GEORGIA FOREST RESEARCH PAPER

82

Nov., 1990

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GEORGIA FORESTRY COMMISSION

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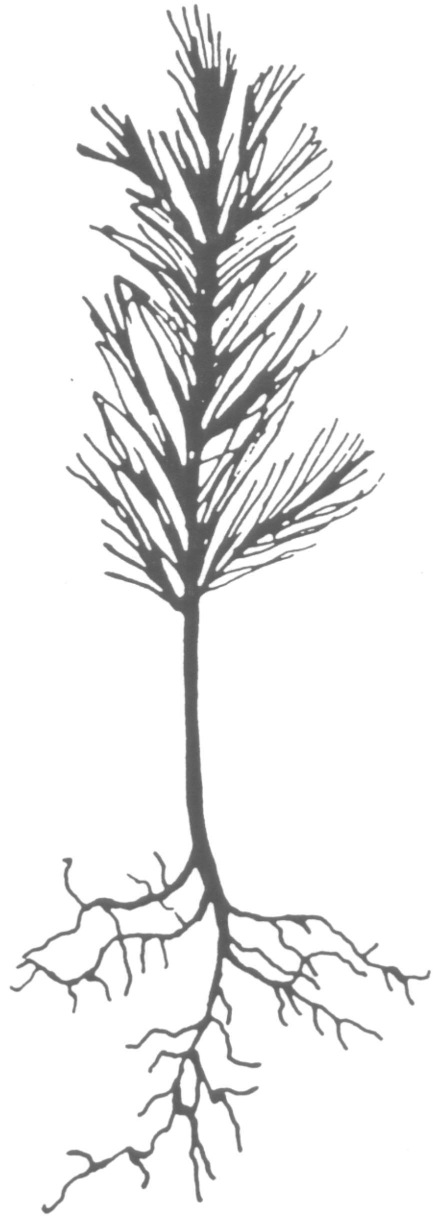

ABSTRACT

Pine seedlings planted on highly erodible agricultural land survived poorly on many sites in the 1986-1988 growing seasons. Georgia Forestry Commission personnel identified several fields in which high seedling mortality had no obvious cause. Soil samples collected around living and dead seedlings in such fields in 1988 did not differ in nutrient content or pH. Nor were herbicide residues or nematode populations detected which might have accounted for high seedling mortality.

Four dinitroaniline herbicides commonly used on agricultural crops were applied at various concentrations to pine seedlings grown in a pot study. They had no measurable effects on the height, groundline diameter, or dry weight production of loblolly pine seedlings.

Low soil moisture combined with other unfavorable factors which varied from one site to another probably caused low survival. White-fringed beetles, improper seedling handling, seedling quality, and inadequate planting might not have resulted in high mortality in a normal year, but proved fatal under dry conditions.

INTRODUCTION

More than 500,000 acres of marginal farmland have been planted to pine in Georgia under the Conservation Reserve Program (CRP) since it was funded in 1986. Commonly soybeans, corn, and cotton were the last agricultural crop before erodible land was converted into slash *(Pinus elliottii)* or loblolly pine *(P. taeda)* plantations. Tree seedlings planted in some of these fields experienced heavy mortality for no readily apparent reason. At times mortality also occurred in strange patterns, such as nearly all seedlings dead in the field but normal survival in surrounding grassy borders or old fence rows. Herbicide residues, high nematode populations, poor seedling handling and planting techniques, fungi, and drought all were suspected as causes. But nobody was certain. Therefore this study was undertaken to examine one of these factors, namely herbicide residues, in more detail.

One common pattern of seedling development right after planting was apparently normal growth up until early summer, then death. This might have corresponded with new roots developing in the topsoil early in the growing season, then reaching the subsoil into which herbicide residues might have leached during the previous fall and winter.

Few herbicides remain active in the soil for more than two months. However, some members of one class of chemicals, the dinitroanilines, persist longer than most and might have caused carryover injury to pine seedlings. Four members of this class of herbicides, commonly used in cotton, peanut, corn, and soybean culture in Georgia are trademarked as Balan, Prowl, Surflan, and Treflan (Brown, 1987; French, 1987; Johnson, 1987). They usually are applied to the soil prior to weed seed germination. Some are strongly absorbed by organic matter and clay (Weed Sci. Soc., 1983). Unless applied at unusually high rates, they are thought to be degraded to non-injurious levels by microorganisms within six months (Murphy and Bullock, 1986). Because of their reported persistence and their use of crops commonly preceding pine seedlings on CRP lands, this study concentrated on these four herbicides.

