Effects of Water Stress and Application Timing on Glyphosate Activity in Forest Trees

by

Peter D'Anieri

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APPROVED:

Shepard M. Zedaker, Chairman

Richard G. Oderwald

Richard E. Kreh

John R. Seiler

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(ABSTRACT)

Field and greenhouse studies were conducted to investigate the role of water stress and time of glyphosate spraying in the variation in glyphosate efficacy. Data on water potential, foliar sugar and starch content, weather, and growth response were gathered for loblolly pine and four of its major competitors on 16 operationally sprayed tracts in Virginia. Glyphosate successfully released loblolly pine on all tracts. Control of white oaks was significantly related to foliar sugar concentration. Water potential and weather variables were not related to glyphosate efficacy for any species.

Seedlings of loblolly pine, red maple, and sweetgum were raised in a greenhouse and nursery environment. At the end of the second growing season, three water stress treatments were imposed on each species at each of four glyphosate application dates. ^{14}C-glyphosate was applied to a subsample of seedlings. Timing of application, water stress, or both significantly affected susceptibility of all three species to glyphosate. Efficacy for all three species corresponded to that expected from field data. Differences in species susceptibility to glyphosate were explained by differences in ^{14}C-glyphosate translocation, but there was no difference among species in absorption

of glyphosate. Efficacy changes across application dates followed seasonal changes in foliar sugar concentration.

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INTRODUCTION

The release of desirable trees from competing woody and herbaceous plants is a well-established forestry practice in Virginia and throughout the southeastern United States. Release is the term commonly used to describe what is technically a silvicultural cleaning operation as applied in southern pine plantations. Cleaning is the removal of stems not past the sapling stage of different species and same age as the crop species that are overtopping, or are deemed likely to overtop, the crop trees. Cleaning is often needed when harvesting and site preparation leave or create a site favorable for species competing with the crop to dominate the site. The objective is to alter species composition in favor of desirable species (Smith 1986). The use of herbicides to accomplish release is the most common method employed because of advantages in cost, safety, degree of site disturbance, and ability to treat large areas in a relatively short time.

Once a release operation has been prescribed, several factors must be taken into account to achieve success. When using herbicides, selectivity relative to crop and target species is essential. Specifically, aerial application of glyphosate [N-(phosphonomethyl) glycine] in the South must be timed to inflict only minor, short-lived injury to pines (Pinus spp.) while suppressing or killing a broad

spectrum of competing hardwood tree species. Although variation in response of crop and target species has been observed, causal stand, plant, and operational conditions have not been identified. Plant factors that could contribute to response variability include metabolic activity, commonly limited by phenology and water stress, and plant size. Operational variables include weather, chemical mixing, application rate, and application pattern. Identification of the importance of these different factors should lead to more consistent pine release using aerially-applied glyphosate.

JUSTIFICATION

Loblolly pine's (Pinus taeda L.) wide utility, extensive geographic range, and competitive ability make it a very valuable southern timber species. Historically, it became the predominant species by rapidly regenerating abandoned farmland in the first half of this century. Fire suppression has also favored loblolly pine over other southern pines throughout the 20th century. Harvest of these mature stands over the past 30 years has placed a burden on landowners to control regeneration of undesirable woody species that were not serious competitors during the period of old-field regeneration. Now, most plantations are on converted hardwood or mixed pine-hardwood lands. Most pure loblolly pine regeneration is now achieved artificially, largely using genetically improved seedlings (Zobel 1982).

Loblolly pine occupies over 1.6 million acres in Virginia. In the Southern Piedmont and Coastal Plain of Virginia, loblolly pine makes up over 60% of the softwood volume, and the proportion is increasing. Over 40% of industry lands and 14% of non-industrial private ownerships in the Coastal Plain and Piedmont are less than 10 years of age. "The single most important action needed to maintain and improve the loblolly pine resource in the Southeast is the regeneration to full stocking of harvested loblolly pine stands" (Sheffield and Knight 1982). The most recent U.S. Forest Service interim survey also listed 102 out of 1592 (6.4%) permanent plots sampled as needing "Cleaning, release, or other stand improvement." Another 341 (21.4%)

were judged to need "stand conversion" or have "no manageable regeneration." Both of these stand classifications would include sites that would be candidates for release in the future if brought under management (Sheffield and Craver 1981). Therefore, silvicultural cleaning is an integral operation in management of plantations, particularly on cutover sites, to maintain the planted level of stocking.

It is on more intensively managed acres that release to protect the planting investment is most justified. Although this means that forest industry is most likely to invest in some site preparation or release operation to control competition, non-industrial lands represent the area where greatest gains can be achieved because these ownerships control 79% of the commercial forest land in Virginia's loblolly pine region (Sheffield and Craver 1981).

Release of pines in the second to fifth years of many plantations is recognized as necessary to assure pine dominance in mature stands. However, an accurate, consistent, and cost-effective treatment does not exist to the satisfaction of many southeastern foresters and landowners.

Decisions on whether to apply herbicides for release are based on some sampling scheme that rates stands based on their amount of hardwood competition and release need. Adequacy of pine stocking, degree of overtopping, age of plantation, growth vigor of brush, and site quality are important in determining if release is required and prioritizing subsequent operations (Todd 1984).

Cost of release is approximately $50 per acre, and compares

favorably, when used in combination with light to medium site preparation, to intensive site preparation that would not require subsequent release (Fitzgerald 1982). Less intensive site preparation combined with release means a lower investment that needs to be carried through the rotation, greater flexibility in site prescription because competition is controlled in two operations (one of which may be found unnecessary), and less site disturbance. Site disturbance increases erosion hazard and potentially reduces the productivity of the site.

Presently, only three herbicides, glyphosate, hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione], and imazapyr {(+/-)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl-5-oxo-(H-imidazol-2-yl)-3-pyridinecarboxylic acid} are registered for aerial release throughout the South. Glyphosate is applied on the majority of lands requiring release because it shows superior selectivity relative to hexazinone (Olinger 1982). Imazapyr was just registered in December, 1987. Operational results, however, indicate that use of glyphosate for release of loblolly pine in the southern Piedmont and Coastal Plain of Virginia is sometimes inadequate. One forest products company that sprayed approximately 1600 acres for pine release in 1984 had to re-spray over 1000 of those acres in 1985 because of poor control of hardwoods after the initial glyphosate treatments. At the same time, some tracts suffered spots of pine injury when sufficient control of woody weeds was achieved. Pine mortality never obviously reduced stocking.

Successful pine release with glyphosate depends on adequate

foliage to intercept the spray solution and metabolic activity within the plant to translocate the chemical throughout root and shoot tissues. Adequate foliage may not be present in late summer on species like black tupelo (Nyssa sylvatica Marsh.) that drop their leaves early. Phenological stage and plant moisture stress reduce metabolic activity (Kramer and Kozlowski 1979), which, in turn, may lessen herbicide efficacy.

Phenological changes, such as formation of the abscision layer, slow or prevent translocation out of leaves in deciduous species. Also, species with an indeterminate growth pattern, such as loblolly pine, may be actively growing, in a resting stage between growth flushes, or have finished its shoot growth for the year. Moisture stress may potentially contribute to reduced foliar herbicide activity by reducing water potential in plants, or decreasing leaf orientation in some species like red maple (Acer rubrum L.) and flowering dogwood (Cornus florida L.). This change in orientation reduces the leaf area available to intercept the herbicide spray. Moisture stress also reduces photosynthesis, possibly slowing translocation of photosynthate and herbicide in water-stressed plants (Badiei et al. 1966).

Measurement of water stress and indicators of phenological stage of both pines and hardwoods may provide the ability to predict results of a release operation on individual plants, plant species within a tract, and entire tracts. The ability to anticipate variation in control and overall success within and between tracts will make scheduling of release operations with glyphosate more efficient.

Restated, an explanation and a way to predict the variable results from aerial applications is needed so that treatments are targeted to conditions conducive to success.

With this background in mind, two general objectives were formulated for this study. The first was to quantify the amount of variability in efficacy that exists in aerial release of loblolly pine with glyphosate. This was accomplished by measuring the growth response of individual loblolly pines and four of its major competitors to commercial release applications of glyphosate.

The second objective was to find accurate and simple pre-treatment plant measurements that can be used to predict the response of vegetation to glyphosate application. This objective was satisfied by measuring plant moisture stress and phenology indicators in seedlings grown and treated in both nursery and field environments, then comparing relationships of plant attributes with herbicide efficacy in these environments.

LITERATURE REVIEW

Prescribing optimum timing of glyphosate application requires knowledge of cellular processes, whole plant physiology, and environmental factors that influence observed efficacy. Biochemical reactions and physiological processes relied on for best herbicide control may or may not be proceeding at optimal rates under all field conditions. Reviewing the action and fate of glyphosate when it is applied is therefore important in hypothesizing how best to predict efficacy.

Mode of action

Glyphosate is a post-emergence herbicide that translocates in both the xylem and phloem (Lund-Hoie 1976, Neal et al. 1985). In the soil, it is strongly adsorbed to soil colloids before it becomes available for root uptake. Foliar applications, such as aerial loblolly pine release, require spraying during the growing season so that the chemical is intercepted and absorbed through the cuticle of target plants. Absorption may vary with leaf angle as it affects interception, and leaf age and growing environment, as they affect cuticle structure. For effective woody weed control, post-emergence herbicides must be translocated to the roots in a phytotoxic form so that re-sprouting does not occur. Translocation in this direction occurs through the phloem tissue, which also carries photosynthetic products. Once glyphosate reaches its site of action, it kills the

plant by interfering with synthesis of the amino acids phenylalanine, tyrosine, and tryptophan (Cole 1985).

Glyphosate is metabolized little by most plants. A summary by Coupland (1985) cited seven studies in which no alteration of the glyphosate molecule by plants was found after 1-2 weeks. An additional nine reports of some degree of metabolism were also reviewed. Five of these indicated less than 11% of absorbed glyphosate was broken down. In studies where high amounts of metabolites were found, it was suggested that many of the compounds were capable of reacting to form the parent molecule again, rather than being broken down to ineffective forms. Effects of environment on metabolism of glyphosate need further study, but the effects are likely the result of changes in enzyme systems that take part in metabolizing the herbicide.

Jaworski (1972) studied glyphosate's mode of action using the aquatic species Lemna gibba L. He found that phytotoxic effects of glyphosate could be reversed by application of phenylalanine but greatest inhibition of toxic effects resulted from a combined application of phenylalanine, tyrosine, and tryptophan. Combining phenylalanine and tyrosine reversed glyphosate's effects in the bacteria Rhizobium japonicum as well, an organism that has the same amino acid pathway. Jaworski hypothesized that glyphosate blocked the activity of two enzymes, chorismate mutase and prephenate dehydratase, that are essential for synthesis of aromatic amino acids.

Using 5-day-old wheat (Triticum aestivum L.) plants grown in nutrient solution, Nilsson (1977) found only 51% as much root growth

for glyphosate-treated vs. control plants after four days and only 78% as much shoot growth. Amino acid analysis showed "conspicuous increases" of glutamic acid (131% of control) and aspartic acid (197%) in treated plants. "Most pronounced decreases" were found for tyrosine, phenylalanine, leucine, and methionine (74-77%). This relationship held for both roots and leaves when considered separately. Because similar amino acid content was found in treated and control plants after four days, interruption of protein synthesis was indicated.

Duke and Hoagland (1977) proposed another explanation for glyphosate's mode of action. They demonstrated that reduced activity of phenylalanine ammonia-lyase was caused by glyphosate and that this enzyme caused a decrease in aromatic amino acids. This effect occurred within 24 hours of treating corn (Zea mays L.) seedlings by placing them in a glyphosate solution. This paralleled a decrease in fresh weight gain of the seedlings. Production of toxic phenolic compounds rather than reduced synthesis of aromatic amino acids and proteins was the hypothesized reason for reduced growth.

In controlled environment studies, Shaner and Lyon (1980) found that soaking the shoots of 10-day-old bean (Phaseolus vulgaris L.) plants in a glyphosate solution decreased transpiration in two to five hours. The speed of this response depended on glyphosate concentration. A concentration sufficient for desirable phytotoxicity caused a peak inhibition of translocation after six hours, at which time phenylalanine and tyrosine were reduced to 50% of control levels. Application of a combination of these compounds reversed the response.

They refuted the hypothesis of phenolic buildup being responsible for transpiration reduction, because artificial application of phenolic compounds produced by phenylalanine ammonia-lyase activity did not inhibit transpiration.

Cole (1985) recently reviewed published literature on glyphosate's mode of action and concluded that inhibition of protein synthesis and secondary compounds were the most important primary effects of the herbicide. Inhibition was accomplished by blocking an enzyme in the shikimic acid pathway, which yields aromatic amino acids used in building plant proteins. Phenolic compound formation is one of the additional processes inhibited, due to reduced availability of phenylalanine. Decreases in auxin levels, particularly indoleacetic acid (IAA), are an effect of reduced levels of phenolics, which normally inhibit the action of IAA oxidases.

Chlorophyll synthesis is halted by a less understood mechanism than described above. Both inhibition of an enzyme needed for chlorophyll molecule formation and destruction of existing chlorophyll through destruction of other protective cell pigments are presumed to occur. Effects on enzymes in the process of nitrate metabolism, important for active meristems, where glyphosate accumulates, are another proposed mode of action. Effects on photosynthesis, respiration, and membrane function are considered secondary modes of action.

Absorption and translocation

Presence of a herbicide at its site of action is necessary for a phytotoxic plant reaction. Once a herbicide has been applied correctly, absorption and translocation are the key processes controlling how much herbicide reaches the site if action. Absorption may be affected by cuticle thickness, hydration, and morphology; herbicide concentration; and surfactants. Translocation is most likely affected by source-to-sink relationships of plant carbohydrates. Water stress and phenology may reduce both absorption and translocation of herbicides in several ways. Water stress could reduce cuticle hydration and photosynthesis, thereby reducing herbicide absorption and potential movement to its site of action, respectively. Late summer application, common in glyphosate use for pine release, means absorption and translocation are influenced by interception by a weathered leaf cuticle. Also, a shift to the roots as a photosynthate sink and the onset of dormancy including formation of the leaf abscision layer occur at this time of year. The direction and magnitude of these conditions on glyposate activity is not known.

Shaner (1978) reported 1- to 3-week-old bean, pea (Pisum sativum L.), and sunflower (Helianthus annuus L.) seedlings grown in a controlled environment chamber had transpiration halted by glyphosate in four to 27 hours, depending on species and dosage. Despite a six-fold greater density of stomata on the lower surface of leaves, effects on transpiration were independent of leaf surface treated. Use of MON 0818 (Monsanto Co., St. Louis, MO) surfactant increased the

rate of penetration of glyphosate into bean leaves. Shaner found that water potential of the leaves was not decreased, but that potassium balance was affected, causing stomatal closure and decreased transpiration. He hypothesized that interruption of transpiration is an early signal that glyphosate has been effective, not that this phenomenon is the immediate cause of death.

In tests on perennial quackgrass (Agropyron repens (L.) Beauv.), radioactive glyphosate was readily translocated to rhizomes and throughout the shoot (Sprankle et al. 1975). The importance of growth stage and surfactants was emphasized. Applying glyphosate at the rate of 2.24 kg active ingredient (a.i.)/ha (0.75 kg acid equivalent per 1.0 kg a.i.), the non-ionic surfactants MON 0027 (Monsanto Co.) and X-77 (Chevron Co., San Francisco, CA) were needed to observe herbicidal effects. Using MON 0818 surfactant provided the fastest absorption of glyphosate: 34% was absorbed within the first four hours, 53% after a total of 48 hours. Therefore, rainfall soon after application may have an important role in effective use. Translocation was immediate: 67% of that applied was recovered in the untreated rhizomes and shoots after 48 hours. Photosynthesis was significantly decreased within 72 hours with MON 0027, but respiration was not decreased until 216 hours (9 days).

There is little data specific to translocation of glyphosate in forest trees, however, so translocation of other herbicides in woody plants may indicate how glyphosate is likely translocated. A translocation study by Leonard et al. (1966) using two phloem-mobile herbicides, 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) and amitrole

(1H-1,2,4-triazol-3- amine), showed poor translocation of the radio-labelled compounds in 3- to 4-year-old red maple and white ash (Fraxinus americana L.) after 30 days. Plants were treated both at the end and during the growing season, but were growing slowly at both times. Over 96% of 2,4,5-T of two formulations and over 76% of amitrole were still in the treated leaves of the two species after 30 days. Although xylem transport was demonstrated, phloem transport was minimal in both species. The high rate applied, which can prevent herbicide mobility by quickly killing treated leaves, was discounted as an explanation of low translocation because even trace amounts applied to cut stem surfaces showed no translocation in the phloem. The importance of application during active growth could explain the ineffective treatment results.

In another study on 2,4,5-T translocation in woody plants, blackjack oak (Quercus marilandica Muenchh.) absorbed less herbicide late in the growing season, and absorption and translocation were lessened as moisture stress increased (Badiei et al. 1966). The study was conducted in a natural stand, by analyzing harvested shoots with treated leaves after 24 hours, and roots after three days. Absorption decreased from 50 - 60% of that applied in monthly May through August applications to about 35% in September. Translocation to the roots had ceased by August but did not clearly follow the root sugar content at treatment time.

Lund-Hoie's (1979) research on Norwegian tree species showed that absorption of glyphosate in birch (Betula) seedlings was only 20% of that applied versus 70% taken up by ash seedlings. Of the total

herbicide absorbed, most was taken up after one week after application in birch, but did not peak until two weeks in ash. Translocation followed the path of carbohydrates in birch, but not in ash, where most glyphosate was recovered in mature untreated leaves despite minimal accumulation of photosynthates there. Movement into the transpiration stream would account for this result. Within two months birch seedlings were able to break down 30 to 40% of the absorbed glyphosate when applied to the foliage, but breakdown was less than 10% when applied to a cut stem surface and accumulated in the roots.

Further study on atmospheric influences showed an increase in translocated glyphosate from about 3% at 12°C, to 6% at 18°C, to 16% at 24°C, at constant 70% relative humidity. Increasing relative humidity from 50% to 70% raised translocation from treated leaves only about 2% at 12°C, but about 15% more was transported when relative humidity increased from 25 to 70% at 24°C (Lund-Hoie 1979).

In an earlier experiment on Norway spruce (Picea abies (L.) Karst.), Lund-Hoie (1976) found 25% absorption in actively elongating shoots versus 4% in inactive shoots. The period of absorption lasted about one week. Translocation followed the path of radio-labelled carbohydrates, accumulating in meristems during shoot growth. Translocation averaged 10-15% of that absorbed. Metabolic analysis showed nearly complete absence of the glyphosate molecule after one month, which correlated with observed necrosis in spruce not persisting beyond the season of application. Rapid metabolism in Norway spruce contrasts with slower glyphosate breakdown in herbaceous plants (Coupland 1985).

Absorption in woody species differs from that in herbaceous ones. The absorption period lasts up to two weeks (Lund-Hoie 1979) and magnitude of absorption appears to be independent of cuticle thickness in broadleaved trees. Because of the long absorption time, temperature and precipitation have a potentially greater influence on treatment efficacy. Translocation occurs mostly in the phloem, but some transfer to xylem also occurs, with consequent transport to aerial meristems. Translocation is most exclusively in phloem in the late summer when root growth is active and stem growth has ceased. Woody plants also apparently metabolize glyphosate more than herbaceous species (Lund-Hoie 1985).

Numerous environmental factors and their roles in absorption and translocation of glyphosate were summarized recently by Caseley and Coupland (1985). The concentration gradient of glyphosate between the inside and outside of a leaf is related to herbicide penetration. Glyphosate is a polar molecule, so conditions of a well-hydrated cuticle with a concentrated chemical solution are best for absorption. Drying of the spray solution will generally halt penetration. Within the plant, the negative charge of the molecule is repelled by cell walls, possibly slowing penetration, but improving availability for non-symplastic translocation. Symplastic entry, as well as cuticular penetration, are most likely to limit phytotoxicity, and make the role of adjuvants important.

