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THE INFLUENCE OF MALEIC HYDRAZIDE AND CERTAIN AUXINS ON GROWTH AND SURVIVAL OF DOUGLAS FIR SEEDLINGS

by

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The Influence of Maleic Hydrazide and Certain Auxins on Growth and Survival of Douglas Fir Seedlings [1]

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INTRODUCTION

Many acres of potential forest land in the United States remain unstocked because landowners are aware of the high incidence of unsuccessful plantations and are unwilling to accept planting risks. Reforestation by planting does involve considerable risk whenever it is practiced, but in northern Idaho the hot, dry summers make the possibility of failure particularly acute. However, the increasing demand for wood and wood products, together with the increasing emphasis on planting as a method of reforestation, requires that techniques be developed to insure the survival of a higher percentage of planted seedlings. Toward this end, most investigations have been concerned with methods of site modification and genetic improvement of planting stock. The possible alteration of the growth characteristics of seedlings with plant growth regulators as a means for increasing survival has, on the other hand, received little attention.

When soil moisture is at or below the wilting point, seedlings with small tops and large root systems should theoretically have a higher potential to survive than seedlings with large tops and small root systems. The root system of the former type of seedling would be able to extract moisture from greater soil volumes while transpiration from their smaller tops would be lower than of seedlings with larger tops. Thus, the internal water relations of such plants should be improved. Since Loewenstein and Pitkin (1961) reported that the major cause of high seedling mortality in northern Idaho is the lack of available soil moisture during the latter part of the growing season, it seems probable that the survival of planted seedlings would be favored if seedlings developed a small top, but large root system, during the first growing season. If seedlings could be chemically treated so that root growth would be favored over shoot growth during the first critical year after planting, the chances of survival under droughty conditions might be improved.

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Maleic hydrazide (MH) is a plant growth inhibitor which has been used in horticultural and agricultural practices for about 25 years. Used primarily to prevent sprouting of stored potatoes and suckering in tobacco, MH more recently has been used to increase the length of the dormant period in citrus trees (Hendershott, 1962). Greulach and Haesloop (1954) had earlier shown that internodal elongation in certain plants was reduced by MH. These findings suggest that MH may reduce the shoot growth of conifer seedlings.

Other workers have reported some root growth inhibition by MH in certain plants, but not in others (Naylor and Davis, 1950). Leopold and Klein (1952) were the first to suggest that this inhibition could be reversed with auxin. Vogt and Cox (1970) recently showed that MH exerts an antagonistic effect on the inhibitory action of high levels of the auxin indolacetic acid (IAA) on stump sprouting by oaks.

The objective of this research was to determine if the growth characteristics of Douglas fir (*Pseudotsuga menziesii* var. *glauca*) seedlings could be altered with MH and certain auxins, and to evaluate the effect of the alteration, if any, on the survival of planted seedlings under field conditions.

METHODS

Two greenhouse experiments and one field study were utilized during the course of the investigation. Field work was not attempted until greenhouse experiments revealed treatments of potential value to seedling survival in the natural environment.

The initial greenhouse experiment was designed to ascertain the effects of MH, when used alone, on growth and development of Douglas fir seedlings. The second experiment again included the MH treatment, but in addition root systems were treated with various auxins. Seedlings were treated by immersing either tops or roots in a glass vessel containing a particular growth substance solution. Treatment solutions of MH were prepared by dissolving the powdered substance in a few milliliters of diethylamine and water and diluting to the desired volume with distilled water. Treatment solutions of the auxins were prepared by dissolving indolebutyric acid (IBA) and/or IAA in a few milliliters of 95 percent ethyl alcohol and diluting to volume with distilled water.

In the first experiment, four groups of twelve 2-0 seedlings were root-pruned to 5 inches and treated with MH (Table 1). During the topsoak period, root systems were enclosed in a moist polyethylene bag to prevent desiccation. Treated seedlings were planted in a completely randomized design in glass-faced root boxes filled with sterile sand. Seedlings were grown under natural photo-periods (mid-June) for one month, during which tap water was added as needed.

Treatments imposed on seedlings in Experiment 2 are also listed in Table 1. Each treatment included 18 seedlings which were planted in pots (completely randomized design) containing equal parts of sand, peat, and soil. Seedlings used in this experiment were 2-1, rather than 2-0, and root systems were pruned to 8 inches instead of 5 inches. The experiment lasted for 2 months, during which natural daylight was supplemented with Gro-Lux lights set for 14- hour photoperiods. Approximately 1 inch of tap water was applied to each pot per week.