METHODS

The study was carried out in two phases. In the first, samples were collected in CRP plantings experiencing high seedling mortality. The second was a pot study to evaluate the effects known concentrations of the four dinitroaniline herbicides on loblolly pine seedling.

Field Phase: About ten problem sites were identified by Georgia Forestry Commission personnel in the summer of 1988. Close examination revealed that larvae of the white fringed beetle *(Graphognathus leucoloma* Boh.) had chewed on the roots at some sites. At others, seedlings were poorly planted, often at an angle, which left the roots too shallow and they dried out in droughty conditions. On about half of the sites the cause of seedling mortality was not obvious. There soil samples were collected from the vicinity of dead seedlings and, when present, around living ones also. Soils were sampled from at least three spots near dead trees, mixed thoroughly in a bucket, and subsampled. The same procedure was followed near living trees. Both the topsoil and subsoil were sampled at each spot, kept cool in an air-conditioned vehicle to prevent nematode mortality, and transported to the laboratory. There the samples were subdivided for immediate nutrient and nematode assays and later herbicide analysis. Samples for herbicide assay were stored at -14° C until residue analysis in December, 1988.

Soils were analyzed for extractable (0.05 N HC1 + 0.025 N H_2SO_4) K, Ca, Mg, Al, Fe, Mn, and Zn by atomic absorption spectrophotometry (Anon. 1971), for available P by colorimetry (Anon. 1975), and for pH (Peech, 1965). Nematode populations were counted with the method reported by Jenkins (1964) and the species identified in the Extension Nematology Laboratory of the University of Georgia.

Soil samples were screened for 26 herbicides (Table 1) by Capillary Gas Liquid Chromatography. Soils were well mixed and a 30 g subsample was soxhlet extracted with acetone for 12 hours. Major interferences were removed by Gel Permeation Chromatography. Extracts were analyzed with a Tracor model 565 gas chromatograph

Table 1. Herbicides for which the soil samples were screened.

Trade Name	Detection Limit[1]	Trade Name	Detection Limit
Atrazine	.007 ppm	Balan	.030 ppm
Bravo	.030	Bromacil	.013
Devrinol	.013	Dimethoate	.034
Dual	.030	Eptam	.027
Goal	.030	Hexazinone	.010
Lasso	.030	Ordram	.045
Oxadiazon	.016	Paarlan	.065
Propachlor	.013	Propazine	.030
Prowl	.030	Roneet	.062
Sencor	.020	Simazine	.020
Sutan	.020	Terbacil	.013
Tillam	.051	Tolban	.065
Treflan	.030	Vernam	.009

[1]Detection Limit calculated by method of Hubaux and Vos (1970) for a 30 g sample weight and total volume of 1.0 ml.

Table 2. Location and planting history of some CRP fields with high seedling mortality in the summer of 1988.

Site#	Georgia County	Planting History
1.	Pulaski	Peanut crop in 1987; about 90% of loblolly seedling planted in winter 1986 died. Replanted in winter of 1987, by July, 1988 about 50% of seedlings dying again.
2.	Dodge	Cotton in 1986; 1987 CRP planting failed completely. Replanted with loblolly in early 1988, failed again by July, 1988.
3.	Dodge	Adjacent to site #2, same history, except that preceding crop was soybeans.
4.	Treutlen	Slash pine planted in early 1988, poor planting job, hardpan at variable depth, bermuda grass competition.
5.	Worth	Peanuts in 1987; loblolly planted in Dec., 1987. 85% mortality in Sept. 1988, many seedlings grew in spring, died in summer.
6.	Laurens	Slash pine planted in early 1987, 50% mortality, replanted in 1988 among the survivors of 1987. Current year's mortality in different areas from last year's, therefore unlikely that residual herbicides are cause of death. This field represents several others in which herbicides were ruled out as major cause for mortality on inspection.

equipped with an NP Detector and dual capillary columns, consisting of Supelco SP-5 and SPB-35.

Pot Study: This phase simulated field conditions where seedling roots might grow first in topsoil from which herbicides had been leached and later in the growing season reach subsoil where residues might have been retained. To achieve this, uniform 1-0 loblolly pine (*Pinus taeda* L.) seedlings were planted in 22 liter plastic tubs filled with sandy loam. Five seedlings were planted in each of 100 tubs in April and placed outdoors on a gravel pad. They were watered as needed with rotary sprinklers which were moved periodically to avoid irrigation patterns. These seedlings were allowed to grow until early June, when their roots began to reach the bottom of the containers.