Once in the phloem, glyphosate is translocated in the same way as photosynthates and is greatest when their transport is highest. Optimum phytotoxicity is achieved when the dominant sink of

photosynthates is the roots, rather than above-ground growing points or reproductive tissues. Temperature affects glyphosate efficacy through its direct relationship with plant biochemical reactions, morphology, transpiration, and inverse relationship with drying of spray droplets. Caseley and Coupland (1985) cite as many studies in which pre- or post-application temperatures influenced efficacy as studies in which temperature had no effect. Such differing results could be due to differences in experimental conditions between studies. Humidity affects leaf morphology, spray drying, and leaf hydration at application time. In all eight papers reviewed regarding relative humidity effects, either before or after application, efficacy increased with increasing humidity. This may be explained by greater photosynthesis rates that generally prevail as relative humidity increases. Similarly, all of six studies reviewed regarding soil moisture showed a direct relationship with glyphosate efficacy. Rainfall and dew effects are quite variable depending on their timing relative to both application and previous or subsequent precipitation. Conditions maintaining a hydrated cuticle are beneficial to glyphosate retention and absorption, but those promoting washing off of the chemical are clearly detrimental. The effect of light on glyphosate efficacy is direct and related to high net photosynthesis and photosynthate translocation. Wind effects have been studied little, but may exert an influence on transpiration, plant morphology, interception and drying of spray, and abrasion of the leaf cuticle.

Water Stress

The availability of water to plants has been proposed as a limiting factor in efficacy of foliar-applied herbicides used in forestry. When plant water stress is great, stomata are closed, and CO_2 uptake for photosynthesis is reduced. Reduced photosynthesis means little carbohydrate production for export from leaves via the phloem, and also less export of herbicides from leaves. The result is poor suppression of target plants.

Seasonal patterns of moisture availability can explain variation in internal water relations of trees and their growth. For example, white oak in Missouri showed decreasing leaf water potential and conductance as soil water potential declined in the summer months. Subsequent increases in water potential and conductance paralleled increases in soil water potential caused by summer rainfall events (Ginter-Whitehouse et al 1983).

Hoover et al. (1953) measured soil moisture continuously for two years in the South Carolina Piedmont beneath a 10-year-old loblolly pine stand. After a winter of low precipitation, current year rainfall was all that was available for tree uptake, and growth did not commence until sufficient water became available in September. After a wet winter, water was stored throughout the soil profile and subsequently used to depths of 66 inches. Soil water was taken up from where it was most available, regardless of depth or concentration of roots.

In both herbaceous and woody species, moisture stress affects

susceptibility to glyphosate. Lauridson et al. (1983) subjected eight-week-old Canada thistle (Cirsium arvense (L.) Scop.) raised in the greenhouse to three levels of plant water potential. Average afternoon water potential for the three treatments was -0.43, -0.97, and -2.01 MPa. Applied at 1.6 kg a.i./ha, glyphosate absorption was reduced 51%, translocation to the shoot was reduced 54%, and translocation to the roots was reduced 85% in severely stressed (-2.01 MPa) vs. unstressed (-0.43 MPa) plants. In field-grown plants treated at 2.5 kg a.i./ha, severe moisture stress decreased shoot control only in the fall application in a year having less soil moisture. In addition, no difference in root kill was measured between severe stress and no stress.

In greenhouse-grown, 10-week-old cogongrass (Imperata cylindrica (L.) Beauv.) and purple nutsedge (Cyperus rotundus L.), plants subjected to an extreme moisture stress treatment (67-73 cm Hg soil water tension) produced significantly less biomass and showed reduced activity at three rates of glyphosate application, as measured by shoot and rhizome growth. Plant growth was improved in the extreme stress treatment when water was applied before and after application (Moosavi-Nia and Dore 1979).

Moisture stress, measured by percent soil moisture, resulted in reduced absorption and translocation of glyphosate in greenhouse-raised barnyardgrass (Echinocloa crus-galli (L.) Beauv.) (Ahmadi et al. 1980). The four levels of stress were 10, 20, 30, and 40% soil moisture. Moisture stress treatments were maintained for three days, pre- and post-treatment. Glyphosate was applied at 0.4 kg/ha.

Reduction in plant dry weight decreased from an average of 90% to an average of 52% in the lowest to greatest moisture stress treatments, respectively. This coincided with decreases in glyphosate absorption of approximately 70% in the most-stressed plants from unstressed plants, and decreases in translocation from the treated portion of the leaf from an average of about 90% of radioactive glyphosate absorbed in unstressed plants to an average of about 50% in most-stressed plants.

Miller and Starr (1963) were among the first to state that site factors influencing moisture availability were important in determining the degree of pine and hardwood kill from 2,4,5-T applications. Current thought was that selectivity for loblolly pine could be achieved by spraying when loblolly had set a resting bud, initiated by moisture stress, and hardwoods had reached the full-leaf stage. Percent kill of different species was plotted against 25 independent variables. For sweetgum (Liquidambar styraciflua L.), the only significant independent variable was number of days since precipitation greater than 0.5 inches (r^2= 0.53). Correlation of kill with moisture variables was best through July, but was poor and unexplained for August and September. For loblolly pine, maximum temperature on the spray date was the only significant variable, explaining 27% of the variation in pine kill. An unexplained positive correlation with number of days since greater than 0.5 inches precipitation increased the r^2 to 0.39. The rate of 2 lbs. a.i./acre in 3 gallons of diesel oil carrier in this study resulted in an excessive average pine kill of 27% throughout the year.

Badiei et al. (1966) conducted tests on the influence of soil moisture and light on 2,4,5-T activity in three-month-old blackjack oak seedlings in a greenhouse. As percent soil moisture increased from 2.8% to 16.0%, absorption of 2,4,5-T increased significantly from 50% to 57% of that applied, and translocation from the treated leaf significantly increased from 13 to 33% of the applied dose. Placing the seedlings in the dark for 24 hours before treatment and kept in the dark afterwards resulted in significantly less translocation (16.4% of that applied) than in plants placed back in the light after treatment (29.8%). This suggests photosynthesis and photosynthate transport activity may be important in herbicide translocation.

Summarizing a study on greenhouse-raised honey mesquite (Prosopis glandulosa Torr.) and winged elm (Ulmus alata Michx.) treated with picloram (4-amino-3,5,6-trichloro-2-pyridinecarboxylic acid) and 2,4,5-T, Davis et al. (1968) found that moisture stress only affected the absorption of picloram by mesquite, reducing it from 36% to 10% when soil water potential changed from -0.05 to -2.08 MPa. In both species, stress reduced translocation and growth similarly. Four soil water potential levels from -1/30-1/20 MPa to -1.8-2.0 MPa were used to measure moisture effects. Growth was steadily decreased to 20% of normal when soil moisture dropped from 0 to -0.5 MPa. In mesquite, both herbicides were taken up and transported to the growing tip within four hours, but the majority of herbicide remained in the treated leaves and little was translocated to the roots after 90 hours. With both herbicides, a decrease in translocation by one-third to one-half occurred largely between 0 and -0.5 MPa. Species

selectivity existed, as picloram was translocated more than 2,4,5-T in mesquite, but the opposite was true for winged elm. Because movement of these herbicides continued up to 90 hours after treatment, persistent stress in the field would be most important in reducing efficacy, as well as short-term stress at the time of application.

Growth Stage

Growth stage may be valuable as an indicator of relative herbicide susceptibility because of its correlation with plant phenology and the internal processes, i.e. current source-sink path, mentioned earlier. Trees with elongating shoots may be more prone to herbicide injury because they have younger leaves that are probably more succulent than late in the growing season. Elongating shoot regions are also sinks for photosynthates, maintaining conditions that are conducive to acropetal herbicide transport. After shoot elongation has ceased, stem diameter and root growth continue and storage of carbohydrates commences. Phloem-mobile herbicides would then be expected to accumulate in the stem and roots. Further, periodicity of growth within a growing season is related to availability of necessary resources (water is most limiting), meaning growth may slow or accelerate depending on the plant-available water supply.

Kramer (1943) reported the period and duration of height growth for planted seedlings of several species grown together in the North Carolina Piedmont. White oak (Q. alba L.) had a growing season lasting 140 days, from late March to early August. Yellow-poplar

(Liriodendron tulipifera L.) grew from April through August, a length of 160 days. Loblolly pine grew from late March into early October, a total of 210 days.

In southern Illinois, Boggess (1956) followed radial growth of white oak in a natural stand through four growing seasons. Over 80% of the year's growth was completed by mid-July, corresponding to the point when available soil moisture was fully depleted. Erratic diameter growth continued until early October as the soil was periodically recharged.

Similar patterns have been found in loblolly pine. In a 30-year-old Arkansas stand monitored for four growing seasons, diameter growth slowed when available soil moisture dropped below 60% in stands with 125 ft^2/ac. basal area. In stands thinned to 55 ft^2/ac., radial growth continued until available water was less than 15%. After June, soil water stored over the winter was depleted, and diameter growth depended on adequate summer rainfall (Bassett 1964).

In screening foliage-applied herbicides for use on the West Coast, Radosevich et al. (1980) concluded that conifers were more tolerant after fall dormancy had set in, and that conifer damage was greatest in summer when moisture was readily available and the trees were photosynthetically active. Of the six conifer species tested, pines showed greater fall selectivity to glyphosate than Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and true firs (Abies spp.). After applying glyphosate at 2.25 kg a.i./ha, growth of hard pines was 87 to 102% of untreated trees for July and September applications, but only 72 to 76% for mid-April application. Survival of hard pines was

87 to 114% for the later spray dates and 71 to 86% for the April application. Trends in photosynthesis rate and xylem water potential at treatment time did not correspond to growth and survival trends of treated trees. Phenological rather than physiological state apparently is more important in predicting glyphosate injury to these western hard pines. Seasonal selectivity was attributed to differential absorption as the leaves mature and differential translocation as xylem water potential decreased during summer drought.

Lanini and Radosevich (1982) studied timing of greatest glyphosate injury on five Pacific Northwest brush species in relation to xylem water potential and photosynthetic activity. Glyphosate was applied at 6.0 kg a.i./ha. Glyphosate's effectiveness was directly related to plant moisture stress as measured by xylem water potential. Significant relationships between photosynthesis and water potential and shrub canopy control were both negative and positive, depending on species. However, absolute values of correlation coefficients were generally lower for photosynthesis (r = 0.16 to 0.85; avg = 0.45) than for water potential (r = 0.07-0.90; avg = 0.60). Predawn water potential ranged fron nearly 0 to approximately -4.0 MPa across all species.

Further tests in the Pacific Northwest concluded that best selectivity for ponderosa pine (P. ponderosa Laws.) was when annual pine growth was completed, xylem water potential was low, and greenleaf manzanita (Arctostaphylos patula Greene) xylem potential was high (Paley and Radosevich 1984). Glyphosate was applied at 6.0 kg

a.i./ha seven times between April 10 and October 16. Pine and manzanita damage were similar through July, then manzanita damage (40 to 80%) exceeded pine damage (5 to 60%) by 15 to 35% for August - October. This coincided with the time when pine growth slowed considerably, but pine growth rate for the whole year was not significantly related to glyphosate damage ($r = 0.74$). Daytime xylem potential was the best predictor of pine damage ($r = 0.85$) and was also significant for manzanita ($r = 0.77$). Photosynthetic rate showed poor association with plant damage and was assumed to be at a sufficient level for other factors such as xylem water potential to be better indicators. Measuring pine growth rate and daytime xylem water potential of both species was recommended to predict herbicide application results and meet the requirements of accuracy and efficiency in operational situations.

King and Radosevich (1985) found that needle or leader growth rate best indicated conifer damage of five species from glyphosate applied monthly at 6.0 kg a.i./ha, especially through July, and that decreasing daytime xylem water potential indicated decreased conifer damage after July. However, multiple regression yielded no significant correlations of herbicide injury to any factors for sugar pine (P. lambertiana Dougl.) or Douglas-fir.

In testing glyphosate tolerance of horticultural woody plants, Neal et al. (1985) treated juniper (Juniperus conferta Parl.) and ligustrum (Ligustrum japonicum Thunb.) at five growth stages: cold acclimation, dormant, bud break, shoot elongation, and shoot termination. Using liquid scintillation spectrometry of

radio-labelled glyphosate, they found that the greatest juniper absorption after 14 days was only 2% of that applied and occurred during the shoot elongation period. In contrast, absorption in ligustrum after one day ranged from 1% during cold acclimation, dormant, and bud break periods to 56% during elongation and growth termination. After 14 days, ligustrum absorption ranged from 8% (cold acclimation) to 70% (elongation). Translocation in ligustrum ranged from 3% in dormant plants, 13% during bud break, 5 to 10% during elongation, up to 7-42 % during termination. Translocation below the treated leaf varied from 2-5%. Vegetative plants absorbed lower amounts in the latter two stages than reproductive plants. One-year-old leaves absorbed glyphosate only at bud break in ligustrum, not thereafter. Leaf age did not affect absorption in juniper. The peak of sensitivity for ligustrum in spring was attributed to translocation from mature leaves to the roots, whereas the greater absorption later in the growing season was followed by translocation mostly upward to the leaf tips, which resulted in tip burn but not plant mortality.

Sugar-starch relationships

In light of the need for phloem transport for effective control of target plants from foliar-applied glyphosate, analysis of the carbohydrate components of the leaves could indicate when plants are susceptible. Starch and sugar levels are known to vary daily and seasonally in woody plants. Donnelly (1976) found a significant

change from 495 mg sugar and 276 mg starch on June 21 to 697 mg sugar and 152 mg starch on September 13 in current-year leaves of sugar maple in Vermont. Diurnal changes also occurred roughly cyclically but were variable and not followed for more than two days in the study. Troughs in leaf sugar occurred in the predawn hours, and peaks occurred sometime in the afternoon.

In water-stressed loblolly pine trees, the seasonal pattern of carbohydrate content in inner bark was changed by drought stress (Hodges and Lorio 1969). Mature trees shielded from rainfall showed a greater loss in starch concentration after July than did control trees. Stressed trees also showed a greater and more rapid accumulation of sugars - peaking soon after soil moisture reached its minimum in June - than did control trees, which peaked in October. Water stress decreased diameter growth by over 50% for the year.

Brady and Hall (1976) related leaf and root sugar changes to phenoxy herbicide susceptibility of 12 southern woody forest weeds. A gradient of decreasing sugar concentration from leaves to roots, encouraging leaf to root transport, explained the accepted susceptibility of half of the species. Leaf and root samples were taken monthly from April 22 to September 22. Susceptible species were most vulnerable when accumulating sugars. Injury patterns characteristic of shining sumac (Rhus copallina L.), sweetgum, black willow (Salix nigra Marsh.), blackjack oak, water oak (Q. nigra L.), and southern red oak (Q. falcata Michx.) were explained by patterns in leaf and root sugar content. American sycamore (Platanus occidentalis L.), green ash (F. pennsylvanica Marsh.), red maple, flowering

dogwood, sweetbay (Magnolia virginiana L.), and winged elm failed to accumulate sugars in their roots. Therefore, this method did not explain the known susceptibility of these species.

In mature white oak, McLaughlin et al.(1980) reported seasonal contents of starch in foliage averaged 1.2% and changed little through the growing season. Accumulation of starch in roots beginning in late summer may indicate transport of sugars for storage at that time, but seasonal sugar data were not presented except on a whole-tree basis over time.

Frost

Another environmental factor that may affect glyphosate performance is frost, as it would interrupt translocation if phloem cells were killed. After showing that fall frosts increased susceptibility of alfalfa (Medicago sativa L.) and quackgrass to glyphosate in a prior study, Davis et al. (1979) simulated spring frosts on the same species in a controlled environment. Alfalfa was significantly more resistant to glyphosate after frost, with dry matter regrowth about 2.5 times as great as control plants after 40 days. Frost had no significant effects on quackgrass growth after glyphosate treatment. Absorption and translocation in quackgrass were increased after frost, but differences were only statistically significant for translocation in more mature plants of two ages examined. Significant reductions in translocation occurred after frost in alfalfa. Lower susceptibility to glyphosate was attributed

to phloem injury of young tissue and contrasted with the maintenance of translocation in fall-treated plants that were conditioned to cold.

Experimental release

Experimental use of glyphosate for pine release began in 1976 (Olinger 1982), and results were first reported in 1980 (Fitzgerald et al. 1980). Fitzgerald evaluated helicopter application of 1.12 and 2.24 kg a.i./ha in 56 L total spray volume per hectare in July. Treatment resulted in suppression of hardwoods and no pine injury, at the larger dose, equal to that of the traditional 2,4,5-T application used as a check. The stand was seven years old and originated by direct seeding following mechanical site preparation.

Olinger (1982) emphasized that glyphosate's tendency to suppress growth of competition for several growing seasons without severely affecting weeds visibly to the extent of other herbicides must be taken into account when evaluating its efficacy. Plot studies initiated in 1976 using mistblower application indicated glyphosate was potentially effective for release of loblolly pine. As a result, more intensive testing to establish the best rate and timing were completed in 1980. Using 1.12, 1.68, and 2.24 kg a.i./ha on five dates from July 21 to October 2, results were tabulated with and without black tupelo, which drops its leaves so early that its interception of spray is reduced. Brush control evaluation with a subjective 0 (no herbicidal effect) - 3 (dead) rating scale defined a rating of 2 (severe effect) as adequate control. Results were

considered satisfactory after one year, despite only two rate-date combinations out of fifteen with average control ratings greater than 2.0. August 21 applications had the highest control rating of the five dates with an average of 1.92, and 2.24 kg a.i./ha proved to be the best rate, with an average rating of 1.75. Pine damage varied but was never great enough to cause concern. High damage on August 20 was attributed to a rainfall-caused growth flush. Aerial trials on three sites in 1980 provided more desirable results: hardwood control rating averaged 2.43 after one year.

Wu et al. (1983) reported comparable results for release of 3- to 5-year-old loblolly pine from Mississippi to Texas. Rates tested were 1.25 and 1.68 kg a.i./ha in 47 to 94 L total spray volume per hectare, applied in September to mid-October. Citing Fitzgerald et al. (1982), white oak was considered tolerant and red maple susceptible to foliar-applied glyphosate, an observation contrary to accepted relative susceptibility of those species. Evaluations were made one year after application using subjective percent control estimates. Fifty to sixty percent control was considered satisfactory for pine release. Timing study indicated the earliest dates caused 13% pine injury for the higher rate; however, burned needles or killed recent flushes were not severe enough to prevent achieving release. When applied before mid-October, glyphosate applied at 1.5 lbs./acre gave the following ranges of control for selected target species from a total of 11 sites: white oak, 25-65%; sweetgum, 55-72%; red maple, 65%; post oak (Q. stellata Wangenh.), 68-71%.

Larsen et al. (1983) reported results of release trials with

glyphosate applied aerially in 3 to 6-year-old stands between August 29 and September 13 in Virginia. Three rates were tested: 1.12, 1.68, and 2.24 kg a.i./ha in 47 to 94 L/ha spray volume. Results were summarized using subjective plot evaluations, with 60% of a hardwood species' rootstocks falling in classes 3 (severe observable injury) or 4 (dead) equalling satisfactory release from that species. Control of selected species was good for white oak (69% of individuals in classes 3 and 4), yellow-poplar (70%), and sweetgum (93%), but unsatisfactory for red maple (42%) at the intermediate rate. At the highest rate, red maple was adequately controlled (65%), while white oak (60%) and yellow-poplar (55%) injury was less. At least 93% of loblolly pines sustained no visible injury, and all pines had recovered at all rates.

A glyphosate release study involving more intensive vegetation sampling was installed in Virginia in 1980 (Minogue et al. 1984). Rates of 1.12, 1.68, and 2.24 kg a.i./ha were applied in 94 L/ha by helicopter on August 29. Evaluation two years later showed no pine mortality, but up to 6% foliar burn. Red maple and white oak basal area were reduced, respectively, from 61% and 23% of hardwood basal area prior to treatment, to 36% and 11% after one year, and remained below pre-treatment levels after two years. Control plots had 32% greater hardwood basal area after two years. Regrowth on treated plots was attributed chiefly to red maple. After two years, pine height and DBH were significantly greater on the 2.24 kg a.i./ha plots than the control. Number of free-to-grow pines was 28, 10, and 8% less and 10% greater compared to pre-treatment levels for control, low, medium, and high treatment rates, respectively (Minogue et al.

1984).

After re-measuring the study by Minogue et al. (1984) after four growing seasons, Zutter et al. (1988) reported that glyphosate-treated pines were 1.7 ft. (13%) taller and 0.56 in. (29%) larger in diameter than control pines. Height-age curves for sprayed treatments were still diverging from those of the control treatment. Four-year pine height growth was significantly and inversely related to hardwood density measured two years after spraying. Hardwood density at the time of spraying and two years later explained 65% of the variation in pine height growth over the four-year period.

