EFFICACY, UPTAKE, AND TRANSLOCATION OF STEM APPLIED TRICLOPYR ESTER IN FOUR FORMULATION SOLVENTS

by

William Grant Schneider

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Approved:

Shepard M. Zedaker

John R. Seiler

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(ABSTRACT)

Two experiments were designed to study efficacy and uptake and translocation of an ester formulation of triclopyr (3,5,6-trichloro-2pyridinyloxyacetic acid) in four solvents, aromatic solvent, aliphatic solvent, vegetable oil, and kerosene following stem applications to red maple (Acer rubrum), white oak (Quercus alba), and Virginia pine (Pinus virginiana). Another objective was to explore correlations between efficacy and uptake and translocation. Additional objectives included examining the influence of concentration, dose, and stem diameter on efficacy and the influence of bark thickness and season of treatment on uptake and translocation. Concentration ranged from 0.25 to 1 lb a.e./gallon in the efficacy study and was 1 lb a.e./gallon in the uptake study. Dose ranged from 0.4 to 2 ml/cm of stem diameter in the efficacy study and was 0.15 ml/cm of stem diameter in the uptake study. Efficacy evaluations were made 14 months following treatment in June. C14triclopyr was used to determine uptake and translocation. Saplings in the uptake study were harvested three weeks following treatments in February, May, and July.

Aliphatic solvent, vegetable oil, and kerosene treatments resulted in excellent crown volume control, largely independent of concentration, dose, and stem diameter. Aromatic solvent gave poorer results, dependent on concentration, dose, and stem diameter, except among the maples. Herbicide uptake with the vegetable oil treatment was greatest. The other solvents provided similar levels of uptake. Uptake was greatest among the maples, the thinnest-barked species, and about equal

in the oaks and pines.

Discrepancies between solvent differences in the efficacy and the uptake studies may have been, in part, a consequence of smaller doses used in the uptake study along with conditions which likely promoted greater solvent evaporation than those under which the efficacy study was performed. Faster evaporation of the kerosene and aliphatic solvents may have reduced their penetration of the outer bark while slower evaporation of aromatic solvent and vegetable oil likely had less influence on their penetration.

Stem diameter correlated negatively with crown volume control, despite basing dose on stem diameter, suggesting that the square of diameter, or stem volume, may be a more appropriate basis for determining doses. Uptake correlated negatively with bark thickness. Uptake did not vary significantly among seasons but translocation did. Movement to the leaves occurred following the May and July treatment but virtually none to leaves or buds following the February treatment. The high degree of sprouting which occurred among saplings in the May treatment compared with none among saplings in the July treatment would suggest that downward translocation of herbicide was greater in July.

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JUSTIFICATION

Since the 1940's, when the manufacture of synthetic, organic herbicides began on a large scale, xenobiotics have been used increasingly in both forestry and utility right-of-way vegetation management. One significant use of herbicides in forestry is for the release of crop trees from undesired woody competition. Because hardwood sprout growth often exceeds that of planted pines (Miller et al. 1991), release is especially important when establishing conifer plantations on sites which previously supported hardwood or mixed conifer/hardwood stands. Release is widely practiced during early stand establishment of conifers, giving documented gains in yield (Stewart et al. 1984). Herbicide applications for conifer release are predominantly accomplished through broadcast foliar applications, whereby all vegetation, including the crop, is sprayed. Broadcast applications, however, have a potential for herbicide drift into non-target areas and much of the herbicide does not actually contact weed species. Non-crop species which will likely not significantly interfere with crop survival and growth and which may be beneficial to wildlife are also affected by broadcast treatments as are rare species which may be present. addition, spraying over crop trees can lead to crop damage due to inadequate selectivity among many herbicides presently labeled for release. Basal stem applications of herbicides do not share these drawbacks. Disadvantages of using basal applications, however, include labor-intensiveness, relatively slow rate of application, necessity of using a petroleum based carrier (due to the impermeability of outer bark to water), and high cost in dense stands. By modifying basal application techniques for speedier application and by determining more precisely how much herbicide and carrier are necessary to apply to achieve acceptable results, material and labor costs might be lowered, making basal applications a more feasible alternative to broadcast

applications.

Herbicides are also important for maintaining rights-of-way free from trees capable of interfering with utility lines. Drift can be a more serious problem because rights-of-way are narrow corridors which often pass through land not owned by the utility company and through more heavily populated areas. In addition, foliage brownout which occurs after foliar applications of many herbicides can be unsightly to utility customers. Brownout can, however, be avoided by using basal applications during the dormant season. While basal applications would be less suited to inaccessible sites or those with rugged terrain, they do not have the above drawbacks, and they allow the selective retention of shrubs, brambles, and small tree species which provide food and cover for wildlife and inhibit the invasion of large tree species.

A distinct disadvantage of basal applications has been the necessity of using fuel oils such as diesel fuel and kerosene as carriers. They are costly, messy, and potentially more hazardous to application personnel and to the environment than the herbicides themselves. With increased usage of basal applications, there is incentive for investigating the effectiveness of less hazardous solvents. Furthermore, quantitative determinations of the relationship between concentration, dose, and efficacy have only recently been made. Previously, techniques involved simply drenching the lower portion of the stem with a dilute solution until it puddled about the base, or more recently, wetting the lower portion of the stem with more concentrated solutions. Better knowledge in this area of concentration and dose might reduce application of unnecessary material.

Finally, it appears that only a few studies have investigated movement of herbicide following basal applications. The factors, inherent in the formulation or the species being treated, that affect uptake and translocation are largely unknown. For instance, a better understanding of physical and chemical properties of solvents which aid

in penetration might help researchers focus their search for better carriers. In addition, uptake studies on various species over a range of stem diameters might explain differential effectiveness in the field if efficacy is correlated with degree of uptake and translocation.

Differential uptake might be related to bark characteristics whereas differential translocation might be related to differences in anatomy and physiology.

With the above considerations in mind, the objectives of this study were:

- 1. To evaluate four solvents for use as carriers in basal applications of triclopyr ester based on efficacy against red maple (Acer rubrum), Virginia pine (Pinus virginiana), and white oak (Quercus alba and Q. prinus) and based on herbicide uptake and translocation in these species, which are often competitors in loblolly pine plantations in the Mid-Atlantic states. These solvents include a vegetable oil, two more highly refined petroleum distillates than traditional fuel oil carriers, and kerosene as a standard of comparison.
- 2. To characterize the relationship between efficacy of basal applications of triclopyr ester and concentration of herbicide, dose applied, and stem diameter.
- 3. To determine the extent of uptake and translocation of triclopyr ester following basal applications to red maple, Virginia pine, and white oak and investigate the possible influence of bark thickness and season of application.
- 4. To investigate the relationship between efficacy of triclopyr ester among the species and degree of uptake and translocation.

LITERATURE REVIEW

Benefits of Pine Release

Along with harvesting, site preparation, and thinning, release treatments are one of the key vegetation management operations carried out in pine plantations in the Southeast. Pines are often planted on sites previously occupied by hardwoods. Because hardwood stump sprouts grow more quickly, initially, than pine seedlings, it is often necessary to suppress the hardwood competition within the first few years after planting, to facilitate seedling establishment.

Many studies have documented the enhanced pine growth which occurs after hardwood competition has been controlled to some degree (Stewart et al. 1984). The Virginia Division of Forestry (VDF) issued a series of reports from 1984 to 1991 (Dierauf) analyzing the gains in loblolly pine growth achieved through release treatments. All the sites in the VDF studies were being converted from mixed hardwood to pine plantations. The final measurements were reported at stand ages ranging from 15 to 23 years old. Most release treatments were applied following the first to fifth growing season after planting. All involved 2 lbs. per acre of 2,4,5-T ((2,4,5 trichlorophenoxy)acetic acid) applied by mistblower or aerially or 2.5 and 5 percent (see Appendix 1) solutions of 2,4,5-T in diesel fuel applied basally. Most of the studies did not involve replication of treatments and, therefore, could not be analyzed statistically. Even so, results consistently showed greater average dbh (mean +34.8%, range -1.5% to +88.1%), basal area (mean +9.45%, range +2.7% to +18.1%), and cordwood volume (mean +46.4%, range +3.6% to +91.3%) for loblolly pine on treated areas as compared to untreated checks.

Zutter et al. (1988) achieved a significant response in height and diameter four years after broadcast spraying three-year-old loblolly

pine with glyphosate. Pine damage was minimal. In another study (Clason 1984), hardwoods in a seven-year-old loblolly pine stand were cut and the stumps treated with a mixture of 2,4,5-T in diesel fuel. The stand was subsequently thinned at ages 12 and 17 years. Radial growth of loblolly pine was 33% greater on the treated plots than on the check plots between ages 12 and 17 years. Significant differences between treated and check plots included dbh at age 17 years, merchantable volume at ages 12 and 17 years, and thinned volume at age 17 years. Sawtimber volume at age 18 years averaged 480 cubic feet on treated plots and only 242 cubic feet on check plots.

Advantages of Basal Applications

Most of the literature discussing the benefits of basal treatments is from a right-of-way managers perspective because these individuals have been utilizing this technique for four decades (Staples 1963). Advantages of basal applications over hand cutting were that they were cheaper, resulted in less resprouting (Olenik 1978), and may have been safer for application personnel. Basal and foliar applications both have advantages and disadvantages (Abbott 1963, Burch et al. 1987, Mann and Aldred 1964, Niering and Goodwin 1974, Olenik 1977, Williams et al. 1970). Broadcast foliar applications are less selective, generally cause an unsightly foliage brownout, are not as effective on some species, and can lead to off-site damage to vegetation if drift occurs; however, they are faster and less labor intensive than basal treatments. Basal applications, on the other hand, necessitate the use of messy and costly oil carriers, are costly when brush is dense, and are limited by terrain and site accessibility; however, their selectivity allows the retention of low growing plants which may reduce establishment and growth of undesired trees and which provide food and cover for wildlife, and off-site drift is less likely. In addition, crop tree damage is

negligible after careful basal applications; whereas, with foliar applications damage is sometimes significant.

Cost comparisons appear favorable when the relatively new streamline basal technique is used. This method involves spraying only a small band on one side of small stems and two sides of larger ones from distances up to a few meters away (Hendler et al. 1987). Zutter et al. (1988) stated that the cost of aerial spraying of glyphosate (N-(phosphonomethyl)glycine) ranged from \$40 to \$60 per acre. al. (1987) estimated the costs from streamline spraying a solution of 20% triclopyr ([(3,5,6 trichloro-2-pyridinyl)oxy]acetic acid), 10% Cidekick (d'l limonene), and 70% diesel fuel on 632 acres of pine plantation to range from \$12 to \$41 per acre; however, control and pine damage were not rated. Schutzman and Kidd (1987) calculated the material costs only in a similar streamline trial to be \$15 to \$19 per acre. Kline (1986) calculated costs from a streamline application on 93 acres of a white pine plantation. Again, a mixture of 20% triclopyr, 10% Cidekick, and 70% diesel fuel was used. All competing hardwoods in a six foot radius around each crop tree were treated. Total costs of labor, supervision, and chemicals averaged \$27.12 per acre.

Basal Application Techniques

In the last decade, efforts have been made to develop basal application techniques which are faster and less expensive but as effective as the conventional method. The conventional method involved applying a 2 to 5% solution of herbicide in diesel fuel. Generally, the lower 1.5 to 2 feet of each stem were sprayed until puddling at the base occurred in order to control sprouting. Accordingly, this type of application required large amounts of solution, which involved large tank trucks to which applicators using backpack sprayers had to return frequently. This also meant large amounts of diesel fuel introduced

into the environment and strong odors from the diesel fuel. Finally, applying this volume of material was extremely time consuming (Melichar and Waggoner 1988).

