling.

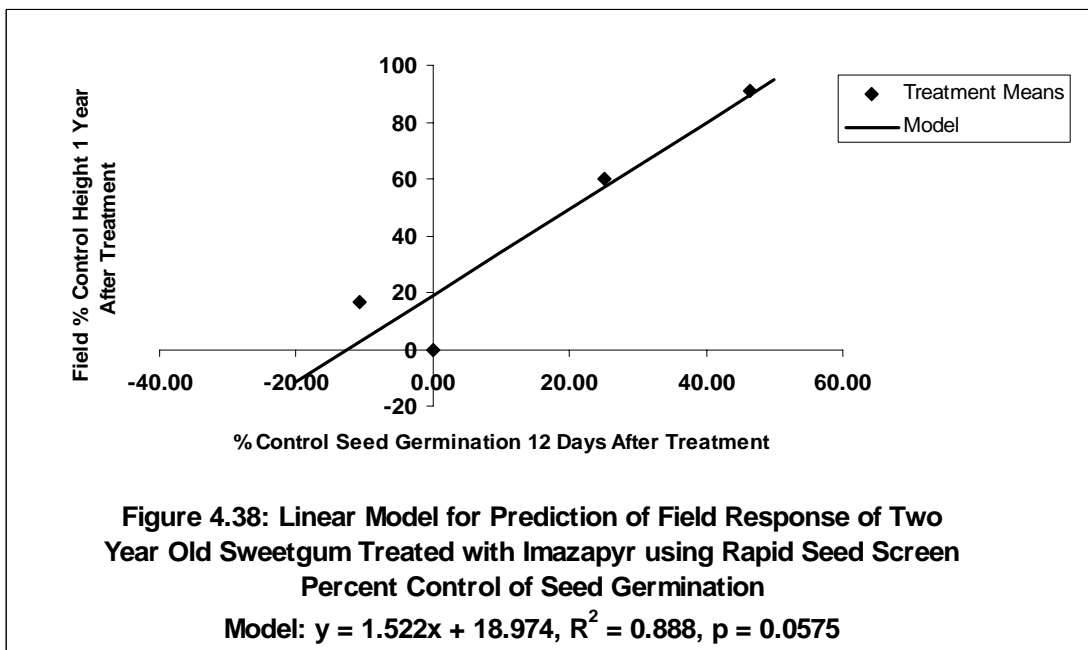
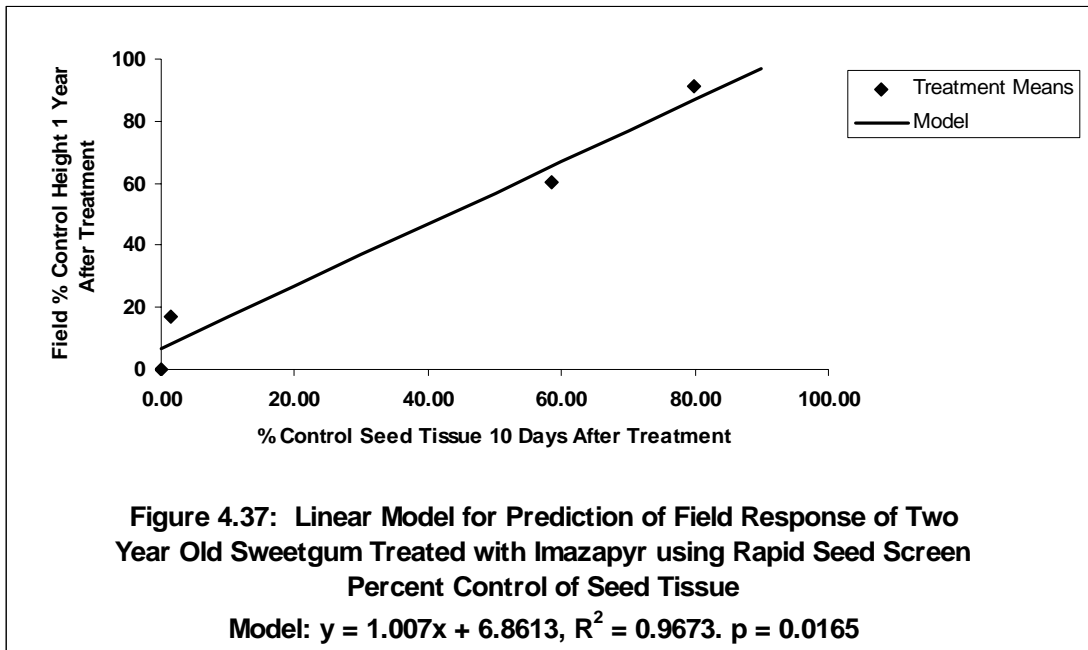
*Sweetgum*

Four significant models were produced using percent control of seed tissue data, from 7 DAT through the end of the screen at 17 DAT, to predict percent control of field height (Figure 4.36). As the screen continued past the 10 DAT interval,  $R^2$  values leveled off as strength of regression decreased. Using percent control of germination only produced two significant models at the 10 and 12 DAT intervals (Figure 4.36).



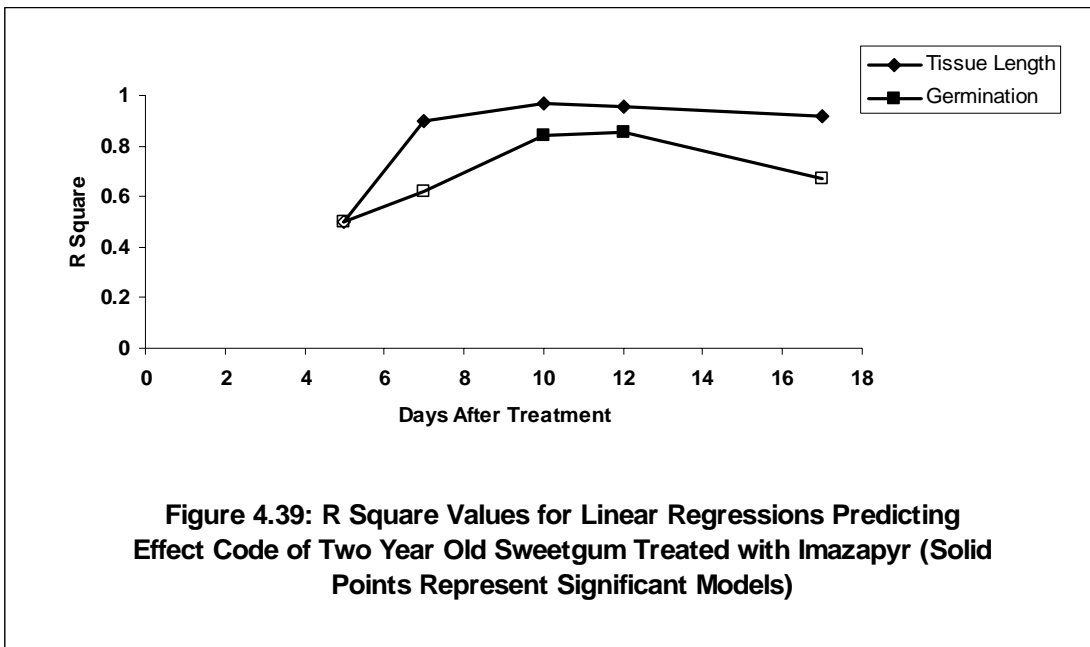
Models predicting percent control of field height 1 YAT had  $R^2$  values of 0.9673 and 0.888 using percent control of seed tissue and percent control of germination as independent variables, respectively (Figures 4.37 and 4.38). Using percent control of seed tissue at 10 DAT

could effectively predict percent control of height of two-year-old saplings from 0 – 100 % (Figure 4.37). Germination data collected at 12 DAT predicted a full range of height control from 0 – 100 % (Figure 4.38).

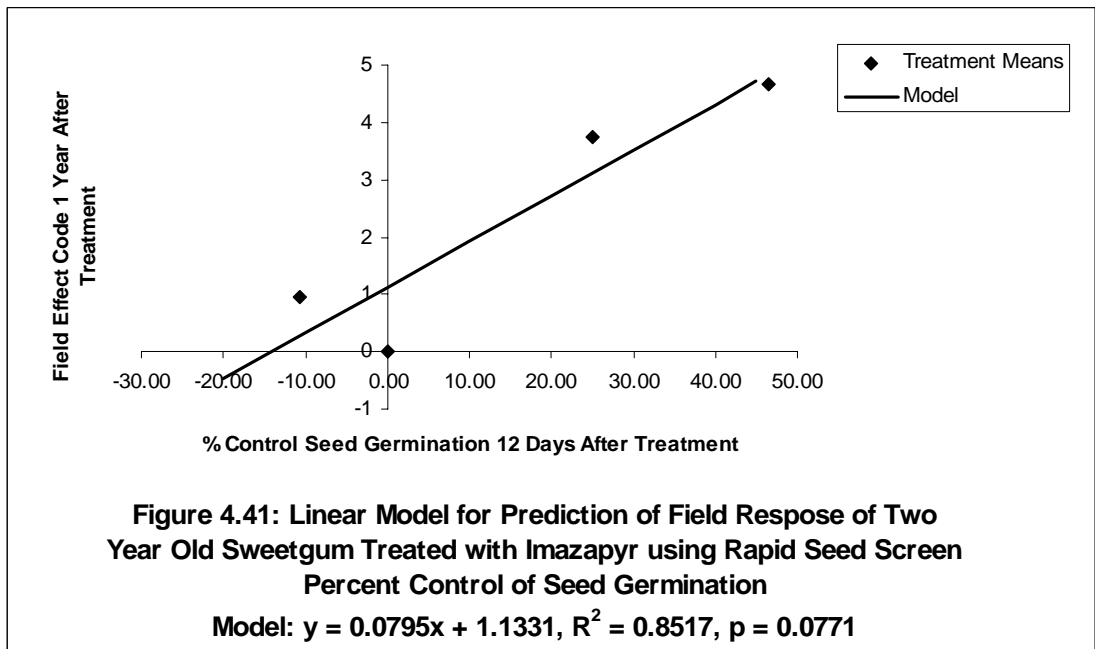
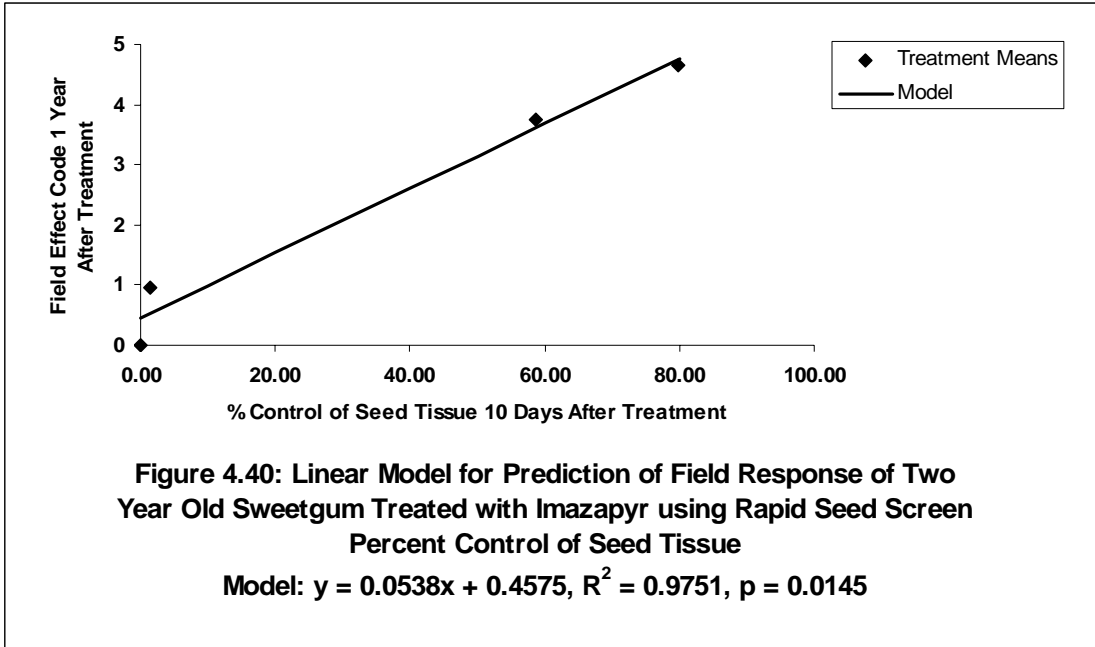




As with predicting percent control of height, there were four significant models that utilized percent control of seed tissue and two that utilized percent control of germination to predict field effect code 1 YAT for two-year-old sweetgum treated with imazapyr (Figure 4.39).  $R^2$  for models utilizing percent control of seed tissue were highest at the 10 DAT interval and began to level off after that point.  $R^2$  values for percent control of germination were highest at the 12 DAT interval.