Tests in the Carolina Piedmont used 1.12, 1.68, amd 2.24 kg a.i./ha of glyphosate in 47 or 94 L/ha carrier applied between August 10 and September 29 (Downs et al. 1984). Loblolly pine mortality up to 4% and foliage burn of 2 to 12% occurred in August applications, but none was suffered in September. Though quantitative hardwood injury data was not presented, after two years, satisfactory release from white oaks and sweetgum was obtained with the two higher rates. Yellow-poplar and red maple were resistant species, and the highest rate was recommended when these species dominate the competition.

Voth and Downs's review (1985) of glyphosate use in pine silviculture in the South recommends application after September 1 to best avoid crop injury. This date may be too late in Virginia, at the northern end of loblolly pine's range. Rates of 1.68 kg a.i./ha for light competition and 2.24 kg a.i./ha for most conditions in a carrier volume of at least 47 L/ha was recommended. Use of surfactants should be avoided due to increases in pine injury. Hardwoods are still

susceptible when leaf color change has commenced, but once leaves are dropped, efficacy is proportionately decreased due to reduced interception surface.

Costs and benefits of release operations

Documentation of the financial worth of releasing southern pines has emerged in the last few years, as the greater cost of herbicides used in place of the banned 2,4,5-T raised questions about their feasibility (Guldin 1984). Based on loblolly pine site index$_{25}$ 58 land, Guldin concluded that $53 per acre could be spent for release at age three, assuming a 10% alternative rate of return, and a commercial thinning at age 18. With slightly better average site quality than this in Virginia and lowering costs of herbicides, glyphosate can be applied for $35 - 45/ac., easily exceeding a 10% return rate.

Trends of major forest practices costs in the South from 1952 - 1979 show costs of removing undesirable vegetation with herbicides increased at an annual rate of 7.8%, costing $40/acre in 1979. This compared to a 9.7% increase for release by cutting and 11.2% increase in mechanical site preparation. The 1979 cost of $93/acre for site preparation would double in less than seven years if historical trends continued (Moak 1982). Under these circumstances, less intensive mechanical site preparation with later release, if needed, will be the most profitable means of controlling weeds to maximize pine growth.

Balmer et al. (1978) studied effects of stand composition on pine growth and profitability. They concluded that increasing the

proportion of hardwoods in young stands decreases pine volume exponentially. Controlling residual hardwoods after harvest doubled pine volume of the succeeding stand at age 20. Further control of all understory hardwoods in mid-rotation (age 14) improved the present net worth at age 20 approximately 60% over stands that had been thinned without hardwood removal. Controlling hardwoods even earlier would allow pines to capture even more of the site resources being utilized by undesirable species.

Bacon and Zedaker (1986) found that control of two-thirds of woody vegetation at plantation ages two and three increased pine volume 42 and 25%, respectively, over untreated stands after three years. This timing and level of control is most representative of operational release programs. Further, the difference in pine stem volume between untreated and treated 2-year-old stands was still increasing after three years.

Burkhart and Sprinz's (1984) model of loblolly pine volume in cutover stands with different levels of hardwood basal area demonstrated that hardwood competition is the most important reason for lower pine yields on cutover vs. old-field sites. This was further documented in a survey by Dickens (1984), which showed that in 35 of 43 forest herbicide studies established, pine volume increased in sprayed areas relative to controls. Pine dominance increased directly with amount of hardwood control. A later survey of 28 sites where herbicides were used to control competition showed 2,4,5-T increased southern pine volume 19% over check areas on the average, and other herbicides used resulted in a 46% average increase. Again,

as the amount of hardwood basal area increased in these stands, pine volume decreased (Glover and Creighton 1985).

An economic analysis by Kline and Kidd (1986) using Burkhart and Sprinz's (1984) model revealed that stand value at harvest is most dependent on percent hardwood basal area, site index, and discount rate. Pine growth lost due to competition is relatively greater at high site indices. For example, using conservative product prices and a 5% discount rate, the net present value of a stand planted with 800 trees per acre on site $index_{25}$ 70 with 10% hardwood basal area is $316/acre but is only $66/acre with 30% hardwood basal area. Corresponding values for site $index_{25}$ 50 are $1/acre and -$94/acre for 10 and 30% hardwood basal area, respectively.

Release by non-chemical methods has also been evaluated in Virginia (Dierauf 1984a, 1984b). Release by hand cutting in a nine-year-old loblolly plantation gave variable results. Three released plots had 4% less, 7% more, and 69% more cord volume than unreleased paired plots by age 23. Though hardwood basal area was essentially the same within paired plots, the plot with the greatest difference had 2.5 times more 3- and 4-inch DBH hardwoods than the others. Removal of these stems could account for a greater response. The less responsive stands had more crop trees per acre, which indicates that pine competition was more important than hardwood competition. Also, greater competition in one untreated plot made the pine volume growth relatively greater in its paired plot. Nearly equal yield from released and unreleased stands indicated that release may not have been necessary (Dierauf 1984a).

A second study by Dierauf (1984b) of release by hand cutting in a 5-year-old loblolly pine stand that was predominantly free-to-grow at treatment time resulted in 27% more cordwood volume at age 18 in released vs. unreleased stands. Difference in pine basal area between treated and untreated plots was increasing from 11.7 ft^2 at age 10 to 15.3 at age 14 to 21.3 at age 18. This indicated a continuing response to the release operation.

Summary

Previous research has shown that glyphosate's chief mode of action is interruption of amino acid synthesis. Glyphosate is absorbed steadily in woody plants for up to two weeks after application, and can be translocated throughout the plant, depending on growth stage and predominant symplastic transport patterns. Moisture stress reduces translocation of other silvicultural herbicides, and is related to susceptibility of some forest species to glyphosate in the Pacific Northwest. Glyphosate efficacy is inconsistent on many hardwood competitors of loblolly pine in the Southeast, but the sources of this inconsistency have not been identified. When successful, aerial release of loblolly pine with glyphosate is a profitable means of increasing pine growth and assuring stand dominance of pine.

Chapter 2

VARIATION IN GLYPHOSATE EFFICACY IN COMMERCIAL LOBLOLLY PINE RELEASE OPERATIONS

Introduction

Aerial application of herbicides has been an accepted technique for silvicultural cleaning in loblolly pine plantations for over three decades. Since the aerial use of 2,4,5-T was suspended in 1979, glyphosate has emerged as the herbicide of choice in most cleaning, or release, operations. Only two other herbicides, hexazinone and imazapyr, are registered for aerial pine release throughout the South. Excessive pine mortality has limited the use of hexazinone, and imazapyr is newly registered and untried on extensive acreages. Reservations regarding glyphosate use, however, center on perceived inconsistent suppression of hardwoods. Explanations of glyphosate's variable efficacy in the Southeast are numerous, but none have been quantitatively investigated.

Several initial investigations into the use of glyphosate for release showed it to be a promising tool when used at the appropriate rate and time (Fitzgerald et al. 1980, Olinger 1982, Wu et al. 1983, Larsen et al. 1983, Minogue et al. 1984, Zutter et al. 1988). Through this work and subsequent experience, commercial applications of 1.68 - 2.24 kg a.i./ha in 47 - 94 L/ha total spray volume are now common. Spraying is done from the second half of August through leaf fall in

late September or early October.

Data from Larsen et al. (1983) probably best represent "average" results in loblolly pine plantations: sweetgum was very susceptible to glyphosate, white oak and yellow-poplar were satisfactorily controlled, and red maple was poorly controlled. Loblolly pine injury was noticeable on 7% of the trees, but recovery was complete one year later.

However, much variability in efficacy is apparent upon further investigation of reported results. In Virginia, Larsen et al. (1983) found poorer control of white oak and yellow-poplar at higher rates, yet better suppression of red maple. From Mississippi to Texas, Wu et al. (1983) reported adequate and approximately equal control of sweetgum and red maple, but variable efficacy on white oaks and injury of up to 13% of loblolly pines. Minogue et al. (1984) recorded increases in number of free-to-grow pines after two years in Virginia with accompanying decreases in white oak and red maple basal area; hardwood regrowth was dominated by red maple. In the same Virginia study, Zutter et al. (1988) reported an increasingly greater pine growth difference between glyphosate-treated and control trees after four years. In the North Carolina Piedmont, white oaks and sweetgum were suppressed, but yellow-poplar and red maple were somewhat resistant to glyphosate injury (Downs et al. 1984). Only Minogue et al. (1984) have quantitatively measured hardwood responses; all others subjectively assessed injury to target species.

Positive identification of causes for these differences in relative hardwood species susceptibility is needed to allow efficient

use and scheduling of aerial glyphosate applications. Actual measurement of responses of sprayed trees and quantification of variability in efficacy will eliminate bias due to subjective ratings of efficacy used in previous studies. Variability in tree response to glyphosate has been attributed to plant water potential and growth activity in western woody species (Radosevich et al. 1980, Lanini and Radosevich 1982, Paley and Radosevich 1984, King and Radosevich 1985), and to weather events and phenological conditions in some southeastern species (Olinger 1982).

In western conifers, photosynthesis rate and xylem water potential were not consistently reliable indicators of susceptibility to glyphosate, but measures of needle or stem elongation rate were significant predictors of injury to Jeffrey pine (King and Radosevich 1985). However, for broadleaved brush species, both xylem water potential and photosynthesis rate were significantly related to broadleaf canopy reduction (Lanini and Radosevich 1982).

In a related study in the Southeast, Miller and Starr (1963) found a significant relationship between number of days since a rainfall event exceeding 0.5 inches and sweetgum injury from 2,4,5-T (r^2=0.53). Maximum temperature on the date of spraying was related to loblolly pine injury (r^2=0.27). These were the only significant relationships from an analysis of 25 independent variables on different species. These relationships were weakest in August and September. Also working with 2,4,5-T in the Southeast, Brady and Hall (1976) could attribute patterns of injury in 6 of 12 woody species, including sweetgum, to gradients in sugar content between foliage and roots.

Red maple was among the species that did not demonstrate a shoot-to-root sugar gradient, so its characteristic pattern of injury was not explained.

Objectives of this study were to document the variation in glyphosate efficacy on several important southeastern tree species, and to measure internal and external plant attributes hypothesized to affect glyphosate activity. Weather, plant water potential, foliar starch and sugar concentrations, and rate of growth were used to predict response of red maple, sweetgum, white oaks, yellow-poplar and loblolly pine to aerial glyphosate application on an individual-plant and tract-wide basis.

Methods and Materials

Sixteen tracts were sampled in 1984 and 1985 in the Piedmont and Coastal Plain physiographic provinces of Virginia. Dates of aerial application of glyphosate to these tracts ranged from August 27 to September 28. Pre-treatment height and two perpendicular crown diameters (CD1, CD2) were measured on at least 20 randomly chosen free-to-grow individuals of each species at each tract. In some instances, low stocking prevented measuring this number. On four tracts, loblolly pine terminal length was measured at the time of spraying and at the end of the growing season. Relative completion of terminal growth was then calculated as the ratio of terminal length at the time of spraying to terminal length at the end of the growing season.

On eight tracts, water potential was measured using a pressure chamber (Scholander et al. 1965) within a day of application on eight or more individuals of each species between the hours of 3:00 and 8:00 a.m. Re-measurement of water potential after 7:00 a.m. indicated no change from measurements made on the same plants before sunrise.

Foliage samples were collected from the same eight sites, from at least eight of the same trees measured for water potential, on the day of spraying for measurement of starch and sugar concentration. Leaf samples were placed in plastic bags, put on dry ice in a cooler in the field, then transferred to a cold room ($2^{o}C$)and later to a freezer until lab analysis. Laboratory procedures for determination of sugar and starch concentrations followed the method of Rowe (1984). Tissue was dried, ground, and weighed, and sugar was extracted with a $95^{o}C$ ethanol solution. After three extractions, the residual starch was hydrolyzed by incubation in a solution of amyloglucosidase enzyme and separated from the tissue residue by vacuum filtration. Sugar was quantified by reaction with anthrone in a boiling water bath and measurement of spectrophotometric absorbance at 620 nm.

On 4 tracts, 30 individuals of each species were measured for crown dimensions before spraying and one year after spraying in an unsprayed block set aside for that purpose. On all 16 tracts, crown dimensions of sprayed trees were re-measured after one growing season. On the 12 tracts sprayed in 1984, crowns were measured again after two years. Crown volume index (CVI) was calculated as:

$$CVI = Height \times [CD1 \times CD2]$$

Unsprayed trees were used to generate regression equations for expected (predicted) CVI of sprayed trees after one year had they not been sprayed, using a method similar to Burch and Zedaker (1988) and Kline et al. (1985). A linear equation of the form

$$\text{Expected CVI at year 1} = b_0 + b_1 \text{ (CVI at year 0)}$$

was fit for each species. Efficacy was then calculated as:

$$\text{Control} = 1 - \left(\frac{\text{CVI at year 1}}{\text{(Expected CVI at year 1)}}\right)$$

and:

$$\text{Relative growth} = \frac{\text{(CVI at year 1)} - \text{(CVI at year 0)}}{\text{CVI at year 0}}$$

Weather data from the weather station nearest the spray tract were gathered from published National Oceanic and Atmospheric Administration monthly summaries. Precipitation and temperature variables (Table 1) were formulated that would potentially relate to plant moisture status at the time of spraying, washing of the herbicide off of foliage, or drying of the spray on the leaf surface. These are among the processes believed or demonstrated to be related to glyphosate susceptibility of plants. Related data pertaining to specific tracts including date of spraying, time of spraying during the day, and presence or absence of dew were obtained from the landowner's operational reports.

Multiple linear regression analysis was then used to assess relationships between efficacy variables and weather variables, water

Table 1. Weather variables tested as independent variables in multiple linear regression analysis.

1.	PREDAYS - Days since > 0.5 inches rainfall before spraying
2.	PREAMT - Amount of last rainfall > 0.5 inches before spraying
3.	PREAMT/PREDAYS
4.	PREAVG - Mean daily rainfall for the 10 days preceding spraying
5.	SPRAMT - Amount of rainfall on the day of spraying + the day after spraying
6.	POSTDAY - Days until > 0.5 inches rainfall after spraying
7.	POSTAMT - Amount of next rainfall > 0.5 inches after spraying
8.	POSTAMT/POSTDAY
9.	POSTAVG - Mean daily rainfall for the 10 days following spraying
10.	PRETEMP - Mean high temperature for the 10 days preceding spraying
11.	SPRTEMP - Sum of the high temperature on the day of spraying and the day after spraying
12.	POSTEMP - Mean high temperature for the 10 days following spraying
13.	PREAVG/PRETEMP
14.	POSTAVG/POSTEMP
15.	SPRAVG/SPRTEMP

potential, foliar sugar content, and foliar starch content. Regression analyses were carried out separately for each species using both individual tree measures and tract means. Starch and sugar analyses were additionally separated by year of treatment because carbohydrate levels may vary from year to year depending on local growing conditions.

Results

Aerial glyphosate application generally provided a successful silvicultural cleaning (Figure 1). Across the 16 tracts, loblolly pine was in the poorest competitive position at the time of spraying. After one growing season, loblolly pine was in a better position relative to all four competing species on the average. However, on seven of 16 sites, crown volume of at least one competing species was still larger than loblolly pine after one year. One of these tracts was re-sprayed the next year because hardwood control was considered inadequate to secure pine dominance. After two years, only red maple was larger than loblolly pine on any tracts (two of 12). Overall, data from the 12 tracts sprayed in 1984 indicated that loblolly pine is increasingly dominating the available growing space.

Regression equations predicting CVI of unsprayed trees after one year, using CVI at the time of spraying as the independent variable, produced coefficients of determination (r^2) of 0.82 to 0.95 for the five species. Calculation of percent control of individual trees using these equations further supports the whole-tract data in Figure

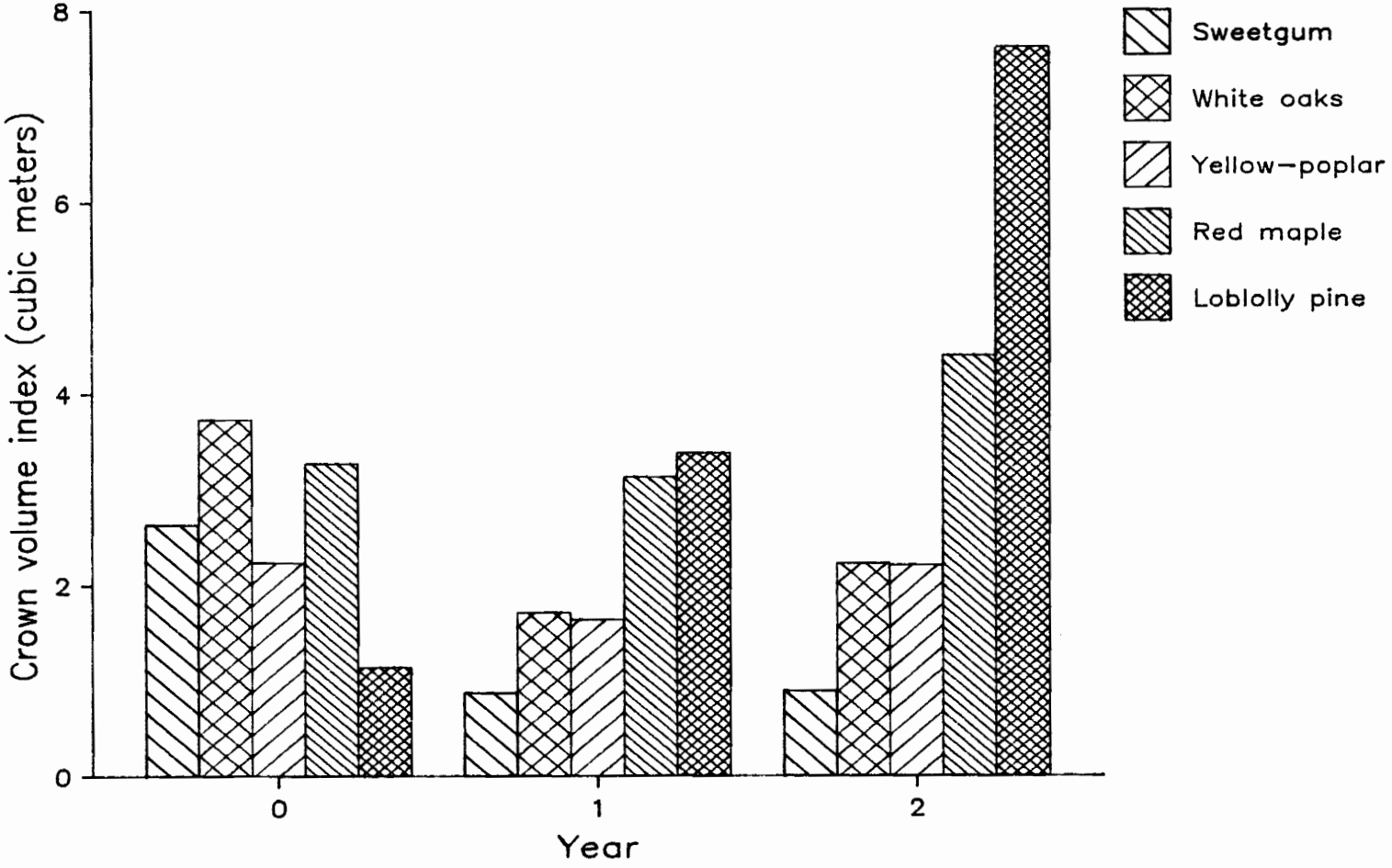


Figure 1. Crown volume response of five species to aerial glyphosate release in 1984 (12 tracts) and 1985 (4 tracts) on the Virginia Piedmont and Coastal Plain.

1 (Table 2). Standard deviation of control exceeds mean control in three of the five species. Variation was lowest in sweetgum, the species most susceptible to glyphosate. Variability among tracts, although less than that among individual trees, was still high.

Loblolly pine growth was slightly suppressed by glyphosate, but gained a competitive advantage relative to all other species. Susceptibility of competitors was in the order red maple = yellow-poplar < white oaks < sweetgum (Table 2). Yellow-poplar may be more susceptible than indicated here because many small individuals were sampled that may not have intercepted a proportionate dose of herbicide due to shading by neighboring stump sprouts.

Using tract averages, white oak control was directly related to foliar sugar content (Figure 2) in both 1984 and 1985. Sugar concentration in white oaks may be an indicator of phloem transport activity or a generally higher metabolic state.

