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Title EFFECTS OF SOME TRANSPIRATION RETARDANTS AND SHADE ON SURVIVAL OF DOUGLAS-FIR SEEDLINGS UNDER DROUGHT CONDITIONS IN THE FIELD

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This study was designed to test the ability of atrazine and 8-hydroxyquinoline sulfate to reduce the transpiration stress and thus prolong the life of potted Douglas-fir seedlings under various levels of shade. Treatment effectiveness was defined by the survival time of seedlings exposed to each combination of chemical treatment and shade.

Shade was very beneficial, but any effects of the chemicals were masked by within treatment variation.

EFFECTS OF SOME TRANSPIRATION RETARDANTS AND SHADE ON SURVIVAL OF DOUGLAS-FIR SEEDLINGS UNDER DROUGHT CONDITIONS IN THE FIELD

by

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EFFECTS OF SOME TRANSPIRATION RETARDANTS AND SHADE ON SURVIVAL OF DOUGLAS-FIR SEEDLINGS UNDER DROUGHT CONDITIONS IN THE FIELD

INTRODUCTION

For many years drought has been cited as a major cause of seedling mortality in the Douglas-fir region. Although drought is often considered an arid-region problem, it also occurs on sites which receive more than 40 inches of precipitation annually. Over much of the natural range of Douglas-fir the summer climate is characterized by long periods of low rainfall and high evaporation potential. These factors make it possible for established vegetation to rapidly remove most of the available moisture from the upper foot of soil. Under such conditions, the summer drought may begin early in June, soon after Douglas-fir growth is initiated, and continue until the fall rains commence in September or October. Drought stresses are most pronounced where Douglas-fir seedlings in full sunlight must compete with herbaceous vegetation for the available moisture. These recurring drought periods are of serious biological and economic importance to foresters who are faced with the task of establishing new forests.

Studies have been conducted at Oregon State University to provide information which may help reduce seedling mortality caused by drought. These studies have been of two general types:

1. Laboratory, greenhouse, and growth chamber studies to answer questions about the mechanisms of drought resistance.

2. Field studies to find effective means of reducing the moisture stress by altering the environment.

The present study was designed to answer the following questions which were suggested by laboratory tests and by the literature:

1. Does three pounds of active atrazine per acre increase survival when competition is not a factor?

2. If so, how does the effect of the dry form compare with that of the foliar spray?

3. Does 8-hydroxyquinoline sulfate increase survival when competition is not a factor?

4. If so, what is the most effective method of application and range of concentrations used in the study?

5. What degree of shading is most favorable for survival?

6. How is survival affected by the interaction of chemical treatments and shade?

REVIEW OF THE LITERATURE

The drought losses which occur in Douglas-fir plantations have inspired considerable research (2, 3, 4, 5, 8, 10, 11, 12, 17). Drought losses result from limited available soil-moisture and transpirational stress (7, p. 354). The available soil-moisture in forest plantations can be increased by scarifying or mulching to control competing vegetation (2, 5, 8) and the transpirational stress on seedlings is often reduced by planting them in the shade of stumps and logs. Perhaps the same results can be obtained with chemicals.

Transpiration Retardants

Atrazine

Several chemicals are used to selectively control unwanted vegetation. Other chemicals can be used to reduce the transpirational stress on the plants because of their ability to control stomata (18, p. 24). Some chemicals will probably do both jobs and atrazine[1] seems to be such a chemical. It is widely used to control grass and some broadleaf weeds, and was found by Smith and Buchholtz (15) to retard transpiration in some crop plants. This dual action could lead to confounding of

[1] 2-chloro-4-ethylamino-6-isopropylamino-s-triazine.

research results where a chemical is applied to both wanted and unwanted plants at the same time. An assumption by Zelitch (18, p. 25) that chemicals would be more effective when applied to the foliage rather than to the roots makes it possible to separate the effects of atrazine in forest plantations. This may be done by planting some trees before the chemical is applied to the competing vegetation and some afterwards, as done by Newton (11, 13). Now that stomatal control has been obtained for short periods with atrazine (15); it seems desirable to attempt to control stomata for prolonged periods in the field.