Techniques have recently been developed which utilize less carrier volume. These include thinline, low volume, low volume banding, and streamline (Hendler et al. 1987). Thinline treatment involves application of undiluted herbicide in a thin stream encircling the stem at about 15 cm above groundline. Low volume treatment involves thorough coverage of the lower 30 cm of stem with solutions containing 20 to 30 percent commercial herbicide formulation without runoff occurring as it does in conventional applications. Low volume banding is similar but generally involves solutions of 50 percent commercial herbicide formulation applied in a mist to a 10 cm band of stem located 1 m above ground. Streamline application is similar to thinline but involves solutions of 20 percent commercial herbicide formulation along with 10 percent Cidekick penetrant. The solution is applied in a straight stream to a small portion of stem located within 15 cm of groundline. Application is made to only one side of small stems and two sides of larger ones. Generally, all of the above techniques have been effective, giving crown reduction or mortality usually exceeding 80% on a range of species as discussed below. Mackay (1988) achieved over 90% control of sugar maple (Acer saccharum) and white ash (Fraxinus americana) using low volume (15% and 25% herbicide formulation) and thinline (50% and 75%) techniques with triclopyr and fluroxypyr ([(4amino-3,5-dichloro-6-fluoro-2-pyridinyl)oxy]acetic acid). Melichar and Waggoner (1988) applied mixtures of triclopyr and triclopyr + picloram (4-amino-3,5,6 trichloropicolinic acid) (total volume of herbicide formulation equaling 25%) using the low volume method. They observed defoliation exceeding 83% of black cherry (Prunus serotina), maple (Acer spp.), oak (Quercus spp.), sassafras (Sassafras albidum), birch (Betula spp.), white ash, and quaking aspen (Populus tremuloides). The amount

of sprouting generally decreased as the relative amount of picloram increased. Warren (1982) found excellent control of bigleaf maple (Acer macrophyllum), sugar maple, and tanoak (Lithocarpus densiflorus) using thinline (100% triclopyr) applications. Using low volume (25% triclopyr) and thinline (100% triclopyr) treatments in naturally regenerated stands of balsam fir (Abies balsamea) and spruce (Picea spp.), Maass and Arsenault (1986) compared efficiency of these two application methods before and after thinning. They found low volume to be more efficient at low stem densities and thinline to be more efficient at high stem densities. Treating before or after thinning did not significantly affect treatment times. Kline (1986) investigated conventional (2, 3, and 4% triclopyr), thinline (100% triclopyr), and streamline (10, 20, or 30% herbicide plus 10% Cidekick) techniques. Triclopyr, triclopyr plus picloram, glyphosate, fluroxypyr, and dicamba (3,6-dichloro-2-methoxybenzoic acid) were used in the streamline treatments. The conventional application gave excellent stemkill at 3 and 4%. The thinline gave good control but appeared slower to apply. Streamline treatments of 20% triclopyr plus 10% Cidekick gave excellent control (70% or better) of 12 woody species. Glyphosate and dicamba were least effective. Hendler et al. (1987) achieved 80% or better stemkill with streamline applications of 20% triclopyr (or triclopyr plus picloram) plus 10% Cidekick on cherry (Prunus spp.), red maple, red oaks (Quercus spp.), dogwood (Cornus florida), hickory (Carya spp.), persimmon (Diospyros virginiana), and sweetgum (Liquidambar styraciflua). Streamline applications are now being recommended for pine release (Va. Coop. Ext. Serv. 1988) because they involve applying herbicide to only one or two sides of a stem and can be made from a few meters away. Thus, streamline applications would appear to be faster than the other techniques and require less solution than the conventional method.

Comparisons of Carriers

Studies comparing efficacy using various carrier solvents have included such solvents as diesel fuel, kerosene, fuel oil, Asplundh Oil, Arborchem Basal Oil (contains lecithin and kerosene), diesel plus water, water alone, and water plus surfactant. Diesel, kerosene, and fuel oil are the traditional carriers used. Melichar et al. (1987) compared Arborchem Basal Oil and kerosene in a low volume treatment using triclopyr ester alone or in combination with picloram. Use of both carriers resulted in effective control. Similarly, Arborchem Basal Oil and fuel oil as carriers in low volume treatments utilizing triclopyr ester alone and with picloram resulted in comparable control (Hall and Hendler 1986). Using water as the carrier in conventional applications of 3% herbicide was shown to give less effective control than using diesel (Kline 1986). Streamline treatments of 20% triclopyr + 10% Cidekick in diesel and Asplundh Oil gave comparable results (Kline 1986). Lichy (1977) installed field trials at four locations involving one or more of the following carriers: diesel, kerosene, and water plus adjuvants. All tests involved conventional applications of triclopyr ester at 0.5, 1, 2, and 4%. Results using water were encouraging, yielding effective control with 2%, whereas diesel and kerosene were generally effective down to 0.5%. Warren (1982) compared conventional applications of triclopyr ester at 1 and 2% mixed with 15, 20, 30, and 100% diesel fuel plus the remaining volume in water plus 0.25 or 0.5% Sponto 712 surfactant. Species treated were tanoak, California black oak (Quercus kellogqii), and ponderosa pine (Pinus ponderosa). Results generally indicated greater control with increasing diesel concentrations. However, triclopyr at 2% in water with Sponto 712 plus diesel was effective on trees less than 3 to 4 inches in diameter. Diesel content of at least 30% was necessary before control approached that of triclopyr at 1% in diesel alone. Goodfellow and Mider (1981)

used various herbicides diluted in oil, oil plus water plus surfactant, water plus surfactant, and water alone. Herbicides included dicamba, dichlorprop (2-(2,4 dichlorophenoxy)propionic acid), glyphosate, hexazinone (3 cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine- 2,4-(1H, 3H) dione), mecoprop (2-(2-methyl-4-chlorophenoxy) propionic acid), picloram, tebuthiuron (N-[5-(1,1-dimethyl)-1,3,4-thiadiazol-2-yl] N,Ndimethylurea), triclopyr, and 2,4-D (2,4 dichlorophenoxyacetic acid). All applications were via the conventional method. Treatments which included water were generally ineffective. Zedaker (1986) compared Asplundh Oil and kerosene using low volume banding (20% herbicide) and low volume (10% herbicide) techniques with triclopyr plus picloram. band applications were made to a 4 inch length of stem. Using both techniques, results after one growing season showed Asplundh Oil treatments to have higher percent mortality than kerosene treatments. However, applications were made during the dormant season and Asplundh Oil appeared more viscous. Observations by application personnel suggested that more mixture may have been applied to stems when using Asplundh Oil as the carrier. Asplundh Oil did not spread around the stem as fast, possibly leading the applicators to apply more in order to assure complete coverage.

Toxicology of Petroleum Carriers

One of the reasons for testing alternatives to petroleum fuels as carrier solvents is concern that fuel oils are health hazards. Many fractions of crude petroleum oil have been shown to induce dermal carcinomas when repeatedly applied to the skin of mice (Bingham et al. 1980). This carcinogenicity has been linked, at least in part, to the presence of polycyclic aromatic hydrocarbons (PAH). The amount of PAH in a particular crude stock varies depending on its origin. PAH can also be formed during a refining process called cracking (Bingham et al.

1980). PAH typically boil at 370°C or above and petroleum products with boiling ranges below this temperature, therefore, contain relatively low amounts. For this reason, most fuel oils, including kerosene and diesel fuel, are not considered to be carcinogenic or to be only marginally so. Also, cracking generally is not involved in the production of these fuel oils. (Bingham et al. 1980). In tests in which jet fuel and diesel fuel were applied to the skin of mice, results indicated no evidence that jet fuel is carcinogenic and only equivocal evidence that diesel fuel is (NIH 1986). However, Biles et al. (1988) suggest that another concern may be the possible presence of co-carcinogens which act to promote the growth of tumors once they have begun to develop. In addition, chronic dermal contact with kerosene has been shown to effect the kidneys in mice (Easley et al. 1982). Therefore, the use of non-petroleum oils or petroleum solvents which are highly refined and composed of fewer numbers or types of compounds may be desirable.

Concentration and Dose

In order to keep costs and use of materials at a minimum, it is important to determine the combinations of herbicide concentration and dose of herbicide mixture per stem which provide acceptable control. The various basal techniques range from dilute concentrations applied at a high dose (conventional) to undiluted herbicide applied at a low dose (thinline). These combinations have all been shown to give effective control. However, for reasons mentioned earlier, researchers interested in forestry applications are concentrating on streamline applications which generally involve concentrations ranging from 10% to 40% herbicide.

Gardiner and Yeiser (1990) applied doses of 1, 2, 3, and 4 ml to stems of blackgum (Nyssa sylvatica), hickory, red maple, red oak, sweetgum, and white oak categorized as 2.5, 5.0, or 7.5 cm in groundline

diameter using a Gunjet. Streamline applications of 20% triclopyr ester plus 10% Cidekick plus 70% diesel fuel were made in March. For 7.5 cm stems, percent crown reduction increased from 22 to 49% from 1 to 4 ml; for 5.0 cm stems, from 48 to 73%; and for 2.5 cm stems, from 76 to 84%.

Kuhns and Lyman (1989) applied doses of 0.5, 1, and 2 ml per inch of stem diameter. Applications consisted of 20% triclopyr ester in Arborchem Basal Oil and were made in March using syringes. After 15 months, 2 ml had provided 100% mortality, while 1 ml had provided 80% mortality, and 0.5 ml had provided 37% mortality of green ash (Fraxinus pennsylvanica) and black birch (Betula lenta).

Yeiser et al. (1989) held dose constant at 2 ml per stem and varied herbicide concentration. Triclopyr ester was tested at 5, 10, 15, and 20% and fluroxypyr at 6, 12, 24, 36, and 48%. All mixtures contained 10% Cidekick and diesel fuel as the carrier. Percent crown reduction of hickory, red maple, sweetgum, white oak, and red oaks was found to increase with concentration except at higher levels of fluroxypyr. Percent crown reduction increased from 38% to 84% between 5% and 20% triclopyr. Percent crown reduction increased from 41% to 71% as fluroxypyr concentration increased from 6% to 24% and crown reduction then decreased to 52% as fluroxypyr concentration increased from 24% to 48%. The authors suggest the decreased crown reduction may be due to reduced penetration because of less carrier in the higher fluroxypyr concentrations.

Yeiser and Boyd (1989) held dose constant at 2 ml per stem and applied triclopyr concentrations of 5, 10, 20, and 40%. All treatments involved streamline applications including 10% Cidekick and diesel fuel and were made in February, June, and September. Percent crown reduction increased from 17% to 70% as herbicide concentration increased from 5% to 40%. Schutzman and Kidd (1987) applied 5 ml of 20% triclopyr ester plus 10% Cidekick in 70% diesel fuel per stems 10 cm or less in groundline diameter. Control was 80% or better for all species.

Worley et al. (1955) varied both concentration (1.5 and 3%) and dose (59 to 590 ml) in basal applications of 2,4,5-T to bear oak (Quercus ilicifolia) stump sprouts. Volumes were recorded on a sprout clump basis with an average of 5 stems per clump. The low dose caused 43% and 73% topkill at the low and high concentrations, respectively. One hundred percent stemkill was achieved with a dose of approximately 250 ml per clump. Sprouting was reduced as dose increased but not as concentration increased.

Uptake and Translocation - Stem applications

Several studies of herbicide translocation following stem applications have been published since the 1950's. Much of the interest has centered on the degree of downward movement via the phloem, since rootkill or inhibition of basal buds is usually desired in order to prevent regrowth of perennial species. Herbicides studied have included 2,4-D, 2,4,5-T, picloram, amitrole (3-amino-1,2,4-triazole), monuron (3-(p-chlorophenyl)-1,1-dimethyl urea), and maleic hydrazide.

Hay (1956) investigated movement of 2,4,5-T in marabu (Dichrostachys nutans), a woody plant, following application of approximately 5 ml of 2, 4, and 8 percent solutions in diesel oil, to a 4 to 6 inch length of basal stem. The stems were 1/2 to 3/4 inch in diameter. Spreading from the treated area was contained by covering it with absorbent cotton and tape. Twelve trials took place throughout the year. Non-metabolized herbicide was extracted and determined in bark and wood samples from roots. Regardless of the concentration or the time of harvest (6 hours to 13 days after treatment), no herbicide was determined to be in the roots. Further trials found only traces in the stem above the treated area and none in the stem below it.

Determinations were made by bioassay, however, and may not have been sensitive to the presence of small amounts or the presence of

metabolites.

Shiue (1958a) found that 2,4,5-T movement apparently occurred primarily in the xylem following application to quaking aspen at a concentration of 24 g/l in diesel oil. Use of an oil soluble anilin red dye mixed in the herbicide solution indicated that entry through the bark occurred mainly through lenticels. After six days, the dye was in the sapwood and after twelve days, light red stripes extended to the top of the sapwood. The dye did not move up the phloem. Assuming that the herbicide followed the path of the dye, then most transport appeared to be occurring in the xylem. Another experiment involved four treatments: girdling without herbicide application, basal application without girdling, basal application with girdling above, and basal application with girdling below. During the first season, girdling alone did not affect growth, basal application killed foliage above and below the treatment area, girdling above also killed foliage above and below, and girdling below killed only foliage above. Therefore, downward movement of 2,4,5-T in the phloem appears to occur in aspen.