In loblolly pine, initial crown volume and relative completion of the last growth flush accounted for 89% of the variability in control:

$$\text{Control} = 0.60 - 0.21(\text{prespray crown volume}) - 0.783\ (\text{percent completion of last terminal flush})$$

This equation indicates that larger pines and pines nearer to fall bud set are less susceptible to glyphosate injury. No potential relationships were evident for the other species.

In general, relationships between weather variables and efficacy (Table 3) were marginally significant ($0.10 < p < 0.20$). Weather

Table 2. Mean percent control (+/- 1 standard deviation) after one year for individual trees across eight tracts sprayed for release with glyphosate. Means differ because the number of individuals of a species measured on each tract ranged from 20 to 40.

	% Control			
	Individual trees		Tracts	
Species	Mean	Stand. dev.	Mean	Stand. dev.
Loblolly pine	14	42	15	29
Red maple	48	53	53	29
Yellow-poplar	47	56	59	24
White oaks	65	54	80	21
Sweetgum	71	39	92	12

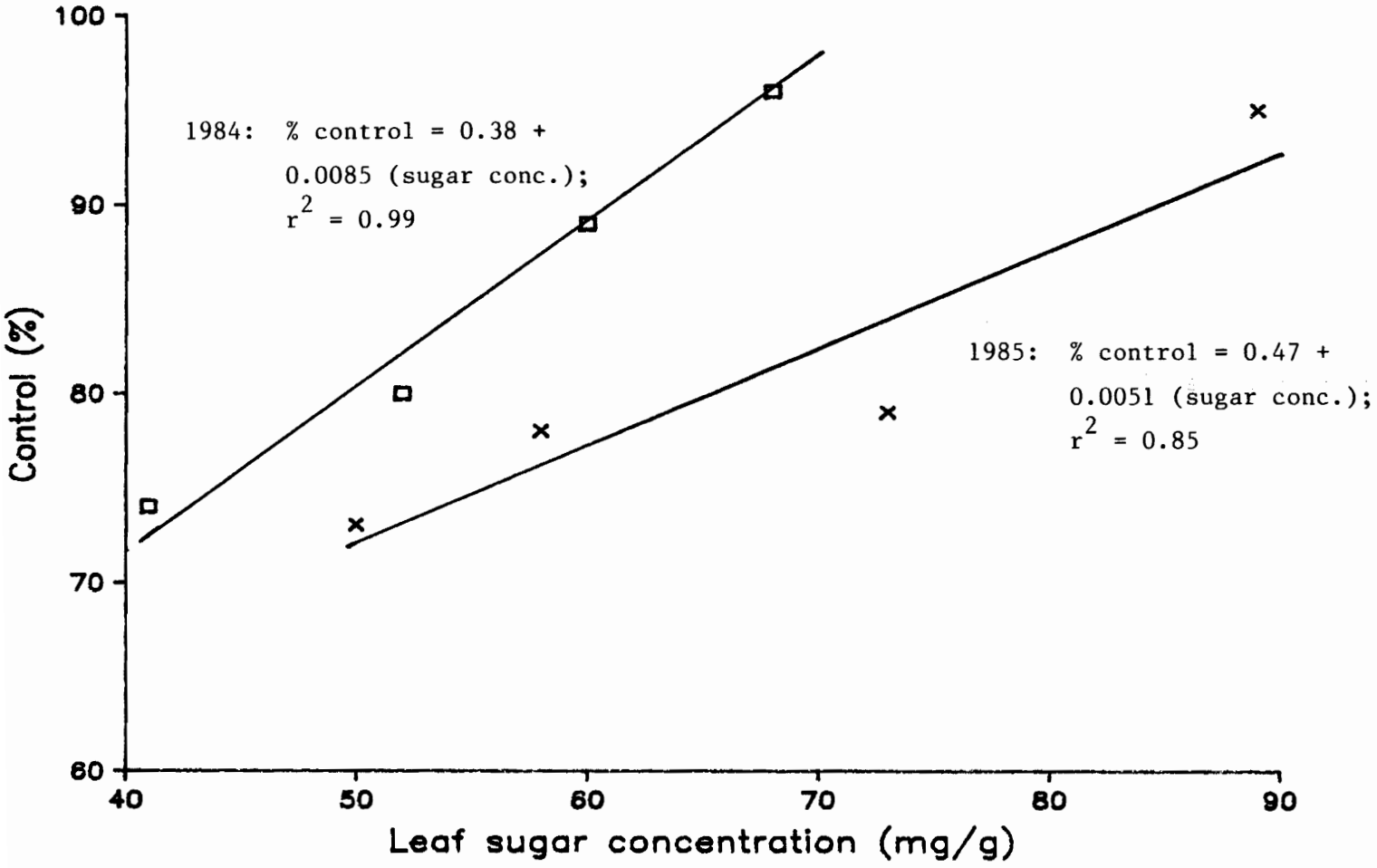


Figure 2. Relationship of mean control of white oaks on eight tracts in Virginia treated with glyphosate and average leaf sugar concentration.

Table 3. Correlation of weather variables with mean species efficacy on 16 tracts sprayed for loblolly pine release with glyphosate in Virginia in 1984 and 1985.

Species	Weather variable	Efficacy variable	Correlation coefficient	p
Loblolly pine	POSTAMT	rel. growth	0.36	0.17
	SPRTEMP	rel. growth	-0.65	0.01
Red maple	PREAVG	% control	-0.37	0.16
	PREAVG/PRETEMP	% control	-0.36	0.16
White oaks	PREDAYS	% control	0.43	0.10
Sweetgum	POSTAVG	% control	-0.40	0.14
	POSTEMP	% control	0.39	0.15
	POSTAMT/POSTDAY	% control	-0.41	0.13
	POSTAVG/POSTEMP	% control	-0.41	0.13

variables that included some measure of precipitation or temperature after spraying (POSTAMT, POSTAVG, POSTAMT/POSTDAY, and POSTAVG/POSTEMP) were related to efficacy inversely. As precipitation after spraying increased, efficacy measured as % control decreased and measured as relative growth increased. This may indicate that washing of herbicide off of foliage and/or relief from water stress are important in glyphosate efficacy.

Correlations between control and initial crown size, water potential, sugar content, starch content, and relative completion of annual height growth of individual trees were not significant ($p > 0.05$).

Discussion

The results of this study provide evidence to further document hypothesized sources of variability in forestry herbicide efficacy. Increasing temperatures after spraying leading to increased efficacy, as found here, are expected because increasing evaporation from spray droplets on the foliage effectively increases the glyphosate concentration gradient between the leaf surface and interior (Caseley and Coupland 1985). However, significant weather variables measuring precipitation before spraying (PREAVG, PREAVG/PRETEMP, and PREDAYS) were related to efficacy in the opposite way than expected, although the correlations were not strong (Table 3). Increasing water supply before spraying should increase efficacy through maintenance of active growth and metabolism.

Miller and Starr (1963) similarly reported increasing pine mortality as temperature on the day of 2,4,5-T spraying increased. They also found a significant positive relationship between sweetgum mortality after 2,4,5-T application and a soil dryness index for the early part of the spray season (May 30 - July 25), but no significant relationship when weekly data through September 12 were included.

The inverse relationship between loblolly pine injury (control) and relative completion of annual growth in this study is somewhat similar to results found for western conifers. Paley and Radosevich (1984) stated that leader growth rate was related to ponderosa pine glyphosate damage from May - July only, although this was not evident from the data they presented. No relationship from August - October was found. King and Radosevich (1985) reported simple correlations between growth rate and glyphosate damage averaging 0.33 - 0.50 for five Northwest conifers.

Less loblolly pine injury from glyphosate when applied to larger trees may have important implications for scheduling release operations. Applications might best be made in the first or second year of the stand to reduce non-crop vegetation as early as possible and maximize pine growth response (Bacon and Zedaker 1987), yet foresters might also prefer to wait until pines are larger, in the third or fourth year, for broadcast applications to reduce loblolly pine injury.

Non-significant relationships between plant water potential and glyphosate efficacy in Virginia are not surprising considering results from the Pacific Northwest. Although Paley and Radosevich (1984)

reported that daytime water potential was significantly related to glyphosate injury in both ponderosa pine and greenleaf manzanita, a prior study (Radosevich et al. 1980) indicated no pattern in glyphosate injury to conifers related to predawn water potential measured before (April), during (July), and after (September) the growing season. Water potential correlations with damage averaged only 0.13 - 0.23 for several northwestern conifers (King and Radosevich 1985). Lanini and Radosevich (1982) found photosynthesis and water potential were significantly correlated with glyphosate efficacy in five northwestern brush species based on three application dates. However, coefficients were both positive and negative for each physiological variable, demonstrating the difficulty in developing a consistent model of glyphosate activity in a mixture of brush species.

The degree of success in explaining species' response to glyphosate in commercial applications may reflect several phenomena. There are obviously many field conditions that cannot be controlled and were not measured in this and other studies. Differences in site productivity, leaf morphology, leaf orientation, and interspecific competition could potentially affect growth status, herbicide absorption, herbicide interception, and utilization of available water on the site. Using weekly 2,4,5-T applications to sweetgum, post oak, and loblolly pine, Miller and Starr (1963) demonstrated that efficacy trends throughout a growing season vary by species in the Southeast. Measures such as water potential are expected to integrate many of these effects, but they may not do so fully.

Although leaf sugar concentration may be a good indicator of

susceptibility in some species, such as white oaks, it is a poor indicator in other species. Although species ranking of sugar concentration was red maple (49.6 mg/g tissue) < loblolly pine (49.7) < white oaks (63.2) < sweetgum (72.3) < yellow-poplar (81.1), differences in absolute levels may be more a species characteristic than an indication of mobilization of sugars out of leaves to growing or storage organs within a species. Also, the principal organs where carbohydrates are being stored at this time may vary by species. If carbohydrates are translocated to be stored in current-year twigs in a tolerant species, at least partially, then crown volume reduction would be less than in plants or species where phytotoxic glyphosate gets all the way to the root system.

Sweetgum was usually controlled so successfully tract-wide (Table 2) at this application rate that sufficient efficacy variation may not have existed for correlation with phenological variables such as leaf sugar concentration. Use of a lower rate might reveal important plant indicators of susceptibility. In white oaks, absorption of the herbicide may have been consistently sufficient, but active plant metabolism, perhaps signalled by high sugar levels, was necessary for the herbicide to be effectively translocated and kill cells. Red maple, which is least susceptible to glyphosate of those hardwoods investigated, may exhibit this response due to low translocation (see Chapter 3). In loblolly pine, the species most likely to be actively growing in late summer, less interception or absorption of the herbicide by needle leaves may be a reason for its greater tolerance than broadleaved species, but Lund-Hoie (1976) attributed late-season

glyphosate tolerance of Norway spruce to reduced herbicide absorption.

Lund-Hoie (1979) has suggested that long-distance herbicide transport necessary in woody plants may be reduced by blocking of the phloem conducting system or binding of the glyphosate molecule. In a companion experiment to the study reported here, efficacy in container-grown loblolly pine, red maple, and sweetgum generally followed leaf sugar concentration in an inverse relationship. This suggests that conjugation of the glyphosate molecule with sugars, a potential means of herbicide detoxification mentioned by Lund-Hoie (1976), may occur. This relationship was not detected in these species in field glyphosate applications because the phenological condition of the container-grown seedlings may have differed from the field seedlings, or variation in field spraying conditions confounded the influence of leaf sugars.

Brady and Hall (1976) were also only partially successful in explaining susceptibility of several woody species, including red maple, to 2,4,5-T using leaf and root sugar concentrations. However, their trees were not treated with herbicide at the time of tissue sampling to relate efficacy closely to sugar gradients. Prior efficacy data was relied on. Also, root tissue samples apparently were not separated into live and dead portions, diluting and biasing root sugar concentrations.

It appears that the mechanisms that determine glyphosate injury, or the conditions that trigger these mechanisms, vary by species. Lund-Hoie (1979) found low absorption (20%) but high phloem translocation in susceptible birch seedlings, yet low root

accumulation despite high absorption (70%) in ash, a tolerant species in the field. Translocation of ^{14}C-sugars to roots was rapid in both species. The action of glyphosate in ash was proposed to be initial phloem translocation, followed by movement into xylem tissue and accumulation in untreated leaves, resulting in a resistant species. Efficient symplastic entry and unimpeded transport was considered the key to glyphosate phytotoxicity.

Many relationships may be masked by inconsistent application. The actual dose of herbicide received by individual plants can be highly variable within a tract due to interacting effects of wind, flight direction, flight speed, and partial shading of smaller trees. Greater success in use of tract means rather than individual tree data may be due to the averaging of this variability in relative herbicide dose received by individuals. Between tracts, differences in ion concentration of water sources, suspended solids in the water source that can partially clog lines, batching of the solution, and flight pattern all can change the quantity of active herbicide falling on a given area or plant surface. Studies in which relationships between plant processes and efficacy have been demonstrated are those in which tighter control on the application system has been maintained.

Summary

Operational application of glyphosate successfully releases loblolly pine from (in order of glyphosate susceptibility) sweetgum, white oaks, yellow-poplar, and red maple. Averaged for each tract,

foliar sugar concentration was directly related to control of white oaks. Control of loblolly pine was inversely related to both crown size at the time of spraying and relative completion of terminal growth. Use of individual tree measures of pre-dawn xylem water potential and foliar sugar and starch content was not successful in predicting response to glyphosate. Weather variables were marginally correlated with glyphosate efficacy.

Chapter 3

EFFECTS OF APPLICATION DATE AND WATER STRESS ON SUSCEPTIBILITY OF RED MAPLE, SWEETGUM, AND LOBLOLLY PINE SEEDLINGS TO GLYPHOSATE

Introduction

Previous studies of application timing have recommended late summer - early fall for best conifer selectivity (Paley and Radosevich 1984). This timing is assumed to take advantage of a period when conifer metabolic activity is low relative to competing broadleaved species. In the Southeast, however, most hardwoods complete annual shoot growth before loblolly pine, which often produces late-summer flushes in response to soil water replenishment (Bassett 1964). Within the late summer spraying window in the southeastern U.S., no gradients in efficacy as the season progresses have been investigated.

Decreases in glyphosate efficacy induced by water stress have been demonstrated in herbaceous species (Lauridson et al. 1983, Ahmadi et al. 1980, Caseley and Coupland 1985). In greenhouse-grown woody plants, water stress affects activity of 2,4,5-T and picloram (Badiei et al. 1966, Davis et al. 1968). Mechanisms of glyphosate activity in forest trees have been studied in greatest detail by Lund-Hoie (1976, 1979), who experimented with Norway spruce, European white birch, and European ash. He reported that absorption ranges from 20-70% between species, absorption continues up to two weeks after application, translocation similarities to photosynthate paths is

species-specific, and metabolism of the glyphosate molecule ranges from one month to greater than four months depending on species.

Plant conditions that affect glyphosate efficacy are often difficult to identify under field conditions, where many potentially important sources of variability cannot be controlled. Use of greenhouse and nursery environments allows tighter control of environmental conditions so that relationships between plant responses and treatment effects can be clarified. This experiment was conducted to:

1) assess effects of date of application and water stress on tree seedling susceptibility to glyphosate,
2) compare responses of three species with different susceptibility to glyphosate based on field observation, and
3) evaluate the relationship between efficacy differences and herbicide absorption and translocation patterns.

Subjective comparison of results with field data was used to indicate whether observed greenhouse relationships are important there as well, or if other sources of variation must be investigated to optimize efficacy in commercial operations.

Materials and Methods

Seedlings of red maple, sweetgum, and loblolly pine were raised from seed at the Reynolds Homestead Agricultural Experiment Station in Critz, VA. Seeds were sown in the early winter of 1985-86 in 98 cm^3 plastic containers (Ray Leach Nursery, Canby, OR) in a 1:1 mix of sand

and Pro-Mix BX^{R}. Seedlings were grown for approximately 10 weeks and put outside in a lathhouse in March, 1986 to induce dormancy. Plants were moved to an unsheltered nursery bed in May, 1986 to resume growth for their second growing season.

In the late summer and early fall of 1986, seedlings were brought into the greenhouse at bi-weekly intervals to impose moisture stress treatments. Three water stress/spray treatments were selected: 1) sprayed after no stress - watered daily, 2) sprayed after moderate stress - no water for approximately seven days before glyphosate application, and 3) sprayed after severe stress - no water for approximately 10 days before glyphosate application. Unsprayed seedlings that were watered daily were used as the experimental control. Predawn water potential was measured during the period stress was imposed to monitor development of the treatments.

At each of four application dates, four replications of 40-49 seedlings were assigned to each stress treatment for each of the three species. The experimental design was completely randomized. Spray dates were 1) August 25-26, 2) September 8-9, 3) September 22-23, and 4) October 4-5. Height of five randomly selected, permanently numbered seedlings in each replication was measured approximately two weeks before spraying and within three days of spraying. Growth rate was calculated as:

(Height 1 - Height 0)/Number of days between height measurements

On the morning of spraying, predawn leaf water potential was measured

with a pressure chamber (PMS Instruments, Corvallis, OR) (Scholander et al. 1965) on the five numbered seedlings in each replication between 3 and 7 a.m. Two mature leaves were taken from each of the five seedlings for determination of sugar and starch concentration, and all 10 leaves for a replication were put in a plastic bag, sealed, placed on dry ice, and put in a freezer.

Trays of seedlings were sprayed in the morning by running them beneath a spray nozzle on a conveyor belt. Prior testing indicated selectivity comparable to field results would be achieved with glyphosate applied at a rate of 0.84 kg a.i./ha in 94 L total volume/ha with distilled water as the carrier. Spray replications consisted of 40 seedlings placed standing in a tray, spaced one inch apart to assure full spray coverage. Up to 14 remaining seedlings for each two sprayed replications were unsprayed as a check on water stress-induced mortality. After spraying, the unsprayed seedlings were returned to the tray with the sprayed seedlings and handled identically until harvest.

On dates 2, 3, and 4, a 0.15 uCi dose of ^{14}C-glyphosate was applied to the base of a single upper mature leaf of five seedlings from each treatment of each species. Seedlings were chosen from among those sampled for water potential, foliage, and growth rate. The ^{14}C solution was mixed to a concentration equal to that sprayed over all seedlings. After application, seedlings were returned to the greenhouse and watered from below every other day by placing containers in a bucket of water. After two weeks, seedlings were harvested by separating each plant into five sections: 1) roots,

2) shoot below the treated leaf, 3) shoot above the treated leaf, 4) treated leaf, and 5) treated leaf rinse. Unabsorbed glyphosate was washed from leaves, following the technique described by Devine et al. (1985), with three consecutive rinses of deionized water totaling 20-25 ml from a wash bottle and collected in a scintillation vial. Tissue samples were stored in a freezer and leaf rinses in a refrigerator.

All other seedlings were returned to the greenhouse, watered from below the day they were sprayed, and put back out in the nursery bed the following day. After natural onset of dormancy and chilling, seedlings were brought into the greenhouse in January, 1987 to induce new growth. Preliminary efficacy was measured by classifying seedlings into one of three or four subjective injury classes (1=no injury, 2=new foliage moderately deformed, 3=new foliage flush inhibited, severely deformed, 4=dead - sweetgum and loblolly pine only) at the end of February and at harvest. At the end of March, terminal extension of up to 10 randomly selected live seedlings was measured per replicate. All seedlings were then harvested by replicate. Tissue from live seedlings was separated into new shoot growth and older growth, which were separate from dead shoots. Tissue was placed directly in a 65^{o}C oven. All tissue samples were dried at least 48 hours to constant weight, then weighed.

^{14}C tissue samples were oven-dried, weighed, and ground. A subsample was weighed and oxidized in a Packard Model 306 Biological Oxidizer (Packard Instrument Co., Downers Grove, IL) using 10 ml of Carbo-Sorb^{R} (Packard Instrument Co.) to absorb $^{14}CO_2$ and 10 ml

Permafluor V^{R} (Packard Instrument Co.) liquid scintillation cocktail. Twenty ml of Ecoscint^{R} (National Diagnostics Inc., Somerville, NJ) liquid scintillation cocktail was added to a one ml subsample of each leaf rinse sample. All vials were then counted in an LKB Wallac Model 1417 (LKB Inc., Gaithersburg, MD) liquid scintillation counter. Corrections from cpm to dpm were made using a quench curve obtained with commercially prepared standards (Ammersham Chemical Co.).

