

PESTICIDE PHYTOTOXICITY

STUDIES WITH SEEDS, GERMINANTS
AND SEEDLINGS OF
BRITISH COLUMBIA CONIFERS

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ABSTRACT

Greenhouse and field experiments were made to determine possible phytotoxic effects of the petroleum weed killers AWK No. 1, AWK No. 1 Special, the insecticides diazinon, Dylox, malathion and Sevin, and the fungicides Benlate, Dexon and Neutro Cop "53" to developmental stages (ranging from seeds to young seedlings) of Douglas-fir (coastal and interior forms), Sitka spruce, white spruce, western hemlock and lodgepole pine. AWK No. 1 and AWK No. 1 Special were often toxic to seedlings in the greenhouse, but not in the field. Dexon was frequently phytotoxic to several developmental stages of most seedling species, both in the greenhouse and the field. Phytotoxicity, when present, usually decreased as seedling age increased. Results are summarized in a table.

RÉSUMÉ

On a procédé à certaines expériences en serre et sur la terrain pour déterminer les effets phytotoxiques possibles des herbicides à base de pétrole AWK No. 1, AWK No. 1 Spécial, les insecticides Diazinon, Dylox, Malathion et Sevin, puis les fongicides Benlate, Dexon et Neutro Cop "53", sur les semences, les plantules et les jeunes semis de Douglas taxifolié (de la côte ou de l'intérieur), de Pin tordu, d'Epinette de Sitka, d'Epinette blanche et de Pruche occidentale. AWK No. 1 et AWK No. 1 Spécial furent fréquemment toxiques pour des semis en serre, mais non sur le terrain. Le Dexon fut ordinairement phytotoxique pour plusieurs espèces de semis et pour les plantes se développant tant en serre que sur le terrain. Lorsque présente, la phytotoxicité diminuait habituellement à mesure que les semis prenaient de l'âge. Le tableau 1 résume sommairement les résultats des diverses expériences.

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INTRODUCTION

Before using pesticides on conifer seedlings, nurserymen usually ask: What chemicals should be used? What possibility is there that the chemical's phytotoxicity will outweigh the benefit of pest control? When the answer to the first question is known, doubt still exists regarding possible phytotoxic effects, especially in relation to seedling species and age. In 1973, greenhouse trials were begun to determine the phytotoxic effects of selected weed killers, insecticides and fungicides on seeds, germinants and young seedlings of coniferous species commonly grown in British Columbia Forest Service (BCFS) nurseries. Some pesticides tested were in current use, but their phytotoxic potential was unknown; others were tested because of their potential usefulness. Greenhouse trials in the winter of 1974 and the spring of 1975 were followed by tests of the materials under nursery conditions, in 1976.

MATERIALS AND METHODS

Greenhouse experiments. On February 11, 1974, and between April 4 - 18, 1975, stratified (5) seeds of Douglas-fir, Sitka spruce, white spruce, western hemlock and lodgepole pine were sown in plastic, 2.5 x 11.4 cm bullets (2) containing an unfertilized, water saturated, 50:50 mixture of sand and peat moss. Dates of sowing the seeds in 1975 were staggered to facilitate subsequent evaluation of the experiment. Scientific names of tree species, germination capacities of the seeds and BCFS seedlot numbers are given in Appendix I. Seeds were covered with sterilized number 2 grit (2) and overhead watering was done as needed. Two days before applying the treatments, one of the two germinants per bullet was removed, ensuring that one germinant per bullet was treated at the proper developmental stage.

The pesticides tested were:

<u>Usages, trade name, and manufacturer</u>	<u>Common Name</u>	<u>Chemical name or composition</u>	<u>Dosage rate (all gallons are Imperial gallons) and details of application</u>
<u>Weed killers:</u>			
AWK No. 1, Shell	Agricultural weed killer	13 to 24%, unsaturated aromatic compounds in 100% petroleum oil	60 gal per surface acre (674 ℓ per ha) in the greenhouse and 45 gal per acre (505.5 ℓ per ha) in the field. Sprayed on soil or seedling shoots.
AWK No. 1, Special, Shell	As above	12 to 18% unsaturated aromatic compounds in 100% petroleum oil	As above
<u>Fungicides:</u>			
Benlate 50 WP, Dupont	benomyl	methyl 1-(butyl-carbamoyl)-2-benzimidazole-carbamate	8 oz a.i. in 100 gal of water per surface acre (560.4 g Benlate in 1.123 kl of water per ha). Sprayed on soil or seedling shoot.
Dexon 35 WP, Laters	fenaminosulf	sodium [4-(dimethyl-amino) phenyl] diazenesulfonate	163.4 lb in 8712 gal of water to one surface acre 183.1 kg in 97.8 kl of water per ha). Drenched onto soil.