Yamaguchi and Crafts (1956) found that labeled herbicides dissolved in 50% ethanol plus 0.1% Tween 20 moved in varying degrees in the phloem when applied directly to the inner bark. Applications were made to manzanita (Arctostaphylos manzanita), toyon (Photinia arbutifolia), and buckeye (Aesculus california), two of which are evergreen, during each month of the year. Activity was detected by autoradiographs of excised bark up to twelve inches above and below the application. 2,4-D and 2,4,5-T appeared to move both up and down with maximum downward movement in the spring and upward movement in the spring and summer. The authors suggested that high transpiration rates in the summer sweep most of the herbicide up in the xylem before it has a chance to be absorbed into the symplast. Monuron showed up least in the inner bark, likely because it is a polar molecule and remained in the apoplast, moving between the cells of the inner bark until it

reached the sapwood.

Crafts (1967) applied 2,4-D, monuron, amitrole, and maleic hydrazide to the epicotyl of soybean (Glycine max) seedlings. Treatment lasted two days at which time the seedlings were freeze-dried and mounted. Autoradiographs showed 2,4-D was transported a limited distance both up and down the stem, monuron moved only upward and into primary leaves, indicating movement in the transpiration stream, amitrole moved up and to a lesser extent down, and maleic hydrazide moved throughout the plant and into the roots. 2,4-D applied to one cotyledon entered the roots and shoot tip but not mature leaves or the other cotyledon. This would suggest that 2,4-D was not entering the transpiration stream and was being transported in the phloem.

Eliasson (1973) applied 2,4-D at 20 g/l in diesel fuel to quaking aspens seven meters tall and 6 cm in diameter. The stem was covered from the root collar up to 30 cm. Herbicide was extracted from the upper, middle, and lower crown and from bark and wood from the groundline and 1 m intervals up the tree. The roots were not sampled. Total recovery was 50 mg out of 400 mg applied as determined by extraction and bioassay. Greatest concentrations were found in the treated area and the upper crown.

Leonard et al. (1966) observed that 2,4,5-T applied to the bark or cut surfaces of red maple and white ash moved upward, particularly to the leaves of red maple, but only trace amounts were found in the stem below or roots of red maple and slightly more in white ash. Prior to application of labeled 2,4,5-T, the entire trees had been sprayed with a dilute aqueous solution. In contrast, amitrole applied similarly moved readily throughout the trees and was found in substantial quantities in the stem below and the roots. The authors suggested upward movement of 2,4,5-T was likely in the xylem and that significant phloem transport did not seem to be occurring judging by the amounts found below the application site.

Eliasson and Hallman (1973) applied picloram and 2,4-D to the cut surface of a main branch of aspen plants. Solutions contained 0.2 g/l of herbicide in 50 ul of water and were placed in a tube on the cut end of the branch. After absorption, another 50 ul of water were added to rinse the tube. Plants were harvested 1, 3, or 9 days after treatment and samples taken from shoot tips, mature leaves above the cut branch, stem above, and roots. After 9 days, 62.8% of the applied picloram was in the growing shoot tip, 9.3% in mature leaves, 8.6% in the stem, and 0% in the roots, though 1.3% was found in the roots of the day one harvest. This compares to 44.8% of 2,4-D in the shoot tip, 27.9% in the mature leaves, 5.3% in the stem, and 1.8% in the roots. The authors suggest that picloram may be more phloem mobile than 2,4-D because it accumulates more in the shoot tip and less in the mature leaves. The root data would suggest otherwise because a higher percentage of 2,4-D was found in the roots.

Sundarum (1965) studied translocation of C¹⁴-labeled 2,4,5-T in the trunks of tropical trees following basal application in diesel fuel. Greater retention of herbicide in the phloem was found in the two susceptible species tested while greater upward movement in the xylem was found in the tolerant species. Therefore, differences in susceptibility may in some cases be related to differences in phloem absorption.

One explanation for any lack of transport in the phloem may be damage or destruction of phloem tissues by the herbicide, rendering them non-functional or reduced in function. Eliasson (1973) noted that bark and cambium of aspen from the area treated with 20 g/l (4%) of 2,4,5-T in diesel fuel was dead one month after treatment. Tissues in the rest of the stem remained alive one year later; however, no leaves developed the second year and after two years the stems were dead. Shiue et al. (1958b) applied a 5% solution of 2,4,5-T in diesel oil to the bark of quaking aspen. Examination of the inner bark showed that phloem

parenchyma had multiplied rapidly causing adjacent sieve tubes to collapse. In another experiment, shortly after foliar application of picloram or 2,4,5-T at 0.04, 0.14, and 0.56 kg/ha to honey mesquite (Prosopis juliflora), Meyer (1970) discovered phloem parenchyma had proliferated throughout tissues in the plants. The higher rates sometimes resulted in less abnormal growth seemingly due to tissue death. Eames (1950) applied 2,4-D to the non-woody hypocotyl of bean (Phaeseolus vulgaris) seedlings and also found abnormal proliferation of tissues located between the primary xylem and the cortex. Growth of phloem parenchyma caused the separation of phloem strands, crushing companion cells and small sieve tubes. Larger sieve tubes lost their cytoplasm. All primary phloem was non-functional after 8 days. No secondary phloem had formed, and no subsequent formation would be expected because the cambium was part of the abnormal tissue. Since triclopyr, like 2,4-D and 2,4,5-T, is a growth regulator, it may cause similar effects to inner bark tissues as those described above.

Uptake and Translocation - Triclopyr

Triclopyr is a relatively new herbicide and has not been studied as extensively as 2,4-D or 2,4,5-T. However, investigations of translocation following foliar application have been made.

Autoradiographs taken after application to tanoak, snowbush ceanothus (Ceanothus velutinus), and bigleaf maple showed activity in untreated leaves, suggesting transport in the phloem from the treated leaf.

Seedlings acclimated for 30 days to warm temperatures (13-26°C as compared to 4-13°C) and long days (16 hour photoperiod as compared to 12 hours) appeared to have translocated more herbicide. Movement to the roots was not evident (Radosevich and Bayer, 1979). Bovey et al (1979) applied the triethylamine salt of triclopyr at 1.12 and 2.24 kg/ha to leaves and stems of huisache (Acacia farnesiana). After 3, 10, and 30

days, most herbicide was in the leaf or stem wash, some was found in the leaves and stem, and very little in the roots (<1 ppm). In applications to horsenettle (Solanum Carolinense), triclopyr was found to increase in the roots over time, and had accumulated to 3-4% of the recovered amount after 16 days (Gorrell et al 1988). Roots were swollen at harvest, indicating activity of an auxin-type herbicide. Plants were also cultivated which had two shoots emanating from the same root. Each shoot was potted separately, connected only by the root bridge. Treatment of one shoot caused herbicidal symptoms in the other in all cases, showing that translocation to the roots had occurred.

Uptake and Translocation - Season of treatment

One advantage of basal treatments is that they can be made throughout the year. A few studies have made comparisons of efficacy achieved from basal applications made at various times throughout the year. Williams et al. (1970) used the conventional method to apply mixtures of picloram and 2,4,5-T during the growing season, during bud break, and during the winter. Assessments of control were mostly made during the following May, June, or July. Results were similar for all application times. Using the conventional technique to apply triclopyr, picloram and 2,4,5-T, Warren (1980) found that winter applications resulted in poorer control of ponderosa pine and tanoak. Contrary to the above results, Burch et al. (1987) achieved slightly better efficacy with dormant season streamline applications. They suggested two possible explanations for their findings. One was that foliage and herbs present during the growing season may have intercepted the herbicide solution, for with streamline applications, treatment involves only a small amount of mixture applied from up to a few meters away. The other was that applications may have been less precise during the growing season. Working conditions were likely less comfortable due to

hotter weather and thicker vegetation. Finally, Yieser and Boyd (1989) found percent crown reduction to be the same following June and September streamline applications and significantly less following a February application. Wet bark from rain the night before, however, may have contributed to the poorer February results.

A hypothesis which has been suggested for some time (e.g. Leonard and Crafts 1956) is that herbicides which are transported in the phloem are moved along with photoassimilates and accumulate at the same destinations, namely active growing points or storage tissues. This hypothesis has been verified for some herbicides. For instance, Dewey and Appleby (1983) showed that labeled glyphosate applied to tall morningglory (Ipomoea purpurea) moved to sinks (roots, shoot tips, and new leaves) in similar proportion as assimilate produced from ¹⁴CO₂; however, glyphosate appeared better able to transfer from the symplast to the apoplast and bypass stem girdles. Both glyphosate and assimilate also moved symplastically to artificial sinks created by applying benzyladenine to mature leaves or cotyledons.

Using an herbicide such as triclopyr which has been shown to be phloem mobile following foliar application (Gorrell et al. 1988), one might expect triclopyr movement in the phloem following basal applications to parallel photoassimilate movement. It is generally accepted that photoassimilates move predominantly upward from storage in the roots and other tissues in the spring to provide substrate for new growth. Then, later in the summer net movement between roots and leaves shifts downward as mature leaves produce excess sugars. Schier (1970) examined the movement of photosynthate in red pine (Pinus resinosa) in May, July, and October and found this pattern. Foliage of six four-year-old seedlings was exposed to 'CO2 during each month. Two seedlings from each treatment were then harvested during January, May, July, and October (excluding the treatment month). At harvests, seedlings were separated into needles, stems, branches, and roots. Results showed that

following the July treatment, more than two times the proportion of C¹⁴ was translocated to the roots (October harvest) than following the May treatment (July harvest). Following the October treatment a relatively large proportion of C¹⁴-assimilate moved to the roots as well, apparently because other sinks had ceased growth. Therefore, if herbicide movement in the phloem is correlated with assimilate translocation, it would be expected that more herbicide would move to the roots in late summer, achieving better root kill or having a greater inhibiting effect on dormant basal buds; whereas, in the spring more herbicide would move upward, achieving faster top-kill. However, upward movement in the phloem may be overshadowed, regardless of the season, by movement in xylem, especially when the transpiration rate is high, as suggested by Yamaguchi and Crafts (1959).

METHODS

This thesis project consisted of two studies. The first examined the effects of the treatments on efficacy following basal applications. The second then investigated the basis for possible treatment differences in the efficacy study, particularly with regard to formulation solvent and species, by determining herbicide uptake and translocation.

Efficacy Study

Sites

This study was installed on two 30 m wide powerline rights-of-way located in the Ridge and Valley Province in Virginia. One was located 8 km north of Blacksburg, VA. It ran generally east and west with slopes ranging from 0 to 40 percent and with a predominantly north-northeast aspect. It was bordered by forest which is predominantly oak. composition was dominated by Virginia pine, red maple, red oak, and rhododendron (Rhododendron spp.). Other species included white and chestnut oak (Quercus prinus), table-mountain pine (Pinus pungens), and black birch. Vegetation appeared to be largely of seed origin. Soils were likely mostly of the Berks-Clymer complex with Berks-Weikert series present on the steeper slopes (USDASCS 1985). The other site was located 1 km southeast of Pulaski, VA. It ran generally north and south with slopes ranging from 0 to 35 percent, including both northern and southern aspects. It too was bordered by a predominantly oak forest. Most of the oaks appeared to be of sprout origin. Vegetation consisted largely of oaks along with greenbriar (Smilax spp.) and blackberry (Rubus spp.). A soil survey was not available for this site.

Thirty-six herbicide treatments consisting of a factorial arrangement of twelve formulations (four solvents containing three herbicide concentrations) and three doses were randomly assigned to 25 stems each of three species — red maple, white (or chestnut) oak, and Virginia pine. Some of the oak and maple stems were in clumps from the same rootstock and were therefore assigned the same treatment. The four solvents are described below (Table 1). Concentrations were 0.25, 0.5, and 1.0 lb a.e. of the butoxyethel ester of triclopyr per gallon. Doses were 1, 3, and 5 ml of solution per 2.5 cm of stem diameter measured at 15 cm above groundline. Concentrations and doses were chosen, based on results from other studies, with the goal of achieving a range of stemkill which would allow comparison of the solvents.

The rights-of-way were divided into four lanes (approximately 8 m wide) to aid locating each tree and decrease the likelihood that applicators would miss a tree. Prior to herbicide application, during May of 1990, 900 stems of each species were tagged; measured for diameter at 15 cm above groundline, height, and crown width; and randomly assigned a treatment. To obtain a good estimate of crown width, two measurements, perpendicular to one another, were made. specific criteria were used to select trees except that trees less than 0.5 cm in stem diameter and those in sprout clumps were avoided if possible. After measuring stem diameter, the precise dose for that tree was calculated and written on the tag. Herbicide applications were made between June 1 and June 14 of 1990. In order to reduce application errors, trees were flagged by color according to the concentration of herbicide they would receive: 0.25, 0.5, or 1.0 lb a.e. per gallon. Each applicator carried four of the twelve formulations, all of the same concentration, and walked along a lane, treating those trees flagged a certain color. Solutions were contained in one liter plastic bottles

Table 1. Chemical and physical properties of formulation solvents used in stem applications of triclopyr ester.