Eight-Hydroxyquinoline Sulfate

Stoddard and Miller (16) found that 8-hydroxyquinoline sulfate will close the stomata of strawberry plants for extended periods. As roots were exposed to higher concentrations of 8-hydroxyquinoline sulfate solution, stomatal closure increased and transpiration decreased. These effects were observed up to the highest concentration used--2,000 ppm. Paskett (14) used a higher concentration, 3,000 ppm, to close the stomata of excised Douglas-fir needles. He was also able to reduce transpiration by submerging the roots of Douglas-fir seedlings in a 3,000 ppm solution for 90 minutes.

Although it is evident that 8-hydroxyquinoline sulfate can close the stomata of Douglas-fir for short periods, the most effective concentration and the best method of application remain unknown. This suggests that a wide range of concentrations should be applied to seedlings in a screening test.

Shade

Shade should complement chemicals which are used to either increase soil-moisture or reduce transpiration. Hofmann (6, p. 43) pointed out the importance of shade for seedling survival on drought sites many years ago. At that time he mentioned brush as a source of shade; and later, McCulloch (9) showed that bracken fern promoted regeneration on certain sites. However, most of the brush species use considerable water--enough in many cases to offset any advantage provided by the shade. For that reason, where soil-moisture is in short supply it is probable that some type of dead shade is more desirable than live shade. Dead shade seems more desirable for experimental work because the shade effect can be more accurately isolated and quantified.

Because chemical transpiration retardants and shade both affect transpiration, the appropriate means of testing for interaction is a factorial experiment.

METHODS AND MATERIALS

Douglas-fir seedlings were planted in gallon cans in the early spring of 1964. The root-pruned, 2-0 seedlings of unknown seed origin were obtained from the Oregon Forest Nursery, Corvallis, Oregon and planted, two per can, in a well-mixed, sandy forest soil from the vicinity of Burnt Woods, Oregon. The potted seedlings were then grown outside at the Oregon Forest Research Laboratory, Corvallis under intermittent shade until they were removed to the field plots on 20 June 1964. During this period all plants were well-watered and all herbaceous plants were pulled to prevent competition for soil-moisture during the drought period which was scheduled to follow. In early June, the seedlings were treated with chemicals and then exposed to drought conditions in the field under various levels of shade.

Experimental Design

In the field, a 4 X 10 factorial design was used to detect changes in survival associated with transpiration retardant treatments, shade treatments, and their interactions. For simplicity, the ten transpiration retardant treatments will be called chemical treatments and the four levels of shade will be called shade treatments. Figure 1 shows that two chemical and one shade treatment were controls. Each of the 40 combinations of chemical and shade treatments is represented by five cans of seedlings. Each can is considered a replication in the analysis. The design is therefore a 4 X 10 factorial with five replications.

Figure 1. Experimental design: 4 X 10 factorial with five cans of seedlings in each combination of transpiration retardant and shade.

The experiment was completely randomized. Treatments were randomly assigned to the cans, cans were assigned random positions within the frames, and the frames were assigned random positions along an arbitrary line.

Experience in the greenhouse and growth chambers indicated that time of death could be estimated to the nearest day (3). In this study, seedlings were observed about every third day for indications of death and survival time was recorded as the number of days from placement in the field to apparent death. Analysis of treatment effects was based on the mean survival time of the two seedlings in each can.

The individual degree of freedom technique was used to test for significant differences among the various treatments. Analysis of variance was used to test for interaction of chemicals and shade.

Transpiration Retardants

Atrazine

Atrazine is commonly used to control grass in Christmas tree and other plantation work. We were interested in its effect on seedlings which are sprayed incidental to grass treatment.

