EFFECTS OF TEMPERATURE ON PHYTOTOXICITY OF MONURON, PICLORAM, CDEC, EPTC, CDAA, AND SESONE TO YOUNG PINE SEEDLINGS[1].)

T. T. KOZLOWSKI [2].), S. SASAKI [2].), AND J. H. TORRIE [3].)

SELOSTE:

LÄMPÖTILAN VAIKUTUKSESTA MONURONIN, PIKLORAMIN, CDEC:N, EPTC:N, CDAA:N JA SESONEN MYRKYLLISYYTEEN NUORISSA MÄNNYNTAIMISSA

The apparent toxicity of soil-incorporated monuron, picloram, CDEC, EPTC, CDAA, and sesone, to young *Pinus resinosa* Ait. seedlings was studied over a temperature range of 10 to 30 C in growth chambers. The herbicides were first applied to the surface of autoclaved soil at 1 1b/A and later mixed into the soil. Thereafter pine seeds were planted and subsequent seedling development was studied. The effects of CDEC, EPTC, CDAA, and sesone were also studied at dosages of 2 and 3 1b/A (soil surface basis). Under the conditions of this study, picloram and monuron were persistent in the soil and toxic to pine seedlings, whereas CDEC, EPTC, CDAA, and sesone appeared to be non-toxic. However, the apparent lack of phytotoxicity of the latter group apparently was caused largely by lack of activation of sesone by autoclaving soil and large losses from the soil of CDEC, EPTC, and CDAA even before seeds were planted. High toxicity of picloram and monuron was shown by reductions in seedling survival, total dry weight increment of plants, and dry weight increment of surviving seedlings. Various temperature regimes greatly affected growth of herbicide-treated plants and controls. In control plants both high and low temperatures adversely affected seedling survival and dry weight increment of roots and shoots. Temperature extremes generally inhibited root growth more than shoot growth. The high temperatures, 25 and 30 C, markedly enhanced phytotoxicity of picloram and monuron.

2.) Department of Forestry, University of Wisconsin, Madison, Wisconsin, U.S.A.
3.) Department of Agronomy, University of Wisconsin, Madison, Wisconsin, U.S.A.

Introduction

Herbicide toxicity appears to be influenced by temperature in a complex manner (MARTH and DAVIS, 1945; MUZIK and MAULDIN, 1964). KOZLOWSKI, SASAKI and TORRIE (1967) concluded that the influence of high temperatures in enhancing triazine toxicity involved complex interactions of physiological activity and temperature effects on herbicide uptake. ÅSLANDER (1950) demonstrated that several species of herbaceous plants were killed more quickly by 2,4-D at air temperatures of 25 than 20 C. Temperature regimes before and after herbicide treatment often influence the sensitivity of plants to herbicides at a specific temperature. KELLY (1949) noted, for example, that when bean plants were maintained at 5, 15, or 25 C for a week before being sprayed with 2,4-D and subsequently maintained at 15 C, those preconditioned at 25 C were most sensitive to the herbicide.

Although many examples are available of seasonal responses of plants to herbicides (HAMNER and TUKEY, 1944; HYDER, 1953), the influence of individual environmental factors on herbicides is difficult to interpret in field experiments. Temperature, precipitation, and stage of plant growth may interact to influence sensitivity of plants to herbicides. The present experiments were conducted in growth chambers to evaluate the effects of 5 herbicides, which varied in structure and persistence in soil, on growth and development of young *Pinus resinosa* seedlings under various constant temperature regimes.

Methods

Herbicides tested included monuron (3-(p-clorophenyl)-1, 1-dimethylurea), picloram (4-amino-3, 5, 6-trichloropicolinic acid, potassium salt), CDEC (2-chloroallyl diethyldithiocarbamate), EPTC(ethyl N, N-di-n-propylthiolcarbamate), CDAA(2-chloro-N, N-diallylacetamide), and sesone(sodium 2,4-dichlorophenoxyethyl sulfate). Each herbicide was applied as a spray with an atomizer at 1 1b/A to the surface of each of 2 greenhouse flats (14 in. × 20 in.) of previously autoclaved greenhouse soil (2 parts loam, 1 part sand) which had been placed in the flats to a 4-inch depth. Three days later the herbicide-treated soils were separately placed in a Patterson-Kelly twin shell blender and thoroughly mixed for 10 minutes. Two control flats of soil which had not received herbicides were similarly mixed. The soil with incorporated herbicide was replaced into flats which were returned to the greenhouse. Three days later the soil plus herbicide was distributed among 57 cylindrical paper cartons of one pint capacity each. Seven days later each carton was planted with 100 red pine *(Pinus resinosa* Ait.) seeds obtained from a northeastern Minnesota source. The seeds were covered with autoclaved sand that was sprinkled with arasan.

The cartons, with holes in the bottom for drainage, were placed on a greenhouse bench and watered daily from above with a sprinkling can. Seed germination began in about 10 days. Thirty one days after planting seeds, 7 randomly selected cartons from each herbicide treatment and controls were selected for counts of live seedlings and harvesting to determine their dry weights at the beginning of the experiment. Ten of the remaining cartons of each herbicide treatment and untreated control were then placed in each of 5 plant growth chambers maintained continuously at 10, 15, 20, 25 and 30° C. Hence, each chamber contained 70 cartons representing 10 replications (cartons) of each of 6 herbicide treatments and 10 control cartons. The light intensity in each chamber was maintained at 1500 foot candles at leaf level on a 19-hour day. Relative humidity was 70 to 90%.