Solvent	Chemical composition	Boiling range
Aliphatic	99% saturated petroleum hydrocarbons, 1% aromatic	193-248 C
Aromatic	Napthalenic petroleum derivative	217-293 C
Kerosene	Mixture of petroleum hydrocarbons	175-325 C
Vegetable	Methylated vegetable oil derivative	>260 C

which were carried in buckets. Tygon tubing was inserted through the lid of the bottles onto which a syringe was connected and solution withdrawn. Applicators were alternated each day between the three concentrations to avoid confounding concentration with applicator. Application was made within the lower two feet of each stem using a 10 ml syringe. No attempt was made to wet the whole two feet of stem; however, an attempt was made to at least encircle each stem with a band of solution. Better coverage of stem surface occurred among maples and small oaks. The solutions tended to soak into the pines and larger oaks and not visibly spread as much. Live crown widths, in two perpendicular axes, and height of each tree were remeasured in August of 1991.

Data Analysis

Trees which showed no signs of herbicidal damage after 14 months were assumed not to have received treatment and not included in the analysis if virtually all other trees receiving the same treatment were dead. It is not unlikely that trees were missed during application due to the density of vegetation on the rights-of-way. After removing the above trees and those not found during remeasurement, 2501 out of 2700 remained plus 64 control trees. Each treatment was represented by at least 19 of the original 25 replicates.

The response variable used in the analyses was crown volume control. Changes in an index of crown volume were used instead of changes in actual crown volume, which is the space encompassed by the perimeter of a tree's crown of leaves. This index equaled the product of two perpendicular crown widths and height, which is proportional to the volume of a cylinder of those dimensions. In addition, pretreatment crown volume indices were adjusted to reflect expected volume at the time of remeasurement if treatment had not occurred (expected crown volume index). Adjustment was accomplished using a regression equation

developed from measurements of untreated trees as suggested in Knowe et al. (1990). The percent change in crown volume index then equaled expected crown volume index minus the posttreatment crown volume index and all divided by the expected crown volume index. Throughout the rest of the document, changes in crown volume index will be referred to simply as crown volume control. Unlike crown reduction, crown volume control accounts for the growth which would have occurred in the absence of treatment. Mortality is also presented in the results. Because mortality paralleled crown volume control and because crown volume control was the main response variable of interest, statistical analysis was largely confined to the latter. In some instances, chi-square tests were performed to test whether or not mortality was independent of the treatments imposed.

Analysis of variance was performed to determine the significance of factors and their interactions in the model. Because the response variable was a percent, arcsinv transformation of the data was performed. Because the data were unbalanced, Type III sums of squares were used for testing significance. Species was included in the model, along with formulation and dose, despite rather large differences in average stem diameter between species. Maples averaged 2.1 cm, oaks 3.6 cm, and pines 5.1 cm (Table 2). Also, many of the oak stems occurred as stump sprouts, whereas none of the pines and few of the maples did. These differences must be considered when interpreting the significance of species effects. Analysis of covariance was considered to remove the possible influences of differences in pretreatment sizes between treatments. Results, however, showed significant interactions (p<0.0001) between the covariate stem diameter and all other factors in the model, violating the assumption of parallelism. In addition, least square means, adjusted for stem diameter, differed only slightly from the unadjusted means. Accordingly, analysis of covariance did not seem appropriate or necessary. Also, solvent and concentration were combined

Table 2. Average dimensions of trees used in studies examining efficacy and uptake of triclopyr ester following stem application.

		Species		
Study	Dimension	Maple	Oak	Pine
Efficacy	Diameter(cm) -average -range	2.13c 0.5-11.0	3.57b 1.0-10.0	5.15a 1.5-13.0
	Height(m) -average -range	2.02c 0.6-6.0	3.16a 0.7-9.0	2.90b 0.2-6.1
Uptake	Diameter(cm) -average -range	2.02b 1.3-3.2	2.80a 1.5-4.6	2.94a 2.1-4.3
	Bark thickness(mm) -average -range	0.83c 0.5-1.5	2.06a 1.0-3.5	1.27b 0.5-2.6

¹Values within a row followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

as formulation because these twelve combinations are being considered for marketing. Duncan's New Multiple Range Test was used to separate means.

In order to characterize the relationship between crown volume control and concentration, dose, and stem diameter, multiple regression was performed. This regression approach is suggested over mean comparisons when analyzing continuous variables (Borders and Shiver 1989; Mize and Schultz 1985).

Uptake and Translocation Study

Design

The experiment was set up as a completely randomized design. It involved the application to three species of a mixture of unlabeled and C¹⁴-labeled triclopyr ester formulated in four solvents in order to compare uptake and translocation. Each treatment was replicated 10 times during July of 1990, and five times each during the following February and May to give an indication of possible differences between seasons of application.

Experimental Procedures

This experiment began at the Reynold's Homestead Agricultural Research Center located in Critz, VA. During the winter of 1989-90, 150 saplings each of red maple, Virginia pine, and white oak were removed from the forest understory. Enough soil was retained with the roots to fill a fifteen liter pot and the roots were clipped to the same size. The branches of the trees were also heavily pruned back due to the smaller root system now available to support them. These saplings were placed in a greenhouse or lathehouse and allowed to grow and become

acclimated to the pots until late July of 1990. At this time approximately 100 of each species were alive. On July 24 the saplings were sorted and 40 of those with the largest and healthiest looking crowns were selected for treatment. Unformulated C14-labeled triclopyr (butoxyethyl ester)(S.A.= 30.5 uCi/umole) was mixed with unlabeled triclopyr ester formulated at 1 lb a.e. per gallon in each of the four solvents. The amount of labeled triclopyr was so small, compared to the unlabeled triclopyr, that it had a negligible effect on concentration. The resulting solutions contained activities of approximately 1 uCi per In preliminary trials difficulties with solution dripping down the stems occurred when doses were too large. Therefore, a small dose using 1 lb a.e. per gallon was chosen (1 lb per gallon is close to the concentration usually recommended for streamline basal applications). It was thought to be desirable to contain the applied solution in a rather small area on the stem. Doing this required using a dose of 150 ul per 1 cm of stem diameter measured at 15 cm above groundline. Containing the solution was deemed important so that dripping on the exterior of the bark would not be confused with translocation, and so that radioactive contamination of the soil could be avoided. addition, in order to investigate the relationship between uptake and bark thickness, it would seem important to apply a constant dose per unit of surface area. However, even with this relatively small dose, dripping on maple stems was still a problem, especially with the vegetable oil formulation. In contrast, the small dose was not enough to encircle the oak and pine stems, so often only one side was treated.

At treatment time, a roll of plastic-backed absorbent paper was placed on the ground. Thin marks encircling the stems were made at 15 and 30 cm above groundline using a grease pencil. These marks delineated the treatment zone and had the added benefit of helping to contain the solution on smooth-barked stems as did laying the saplings down and treating the stems horizontally. After the solutions had

adequately penetrated or the solvent had evaporated so that dripping was no longer a problem, the saplings were returned to a greenhouse. Saplings receiving the vegetable oil formulation had to remain horizontal for a longer period to prevent dripping. All saplings were watered at the end of the day and periodically thereafter. Watering was performed such that minimal amounts of water contacted the stems. procedures were repeated in February and May of 1991 on the remaining trees. Average minimum and maximum temperatures in the greenhouse during the February and May treatment were 1.4°C & 20.5°C and 14.7°C & 33°C, respectively. Daily temperature extremes were not recorded in the greenhouse during the July treatment. However, comparing the February and May temperatures in the greenhouse with those at the nearby weather station, temperature minimums appear very similar while maximums are approximately 9°C higher in the greenhouse. Estimates for average minimum and maximum temperatures in the greenhouse in July would have been 19°C and 37°C, respectively.

Harvests occurred three weeks after treatment, since by this time trees treated in the efficacy study were showing definite herbicidal effects and the leaves of the maples were dying. Harvest involved cutting the stem into segments including the treated stem section, the roots, the leaves, and stem segments above and below the treatment area. All the sections were placed in a common paper bag except the treated stem section which was placed in a separate bag. Finally, the soil was washed from the roots, and they were set out to dry and later bagged. Due to the high clay content of the soil, washing it off the roots was difficult and many of the more fragile root tips where herbicide might be expected to collect were likely lost with the soil. All material but the treated stem sections was placed in an oven at 60°C to dry for two weeks. The treated sections were kept frozen in an attempt to reduce further herbicide penetration and reduce evaporation of herbicide from the bark surface. Later, the treated sections were divided into three

fractions: the outer bark, the inner bark, and the wood. The bark was sectioned and removed by a combination of peeling and cutting with a razor blade. The dark outer bark was separated from the lighter inner bark. Usually some inner bark remained with the outer bark. thicknesses on both ends of the treated section or on the corresponding ends of the adjacent stem sections were measured with a ruler to the nearest 0.5 mm and averaged for each sapling. Total weight of outer bark, inner bark, and wood from the treated sections and total weight of leaves were determined for each sapling. The whole bark fractions were ground in a blender, using a 250 ml steel cup. The outer bark ground fairly well, but it was necessary to cut the inner bark lengthwise due to its fibrous nature. The wood from the treated section was cut into one inch pieces and then further split using pruning shears. pieces were then mixed together and a 33 percent subsample further cut up and ground in the blender. All leaves were removed from each sapling and mixed together. Two subsamples from the leaves of each sapling were ground. Samples of root bark were also removed, using a chisel because the bark had dried out and become very hard. The bark was removed from around the circumference in a 2-3 cm band located between 3-10 cm below groundline. Bark was sampled because downward movement of herbicide would likely take place in the phloem. Occasionally these "root" bark samples were actually lower stem bark samples, for when the saplings were potted this area had been buried. Additionally, some fine roots were sampled and ground, particularly from saplings in which activity was present in the root bark. In general, grinding was more complete on larger samples and on non-fibrous material (i.e. the outer bark and the leaves).

Two subsamples were taken from each ground fraction, except where two portions were ground (i.e. in the case of the leaves). Also, because so little activity was found in the first root bark subsamples, a second was only analyzed when activity was found in the first to

determine if the result was reproducible. Each subsample was weighed to the nearest mg and then combusted in an OX-500 biological material oxidizer (R.J. Harvey Instruments Corp., Hillsdale, N.J.). combustion gases, which contained the C14 as 14CO2, were bubbled through C14 Cocktail (R.J. Harvey Instruments Corp., Hillsdale, N.J.), absorbing the 14CO2 in solution. This solution was then assayed in a Beckman LS-250 liquid scintillation counter (Beckman Instruments, Inc., Columbia, MD). After subtracting a background level of 60 cpm's as determined from leaf material, subsample cpm's were divided by the counter efficiency to convert to dpm's and then multiplied by the oxidizer correction factor and by the ratio of the weight of the whole section to the weight of the subsample to get activity present in a given section. The percent of applied activity found in a section was then assumed to be proportional to the amount of unlabeled herbicide present. The two subsample values were then averaged for the analysis. One untreated tree was also harvested from each species during each season in order to quantify possible contamination through equipment and handling.

Because solvent evaporation was noted following treatment, there was concern over the effect evaporation of C¹⁴-triclopyr might have had on recoveries; therefore, a test of triclopyr evaporation was carried out in September of 1991 at the Reynold's Homestead. The same solutions applied to the saplings were used in the test. A 10 ul drop of each solution was placed on each of three glass slides and three slices of maple bark, approximately 1 cm x 2 cm, each resting on a glass slide. A 10 ul drop was also placed directly into each of three glass scintillation vials as a standard and 18 ml of Ecoscint scintillation cocktail (National Diagnostics, Manville, N.J.) added. The slides were placed on a cardboard tray and set outside approximately 20 feet from the Reynold's weather station. They remained there from 11:30 AM to 5:30 PM on a warm, sunny day (see Table 10b). After this period, the slides were washed with the same solvent in which the triclopyr had been

diluted. A 1 ml wash was followed by a 0.5 ml wash and then the slide was wiped with a tissue. Each wash and the tissue were placed in a scintillation vial to which 18 ml of Ecoscint were added. The slice of bark was placed in a scintillation vial and later oxidized as described earlier for tissue samples.

Relative rates of solvent evaporation were also determined because of their potential relevance for explaining differences in uptake. Similar amounts of each formulation solvent, weighing approximately four grams, were placed into three petri dishes with a surface area of 60 cm². Weights were remeasured after 1, 3, 7, and 24 hours. The experiment was performed in a fume hood at 26°C.