Laboratory procedures for measurement of starch and sugar concentrations followed the method of Rowe (1984). Tissue was dried, ground, and weighed. Sugar was extracted with in a 95^{o}C bath with 20% ethanol. After three extractions, residual starch was hydrolyzed by incubation in a solution of amyloglucosidase enzyme and separated from tissue residue by vacuum filtration. Sugar was quantified by reaction with anthrone in a boiling water bath and measurement of spectrophotometric absorbance at 620 nm.

Data was analyzed using two-way analysis of variance employing the General Linear Models procedure of the Statistical Analysis System (SAS Institute, Cary, NC). Effects of date and water stress treatment and their interaction on efficacy were tested with four dates and three treatments. Sweetgum data for date 2 were discarded because seedlings were flooded with water on the day of spraying in the course of watering from below. Efficacy on this date was clearly diminished.

Non-herbicide injury to three replications of sweetgum detected in the unsprayed 14 seedlings in each tray caused exclusion of those replications from efficacy data analysis. Despite similar injury to loblolly pine, all replications were included in data analysis because

mortality patterns were uniform among replications, so results were not affected. Non-herbicide mortality was visually symptomized by a medium brown color of normally formed foliage, while herbicide mortality was characterized by dark brown, shriveled, malformed leaves. The criterion for exclusion of replications was survival of sprayed trees exceeding that of the unsprayed trees in that replication. This resulted in omission of 3 of 42 sweetgum replications (date 2 already excluded).

Effects of date and water stress on ^{14}C-glyphosate action were tested with three dates and three stress treatments. Comparison of absorption and translocation with efficacy were made omitting the date 1 efficacy data because no ^{14}C application was made on that date.

Results

Species comparison: Response to the water stress treatments was dependent on species (Table 4). Loblolly pine pre-dawn water potential gradually decreased as water was withheld for a longer period, and significant differences developed at spray time. Red maple showed a similar response, but only reached approximately half the water potential of loblolly pine for the same treatment. Much smaller differences in leaf water potential were significant for sweetgum. Despite these high levels of water potential measured in the early morning, visual wilting in the afternoon developed for some seedlings in both treatments 3 and 4, particularly in sweetgum.

Species differences in pre-spraying physiological condition were

Table 4. Mean leaf water potential of container-grown seedlings for each water stress treatment by species, combined over four application dates (Aug. 25 - Oct. 5, 1986).

	Treatment[1]			
Species	Control	1	2	3
	- - - - - - - - - - (MPa) - - - - - - - - - -			
Sweetgum	-0.16 bc[2]	-0.15 c	-0.19 ab	-0.19 a
Red maple	-0.22 b	-0.20 b	-0.39 a	-0.52 a
Loblolly pine	-0.46 c	-0.52 c	-0.75 b	-1.03 a

[1] Treatments: 1 = watered daily + sprayed, 2 = water withheld approx. 7 days + sprayed, 3 = water withheld approx. 10 days + sprayed.

[2] Means within a row followed by the same letter are not signifciantly different (Duncan's Multiple Range Test, alpha=0.05).

also apparent (Table 5). Foliar starch concentration was the same for all species, but foliar sugar concentration was significantly different between species. Pre-spray growth rate was slower in red maple than sweetgum and loblolly pine. Differences in water potential, as discussed above, also existed.

Efficacy of glyphosate was measured by new growth in tissue dry weight, survival, and terminal growth after spraying (Table 6). Data are expressed relative to growth or survival of the control treatment for that species. Glyphosate was clearly more phytotoxic to sweetgum than red maple and loblolly pine. Survival of red maple was very high. Survival of loblolly pine was excessively low for a commercial application, but reflects additional non-herbicide mortality. Superior relative terminal growth of loblolly pine indicates more consistent kill of the terminal bud in sweetgum and red maple. Terminal growth measurement does not account for growth of laterals, which may overcome the loss of the previous terminal within one year.

Translocation patterns based on recovery of ^{14}C-glyphosate in different plant sections explains these efficacy differences (Table 7). All three species absorbed similar quantities of glyphosate into the leaf. Sweetgum, the most susceptible species, transported nearly triple the amount of glyphosate in the phloem (roots + stem below treated leaf) relative to red maple and loblolly pine. Only loblolly pine showed appreciable translocation in the xylem (stem above treated leaf).

Table 5. Pre-spraying levels of foliar sugar concentration, foliar starch concentration, water potential, and growth rate of container-grown seedling sweetgum, red maple, and loblolly pine, for all treatments combined.

	Species		
Seedling attribute	Sweetgum	Red maple	Loblolly pine
Sugar concentration (mg/g)	71.7 a[1]	45.5 c	63.0 b
Starch concentration (mg/g)	4.0 a	4.5 a	3.1 a
Leaf water potential (MPa)	-0.18 c	-0.37 b	-0.75 a
Growth rate (mm/day)	1.01 a	0.62 a	0.93 a

[1] Means within a row followed by the same letter are not signifciantly different (Duncan's Multiple Range Test, alpha=0.05).

Table 6. Differences in efficacy between container-grown seedlings of sweetgum, red maple, and loblolly pine sprayed with 0.84 kg a.i./ha glyphosate on four dates from Aug. 25 - Oct. 5, 1986.

	Species		
Efficacy measure	Sweetgum	Red maple	Loblolly pine
	- - - - (% of control) - - - - - - -		
Dry wt. of new tissue per seedling (of surviving trees)	17 b[1]	47 a	43 a
Survival	50 b	96 a	84 a
Terminal growth per seedling (of surviving trees)	31 b	37 b	68 a

[1] Means within a row followed by the same letter are not signifciantly different (Duncan's Multiple Range Test, alpha=.05).

Table 7. Translocation patterns and absorption of ^{14}C-glyphosate in container-grown seedlings of sweetgum, red maple, and loblolly pine.

Plant section	Species		
	Sweetgum	Red maple	Loblolly pine
	- - - - (% of ^{14}C absorbed) - - - - - -		
Roots	48 a[1]	13 b	3 c
Stem below treated leaf	26 a	12 b	22 a
Stem above treated leaf	4 b	1 b	12 a
Treated leaf	22 c	74 a	63 b
	- - - - (% of ^{14}C applied) - - - - - - -		
Total	51 a	42 a	49 a

[1] Means within a row followed by the same letter are not significantly different (Duncan's Multiple Range Test, alpha=.05).

Date and water stress effects: Date of glyphosate application and water stress treatment significantly interacted to affect dry weight of new tissue produced in red maple ($p = 0.0002$) and loblolly pine ($p = 0.004$). However, the interaction pattern is not orderly in either species (Figure 3). In loblolly pine, increasing water stress decreased glyphosate efficacy on date 1, but as internal physiological changes associated with fall dormancy occur at the later application dates, there was little relationship between water stress treatment and efficacy. In red maple, efficacy on date 1 was also reduced by water stress. Efficacy for all treatments is highest on date 3, perhaps coinciding with the time of greatest reabsorption of nutrients and carbohydrates into perennial portions of the plant. Formation of the leaf abscision layer by date 4 may be responsible for the associated decrease in efficacy for all treatments at that time. The effect of application date in reducing sweetgum efficacy was marginally significant ($p = 0.10$). Although water stress had no significant effect on loblolly pine efficacy as measured by dry weight of new tissue, water stress did increase pine survival ($p = 0.0001$).

Seasonal patterns in efficacy are most closely associated with foliar sugar concentration (Figure 4). Although sugar levels were not statistically different among dates, the direction and magnitude of changes in leaf sugars roughly paralleled changes in efficacy for all three species. This suggests that sugars may play a role in metabolically detoxifying the glyphosate molecule.

Main effects of water stress on ^{14}C-glyphosate absorption and phloem translocation in sweetgum were highly significant ($p = 0.03$ and

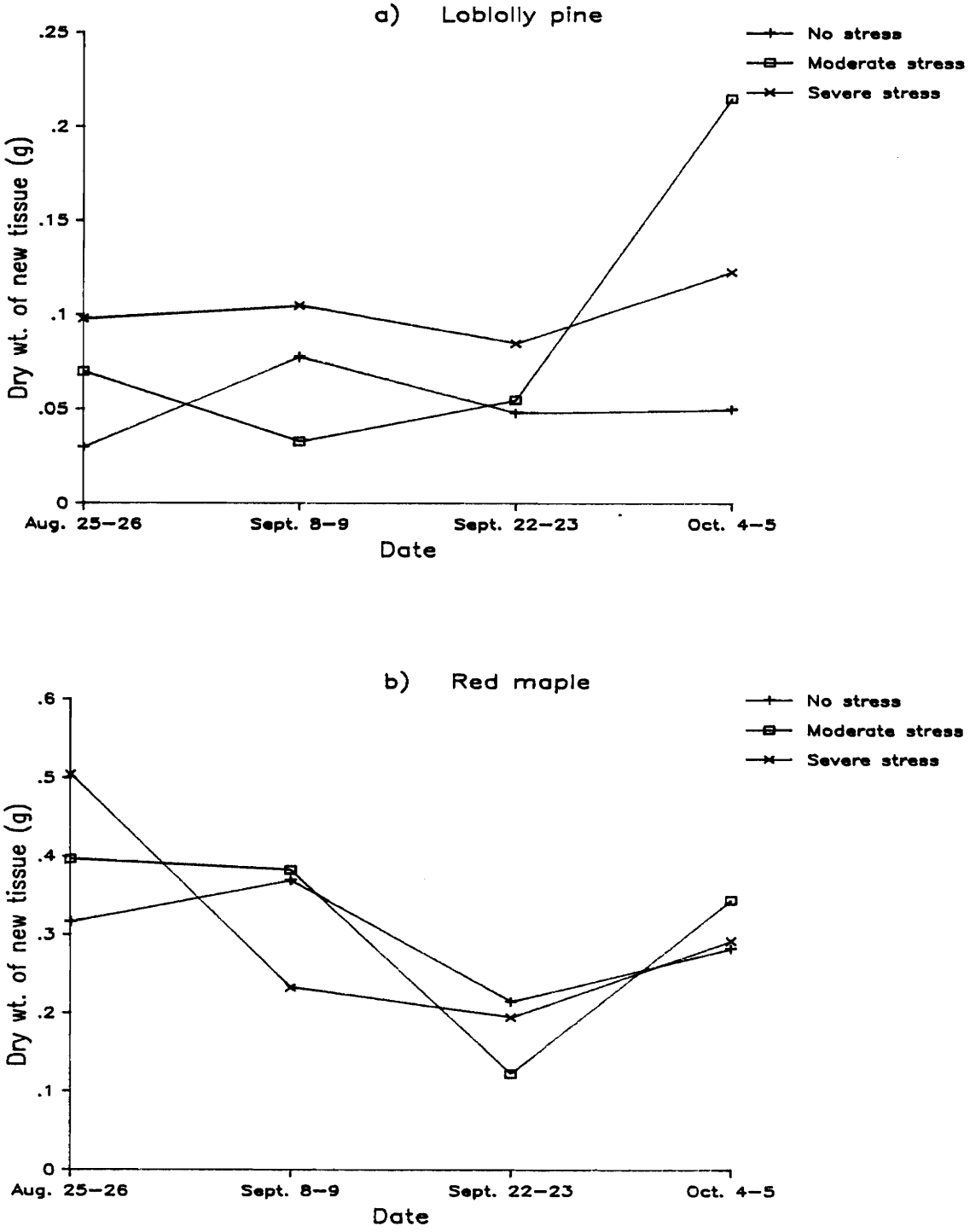


Figure 3. Interacting effects of application date and water stress treatment on glyphosate efficacy in container-grown a) loblolly pine and b) red maple.

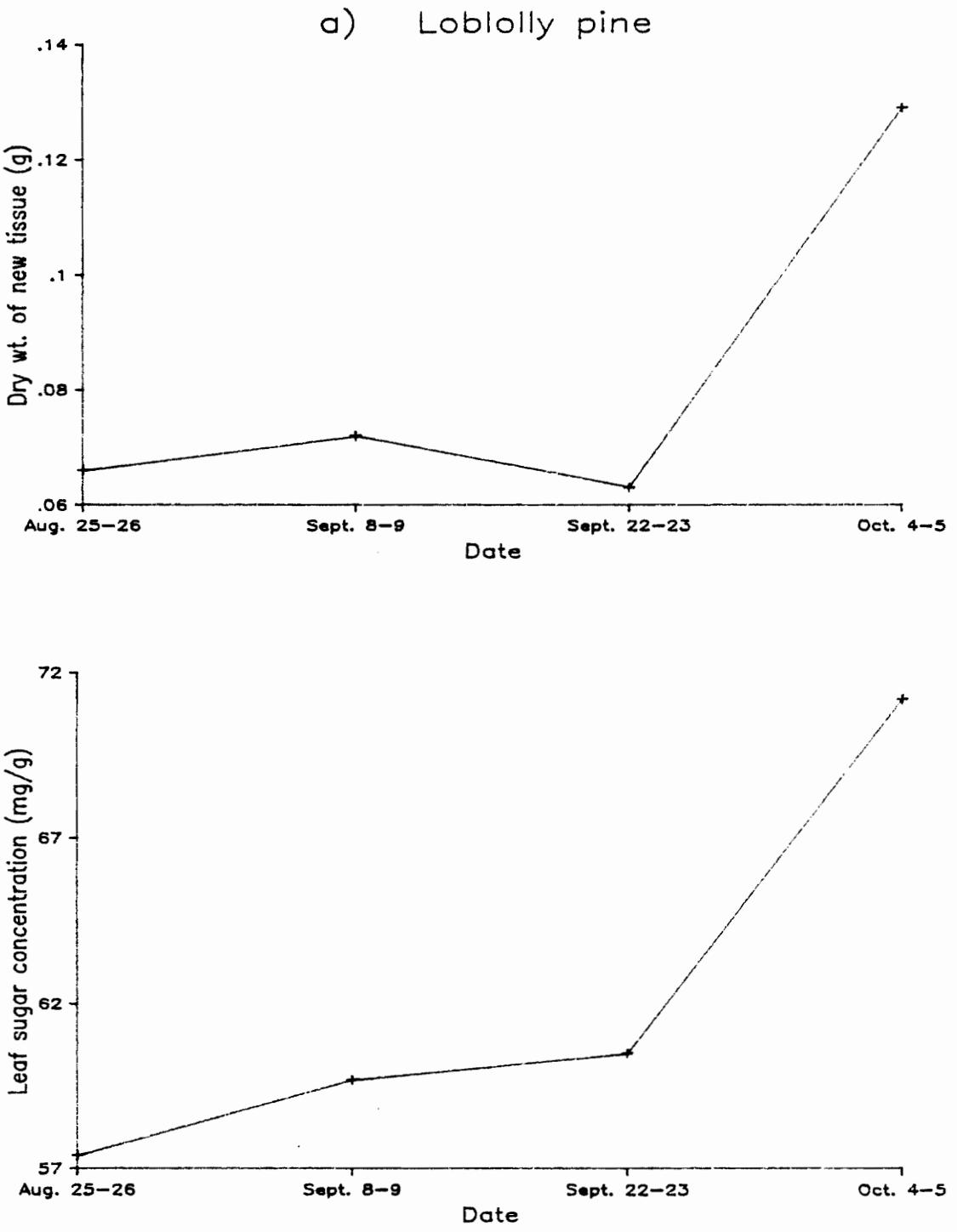
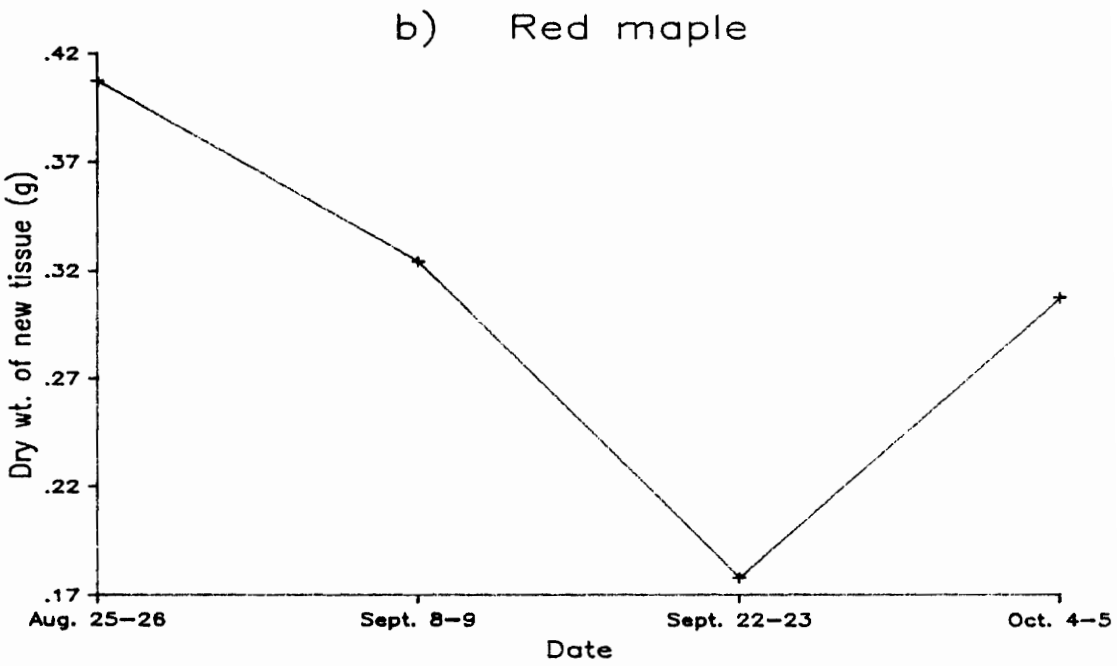
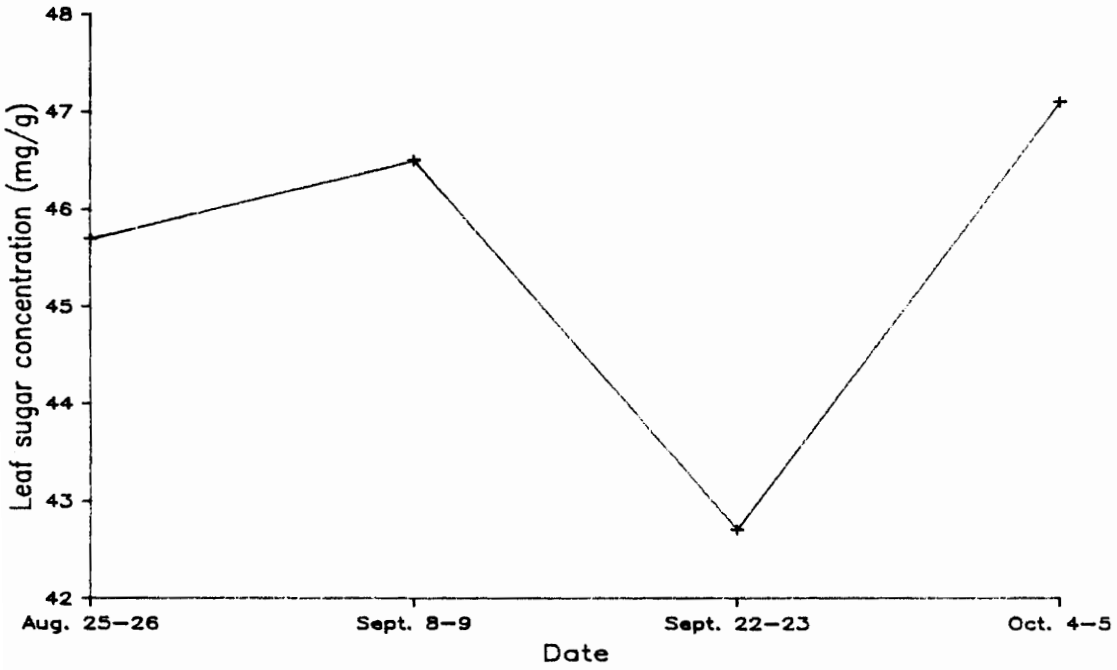


Figure 4. Seasonal changes in glyphosate efficacy, measured by dry weight of new tissue produced, and leaf sugar concentration in container-grown a) loblolly pine, b) red maple, and c) sweetgum.