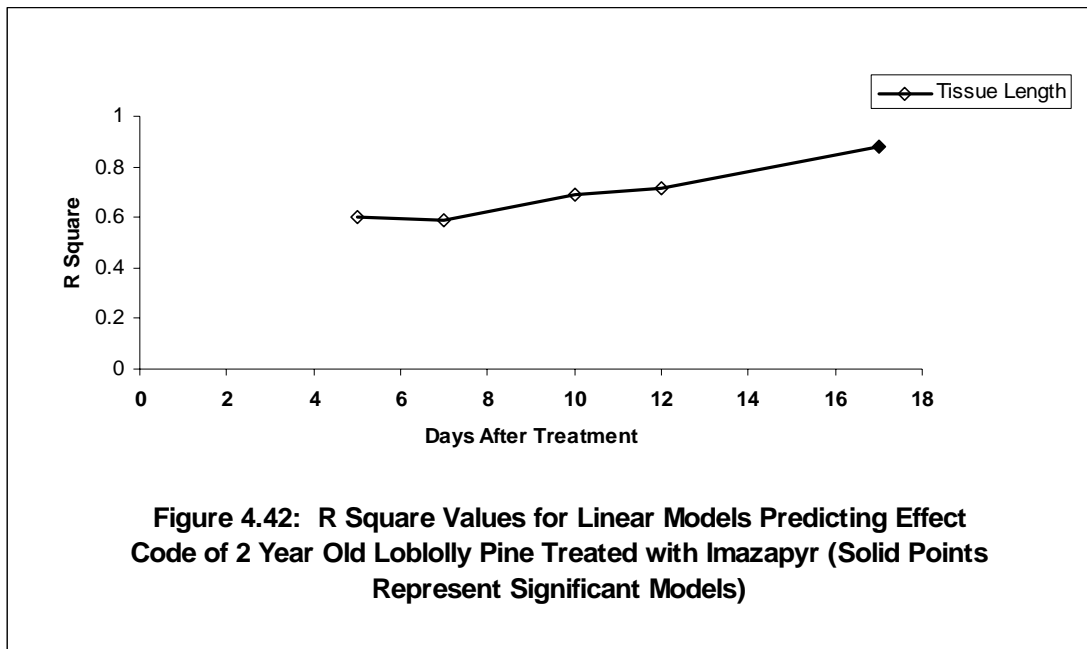
Models for predicting effect code 1 YAT had  $R^2$  values of 0.971 and 0.851 using percent control of seed tissue and percent control of germination, respectively (Figures 4.40 and 4.41). Percent control of seed tissue at 10 DAT can predict the full range of effect codes using tissue control data from 0 – 80 % (Figure 4.40). Percent control of germination data from -20 % to 50% can predict the full range of effect code in two-year-old saplings (Figure 4.41).



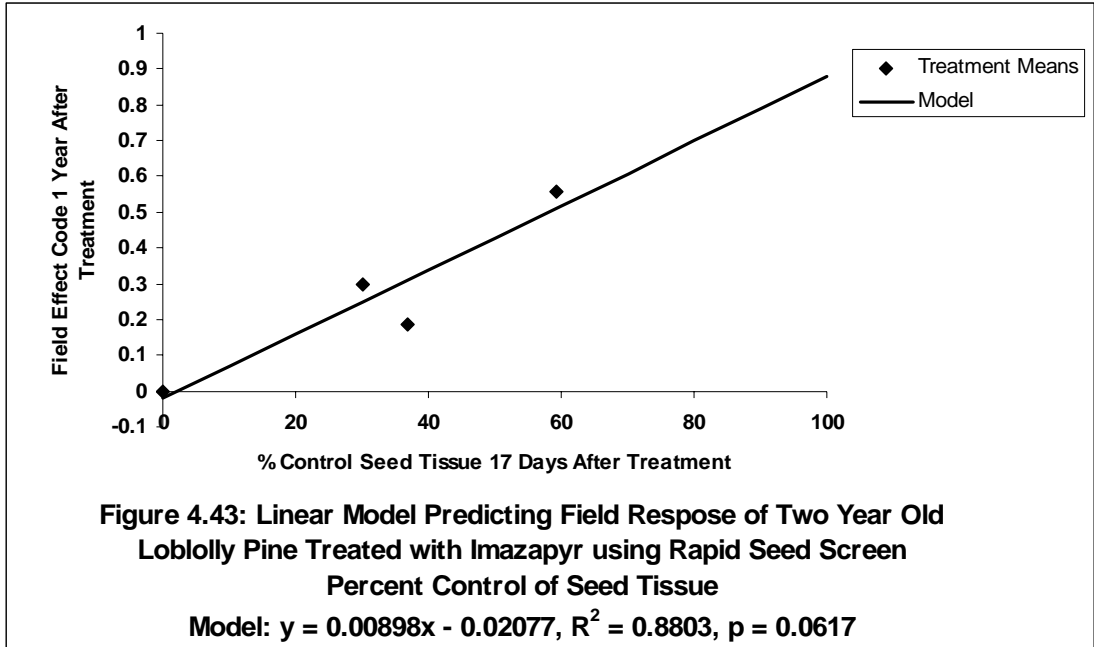
There was no significant model that could predict any field response measured of two-year-old sweetgum treated with triclopyr using the seed data as the independent variable.

## Loblolly Pine

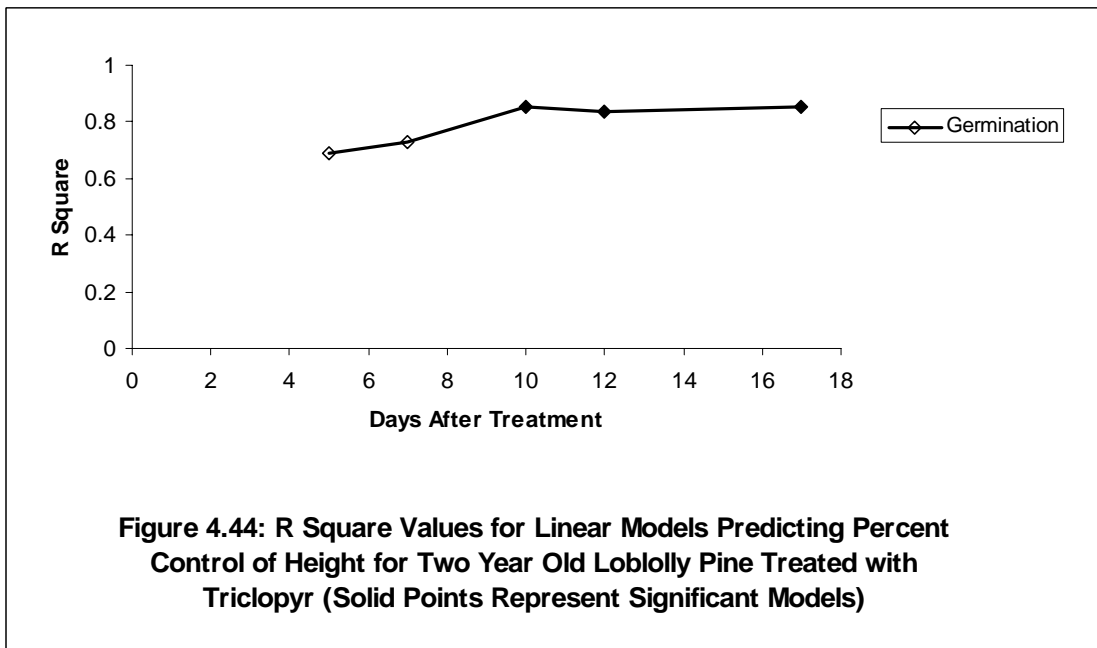
Only one significant model was produced that could predict the field response of loblolly pine treated with imazapyr. The model used percent control of tissue length to predict effect code using data collected at the end of the seed screen (Figure 4.42). There were no significant models predicting field effect code using seed tissue data collected before the 17 DAT interval.



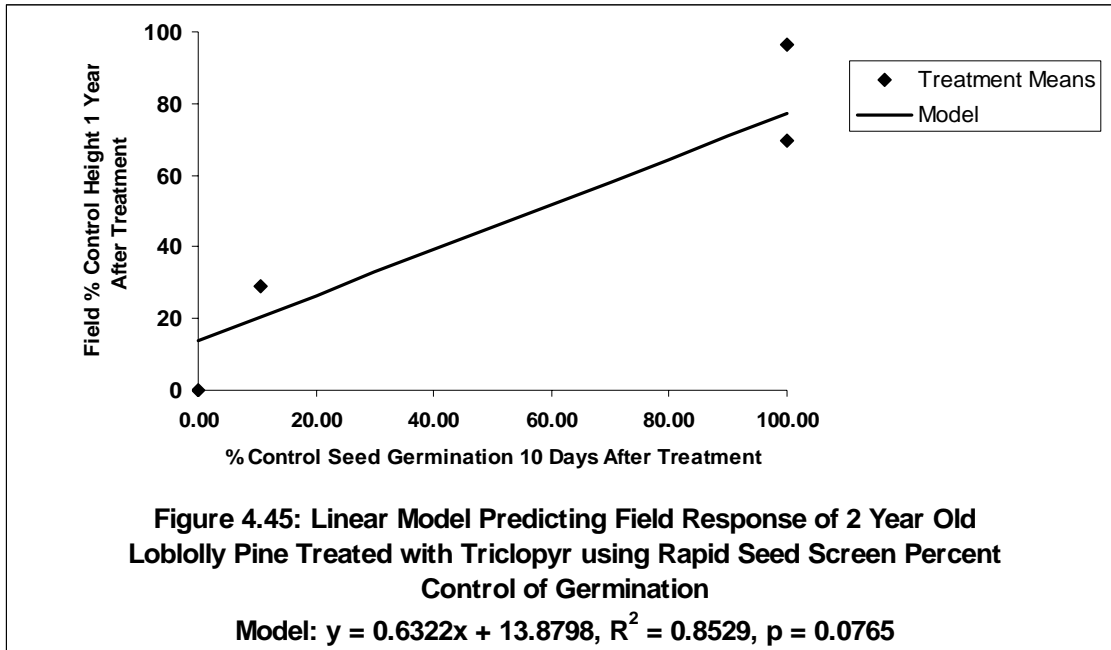
The model for predicting field effect code 1 YAT had a  $R^2$  value of 0.8803. Due to loblolly pine's tolerance to imazapyr, effect code can only be predicted up to 1 (Figure 4.43).



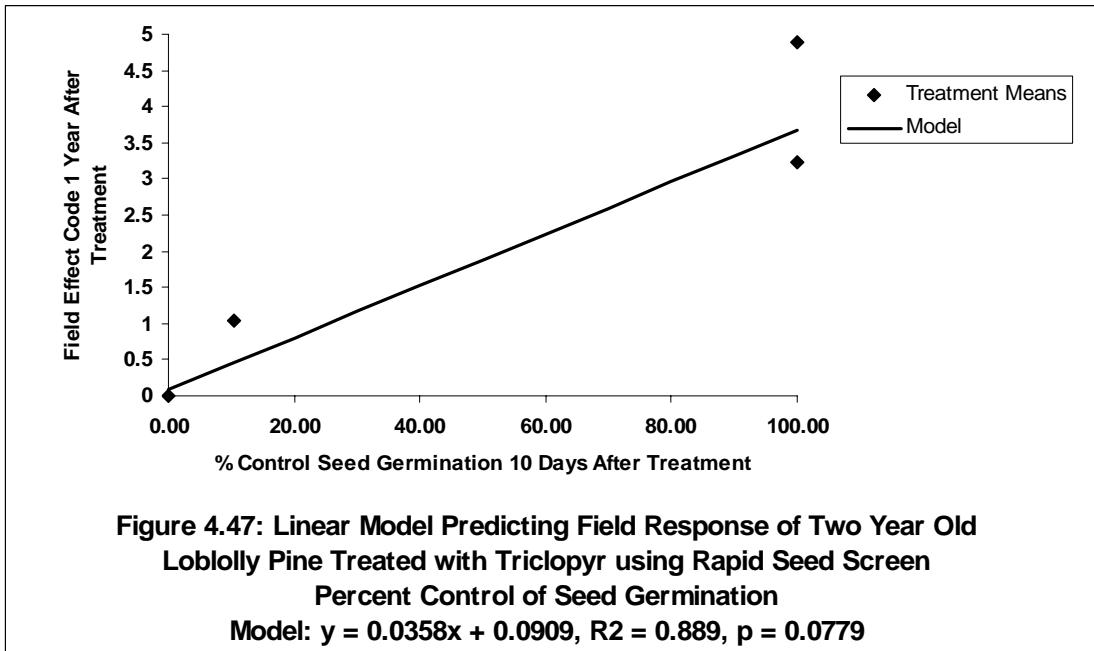
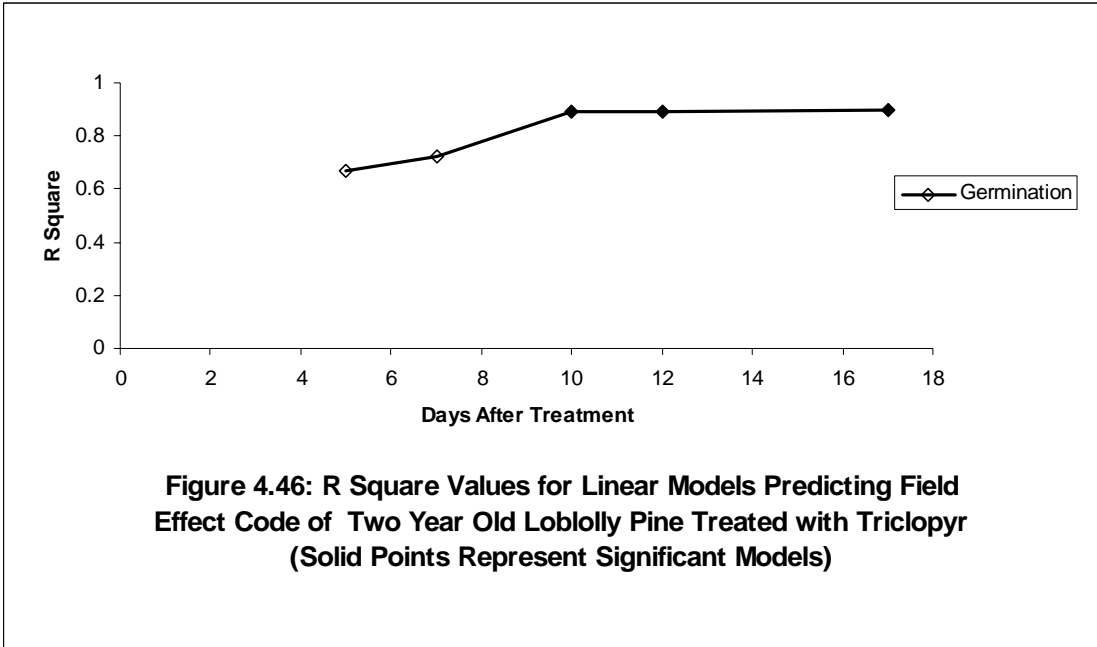
The triclopyr seed screen predicted percent control of height of two-year-old loblolly pine using percent control of seed germination as early as 10 DAT (Figure 4.44).