Usages, trade name, and manufacturer	Common Name	Chemical name or composition	Dosage rate and details of application
Neutro Cop "53", Stauffer	-----	basic copper sulfate 100%, metallic copper 53%	4 lb a.i. in 100 gal of water per surface acre (4.48 kg in 1.123 kl of water per ha). Sprayed on soil or seedling shoot.
<u>Insecticides:</u>			
Basudin 50 EC, Ciba-Geigy	diazinon	O,O-diethyl O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate	2 lb a.i. in 100 gal of water per surface acre (2.24 kg in 1.123 kl of water per ha). Sprayed on soil or seedling shoot.
Dylox 80 SP, Chemagro	trichlorfon	dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate	As above
Cythion 50 EC, Chipman	malathion	diethyl mercaptosuccinate S-ester with O, O-dimethyl phosphorodithioate	3 lb a.i. in 100 gal of water per surface acre (3.36 kg in 1.123 kl of water per ha). Sprayed on soil or seedling shoot.
Sevin 50 WP, Ciba-Geigy (Green Cross)	carbaryl	1-naphthyl-methylcarbamate	1.5 lb a.i. in 100 gal of water per surface acre (1.68 kg in 1.123 kl of water per ha). Sprayed on soil or seedling shoot.

Because earlier phytotoxicity studies (1) had been made with diazinon and malathion on white and Sitka spruces and western hemlock, only fenamiosulf (Dexon) was applied to those species in 1974; the same year, diazinon, malathion and fenamiosulf were tested on Douglas-fir and lodgepole pine. The following year, the remaining chemicals were tested on all seedling species (Appendix I).

Sprays were applied with a tower, similar to one described by Potter (3), with the nozzle set 75 cm above the soil surface and operating at 26.6 K Pa pressure. Drenches were administered with a syringe. Treatments were given once at each of the following developmental stages: (i) seed (immediately after sowing), (ii) radicle just starting to emerge, (iii) radicle 1.25 cm long, (iv) post-emergence stage of seedling with the seedcoat still covering, or partially covering the primary needles, and (v) post-emergence

stage of seedling with the seedcoat shed and the primary needles expanded. In both years, distilled water was added to control (untreated) seedlings.

In 1974, each treatment (pesticide-seedling species-developmental stage combination) was replicated four times with 10 observations per replicate, while in 1975, each treatment was replicated eight times for the seed stage and four times for the other stages. The "bullets" were arranged in a completely random design (4) on a greenhouse bench. Each week, they were re-randomized to eliminate possible greenhouse effects. Daily greenhouse temperatures in 1974 averaged 23°C, range 17-30°C. Comparable values for 1975 were 22°C, range 11-30°C. The relative humidity averaged 63% in 1974 and 66% in 1975. Seedling mortality was recorded 13 weeks after sowing and seedling shoot growth was measured (soil line to tip of apical bud); the oven-dry (105°C

for 24 hours) weights of shoots and roots were determined gravimetrically 12 weeks after applying the pesticides. Data were subjected to tests for homogeneity of variance and normality (4) and, where needed, transformed for the analyses of variance. The Student-Newman-Keul's multiple-range test (4) was used to compare the significance of mean differences.

Field experiments. These experiments were made during the 1976 growing season at the BCFS nurseries at Koksilah with coastal Douglas-fir and Sitka spruce, and at Surrey with interior Douglas-fir, white spruce and lodgepole pine. The seedlot numbers, sowing rates, etc., are given in Appendix I. The pesticides, dosage rates, etc., were the same as in the greenhouse experiments except that the sprays were applied as a fog-spray to the soil or seedling shoots using a portable sprayer operated at 152 K Pa pressure; fenamino-sulf (Dexon) was drenched into the soil with a sprinkling can. To ensure accurate dosages, each material was prepared in a small bottle and the contents sprayed to exhaust on each specified replicate. All pesticides were applied within 72 hours of preparation. Control seedlings were treated with water. The experimental design was a randomized complete-block (4), with each treatment and control (pesticide x seedling developmental stage) replicated seven times. Each 30-m-long block contained 50-, 60-cm long x 108-cm wide, replicates. Five seedling developmental stages were treated: (i) seed, within 48 hours of sowing, (ii) germinant just emerging from soil, (iii) germinant emerged, erect, seedcoat *in situ*, (iv) seedling seedcoat shed, secondary needles 2-mm long, and (v) seedling epicotyl 2- to 3-cm long. At each treatment, about 75% of the seedlings were at the desired developmental stage. The plots received routine nursery care, except that they were hand-weeded.