In the first week of June, the bottom was cut out of the tubs and they were placed on top of other tubs containing a 20 cm deep layer of soil. The surface of the soil in the lower containers had been sprayed with known concentrations of herbicides. The upper and lower tubs were sealed together with duct tape and the roots allowed to grow into the soil containing four dinitroaniline herbicides.

Six concentrations of each herbicide were applied, starting with the basic rate recommended for field application, i.e. 1.5 pints per acre for both Prowl and Treflan; 2 quarts/acre of Surflan; and 3 quarts/acre of Balan. The other treatments were dilutions of 1/10, 1/100, 1/1,000, 1/10,000 and 1/100,000 of these basic rates. There were 24 tubs (4 herbicides each at 6 concentrations) plus one control, or 25 tubs in each block.

The statistical design of the pot study was a randomized complete block with four replications, each consisting of 25 treatments as described above, for a total of 100 pots containing five seedlings each.

The pot study was dismantled in the first week of October. One block was sacrificed in developing sampling methods. The upper and lower tubs were separated by removing the duct tape seal and slicing the soil and roots in the lower tub off with a knife. Then the roots of the seedlings in each tub were washed free with a gentle stream of tapwater. The shoots, roots in the upper tub, and roots in the lower tub of the five seedlings in each tub were then cut apart and placed in paper bags and dried at 70° C. to constant weight. Dry weights were recorded and all data subjected to analysis of variance.

RESULTS AND DISCUSSION

Field Phase: Georgia Forestry Commission personnel determined CRP planting survival during the summer of the first growing season. They reported several fields with unusually high mortality. The location and planting history of a sample of such fields are listed in Table 2.

In order to screen whether soil nutrient deficiencies existed, the topsoil and subsoil were sampled near living and dead pine seedlings and analyzed for their nutrient content. The soil nutrient levels considered adequate for growing Christmas trees in the Georgia Coastal Plain are also listed for comparison (Table 3).

One would hardly suspect that agricultural fields, even marginal ones such as these, would be so infertile as to cause pine seedling mortality. Even on infertile forest lands, nutrients previously absorbed in the nursery and those present on the site are nearly always adequate to support pine seedling growth during the first growing season. There are some instances of less than recommended soil potassium (sites #2 and 3) and phosphorus levels (sites #2, 3, and 5) and one instance (site #2) where the Ca:Mg ratio is greater than ten, considered undesirable. But there are no obvious differences between soil nutrient levels around living and dead seedlings so it is unlikely that lack of nutrients led to seedling mortality.

A range of soil pH from 5.5 to 6.0 is recommended for best pine growth (Plank, 1985), although conifers are commonly grown in the range between pH 4.5 and 6.5 (Pritchett, 1979). Soil acidity at the five sites ranged from pH 4.6 to 6.3. Like fertility, soil acidity is an unlikely factor in the seedling mortality in these fields.

Nematode populations were assayed in the Nematology Laboratory of the Cooperative Extension Service at the University of Georgia. Soil samples were screened for fourteen kinds: root-knot, sting, lance, reniform, lesion, stubby-root, ring, stunt, spiral, dagger, sheath, cyst females, cyst larvae, and pine cystoid nematodes. Of these, lance and pine cystoid are the most detrimental to pine roots; sting, stubby-root, and stunt affect pines moderately; and spiral, dagger and sheath must be present in very high numbers to influence pine growth (Personal communication, Dr. J. L. Ruehle, U.S.D.A. Forest Service, Athens, GA). None of the most injurious nematodes were found at any of the five sites. Only low numbers (4 to 20 individuals per 100 cc of soil) of the spiral and dagger nematodes, which are least injurious to pine, were found at sites #2, 3, and 4. Nematode populations generally peak in late spring and early summer. These soils were sampled in July through September, 1988. It is unlikely that high nematode populations at these sites were missed due to sampling season. These results agree with those from nematode assays for 42 farms conducted in 1988 from which the investigators concluded that "the causes for planting failures are complex and factors besides nematodes must be considered" (Sharma et al., 1989).

All soil samples collected at these fields were screened for the 26 herbicides listed in Table 2. Only three samples contained residues. Topsoil around living trees in Pulaski County contained .02 and .05 ppm, and the subsoil under it .03 and .18 ppm of atrazine and hexazinone, respectively. Topsoil in Dodge County contained .26 ppm of Goal. Atrazine and hexazinone were probably applied to control herbaceous competition in the pine plantings. None of them should have injured the pine seedlings.