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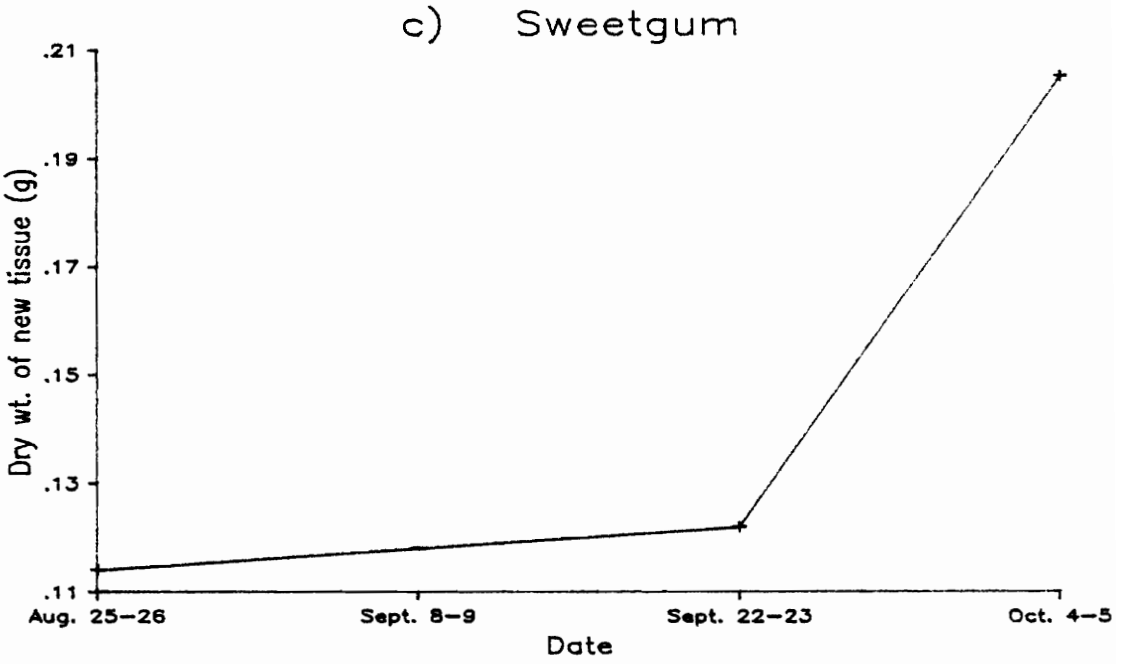

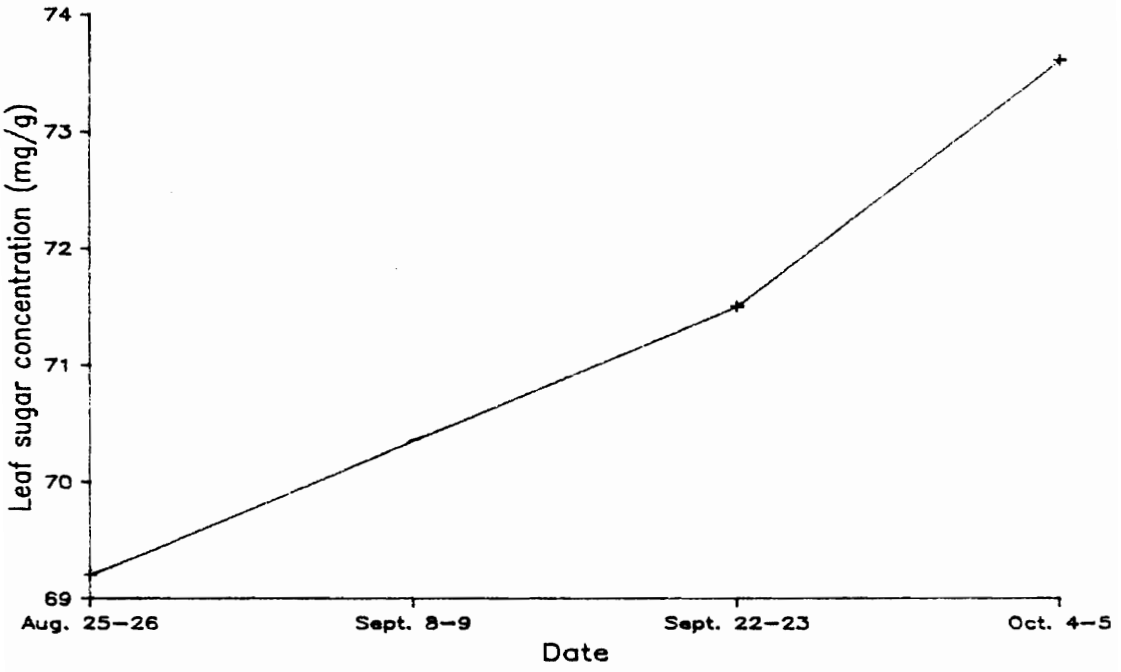

Figure 4 (continued).

0.01, respectively). Greatest absorption and phloem translocation occurred on date 2, least on date 3, with intermediate levels on date 4. The reversal on dates 3 and 4 of the expected pattern associated with plant phenology changes suggests that a non-plant factor, such as weather conditions the day of spraying, may have affected glyphosate efficacy.

Effects of application date on glyphosate activity were more important than water stress in red maple. Significantly less herbicide was transported out of the treated leaf ($p = 0.01$) into the phloem as the season progressed. In loblolly pine, date was generally more important than water stress in herbicide movement, following the pattern in red maple. Less glyphosate was transported both above and below the treated leaf in loblolly pine after date 2.

Discussion

Characteristics of efficacy and translocation patterns among species raised in the nursery agree well with observed responses to glyphosate in the field. Approximately 50% control of red maple in this study agrees closely with field efficacy (see Chapter 2). Further, red maple was a very difficult species to kill at rates that are highly toxic to sweetgum. Although sweetgum translocation did not respond directly to date of application, as expected, the large amount of active herbicide in sweetgum or its relatively high susceptibility to glyphosate may have masked these effects. Loblolly pine was the only species with appreciable xylem transport of glyphosate, which may

explain the characteristic kill of only the most recent terminal flush in field applications.

Lund-Hoie (1976) attributed glyphosate tolerance in Norway spruce seedlings to reduced herbicide absorption late in the growing season. ^{14}C-glyphosate movement followed that of $^{14}CO_2$ during shoot elongation, but not after shoot growth had ceased. Biochemical conjugation of glyphosate with plant sugars, indicated by the present study, was suggested as a likely mechanism of detoxification of glyphosate within the plants. Greater translocation of glyphosate in birch was responsible for its greater susceptibility than ash (Lund-Hoie 1979). Glyphosate accumulated in the roots with carbohydrates in birch, but glyphosate did not follow carbohydrate translocation to the roots in ash. Lund-Hoie also suggested that detoxification of glyphosate proceeds faster in leaves than in roots, at least in birch. Leaf application resulted in 30 - 40% metabolism of the 20% of ^{14}C-glyphosate absorbed. Stem application resulted in approximately 80% absorption, with only about 10% of this metabolized within two months. Some of these phenomena may similarly explain the overall differences in glyphosate injury in observed sweetgum, red maple, and loblolly pine.

Effects of timing and water stress were important contributors to response of these three species to glyphosate. Both of these factors and their interaction were important in red maple and loblolly pine (Figure 3). In sweetgum, date alone apparently was the key factor affecting response. Paley and Radosevich (1984) have also reported significant relationships between water stress and glyphosate injury

in monthly April - October treatments in ponderosa pine and greenleaf manzanita. This relationship was not apparent in a previous study including ponderosa pine sprayed in April, July, and September (Radosevich et al. 1980). Water stress induced slower growth and coincident lower translocation of picloram in winged elm and honey mesquite (Davis et al. 1968).

Coincidence of slower growth at time of spraying in red maple, and higher growth rate in sweetgum and loblolly pine (Table 5) with herbicide tolerance (Table 6) agrees with hypothesized relationships of these variables in forest trees (King and Radosevich 1985). Paley and Radosevich (1984), however, did not find a significant relationship between growth rate and glyphosate damage to ponderosa pine. Five other western conifers' susceptibility to glyphosate was also not significantly related to growth rate or water potential data, except for Jeffrey pine (P. jeffreyi Grev. & Balf.) (King and Radosevich 1985). Decreasing species efficacy caused by water stress coincides, however, with existing documentation of this phenomenon in herbaceous species (Ahmadi et al. 1980, Lauridson et al. 1983). Species differences in translocation rather than absorption of glyphosate support Caseley and Coupland's (1985) conclusion that symplastic entry is the key barrier to glyphosate phytotoxicity.

Summary

Phenological condition and water stress are significant factors in explaining selectivity among species and variation in efficacy within

species to glyphosate. Gross similarities in efficacy, supported by translocation patterns, between greenhouse and field data indicate that variation in field applications of glyphosate may be affected by timing and water stress. Because timing and water stress were not closely related to efficacy in an accompanying field study (see Chapter 2), effects of these factors must be overridden by other field conditions. Careful study of uniformity of applied dose to individual seedlings in the field may be the next step in accounting for variability in commercial operations.

Chapter 4

SUMMARY

Initial research on the suitability of glyphosate as a pine release herbicide produced mixed efficacy results on many species (Wu et al. 1983). Nevertheless, glyphosate was generally successful in suppressing competition enough to allow planted trees to dominate the young stand (Olinger 1982). With few other chemicals labelled for pine release, glyphosate became the dominant herbicide for this use.

Despite several studies regarding glyphosate's mode of action, absorption characteristics, and translocation patterns, no previous research has been done to expressly relate observed field variation to plant phenology and metabolism. Biochemical studies have shown that the key process affected by glyphosate is amino acid synthesis (Cole 1985). Translocation to actively growing meristems seems to be the primary pathway of mobilization of glyphosate out of leaves (Lund-Hoie 1976).

Translocation does not, however, necessarily follow the path of photosynthates, particularly in perennial plants once they have ceased shoot elongation (Lund-Hoie 1979, Badiei et al. 1966). An explanation for how glyphosate efficacy is related to foliar carbohydrate levels in late summer, when shoot growth has ceased and leaf abscision approaches, is confounded by whether sugars indicate increased efficacy through greater translocation, or contribute to reduced efficacy by chemical conjugation with the active herbicide molecule.

Further, the relative importance of these opposing factors is probably species-specific.

Translocation of photosynthates to roots occurs because roots are often still growing in the fall, and may grow throughout the winter in the South. However, measuring foliar sugar and starch levels does not reveal whether those compounds are destined to be moved to the roots, stored in other perennial tissue, or lost in leaf fall. A more complete plant carbohydrate budget is needed to more fully explain the pathways of phloem-mobile herbicides.

In West Coast pines, King and Radosevich (1985) considered growth activity most reliable as an indicator of glyphosate susceptibility. Growth activity appears to be a better surrogate measure of phloem activity than carbohydrate concentrations. In this study, low tolerance to glyphosate in containerized sweetgum corresponded to its faster growth rate at the time of spraying and higher foliar sugar content relative to the other species.

Interpretation of field and greenhouse water stress data also indicates areas where research could be extended. Water stress often reduces glyphosate efficacy in herbaceous species (Lauridson et al. 1983, Moosavi-Nia and Dore 1979), presumably because insufficient water initiates stomatal closure, thereby preventing uptake of CO_2 needed for photosynthesis. Reduced photosynthesis translates to less symplastic transport. In the short term, as in greenhouse experiments, effects of water stress on photosynthesis might be the dominant moisture-related factor bearing on efficacy. Over the longer term, moisture stress might be expected to decrease herbicide efficacy

if stress contributes to a thicker leaf cuticle or steeper leaf orientation. If morphological variables such as these come into play, then it is not surprising that pre-dawn water potential might not be detectable, as in this study, as an important predictor of efficacy in the field. West Coast brush species's susceptibility to glyphosate was significantly related to water potential, but relationships were both direct and inverse, depending on species (Lanini and Radosevich 1982).

The more significant relationships of leaf sugar concentration in white oak, and size and relative completion of terminal growth in loblolly pine, obtained using tract means of efficacy rather than individual stem efficacy probably indicate a balancing of relative dosages received by each sprout or seedling in the field. Inability to find significant relationships in individual greenhouse seedlings compared to whole replications can be partially attributed to the small range in water potential encountered in the early morning in red maple and sweetgum.

In the absorption and translocation study, ^{14}C-glyphosate movement patterns induced by treatment did not necessarily follow efficacy trends, but low sampling intensity might be part of the reason. Water stress measurements for a replication were based on a 12.5% subsample, then absorption and translocation were based on 25% of that subsample. Observation of varying degrees of wilting in the afternoon of individuals within a replication made it apparent that much within-replicatione variability existed. This could be due to plant crown and root system size, tray position, soil texture, or a

combination of these.

Although many potential sources of variability in the greenhouse study have been mentioned, significant application timing and water stress treatment effects were found. Greenhouse seedlings were also raised out in a nursery bed with irrigation rather than inside for as much of the study as possible to approximate characteristics of field seedlings. If greenhouse seedlings did match field seedlings in characteristics like cuticle development, leaf arrangement, and response to water stress, then the factor most likely to explain the lack of significant moisture stress relationships with field efficacy is the difference in application system. The spray hood used in the greenhouse study was repeatedly calibrated for application rate, and the spray pattern was watched closely. However, a helicopter that can apply 5 gal./ac. consistently may not be able to apply a uniform amount to every 100 ft^2. Clogging of the spray apparatus, wind, flight direction, and topography all may increase the herbicide dose in one spot at the expense of another, resulting in variable control simply due to varying dosage.

In conclusion, this research has shown that water stress and application timing, as an indicator of phenology, significantly affect susceptibility of loblolly pine, red maple, and sweetgum to glyphosate. Further, species differences in herbicide injury are explained by ^{14}C-glyphosate translocation. Insignificant effects of water stress and spray timing in the field are attributed to overriding influences of other site and stand factors and application variability.

LITERATURE CITED

Ahmadi, M. S., L. C. Haderlie, and G. A. Wicks. 1980. Effect of growth stage and water stress on barnyardgrass (Echinocloa crus-galli) control and on glyphosate absorption and translocation. Weed Sci. 28:277-282.

Bacon, C. G. and S. M. Zedaker. 1986. Third year growth response of loblolly pine to eight levels of competition control. South. J. Appl. For. 11:91-95.

Badiei, A. A., E. Basler, and P. W. Santelmann. 1966. Aspects of movement of 2,4,5-T in blackjack oak. Weed Sci. 14:302-305.

Balmer, W. E., K. A. Utz, and O. G. Langdon. 1978. Financial returns from cultural work in natural loblolly pine stands. South. J. Appl. For. 2:111-117.

Bassett, J. R. 1964. Diameter growth of loblolly pine trees as affected by soil-moisture availability. USDA For. Serv. Res. Pap. SO-9. 7 pp.

Boggess, W. R. 1956. Weekly diameter growth of shortleaf pine and white oak as related to soil moisture. Proc. Soc. Amer. For. 1956:83-89.

Brady, H. A. and O. Hall. 1976. Relation of sugar changes and herbicide susceptibility in woody plants. Proc. South. Weed Sci. Soc. 29:276-283.

Burch, P. L. and S. M. Zedaker. 1988. Timing of disturbance after herbicide applications for chemical site preparation. South. J. Appl. For. (in press).

Burkhart, H. E. and P. T. Sprinz. 1984. A model for assessing hardwood competition effects on yields of loblolly pine plantations. VPI & SU School of For. and Wildl. Res. Publ. No. FWS-3-84, Blacksburg, VA. 56 pp.

Caseley, J. C. and D. Coupland. 1985. Environmental and plant factors affecting glyphosate uptake, movement and activity. Chap. 7 in E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, London. 490 pp.

Cole, J. C. 1985. Mode of action of glyphosate - a literature analysis. Chap. 5 in E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, London. 490 pp.

Coupland, D. 1985. Metabolism of glyphosate in plants. Chap. 3 in E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, London. 490 pp.

Davis, F. S., M. G. Merkle, and R. W. Bovey. 1968. Effects of moisture stress on the absorption and transport of herbicides in woody plants. Bot. Gaz. 129:183-189.

Davis, H. E., R. S. Fawcett, and R. G. Harvey. 1979. Effects of frost and maturity on glyphosate phytotoxicity, uptake, and translocation. Weed Sci. 27:110-114.

Devine, M. D., H. D. Bestman, C. Hall, and W. H. Vanden Born. 1984. Leaf wash techniques for estimation of foliar absorption of herbicides. Weed Sci. 32:418-425.

Dickens, D. F. 1984. Impact of vegetation control on loblolly pine yield. Proc. South. Weed Sci. Soc. 37:255.

Dierauf, T. A. 1984a. Loblolly pine release study report number 1. Va. Div. For. Occas. Rep. 60. 8 pp.

Dierauf, T. A. 1984b. Loblolly pine release study report number 2. Va. Div. For. Occas. Rep. 61. 6 pp.

Donnelly, J. R. 1976. Carbohydrate levels in current-year shoots of sugar maple. USDA For. Serv. Res. Pap. NE-347. 6 pp.

Downs, J. P., A. P. Hagerbaumer, L. J. Bloomer, R. H. Blackwelder, D. Todd, and S. Dudley. 1984. Glyphosate for release of loblolly and eastern white pine in the Carolina Piedmont. Proc. South. Weed Sci. Soc. 37:243.

Duke, S. O. and R. E. Hoagland. 1978. Effects of glyphosate on metabolism of phenolic compounds. I. Induction of phenylalanine ammonia-lyase activity in dark-grown maize roots. Plant Sci. Let. 11:185-190.

Fitzgerald, C. H. 1982. Chemical site preparation and release in loblolly pine stands. pp. 78-85 in Proc. Symp. on the Loblolly Pine Ecosystem (East Region). North Car. St. Univ., Raleigh. 335 pp.

Ginter-Whitehouse, D. L., T. H. Hinckley, and S. G. Pallardy. 1983. Spatial and temporal aspects of water relations of three tree species with different vascular anatomy. For. Sci. 29:317-329.

Glover, G. R. and J. L. Creighton. 1985. Effects of hardwoods on pine yield, summary of data. Proc. South. Weed Sci. Soc. 8:256.

Guldin, R. W. 1984. Economic returns from spraying to release loblolly pine. Proc. South. Weed Sci. Soc. 37:248-254.

Hodges, J. D. and P. L. Lorio Jr. 1969. Carbohydrate and nitrogen fractions of the inner bark of loblolly pines under moisture stress. Can. J. Bot. 47:1651-1657.

Hoover, M. D., D. F. Olson Jr., and G. E. Greene. 1953. Soil moisture under a young loblolly pine plantation. Soil Sci. Soc. Amer. Proc. 17:147-150.

Jaworski, E. G. 1972. Mode of action of N-phosphonomethylglycine: inhibition of aromatic amino acid biosynthesis. J. Agric. Food Chem. 20:1195-1198.

King, S. P. and S. R. Radosevich. 1985. Herbicide tolerance in relation to growth and stress in conifers. Weed Sci. 34:472-478.

Kline, W. N., R. D. Fears, and S. M. Zedaker. 1985. Procedures for evaluating woody plant control in forest management systems. Down to Earth 41:9-15.

Kline, W. N. and F. A. Kidd. 1986. Economics of brush control in loblolly pine plantations. Proc. South. Weed Sci. Soc. 39:324-334.

Kramer, P. J. 1943. Amount and duration of growth of various species of tree seedlings. Plant Phys. 18:239-251.

Kramer, P. J. and T. T. Kozlowski. 1979. Physiology of woody plants. Academic Press, Orlando, FL. 811 pp.

Lanini, W. T. and S. R. Radosevich. 1982. Herbicide effectiveness in response to season of application and shrub physiology. Weed Sci. 30:467-475.

Larsen, T. E., J. D. Gnegy, and H. L. Olinger. 1983. Glyphosate for release of loblolly pine. Proc. South. Weed Sci. Soc. 36:235-238.

Lauridson, T. C., R. G. Wilson, and L. C. Haderlie. 1983. Effect of moisture stress on Canada thistle (Cirsium arvense) control. Weed Sci. 31:674-680.

Leonard, O. A., D. E. Bayer, and R. K. Glenn. 1966. Translocation of herbicides and assimilates in red maple and white ash. Bot. Gaz. 127:193-201.

Lund-Hoie, K. 1976. The correlation between tolerance of Norway spruce (Picea abies) to glyphosate (N-phosphonomethylglycine) and the uptake, distribution, and metabolism of the herbicide in the spruce plant. Sci. Rep. Agric. Univ. Norway 55:1-26.