In the first experiment the total number of buds broken at the end of one month was recorded. Seedlings were then lifted from their containers and top and root growth measured. The longest new shoot and root was measured from the point of growth inception to the tip of the organ. In the second experiment, the number of broken buds was recorded at intervals of several days. Also, the number of roots with new growth in excess of 0.5 inches were counted on each seedling.

Table 1. Growth substance treatments applied to Douglas fir seedlings in greenhouse experiments I and II.

	Treatment[1]	Top-soak	Root-soak[1]
Experiment I	A	5000 ppm MH	None
	B	3000 ppm MH	None
	C	1000 ppm MH	None
	D	Diethylamine[2]	None
Experiment II	A	1000 ppm MH	None
	B	1000 ppm MH	1 ppm IBA 10ppm IAA
	C	1000 ppm MH	10ppm IBA 10ppm IAA
	D	1000 ppm MH	10ppm IAA
	E	Diethylamine	None
	F	None	None

[1] Top-soak period in Experiment I was 1 hour, in Experiment II it was 15 minutes. Root-soak period in Experiment II was 2 hours.

[2] A few milliliters of diethylamine (solvent for MH) in 1 liter of water.

After evaluating results of greenhouse experiments, certain treatments were selected to be field tested for their effect on survival of planted seedlings. The field study site was located on the Rathdrum Prairie near Athol, Idaho in Kootenai County. The average annual precipitation is about 22 inches with only about 1 inch coming during the critical months of June, July, August, and September. The soil is derived from sandy and gravelly glacial outwash composed mainly of granite, schist, slate, and quartzite. The texture of the surface soil is a gravelly sandly loam with a pH of about 6.1.

Field treatments (Table 2) were applied in a randomized complete block design. Each treatment consisted of 100 2-1 seedlings which were root-pruned to 9 inches prior to treatment in the field with growth substances. Seedlings were planted in early April, 1967, about one week after the planting site had been plowed. At weekly intervals, beginning on April 15 and continuing until July 12, the total number of broken buds per seedling was counted. Seedling survival and vigor were recorded through October 10.

Table 2. Growth substance treatments applied to Douglas fir seedlings in the field experiment.

Treatment	Top-soak (15 min.)	Root-soak (2 hours)
A	1000 ppm MH	1 ppm IBA 10 ppm IAA
B	1000 ppm MH	10 ppm IAA
C	1000 ppm MH	10 ppm IAA 10 ppm GA
D	10 ppm MH	1 ppm IBA 10 ppm IAA
E	None	None

RESULTS AND DISCUSSION

Results of the first experiment indicated that the three concentrations of MH did inhibit bud opening (Table 3). Wide variation in percent bud break among seedlings within a given treatment was noted. For example, two seedlings in treatment A had no broken buds after one month while another seedling in the same treatment had 100 percent broken buds. There was less variation in bud break among seedlings in the control group. Results of this experiment demonstrated the large inherent differences in sensitivity of individual seedlings to plant growth substances.

Shoot growth after one month was significantly reduced by the MH treatments (Table 3). Shorter shoots on seedlings treated with MH are probably the result of two factors. First, since bud break was delayed, the shoots of MH treated plants grew for a shorter period than did shoots of control plants. Secondly, Greulach and Haesloop (1954) reported that MH not only delays bud break but also inhibits elongation of internodes.

All three MH concentrations were detrimental to root growth (Table 3). Actually, in most cases, there was no new root growth at all on MH treated plants. In comparison, the control seedlings showed, on the average, considerably greater root growth. It seems likely that MH was transported to the root system where it adversely affected both root initiation and growth. Crafts (1961) has demonstrated that radioactively labeled MH applied to the tops of barley plants would accumulate in the roots. The mechanism by which MH interferred with root growth is not known. However, Pilet (1957) found that high concentrations of MH 1-10^{-3} M) increased the activity of IAA-oxidase, an enzyme which destroys IAA in *Lens culinaris*. Thus, MH may be reducing the level of endogenous auxin to a growth limiting level.

Although the inhibition of top growth by MH was the desired result, the inhibition of root growth was unsatisfactory. The two higher concentrations of MH also produced some chlorosis and necrotic spotting at the needle tips. It was decided, therefore, to reduce the MH concentration to 1000 ppm and the length of the top-soak period to 15 minutes in the next experiment. In addition, roots were soaked in auxin solutions in the hope of counteracting the detrimental effect of MH on root growth.