Mid-summer seedling survival was determined 2 weeks after the last treatment by counting the live seedlings in the center 30 cm of seedling rows three, four and five of each replicate. Counts were made at Koksilah on July 16 and 26, for coastal Douglas-fir and Sitka spruce, respectively, and at Surrey on July 20 for interior Douglas-fir and on August 4 for white spruce and lodgepole pine. End of season survival counts were made during the second week of October. Concurrently, seedling heights (soil line to tip of the apical bud) were determined for 20 randomly selected seedlings from within each counting site of each replicate. Data were treated the same as the greenhouse study.

RESULTS AND DISCUSSION

The results are summarized in Table 1.^{1/} Overall, more phytotoxicity was observed in the greenhouse than in the field, demonstrating that a candidate pesticide should not be rejected solely on the basis of greenhouse experiments. Phytotoxicity also tended to decrease, in the greenhouse and nursery as seedling age increased.

Petroleum week killers AWK No. 1 and AWK No. 1 Special exhibited some degree of phytotoxicity to seeds, germinants and young seedlings of most seedling species in the greenhouse; but, in the field, they caused damage only when sprayed onto some developmental stages of coastal and interior Douglas-fir, Sitka spruce and white spruce. Mostly, their effects were related to reductions in end-of-season shoot growth, which were statistically significant but of no practical importance.

Benlate (benomyl) fungicide was one of the least phytotoxic materials tested. In general, it was not phytotoxic in either the greenhouse or field. It reduced shoot growth when applied in the field to certain post-emergence stages of coastal and interior Douglas-fir and white spruce, and to Sitka spruce seeds, but not enough to have any practical importance.

Dexon, the most phytotoxic of the nine pesticides tested, caused mortality and reduced seedling growth of all test species under greenhouse and field conditions. Only nursery-grown Douglas-fir (two oldest stages) and the oldest stage of Sitka spruce were unaffected. This fungicide, recommended for control of species of *Pythium* and *Phytophthora*, should be used with extreme caution, and perhaps at lower dosages, in local forest nurseries.

Diazinon (Basudin) insecticide affected mid-season, but not end-of-season survival when applied to two of the three post-emergence stages of field-grown Douglas-fir seedlings. It reduced shoot growth of greenhouse-reared lodgepole pine when applied to seeds. Although of no practical significance, it also reduced shoot growth when applied to various developmental stages of coastal and interior Douglas-fir, white spruce and Sitka spruce in the nursery.

Mortality of western hemlock was increased

^{1/} The quantitative data and results of the multiple-range tests are available from the authors.

in the greenhouse, when Dylox (trichlorfon) insecticide was sprayed on seedlings with the seedcoat in situ. Western hemlock was used only in the greenhouse; thus, the effects this insecticide would have on this species under bareroot conditions is not known. The results indicate the potential for damage if Dylox is used on western hemlock container seedlings. Although Dylox had a detrimental effect on shoot growth when applied to certain developmental stages of coastal and interior Douglas-fir and Sitka spruce in the nursery, these effects were not of sufficient magnitude to prevent use of this insecticide on bareroot stock.

Shoot length and root weight of lodgepole pine seedlings were reduced when the insecticide malathion (Cythion) was applied in the greenhouse to lodgepole pine seeds and emerged seedlings which had shed their seedcoats. Slight reductions in shoot growth of nursery-grown Douglas-fir (coastal and interior) and Sitka and white spruce resulted from applying malathion to various developmental stages of these species; however, the reductions were not large enough to be of practical concern.

In the greenhouse experiments, Neutro Cop "53" fungicide increased mortality when applied to seeds and seeds with the radicle just emerging of all species, except Douglas-fir. The fungicide was slightly harmful to shoot growth when applied in the nursery to Sitka spruce seeds and Douglas-fir seedlings with the seedcoat still intact. These results indicate that while this material may be safe for field use, it should not be used on seeds or germinants in container nurseries.

Although Sevin (carbaryl) affected shoot length of all test species, in the greenhouse and nursery, there was no definite pattern with developmental stage affected. As the effects of Sevin were not of any magnitude, they should not prevent its use on forest nursery seedlings.

These findings should be used only as guidelines. For example, the data apply to one dosage rate and one application of each pesticide. Factors such as interaction with other pesticides, unfavorable weather or physiological condition of the seedling may also influence pesticide phytotoxicity. The results do not preclude the nursery manager trying out a pesticide on a small scale several days before treating a large area.