The model using percent control of seed germination data from 10 DAT had a  $R^2$  value of 0.8529 but could only predict up to 60 percent control of height 1 year after treatment for two-year-old loblolly pine treated with triclopyr (Figure 4.45).



The triclopyr seed screen was also effective in using germination data to predict field effect code. Three significant models were produced using data collected at the 10, 12, and 17 DAT intervals (Figure 4.46). Germination data collected at the 10 DAT predicted field effect code and produced a model with a  $R^2$  value of 0.899, the highest  $R^2$  observed for the germination data. The linear model for predicting effect code was capable of predicting effect code up to approximately 4 given the full range of germination control of 0 – 100 % (Figure 4.47).



Section 3: Leaf Area – Biomass Ratios and Dose Response Curves

This section will present results of the dose response curves captured in the two field screens and the second rapid greenhouse screen by species and chemical. Text, figures, and tables will relate the slope of the linearized dose response curves to the leaf area – biomass ratios calculated for each species in its’ respective age class.

*Three-Year-Old Plantation*

As previously stated, there was one less rate tested per chemical in the three-year-old plantation as compared to the other screens. Also, there was no control treatment available in the three-year-old plantation. This caused measurements to remain in mean height difference or percent change in height units. There were no true dose responses present in the three-year-old sapling screen.

*Two-year-old Plantation versus One-year-old Seedlings from Rapid Greenhouse Screen 2*

Leaf area – biomass ratios consistently and significantly decreased across species as age increased (Table 4.2). This shows that biomass increases at a faster rate compared to unit leaf area as the seedling matures and thus decreases the ratio.

*Table 4.2: Leaf Area – Biomass Ratios (sq cm / g) for Yellow-poplar, Sweetgum, and Loblolly Pine as influenced by age (t values for paired t test for significant difference within species)*

<i>Species</i>	<i>two-year-old</i>	<i>one-year-old</i>	<i>t value</i>	<i>Pr &gt; t</i>
Yellow-poplar	96.09	139.33	3.02	0.0126
Sweetgum	81.72	184.84	9.51	< 0.0001
Loblolly	33.25	45.2	4.69	0.0004

*Yellow-poplar*

One-year-old yellow-poplar consistently had a higher percent control of height by comparison to two-year-old yellow-poplar for the same rates (Figure 4.48). However, the rate at

which response changes across rates remained the same between the two age classes and is not significantly different as tested with the interaction term, ratio\*logdose (Table 4.3).

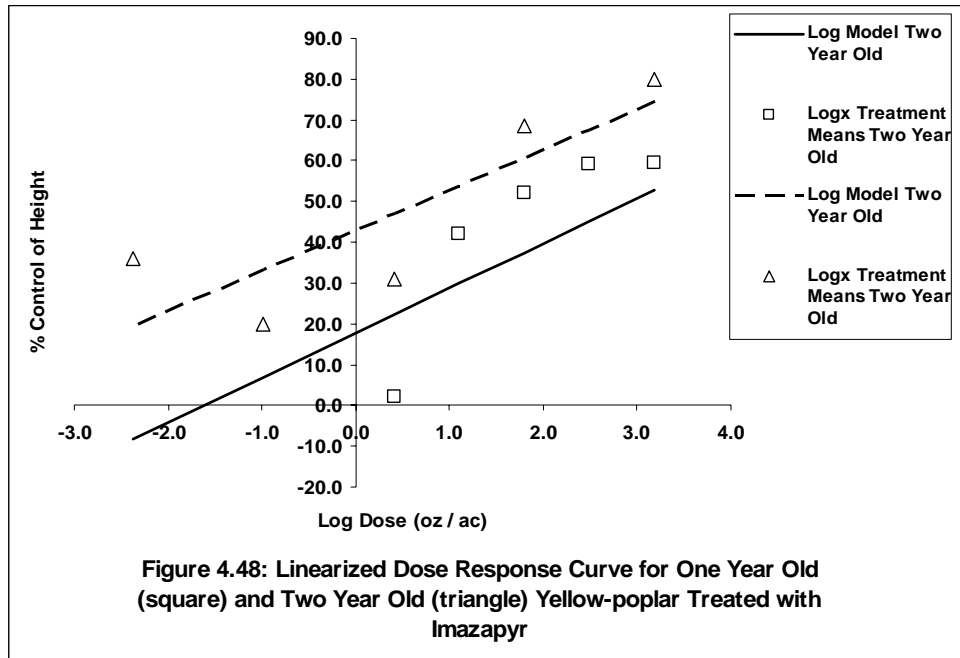
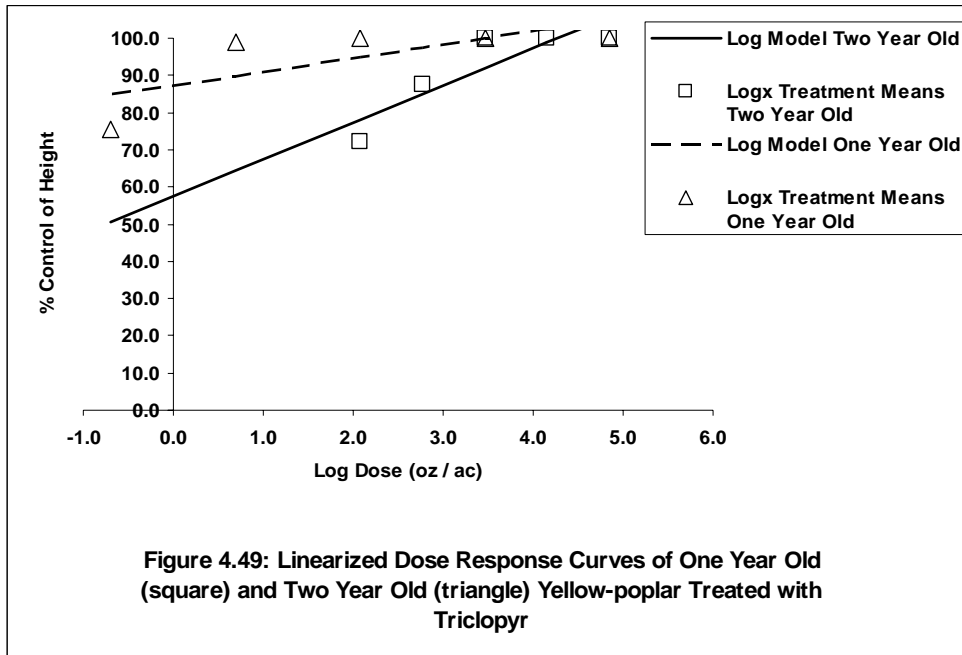


Table 4.3: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Yellow-poplar Treated with Imazapyr

Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	14.387	6.112	2.35	0.0197
logdose	1	23.01716	2.8419	8.1	< 0.0001
ratio	1	-11.492	6.887	-1.67	0.097
ratio*logdose	1	-0.5996	0.4685	-1.28	0.2021

Yellow-poplar treated with triclopyr responded differently than to imazapyr treatments. One-year-old seedlings had higher percent control of height at lower rates of imazapyr compared to the two-year-old sapling response (Figure 4.49). Dose response was more constant than the two-year-old saplings resulting in a more horizontal slope as compared to the two-year-old dose response. This difference in slopes, or interaction between the two variables, was significant at the  $p < 0.05$  level indicating a statistical significant difference in slopes (Table 4.4).



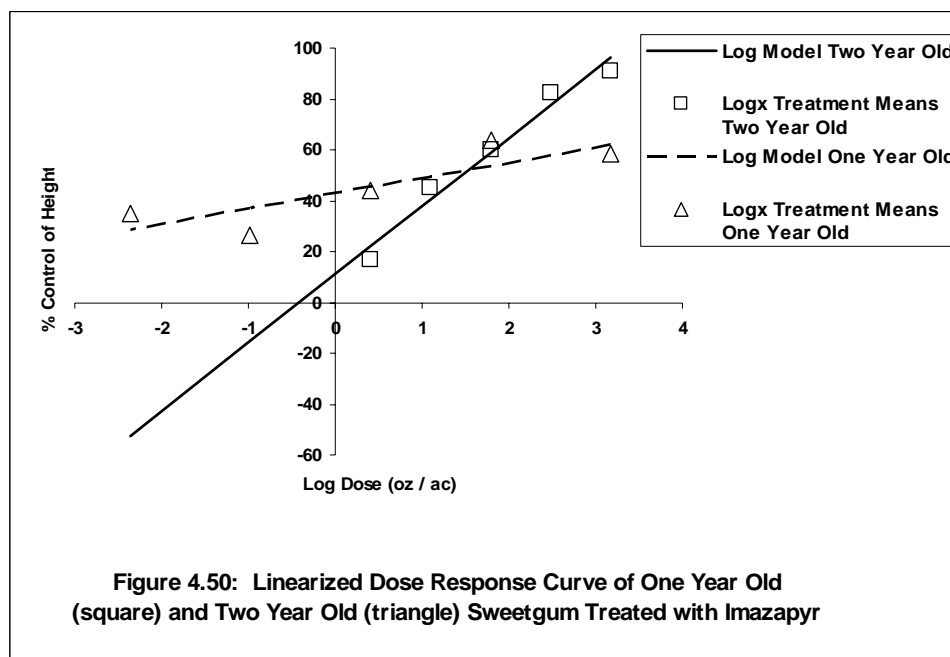


*Table 4.4: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Yellow-poplar Treated with Triclopyr*

Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	91.1418	4.137	22.03	< 0.0001
logdose	1	2.556	1.129	2.26	0.0249
ratio	1	-15.9533	2.738	-5.82	< 0.0001
ratio*logdose	1	0.11673	0.0354	3.3	0.0012

*Sweetgum*

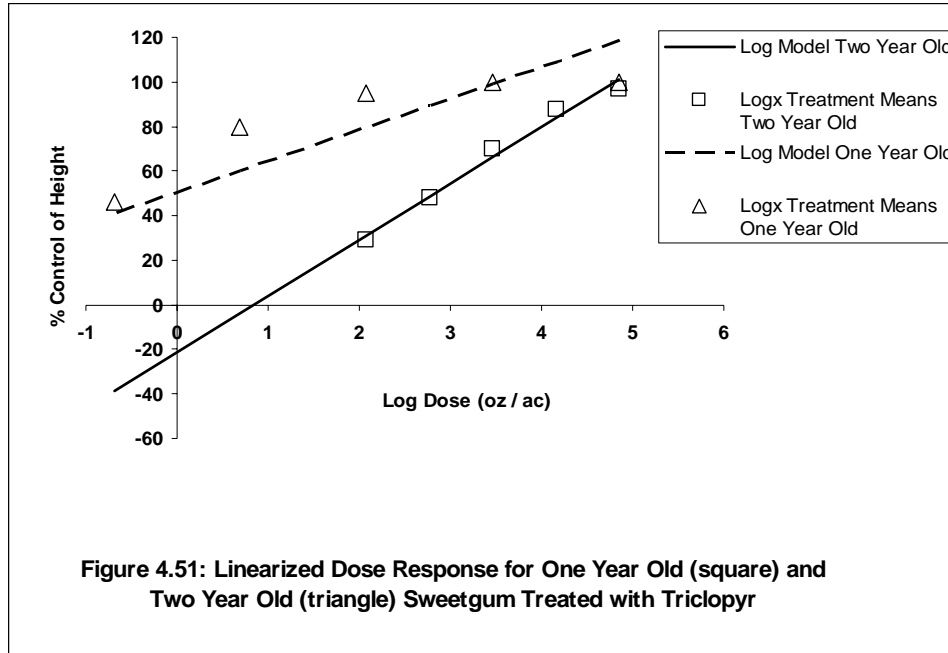
One-year-old sweetgum seedlings treated with imazapyr also exhibited a higher dose response rate at the lower rates of imazapyr as compared to the two-year-old saplings (Figure 4.50). At the second highest rate, 6 oz / ac, there was an increase in efficacy in two-year-old saplings that is greater than that of one-year-old seedlings. The two slopes were significantly different as two-year-old saplings have a higher rate of efficacy increase than one-year-old seedlings (Table 4.5).



