1972

Lethal and Nonlethal Effects of the Organic Horizons of Forested Soils on the Germination of Seeds from Several Associated Conifer species of the Rocky Mountains

T. W. Daniel
Utah State University

Josef Schmidt
Utah State University

Recommended Citation
Daniel, T. and Schmidt, J. (1972). Lethal and nonlethal effects of the organic horizons of forested soils on the germination of seeds from several associated conifer species of the Rocky Mountains. Can. J. For. Res. 2(3): 179–184.

Lethal and Nonlethal Effects of the Organic Horizons of Forested Soils on the Germination of Seeds from Several Associated Conifer Species of the Rocky Mountains[1]

T. W. DANIEL AND JOSEF SCHMIDT[2]

Forest Science Department, College of Natural Resources, Utah State University, Logan, Utah 84321

Received October 26, 1971

DANIEL, T. W., and SCHMIDT, J. 1972. Lethal and nonlethal effects of the organic horizons of forested soils on the germination of seeds from several associated conifer species of the Rocky Mountains. Can. J. Forest Res. **2**, 179–184.

The study tests whether the lethal effect of overwintering Engelmann spruce seed in its O-horizon affects Douglas-fir, lodgepole pine and subalpine fir seeds and whether the O-horizons of the three associated species have the same lethal effect on seeds. All seed treatments of each species were stratified in petri dishes in the pure O-horizon (unsterilized and autoclaved) of each species. Seed treatments were untreated, 10% slurry of 50% Captan and powdered 75% Captan.

All untreated seeds demonstrated the lethal quality of unsterilized Engelmann spruce O-horizon. Its effects on untreated seeds and powder treated seeds were as follows: Engelmann spruce seed 3.5 and 77.3% (germination of untreated and powdered respectively), subalpine fir seed 1.3 and 20.8, Douglas-fir seed 9.5 and 84.8 and lodgepole pine seed 11.9 and 85.7. Subalpine fir O-horizon was lethal to its own seeds but only moderately harmful to seeds of other species. Douglas-fir O-horizon had a significant adverse effect on its own seeds and was only moderately harmful to seeds of other species. Lodgepole pine O-horizon was almost neutral to all seeds. Autoclaving of the O-horizons and powder treatment of seeds produced similar results.

DANIEL, T. W. et SCHMIDT, J. 1972. Lethal and nonlethal effects of the organic horizons of forested soils on the germination of seeds from several associated conifer species of the Rocky Mountains. Can. J. Forest Res. **2**, 179–184.

Le but de cette étude est de tester s'il y a un effet léthal dû à la présence des graines de l'épinette d'Engelmann qui ont passé l'hiver dans leur horizon O, sur les graines de sapin de Douglas, de pin à feuilles tordues et de sapin concolore et inversement. Les lots de graines de chaque espèce furent stratifiés dans des plats de Petri contenant l'horizon O de chaque espèce, non stérilisé et traité à l'autoclave. Les graines (1) non-traitées, (2) traitées avec une suspension à 10% de captan à 50% et (3) saupoudrées avec captan à 75%. Toutes les graines non-traitées ont prouvé que l'horizon O non-stérilisé, de l'épinette d'Engelmann possèdent des qualités léthales. Ses effets sur les graines non-traitées et traitées à la poudre furent les suivants (les chiffres indiquent le pourcentage de germination chez les graines non-traitées et traitées à la poudre respectivement): graines d'épinette d'Engelmann 3.5 et 77.3, graines de sapin concolore 1.3 et 20.8; graines de sapin de Douglas 9.5 et 84.8 et graines de pin à feuilles tordues 11.9 et 85.7. L'horizon O du sapin concolore avait des effets léthaux sur ses propres graines, mais des effets modérément nocifs sur les graines des autres espèces. L'horizon O du pin à feuilles tordues avait un effet neutre sur toutes les graines. Enfin, le traitement à l'autoclave des horizons O et le traitement à la poudre des graines ont donné des résultats similaires.

[Traduit par le journal]

In the complex ecosystem of a forest stand, many factors influence whether a tree seed will germinate and grow to become a part of the next generation. In the Rocky Mountains, a seed overwinters on the forest floor where the moist, cool storage (stratification) prepares the seed for rapid germination in the spring. Yet this same environment can be very hostile. Just how and in what form the hostility is expressed depends upon the characteristics of the seed bed, the insolation, the density of the rodent population and many other elements.

One of the characteristic seedbeds of the forest floor is the organic layer above mineral soil, the O-horizon. The O-horizon is defined by the latest soil classification as the horizon (1) formed or forming in the upper part of a mineral soil and above the mineral part and (2) dominated by fresh or partly decomposed

[1]Publication approved by Director of Utah State Agricultural Research Station.

[2]Present address: Institut fuer forstliche Standortsforschung, Hochschule fuer Bodenkultur, Wien, Austria.

organic material. The O-horizons used in this study consisted of leaves or needles, small twigs and possibly faunal remains, all in various stages of decomposition. The general concensus (Moore 1926; Barr 1930; Baldwin 1942; Smith 1954; Roe and Schmidt 1967) is that this seedbed is an unfavorable one for conifer seeds, though in mixed stands reproduction often occurs under trees of a different species (Dengler 1944; Koestler, 1950). If the biological entity of a forest type is considered a product of the coevolution of its constituents, then the possibility cannot be overlooked that the forest O-horizon, itself a complex of dead and living matter, contributes some selective influence.

This study was designed to explore whether the lethal effect of the Engelmann spruce (*Picea engelmannii* Parry) O-horizon on overwintering Engelmann spruce seed ((Daniel and Glatzel 1966) (confirmed a series of field trials on overwintering seed in the O-horizon and bare soil)) was selective only for Engelmann spruce seeds and whether the O-horizons of associated species produced similar reactions.