A foliar spray was used to apply atrazine to one group of seedlings under simulated field conditions at the rate of three pounds of active ingredient per acre (see appendix for details). An equal amount of the chemical was applied to another group in dry form to eliminate any direct effect on the foliage. Such effects should be indicated by comparison of the two methods of application.

Eight-Hydroxyquinoline Sulfate

Three concentrations of 8-hydroxyquinoline sulfate were used. Aqueous solutions of each concentration were applied at the rate of 100 ml per can either one time (Rows 5, 7, and 9 Figure 1), or four times (Rows 6, 8, and 10 Figure 1). A wide range of total quantities of 8-hydroxyquinoline sulfate was applied:

Row	Concentration in ppm X 10^3	Number of Applications	Total per can	
			Solution (ml)	HQS (g)
5	2	1	100	0.2
7	4	1	100	0.4
9	8	1	100	0.8
6	2	4	400	0.8
8	4	4	400	1.6
10	8	4	400	3.2

A single application of the highest concentration contained the same amount of active ingredient as four applications of the lowest concentration. Therefore, it was possible to test the effects of the number of applications. The multiple applications were at three-day intervals and all cans which did not receive 100 ml of solution were given 100 ml of tap water each time the solutions were added. After the chemical treatments were completed on 20 June 1964 the cans were moved immediately to the field plots.

The field plots were located on a 20 percent south slope in the Hospital Lot--a portion of Oregon State University's McDonald Forest north of Corvallis, Oregon. After chemical treatments were completed, the potted seedlings were arranged in eight groups of 25 cans each and placed into holes dug about six inches deep (Figure 2). Soil was replaced around the cans to reduce heating by radiation.

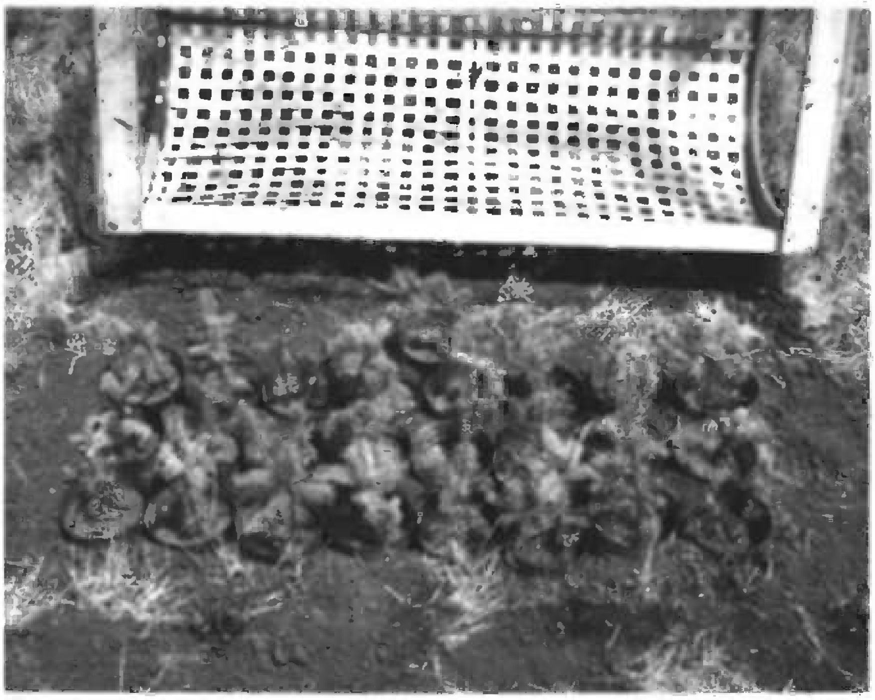


Figure 2. Seedlings in the field: cans are buried in the soil. Shade frame in background provides 3/4 shade when placed over the seedlings.

Various levels of shade were provided by the sheet-metal shade frames shown in Figure 3. The levels may be designated by the fraction of full shade or as a fraction of full sunlight which passes through.