The cartons were watered daily and the condition of the seedlings in each temperature regime periodically noted. Survival counts were taken weekly for each carton for 15 weeks. The shoots of live seedlings were then harvested for dry weight determinations. In addition the roots in each carton were carefully washed out and their dry weights determined. Shoot-root ratios were calculated for plants of each carton at the end of 15 weeks.

As no marked herbicidal activity of CDEC, EPTC, CDAA, and sesone at 1 1b/A was shown, the dosage of these herbicides was increased to 2 and 3 1b/A and the experiment was repeated. However, after the herbicides were sprayed on the soil surface, the flats were left exposed in the greenhouse for 4 days and then mixed. After mixing, the soils plus herbicide were put back in the flats and left exposed in the greenhouse for 5 weeks after which they were placed in cartons and planted with red pine seeds. After cartons were placed in the growth chambers no obvious phytotoxicity was observed after 5 weeks at various temperature regimes and plants were harvested at that time.

The numbers of live seedlings in certain cartons were extremely low at the time they were placed in the growth chambers. Such cartons occurred in most treatments including controls. No reason can be offered for the low germinations. A procedure developed by DIXON (1950) was used to determine which cartons differed significantly at the 5% level for number of live seedlings from other cartons within a treatment. These were not included in the analysis of the data and amounted to 4% of the cartons.

Results

Effects of monuron, picloram, CDEC, CDAA, EPTC, and sesone at 1 lb/A. The differences in average number of live seedlings for various herbicide treatments were not significant before plants were placed in growth chambers. However, the original dry weights of picloram-treated seedlings were significantly higher than those of other treatments. Among the other treatments there were

no striking differences in dry weights, although those of CDEC-treated seedlings were slightly lower than in controls.

Pronounced effects of temperature on development of pine seedlings were observed under herbicide treatments and in controls. In control plants both high and low temperatures adversely affected various aspects of seedling growth including survival and dry weight increment of various tissues (Fig. 1, 8, 9, 10, 11, 12). After 15 weeks, lowest mortality and highest dry weight production of control seedlings occurred at 20 and 25 C respectively. For the first four weeks the number of live seedlings varied little in the different temperature regimes. At 15 weeks, however, the number of live seedlings at 10 and 30 C was significantly lower than at 20 C. Dry weight production of control plants at harvest was greatest at 25 C and was reduced much more at temperatures of 10 and 15 C than at 30 C (Fig. 8).

Apparent herbicidal activity varied greatly under the different temperature regimes. Whereas monuron and picloram showed marked phytotoxicity at high temperatures, the other herbicides generally showed little or none (Fig. 2 to 7). The overall high phytotoxicity of picloram and monuron was variously shown. For example, numbers of live seedlings were lower in picloram treatment at all temperature regimes, and in monuron treatment at temperature regimes 20 C and higher, than for controls (Fig. 2, 3). Likewise plant dry weights and shoot dry weights were significantly lower than controls at all temperature regimes greater than 10 C for both picloram and monuron (Fig. 8 and 9). Plants treated with either of these herbicides had lower total dry weights of roots (per carton) than controls at all temperatures (Fig. 10). Furthermore, dry weights of shoots of surviving seedlings were lowered by picloram or monuron, except at the 2 highest temperatures. In contrast, dry weights of shoots of sesone-treated surviving plants were greater than those of controls (Fig. 11).

The high temperatures, 25 and 30 C, increased toxicity of both picloram and monuron greatly, but monuron toxicity was increased more. This is shown by more rapid killing of monuron-treated seedlings over those treated with picloram (Fig. 2, 3).

At the time plants were placed in the growth chambers the number of seedlings was lower under picloram than under monuron treatment. Nevertheless, the seedling mortality rate thereafter was higher in the monuron-treated plants. At low temperatures, 10 and 15 C, neither monuron nor picloram killed many seedlings. Yet both of these herbicides were toxic at the low temperatures as shown by reductions at 15 weeks in dry weights of herbicide treated plants (Fig. 8).