*Table 4.5: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Sweetgum Treated with Imazapyr*

Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	40.104	5.771	6.95	< 0.0001
logdose	1	8.996	2.702	3.33	0.0011
ratio	1	-21.6558	6.703	-3.23	0.0015
ratio*logdose	1	1.194112	0.4474	4.34	< 0.0001

One-year-old sweetgum again showed higher dose response ratings, especially at the lower rates of triclopyr tested. As biomass increases per unit leaf area, there was a greater reduction in efficacy rating as treatment rate decreases compared to a lower biomass (Figure 4.51). The difference in rate of efficacy loss, or the difference in slopes of the two age classes (leaf area – biomass ratios), was significant at the  $p < 0.05$  level (Table 4.6).



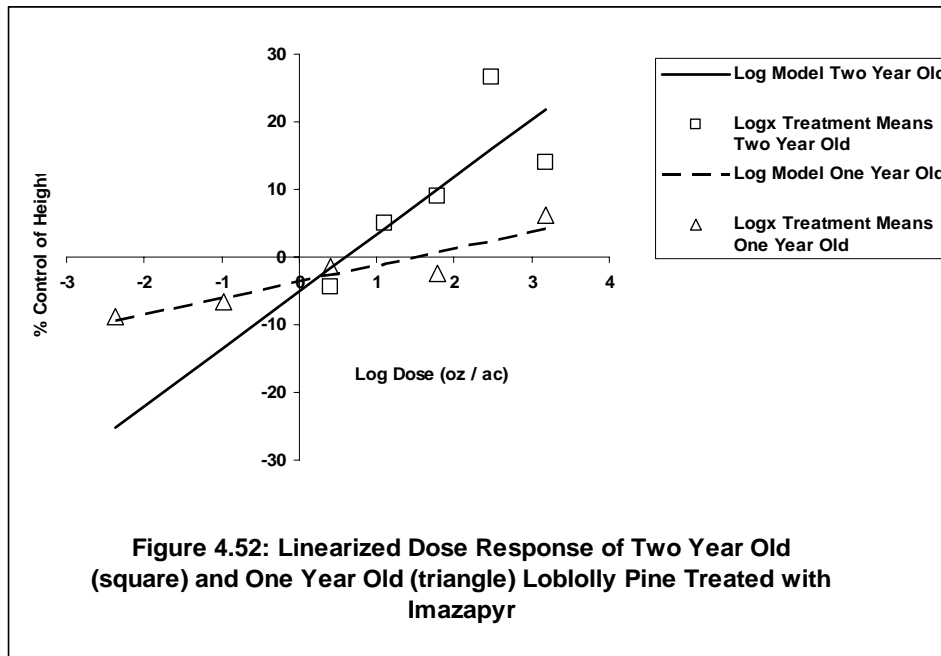
*Table 4.6: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Sweetgum Treated with Triclopyr*

Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	81.513	5.342	15.26	< 0.0001
logdose	1	4.8348	1.453	3.33	0.0011
ratio	1	-54.697	3.518	-15.55	< 0.0001
ratio*logdose	1	0.3859	0.0453	8.51	< 0.0001

### *Loblolly Pine*

There was a graphical difference in rate of response of two-year-old loblolly pine and one-year-old loblolly pine (Figure 4.52). Overall, one-year-old loblolly pine, with a higher leaf area biomass ratio, was not as greatly affected by imazapyr applications as two-year-old loblolly pine. It should be noted that the response variable, percent control of height, is influenced by the size of the one-year-old seedlings and the amount of height control they could have undertaken as compared to the two-year-old loblolly pine. Interestingly, the difference in slopes, or rate of efficacy rating increase, was deemed not statistically significant and therefore not different for

the two age classes (Table 4.7). It should be noted that the control response for each age class (which was 0 since percent control of the control is 0), was included in statistical analysis but not shown on the model figures (log (0) is undefined). The tolerance of loblolly pine to imazapyr may have contributed to these findings as well.

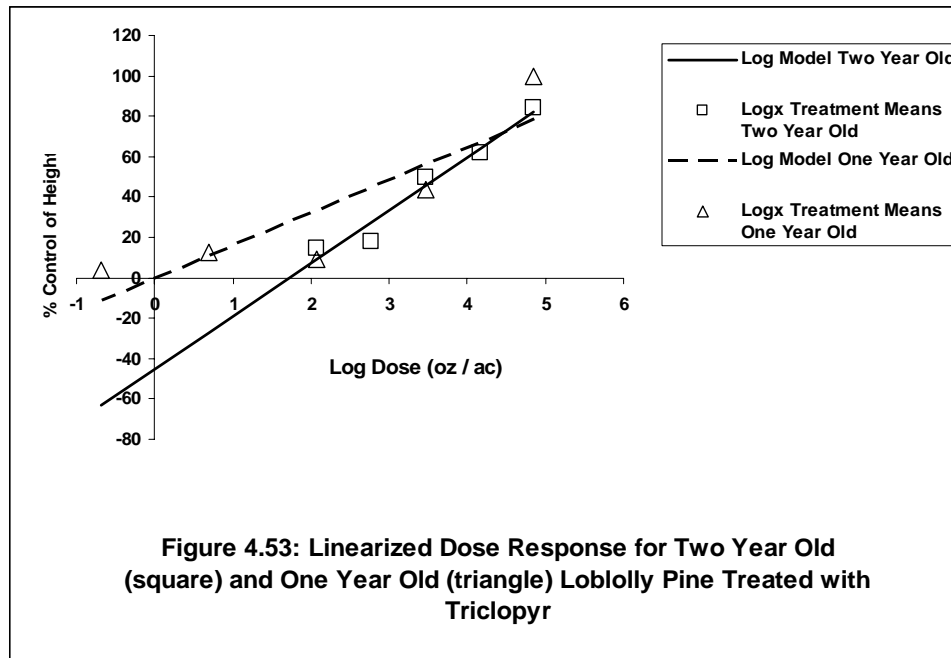


*Table 4.7: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Loblolly Pine Treated with Imazapyr*

Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	-5.729	4.279	-1.34	0.1824
logdose	1	3.649	1.99	1.83	0.0684
ratio	1	2.49	4.846	0.51	0.6077
ratio*logdose	1	0.273	0.3353	0.82	0.4161

One-year-old loblolly pine treated with triclopyr responded in a similar fashion as the two-year-old loblolly pine treated at the same rates (Figure 4.53). As rates continued to decrease, predicted response of two-year-old loblolly was lower than the response measure for

the one-year-old seedlings. This shows that there was a degree of positive control response at lower rates for the one-year-old seedlings (less biomass per unit leaf area) as compared to the negative control response for the two-year-old saplings (higher biomass per unit leaf area) for the same rates. This difference in response rate was significant at the  $p < 0.05$  level (Table 4.8).



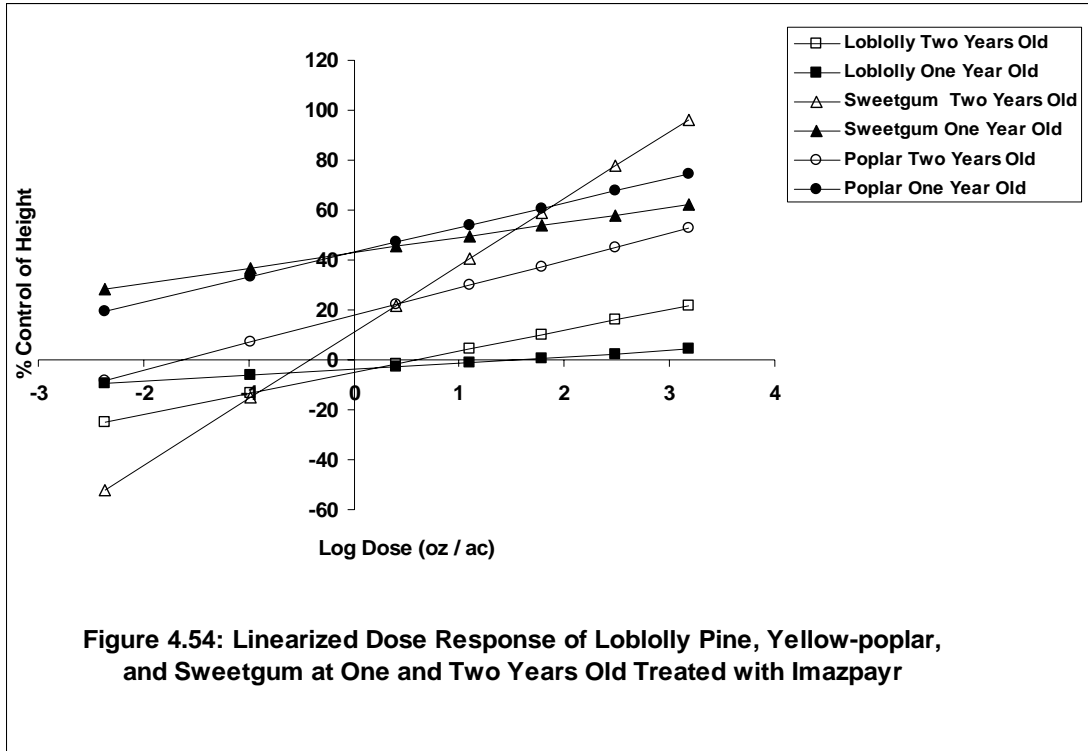
*Table 4.8: Parameter Estimates and t Statistics for Comparing Slopes of Dose Response Models for Loblolly Pine Treated with Triclopyr*

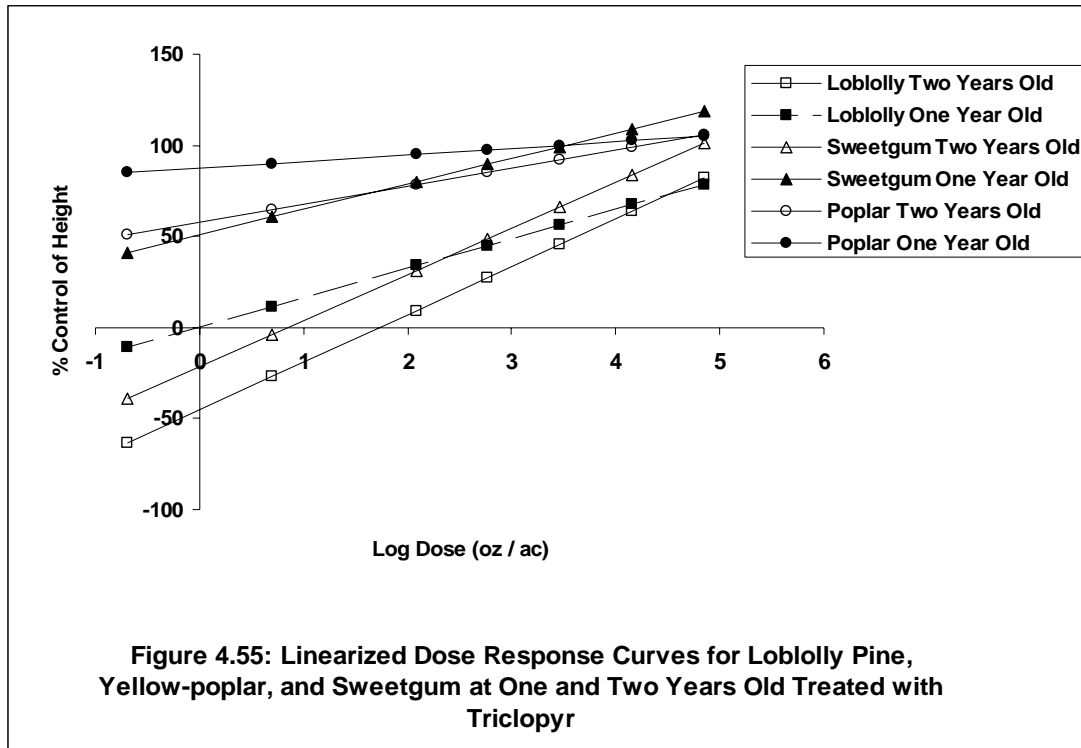
Variable	df	Parameter Estimate	Standard Error	t Value	p
Intercept	1	-60.401	8.763	-6.89	< 0.0001
logdose	1	32.112	2.382	13.48	< 0.0001
ratio	1	6.951	5.765	1.21	0.2296
ratio*logdose	1	-0.1485	0.073	-2.02	0.044

Comparison of Dose Responses across Species

Species differentiation was exhibited by comparing the dose response curves for both age classes for the three species examined (Figures 4.3.7 and 4.3.8). Imazapyr responses were not as separated as the triclopyr screens by comparison but still exhibited some differentiation. The triclopyr dose response curves showed a consistently higher response for yellow-poplar as

compared to the other species. Loblolly pine exhibited a consistently lower response overall as compared to the other species treated with triclopyr.