Letter Assigned	Fraction of Full Sun	Fraction Shaded
A	1/1	0
B	1/2	1/2
C	1/4	3/4
D	1/8	7/8

The fraction of full sun clearly shows the geometric relationship among the levels. However, since the treatments are referred to as shade, it seems more appropriate to designate the levels by the fraction shaded. Levels A, B, C, and D are therefore synonymous with 0, 1/2, 3/4, and 7/8 shade respectively.

Figure 3. Shade frames in position.

The hemi-cylindrical frames were so constructed and oriented that the surface of the sheet-metal strips was nearly perpendicular to the sun's rays most of the time. Details of construction are given in the appendix. There was no attempt to quantify the radiation load on the seedlings--shading was considered relative. Light intensity was checked under the various frames at midday on two clear days during the summer. A Weston model 756 illumination meter indicated that relative light intensities were very close to the expected values.

Each shade treatment was applied to 50 cans of seedlings. The 50 cans were randomly divided between two shade frames. Unshaded control plots were screened against rodent damage with wire netting which did not provide measureable shade.

All cans were watered uniformly until 20 June 1964. After that date no water was added and no significant precipitation occurred. Therefore, it is assumed that soil moisture was uniform among the cans and survival was tallied from that date.

RESULTS

Shade delayed the death of seedlings, but the magnitude of the shade-effect varied among the chemical treatments. Variation within the chemical treatments was great enough to mask most of the variation among them. As a consequence, some real effects of the chemicals and interactions may have escaped detection in this study.

Transpiration Retardants

Analysis of variance was not used to test for differences between or among the chemical treatments within any given level of shade because each level was represented by only five observations.

Atrazine

Analysis of variance shows no difference between the two methods of application, nor between the atrazine treatments and the controls.

Eight-Hydroxyquinoline Sulfate

The individual degree of freedom technique was used to test for differences among the 8-hydroxyquinoline sulfate treatments and to compare them with the controls. The only difference detected was between treatments six and nine (Figure 4). Both groups received the same amount of chemical, but in different concentrations. Plants which were treated four times with dilute solution lived significantly longer than those which were treated only once with concentrated solution.

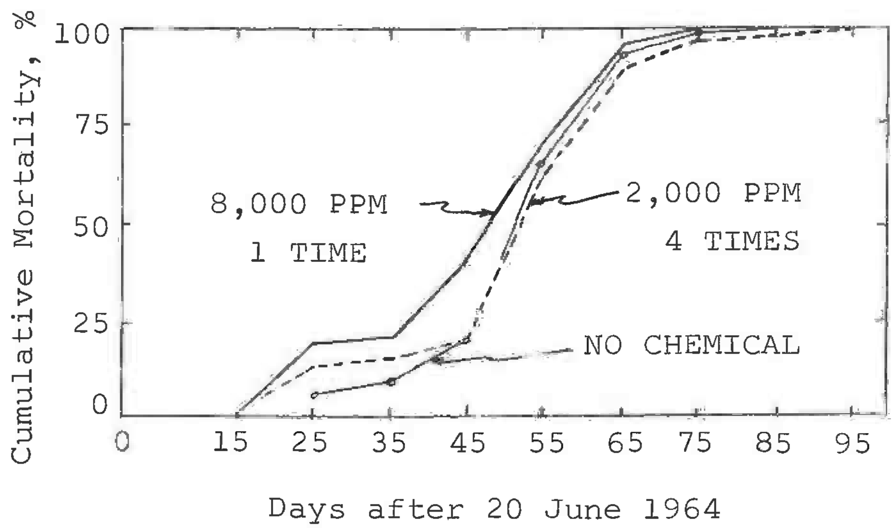


Figure 4. Effects of 0.8 g 8-hydroxyquinoline sulfate on mortality of Douglas-fir seedlings.