Temperature extremes, in both herbicide treatments and controls, generally inhibited root growth more than shoot growth (Fig. 12). However, greater inhibition of root growth relative to shoot growth occurred at the lowest (10 C) than the highest (30 C) temperature. When herbicide toxicity was evident it usually was reflected more in decreased growth of roots than of shoots. Whereas
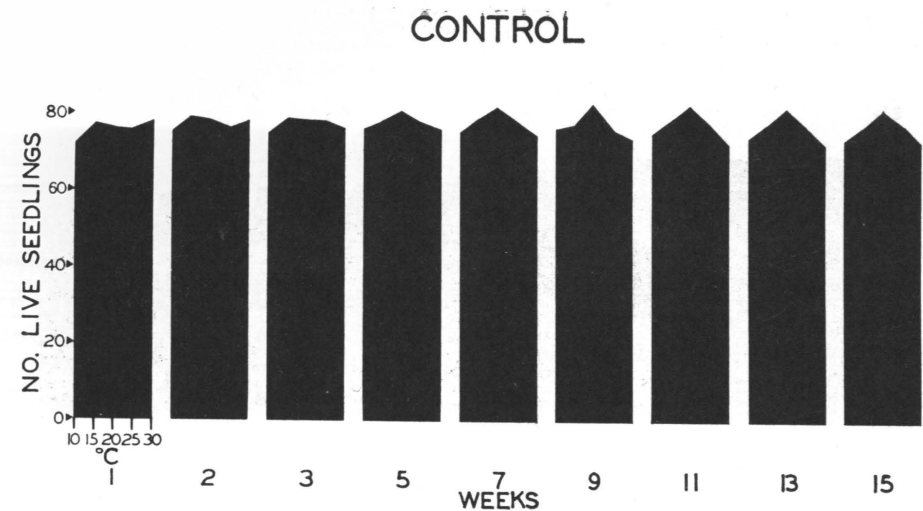
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Figure 1. Seedling survival of control plants at various temperature regimes.
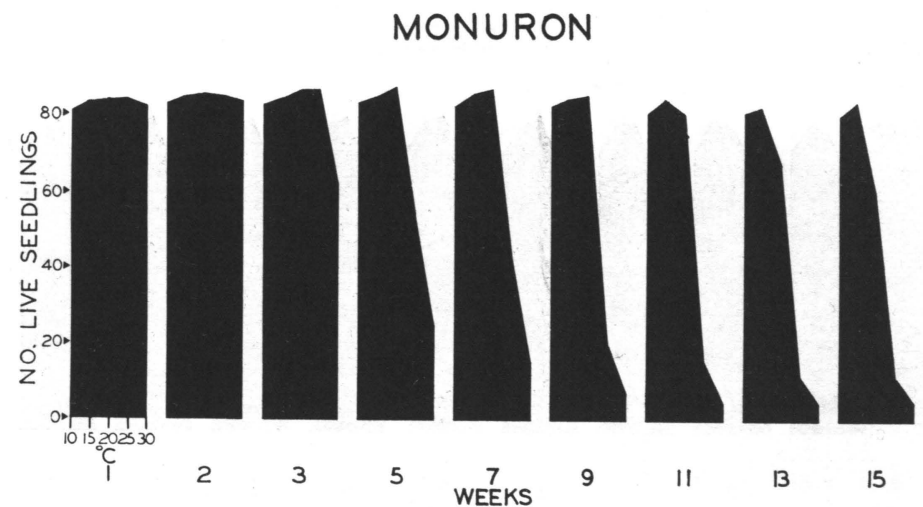*Kuva 1. Vertailutaimien eloonjääminen erilaisissa lämpötiloissa.*

Figure 2. Effect of monuron at 1 lb/A (soil surface basis) incorporated in soil on seedling survival at various temperature regimes.
Kuva 2. Monuronin vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annostusta eekkeriä kohden (n. 1 kg/ha) maahan sekoitettuna.
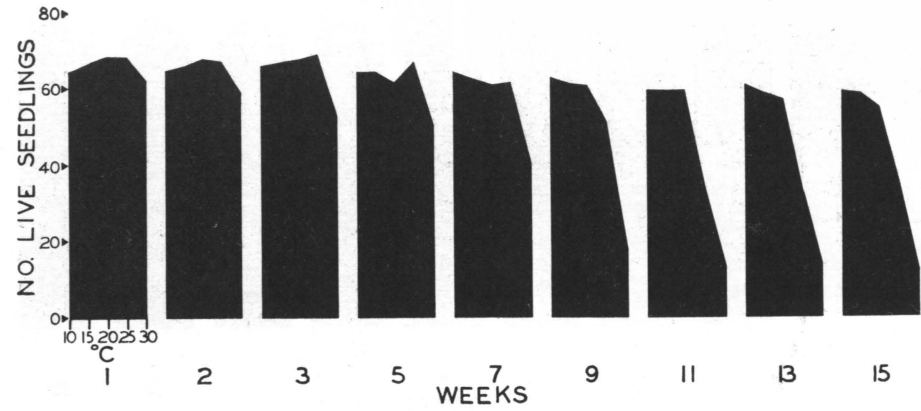
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PICLORAM

Figure 3. Effect of picloram at 1 lb/A (soil-surface basis) incorporated in soil on seedling survival at various temperature regimes.

Kuva 3. Pikloramin vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annotusta eekkeriä kohden maahan sekoitettuna.

CDEC

Figure 4. Effect of CDEC at 1 lb/A (soil-surface basis) incorporated in soil on seedling survival at various temperature regimes.

Kuva 4. CDEC:n vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annostusta eekkeriä kohden maahan sekoitettuna.
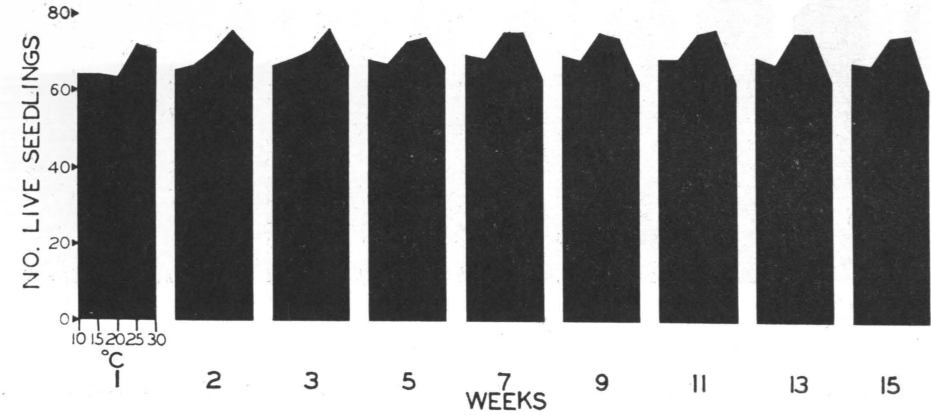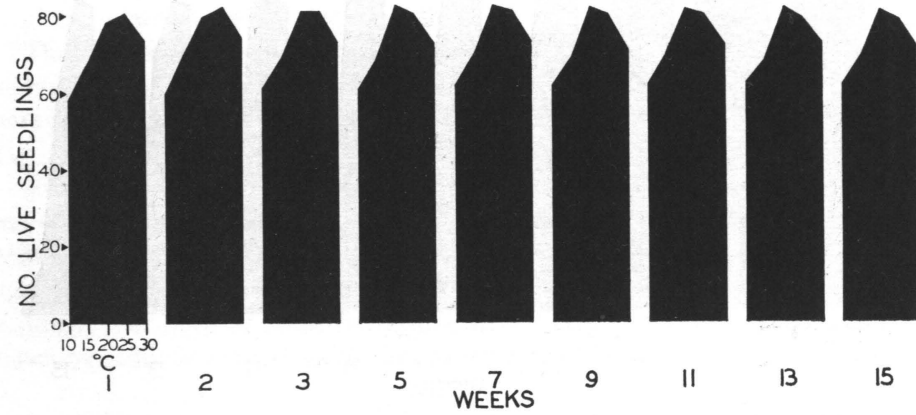
CDAA