Pot Study: The field and pot studies were carried out simultaneously. The latter was designed to test the hypothesis that herbicides applied to preceding agricultural crops had leached out of the topsoil and become absorbed by the subsoil where they might be injurious to seedling roots. To simulate this situation, seedlings were allowed to grow in tubs filled with herbicide free soil from April to June. When their roots began to reach the base of the plastic tubs, the bottoms were cut off and the tubs placed on soil treated with a series of dilutions of four dinitroaniline herbicides as outlined under Methods. The seedlings continued to grow well, however, and exhibited no signs of herbicide injury. Needle color remained normal, no deformities occurred, and shoots continued to

Table 3. Soil nutrient levels at sites with high pine seedlig mortality.

Site number	Soil horizon	Seedling status	K	Ca	Mg	P	pH
			---------- lbs/acre	----------	----------	----------	
1	topsoil	living	90	528	50	132	6.2
1	topsoil	dead	96	600	54	172	6.3
1	subsoil	living	50	296	38	40	5.4
1	subsoil	dead	56	320	42	38	5.6
2	topsoil	living	23	390	32	9	4.9
2	topsoil	dead	28	736	56	22	5.8
2	subsoil	living	23	177	21	1	4.6
2	subsoil	dead	10	224	18	2	6.1
3	topsoil	dead[1/]	36	308	31	12	5.3
3	subsoil	dead	32	118	19	2	4.7
4	topsoil	dead[1/]	161	864	122	48	5.7
4	subsoil	dead	173	560	124	4	5.1
5	topsoil	living	60	332	68	10	5.6
5	topsoil	dead	94	602	93	15	5.0
5	subsoil	living	103	917	140	9	5.3
5	subsoil	dead	116	724	124	30	4.6
Recommended levels [2/]			61-150	200+	31-60	31-60	5.5-6.0

[1/] On sites #3 and 4 no living seedlings were found.

[2/] Recommended levels for Virginia and white pine Christmas tree plantations in the Coastal Plain of Georgia (Plank, 1989)..

expand.

Shoot dimensions: Growth of the terminal shoots and ground line diameter were measured for all seedlings. Only the data collected in October, just prior to harvest, are discussed here. Analyses of covariance, using initial seedling height as covariate, were performed for new shoot growth and ground line diameter (GLD).

At harvest seedlings in the entire experiment averaged .70 cm stem diameter at groundline. There were significant (.05 level of confidence) differences among treatments. Duncan's Multiple Range Test showed that seedlings treated with 1/10 of the recommended rate of Prowl had a significantly larger ground line diameters, and those treated with 1/100,000 dilution of Surflan significantly smaller GLDs than all other seedlings. All other 23 treatments showed no significant differences in GLDs.

Seedlings averaged 41.5 cm in total height with 21.6 cm of that in new shoot growth. There were significant (.05 level of confidence) differences among treatments in the current season's shoot growth. Duncan's Multiple Range Test showed several broadly overlapping groups of means without significant differences.

It is logical to assume that the full, recommended rate of herbicide, rather than any of the dilutions, would have had the greatest effect on seedling growth. In both stem diameter and shoot growth, however, the seedlings treated with the full, recommended rate of herbicides did not differ significantly from a group of 23 and 18 other treatments, respectively. There were no obvious trends in the data due to either the kind or the concentrations of herbicide applied. Apparently the statistical differences were not biologically significant.

Dry Weight Components: At harvest the seedlings were cut apart into shoots, roots in the upper tub, and roots in the lower tub, i. e. roots that had grown through the herbicide treated surface after June. There were no significant differences among the dry weights of each seedling component. The oven-dry weight of the average seedling was 264 g distributed as follows: 163.1 g stem and needles, 72.2 g roots in upper tub, and 28.6 g roots in lower tub.

Seedlings in one block produced significantly less root and shoot weight, even though the filling of pots with well mixed soil, planting with seedlings, and assignment of blocks and treatments all had been at random. The rotary sprinkler used to water the pots was moved frequently to prevent an irrigation pattern. There was no obvious explanation for this difference. Perhaps a building about 10 m distant caused some consistent air turbulence which affected the microclimate around one block.

Herbicide residues were determined in the soils of this pot study. The soil surface of the lower tubs had been treated with the various herbicide concentrations in the first week of June and the study was dismantled four months later in early October. Balan residue was not found in any treatment, and Prowl was detected in only one of the three tubs treated with the full, recommended rate. Surflan and Treflan were more persistent. The recommended rate treatment of Surflan in June resulted in 0.4 ppm residue in October (average of three tubs) and two of the three tubs treated with the recommended rate of Treflan contained an average residue of 0.06 ppm. None of these residual levels had any apparent effect on the seedlings.