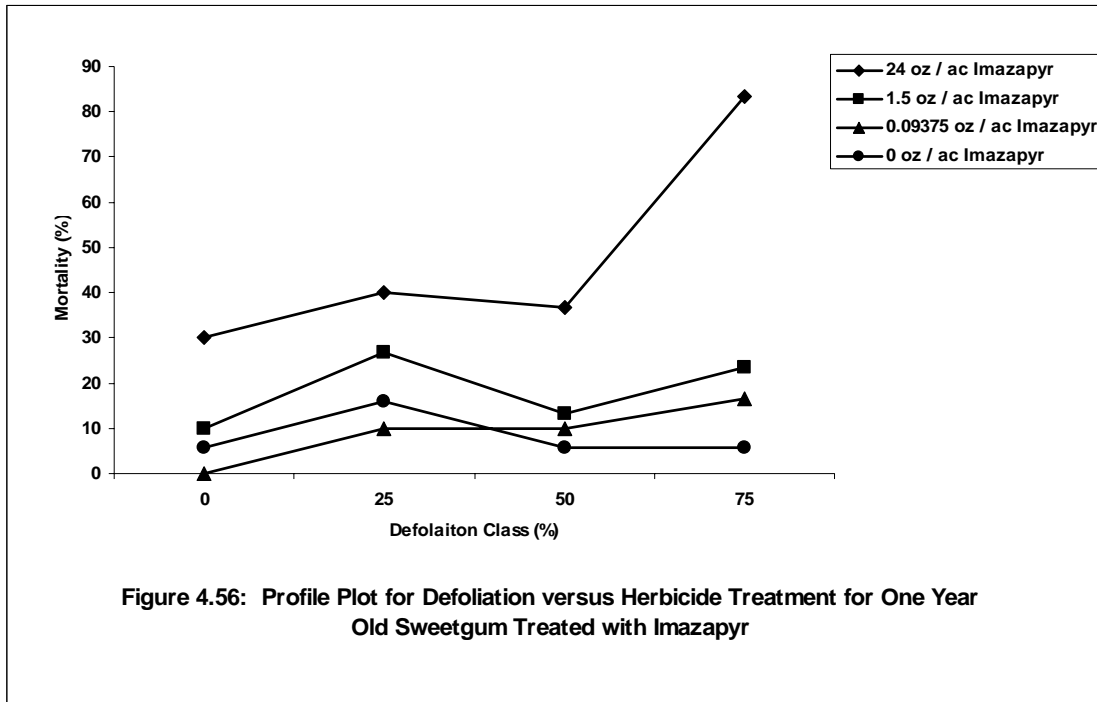


#### Section 4: Hand Defoliation Screen

Efficacy ratings for the hand defoliation screen were measured in height (cm) and mortality (%) and analyzed. Results using mortality ratings are presented here as it is potentially not as affected by pre-application condition as much as height and is representative of the response due to changes in leaf area.

#### *Sweetgum*

Percent mortality rates for sweetgum treated with imazapyr consistently remained lower than 30 % as defoliation increased for all treatments except the highest, 24 oz / ac (Figure 4.56). Mortality rates for this treatment were between 30 and 40 % for the first 3 levels of defoliation then approached 90 % for the highest defoliation class, 75 %. There was a significant interaction between percent defoliation and application rate (Table 4.9). The defoliation\*herbicide interaction was significant at the  $p < 0.05$  level. This significance indicates the presence of a reduced leaf area and herbicide rate interaction.



*Table 4.9: ANOVA for Testing Interaction of Defoliation and Herbicide Rate for Sweetgum Treated with Imazapyr*

Source	df	Type III SS	Mean Square	F Value	p
replication	2	0.7167	0.3583	2.64	0.0724
defoliation	3	1.9486	0.6495	4.79	0.0027
rate	3	10.45	3.4833	25.7	< 0.0001
d*r interaction	9	2.931	0.3256	2.4	0.0117

Sweetgum seedlings treated with triclopyr had consistent increase in mortality response as herbicide rate increased (Figure 4.57). The addition of the defoliation factor did not appear to affect the response rate until the 75 % defoliation class. This 75 % defoliation rate appeared to increase the response for the 8 oz / ac rate to approach the response for the 128 oz / ac treatment. As with one year old sweetgum treated with imazapyr, a significant interaction existed between herbicide and defoliation rates (Table 4.10).



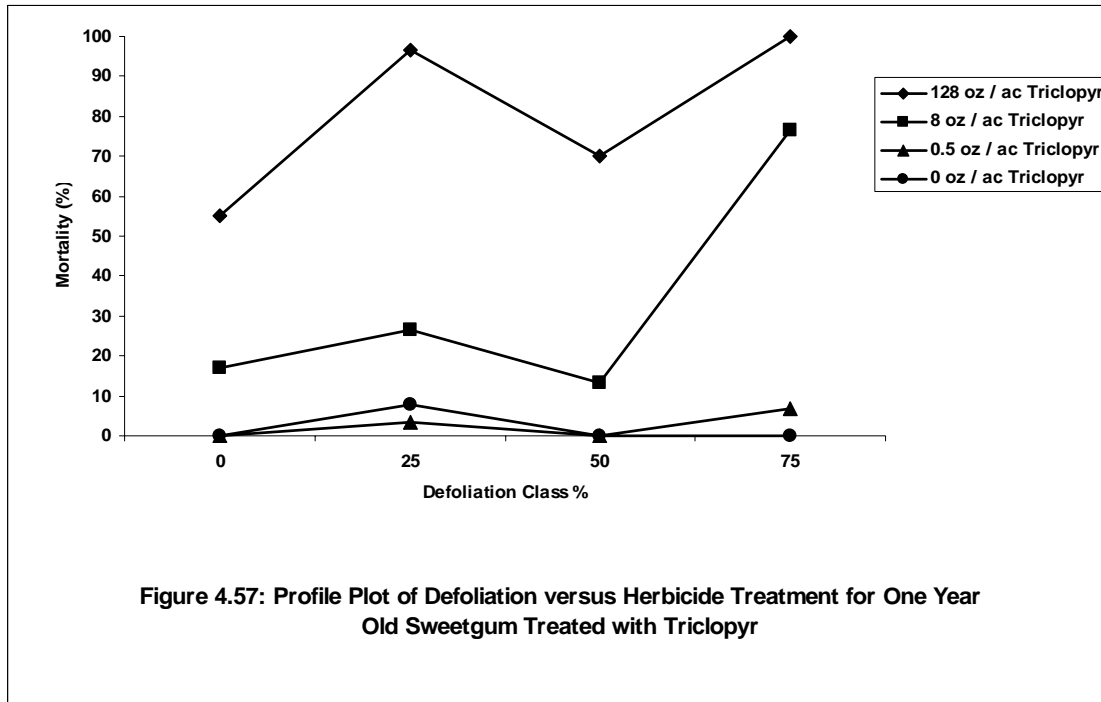


Table 4.10: ANOVA for Testing Interaction of Defoliation and Herbicide Rate for Sweetgum Treated with Triclopyr

Source	df	Type III SS	Mean Square	F Value	p
replication	2	0.1042	0.052	0.53	0.5892
defoliation	3	3.9812	1.32	13.5	< 0.0001
rate	3	40.06	13.353	135.8	< 0.0001
d*r interaction	9	5.305	0.5894	5.99	< 0.0001

### Yellow-poplar

As defoliation rates increased, the response rate for yellow-poplar treated with imazapyr increased for the highest two rates tested, 24 and 1.5 oz / ac, until defoliation reached 75 % (Figure 4.58). At that point, response for the two highest herbicide rates decrease. The lowest rate tested, 0.09375 oz / ac, resulted in a decrease in response as defoliation increased to 50 % then experienced a sharp increase in control at the 75 % defoliation level. The interaction between the herbicide rate and defoliation class was significant at the  $p < 0.05$  level (Table 4.11). This interaction appeared to be occurring mostly between the 1.5 and 0.09375 oz / ac rate (Figure

4.58). Interestingly, the effect of defoliation was not significant alone in the model tested as the observed F value is 0.43.

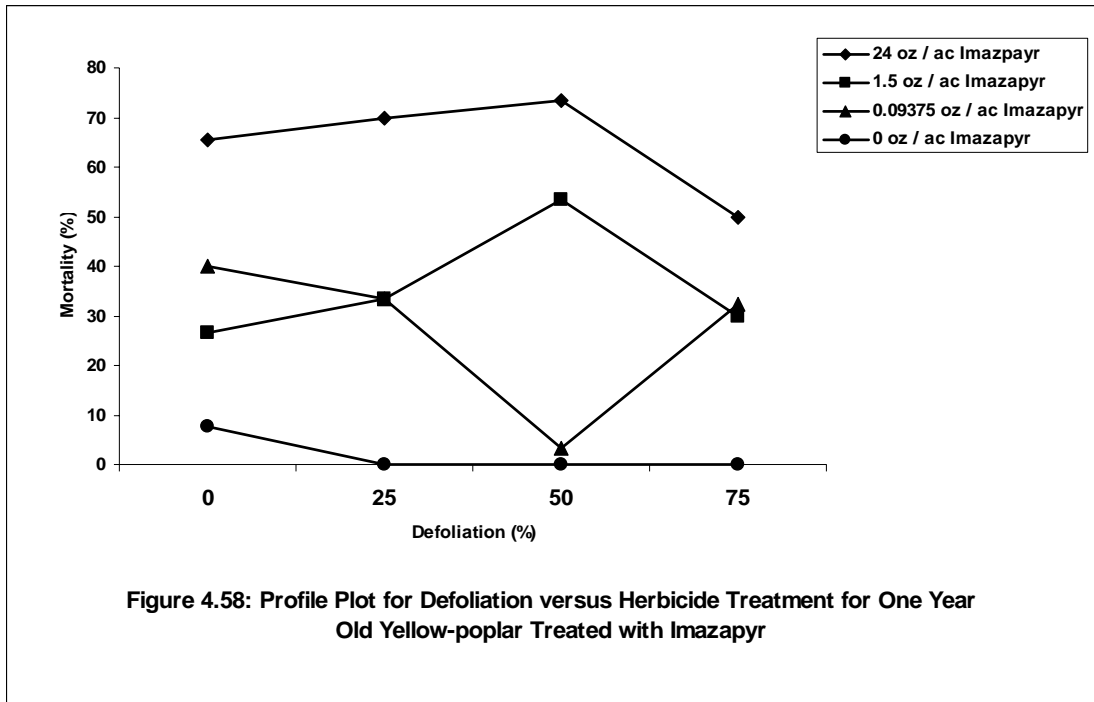


Table 4.11: ANOVA for Testing Interaction of Defoliation and Herbicide Rate for Yellow-poplar Treated with Imazapyr

Source	df	Type III SS	Mean Square	F Value	p
replication	2	0.1043	0.0521	0.27	0.7665
defoliation	3	0.2529	0.0843	0.43	0.7317
rate	3	14.633	4.877	24.87	< 0.0001
d*r interaction	9	4.383	0.487	2.48	0.0091

Response for sweetgum treated with triclopyr was affected by the interaction between herbicide dose and reduction in leaf area. The highest two rates of triclopyr tested, 128 and 8 oz / ac, had consistently high response rates as defoliation percentages increased (Figure 4.59). The lowest rate tested, 0.5 oz / ac, had a decrease in response as defoliation increased from 0 to 25 % and then experienced a sharp increase in response as defoliation reached 50 %. The herbicide control treatment realized little mortality as defoliation increased. The interaction between

defoliation and herbicide rate for the full model proved to be significant at the  $p < 0.05$  level (Table 4.12).

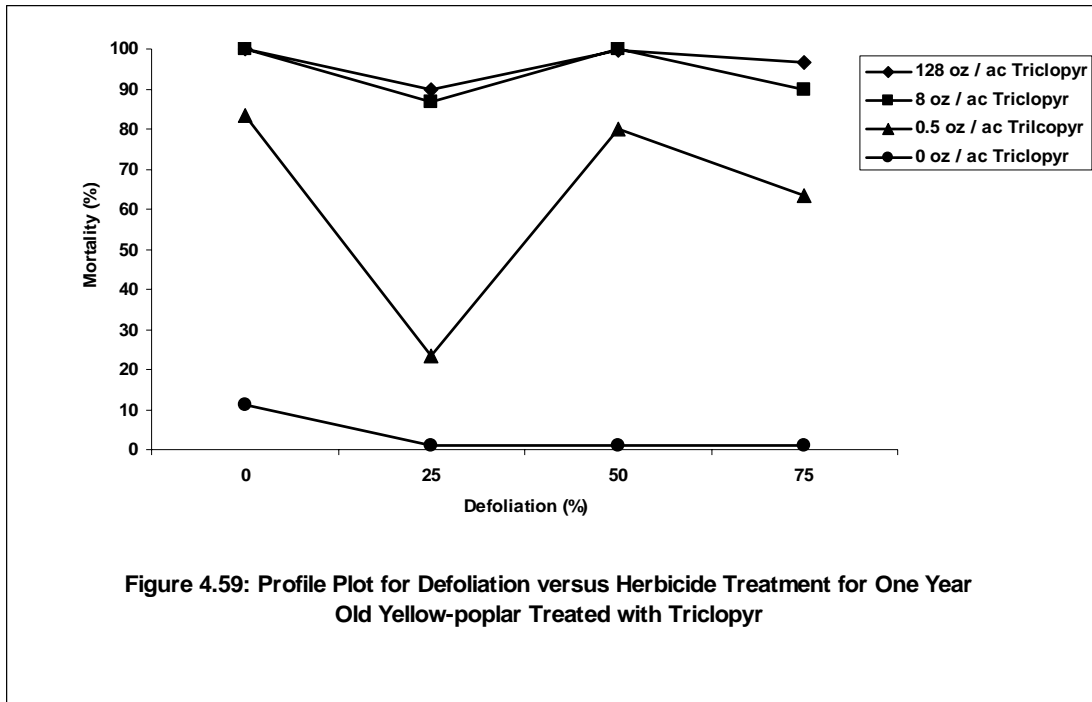


Table 4.12: ANOVA for Testing Interaction of Defoliation and Herbicide Rate for Yellow-poplar Treated with Triclopyr

Source	df	Type III SS	Mean Square	F Value	p
replication	2	0.0898	0.0449	0.54	0.5848
defoliation	3	2.577	0.859	10.27	< 0.0001
rate	3	28.175	9.3917	112.31	< 0.0001
d*r interaction	9	3.331	0.3702	4.43	< 0.0001

## **Chapter 5:** **Discussion**

### *Section 1: Prediction of Field Response using Rapid Greenhouse Screen*

#### *Three-year-old Plantation*

There were fewer significant models predicting field response of three-year-old saplings than the response of two-year-old saplings; a result of having only two overlapping data points in regressions involving RGS2 data. The three-year-old plantation was also trimmed to 3 feet to allow the sprayer to operate properly. The plantation was given 6 weeks to regrow but some saplings, especially green ash, did not recover as quickly from the trimming and had a much lower leaf area for herbicide uptake. This lower leaf area may have reduced efficacy and may have also influenced the degree to which predictions of field response could have been made.