Figure 5. Effect of CDAA at 1 lb/A (soil surface basis) incorporated in soil on seedling survival at various temperature regimes.

Kuva 5. CDAA:n vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annostusta eekkeriä kohden maahan sekoitettuna.
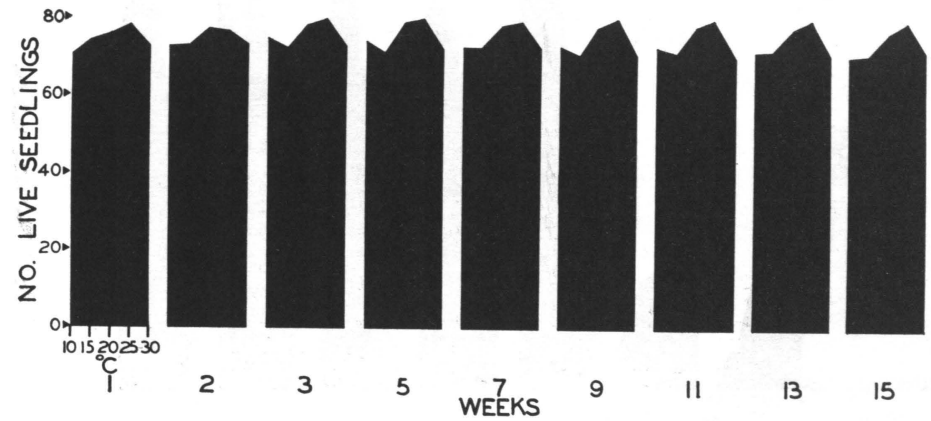
EPTC

Figure 6. Effect of EPTC at 1 lb/A (soil surface basis) incorporated in soil on seedling surviva at various temperature regimes.

Kuva 6. EPTC:n vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annostusta eekkeriä kohden maahan sekoitettuna.
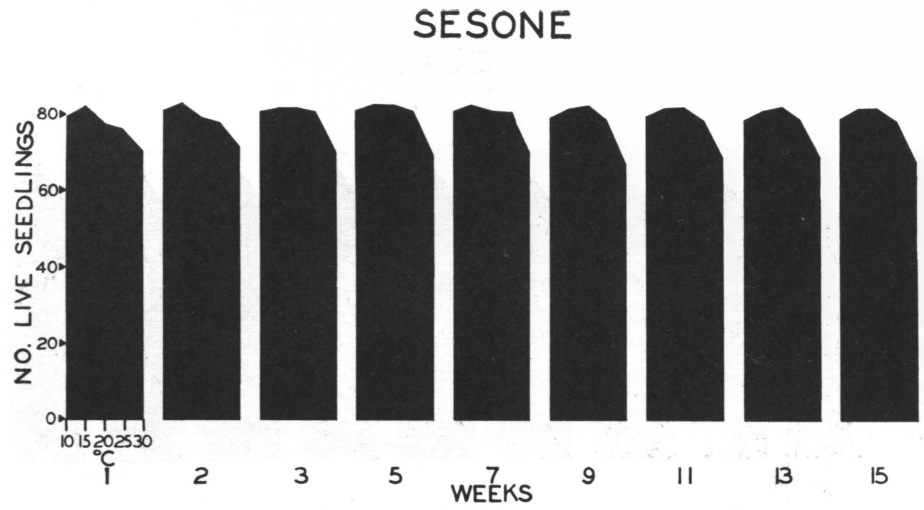
SESONE

Figure 7. Effect of sesone at 1 1b/A (soil surface basis) incorporated in soil on seedling survival at various temperature regimes.

Kuva 7. Sesonen vaikutus taimien eloonjäämiseen eri lämpötiloissa käytettäessä 1 naulan annostusta eekkeriä kohden maahan sekoitettuna.

DRY WEIGHT PLANTS

Figure 8. Effect of herbicides on total dry weights of plants at various temperature regimes.
Kuva 8. Kasvimyrkkyjen vaikutus taimien kokonaiskuivapainoon eri lämpötiloissa.