Data Analysis

The response variable was percent of applied C¹⁴ found in a each section of the sapling. Analysis of variance was performed by section (i.e. leaves; outer bark, inner bark, and wood from the treated section; and roots) on arcsin transformed data. Because the data were unbalanced, Type III sums of squares were used for testing significance. Class variables tested for significance in the analyses were species, formulation solvent, and treatment month, though there was a restriction on randomization with regard to treatment month. Duncan's New Multiple Range Test was used to compare treatment means. The relationships between percent triclopyr uptake and bark thickness were modeled using regression.

Analysis of covariance was attempted to account for the variability caused by differences in tree size. Stem diameter was used as the covariate. Results showed that the interaction between species and diameter was significant at the 0.10 level in all except the leaf analysis. Therefore, analysis of covariance was likely not appropriate unless a separate analysis was performed for each species. Because of

interest in comparing results among species, separate analyses were not ultimately performed, and therefore analysis of covariance was not appropriate. Accordingly, when interpreting the results, the following should be considered: diameter ranges were very similar within species for each treatment; however, among the species, maples were consistently smaller. Maples averaged 2.0 cm, while oaks averaged 2.8 cm and pines 2.9 cm in diameter (Table 2).

Some observations were lost or removed from the data set prior to analysis. Two July treatments were replicated only nine times and two February treatments and one May treatment only four times due to mistreatment or mislabeling of bags. Also, three uptake values appeared to lie far outside the range of the other values when plotted by solvent against bark thickness. In addition, studentized residuals for these apparent outliers, which would be expected to follow a Student's t-distribution and, therefore, lie mainly between -2 and 2, were greater than two, and their covariance ratios were less than those for all other observations, providing further indication that the outlying values might be having a disproportionate influence on the fit of the regression lines (Belsley et al. 1980). Therefore, these observations were not included in the analysis.

RESULTS AND DISCUSSION

Efficacy

The analysis of variance showed species, formulation and dose to be significant factors in the model (p<0.0001). In addition, all interactions between formulation, species, and dose were highly significant (p=0.0007 or less).

Overall, the maples were the easiest to control or kill, oaks intermediate, and pines most difficult (Table 3), but differences were mainly confined to trees treated with the aromatic formulations (Tables 4-7). The other formulations provided nearly 100 percent control and mortality of all species at the concentrations and doses used (Table 8). Previous studies have also shown all to be relatively easily controlled with basal applications (Kline 1986, Burch et al. 1987, Melichar et al. 1987). The species differences with the aromatic treatments may have been, in part, a reflection of the relative average size of each species used in the study (Table 2).

Among the maples, control of crown volume was 100 percent and mortality nearly 100 percent, regardless of formulation or dose, and no significant differences were found between treatments. Among the oaks, the aliphatic solvent, vegetable oil, and kerosene formulations gave control levels which were not significantly different and were similar in percent mortality (Tables 4 and 5). Control and mortality were reduced, however, at the lowest dose with minimums of 94 percent control and 67 percent mortality. The aromatic formulations gave significantly poorer control and less mortality than the others, ranging from 16-100 percent and 12-100 percent, respectively. Within the aromatic formulations, each concentration was significantly different, with the highest giving the best control and most mortality and the lowest the least. Only at the high concentration with all doses and at the middle

Table 3. Mean percent crown volume control and percent mortality across formulations and doses in August 1991 among red maples (<u>Acer rubrum</u>), white oaks (<u>Quercus alba</u>), and Virginia pines (<u>Pinus virginiana</u>) treated in June 1990 with triclopyr ester.

Species	Crown volume control	Mortality
		- %
Maple	100a ¹	99
Oak	96b	81
Pine	93c	70
		$(p < 0.001)^2$

¹Values followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

P-value, determined by chi-square test, for the independence of

mortality and species.

Table 4. Percent crown volume control in August 1991 among white oaks (<u>Quercus alba</u>) treated in June 1990 with triclopyr ester formulated in four carrier solvents at three concentrations and applied at 3 doses.

For	mulation	Dose (ml/2.5 cm) ²			
Carrier solvent	Concentration (lb/gal)	1	3	5	mean
				8	
Aromatic	1.0	81	86	100*	93b
	0.5	48	53	96*	68c
	0.25	16	19	46	26d
Aliphatic	1.0	100	100	100	100a
	0.5	94	99	100**	98a
	0.25	99*	100	100	100a
Vegetable	1.0	100	100	100	100a
	0.5	99*	100	100	100a
	0.25	100	100	100	100a
Kerosene	1.0	100	100	100	100a
	0.5	97*	100	100	100a
	0.25	96*	100	100	99a

Table values, except the means, are averages from 19-25 replicates. Formulation means followed by the same letter are not significantly different. Values followed by * are significantly different from other values in the same row. ** indicates the extreme values in a row are significantly different from one another. (Duncan's NMRT, alpha =0.05).

Dose in ml of solution per 2.5 cm of stem diameter at 15 cm above groundline.

Table 5. Percent mortality in August 1991 among white oaks (<u>Ouercus alba</u>) treated in June 1990 with triclopyr ester formulated in four carrier solvents at three concentrations and applied at 3 doses.

Formulation			Dose (ml/	2.5 cm) ¹	
Carrier solvent	Concentration (lb/gal)	1	3	5	<u>mean</u>
				%	
Aromatic	1.0	68	63	100	77
	0.5	33	35	70	46
	0.25	12	13	30	18
Aliphatic	1.0	100	100	100	100
	0.5	68	91	100	85
	0.25	78	100	96	92
Vegetable	1.0	95	100	100	98
	0.5	78	100	100	93
	0.25	88	96	100	94
Kerosene	1.0	85	95	100	93
	0.5	67	96	100	88
	0.25	79	88	96	88

Dose in ml of solution per 2.5 cm of stem diameter at 15 cm above groundline.

Table 6. Percent crown volume control in August 1991 among Virginia pines (<u>Pinus virginiana</u>) treated in June 1990 with triclopyr ester formulated in four carrier solvents at three concentrations and applied at 3 doses.

For	mulation		Dose (ml/	2.5 cm) ²	
Carrier solvent	Concentration (lb/gal)	1	3	5	mean
Aromatic	1.0 0.5 0.25	32 33 18	54 50 29	\$ 53 51 56*	47c 45c 34d
Aliphatic	1.0	100	100	100	100a
	0.5	99*	100	100	100ab
	0.25	93*	99	98	99ab
Vegetable	1.0	100	100	100	100ab
	0.5	99*	100	100	100ab
	0.25	86*	100	100	98b
Kerosene	1.0	100	100	100	100ab
	0.5	98*	100	100	100ab
	0.25	95*	100	100	99ab

Table values, except the means, are averages from 19-25 replicates. Values followed by the same letter are not significantly different. Values followed by * are significantly different from other values in the same row. (Duncan's NMRT, alpha = 0.05).

Dose in ml of solution per 2.5 cm of stem diameter at 15 cm above groundline.

Table 7. Percent mortality in August 1991 among Virginia pines (Pinus virginiana) treated in June 1990 with triclopyr ester formulated in four carrier solvents at three concentrations and applied at 3 doses.

For	mulation	Dose (m1/2.5 cm)			
Carrier solvent	Concentration (lb/gal)	1	3	5	mean
				£	
Aromatic	1.0	0	16	29	15
	0.5	8	20	17	15
	0.25	0	8	13	7
Aliphatic	1.0	95	100	100	99
	0.5	74	100	100	91
	0.25	60	96	92	83
Vegetable	1.0	91	100	100	97
	0.5	80	100	100	93
	0.25	49	96	100	81
Kerosene	1.0	91	96	100	96
	0.5	65	100	100	89
	0.25	64	92	96	84

Dose in ml of solution per 2.5 cm of stem diameter at 15 cm above groundline.

Table 8. Mean percent crown volume control and percent mortality across species, concentrations, and doses in August 1991 among trees treated in June 1990 with triclopyr ester formulated in four carrier solvents.

Solvent	Crown volume control	Mortality
		%
Aromatic	75b ¹	51
Aliphatic	100a	94
Vegetable	100a	95
Kerosene	100a	93
		$(p < 0.001)^2$

¹Values followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

²P-value, determined by chi-square test, for the independence of

concentration along with the highest dose did the aromatic formulations approach the performance of the others.

Results among pines were similar to those among oaks, with a minimum control and mortality among the non-aromatic formulations of 86 and 49 percent, respectively (Tables 6 and 7). However, whereas control and mortality reached 100 percent among the oaks when the high concentration aromatic formulation was used along with the highest dose, control and mortality only reached 53 and 29 percent, respectively, with that same combination applied to pines. Again, control decreased at the lowest dose.

Overall, the aliphatic solvent, vegetable oil, and kerosene formulations gave very similar control and mortality whereas the aromatic treatments produced about three-quarters the control and half the mortality (Table 8). In fact, from observations, many of the pines and oaks treated with the aromatic formulations showed no visible signs of herbicide damage. This was especially true of the trees of larger diameter, for, as will be discussed later, diameter was a significant determinant of crown control, especially when the aromatic formulations were used. One sign of herbicidal effects often seen among the oaks, but seemingly to a greater degree among those treated with the aromatic solvent, was abnormal tissue growth at the site of application. This may suggest that, particularly when using the aromatic formulations, enough herbicide was penetrating to induce cell proliferation but not enough to cause pronounced tissue death.

Uptake and Translocation

Solvents and species

Each analysis showed significant species and solvent main effects (p<0.007) except for solvent effects for total recovery (p=0.85) and root recovery (p=0.49). All interactions between species and solvents were significant (p<0.03) except for root recovery (p=0.60). triclopyr activity recovered in the saplings did not vary between the four formulations (Table 9). Recoveries were above 80 percent of applied C14-triclopyr. Most of the 20 percent which was unaccounted for probably was located in the relatively large mass of unsampled stems and branches. Less was recovered in the outer bark and, correspondingly, more inside the saplings with the vegetable oil formulation than with the others. Among the other solvents, about the same distribution of activity was found. In particular, activities in the inner bark, wood and leaf fractions were greater with vegetable oil. Therefore, penetration or uptake of herbicide was greater when formulated in the vegetable oil carrier than with the other solvents. The amount of activity recovered in the leaves was least for the aromatic solvent, approximately equal for the aliphatic solvent and the kerosene, and greatest with the vegetable oil. Activity levels in the root bark were very low, regardless of formulation. In addition little or no activity was found in the few fine root samples analyzed (data not presented). Because the lack of downward movement suggests phloem translocation was not great, translocation to the leaves likely occurred in the xylem. experiments involving foliar application of 2,4-D and 2,4,5-T to leaves of quaking aspen plants, Eliasson (1965) discovered that most of the movement up the stem could be attributed to movement in the xylem and not the phloem. He also suggested that because the herbicides apparently easily transferred between the phloem and the xylem, that

Table 9. Mean percent of applied activity across species and treatment months recovered in saplings three weeks after stem applications of triclopyr ester formulated in four solvents.

		Formulation	n solvent	
Tree Section	Aromatic	Aliphatic	Vegetable	Kerosene
<u>Beccion</u>	ALOMACIC		vedecapte	Kerosene
			%	
Outer bark ³	78.62a²	78.01a	69.91b	77.71a
Inner bark⁴	1.23b	1.23b	2.91a	1.21b
Wood ⁵	0.42b	0.67b	2.12a	0.50b
Leaves ⁶	0.21c	0.48b	1.82a	0.44b
Roots ⁷	<u>0.001a</u>	0.005a	0.013a	0.002a
Total	81.94a	82.21a	81.05a	81.05a
Uptake ⁸	2.41b	3.14b	8.96a	2.85b

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

Values within a row followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Outer bark of treated stem section.

⁴Inner bark of treated stem section.

Wood of treated stem section.

⁶Total leaves (terminal buds of maples and oaks were sampled in February).

Band of root bark 2-3 cm wide removed from 3-10 cm below groundline.

downward movement in the phloem is likely hampered by transfer to the xylem and subsequent upward movement. Because movement of solutes in the xylem is generally faster than that in the phloem, the net result would be less downward translocation. Movement in the xylem being the case, it is likely that movement to the leaves simply depended on herbicide penetrating the bark and reaching the wood. How much influence the solvent has on translocation after the herbicide has penetrated the bark is unclear, but could be investigated by applying solution to exposed inner bark or wood.