Shade

Analysis of variance indicated that all levels of shade prolonged survival under the experimental conditions. At the one percent level of significance there was no difference among the 1/2, 3/4, and 7/8 shade treatments. However, at the five percent level of significance there was a difference between 1/2 and 7/8 shade. In each case, the survival period increased with the level of shade.

Table 1. Differences among shade treatments.

Treatment	A	B	C	D
Level of shade	0	1/2	3/4	7/8
Mean days of survival	48.0	55.2	56.5	59.8
1% level of significance	———	———	———	———
5% level of significance	———	———	——— ———	———

Solid lines connect treatments which were not different at the given level of significance with the individual degree of freedom technique.

These figures do not show the distribution of mortality with time. To illustrate this, mortality was tallied by ten-day intervals and plotted over the mid-point of the intervals. Figure 5 shows that seedlings without shade began to die sooner than those with shade, and that during the first 60 days after the drought period began the mortality in 7/8 shade was only about one-half that in full sun. The mortality curves for 1/2 and 3/4 shade fall between the curves shown.

There was no detectable interaction between chemicals and shade.

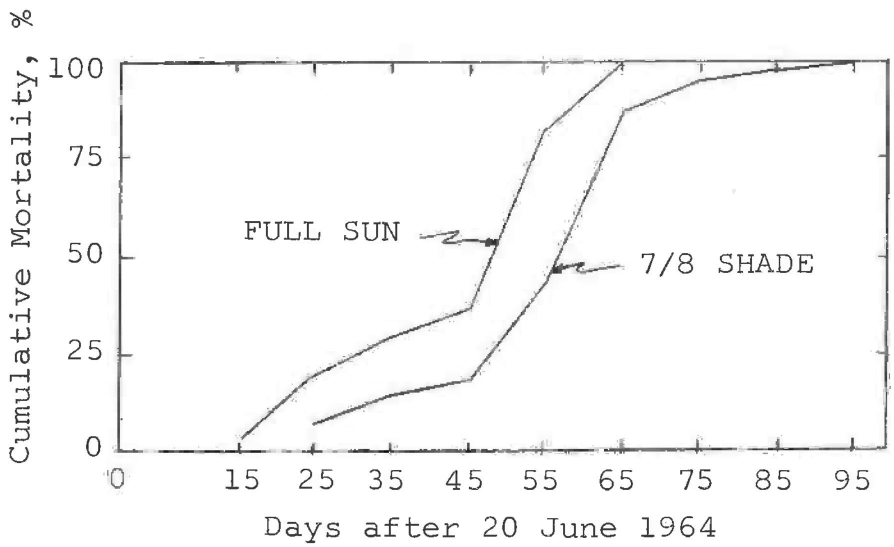


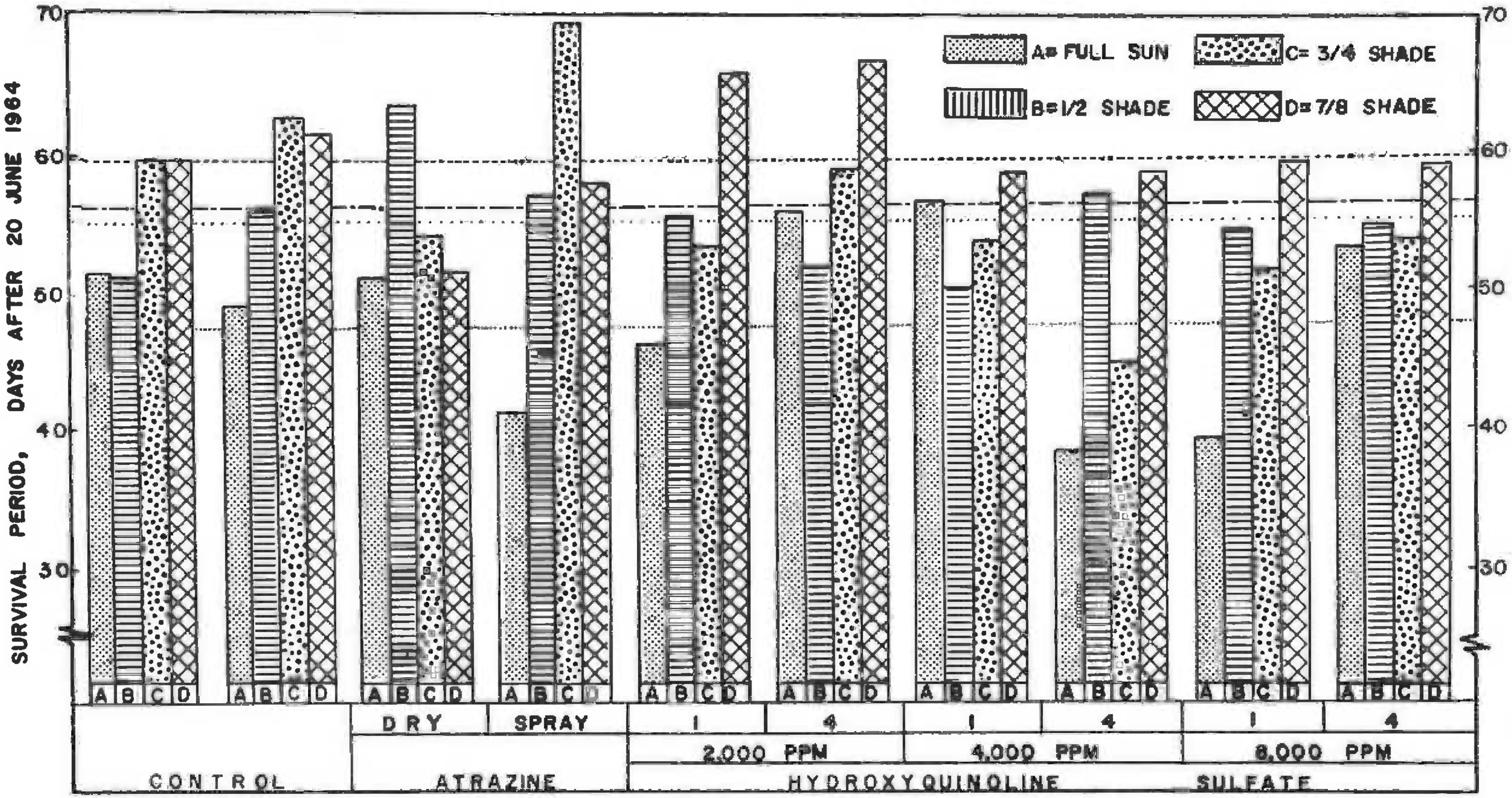
Figure 5. Effects of shade on mortality of Douglas-fir seedlings.

DISCUSSION

The results of this study verify once again a phenomonon which was probably observed by many people long before any experimental work was undertaken in Douglas-fir regeneration--simply that shade postpones the death of seedlings which lack available soil-moisture during hot, dry weather. The benefits of shade are significant and should not be overlooked.

Although no effects of any of the chemical transpiration retardants could be detected statistically, a perusal of Figure 6 reveals differences among the means of the various treatments. Several sources of variations within the treatments could have obscured any real treatment effects. However, since care was taken to select uniform-size seedlings, to completely randomize throughout the experiment, and to maintain uniform soil-moisture prior to the drought period, it is assumed that some of the variation can be attributed to the genetic variation among the seedlings. The remaining variation probably resulted from experimental techniques.