DRY WEIGHT SHOOTS

Figure 9. Effects of herbicides on total dry weights of shoots of plants at various temperature regimes.
Kuva 9. Kasvimyrkkyjen vaikutus taimiversojen kokonaiskuivapainoon eri lämpötiloissa.
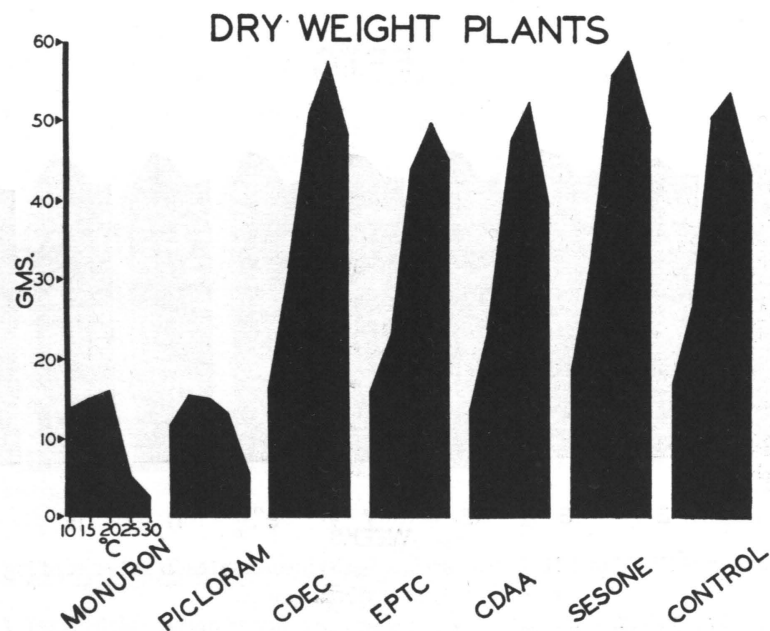
DRY WEIGHT ROOTS
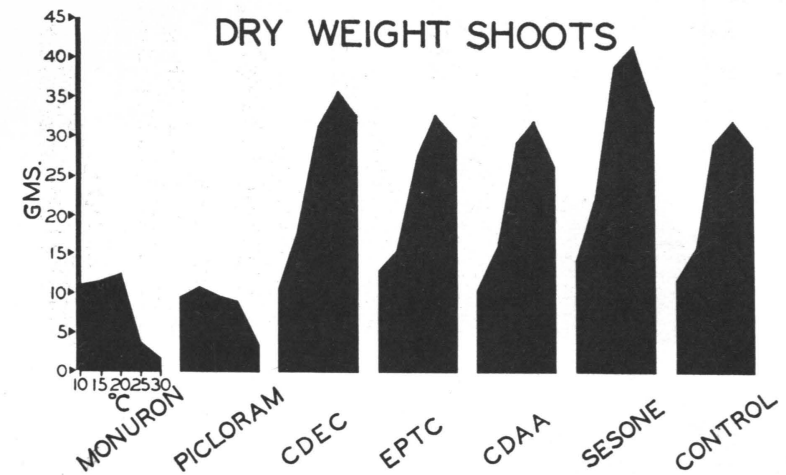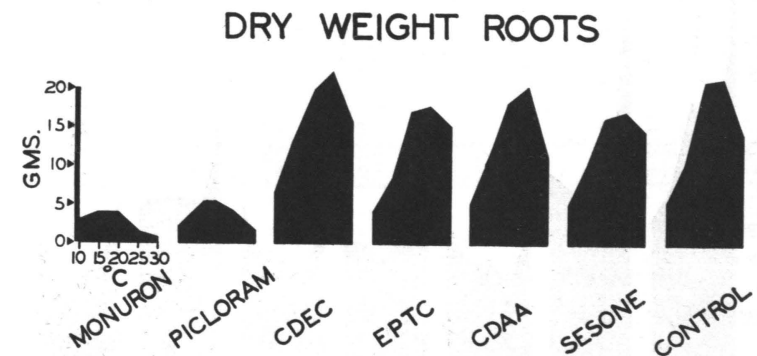

Figure 10. Effects of herbicides on total dry weight of roots of plants at various temperature regimes.
Kuva 10. Kasvimyrkkyjen vaikutus taimien juurten kokonaiskuivapainoon eri lämpötiloissa.
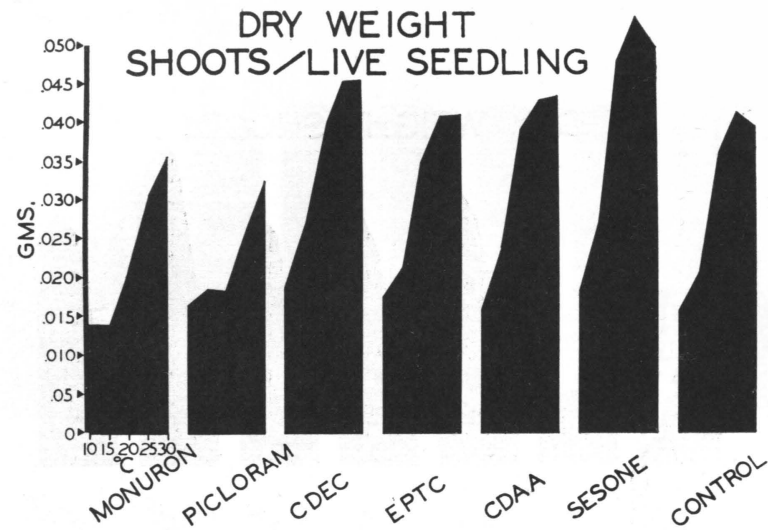
Figure 11. Effects of herbicides on average dry weight of shoots of surviving seedlings at various temperature regimes.