The only model that predicted field response of green ash treated with imazapyr used RGS1 mortality to predict mean height difference (Figure 4.1), an important variable to forest and rights-of-way managers. The imazapyr rates tested for green ash screenings may have been too low, at  $x = 24 \text{ oz} / \text{ac}$ , to allow for a full rate response differentiation. Site preparation rates for woody plant control including green ash, in loblolly stands range from 24 – 40 oz / ac (BASFS 2000). The rapid greenhouse screen is capable of treatment response differentiation for green ash treated with imazapyr; however, rates tested here may not cover an adequate spectrum.

No model was produced that could predict efficacy of triclopyr applied to three-year-old green ash. Triclopyr is labeled for ash control in forestry site preparation at 4 – 8 quarts / ac (128 – 256 oz / ac), so it is unlikely that ash is tolerant to triclopyr. (Dow Agrosiences 1997). The rates tested may have been too low (128 – 0.5 oz / ac); however, large scale mortality was seen at the higher rates tested for triclopyr on the RGS1 and RGS2. It is more likely that the lack of significant prediction models was due to the effect trimming the green ash.

Response of three-year-old sweetgum treated with imazapyr was consistently predicted using RGS1 data. RGS1 data readily predicted mean height difference 1 YAT using effect code, percent mortality, and percent control of height as independent variables (Figures 4.2 – 4.4). Response rates for three-year-old sweetgum saplings treated with triclopyr could not be as readily predicted as imazapyr. This may be due to sweetgum's higher susceptibility to imazapyr compared to triclopyr. Miller (1990) showed an approximate 20 % increase in crown volume reduction and a 70 – 80 % reduction in root stock with a mid - summer application of imazapyr on sweetgum. As shown in Figure 4.51, the slope of a dose response curve for sweetgum treated with triclopyr increases from age 1 to age 2. The older (and larger) a sweetgum sapling gets, the faster the dose response (steeper slope) yet it takes more chemical to get the same response. This difference in dose response may affect the prediction of field response of sweetgum treated with triclopyr.

No models predicted field response of three-year-old loblolly pine treated with either imazapyr or triclopyr. Loblolly pine is very tolerant to imazapyr applications. Imazapyr applications at 2 and 4 lb / ai resulted in less than 3 % pine mortality and less than 14 % needle burn (Minogue et al 1985). This tolerance may confound screening results and may hinder regression modeling because so many doses had zero efficacy. The slope of the dose response lines tested here for loblolly treated with triclopyr increased as age increased (Figure 4.53).

Even given the limitations of the models for the three-year-old field screen described above, rapid greenhouse screens can still predict field efficacy in a relatively short period of time. More significant models predicting response of the three-year-old saplings would have been produced using RGS2, as with the two-year-old saplings, if rate titrations would have overlapped.

### *Two-year-old Plantation*

Yellow-poplar is susceptible to both imazapyr and triclopyr applications. Miller (1990) had a crown volume reduction of 100 % for imazapyr treatments and greater than 90 % for triclopyr, formulated as Garlon 4, for applications made in May, July, and September. These results indicate the ability for the two chemicals to control yellow-poplar which indicates the *potential* success of treatment response and field prediction using rapid greenhouse screening.

The field response of yellow-poplar treated with imazapyr was consistently predicted using log y data transformations. All models presented, whether using RGS1 or RGS2 data had a log transformation to fit the data. This log response shows the quicker response rate of the smaller seedlings as compared to the field grown trees as imazapyr rates increased. This can be further explained by examining the linearized dose response curve for yellow-poplar treated with imazapyr (Figure 4.48). The two parallel lines show that the one-year-old seedling consistently has a higher response for the same rate as compared to the two-year-old. The full range of field effect code 1YAT was also predicted using % necrosis at 8WAT. This was effective only when % necrosis is greater than 30 % (Figure 4.8) but shows the ability of the rapid greenhouse screen technique to quickly determine herbicide activity.

A linear relationship was predicted for two-year-old yellow-poplar treated with triclopyr using RGS1 % control of height and effect code, both 1 YAT, as independent variables to predict % control of field height. This shows a linear relationship in height control for two-year-old yellow-poplar and one-year-old yellow-poplar treated with triclopyr. RGS2 predicted a log relationship between the independent variable % necrosis 8 WAT and % control of height 1YAT and % mortality 1 YAT. The models incorporating RGS2 data appear to be predictive only when the independent variable response exceeds 80%, thereby limiting its potential (Appendix

Figures 1 and 2) The difference in relationships between the two independent variables (RGS1 and RGS2) may be due to the physiological differences in seedling batches used in the individual screens. Yellow-poplar seedlings in the RGS1 screen were more developed at the time of herbicide application than RGS2, possibly causing a response similar to the yellow-poplar field screen. The seedlings used in RGS1 were lifted at the end of the growing season, shipped to the research site, transplanted, and allowed to grow in controlled conditions for approximately 6 – 8 months until treated. The seedlings used in the second screen were lifted at the end of the growing season, stored in a cooler till shipped in mid-summer, transplanted into D40 pots when received, and allow to flush and grow in the greenhouse for only approximately 2 months and may have been stressed due to shipment and transplanting. Uniform physiological status of the greenhouse seedlings is necessary for consistent results in rapid greenhouse screens (Zedaker and Seiler 1989). Models were created using both screens; however, results and strengths of predictions varied.

Percent control of field height and effect code for two-year-old sweetgum treated with imazapyr was predicted by a linear model using RGS1 percent control of height as the independent variable (Figures 4.13 and 4.14). These linear models express the excellent treatment response differentiation over the rates of imazapyr tested and predicted the full response (0 – 100 % for height control and 0 – 5 for effect code). RGS2 consistently predicted the log response of two-year-old sweetgum treated with imazapyr (Figures 4.15 – 4.17). This difference in models between the two independent variables may again be explained by the physiological difference in the two seedlings batches used in the screens. The stress induced by shipping and transplanting the second batch of sweetgum seeds *may* have increased sweetgum's susceptibility to herbicide application. This increase in susceptibility *could* cause a quicker

response rate resulting in a log trend. Differences in response between the two rapid greenhouse screens may also have been caused by the time of year that each screen took place, the environments in which rotations occurred, or other non – herbicidal effects.

Field response of two-year-old sweetgum treated with triclopyr could only be predicted using RGS1 data. These models predicted the log response of percent control of height and effect code using percent control of height and effect code as independent variables (Figures 4.18 – 4.20). The difference in models between imazapyr and triclopyr may be attributed to the rapid expression of triclopyr symptomology. This rapid symptomology, most likely, is a function of the mode of action and formulation of the chemical. The amine formulation of imazapyr can be foliarly absorbed in 24 hours while the ester formulation of triclopyr, on the other hand, can be absorbed in 4 hours (Aherns 1994). The expression of symptomology for imazapyr is very gradual as meristematic regions become chlorotic and are slowly followed by leaf necrosis (Aherns 1994). Total plant control occurs very slowly by impeding plant growth followed by death of meristematic regions (Bell 1997). Triclopyr is readily translocated and symptomology is almost immediate with signs of stress occurring as quickly as 24 hours after treatment. Complete death from triclopyr applications may occur as soon as 3-5 weeks after treatment. This quick response of young seedlings treated with triclopyr, especially under transplant stress, *may* explain the log response in the model.

Response of two-year-old loblolly treated with imazapyr was predicted using effect code measured in RGS2 (Figure 4.21). This was surprising considering loblolly pine's tolerance to imazapyr (Minogue et al 1985). The scales on the two axis are similar as small changes in effect code with increasing dose allowed for the prediction of field response and subsequent tolerance.



RGS1 and RGS2 were successful in predicting field response of loblolly pine treated with triclopyr. All models were linear regardless of independent or dependent variables. Two of the most interesting models use % necrosis 8WAT (Figure 4.25) and % control of height 1YAT (Figure 4.27) to predict field % control of height. These two models have a near perfect linear relationship as  $R^2$  values are 0.9855 and 0.9728, respectively. The independent variable % necrosis 8WAT is successful due to triclopyr's quick time to visual symptomology and the response differentiation by rate for both field height and seedling leaf necrosis. Loblolly pine is susceptible to triclopyr applications in the field (Miller 1990) and spectrum of height control in the field is well correlated with greenhouse screens (Zedaker and Seiler 1989).

The models presented above show the ability of the second rapid greenhouse screen to adequately predict field response of the two-year-old woody species tested. The models for the two-year-old screen, combined with the three-year-old field screen, show the adequacy of the rapid greenhouse screening technique as a method to determine initial activity and control response of species in question. Regardless of parameters or model transformations used, or at what time parameters were measured, the significant regressions show the ability for this screening technique to predict traditional field screens and can enable herbicide developers to streamline research options in a cost and time effective manner.

### Section 2: Effective Woody Plant Seed Screening Techniques and Prediction of Field Response Using Rapid Seed Screen

Several important observations can be made based on the results of the rapid seed screening. Seed germination is inhibited by the use of a non-ionic surfactant in the mixes. Removal of surfactant from the screen increased germination and survival of all seeds tested. Seeds were extremely susceptible to fungal infestations during the growth germination phase. This was caused by the constant wet environment the seeds are exposed to. The Banrot®

treatment 24 hours prior to the herbicide treatment appeared to alleviate this problem while having no apparent antagonistic effect on the herbicide treatment. The last screen performed included the Banrot treatment and the removal of surfactant and resulted in higher success rates than the previous screens. Watering levels are also critical to decrease the amount of fungal growth. Maintaining a constant humidity in the chamber during screening helps to reduce the rate of seed drying; however, it is still critical to monitor watering levels. In the case of the final screen for all species tested, the watering regime consisted of 1 – 2 mL of water per petri dish every other day. It is critical to water each petri dish the same to keep the integrity of the replications while minimizing over watering.

Using 10 mL of herbicide solution per Petri dish may have been too high of an application rate. Triclopyr screens were not as successful as imazapyr screens which indicates chemical selectivity; yet, it may be possible to decrease the rate of application and still maintain the success rate of imazapyr and increase the success rate of triclopyr. There were also different success rates for different species which also indicates the selectivity of the seed screen on a species basis.

### *Green Ash*

At low doses, the green ash – imazapyr seed screen resulted in a tissue growth and germination stimulation response (Figures 4.28 A and 4.29 A). This stimulation effect, called hormesis, is common in toxicology and is defined as the presence of a modest stimulation of response occurring at low rates and inhibition of response occurring at higher rates (Calabrese and Baldwin 2003). It is not known why the response for the 0.375 oz / ac treatment did not respond as well as the 0.09375 oz / ac in terms of growth stimulation, although it did mimic the control response for germination (Figure 4.29 A). There was the presence of treatment

differentiation at the end of the seed screen as the highest rate tested, 24 oz / ac, provided the highest degree of tissue and germination inhibition with lower rates providing less. This shows the ability of a rapid seed screen with green ash using imazapyr to determine initial probable herbicide activity and provide information to make the decision on whether or not to continue the screening process in the greenhouse.

The green ash-triclopyr seed screen resulted in a high degree of germination inhibition and subsequent tissue growth (Figures 4.28 B and 4.29 B). This difference in response by comparison to the imazapyr screen may be attributed to the difference in modes of action. Triclopyr, an IAA transport inhibitor, can easily affect seed growth by inhibiting the transport of IAA across cell walls which is crucial for seed tissue development. Imazapyr; however, is an ALS inhibitor and does not immediately inhibit cell elongation, growth, and transport of growth hormone; thus resulting in higher seed germination rates. It is not clear if lower rates of triclopyr would increase success rates of a seed screen with green ash. Unfortunately, no regressions could be run predicting field response of green ash due to the lack of rate overlap between the seed screen and the three-year-old plantation screen.

#### *Yellow-poplar*

The yellow-poplar – imazapyr screen also had a hormetic response observed with the 0.09375, 0.375, and 6 oz / ac treatments for mean tissue growth by the last day of the screen (Figure 4.30 A). The germination response was stimulated above the control for all rates except the highest (24 oz / ac) (Figure 4.31 A). The scale of the response must be observed in order to keep the results in context. Mean tissue length ranged from 0.5 to 4.5 mm while germination never passed 16 % (control treatment only germinated to a 10 % level by 21 days). It appears that the success of a yellow-poplar seed screen is dependent on the ability to effectively

germinate seeds. The stratification process and treatment methods should be examined to increase success rates. Studies have shown that soaking seeds in a gibberellin solution can decrease stratification times and using the excised embryo method may increase germination (Young and Young 1992). Germination of yellow-poplar seeds is often low and may be due to the naturally low proportion of filled seeds to empty seeds common to yellow-poplar (Bonner 2004). Empty seeds were not removed for this study which would have severely decreased the germination rates. When empty seeds are removed from germination tests, germination rates can approach 80 – 90 % (Bonner and Russell 1974).