Some bias may have favored the shade treatments over the unshaded control. In the greenhouse it is possible to estimate the time of drought-induced death with considerable accuracy (± one day (3)). Seedlings which die under a given external environment develop characteristic



EFFECTS OF CHEMICAL TREATMENTS AND SHADE ON SURVIVAL OF DOUGLAS-FIR SEEDLINGS

FIGURE 6

symptoms prior to death. These symptoms can be used to predict death under the given conditions; but when plants are observed under four different environments the symptoms may vary among the environments. The seedlings exposed to full sun in this study became chlorotic within the first three weeks of exposure even though they were alive. However, shaded seedlings, especially those in heavy shade, generally did not become as chlorotic even at the time of death. This suggests that there may be a tendency to underestimate the survival time for the chlorotic, exposed plants and to overestimate the survival time for the healthier-looking, shaded plants.

Another type of potential bias could be eliminated by expressing mortality, or survival, as a function of one or more environmental variables. Relative levels of shade would be adequate for comparison if all days were comparable--but the benefits of shade diminish on cloudy days. When each day is given equal weight in survival calculations the range of values and the experimental error tend to increase. For example, plants which barely survive a brief period of high potential evapotranspiration, and could not survive another similar day, may be able to survive many additional days which are less severe. Temperature or insolation data could be used to weight each day in the survival calculation if such data had been collected during the study.

This is a pot study and the results should not be extrapolated to the field. It must be remembered that the seedlings were potted in gallon cans to restrict roots to similar volumes of uniform soil. It would be very hazardous to estimate survival time for field planted seedlings under similar conditions because the cans not only limited the rooting space but may have caused increased heating at the surface by restricting air movement. In addition to the surface heating there may have been some conduction of heat down the side of the cans to where some of the roots came in contact with the can.

Each of these pot effects should tend to decrease survival in the unshaded plots more than in the shaded plots during the periods, but the bias should diminish on cooler days. The fact that these effects would be randomly distributed among the transpiration retardant treatments but not among the shade treatments may partially explain the experimental results.

It is unlikely that these effects are any more serious than in conventional pot studies, but they are adequate reasons for differentiating between pot studies and field studies.

Atrazine has often been credited with increasing seedling survival; yet this test reveals no such effect.

The beneficial effects of atrazine are largely the result of reducing the competition for soil-moisture. However, the only competition for soil-moisture in this study was between the two seedlings in any given can--all other vegetation was removed before the drought period began. The results of statistical analysis show that the survival of the 80 trees which were treated with atrazine was not unlike the survival of the 80 untreated trees. From this, we can assume that in commercial application of three pounds of active atrazine per acre, the only effect on survival is through control of competition.

Very little was learned about the range of concentrations of 8-hydroxyquinoline sulfate which would be most effective in retarding transpiration in Douglas-fir seedlings. Although four applications of 2,000 ppm provided better results than an equal volume of active ingredient applied at one time in a concentration of 8,000 ppm; the results were no better than with the untreated seedlings. Other methods of applying the chemical to the seedlings may be more satisfactory.

Any method of treating the seedlings prior to planting would have to impart very long-term effects to aid survival during the summer months. If it is possible to obtain such long-term residual effects, there may be some accompanying drawbacks. Arkley (1) has pointed out that under some conditions, dry-matter production varies with transpiration. In a situation where animal damage or competition for light are major causes of mortality it is conceivable that reduced height growth would be tantamount to death--but on most droughty sites, the possibility of succumbing to these threats is preferable to the alternative of almost certain death by dehydration.

SUMMARY

Many Douglas-fir seedlings succumb to the annual summer drought periods which occur over much of the natural range of this important timber species. Laboratory and field studies have been conducted at Oregon State University in an effort to reduce these losses. This study was designed to test the ability of two chemicals to prolong the life of Douglas-fir seedlings which were exposed to drought conditions in the field.

Atrazine and 8-hydroxyquinoline sulfate can retard transpiration in certain plants for short periods in the laboratory, but the long-term effects are not known. To investigate these effects, potted seedlings were treated with the chemicals in various ways early in the summer. After the chemical treatments were completed, the seedlings were moved to the field where water was withheld and artificial shade was provided; they were then allowed to dehydrate and die. Seedling survival time was tallied as a measure of chemical and shade effectiveness.