Kuva 11. Kasvimyrkkyjen vaikutus eloonjääneiden taimiversojen keskimääräiseen kuivapainoon eri lämpötiloissa.
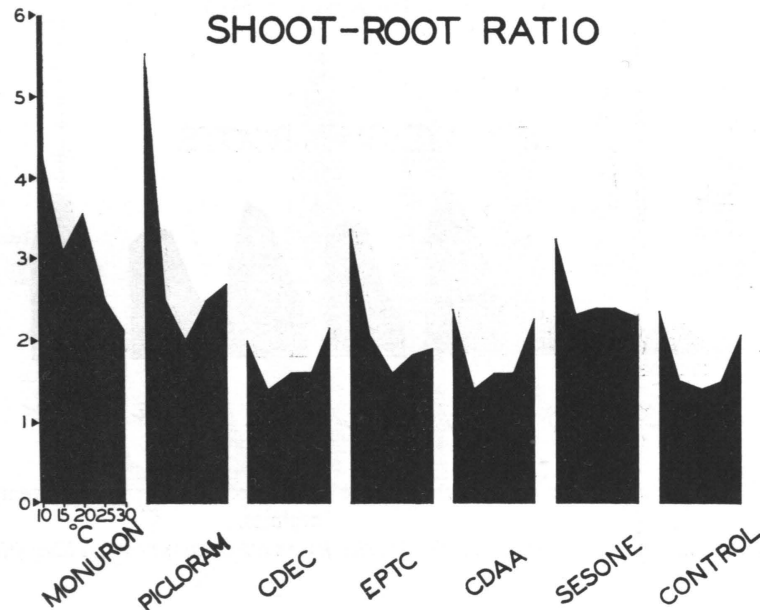

Figure 12. Effects of herbicides on shoot-root ratios of plants at various temperature regimes.

Kuva 12. Kasvimyrkkyjen vaikutus taimien verson ja juuriston suhteeseen eri lämpötiloissa.

picloram and monuron significantly depressed shoot growth at all temperatures except 10 C, these two herbicides depressed root growth at all temperatures and to a greater degree. Monuron and picloram inhibited root growth over shoot growth to a greater extent than the other herbicides tested. This was especially true at low temperatures (Fig. 12). At 10 C shoot-root ratios of plants at harvest were the following for various treatments: picloram, 5.6; monuron, 4.3; EPTC, 3.4; sesone, 3.3; CDAA, 2.4; CDEC, 2.0; and control 2.4.

Effects of CDEC, EPTC, CDAA, and sesone at 2 and 3 lb/A. The differences for numbers and dry weights of seedlings for various herbicide treatment at 2 or 3 1b/A were not significantly different before plants were placed in the growth chambers.

Table 1. Effects of EPTC, sesone, CDEC, and CDAA at 2 and 3 1b/A applied to soil surface and then incorporated in soil on various aspects of growth of red pine seedlings. Plants were harvested after 5 weeks at indicated temperatures.

Taulukko 1. EPTC:n, sesonen, CDEC:n ja CDAA:n vaikutus Pinus resinosan taimien kasvuun annostuksen ollessa 2 sekä 3 naulaa eekkeriä kohden (n. 2 ja 3 kg/ha). Aineet levitettiin maan pinnalle, minkä jälkeen ne sekoitettiin maahan. Taimet korjattiin viiden viikon kuluttua.

Treatment Käsittely	Temperature (C) — Lämpötila (°C)									
	10	15	20	25	30	10	15	20	25	30
	Average No. of live seedlings Elävien taimien lukumäärä					Dry Weight of shoots (g) Versojen kuivapaino (g)				
EPTC, 2 1b/A	73.2**	74.1	62.7	63.6	55.4	1.11	1.56	1.96*	2.14	1.61
EPTC, 3 1b/A	55.2	69.6	60.3	59.1	53.4	0.82	1.86*	2.05*	1.87	1.56
Sesone, 2 1b/A	62.5	56.0*	61.8	71.2**	61.7*	0.95	1.29	1.94*	2.31	1.78
Sesone, 3 1b/A	70.4**	76.3	69.8	56.5	66.8**	1.06	1.97**	2.33**	1.99	2.00**
CDEC, 2 1b/A	75.0**	76.2	74.0	64.7	56.8	1.22	2.03**	2.72**	2.27	1.96*
CDEC, 3 1b/A	70.0**	68.8	67.7	72.0**	70.2**	1.11	1.90*	2.43**	2.66**	2.11**
CDAA, 2 1b/A	66.3*	75.3	64.4	66.4*	65.3*	1.03	1.92*	1.88	2.06	1.93*
CDAA, 3 1b/A	70.7**	61.7	54.8	67.2*	59.5	1.04	1.62	1.55	2.23	1.75
Control, no herbicide	53.3	70.0	64.1	54.3	48.1	0.81	1.50	1.61	2.03	1.51
	Dry Weight of roots (g) Juurten kuivapaino (g)					Shoot-root ratio Versojen suhde juuriin				
EPTC, 2 1b/A	0.21	0.32**	0.47*	0.52	0.39	5.29	4.88**	4.17**	4.12**	4.13**
EPTC, 3 1b/A	0.17	0.43	0.68	0.76*	0.48	4.82	4.33**	3.01	2.46**	3.25
Sesone, 2 1b/A	0.20	0.37*	0.64	0.71	0.52	4.75	3.49**	3.03**	3.25	3.42
Sesone, 3 1b/A	0.21	0.54	0.75	0.61	0.56	5.05	3.65**	3.11**	3.26	3.57**
CDEC, 2 1b/A	0.27	0.46	0.78	0.57	0.51	4.51	4.41**	3.49**	3.98**	3.84**
CDEC, 3 1b/A	0.19	0.50	0.73	0.81*	0.62	5.84**	3.80**	3.33**	3.28	3.40
CDAA, 2 1b/A	0.23	0.56	0.62	0.47*	0.52	4.47	3.43*	3.03*	4.38**	3.71
CDAA, 3 1b/A	0.17	0.42	0.57	0.60	0.51	6.11**	3.86**	2.72	3.72**	3.43
Control, no herbicide	0.16	0.51	0.65	0.62	0.47	5.06	2.94	2.48	3.27	3.21