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Table 1. Summary of results from pesticide phytotoxicity studies made in the greenhouse and nursery

Pesticide tested 1/	Seedling developmental stages treated					Species affected and phytotoxic effects 2/				
	Greenhouse experiments					Nursery experiments				
	Seed	Radicle just emerged	Radicle 1.25 cm. long	Seedling emerged, seedcoat in situ	Seedling emerged, seedcoat shed	Seed	Germinant emerging from soil	Post emergence, seedcoat in situ	Post emergence, seedcoat shed, secondary needles 2 mm long	Post emergence, epicotyl 2.3 mm long
AWK No. 1 (agricultural weed killer)	CDF ^a	CDF ^a	CDF ^a	CDF ^b	CDF ^a	SS ^{a,c}	SS ^c	CDF ^c	WS ^c	IDF ^c
	Lp ^a	Lp ^a	Lp ^b	WS ^b	SS ^c					
	WS ^a	WS ^a	WS ^a	SS ^{b,c}	WH ^a					
	SS ^a	SS ^a	SS ^a	WH ^a						
	WH ^a	WH ^a	WH ^a							
AWK No. 1 Special (agricultural weed killer)	CDF ^a	CDF ^a	CDF ^a	CDF ^b	CDF ^a	SS ^c	SS ^c	CDF ^c	WS ^c	IDF ^c
	Lp ^a	Lp ^a	Lp ^a	WS ^{b,c}	SS ^c					
	WS ^a	WS ^a	WS ^a	SS ^{b,c}						
	SS ^a	SS ^a	SS ^a	WH ^a						
	WH ^a	WH ^a	WH ^a							
Benlate (benomyl)						SS ^c		CDF ^c	WS ^c	IDF ^c
Dexon (fenamiphosulf)	CDF ^{b,c,d}	CDF ^{b,c}	CDF ^{b,d}	CDF ^d	CDF ^d	CDF ^b	CDF ^b	CDF ^c	Lp ^c	Lp ^c
	Lp ^{b,d}	Lp ^{c,d}	Lp ^d	Lp ^{a,c,d}	Lp ^{c,d}	Lp ^{a,c}	SS ^c	Lp ^{b,c}	WS ^c	SS ^c
	WS ^{b,c,d}	WS ^{b,c,d}	WS ^{a,c,d}	WS ^{b,c,d}	WS ^{b,c,d}	WS ^c		WS ^{b,c}	SS ^c	IDF ^c
	SS ^{a,b,c,d}	SS ^{b,c,d}	SS ^{a,b,c,d}	SS ^{b,c,d}	SS ^{c,d}	SS ^{a,c}		SS ^c	IDF ^c	
	WH ^{b,c,d}	WH ^{b,c,d}	WH ^{a,b,c,d}	WH ^d	WH ^d	IDF ^c		IDF ^c		
Basudin (diazinon)	Lp ^b					SS ^c	SS ^c	CDF ^c	WS ^{b,c}	CDF ^c IDF ^c
Dylox (trichlorfon)			WH ^a			SS ^c	SS ^c	CDF ^c	WS ^c	IDF ^c
Cythion (malathion)	Lp ^b			LP ^d			SS ^c	CDF ^c	WS ^c	IDF ^c
Neutro Cop "53"	Lp ^a	Lp ^a				SS ^c		CDF ^c		
	WS ^a	WS ^a								
	WH ^a	SS ^a								
		WH ^a								
Sevin (carbaryl)		Lp ^c	WS ^{b,c}	WH ^{b,c}		SS ^c	SS ^c	CDF ^c		

1/ A trade name and a common name.

2/ The symbols CDF, IDF, LP, WS, SS and WH denote coastal Douglas-fir, interior Douglas-fir, lodgepole pine, white spruce, Sitka spruce and western hemlock, respectively. For the greenhouse experiments, the superscripts indicate that the pesticide increased mortality (a), decreased shoot length (b) or shoot weight (c) or root weight (d) or end-of-season survival (a), end-of-season survival (b) or end-of-season shoot length (c).

APPENDIX I

Names, B.C. Forest Service seedlot numbers, and germination capacities or numbers of viable seeds sown of tree species used.

Common and Scientific names	Seedlot number	Germination capacity (%) of greenhouse sown seeds or viable seed per linear foot (VS/ft; VS/0.3 m) of seedbed for field sown seeds
Douglas-fir, <u>Pseudotsuga menziesii</u> (Mirb.) Franco	315 coastal form 1647 coastal form 2046 interior form	87% 155 VS/ft 145 VS/ft
Sitka spruce, <u>Picea</u> <u>sitchensis</u> (Bong.) Carr	951 950	87% 190 VS/ft
White spruce, <u>Picea</u> <u>glauca</u> (Moench) Voss	1848 2211 2211	73% (1974 experiment) 90% (1975 experiment) 170 VS/ft
Western hemlock, <u>Tsuga</u> <u>heterophylla</u> (Raf.) Sarg.	960	67.5%
Lodgepole pine, <u>Pinus</u> <u>contorta</u> Dougl.	1975 2203 2203	58% (1974 experiment) 87% (1975 experiment) 125 VS/ft