The triclopyr seed screen effectively inhibited germination and subsequent tissue growth of yellow-poplar seeds (Figures 4.30 B and 4.31 B). As with the green ash screen, it is unclear if a lower spectrum of rates could be used to increase the success of a rapid seed screen. The Miller 1990 study used a 2 % rate of Garlon 4 that resulted with at least a 95 % reduction in crown volume. This rate, which is equivalent to 51.2 oz / ac for a 20 GPA application, should be considered as the high – end starting rate for titrations in a seed screen involving yellow-poplar seeds. There were no significant regressions established to predict field response of two-year-old yellow-poplar treated with either imazapyr or triclopyr. This may be due to the low germination success for the imazapyr screen and the almost complete lack of germination and growth for the triclopyr screen.

### *Sweetgum*

A hormetic response occurred for the lowest rate of imazapyr tested (Figure 4.32 A). By the end of the screen, the rate titration for the remaining four rates exhibited consistent negative responses by rate. Germination percentages were also affected by lower rates as the 0.09375, 0.375, and 1.5 oz / ac rates resulted in higher germination percentages than that of the control.

This hormetic response does not appear to affect the ability of the sweetgum – imazapyr seed screen to predict field response of two-year-old saplings as percent control of seed tissue and percent control of germination predicted both percent control of height and effect code, both 1 YAT, for two-year-old sweetgum saplings treated with imazapyr. The earliest point in the seed screen that percent control of tissue can predict percent control of field height is 7 DAT; however, a stronger model was produced at the 10 DAT interval (Figure 4.36). This is because of the response separation that occurs between the 7 and 10 DAT intervals (Figure 4.32 A). After the 10 DAT interval, response differentiation does not increase to the point to strengthen the model as  $R^2$  values remained consistent to that of the 10 DAT model. Percent control of germination as an independent variable produced a weaker model to predict height earlier than did percent control of seed tissue. This may be due to the non-declining value of germination. Once a seed germinates, it stays germinated. This type of response may begin to level out and not be the best to predict field response. Prediction of effect code was best at the same time intervals using seed data as compared to prediction of field height control (10 DAT for seed tissue data and 12 DAT for germination data). These models also had  $R^2$  values comparative to those of the prediction of % control of height models. This shows the effectiveness of the effect code parameter to differentiate in visual response measurements as well as a quantitative variable such as height.

There was complete inhibition of germination and growth of sweetgum seeds treated with triclopyr. This difference in results between the two chemicals tested on sweetgum may, again, be explained by mode of action and dosage. The IAA transport inhibition mechanism inhibited the transport of growth hormone during the developmental phase of seed tissue inside the seed and prevented any response. The rate spectrum tested should be lowered to determine if a

triclopyr seed screen can be performed or if any rate, no matter how low, can inhibit sweetgum germination. There were no significant models produced to predict field efficacy of triclopyr for sweetgum due to the nondifferentiation of response ( $x = 0$ ).

### *Loblolly Pine*

Loblolly pine was the only species tested in the seed screen that did not exhibit a hormetic response for mean tissue length. There was a consistent reduction in response as rates increased from 0 to 24 oz / ac (Figure 4.34 A). Even though loblolly pine is tolerant to imazapyr, imazapyr applications can still stunt growth of field grown saplings (Quicke and Lauer 1993). This response is observed in the seed screen. Germination rates were not inhibited to the degree as the other species. All rates tested resulted in nearly 100 % germination percentages (Figure 4.35 A).

Only one model was produced using rapid seed screen data to predict field response of two-year-old loblolly pine treated with imazapyr. The model predicted field effect code, the same parameter predicted in the greenhouse screens, using percent control of seed tissue at the end of the seed screen (Figure 4.43). This model only predicted values of effect code less than 1. This model predicted visual symptomology akin to treatment tolerance, since the value of effect code = 1 is “slight chlorosis of needles”. This model would be of little use since seed tissue control covers the entire range of values (0 – 100 %) to predict field tolerance.

The loblolly – triclopyr seed screen was the only triclopyr seed screen performed in which lower rates exhibited a measurable response. The three lowest rates, 0.5, 2, and 8 oz / ac, exhibited treatment differentiation for both seed tissue and seed germination (Figures 4.34 B and 4.35 B). It is not clear why loblolly had higher response rates to triclopyr treatments compared

to hardwood responses. There may be a physiological difference between gymnosperm and angiosperm seeds that needs to be investigated.

A linear model was created to predict % control of height 1 YAT for two-year-old loblolly pine treated with triclopyr using % control of seed germination as the independent variable. Data collected at the 10 DAT interval provided a model with a  $R^2$  of 0.8529. The use of later germination data did not significantly increase the strength of the regression (based on  $R^2$  and p values). Responses for the triclopyr after the 10 DAT were at their highest level (80 – 90%) for the entire screen (Figure 4.35 B). This data was similar to the field response of the two-year-old loblolly screen in that a higher degree of control was observed with the highest triclopyr rate tested while lower rates had little to no effect. Percent control of germination data predicted field effect code 1 YAT. Seed data collected at 10 DAT was used to create a model that could predict the full range of effect code in the field (Figure 4.47). This model shows the treatment means in which the drastic differences in response by rate is evident thus allowing a significant model to be produced for triclopyr, as opposed to the other species tested.

The rapid seed screening technique determined initial activity and species spectrum in an extremely short period of time. Time to initial treatment effect occurred anywhere from 5 to 16 days after treatment. This is extremely important to those in the herbicide development industry. It can be used to make decisions on future research requirements and protocols for a certain compound by performing a rapid seed screen, utilizing minimal active ingredient as compared to a traditional field screen, and provide results in as little as 5 days after treatment. This screening technique, in combination with the rapid greenhouse screen, may be used to streamline development decisions in a cost and time effective manner. This may move researchers to re-evaluate existing chemistries or investigate new chemistries for woody plant vegetation

management since it is now possible to do initial activity and species spectrum research in a short period of time.

### Section 3: Leaf Area – Biomass Ratios and Their Effect on Herbicide Efficacy

Leaf – area biomass ratios decreased as seedlings progressed from one-year-old greenhouse seedlings to two-year-old field planted saplings (Table 4.2). This coincides with the previous studies that show the biomass partitioning changes over time (Madgwick 1977).

#### *Dose Response Curves and Leaf Area Biomass*

One-year-old yellow-poplar was affected by imazapyr in the same manner as the two-year-old saplings. The overall efficacy rating for the one-year-old seedlings was higher but the rate at which control increased remained the same between the two age classes (Figure 4.48). Poplar only a 26 % reduction in the leaf area - biomass ratio as the plant matured. The change in physiology between the two age classes is small and may not be sufficient to affect the efficacy of imazapyr.

The leaf area – biomass ratios were based on total above ground biomass. The majority of above ground stem biomass is composed of dead cells while only a small percentage of the stem biomass is living tissue (sapwood). Studies have shown that there is a consistent positive linear relationship between the leaf area – sapwood area relationship within species; however, large differences exist between species (Kramer and Boyer 1995). This large difference across species may influence the efficacy of certain compounds across species as it is the living tissue being controlled.

Sweetgum showed a significant difference in dose response slopes between the two age classes for treatments of imazapyr while at the same time experiencing a 55 % decrease in its leaf area – biomass ratio as it matured. Responses for the one-year-old seedlings did not increase



at the same rate as the two-year-old response and was actually lower for the highest rate tested than the two-year-old saplings. This shows that at the lower rates tested, imazapyr had lower efficacy in the saplings with a lower leaf – area biomass ratio as compared to the higher biomass ratio which had higher response ratings for the same rates. As rates of imazapyr increased, levels of control for the higher ratio saplings increased to that above the small seedlings, whose control ratings increased but not at the rate of the saplings.

Loblolly treated with imazapyr also failed to test for significant slopes in dose response curves across the two age classes. The figure; however, shows a trend in which the slopes appear different. As stated before, the tolerance of loblolly pine to imazapyr may have confounded the response.

All species screened with triclopyr showed the same pattern of response between age classes (Figures 4.49, 4.51, 4.53). These slopes between age classes for each species were significantly different. Overall control was higher at the lower rates of triclopyr for the younger seedlings with higher biomass ratios. As herbicide concentration increased, response of the two-year-old sapling consistently increased to match that of the one-year-old seedling treated at the same highest rate. The three relationships by species presented showed a decrease in efficacy at lower rates of triclopyr tested for the plants with the lower leaf area – biomass ratio as compared to the plants with the higher leaf area – biomass ratios but efficacy increases as concentration increases so response of the two-year-old saplings is similar to that of the one-year-old saplings. In essence, one needs more to control more.

Comparing the linearized dose response curve across species can give an indication of the difference in response by species (Figures 4.54 and 4.55). For species treated with triclopyr, there is a consistent lower response rate across species at the lower rates for the two-year-old

saplings compared to the one-year-old seedlings. Species differentiation also is present as both age classes of yellow-poplar are more susceptible at the lowest rate tested, indicating the need to adjust rate spectrums to be species specific. Two-year-old sweetgum and loblolly pine are least susceptible at the lowest rate tested. All responses approach the same point at the highest rate tested, regardless of age class. This indicates that rates tested for triclopyr may be approaching the upper limit of response.

This study and the Miller 1990 study are consistent in that the degree of control for the three species by each chemical is similar. Miller evaluated complete rootstock control for the same three species tested here and triclopyr ester at a 2 % v/v solution and imazapyr amine at 0.5 % v/v solution. The results for the July application of the Miller study can be ranked by degree of control (Table 5.1). Dose response results for the rapid greenhouse screen study can be grouped and summarized by rankings as well (Table 5.1). There are some similarities to the two rankings of efficacies. In Miller's study; however, sweetgum rootstocks were very susceptible to imazapyr applicaitons as compared to the rapid screen study. Overall, the results from both studies are comparable indicating the ability for the rapid greenhouse screen to predict initial herbicide activity. These results, as well as the time to significant treatment effect and strength of regression models produced with the rapid seed screens, show that the entire rapid screen process may be used as a dichotomous key for a more inexpensive primary screen for woody plant herbicide research and development. This dichotomous key would allow for the question presented in the beginning of this thesis (Should screening continue to the next stage or be terminated?) to be answered in a cost and time efficient manner.

*Table 5.1: Comparison of Efficacy of Imazapyr and Triclopyr for Three Species of Interest*

	Miller 1990		RGS 2	
Rank	Species	Chemical	Species	Chemical
1	Sweetgum	Imazapyr	Yellow-poplar	Triclopyr
2	Yellow-poplar	Imazapyr	Sweetgum	Triclopyr
3	Yellow-poplar	Triclopyr	Yellow-poplar	Imazapyr
4	Loblolly Pine	Triclopyr	Sweetgum	Imazapyr
5	Sweetgum	Triclopyr	Loblolly Pine	Triclopyr
6	Loblolly Pine	Imazapyr	Loblolly Pine	Imazapyr

This study is also comparable to the efficacy matrix utilized in the Chemical Expert Silvicultural System (ChESS) (Zedaker 1992). ChESS uses data compiled from research and uses this database to make herbicide prescription recommendations. Although the comparison is not identical, there is a consistent pattern when comparing the efficacy of RGS 2 (average of 5 rates) and ChESS (highest labeled rate) (Table 5.2). The ChESS system utilized a broader data set to obtain control values by comparison to the RGS2 control values. This explains the differences in control values as the ChESS data is more robust.

*Table 5.2: Comparison of Efficacy Data between Rapid Greenhouse Screen 2 and ChESS*

ChESS				RGS2			
Rank	Species	Chemical	Percent Control	Rank	Species	Chemical	Percent Control
1	Yellow-poplar	Triclopyr	95	1	Yellow-poplar	Triclopyr	95
1	Sweetgum	Imazapyr	95	2	Sweetgum	Triclopyr	85
2	Yellow-poplar	Imazapyr	85	3	Yellow-poplar	Imazapyr	47
2	Sweetgum	Triclopyr	85	4	Sweetgum	Imazapyr	45
2	Loblolly Pine	Triclopyr	85	5	Loblolly Pine	Triclopyr	34
3	Loblolly Pine	Imazapyr	20	6	Loblolly Pine	Imazapyr	-2

#### *Hand Defoliation – Herbicide Interactions*

The sweetgum screen for defoliation classes and herbicide rates for imazapyr produced different results than that of the yellow-poplar screen for imazapyr. The two low rates, 1.5 and 0.09375 oz / ac, exhibited low response rates (< 30 % mortality) across all the defoliation

classes. The highest rate, 24 oz / ac, exhibited a consistent response of 30 – 40 % mortality until the highest defoliation class, 75 % (Figure 4.56). This can be explained by the high dose rate being taken up by a lower leaf area but still having a high enough concentration of active ingredient to translocate to the site of action. The lower rates of imazapyr did not have a high enough concentration at application to provide enough material for uptake and translocation to be effective in controlling one-year-old sweetgum.

The yellow-poplar – imazapyr screen had a decrease in efficacy for the highest rate at the highest defoliation class. The lower two rates experienced relatively the same amount of control at the 0 and 25 % defoliation class then a sharp increase in efficacy in the 1.5 oz / ac rate and a sharp decrease in the 0.09375 oz / ac rate. The interaction between herbicide rate and defoliation rate appears to lower the efficacy of the lower rate of herbicide applied to a reduced leaf area to control the same biomass. The two lower rates of herbicide have similar response rates at the 75 % defoliation class. It appears that this large reduction still leaves enough leaf area (yellow-poplar leaves are relatively larger than sweetgum) to provide 30 % mortality for a given biomass with rates 1.5 oz / ac and lower.