Table 3. The effect of maleic hydrazide on bud break and growth of shoots and roots of 2-0 Douglas Fir seedlings (Greenhouse experiment I). After the indicated treatments had been applied, the plants were grown in glass-faced root observation boxes for a period of one month.

Treatment [1]	Buds broken after one month (%)	Percent inhibition [2]	Ave. length of longest new shoot per seedling (inches)	Ave. no. of roots per seedling with new growth greater than 0.25 inches	Ave. no. of roots per seedling with new growth less than 0.25 inches
A	27.3	63	0.08	0.33	0.25
B	56.8	22	0.19	0.66	0.66
C	43.2	41	0.60	1.42	0.83
D	73.6	--	1.08	11.10	4.80

[1] Treatment Code:

- A One hour top-soak in 5000 ppm MH
- B One hour top-soak in 3000 ppm MH
- C One hour top-soak in 1000 ppm MH
- D Control—Seedlings soaked in solution containing few milliliters of solvent for MH

$$\text{Percent inhibition} = \frac{\%\text{broken buds in control} - \%\text{ broken buds in treatment}}{\%\text{broken buds in control}} \times 100$$

Results of the second experiment showed this concentration of MH and the shorter top-soaking period were still effective in retarding bud break (Table 4). After one month significantly fewer buds had opened on seedlings treated with MH as compared to seedlings in the two control treatments (E AND F). However, by the end of the second month only treatment D had significantly fewer broken buds. Thus, the effect of MH on bud break, under the conditions of this experiment, was to generally delay, rather than completely inhibit opening. As far as shoot growth was concerned, all groups of seedlings treated with MH, with the exception of treatment B, produced significantly shorter new shoots than did seedlings in the two control treatments.

Maleic hydrazide, when applied alone (treatment A), reduced root growth but not nearly to the extent as in the first experiment. There are several reasons for this. First, duration of the top-soak was only fifteen minutes in the second experiment rather than one hour, so less MH was absorbed. Also, root-pruning was less severe and the roots had an extra month to grow in this experiment. It is also probable that the mixture of sand, peat, and soil was a more favorable rooting medium than sand alone. In three of the four treatments where MH was used, the number of roots with new growth greater than 0.5 inch was greatly reduced from that of control seedlings (Table 4). However, treatment B, which employed a root-soak in 1 ppm IBA and 10 ppm IAA in combination with the top-soak in MH, produced an average of 100 such roots, the highest number of any treatment. There was no significant difference for this parameter of root growth among treatment B and the two controls, indicating that the negative effect of MH on the regenerating potential of the root system had been eliminated. The average length of the longest new root ranged from 5.3 to 8.1 inches (Table 4). The two control treatments produced the longest roots, but becuase of wide variation within treatments, no significant differences were indicated.

It was noted that the top-growth of the control group and the group treated with the solvent for MH was slightly more vigorous than that of other groups of seedlings. A subjective rating of seedling vigor gave the former two groups a rating of 4.0 out of a best possible score of 5.0. The average vigor of the latter groups of seedlings, all of which had been treated with MH, ranged from 3.4 to 3.9. The main reason for the lower rating was the development of a slight chlorosis on the older needles.

The greenhouse experiments showed that top growth of Douglas fir seedlings could be reduced temporarily without signigicantly affecting root growth if the proper combination of MH and auxins was employed. Under the conditions of the greenhouse experiments in this study, the proper combination was 1000 ppm MH for the top-soak solution and a mixture of 1 ppm IBA and 10 ppm IAA for the root-soak solution. It was decided to evaluate the effects of this treatment, along with several others, on the survival of field planted seedlings. One of the field treatments (C) had gibberellic acid included in the root-soak because Brian and Hemming (1957) postulated that MH inhibits the action of a "gibberellin-like" hormone in dwarf pea plants.

The summer of 1967 in northern Idaho was one of the hottest and driest on record. Daily air temperatures in excess of 100 degrees F and relative humidity readings of about 10 percent were common from late June to the middle of September. By late July the soil moisture content had dropped to the wilting

Table 4. The effect of maleic hydrazide, indolebutyric acid, and indoleacetic acid on bud break and growth of shoots and roots of 2-1 Douglas Fir seedlings (Greenhouse experiment II). After the indicated treatments had been applied, the plants were grown in pots for two months.