One possible explanation of the enhanced penetration provided by the vegetable oil is better compatibility with the fatty substance, suberin, with which cork cells in the outer bark are lined, and which makes them impermeable to water. Perhaps the vegetable oil more easily solubilized or mixed with these fats than the petroleum solvents, making the outer bark a more porous structure through which to diffuse. addition, the vegetable oil was determined to be much less volatile than the other solvents, particularly the aliphatic solvent and the kerosene (Figure 1). Triclopyr ester does not evaporate (volatilize) as readily as the solvents as evidenced by the results of an evaporation test (Tables 10a and 10b). These results showed little or no evaporation of triclopyr after six hours following application to bark or the surface of a glass slide. Also, the high average total recoveries exceeding 80 percent in the uptake experiment (Table 9) indicate the same. Therefore, as the solvents evaporated, the triclopyr was concentrated and less medium was present to assist in penetration. In addition, with higher concentrations likely due to evaporation of the solvents, phloem cell damage or death may have been greater which may have reduced phloem translocation. Why the aromatic solvent, which was intermediate in volatility, did not give intermediate levels of uptake would suggest the involvement of other factors. It may be that the compounds in the aromatic solvent do not dissolve or mix well with the fats of the outer

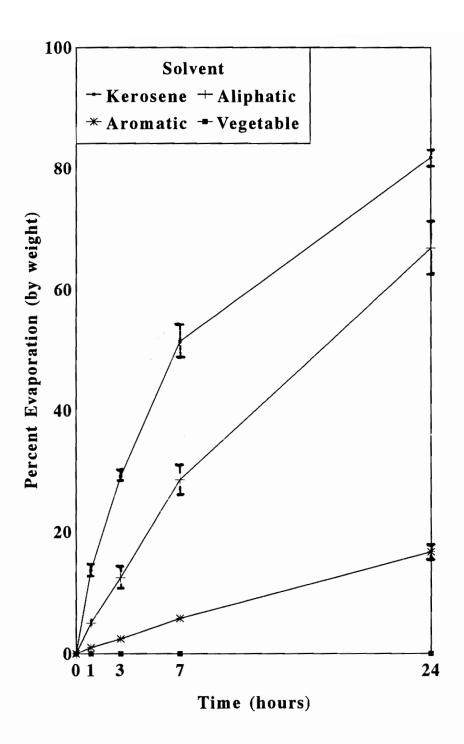


Figure 1. Mean evaporation of formulation solvents from three replicate petri dishes in a fume hood at 26°C. Standard errors about each mean are presented.

Table 10a. Amount of C¹⁴-labeled triclopyr ester formulated in four solvents remaining on a slide or a piece of bark after six hours as determined by washing the slides and oxidizing the bark. ¹

	C ¹⁴ activity	(dpm ~ 1 standard o	deviation)
Solvent	Standard ²	Slide	Bark
Aliphatic Aromatic Kerosene Vegetable	19,801 ~ 380 18,967 ~ 236 20,187 ~ 165 20,555 ~ 3157	19,311 ~ 291 19,437 ~ 782 18,625 ~ 744 20,092 ~ 3258	20,422 ~ 682 20,958 ~ 2040 21,305 ~ 174 23,696 ~ 3163

¹Three replicate ten ul drops of solution containing both C¹⁴-labeled and unlabeled triclopyr were placed on slides or bark surfaces.

²Drops were placed directly into scintillation vials to which scintillation fluid was immediately added.

Table 10b. Weather parameters during six-hour evaporation test.

Parameter	minimum	maximum
Temperature (°C) Relative Humidity Wind speed (mph) Solar radiation (uM/m ² s)	25.1 4.7 (at 27.8°C) 0 394	28.1 19.4 (at 25.1°C) 8.4 1605

Photosynthetically active radiation.

bark. Observations following treatment support this explanation. Aromatic solvent appeared to drip more on the surface of the stems than the aliphatic solvent or kerosene, indicating that penetration of the outer bark was occurring more slowly with the aromatic formulation. If so, though less aromatic solvent evaporates, this may be offset by less penetrability and therefore result in similar uptake as with the aliphatic solvent and kerosene.

Among the species, significantly more uptake occurred in the maples than in the pines and oaks, which took up about equal amounts (Table 11). Activities in the outer bark differed among all three species, with pine showing the most. This may indicate that more actually entered the oaks but was not accounted for, perhaps because it was in the unsampled stemwood above the treated section. Less penetration of the pine outer bark might be explained by its structure. As the pines get older, the bark becomes platy. These plates do not always appear solidly attached, so herbicide applied to a poorly attached plate would soak in but might have difficulty moving from the plate to the underlying bark layers.

The results also indicate that the small activities found in the roots are mostly confined to the maples (Table 11). This would correspond with the generally greater uptake and translocation found in the maples, however, an alternative explanation may involve movement over the surface of the stem rather than in the phloem. Dripping down the stems was a problem largely confined to the maples, presumably due to their smooth, thin outer bark. Sometimes solutions reached the groundline, just a few centimeters from where the root bark samples were removed. Even if movement over the surface stopped short of the sampling area, the decreased distance the herbicide would have to travel in the phloem may have been critical due to the relatively slow rate of movement which occurs in the phloem and the short duration of the experiment. This suggests that application near the groundline is

Table 11. Mean percent of applied activity across solvents and treatment months recovered in red maple (<u>Acer rubrum</u>), white oak (<u>Quercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester.

_		Species	
Tree Section	Maple	Oak	Pine
-		%	
Outer bark ³	64.00c ²	77.01b	85.32a
Inner bark ⁴	2.47a	1.02c	1.43b
Wood⁵	2.70a	0.40b	0.22b
Leaves ⁶	2.95a	0.04c	0.23b
Roots	_0.02a	_0.00b	0.00b
Total	76.17b	79.38b	88.05a
Uptake ⁸	10.15a	1.87b	2.22b

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C¹⁴-triclopyr/ml.

Values within a row followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Outer bark of treated stem section.

⁴Inner bark of treated stem section.

Wood of treated stem section.

⁶Total leaves (terminal buds of maples and oaks were sampled in February).

Band of root bark 2-3 cm wide removed from 3-10 cm below groundline.

⁸Uptake = inner bark + wood + leaves + roots.

important if enough herbicide is to reach dormant buds around the root collar. Worley et al. (1955) found that placement of 2,4,5-T at the root collar was critical for reducing subsequent sprouting of bear oak (Quercus ilicifolia).

Previous studies have also shown a lack of downward movement when other growth regulators, 2,4-D and 2,4,5-T, were applied to the stems of trees (Eliasson 1973, Eliasson and Hallman 1973, Hay 1956, Leonard et al. 1966, Schiue 1958a). It would appear that triclopyr also moves in small quantities to the roots, judging from the results of the present experiment and foliar applications to woody plants (Radosevich and Bayer 1979, Bovey et al. 1979). In fact, Burch (1985) found that triclopyr applied to the leaves of red maple seedlings moved to the roots in much smaller quantities than 2,4-D.

In general, differences in uptake among species may be related to the thickness of the outer bark, the barrier to movement into living inner bark tissues or into the xylem and subsequent translocation to living tissues in the leaves or along the stem. Maple bark was thinner than oak and pine bark (Table 2), and though not measured, the outer bark appeared to make up a smaller portion of the total bark thickness among the maples. It seems reasonable that the thinner the outer bark, the more easily it is crossed. Results from a study by Leonard et al. (1966) also showed greater movement of ¹⁴C-2,4,5-T to the leaves of red maple (36%) than to those of another, thicker-barked species, white ash (10%). In contrast, greater amounts were found in the stem below and in the roots of white ash (2.7%) than red maple (0.2%). Perhaps in species like red maple which have thinner bark, more herbicide is drawn into the transpiration stream, thereby reducing the opportunity for absorption into phloem cells. Alternatively, with such a thin outer bark, the amount or concentration of herbicide contacting the living inner bark cells may be greater, leading to more phloem damage. The relationship between bark thickness and uptake will be explored further in a later

section. Other factors such as the texture (e.g. smooth vs. platy), internal structure, and the chemical composition of the outer bark would seem to be important, also, but were not investigated in this study.

Analysis of variance indicated significant interactions between species and solvents (p=0.005 or less) for activities found in all sections except the roots. Tables 9 and 11 compared main effect means and showed the maple and vegetable oil treatments to result in a significantly lower percentage of activity in the outer bark. Table 12 shows that for activity recovered in the outer bark, the vegetable oil effect was confined to the maples. In the inner bark, wood and leaves, however, while the greater activities found with the vegetable oil treatment were most dramatic among the maples, they also generally occurred among the pines and oaks (Tables 13-16). In addition, the aromatic solvent was generally lowest in recoveries inside the saplings among the pines and oaks and lowest in the leaves and in total uptake in the maples (though not always significantly).

Overall, it appeared that among oaks and pines, uptake was very small (Table 11). This however, may be due to the rather small doses which were applied. Generally, among the oaks and pines, the applied solution only partially covered one side of the stem in the treatment area and did not encircle it. In contrast, this dose was enough to encircle and drip down the maple stems. Because holding dose constant (for a given diameter) among the species seemed desirable, it was necessary to make a compromise and choose a dose which would not lead to too much dripping down maple stems, yet would at least partially cover the oak and pine stem treatment section. If doses had been based on the amount required to wet a certain surface area, as is commonly done in practice, perhaps oak and pine uptake would have been higher. As it was, the small doses may not have provided enough solution to allow much movement through the bark, thus, trapping herbicide in the outer bark. With so little solution in such a big volume of outer bark, herbicide

Table 12. Mean percent of applied activity across treatment months recovered in the outer bark of red maple (<u>Acer rubrum</u>), white oak (<u>Quercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester.

		Formulation solvent				
Species	Aromatic	Aliphatic	Veqetable	Kerosene		
			8			
Maple	$71.07aB^2$	73.41aB	39.80bC	71.80aB		
Oak	79.56aA	75.56aB	76.49aB	76.41aAB		
Pine	84.48aA	84.44aA	88.64aA	83.43aA		

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

²Values within a row followed by the same lowercase letter and values within a column followed by the same uppercase letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Table 13. Mean percent of applied activity across treatment months recovered in the inner bark of red maple (<u>Acer rubrum</u>), white oak (<u>Quercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester.

		Formulation solvent				
Species	Aromatic	Aliphatic	Vegetable	Kerosene		
			%			
Maple	2.02bA ²	1.52bA	5.09aA	1.78bA		
Oak	0.68bB	0.96bA	1.77aB	0.84bB		
Pine	1.17bB	1.25bA	2.27aB	1.15bB		

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

²Values within a row followed by the same lowercase letter and values within a column followed by the same uppercase letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Table 14. Mean percent of applied activity across treatment months recovered in the wood of red maple (<u>Acer rubrum</u>), white oak (<u>Ouercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester.

Species	Formulation solvent				
	Aromatic	Aliphatic	Vegetable	Kerosene	
			8		
Maple	1.30bA ²	1.85bA	8.29aA	1.14bA	
Oak	0.25aB	0.38aB	0.48aB	0.53aAB	
Pine	0.09bB	0.22abB	0.53aB	0.14bB	

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

²Values within a row followed by the same lowercase letter and values within a column followed by the same uppercase letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Table 15. Mean percent of applied activity across treatment months recovered in the leaves of red maple (<u>Acer rubrum</u>), white oak (<u>Quercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester.

Species	Formulation solvent				
	Aromatic	Aliphatic	Vegetable	Kerosene	
			8		
Maple	$1.08bA^2$	1.72bA	8.71aA	2.17bA	
Oak	0.006bB	0.05abC	0.11aB	0.04abB	
Pine	0.07bB	0.29abB	0.45aB	0.18abB	

Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

²Values within a row followed by the same lowercase letter and values within a column followed by the same uppercase letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Table 16. Mean percent of applied activity across treatment months recovered inside red maple (<u>Acer rubrum</u>), white oak (<u>Quercus alba</u>), and Virginia pine (<u>Pinus virginiana</u>) saplings three weeks after stem applications of triclopyr ester².

		Formulatio	n solvent	
Species	_Aromatic	Aliphatic	Veqetable	Kerosene
			8	
Maple	5.32bA ³	6.47bA	26.31aA	6.29bA
Oak	1.20bB	1.79abB	2.89aB	1.77abB
Pine	1.55bB	2.03bB	3.78aB	1.75bB

¹Includes recoveries from inner bark, wood, leaves, and root bark.
²Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C¹⁴-triclopyr/ml.

³Values within a row followed by the same lowercase letter and values within a column followed by the same uppercase letter are not significantly different (Duncan's NMRT, alpha = 0.05).

likely had difficulty diffusing into the inner bark and stem in large quantities during the three week period. Given more solution or a longer period of time, uptake may have been enhanced and perhaps approached that among maples.

Timing of application

The analyses indicated significant treatment month effects (p<0.0002) except for total uptake (p<0.11). Therefore, it appeared that uptake was not affected by timing, for total recovered activity inside the saplings was approximately equal across seasons (Table 17). This is despite the lack of translocation to the buds and leaves in February. Instead, activity generally appeared in greater amounts in the inner bark and wood of the treated section. If herbicide penetration of the outer bark were occurring by diffusion alone, results would likely have shown enhanced uptake in May and July due to translocation away from the treated section continually maintaining a higher gradient. It may have been, though, that differences in the gradient due to translocation were not enough to noticeably affect diffusion. These results also indicate that in the spring, herbicide located in the wood and inner bark following winter application would likely move up the xylem and in the phloem as if treated in spring. However, it is possible that the herbicide present in the phloem of the treated section for several months may damage living tissues more over this period than if the application had taken place in the spring. this is so, translocation in the phloem, if it does occur in appreciable amounts, might be hampered more with winter treatments.