*, ** indicate different from the control at the 5 % and 1 % levels respectively

*, ** *osoittavat eroja vertailuaineistosta 5 ja 1 %:n tasoilla.*

After 5 weeks no marked phytotoxicity of the herbicides was shown at either 2 or 3 1b/A. No general pattern emerged in the effect of herbicides on numbers of seedlings, shoot dry weight, root dry weight, or shoot-root ratios at harvest (Table 1). Effects of temperature regimes were apparent, however, with growth generally reduced at the highest and lowest temperature over intermediate ones. Growth of seedlings was not consistently greatest at a particular intermediate temperature.

Discussion

Marked variations were shown in apparent toxicity of soil-incorporated herbicides under the conditions of these experiments. The differences in plant response to various herbicide treatments undoubtedly largely reflected rapid early losses of certain herbicides and not others. Hence, the differences in apparent toxicity probably indicate variations in herbicide persistence to a greater extent than differences in absolute toxicity among the compounds tested.

Whereas picloram and monuron at 1 1b/A (soil surface basis) were very toxic to red pine seedlings, particularly at high temperatures, sesone, CDEC, CDAA, and EPTC did not appear to be toxic, but sesone was inactivated by autoclaving the soil and the other herbicides undoubtedly were largely lost from the soil even before seeds were planted.

Even the most persistent and toxic herbicides of the present study, picloram and monuron, were considerably less toxic and slower acting than were certain triazines. For example, in a comparative study, Kozlowski, Sasaki, and Torrie (1967) found that several triazine herbicides killed red pine seedlings much faster than picloram or monuron did in the present work. For example, at 30 C seedling mortality at the end of 7 weeks was 100% under atrazine or simazine treatment at 1 1b/A, 92% for prometone, 57% for propazine, 55% for prometryne, and 84% for ipazine. Whereas, with picloram and monuron phototoxicity was restricted largely to high temperatures, the triazines were toxic over a considerably greater temperature range. Furthermore, monuron and picloram did not begin to kill appreciable numbers of seedlings at high temperatures until 3 weeks after they were put in the growth chambers whereas the triazines at the same dosages often killed seedlings within a week.

In the present experiments the concentrations of non-persistent herbicides undoubtedly rapidly decreased after application whereas those of persistent ones did not. After herbicides are applied to soils they are subjected to the action of several processes which promote their inactivation. These processes include metabolism by soil microorganisms, adsorption to mineral and organic colloids, leaching by rain or irrigation water, non-biological chemical reactions, photochemical alterations, volatilization, and absorption by higher plants. These processes are directly and indirectly influenced by various soil properties, herbicide properties, and environmental variables (Sheets, 1964).

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The relatively high toxicity of soil-incorporated picloram and monuron in the present experiments is in accord with observations on persistence of these herbicides. Problems with monuron persistence sometimes occur. When the herbicide is applied for selective weed control in resistant crops, sufficient residues sometimes remain in the soil to injure susceptible crops the following year (Sheets, 1958; Arle, Miller, and Sheets, 1965). Monuron applied at 1 to 2 1b/A to irrigated cotton soils appeared to persist from one application to the next. Differences between populations on control and monuron plots during the third and subsequent years appeared to be due to residual activity of the herbicide. However, Hill et al (1955) presented data from the humid cotton growing region indicating that monuron applied at 1 to 2 1b/A 2 years in succession disappeared from the soil in 4 to 8 months after application. In the present experiments the soil was autoclaved before herbicides were applied. Inactivation of phenylurea herbicides occurs under soil conditions favorable for growth of microorganisms, but in dry or autoclaved soils activity is retained for a long time (Ogle and Warren, 1954; Sheets and Crafts, 1957). Loss of phenylureas by volatilization is low and apparent only when the herbicide remains on the surface of the soil in a hot and dry environment (Hill et al, 1955). Picloram also is somewhat persistent. Herr, Stroube and Ray (1966) found little or no residues of picloram in silt loam 15 months after application up to 8 oz/A. When applied at higher dosages, however, herbicide residues were found after 15 months.

Under the conditions of the present experiment EPTC, CDEC, sesone, and CDAA at 2 and 3 1b/A appeared to show no obvious toxicity (Table 1). Significant increases in final number of seedlings, dry weights of shoots, dry weight of roots, and shoot-root ratios over controls probably were traceable to unexplainable low averages for control seedlings at the time plants were placed in the growth chambers rather than to stimulation of growth by these herbicides.

The lack of apparent toxicity of EPTC, CDEC, CDAA, and sesone to young red pine seedlings at 2 and 3 1b/A in the present study was at variance with other studies showing that these herbicides injured pine seedlings (Kozlowski and Kuntz, 1963; Kozlowski and Torrie, 1965). This observation together with published reports indicates that, under the conditions of the present experiment, much of the nonpersistent herbicides, was lost before the plants were placed in the growth chambers. The use of autoclaved soil accounted for lack of sesone toxicity, as sesone requires activation by soil organisms.