SUMMARY AND CONCLUSIONS

The central question for this study was whether residual herbicide levels in farm land might have caused high pine seedling mortality. Top- and sub-soils from the seven CRP sites on which pine seedlings died for no obvious reason had no detectable levels of a suite of 26 commonly used herbicides. Seedlings in the pot study showed no ill effects from four relatively persistent herbicides. Green and Metcalfe (1986) reached the same conclusion for loblolly pine planted in an old soybean field which had been treated with Oust. However, in reforesting marginal cropland which had been treated with the herbicide Classic at the recommended rate, loblolly pine seedlings suffered 90% mortality in Maryland (Anon., 1988).

The nematodes found at these sites do not seem either the type or numerous enough to cause significant seedling mortality. Neither severe nutrient deficiencies nor imbalances existed in the soils of the sites.

So what killed the loblolly and slash pine seedlings on these old agricultural fields? It would be neat to be able to identify a single cause, but reality is more complex. Every field has its peculiarities. The droughty conditions of the 1988 growing season undoubtedly contributed to heavy mortality. In an average year, the innate hardiness of loblolly and slash pine will overcome many unfavorable factors such as small seedling size, poor root systems, and handling or planting mistakes. Drought, however, can cause otherwise tolerable mistakes to become fatal ones.

Georgia Forestry Commission personnel were alarmed by 45 and 35% mortality rates in 1986 and 1987 pine plantings, respectively (Green, 1989). A survey showed that only two-thirds of the seedlings had been planted properly. The others were J-rooted, severely rootpruned, not planted deep enough, or otherwise mishandled. This prompted the Commission to teach a series of workshops for planting contractors and landowners. Survival for loblolly and slash pine increased to 75% in 1988, in spite of continued drought conditions. Another survey in early 1989 showed that 76% of the seedlings planted by contractors who had attended workshops should survive, whereas only 63% survival was predicted for seedlings planted by those who had not been certified (Personal communication, L. Thompson, Ga. For. Comm.).

In several fields visited in the course of this study, pine seedling roots were attacked by larvae of the white-fringed beetle. These beetles also destroyed many seedlings when abandoned agricultural fields were converted to pine during the start-up of the Savannah River Plant near Aiken, SC (Pesonal communication, Dr. J. T. May) in the 1940's. In the meantime, foresters have largely forgotten about this insect, therefore I summarize the scant information found in the literature.

Adults of the white-fringed beetle are about 11mm long, with dark gray or grayish-brown scales. They have two light stripes that run the length of the scales and a fringe of white hairs along their lower margins, giving them their common name. Only females are known to exist; they cannot fly. The beetles usually overwinter as yellowish-white larvae, up to 13 mm long, with a light-brown head and slightly curved body. From March through May these larvae feed on the taproots and large laterals of nearly 400 plant species. Legumes like

Figure 1.--Pot study about two months after the bottoms were cut off the upper tubs. They were then placed atop other plastic containers filled with soil which had ben treated with herbicides. Duct tape was used to seal the tubs.

peanuts, soybeans, and velvet beans are preferred; plants with fibrous root systems, e.g.grasses and small grains, are poor hosts for this beetle. Many of the marginal agricultural fields in the CRP program had been in soybeans recently.

The first adult beetles appear above-ground in May, most emerge in June and July with stragglers in August (Hunt, 1982). Egg laying begins within a month of emergence. They are generally deposited on plants and other objects at or near groundline in masses of sixty or more white, oval eggs less than 1mm in length. They hatch in midsummer and the larvae feed underground until the following spring. There is only one generation per year (Davidson and Peairs, 1966). White-fringed beetles strip the bark and phloem from large laterals and taproots of pine seedlings, leaving the yellowish-white xylem exposed.

In summary, no single cause for pine seedling mortality could be identified in the fields included in this study. There were no obvious nematode problems, nutrient deficiencies or imbalances, or harmful levels of herbicides. Applications of four dinitroaniline herbicides in a pot study produced no measurable effects on the growth of loblolly pine seedlings. Heavy seedling mortality could have been caused either by incorrect seedling handling, by subtle combinations of minor mistakes in planting, or unfavorable site factors. In an average growing season the hardy pine seedlings might have survived in spite of these unfavorable factors. In a drought season they often proved fatal.

■

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