While little herbicide was recovered in the root bark (Table 17), 11 of the 14 saplings which showed activity in the root bark were from the July application and none were from May. In addition, no saplings were observed to be sprouting from the root collar or stem below the

Table 17. Mean percent of applied activity across species and solvents recovered in saplings following stem applications of triclopyr ester in February, May, and July.

		Treatment month	
Tree Section	February	May	July
		%	
Outer bark ³	70.57b ²	83.01a	75.08b
Inner bark⁴	1.97a	2.24a	1.15b
Wood ⁵	1.51a	0.50b	0.73b
Leaves ⁶	0.001b	1.09a	1.08a
Roots ⁷	<u>0.003a</u>	<u>0.00a</u>	<u>0.01a</u>
Total	75.81b	88.51a	80.46b
Jptake ⁸	3.89ab	4.83a	3.76b

 $^{^1}$ Solution was applied at a dose of 150 ul/cm of stem diameter and contained primarily unlabeled triclopyr (1 lb a.e./gal) along with 0.8-0.9 uCi of C^{14} -triclopyr/ml.

Values within a row followed by the same letter are not significantly different (Duncan's NMRT, alpha = 0.05).

Outer bark of treated stem section.

Inner bark of treated stem section.

Wood of treated stem section.

⁶Total leaves (terminal buds of maples and oaks were sampled in February).

Band of root bark 2-3 cm wide removed from 3-10 cm below groundline.

treated stem section at the time of the July harvest; however, 85 percent of the maples and 42 percent of the oaks were sprouting at the time of the May harvest. Some sprouts were even emanating from the stem directly below the treatment area. Both the questionable greater activities recovered in root bark from July treatments and sprouting following May treatments would support the hypothesis that greater downward translocation of herbicide occurs in the summer than in the spring.

All interactions between species and treatment month were highly significant (p<0.006) as were interactions between solvent and treatment month among the leaves (p<0.0001) and to a lesser degree the inner bark (p=0.04). These interactions seem largely attributable to the virtual absence of translocation to the buds or leaves in February, making the species and solvent effects dependent on the month treated. It did not appear that interactions were such that subclass effects were not visible in the main effects due to cancellation, however, so comparisons between the main effect means were presented.

Comparison of Efficacy and Uptake and Translocation

It seems logical that uptake is related to efficacy and that better uptake of a certain formulation or in a certain species would suggest better efficacy with that formulation or against that species, although Sundarum's (1965) results suggested that a species' susceptibility was related not so much to total uptake as to the amount absorbed into the phloem. The uptake study would suggest that the vegetable oil formulations would provide the best control and the others would give about equal control and considerably less than the vegetable oil formulations. However, actual results showed the aliphatic and the kerosene formulations to give control equal to the vegetable oil formulation and the aromatic to give considerably poorer control (Table

8). One explanation for this discrepancy may be that since control was so high among the aliphatic, vegetable, and kerosene formulations at the concentrations and doses used, it was not possible to separate the performances of the solvents, and at lower concentrations and/or doses, such as the dose used in the uptake study, better performance by the vegetable oil formulation might occur. An alternative explanation may involve the matter of differences in solvent volatility. If evaporation was an important factor in the uptake study, it likely would have hampered the performance of the aliphatic solvent and the kerosene because they appeared to evaporate fastest. Results from the test of relative solvent evaporation rates support this observation (Figure 1). Loss of carrier through evaporation may in part account for the reduced uptake observed relative to the vegetable oil. Evaporation was likely more pronounced in the uptake study, where saplings were laid horizontally, exposing the treated stems to direct sunlight. efficacy study was carried out in the field with trees standing vertical and treated stems usually shaded by a canopy of leaves. Also, vegetation often surrounded the trees which likely reduced air flow that would otherwise aid in evaporation. The aromatic solvent did not appear to evaporate nearly as quickly as the aliphatic and the kerosene, however, uptake using the aromatic solvent was the same or slightly less than with the aliphatic and kerosene (Table 9). It seems plausible that if the uptake study's applications had occurred under conditions similar to those under which the efficacy study was performed and at similar doses, the kerosene and aliphatic would have evaporated less, or evaporation would have been less critical at the higher doses, and uptake would have surpassed the aromatic, thus, correlating more closely with the efficacy results.

Comparisons between species gave similar results in both the uptake and the efficacy studies. Maple had the greatest uptake and the greatest control. Pines had the most herbicide tied up in the outer

bark and the poorest control. In addition, pines and oaks in the uptake study were approximately equal in stem diameter, while in the efficacy study the pines were larger. This may explain similar uptake results while efficacy results showed pines more difficult to control (Table 3).

Efficacy and Concentration, Dose, and Stem diameter

One objective of the efficacy study was to model the relationship, if any, between concentration, dose and efficacy for each solvent/species combination. Another was to examine the relationship between stem diameter and efficacy. The results from Tables 4 and 6 indicate that the only strong relationships with concentration and dose and efficacy are for the aromatic formulations when applied to Virginia pine and white oak. It seems likely that better relationships would exist for red maple and for pine and oak with the other carrier solvents; however, concentration and dose levels used in this study are in the region of that relationship where control has plateaued near 100 percent. Among the maples, the application often resembled a conventional treatment with solution puddling at the base of the trees. Conventional treatments generally used 4-5 percent herbicide solutions, though, where solutions in this study ranged from 6.25 to 25 percent. Therefore, it is not surprising that control among maples was high regardless of dose or concentration. The oak and pine applications more closely resembled low volume banding, since faster penetration and, therefore, less dripping and less coverage occurred. Banding applications generally use concentrations higher than 6.25 percent, so the reduced control at the low concentration and low dose seem reasonable (Tables 4 and 6).

Other studies have typically used smaller doses and achieved a greater range in crown reduction or control using a fuel oil carrier. Gardiner and Yieser (1990) applied 20% solutions of triclopyr to red

maple, white oak, blackgum, hickory, red oak, and sweetgum. Doses of 1 to 4 ml/2.5 cm were applied to 2.5 cm stems, achieving 76-84% crown reduction. Doses of 0.5-2 ml/2.5 cm were applied to 5 cm stems, giving 48-73% crown reduction. Doses of 0.33-1.33 ml/2.5 cm were applied to 7.5 cm stems, giving 22-49% crown reduction. In one study, Yieser et al. (1989) applied 2 ml of solutions ranging in concentration from 5 to 20%, and achieved 38-84% crown reduction of red maple, white oak, hickory, red oak, and sweetgum. In another study, Yieser and Boyd (1989) applied 2 ml of solution ranging in concentration from 5-40% and achieved 17-70% crown reduction of white oak, hickory, and red oak. Because doses in the last two studies were not based on diameter, doses per stem diameter were on average likely at the low end or below doses used in the present study. These lower doses along with applications which were likely less precise than those in the present study, may explain the lower levels of crown reduction achieved in these other studies. In addition, red maple and white oak were never the most difficult to control in the above studies. Therefore, the presence of other more difficult to control species may also explain the lower crown reduction as compared to this study.

The bear oaks in Worley et al.'s (1955) experiment seem to have required an unusually high dose of approximately 250 ml to achieve 100% stem kill when compared to the present study and others. However, the dose was per clump of sprouts and the herbicide concentrations were low (1.5 and 3%). In addition, according to the authors, bear oaks sprout vigorously and one of the hypotheses of their experiment was that high doses would be required for enough herbicide to reach the root collar and prevent dormant buds there from sprouting.

In a study similar to the present one, Kuhns and Lyman (1989) applied 20% triclopyr with syringes and achieved mortality of 80% and 100% with doses of 1 and 2 ml/2.5 cm, respectively, which are results similar to those of the present study. When the dose was cut to 0.5 ml,

mortality dropped to 37%. Comparing results from the present study across species, the treatments with 25% triclopyr (1 lb/gal) at doses of 1 ml and 3 ml/2.5 cm averaged above 90% and close to 100% mortality, respectively. It would appear that a dose of 1 ml/2.5 cm is on average the level below which crown reduction and mortality begin to drop off; however, the dose necessary to achieve a certain level of control is almost certainly species specific. Observations and results would suggest that the thinner-barked maples would require a much smaller dose than the pines and oaks. As mentioned earlier, doses used in the present study were too high to determine what these relationships are for each species, except in two instances. The poor performance of the aromatic formulations on the oaks and pines did allow an examination of concentration and dose and, in addition, an investigation of whether or not efficacy was related to diameter even when the dose was increased linearly with the diameter.

Indeed, with concentration and dose in regression models, diameter still explained a significant amount of variation in crown volume control (Table 18). Among pines, the amount of variation explained by diameter was much greater than that explained by concentration or dose, judging by the partial F values. Among oaks, all three variables are of approximately equal importance in the model. Additionally, at the low dose, where control was not complete with the aliphatic, kerosene, and vegetable oil formulations, control also appeared negatively related to diameter (data not presented). The significance of the negative relationship between control and diameter, despite basing the dose on diameter, suggests that if dose were held constant, percent control might actually decrease with the square of the diameter. If so, this would indicate that tree volume, which increases with the square of the diameter, may be a better basis for determining dose. logical because the dose becomes diluted throughout the mass or volume of the tree. This approach would be equivalent to the manner in which

Table 18. Regression models of percent crown volume control of white oaks and Virginia pines when treated with triclopyr ester in aromatic solvent.

Species	Statistic	Intercept	Conc ²	Dose ³	Diam ⁴	R-square
Oak	Coefficient Partial F P-value	36.7 20 <0.0001	52.9 48 <0.0001	8.2 31 <0.0001	-8.8 46 <0.0001	0.39
Pine	Coefficient Partial F P-value	73.8 114 <0.0001	8.3 2 0.15	4.2 14 0.0002	-9.6 120 <0.0001	0.40

¹General form of model: % CVC = b_0 + b_1 (conc) + b_2 (dose) + b_3 (diam).

²Concentration (lb a.e./gal).

³Dose (m1/2.5 cm).

⁴Diameter (cm).

doses are determined for animals, namely on a volume/volume or weight/weight basis. Likewise, outer bark volume also likely increases faster than stem diameter. If this is so and uptake and efficacy are linearly related to bark volume, then this would support the significance of stem diameter in the models. Therefore, basing dose on volume or the square of the diameter would appear to be a more proper approach.

Uptake and Bark thickness

Because trees of each species used in the study did not cover the same ranges of bark thickness (Figures 2-5) and because slopes and intercepts of the relationship between the natural log of percent uptake and bark thickness were sometimes different among species when compared using dummy variable regression (as is evident from coefficients in Table 19), results were presented within as well as across species. In general, even within species and solvents, there was substantial variation which was unexplained by a linear relationship with bark thickness, leading to low R2 values. The imprecision inherent in some of the techniques used in this experiment along with the narrow range of bark thicknesses among the maples and to a lesser degree the oaks and pines probably contributed to the poor relationships observed. Results either indicated no clear pattern or one of large variation in percent uptake with thinner bark and less variation and less uptake with thicker bark (Figures 2-5). Most trends indicated a negative relationship. maples typically exhibited the former pattern, that is, a scatter of data points. The pines and oaks typically exhibited the latter pattern and, thus, a natural log transformation on percent uptake was used to reduce the heterogeneity of variance. Using an alpha level of 0.05, none of the maple models was significant (Table 19). Among the oaks and pines, only the aromatic formulation gave significant models.

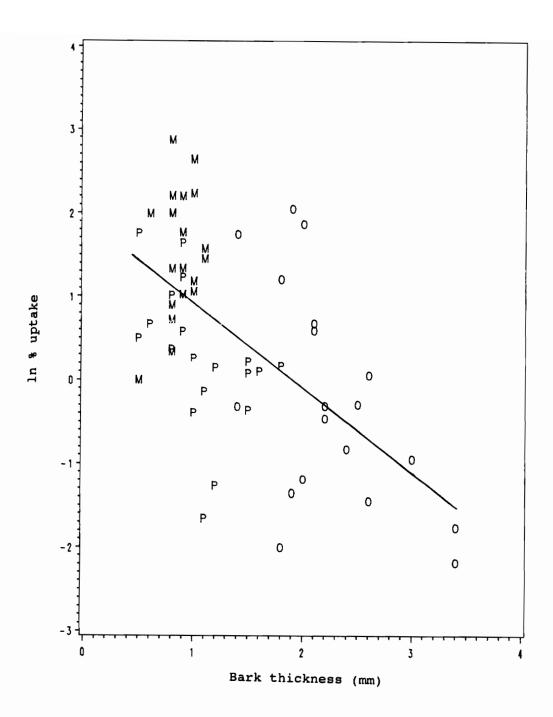


Figure 2. Percent uptake of triclopyr, formulated in aromatic solvent (1 lb a.e./gal), versus bark thickness. M, O, and P refer to red maple, white oak, and Virginia pine, respectively. Regression line fitted across species. Model: ln % uptake = $b_0 + b_1$ (bark thickness). R^2 =0.33. P<0.0001.