Treatment [1]	Buds broken after one month (%)	Buds broken after two months (%)	Ave. length of longest new shoot per seedling (inches)	Ave. no. of roots per seedling with new growth greater than 0.5 inches	Ave. length of longest new root per seedling (inches)
A	72.2	88.8	1.0	57.6	6.5
B	69.4	78.9	1.2	100.2	6.4
C	64.0	84.9	0.8	59.1	6.3
D	47.0	65.9	0.6	49.4	5.3
E	95.4	99.8	1.2	82.1	7.7
F	91.0	97.4	1.5	87.8	8.1

[1] Treatment Code:

A - 15 minute top-soak in 1000 ppm MH only
B - 15 minute top-soak in 1000 ppm MH, 2 hour root-soak in 1 ppm IBA and 10 ppm IAA
C - 15 minute top-soak in 1000 ppm MH, 2 hour root-soak in 10 ppm IAA and 10 ppm IBA
D - 15 minute top-soak in 1000 ppm MH, 2 hour root-soak in 10 ppm IAA
E - 15 minute top-soak in solution containing only 2 ml of diethylamine (solvent for MH) and distilled water
F - Control - Seedlings were planted with no soaking period for tops or roots

point and remained there the rest of the summer. Thus, it was an ideal situation for testing the effects of the growth substance treatments on increasing drought resistance of planted seedlings.

During the early part of the summer the only seedling mortality was cuased by the northern pocket gopher, *Thomomys talpoides*, and the Columbian ground squirrel, *Citellus columbianus*. A total of 35 seedlings of the 500 plants were destroyed by these animals. After June there was no further loss to this source, probably because forbs and grasses had become abundant on the planting site by that time.

Fig. 1 graphically depicts survival percentages of seedlings from the various treatments. On August 2, seedling survival exceeded 95 percent regardless of treatment. During the early weeks of August, seedling survival in treatments A, B, and D ranged from 10 to 20 percent higher than that of the control. The relatively lower survival rate for the control during early August was actually treatments A and D still exhibited survival rates higher than that of the control. However, because of the wide variation among replications within treatments, these differences were not significant. By September 26 only treatment D seedlings had a higher survival rate than the control seedlings. The final survival count was taken on October 10, at which time survival ranged from a low of 0.0 percent for treatment C to a high of 16.5 percent for treatment D.

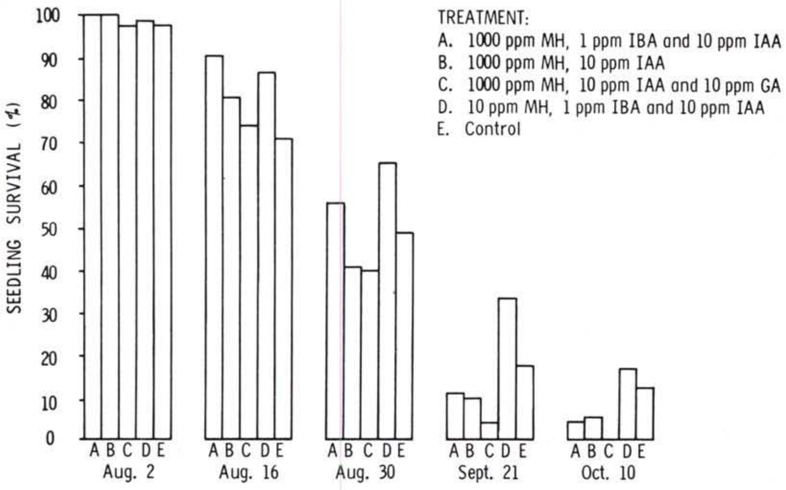

Fig. 1.
Survival of 2-1 Douglas Fir seedlings as affected by treatment with growth substances. Trees were planted at the Athol experimental site immediately after the indicated treatments had been applied.