Shade delayed the death of seedlings, but the magnitude of the shade-effect varied among the chemical treatments. Variation within the chemical treatments was great enough to mask most of the variation among them. Plants which were treated with dilute solution of 8-hydroxyquinoline sulfate survived longer than those which

received the same amount of active chemical in concentrated solution. No other chemical effects or interactions were detected. The results would probably be more meaningful if survival periods could be weighted by an appropriate environmental variable.

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Chemical Applications

Atrazine

Three pounds of active ingredient per acre is commonly used to control grass. For each group of 20 cans, this rate requires 0.154 g of 80 percent active commercial atrazine. It is relatively easy to obtain uniform distribution of chemicals over large areas, but special techniques are required to assure uniform distribution among cans which are only six inches in diameter. The techniques used in this study are described below.

Dry Application. Ten times the required amount of atrazine (1.54 g) was thoroughly mixed with 198.46 g of oven-dry clay which had passed a #40 sieve. One-tenth of the 200 g mixture was then applied to the 20 cans at the rate of one gram per can.

Foliar Application. An overhead sprayer in greenhouse section 16-5 was calibrated to deliver 25 ml of suspension to each can during a series of ten passes. After each pass of the sprayer the cans were systematically moved to different positions to insure equal distribution of the solution among the cans. To increase the accuracy of weighing and mixing the components, 4.62 g of 80 percent active commercial atrazine was mixed in 15 liters of water. On the basis of the calibration, it is assumed that 500 ml of suspension, containing 0.154 g of atrazine, reached the plants or soil in the 20 cans which were treated.

Eight-Hydroxyquinoline Sulfate

Ten liters of 8,000 ppm solution of 8-hydroxyquinoline sulfate was obtained by adding 140 g of 8-hydroxyquinoline sulfate (Penick Chemical Company) to 17.5 liters of water. The other concentrations (4,000 ppm and 2,000 ppm) were obtained from this stock solution by serial dilution.

One-hundred milliliters of a given solution was poured on the soil in each of 40 cans--a total of 120 cans for the three solutions. One-half of the cans in each group were then given three additional similar treatments at three-day intervals.

Shade Frame Details

The various levels of shade were provided by sheet-metal strips one-inch wide. The strips were attached to plywood arches which were nailed to the ends of rectangular wooden frames 30 inches wide and 60 inches long. Each of the frames had strips spaced one inch apart parallel to the long axis of the frame--this provided 1/2 shade. To provide more shade, additional strips were placed at right angles to the long axis. Figure 2 shows a frame, which provides 3/4 shade, tipped sideways to provide access to the seedlings.

Data and Analysis

Table 2. Treatment Averages--each figure represents five observations.

Retardant Treatments*	Levels of Shade A 0	B 1/2	C 3/4	D 7/8	Means
1	51.0	51.7	59.7	59.7	55.5
2	48.7	55.5	62.4	61.2	57.0
3	50.9	63.5	54.0	51.5	55.0
4	41.3	56.8	69.4	58.0	56.4
5	46.2	55.4	53.4	65.8	55.2
6	55.8	51.9	59.0	66.3	57.8
7	56.4	50.2	53.8	58.8	54.8
8	38.4	56.7	47.1	58.7	50.2
9	39.3	55.3	57.0	59.6	52.8
10	53.5	55.0	53.8	59.2	55.4
Means	48.0	55.2	56.5	59.8	54.9

*Description of transpiration retardant treatments:

1. Control--no chemical
2. Control--no chemical
3. Atrazine applied dry--directly to the soil
4. Atrazine applied as foliar spray
5. 2,000 ppm 8-hydroxyquinoline sulfate applied one time
6. 2,000 ppm HQS applied four times
7. 4,000 ppm HQS applied one time
8. 4,000 ppm HQS applied four times
9. 8,000 ppm HQS applied one time
10. 8,000 ppm HQS applied four times