Evidence for herbicide loss exists in the lack of demonstrated decrease in seedling growth at a 3 1b/A dosage over 2 1b/A. Furthermore, EPTC and CDAA at 1 1b/A reduced root growth over controls at certain temperatures but did not do so at the higher dosages. This discrepancy undoubtedly is the result of much greater loss of the herbicides when they were applied at the higher dosages. Flats of soil that were surface treated with 2 or 3 1b/A of these herbicides were left longer on greenhouse benches before mixing than were flats treated at 1 1b/A. Also more time elapsed between mixing of soil and herbicide and planting

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seeds. Hence, losses by volatility undoubtedly were great for non-persistent herbicides applied at the high dosages.

Considerable evidence is available that EPTC is readily lost from soils by volatilization. GRAY (1965) found that 22 to 38% of EPTC applied to the surface of wet soils was lost depending on the soil type. Much more herbicide was lost from moist than from dry soils. Letting the surface of freshly worked moist soils dry out before spraying with EPTC greatly reduced loss by vaporization. Immediate incorporation prevented any loss of EPTC from dry soil and greatly reduced the loss from moist soil. After spraying EPTC on dry soil, sprinkling with small amounts of water increased loss of the herbicide. GRAY and WEIERICH (1965) sprayed EPTC to the surface of dry, moist, or wet loamy sand contained in small aluminum flats in the greenhouse. The rate of loss of EPTC was greatly increased as soil moisture was increased. The greatest rate of loss on dry, moist, and wet soils occurred during the first 15 minutes after spraying EPTC on the soil. On dry soils, the rate of loss of EPTC slowed down after the first 15 minutes so that little more loss occurred the first few hours, or even in 24 hours. This indicated that most of the vaporization of EPTC from dry soil occurred while the spray was drying, which usually required about 10 minutes. After the spray dried, EPTC appeared to be adsorbed rather strongly to the dry soil. Loss of CDAA and CDEC in the present study apparently also occurred. When KOZLOWSKI and TORRIE (1965) rapidly incorporated CDAA or CDEC at 2 1b/A in Plainfield sand in which red pine seedlings were grown, both seedling survival and dry weight increment were reduced. Although excellent weed control often is obtained from CDAA it is susceptible to both evaporation and leaching. The herbicide is relatively volatile but is either rapidly adsorbed by soil and gradually released or very small quantities of CDAA must be required to control weeds effectively. When left on the soil surface CDAA is rapidly lost. Since most of this loss occurs within a few hours after application, the herbicide should be incorporated in the soil as soon as possible after application (DEMING, 1963).

The greater inhibition of root growth than shoot growth by toxic herbicides in the present study was striking. This general effect deserves further study because it suggests that certain mildly toxic herbicides, which do not kill seedlings, might affect their plantability by causing them to develop stunted root systems that will be inadequate for normal growth and survival after outplanting.

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SELOSTE:

LÄMPÖTILAN VAIKUTUKSESTA MONURONIN, PIKLORAMIN, CDEC:N,
EPTC:N, CDAA:N JA SESONEN MYRKYLLISYYTEEN NUORISSA
MÄNNYNTAIMISSA.

Maahan lisätyn monuronin, pikloramin, CDEC:n, EPTC:n, CDAA:n ja sesonen silmin havaittavaa myrkyllisyyttä *Pinus resinosan* Ait. taimille tutkittiin
kasvihuoneessa 10—30 C asteen lämpötiloissa. Kasvimyrkyt levitettiin ensiksi
autoklaavissa steriloidun maan pinnalle ja sekoitettiin myöhemmin maahan.
Käytetty annostus oli 1 naula eekkeriä kohden (n. 1 kg/ha). Tämän jälkeen
männyntaimet istutettiin ja ryhdyttiin seuraamaan niiden kehitystä. CDEC:n,
EPTC:n, CDAA:n sekä sesonen vaikutusta tutkittiin myös annostuksen ollessa
2 ja 3 naulaa eekkeriä kohden (n. 2 ja 3 kg/ha).

Tämän tutkimuksen olosuhteissa piklorami ja monuroni osoittautuivat sekä
maassa pysyviksi että myrkyllisiksi männyntaimille, kun taas CDEC, EPTC,
CDAA ja sesone eivät aiheuttaneet myrkytysoireita. Myrkyllisyyden puuttuminen jälkimmäisestä ryhmästä johtui ilmeisesti suureksi osaksi siitä, että sesone
ei aktivoitunut autoklaavissa steriloidussa maassa, sekä CDEC-, EPTC- ja
CDAA-häviöstä maasta jo ennen taimien istuttamista. Pikloramin ja monuronin
suuri myrkyllisyys ilmeni taimien kuolleisuudessa, kasvien alhaisessa kuivapainossa sekä eloon jääneiden taimien kuivapainon hitaassa lisääntymisessä.

Erilaiset kasvuympäristön lämpötilat vaikuttivat suuresti sekä kasvimyrkyillä käsiteltyihin että vertailutaimiin. Vertailutaimien kohdalla sekä korkeat että
matalat lämpötilat vaikuttivat kuolleisuuteen ja juurten ja versojen kuivapainon kasvuun, joskin vastakkaisiin suuntiin. Äärilämpötilat haittasivat yleensä
juurten kasvua enemmän kuin verson kasvua. Korkeat lämpötilat, 25 C ja 30 C
asetetta lisäsivät huomattavasti pikloramin ja monuronin myrkyllisyyttä.