Lund-Hoie, K. 1979. The physiological fate of glyphosate-^{14}C in Betula verrucosa and Fraxinus excelsior - the effect of ammonium sulphate and the environment on the herbicide. Sci. Rep. Agric. Univ. Norway 58:1-24.

Lund-Hoie, K. 1985. Efficacy of glyphosate in forest plantations. Chap. 21 in E. Grossbard and D. Atkinson, eds. The Herbicide Glyphosate. Butterworths, London. 490 pp.

McLaughlin, S. B., R. K. McConathy, R. L. Barnes, and N. T. Edwards. 1980. Seasonal changes in energy allocation by white oak (Quercus alba). Can. J. For. Res. 10:379-388.

Miller, W. F. and J. W. Starr. 1963. The role of moisture stress in pine and hardwood kill. Proc. South. Weed Sci. Soc. 16:223-231.

Minogue, P. J., M. R. Kuhns, and B. R. Zutter. 1984. Pine release from hardwood competition with three rates of glyphosate. Proc. South. Weed Sci. Soc. 37:242.

Moak, J. E. 1982. Forest practices cost trends in the South. South. J. Appl. For. 6:130-132.

Moosavi-Nia, H. and J. Dore. 1979. Factors affecting glyphosate activity in Imperata cylindrica (L.) Beauv. and Cyperus rotundus L. I. Effect of soil moisture. Weed Res. 19:137-143.

Neal, J. C., W. A. Skroch, and T. J. Monaco. 1985. Effects of plant growth stage on glyphosate absorption and transport in ligustrum (Ligustrum japonicum) and Blue Pacific juniper (Juniperus conferta). Weed Sci. 34:115-121.

Nilsson, G. 1977. Effects of glyphosate on the amino acid content in spring wheat plants. Swed. J. Agric. Res. 7:153-157.

Olinger, H. L. 1982. How do you spell release. Proc. South. Weed Sci. Soc. 35:216-223.

Paley, S. P. and S. R. Radosevich. 1984. Effect of physiological status and growth of ponderosa pine (Pinus ponderosa) and greenleaf manzanita (Arctostaphylos patula) on herbicide selectivity. Weed Sci. 32:395-402.

Radosevich, S. R., E. J. Roncoron, S. G. Conard, and W. B. McHenry. 1980. Seasonal tolerance of six coniferous species to eight foliage-active herbicides. For. Sci. 26:3-9.

Rowe, M. L. 1980. The effect of paraquat treatment on the starch content of loblolly pine seedlings (Pinus taeda L.). M.S. thesis, Univ. Tenn., Knoxville. 57 pp.

Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148:339-346.

Shaner, D. L. 1978. Effects of glyphosate on transpiration. Weed Sci. 26:513-516.

Shaner, D. L. and J. L. Lyon. 1980. Interaction of glyphosate with aromatic amino acids on transpiration in Phaseolus vulgaris. Weed Sci. 28:31-35.

Sheffield, R. M. and G. C. Craver. 1981. Virginia's pine resource: an interim assessment. USDA For. Serv. Res. Bull. SE-60. 12 pp.

Sheffield, R. M. and H. A. Knight. 1982. Loblolly pine resource - southeast region. pp. 7-24 in Proc. Symp. on the Loblolly Pine Ecosystem (East Region). North Car. St. Univ., Raleigh. 335 pp.

Smith, D. M. 1986. The Practice of Silviculture. J. Wiley and Sons, New York. 527 pp.

Sprankle, W. P., W. F. Meggitt, and D. P. Penner. 1975. Absorption, action, and translocation of glyphosate. Weed Sci. 23:235-240.

Todd, S. D. 1984. A method for evaluating and prioritizing loblolly pine plantations for release. Champion Res. Note SE-84-01. 10 pp.

Voth, R. D. and J. P. Downs. 1985. Forestry review on glyphosate uses and performance in the Southeast. Proc. South. Weed Sci. Soc. 38:136-139.

Wu, C. H., G. A. Dixon, S. R. Muench, and C. L. Sandberg. 1983. The use of glyphosate for pine release in the Midsouth. Proc. South. Weed Sci. Soc. 36:230-234.

Zobel, B. 1982. Loblolly pine - in retrospect. pp. 1-6 in Proc. Symp. on the Loblolly Pine Ecosystem (East Region). North Car. St. Univ., Raleigh. 335 pp.

Zutter, B. R., P. J. Minogue, and D. H. Gjerstad. 1988. Response following aerial applications of glyphosate for release of loblolly pine in the Virginia Piedmont. South. J. Appl. For. 12:54-58.

Appendix I

Methods used for plant tissue ^{14}C analysis

Sample seedlings were sectioned into four parts at harvest: treated leaf (TL), stem above the treated leaf (SAL), stem below the treated leaf (SBL), and roots. All sections were immediately placed in plastic bags and put in a freezer or placed on dry ice, then transferred to a freezer for storage.

Frozen sections were dried to constant weight in a 65°C oven, placed in a desiccator to cool to room temperature, weighed to the nearest 0.0001 g, transferred to paper coin envelopes, and returned to the oven. TL and SAL were ground by gently pressing and folding the envelopes containing the tissue by hand. SBL and roots were ground with a mortar and pestle, using liquid nitrogen as a grinding medium.

Subsamples of each tissue section were weighed into paper Combusto-cones^{R} (Packard Instrument Co.) to aid oxidation, placed in a Combusto-tray^{R} holder, covered with plastic film, and transported to the oxidizer. Subsamples were weighed in lots of 100 for oxidation.

All samples were oxidized in a Packard Model 306 biological oxidizer. The oxidizer was set to add 10 ml of Carbo-Sorb^{R} (Packard Instrument Co.) carbon dioxide absorbent and 10 ml of Permafluor V^{R} (Packard Instrument Co.) liquid scintillation cocktail into a glass scintillation collection vial. Oxidation times were set to one minute for roots and SBL, 1/2-minute for SAL, and 1/4-minute for TL. Preliminary testing established these times as sufficient for complete

combustion of each respective plant section.

Radioactivity of oxidized samples was counted on an LKB Wallac Model 1217 liquid scintillation counter. Counter settings programmed were:

Program mode = 1	Channel 1 = 50 - 165
Count mode = 4	Channel 2 = 0 - 0
Listing = Y	Channel 3 = 100-135
Time = 120	Channel 4 = 135 - 184
Counts 1 = 900000	External std time = 30
Auto window = N	External std counts = 900000
Ratio monitor = N	Print = 1,2,5,7,21,22,23

The external standard channels ratio (ESR) method was used to convert machine readings in counts per minute (CPM) to disintegrations per minute (DPM). A multiple regression equation fit to the % counting efficiency - ESR data was used to calculate % counting efficiency from the ESR readings for each sample. DPM were then calculated as CPM divided by counting efficiency, less background DPM. DPM for the entire tissue sample was then calculated using the ratio of sample weight oxidized to total sample weight.

Leaf rinse samples were stored in a refrigerator after harvesting. To determine ^{14}C content, 1.00 ml of each sample was pipetted into a glass scintillation vial, and 20 ml of Ecoscint^{R} liquid scintillation cocktail were added. CPM were converted to DPM by correcting for quenching and background radiation, as described for tissue samples. DPM for the entire leaf rinse were then obtained by weighing the remaining leaf rinse in the vial, then subtracting the mean weight of

the vial and vial cap.

Appendix II

Methods used for foliage sugar and starch analysis

1. Sample preparation

Fresh leaf samples were sealed in plastic bags and placed in a cooler containing dry ice at the time they were collected. Field samples were stored in a cold room (2-4°C) for 1-2 months, then stored in a freezer. Frozen samples were freeze-dried and stored back in the freezer until analysis. Greenhouse foliage samples were all transferred from dry ice to a freezer within one hour of collection.

All samples were ground in a Wiley mill through a 40 mesh screen, collected in paper coin envelopes, and kept in a 65°C oven. Samples were placed in a desiccator at room temperature for at least one hour prior to weighing.

2. Sugar extraction

The following reagents were prepared ahead of time:

Ethanol solution - 20% ethanol v/v in deionized water (20% gave the same extraction results as using 80% ethanol).

Citrate buffer - 5.25 g citric acid monohydrate was dissolved in 500 ml deionized water; 7.40 g sodium citrate was dissolved in 500 ml deionized water; the citric acid solution was added to the sodium citrate solution until the pH came down to 4.5. The solution was stored

in a refrigerator.

One hundred mg of each sample were weighed into 40-50 ml glass or plastic centrifuge tubes. Samples were weighed in groups of 16 because the centrifuge capacity was eight for tubes of this size. Two groups of 16 were usually weighed out together ahead of time. Ten mg of glucose was added to a sample in each pair of groups to quantify sugar recovery.

Twenty five ml of ethanol solution were added to the first eight samples. These samples were placed in an 80-85^{o}C water bath for 10 minutes, manually shaking them twice during the period. The samples were then centrifuged at 7500 rpm for 10 minutes, while ethanol solution was added to the remaining eight samples, which were then placed in the water bath.

While all samples were either in the water bath or centrifuge, the amyloglucosidase solution was prepared (it should be prepared fresh for each group of samples). The solution was prepared by adding 1.000 g amyloglucosidase powder per ml of citrate solution. One hundred ten to 125 ml of solution were prepared for each set of samples in a 125 ml erlenmeyer flask. The flask was shaken vigorously, then allowed to settle while the sugar extraction procedure was completed.

After centrifugation, the supernatant was poured through a glass funnel lined with glass wool and collected in a 100 ml volumetric flask. The sugar extraction steps (starting with addition of 25 ml ethanol solution) were repeated twice more for each sample.

The supernatant was brought up to 100 ml volume with ethanol solution, shaken thoroughly, and approximately 20 ml were poured into

plastic vials, sealed, and stored in the refrigerator for further analysis.

3. Starch extraction

Five ml of deionized water were pipetted into each sample tube containing the tissue residue plus two additional tubes: one with 10 mg starch (control) and one empty (blank). All 18 tubes were placed in a 90-95°C water bath for 30 minutes, then cooled to room temperature.

During this time, the 100 ml volumetric flasks were cleaned by triple-rinsing with ethanol solution to prepare for the next group of 16 samples.

The supernatant of the amyloglucosidase solution was then carefully poured into another container, leaving the residue behind. Five ml of amyloglucosidase solution were pipetted into each sample, and the samples were placed in a 40°C water bath for two hours. Each tube was swirled by hand three times during this time (1/2-hour intervals).

While the first group of samples were incubating, the sugar extraction procedure was completed on the second group of 16 samples previously weighed out.

After two hours, the incubating samples were placed in a 95°C water bath for five minutes to stop the enzyme reaction. The second group of samples were then placed in the incubating bath for two hours.

The incubated samples were then vacuum-filtered through #1 Whatman filter paper. A stirring rod was used to loosen the residue and

each tube was triple-rinsed with deionized water. The filtrate was transferred to 50 ml volumetric flasks, brought to volume with deionized water, and shaken thoroughly. Approximately 15-20 ml were poured into vials, which were stored in a freezer until further analysis.

All groups of samples were stored together with their associated blank and control.

4. Anthrone colorimetric sugar analysis

Standard sugar solutions were made up ahead of time and stored in the refrigerator. Two, 5, 10, and 20% (w/v) glucose solutions in 20% ethanol were used for the sugar samples; and 2, 5, and 10% solutions in deionized water were used for the starch samples. Preliminary testing indicated these standards would cover the range of sample concentrations.

Sulfuric acid reagent was prepared by adding 760 ml sulfuric acid concentrate (specific gravity = 1.84) to 240 ml deionized water in a one liter volumetric flask in an ice bath.

Anthrone solution was prepared by adding one liter of 72% sulfuric acid to 1.000 g anthrone. After mixing thoroughly, the anthrone dissolved in approximately 1/2 hour. Anthrone solution more than 24 hours old was discarded.

Batches of 36 samples were taken from storage (starch samples from the freezer were thawed - this took approximately one hour). Duplicate subsamples of 0.5 ml were pipetted from each sample vial, each standard glucose solution, and a blank solution into glass test tubes (approximately 15 ml volume) in an ice bath. Using a repipet,

10 ml of anthrone solution were added to each test tube. Test tubes were then capped, mixed with a vortex mixer, and placed in a 95°C water bath for 15 minutes.

The spectrophotometer was turned on to allow it to warm up.

Samples were immediately placed in an ice bath and brought to room temperature.

Samples were then read on a Cary spectrophotometer set at 620 nm. The spectrophotometer was zeroed with the blank, then the standard samples were read, then the leaf samples were read. The 80 test tubes in a batch could be read in approximately two hours. Preliminary testing indicated there was no change in sample color during this period.

For consistent readings on the spectrophotometer, the same cuvets were always used for the blank and the samples. The cuvets were always set in the machine compartment facing the same direction. The faces of the cuvet were kept clean by wiping with acetone as often as necessary.

All test tubes were shaken thoroughly immediately before pouring the solution into the cuvet. The cuvet was rinsed twice with the new sample before reading each sample.

Spectrophotometer readings for sugar samples were converted to sugar concentrations using a linear equation provided by the standards for each batch, then dividing by the mean recovery of 87% (based on 28 samples with a known quantity of sugar added to the sample at the beginning of the extraction). For starch samples, machine readings were converted to concentrations using the equation for the standards

in each batch. The mean blank enzyme concentration was then subtracted from each sample concentration, and corrections for recovery were made by applying the recovery value for each extraction batch.

Appendix III

Data documentation

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ALLSPP84 FIELD    A1 05/02/88 14:44     F 80  1554 RECS 05/02/88 19:35 PAGE      1


*** FILE: ALLSPP84 FIELD ***
THESE DATA WERE COLLECTED FROM TREE MEASUREMENTS FROM 12 TRACTS SPRAYED
WITH GLYPHOSATE FOR LOBLOLLY PINE RELEASE IN 1984 ONLY. DATA ARE
FROM 20-40 INDIVIDUALS PER SPECIES PER TRACT.  SEE PETER D'ANIERI'S
THESIS FOR SAMPLING INFO. CONTACT PETER D'ANIERI OR SHEP ZEDAKER FOR MORE
INFORMATION.  TREES WERE MEASURED FOR PRE-SPRAYING CROWN SIZE, AND
CROWN SIZE ONE AND TWO YEARS POST-SPRAYING.

VARIABLE DEFINITIONS:
TRACT=TRACT I.D. NO., SPECIES, AND TREE #
SPECIES CODES:
   LP=LOBLOLLY, RM=RED MAPLE, WO=WHITE OAKS, SG=SWEETGUM,
   YP=YELLOW-POPLAR
HT84=MEAN HEIGHT BEFORE SPRAYING: ALL MEASURES ARE IN METERS
C184=MEAN CROWN DIAMETER 1, BEFORE SPRAYING
C284= MEAN C.D. 2, BEFORE SPRAYING
HT85= MEAN HT., 1 YEAR AFTER SPRAYING
C185=MEAN C.D. 1, 1 YEAR AFTER SPRAYING
C285=MEAN C.D. 2, 1 YEAR AFTER SPRAYING
HT86= MEAN HT., 2 YEARS AFTER SPRAYING
C186=MEAN C.D. 1, 2 YEARS AFTER SPRAYING
C286=MEAN C.D. 2, 2 YEARS AFTER SPRAYING
WP=MEAN XYLEM WATER POTENTIAL, IN -BARS
SUGAR=MEAN LEAF SUGAR CONCENTRATION, IN MG/G LEAF TISSUE
STARCH=MEAN LEAF STARCH CONC., IN MG/G DRY LEAF TISSUE
TRT= SPRAYED (1) OR UNSPRAYED (0)

DATA LP84;
INPUT TRACT 1-2 HT84 8-11 2 C184 13-16 2 C284 18-21 2
           HT85 23-26 2 C185 28-31 2 C285 33-36 2
           HT86 38-41 2 C186 43-46 2 C286 48-51 2
           WP 53-56 1 SUGAR 58-62 1 STARCH 64-68 1;
CV0=HT84*C184*C284;
CV1=HT85*C185*C285;
CV2=HT86*C186*C286;
CARDS;
10LP01 2.15 0.80 0.65 2.58 1.19 1.08 3.51 1.32 1.78   .    .    .
10LP02 2.10 0.90 0.90 2.61 0.96 1.35 3.50 1.85 1.78   .    .    .
10LP03 2.20 1.50 0.90 2.54 1.99 1.72 3.45 2.61 1.69   .    .    .
10LP04 2.20 0.80 0.60 2.68 0.76 0.73 3.52 1.21 0.98   .    .    .
10LP05 2.25 1.30 0.90 2.92 1.29 2.25 3.54 2.02 2.22   .    .    .
10LP06 2.00 1.30 1.10 2.29 1.59 1.39 2.39 1.82 1.44   .    .    .
10LP07 2.80 1.60 1.50 2.24 1.76 1.72 4.00 2.46 2.52   .    .    .
10LP08 1.90 1.20 1.40 2.53 1.29 1.58 3.46 1.66 1.92   .    .    .
10LP09 2.60 1.50 1.10 2.33 1.27 1.98 4.03 2.26 1.79   .    .    .
10LP10 2.50 1.00 0.80 2.86 1.43 1.42 3.78 1.59 1.88   .    .    .
10LP11 1.70 1.00 0.90 1.98 1.58 1.54 2.45 1.79 1.72   .    .    .
10LP12 1.86 0.90 0.80 2.07 0.69 1.04 2.64 2.48 1.21   .    .    .
10LP13 1.50 0.50 0.60 0.00 0.00 0.00 0.00 0.00 0.00   .    .    .
10LP14 0.80 0.30 0.20 0.00 0.00 0.00 0.00 0.00 0.00   .    .    .
10LP15 2.25 1.05  .   2.89 1.26 1.63 3.72 1.77 1.66   .    .    .
10LP16 0.60 0.25 0.15 0.99 0.38 0.24 1.71 0.75 0.64   .    .    .
10LP17                1.82 0.64 0.92 2.52 1.09 0.78   .    .    .
10LP18 1.85 0.80 0.60 2.69 1.51 1.42 3.29 1.64 1.52   .    .    .
10LP19 2.70 1.30 1.10 3.18 1.89 1.72 3.57 2.36 1.74   .    .    .
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ALLSPP85 FIELD    A1 05/02/88 16:31    F 80  1478 RECS 05/02/88 19:35 PAGE

*** FILE: ALLSPP85 FIELD ***
THESE DATA WERE COLLECTED FROM TREE MEASUREMENTS FROM 4 TRACTS SPRAYED
WITH GLYPHOSATE FOR LOBLOLLY PINE RELEASE IN 1985 ONLY. DATA ARE
FROM 50-60 INDIVIDUALS PER SPECIES PER TRACT.  SEE PETER D'ANIERI'S
THESIS FOR SAMPLING INFO. CONTACT PETER D'ANIERI OR SHEP ZEDAKER FOR MORE
INFORMATION.  TREES WERE MEASURED FOR PRE-SPRAYING CROWN VARIABLES AND
CROWN SIZE ONE YEAR AFTER SPRAYING.