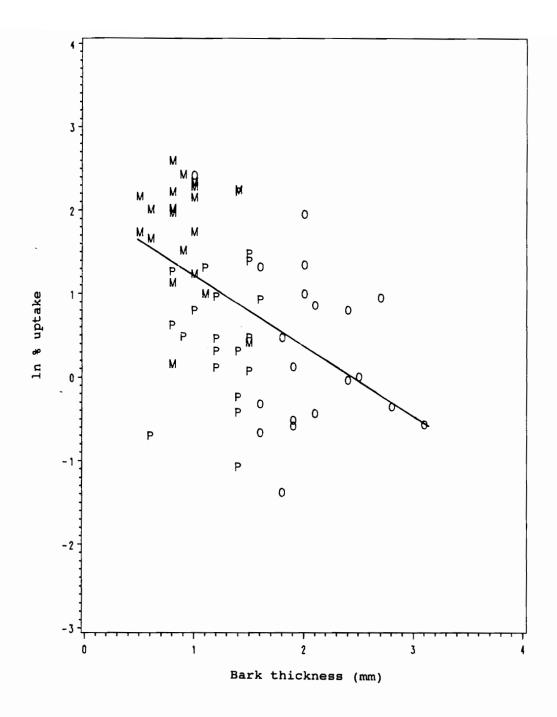


Figure 3. Percent uptake of triclopyr, formulated in aliphatic solvent (1 lb a.e./gal), versus bark thickness. M, O, and P refer to red maple, white oak, and Virginia pine, respectively. Regression line fitted across species. Model: ln % uptake = $b_0 + b_1$ (bark thickness). R^2 =0.24. P<0.0001.

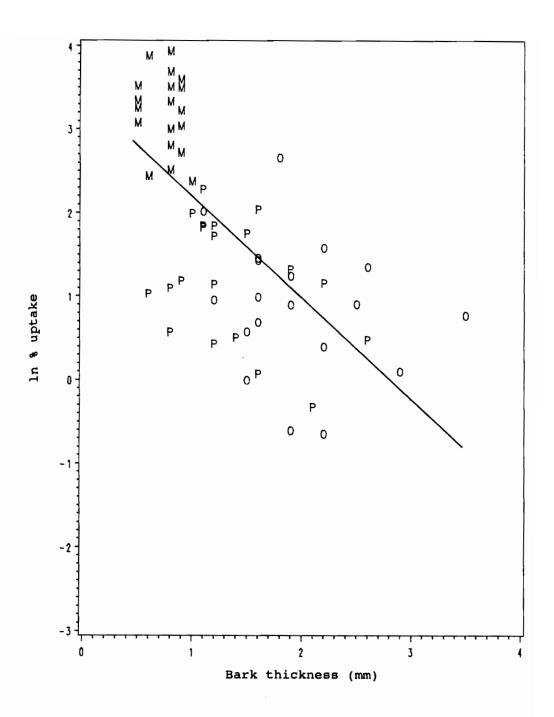


Figure 4. Percent uptake of triclopyr, formulated in vegetable oil (1 lb a.e./gal), versus bark thickness. M, O, and P refer to red maple, white oak, and Virginia pine, respectively. Regression line fitted across species. Model: ln % uptake = b₀ + b₁(bark thickness). R²=0.45. P<0.0001.

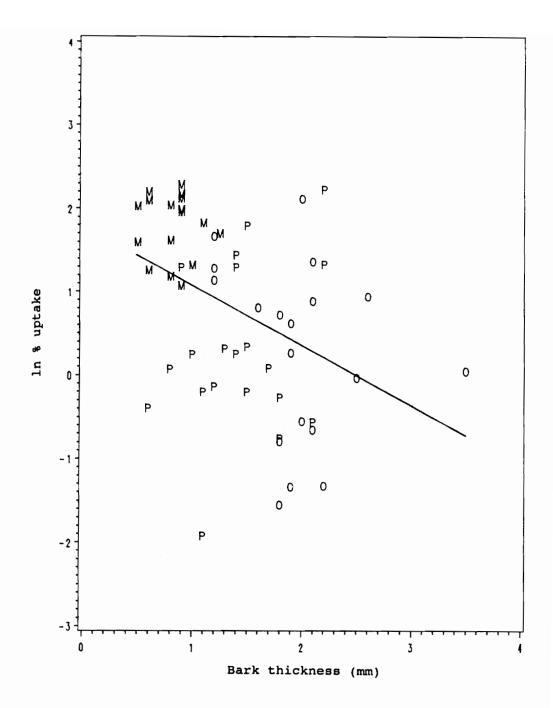


Figure 5. Percent uptake of triclopyr, formulated in kerosene (1 lb a.e./gal), versus bark thickness. M, O, and P refer to red maple, white oak, and Virginia pine, respectively. Regression lines fitted across species. Model: ln % uptake = $b_0 + b_1$ (bark thickness). R^2 =0.17. P=0.002.

Table 19. Regression models of the relationship between the natural log of percent uptake and bark thickness (mm).

	Formulation	Coefficients			
Species	Solvent	Intercept	Slope	<u>P-value</u>	<u>R-square</u>
					0.00
Maple	Aromatic	0.31	1.41	0.24	0.08
	Aliphatic	2.31	-0.61	0.31	0.06
	Vegetable	3.52	-0.42	0.51	0.02
	Kerosene	1.91	-0.13	0.79	0.005
Oak	Aromatic	2.61	-1.27	0.01	0.30
	Aliphatic	1.51	-0.57	0.23	0.08
	Vegetable	1.50	-0.31	0.36	0.05
	Kerosene	1.28	-0.49	0.31	0.06
	Relobene	1.20	0.15	0.01	0.00
Pine	Aromatic	1.39	-1.04	0.05	0.21
	Aliphatic	0.18	0.30	0.65	0.01
	Vegetable	1.86	-0.48	0.14	0.12
	Kerosene	-0.51	0.59	0.26	0.07
All	Aromatic	1.94	-1.02	<0.0001	0.33
	Aliphatic	2.04	-0.83	<0.0001	0.24
	Vegetable	3.42	-1.22	<0.0001	0.45
	Kerosene	1.81	-0.73	0.002	0.17
	VETOBELIE	1.01	-0.75	0.002	0.17

¹General form of model: $ln % uptake = b_0 + b_1(bark thickness).$

models were combined across species, R-squares and model significances increased. The coefficients of the regression lines for the aromatic, aliphatic, and kerosene treatments indicate that, while the intercepts are similar, uptake with the aromatic solvent decreases most rapidly as bark thickness increases. This result provides further evidence that the aromatic solvent has greater difficulty penetrating bark.

Yeiser and Boyd (1989) found significant correlations between mortality and bark thickness (R=-0.25) and groundline diameter (R=-0.31) following streamline applications to oaks and hickories; however, the correlation coefficients were not very large. They were negative, though, which concurs with the above results. Gardiner and Yeiser (1990) found that among 5 of 6 species treated, average percent crown reduction by species correlated negatively with the average bark thickness by species. Similar results were found in the present uptake study, with greater uptake into the thinner-barked maples than into the thicker barked oaks and pines (Table 11). Also, in the efficacy study average crown volume control was greatest among the maples (Table 3).

It seems likely that the movement of carrier solvents and herbicide through the outer bark occurs, in part, by capillary action because there are no living cells in the outer bark to perform active transport and no liquid medium through which herbicide molecules could diffuse. Whether the herbicide and carrier infiltrate the bark at the same rate would depend on the relative values of the adhesive forces between them and the constituents of the outer bark. If the carrier has greater adhesive attraction and moves faster, then diffusion down the concentration gradient thus created may also be involved in herbicide movement across the bark. Diffusion likely cannot occur until the carrier solvent has infiltrated the bark and, thus, provided a liquid medium. If diffusion does play a role, then a version of Fick's second law (Nobel 1974), below, may help explain the relationship between bark thickness (distance travelled, x) and uptake.

$\ln C = \ln [M/2(\pi Dt)^{1/4}] - [(1/4Dt)*x^2]$

The equation shows the relation between distance, x, and the concentration, C, of a solute at x at a given time, t, when a given amount of solute per unit area, M, is placed at x=0 (the surface of the bark in the present discussion). D is the diffusion coefficient which is specific to the solute in a particular medium. Concentration was not measured in this experiment but rather total amount of herbicide which penetrated the outer bark, so the equation does not apply exactly. However, it suggests that the natural log of uptake might be negatively related to the square of bark thickness. The actual data was too variable to determine if the relationship found was actually curvilinear as Fick's law suggests. If capillary action is important, then the law would not strictly hold for herbicide penetration through bark. addition, the equation assumes no obstructions in the medium as would be present in the outer bark (e.g. cell walls). Binding of herbicide to these obstructions might hamper diffusion. What the relative contributions of diffusion and capillary action are, and how the properties of the carrier solvent affect these two processes is unclear to this author, except that diffusion is slower as the viscosity of the medium increases. Uptake, however, was not less when triclopyr was formulated in the seemingly more viscous vegetable oil. The rate of diffusion would likely also be dependent on the ability of the solvent to permeate the outer bark, providing a continuous medium through which diffusion could occur. If this is the case, then possibly greater infiltration of the outer bark by the vegetable oil explains greater triclopyr uptake with that formulation. Capillary movement of the herbicide would likely be independent of movement of the carrier. significant carrier solvent effects would suggest that diffusion in the solvent may be an important mechanism for herbicide penetration.

CONCLUSIONS

Results from the uptake and translocation study indicated that the vegetable oil is a superior carrier solvent to the others tested; however, the experiment was performed under conditions which appeared to promote a relatively high degree of evaporation and using relatively small doses as compared with those used in the efficacy study. ranking of the solvents by rate of evaporation, from fastest to slowest, was kerosene > aliphatic > aromatic > vegetable. In commercial applications, if conditions are such that less evaporation of the carrier occurs or evaporation does not have a large influence because larger doses are used, perhaps uptake would not be so much greater with the vegetable oil formulation. Results from the efficacy study would support this conjecture, for crown volume control was about equal when triclopyr was formulated in kerosene, aliphatic solvent, or vegetable oil. The aromatic formulations provided relatively poor crown control, indicating that evaporation probably does not explain their lesser uptake than the vegetable oil formulation. Therefore, the vegetable oil and the aliphatic solvent would appear to be plausible candidates to use in place of fuel oils like kerosene. The vegetable oil has the advantage of not being a petroleum product. Efficacy results suggest that 0.25 lb a.e./gal formulations with all but the aromatic solvent give excellent control, decreasing only slightly at the lowest dose used in the experiment. Results also indicate that dose should be dependent on species as well as stem diameter or tree volume. In particular, it would appear that thin-barked species would require smaller doses than thicker-barked.

Uptake appeared to decrease with bark thickness; however, techniques used in this experiment were not precise enough to find a well fitting relationship. Similarly, when treatments did not produce complete crown volume control, stem diameter was a significant

determinant of percent crown volume control, despite basing doses on diameter. Therefore, basing doses on stem volumes would likely be a better approach. Basing doses on volumes would also be comparable to the manner in which they are determined, by weight, when giving drugs to animals.

Data showing possible translocation to roots along with data on sprouting would suggest that triclopyr movement to the roots causing reduction in sprouting was greater following summer application than spring application.

APPENDIX 1

All percentages of herbicide in solution refer to percent of commercial formulation as follows:

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Dicamba (Banvel 520)

Fluroxypyr (1.67 lbs a.e./gallon)

Glyphosate (3 lbs a.e./gallon)

Triclopyr ester (4 lbs a.e./gallon)

Triclopyr (2 lbs a.e./gallon)

+ Picloram (1 lb a.e./gallon)

2,4-D ester (4 lbs a.e./gallon)

2,4,5-T ester (4 lbs a.e./gallon)
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VITA

William G. Schneider was born in Washington, D.C. on April 9, 1965. He attended public schools in Kensington, MD. In May 1987 he graduated from the University of MD with a Bachelor of Science degree in Biology. He entered graduate school at Virginia Tech in August 1989 and received a Master of Science degree in Forestry in December 1991.

William V. Schneider