The two triclopyr screens exhibited contrasting results as well. Sweetgum had an overall lower response rate across defoliation classes than yellow-poplar (Figures 4.57 and 4.59 respectively). The two low rates and control treatment exhibited responses lower than 20 % mortality for the 0 – 50 % defoliation classes while the highest rate of triclopyr maintains a 50% and greater mortality rate for the same defoliation classes. At the 75 % defoliation rate, an increase in efficacy is observed with the 128 and 8 oz / ac rates, signaling that there is enough active ingredient for uptake and translocation to provide control. It is unclear why the lower rates of defoliation did not receive the same amount of control for the lower two rates as the 75 %

defoliation. It is possible that there is enough remaining leaf area to continue photosynthesis and sugar production to counteract the lower rates of herbicide until the 75 % defoliation class. The highest rate of triclopyr may be high enough to overcome plant metabolism of the active ingredient to provide control.

The highest two rates of triclopyr provide > 80 % mortality for yellow-poplar over all defoliation classes. The lowest rate tested experienced a decrease in efficacy as the defoliation percentage went from 0 to 25. This is the response expected as a lower surface area for herbicide uptake would decrease efficacy of the same biomass at a certain rate of herbicide. This does not hold true as there is an increase in efficacy for the same rate as defoliation increases to 75%. This may be caused by the relatively large size of the yellow-poplar leaves allowing for uptake.

In summary, the response expected when there is a lower leaf area for herbicide uptake given the same biomass would be a decrease in efficacy (less active ingredient being absorbed and translocated to the site of action). This response was seen with yellow-poplar at the highest defoliation class at the highest two rates of imazapyr tested while there was consistently high mortality for yellow-poplar treated with the highest two rates of triclopyr across all defoliation classes. The opposite response was seen with sweetgum treated with both imazapyr and triclopyr at the highest defoliation class. There may be an interaction between the levels of defoliation that reduces sweetgum's ability to metabolize the active ingredients and therefore increasing its susceptibility to herbicide applications at the high defoliation level tested. The Bovey 1998 study showed no decrease in efficacy of clopyralid in defoliated honey mesquite. This is a result similar to this study as there was no decrease in efficacy; however, Bovey did not show an increase in efficacy as seen here. This shows that the complex relationship between

uptake kinetics and herbicide efficacy is one that is not fully understood in woody plants and needs to be examined further.

## **Chapter 6: Conclusions, Applications, and Future Research**

The data presented here shows that the rapid screens are species specific and more importantly chemically specific. Rapid screening techniques should be developed to be specific to the modes of action of chemicals used in woody plant control. There are a select few families of chemistries that are commonly used in woody plant control. These include aromatic amino acid inhibition (glyphosate), ALS inhibition (imazapyr, metsulfuron), and growth hormone regulation (triclopyr, dicamba, 2,4-D). Rapid screening techniques might be developed to be specific to each family of chemistry to improve the statistical significance of prediction models.

Rapid greenhouse screens involving an ALS inhibitor need to account for the long time to symptomology and species response difference. For the hardwood species tested here, rates should be adjusted to allow a broader dose response curve. The time constraints in this study did not allow for a longer dormancy period and more importantly a final evaluation past 6 weeks after seedlings were placed back in the greenhouse. This may not be enough time to allow for the full range of effect for an ALS inhibitor, such as imazapyr, to develop on rapidly grown and screened seedlings.

Rapid greenhouse screens involving a growth regulator, such as triclopyr, appear to be more effective in developing models for prediction. Screens with this chemical are also species dependent. Across both age classes tested in the dose response curves, poplar was consistently more susceptible to triclopyr than sweetgum while sweetgum was consistently more susceptible than loblolly. Rate titrations should be examined, adjusted, and applied on a species basis both in the rapid screen and the field screen to attempt to strengthen the regression models and further understand the chemical – species interaction for both imazapyr and triclopyr in rapid screens.

Overall physiological status of seedlings can affect the results of a rapid screen. Variance in seedling physiology can greatly affect the response means. Future screens should begin from a seed source so that the seedlings used for the screen are more physiologically similar.

Effect code became a useful parameter to use in prediction of field response. This parameter, used in both the field screens and rapid greenhouse screens, allowed for visual differentiation of effect in a qualitative manner. This qualitative variable should be included in future research to strengthen the rapid screen process due to its rapid manner of measuring and its ability to qualitatively differentiate among treatment effects.

Seed screening provided initial determination of herbicide activity by observing responses over the entire screen. The rapid seed screen also provided parameters as early as 10 DAT to be used to predict field response. Percent control of seed tissue provided a slightly more consistent variable as percent control of germination is an absolute value (once a seed germinates, it can not reduce). Seed screening is an extremely sensitive process. The use of surfactant can severely inhibit a seed screen while the addition of a fungicide treatment, such as Banrot, before herbicide application, can reduce fungal infestation due to the moist environment. Banrot did not appear to have a negative effect on seed germination. Germination techniques for hard seed species, such as yellow-poplar, should be examined to increase germination potential to decrease non-herbicidal nonresponse. The ability to control the environment where the seeds are germinating in is critical as well as watering levels and relative humidity can affect germination potential. Seed screen data shows that the seed screening process is extremely sensitive to mode of action. Future research should test a compound with the same mode of action as triclopyr, such as dicamba, to see if these results are duplicated.



The rapid seed screening technique had the ability to determine initial activity and species spectrum in an extremely short period of time. Time to initial treatment effect occurred anywhere from 5 to 16 days after treatment. This fact is of extreme importance to those in the herbicide development industry as one can make decisions on future research requirements and protocols for a certain compound by performing a rapid seed screen, which requires minimal active ingredient as compared to a traditional field screen, and have results from the seed screen in as little as 5 days after treatment. This screening technique, in combination with the rapid greenhouse screen, may be used to streamline research options for a herbicide developer in a cost and time effective manner. This may possibly move researchers to re-evaluate existing chemistries or visit new ones for woody plant vegetation management since it is now possible to do initial activity and species spectrum research in a short period of time.

Rapid screening techniques were also extremely effective in lowering the amounts of active ingredient required for screening as compared to the traditional field screen. Table 6.1 summarizes the amount of active ingredient used in each screen. There is up to a 18 fold decrease in the amount of active ingredient used for rapid seed screens as compared to the traditional field screen.

*Table 6.1: Total Active Ingredient (milligrams) Used for Screening Techniques*

Screen Technique	Imazapyr	Triclopyr
Rapid Seed Screen	1812.81 mg	11,084 mg
Rapid Greenhouse Screen	3434.1 mg	20,714.5 mg
Traditional Field Screen	31,600 mg	188,521 mg

The differences in leaf area – biomass ratios also influence the different responses seen here between the one-year-old seedlings and the two-year-old saplings. A majority of the triclopyr screens appeared to have rates too high to allow for lower response rates for the

greenhouse seedlings as one would see in field grown, larger trees. It may be possible to create two different rate titrations, a lower one for the greenhouse and similar one as here for the field that provides stronger statistical models. For example, the difference in rate titrations may be added to the model to allow for the prediction of rate  $x$  in the field by using rate  $0.5 x$  in the greenhouse.

An interaction is present between herbicide rate and defoliation for the two species and chemicals tested. Individual tests comparing two rates at a time should be performed to determine at approximately what rate of defoliation the interaction affects herbicide efficacy. This study was performed on young containerized seedlings. Field trials should be established incorporating larger, more developed saplings and root biomass data to further examine the influence that changes in leaf area per unit biomass have on herbicide efficacy.

Overall, rapid greenhouse and seed screening is possible. The screens are species and chemically dependent and should be developed to reflect this. The fact that rapid greenhouse screens are capable of producing statistically significant regressions in less than 9 months to predict field response and the rapid seed screening technique can show treatment effect in as little as 5 days after treatment are important findings in this work and hopefully give incentive to future researchers to refine the rapid screening techniques for species and family of chemistry.

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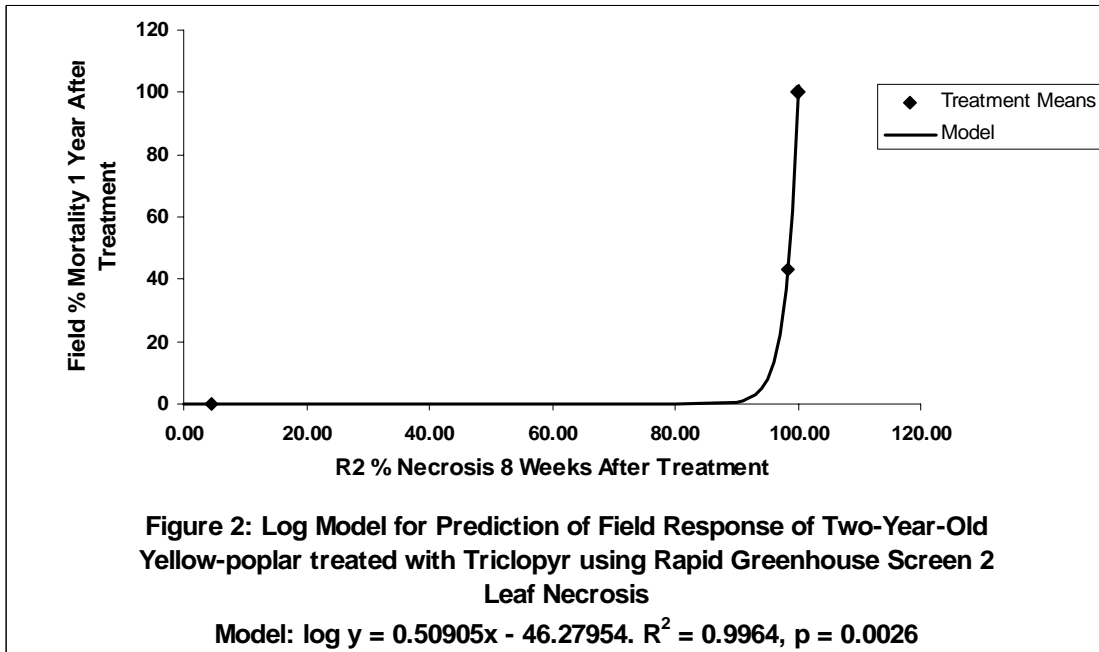
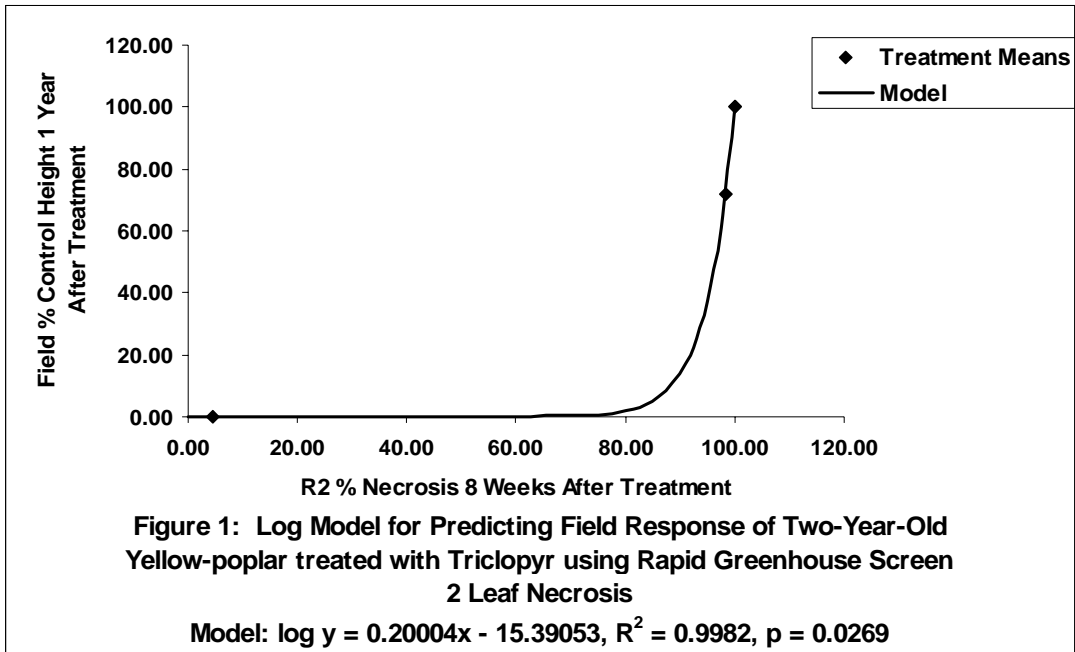
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**Appendix A: Figures**





## **Vita**

Mitchell Phillip Blair was born in Prince George's County, Maryland on July 7, 1974. His family moved to Virginia in 1979 where he attended Roanoke Catholic High School in Roanoke, VA and graduated in 1992. After many years attending community college and working jobs in construction and forestry, Mitch transferred to Virginia Tech in August of 1999. He graduated Cum Laude with a B.S. in Forestry (concentration in Environmental Resource Management) in December of 2001. In January of 2002, he began his Master's program under the advisement of Dr. Shep Zedaker. Mitch completed the final draft of his thesis in March of 2004 and officially graduated with a M.S in Forestry in May of 2004. He is currently employed as a Vegetation Management Research Specialist for the University of Kentucky's Department of Agronomy in Lexington, KY.