VARIABLE DEFINITIONS:
TRACT=TRACT I.D. NO.
ID=SPECIES AND TREE #
SPECIES CODES:
   LP=LOBLOLLY, RM=RED MAPLE, WO=WHITE OAKS, SG=SWEETGUM,
   YP=YELLOW-POPLAR
TRT=SPRAY TREATMENT, 0=UNSPRAYED, 1=SPRAYED
GROWY0=CURRENT YEAR HEIGHT GROWTH, AT THE TIME OF SPRAYING
TRCFG=CURRENT-YEAR HEIGHT GROWTH OF THE MOST RECENT PINE LEADER FLUSH,
      AT THE TIME OF SPRAYING
TRNEEDL=NEEDLE LENGTH ON THE LATEST PINE LEADER FLUSH, AT THE
       TIME OF SPRAYING
HTY0= HEIGHT., AT THE TIME OF SPRAYING
C1Y0= CROWN DIAMETER 1, AT THE TIME OF SPRAYING
C2Y0= C.D. 2, AT THE TIME OF SPRAYING
WP= XYLEM WATER POTENTIAL, IN -BARS
ENDHTY0= HT., AT THE END OF THE GROWING SEASON
ENDC1Y0= C.D. 1, AT THE END OF THE GROWING SEASON
ENDC2Y0= C.D. 2, AT THE END OF THE GROWING SEASON
ENDCFGY0=CURRENT-YEAR HEIGHT GROWTH OF THE MOST RECENT PINE LEADER FLUSH,
      AT THE END OF THE GROWING SEASON
ENDNEEDL=NEEDLE LENGTH ON THE LATEST PINE LEADER FLUSH, AT THE END OF THE
       GROWING SEASON
HTY1= HT., 1 YEAR AFTER SPRAYING
C1Y1= C.D. 1, 1 YEAR AFTER SPRAYING
C2Y1= C.D. 2, 1 YEAR AFTER SPRAYING

DATA LPCROWN;
INPUT TRACT 2 ID $ 3-6 TRT 8 GROWY0 10-13 2 TRCFG 15-17 3 TRNEEDL 19-21
      HTY0 23-25 2 C1Y0 27-29 2 C2Y0 31-33 2 WP 35-37 1 ENDHTY0 39-41 2
      ENDC1Y0 43-45 2 ENDC2Y0 47-49 2 ENDNEEDL 51-53 ENDCFGY0 55-57 2
      HTY1 59-61 2 C1Y1 63-65 2 C2Y1 67-69 2;
CV0=HTY0*C1Y0*C2Y0;    CV1=HTY1*C1Y1*C2Y1; ENDCV0=ENDHTY0*ENDC1Y0*ENDC2Y0;
EXPCV0=0.100 + 1.04*CV0; GROCONTR=ENDCV0/EXPCV0;
IF ENDCFGY0=0 THEN DELETE;  IF ENDNEEDL =0 THEN DELETE;
CFCOMP=TRCFG/ENDCFGY0; IF CFCOMP>1 THEN CFCOMP=1;
NEEDCOMP=TRNEEDL/ENDNEEDL; IF NEEDCOMP>1 THEN NEEDCOMP=1;
IF TRT=0; IF HTY0=.22 THEN DELETE;
IF HTY1<HTY0 THEN DELETE;
CARDS;
01LP01 0   911149  43 259 103  86  60 264 106  87  64  15 373 166  93
01LP02 0   789164  42 159  54  43  25 161  76  49  66  15 243 126  74
01LP03 0   708272 131 181  57  59  30   0   0   0   0   0   .   .   .
01LP04 0   912239  49 241  76  57  65 240  72  83  94  23 339 134  93
01LP05 0   788395  69 171  46  45  60 167  74  68 108  35 262 111 108
01LP06 0   851256  96 227  64  62  50 226  98  85 123  24 333 123  96
01LP07 0   522134  71 139  52  44  40 139  45  65  93  14 224  69  89
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WEATHER  DATA     A1 05/02/88 13:00    F 80    48 RECS 05/02/88 19:36 PAGE

*** FILE: ALLSPP WEATHER ***
THESE DATA WERE COLLECTED OR CALCULATED FROM NOAA MONTHLY WEATHER
SUMMARIES FOR 1984 AND 1985.  THEY REPRESENT WEATHER CONDITIONS FROM
THE NEAREST WEATHER STATION TO TRACTS SPRAYED FOR AERIAL LOBLOLLY PINE
RELEASE WITH GLYPHOSATE.  CONTACT PETER D'ANIERI OR SHEP ZEDAKER FOR MORE
INFORMATION.
VARIABLES DEFINITIONS:
TRACT=TRACT I.D. NO.
PRPRDAYS=# OF DAYS SINCE > 0.5 IN. RAIN PRIOR TO SPRAYING
PRPRAMT=INCHES OF LAST RAIN > 0.5 INCHES BEFORE SPRAYING
PRPRAVG=AVG. INCHES OF RAIN FOR THE 10 DAYS PRIOR TO SPRAYING
TRTPR=INCHES OF RAIN ON THE DAY OF SPRAYING + THE NEXT DAY
POPRDAYS=# OF DAYS UNTIL >0.5 IN. OF RAIN AFTER SPRAYING
POPRAMT=INCHES OF FIRST RAIN AFTER SPRAYING > 0.5 IN.
POPRAVG=AVG. INCHES OF RAIN FOR THE 10 DAYS AFTER SPRAYING
PRTE=SUM OF THE HIGH TEMPERATURE FOR THE 10 DAYS BEFORE SPRAYING
TRTTE=SUM OF HIGH TEMPS. FOR THE DAY OF SPRAYING + THE NEXT DAY
POTE=SUM OF HIGH TEMPS. FOR THE 10 DAYS AFTER SPRAYING
SPP=SPECIES- LP=LOBLOLLY, RM=RED MAPLE, WO=WHITE OAKS,SG=SWEETGUM,
YP=YELLOW-POPLAR

DATA RAIN;
INPUT TRACT 1-2 PRPRDAYS 9-10 PRPRAMT 12-14 PRPRAVG 16-18
      TRTPR 20-21 POPRDAYS 23-24 POPRAMT 26-28 POPRAVG 30-32 PRTE 34-36
      TRTTE 38-40 POTE 42-44;
* COLUMNS 4-7 = TREATMENT DATE;
PRHALFAV=PRPRAMT/PRPRDAYS;
POHALFAV=POPRAMT/POPRDAYS;
PREVAP=PRPRAVG/PRTE;
TRTEVAP=TRTPR/TRTTE;
POEVAP=POPRAVG/POTE;
CARDS;
01 9/15 20 175 000 00 12 102  47 854 150 805
02 9/14 19 175 000 00 13 102  47 879 141 797
03 9/16 21  60 000 00 11 113  27 882 159 824
06 9/28  1 445 579 00  4  99 335 815 156 736
10 8/27 13  91 000 00  8  76  88 839 170 844
11 9/19 36 182   3  0 12 149  43 807 161 782
12 9/18 35 182   3  0 13 149  22 815 151 801
13 9/17 34 182   3  0 14 149   0 820 146 820
14 9/15 11 100   6 17 16  62   0 812 158 823
15 9/ 7  3  66 103 00 24 156  11 838 145 796
16 9/14 26  70  15 18 17 156  18 792 171 787
17 9/ 8  4  50 197 00 23 140   1 851 160 840
18 9/ 6  2  50 187 10 25 140   1 865 159 846
19 9/ 7  3  50 197 00 24 140   1 859 157 843
20 9/ 6  8 235 273 00  7  50   5 800 152 758
21 9/ 5  1  76  76  2 26 144   4 869 158 811
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WTEFFSS  DATA     A1 05/02/88 13:42     F 80    227 RECS 05/02/88 19:36 PAGE

*** FILE: WTEFFSS DATA ***
THESE ARE COMPLETE DATA FOR ALL REPLICATIONS OF LOBLOLLY PINE, RED
MAPLE, AND SWEETGUM USED IN AN EXPERIMENT TO TEST THE EFFECTS OF
WATER STRESS AND DATE OF GLYPHOSATE APPLICATION ON GLYPHOSATE
SUSCEPTIBILITY IN THESE THREE SPECIES.  THE STUDY DESIGN WAS
COMPLETELY RANDOMIZED WITH 4 (APPLICATION DATES) X 3 (WATER
STRESS TREATMENTS) FACTORIAL TREATMENTS.  THE STUDY WAS DONE AT
REYNOLDS HOMESTEAD AGRICULTURAL EXPERIMENT STATION.  SEE
PETER D'ANIERI, SHEP ZEDAKER, OR RICHARD KREH FOR FURTHER
INFORMATION.
VARIABLE DEFINITIONS:
SPP=SPECIES, LP=LOBLOLLY PINE, RM=RED MAPLE, SG=SWEETGUM
DATE=DATE OF 1986 GLYPHOSATE APPLICATION, 1=8/25-26, 2=9/8-9,
     3=9/22-23, 4=10/4-5
TRTMT=WATER STRESS TREATMENT, 1=CONTROL (UNSPRAYED, NO STRESS)
     2= NO STRESS, 3=MODERATE STRESS, 4=SEVERE STRESS
REP=REPLICATION-4 PER TREATMENT COMBINATION
ALL WEIGHTS ARE SEEDLING MEANS, BASED ON HARVESTS IN MARCH, 1987,
IN DRY GRAMS
PRWT= WT. OF LIVE WOODY STEM SECTIONS
NEWWT= WT. OF LIVE GREEN STEM SECTIONS + NEW FOLIAGE
DEADWT= WT. OF DEAD STEMS
SURV=% SURVIVAL
USPRWT= WT. OF LIVE WOODY STEM SECTIONS OF AN UNSPRAYED SUBSAMPLE
USNEWWT= WT. OF LIVE GREEN STEM SECTIONS + NEW FOLIAGE OF AN UNSPRAYED
     SUBSAMPLE
DEADWT= WT. OF DEAD STEMS OF AN UNSPRAYED SUBSAMPLE
USSURV=% SURVIVAL OF AN UNSPRAYED SUBSAMPLE
AVGHT1= AVG. LENGTH OF NEW TERMINAL GROWTH (CM), MEASURED IN
     FEB., 1987 (TIME 1)
CLAS1T1=% OF SEEDLINGS IN HERBICIDE INJURY CLASS 1=NO APPARENT
     INJURY, AT TIME 1
CLAS2T1=% IN CLASS 2=NEW FOLIAGE EMERGING, BUT DEFORMED, AT TIME 1
CLAS3T1=% IN CLASS 3=PLANT LIVE WITH GREEN BUDS, BUT FOLIAGE
     EMERGENCE SUPPRESSED OR SEVERELY DEFORMED, AT TIME 1
CLAS4T1=% IN CLASS 4=DEAD, AT TIME 1
CLAS1T2 - CLAS4T2= SAME AS ABOVE, BUT ASSESSED AT HARVEST IN
                   MARCH, 1987=TIME 2
STARCH=LEAF STARCH CONCENTRATION, IN MG/G DRY LEAF TISSUE
SUGAR=LEAF SUGAR CONCENTRATION, IN MG/G DRY LEAF TISSUE
WP=WATER POTENTIAL, IN -BARS
DATA REPWT;
INPUT SPP $ 1-2 DATE 3 TRTMT 4 REP 5 PRWT 7-9 2 NEWWT 10-12 2
  DEADWT 14-16 2 SURV 17-19 2 USPRWT 21-23 2 USNEWWT 24-26 2
  USDEADWT 28-30 2 USSURV 31-33 2 AVGHT1 35-36 CLAS1T1 37-39 2
    CLAS2T1 40-42 2 CLAS3T1 43-45 2 CLAS4T1 46-48 2 AVGHT2 50-51
    CLAS1T2 52-54 2 CLAS2T2 55-57 2 CLAS3T2 58-60 2 CLAS4T2 61-63 2
    STARCH 64-66 1 SUGAR 67-70 1 WP 71-73 1;
IF TRTMT=1 THEN DELETE;
IF SPP='SG';


CARDS;
LP111 209 12 143 18                    21 36  1 13 50  5 16  0  2 82 23 589 62

LP112 167 13  70 29                    19 32  4 16 47 33 25  2  2 71        36
LP121 220  9  76 10                 15  0  0 15  2 82 11  2  7  0 90 29 545 60
LP122  98  0  80  2 311 34 100      15  0  0  2  2 95  0  0  0  2 97 36 440 75
LP123 187  2  82 20                 29  9  3 19  5 73 19  2 15  2 80 67 578 67
LP124 209  1  89 22 173 14 133      29 12 10  7 27 55  0  0 13 10 77  0 491 57
LP131 139 12  70 31                 43 20  5 25 15 55 37  3 23  5 69 19 474 76
LP132 177  8  89 31 193 17 122      43 37  7 17 17 57 22  5 25  0 70110 586 74
LP133 177  6  78 10                 14  8  5  0  5 90  9  2  5  2 90 66 659 89
LP134 112  2  93 10 301 22 154      14  0  0  7  7 85  0  0  5  5 90157 685 74
LP141 195 10  57 33                 43  7 20  7 22 50 12 13 13 13 63 44 688187
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C14       DATA      A1 05/02/88 17:44     F 80    714 RECS 05/02/88 19:34 PAGE

*** FILE: C14 DATA ***
THESE ARE DATA FROM AN EXPERIMENT TO TEST THE EFFECTS OF WATER STRESS
AND DATE OF GLYPHOSATE APPLICATION ON ABSORPTION AND TRANSLOCATION
OF THE HERBICIDE USING C-14-LABELLED GLYPHOSATE.  FIVE SEEDLINGS OF EACH
OF THREE SPECIES RECEIVED C-14-GLYPHOSATE FOR EACH TREATMENT COMBINATION
OF THREE APPLICATION DATES X THREE WATER STRESS TREATMENTS.  PLANTS WERE
HARVESTED AFTER 2 WEEKS, THE DRIED TISSUE SAMPLE WERE OXIDIZED, AND C-14
ACTIVITY WAS COUNTED USING LIQUID SCINTILLATION SPECTROMETRY.  SEE
PETER D'ANIERI OR SHEP ZEDAKER FOR MORE INFORMATION.

NUMBER=SEEDLING I.D.: THE FIRST DIGIT IS THE SPECIES - 1=SWEETGUM,
       2=LOBLOLLY PINE, 3=RED MAPLE.  THE SECOND DIGIT IS THE APPLICATION
       DATE - 2=SEPT. 8-9, 3=SEPT. 22-23, 4=OCT. 4-5.  THE LAST TWO
       DIGITS ARE THE NO. (1-80)
TRTMT=WATER STRESS TREATMENT - 2=NO STRESS, 3=MODERATE STRESS,
      4=SEVERE STRESS
SECTION= PLANT SECTION: 1=ROOTS 2=STEM BELOW TREATED LEAF
       3=STEM ABOVE TREATED LEAF 4=TREATED LEAF 5=LEAF RINSE
TOTWT=TOTAL DRY WT. OF THE PLANT SECTION (G)
CTWT=DRY WT. OF THE SECTION THAT WAS SAMPLED FOR C-14 ACTIVITY
CPM=COUNTS PER MINUTE, FROM THE LIQUID SCINTILLATION COUNTER PRINTOUT
ESR=EXTERNAL STANDARD RATIO, FROM THE LSC PRINTOUT
VOL=TOTAL VOLUME OF LEAF RINSE (ML)

DATA C14;
INPUT NUMBER 1-4 TRTMT 6 SECTION 8 TOTWT 10-15
      CTWT 17-22   CPM 24-29 ESR 31-34 3 VOL 36-39 2;
IF CTWT>TOTWT THEN TOTWT=CTWT;
EFF=ESR**(-156.720)+(ESR**2)*12.305+(ESR**0.5)*310.891-84.047;
BKG=51; VIAL=4.75; CAP=3.52;
IF SECTION=5 THEN VOL=VOL-VIAL-CAP+1;
IF SECTION=5 THEN DPM=((CPM/EFF*100)-BKG)*(VOL);
ELSE DPM=((CPM/EFF*100)-BKG)*(TOTWT/CTWT);
DPMPERG=DPM/TOTWT;
CARDS;
1202 2 1  .6839  .4218  44888  542
1209 2 1 1.0607  .4388  27206  534
1216 2 1 1.5831  .5225   9243  543
1217 2 1 1.1666  .5187   3922  534
1220 2 1 1.3855 1.0632  36171  536
1233 3 1 1.0982 1.0032   7201  535
1236 3 1 1.5310  .9766  21636  516
1242 3 1  .5811  .5493  14742  553
1248 3 1  .7576  .4593  11833  537
1249 3 1  .7829  .4859   4623  470
1262 4 1  .3825  .3348  66093  526
1268 4 1  .7849  .3935  19450  523
1273 4 1  .5661  .4290  25112  539
1277 4 1  .6865  .4361  38418  525
1278 4 1 1.0835  .9640  53206  542
1301 2 1 1.0683  .4479  35758  529
1308 2 1  .9334  .4599  49983  531
1334 2 1  .8140  .4613  44150  520
1338 2 1 1.2551  .5044  36096  524
1340 2 1 1.0036  .5350  43568  527
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REYNOLDS ALLDATA  A1 05/02/88 16:44     F 80  1032 RECS 05/02/88 19:35 PAGE

*** FILE: REYNOLDS ALLDATA ***
THESE ARE DATA FOR INDIVIDUALLY TAGGED SEEDLINGS MONITORED IN AN
EXPERIMENT TESTING THE EFFECTS OF WATER STRESS AND DATE OF HERBICIDE
APPLICATION ON GLYPHOSATE EFFICACY AT REYNOLDS HOMESTEAD AGRICULTURAL
EXPERIMENT STATION.  TEN SEEDLINGS IN EACH 1/2-TRAY REPLICATION
(49 SEEDLINGS) WERE RANDOMLY SELECTED 4 WEEKS PRIOR TO SPRAYING TO
MEASURE GROWTH RATE, WATER POTENTIAL, AND COMPOSITE LEAF SUGAR AND STARCH
CONCENTRATION BEFORE SPRAYING TO RELATE TO POST-TREATMENT GROWTH RESPONSE
IN TERMS OF HEIGHT AND DRY WT. GROWTH.

SPP: 1=SWEETGUM 2=LOBLOLLY PINE 3=RED MAPLE
DATE (OF SPRAYING): 1=8/25-26/86  2=9/8-9/86  3=9/22-23/86  4=10/4-5/86
NUMBER: SEEDLING NUMBER (1-80)
TRTMT: WATER STRESS TREATMENT: 1=UNSTRESSED, UNSPRAYED  2=UNSTRESSED
                               3=MODERATE STRESS  4=SEVERE STRESS
REP: REPLICATION
HT1: HEIGHT IN MILLIMETERS OF SEEDLING 4 WEEKS BEFORE SPRAYING
HT2: HEIGHT 2 WEEKS BEFORE SPRAYING
HT3: HEIGHT AT SPRAYING
WP: WATER POTENTIAL IN -BARS AT SPRAYING
  FOR ALL 'NEW..' VARIABLES, '.'= A DEAD SEEDLING.
NEWHT1: HEIGHT GROWTH IN MILLIMETERS THE NEXT GROWING SEASON, APPROX.
        4 WEEKS BEFORE HARVEST
NEWHT2: HEIGHT GROWTH 0-1 WEEK BEFORE HARVEST
NEWWT: WEIGHT IN GRAMS OF NEW GROWTH AT HARVEST
OLDWT: WEIGHT IN GRAMS OF LAST YEAR'S (PRE-SPRAYING) TISSUE
********************************************************************** ;

DATA SEEDLING;
INPUT SPP 1 DATE 2 NUMBER 3-4 TRTMT 7 REP 8 HT1 11-13 HT2 15-17
  HT3 19-21 WP 23-25 1 NEWHT1 27-28 NEWHT2 30-32 NEWWT 34-37 3
  OLDWT 39-41 2;
DEADWT=.;
IF NEWWT=. THEN DEADWT=OLDWT;
IF NEWWT=. THEN OLDWT=.;
IF NEWWT=0 THEN NEWWT=.;
IF NEWHT1=0 THEN NEWHT1=.;
IF NEWHT2=0 THEN NEWHT2=.;
IF DATE=1 THEN DAYGROW2=(HT3-HT2)/16;
IF DATE=2 AND SPP=1 THEN DAYGROW2=(HT3-HT2)/11;
IF DATE=2 AND SPP=2 THEN DAYGROW2=(HT3-HT2)/9;
IF DATE=2 AND SPP=3 THEN DAYGROW2=(HT3-HT2)/10;
IF DATE=3 THEN DAYGROW2=(HT3-HT2)/16;
IF DATE=4 THEN DAYGROW2=(HT3-HT2)/13;
IF DAYGROW2 <=0 THEN DAYGROW2 = .;
IF DATE=1 THEN DAYGROW4=(HT3-HT1)/29;
IF DATE=2 THEN DAYGROW4=(HT3-HT1)/27;
IF DATE=3 AND SPP=1 THEN DAYGROW4=(HT3-HT1)/27;
IF DATE=3 AND SPP=2 OR SPP=3 THEN DAYGROW4=(HT3-HT1)/25;
IF DATE=4 THEN DAYGROW4=(HT3-HT1)/29;
IF DAYGROW4 <=0 THEN DAYGROW4 = .;
CARDS;
1121  11  174 168 196  20 24  63  484  36
1122  11  172 203 255  20 28 178 1275  93
1123  11  170     210      46 224  973  52
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VITA

Peter D'Anieri was born in Schenectady, New York, in 1963 to John E. and Mary R. D'Anieri. He graduated from Niskayuna High School in 1981. He graduated from the University of Maine in 1985 with a Bachelor of Science degree in Forest Management and Wildlife Management. He completed his Master of Science degree in Forestry at Virginia Polytechnic Institute and State University in 1988.