Although final survival rates were extremely poor in all treatments, it is noteworthy that, for most of the season, treatments A and D produced the most favorable results. Treatment A had also yielded the most favorable results in the greenhouse. Treatment D had the same root-soak solution (1 ppm IBA and 10 ppm IAA) as treatment A, but the top-soak solution was cut to only 10 ppm MH. At this low concentration, there was no significant inhibition of bud break (Fig. 2), although the rate of bud break was significantly reduced in the three treatments where 1000 ppm MH was used. For example, on May 28, only 39.8, 33.2, 31.4 percent of the buds in treatments A, B, and C had opened, whereas 90.1 and 81.7 percent had opened in treatment D and the control. Despite the fact that 10 ppm MH did not delay bud break, the auxin combination used in the root soak solution may have stimulated root growth, thereby increasing the ability of seedlings to survive.

The length of the longest new shoot on each seedling was measured on June 21. This parameter of shoot growth averaged from .80 to .85 inches for treatment D and the control, respectively. The latter two treatments did not significantly differ from each other, but were significantly longer than the former three treatments. Thus, the field results supported findings from greenhouse experiments that MH concentrations of 1000 ppm delay and inhibit bud break of Douglas fir seedlings, as well as suppress shoot growth.

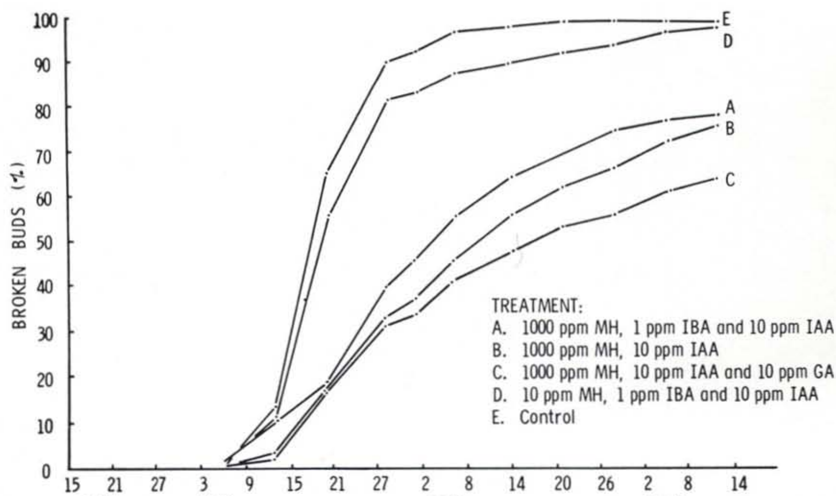

Fig. 2

The rate of bud break of 2-1 Douglas fir seedlings as influenced by various plant growth substances (field experiment). Trees were planted at the Athol experimental site immediately after the treatments had been applied.

Since none of the growth substance treatments significantly increased survival rates of planted seedlings, this aspect of the study was a failure. However, it is doubtful that any type of treatment, excluding irrigation, would have greatly increased seedling survival rates during this extremely dry summer. Treatments A and D perhaps would have increased survival rates under moderately droughty conditions, since for much of the growing season their survival rates were higher than that of the control seedlings.

SUMMARY

The effects of MH, a plant growth inhibitor, and two auxins (IBA and IAA) on growth and survival of Douglas fir seedlings were examined in greenhouse and field experiments. In the first greenhouse experiment, it was found that top-soaking seedlings for one hour in concentrations of 1000 to 5000 ppm MH would inhibit budbreak and shoot growth. However, root growth was also seriously curtailed and seedling vigor declined.

An attempt was made in the second greenhouse experiment to overcome these negative effects of MH on root growth and vigor by utilizing a shorter soaking period and by supplementing the MH treatment with a root-soak in certain auxin solutions. MH at a concentration of 1000 ppm was still effective in delaying bud break and reducing shoot growth, even after the roots were soaked in auxin solutions. Furthermore, a root-soak in a combination of 1 ppm IBA and 10 ppm IAA prevented the reduction in root growth caused by MH in other treatments.

Theoretically a reduction in top-growth coupled with an adequate root system should provide seedlings with greater ability to withstand drought. This hypotheses was tested with a field experiment at Athol, Idaho, in 1967. As in the greenhouse, bud break was suppressed by top-treatment with 1000 ppm MH. The effects of the root-soak treatments on root growth of field planted seedlings is not known. However, in those treatments where a combination of 1 ppm IBA and 10 ppm IAA were used, survival rates of seedlings for much of the growing season were higher than that of control seedlings. The growing season in 1967 in northern Idaho was so severe that it is unlikely that any growth substance treatment could have significantly increased survival of planted seedlings. Further study will be required to determine if alterations in the seedling's growth pattern with plant growth substances would be beneficial for survival.

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