Materials and Procedure

As the study is a part of a continuing investigation of spruce–fir regeneration on the College Forest of Utah State University, the materials used were collected there (T.13N R.4E Sec. 10 and 21, S.L.M.) except for the Douglas-fir O-horizon and seed. The elevations of the stands varied from 8000 to 8500 ft (2440 to 2975 m). The O-horizon was collected under pure canopies of Engelmann spruce, subalpine fir (*Abies lasiocarpa* [Hook.] Nutt), lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.) and Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco). The entire organic layer of each sample except for the coarse twigs and debris was thoroughly mixed. Because no pure Douglas-fir stands grow in the College Forest, the O-horizon sample was collected at a 7000 ft (2135 m) elevation in the vicinity. Engelmann spruce and subalpine fir seeds were collected in the fall of 1967 and lodgepole pine seed in 1968. The Douglas-fir seed was collected in 1964 in Provo Canyon near Provo, Utah, at 8000 ft (2440 m) elevation.

For comparative purposes, two other substrates were selected for study: (1) subsoil (actually the top of the B-horizon, silty clay loam texture with gravel) was collected, after removing the top 6 in. of mineral soil, from the place where the Engelmann spruce O-horizon had been gathered; and (2) a coarse granitic sand was obtained from a commercial sand source. The subsoil was screened to remove all gravel.

To simulate the overwintering process in the laboratory, the seeds were subjected to stratification (at 2 °C for 90 days) in the various substrates. The O-horizon substrates were soaked for 24 h and the excess water was squeezed out by hand. Moisture in the mineral soil substrates was raised to field capacity. All of the sand substrate, one-half of each of the O-horizon substrates and none of the subsoil were autoclaved for 20 min at 120 °C.

Seeds were discarded for cracks or chips and then 50 seeds were counted into each small bag of nylon netting. The bags were to simplify removal of the seeds from the substrates without prohibiting close contact between the seeds and substrates. In the first experiment, one-half the seeds were untreated and one-half were treated by agitating the bags individually in a 10% slurry of the fungicide Captan 50-WP. In the second experiment, three treatments were given the seeds, the same two as for experiment 1 plus a powder fungicide treatment using Orthocide 75 which has 75% Captan. In addition, the O-horizons used in each experiment were collected just previous to each experiment.

For stratification, the appropriate substrate and the bags containing the seeds were put into petri dishes (100 mm × 20 mm). Each bag was assured firm contact with the substrate. To assure aeration, a narrow masking tape ridge was spotted at one point along the rim of the lower dish. The dishes were randomly placed in boxes, one box for each species of seed in the first experiment, and a wet cloth over the dishes was maintained to prevent drying out during stratification.

After stratification, the bagged seeds were removed from the substrate, rinsed and cleaned free of substrate in distilled water, dipped into a 10% slurry of Captan 50-WP and drained on a blotter. A bag was then emptied into a petri dish containing 1.5% Bacto-Agar and germinated at 25 °C. A seed was considered germinated and was removed when the radicle extruded 1 mm or more and showed geotropic response.

In both experiments, a determination was made of the moisture contents of all the substrates from the petri dishes from which the sacks were removed and of the pH of all the substrates.

The design of the first experiment was as follows: (1) The two treatments of each species of seed were stratified in each of the substrates; (2) there were eight substrates of the O-horizons (the four O-horizons, sterilized and unsterilized), one of subsoil, one of sand; (3) the sampling for each treatment consisted of five petri dishes with five 50-seed sacks in each petri dish; (4) total degrees of freedom were 2000 (10 substrates) (5 dishes) (5 sacks) (2 seed treatments) (4 species).

The design of the second experiment was in two parts: (1) The four species of seeds with the three seed treatments were stratified in unsterilized Engelmann spruce O-horizon, the total degrees of freedom being 288 (4 seeds) (3 seed treatments) (8 petri dishes) (3 sacks in each petri dish); (2) the four species of seeds with three seed treatments were stratified in their own sterilized and unsterilized O-horizon, the total degrees of freedom being 576 (4 species) (3 seed treatments) (2 O-horizon treatments) (8 dishes) (3 sacks).

The brief descriptions of the designs are supplementary to the more diagrammatic presentation given by Tables 1, 3 and 4. The change in number of petri dishes per treatment from five with five sacks in each in the first experiment to eight with three sacks in each in the second experiment was made to give more degrees of freedom to the experimental error.

Since the total germination data were samples from binomial distributions, an arcsine transformation was performed for the statistical analyses. In the results, the actual means of the germination percentages of each treatment are given and the statistical validity among the comparisons is indicated.

Results

The germination data from the first experiment are given in Table 1 and the analysis of variance by species of seed is given in Table 2. The results of the second experiment and their statistical significance are given in Tables 3 and 4. The seed of all species except subalpine fir shows a high germination capacity. However, the changes in germination capacity between the first and second experiment for Engelmann spruce and subalpine fir are the result of changing seed lots and not the effect of treatments.

Discussion

Seed treatments, substrates and sterilization of substrates had in most instances very highly significant effects on the germination of seed from all the species though the seed treatments were only significantly or highly significantly effective in influencing the germination of Engelmann spruce and Douglas-fir seeds (Table 2). For the most part, the effects of seed treatments and substrates were independent of each other. The only reason for a significant interaction between seed treatments and subtrates for subalpine fir seed, aside from the poor germination, was the failure of the slurry treated seed to show any significant effect in the autoclaved lodgepole pine O-horizon. The effects of the seed treatments and of autoclaving of the substrates were independent of each other for Engelmann spruce and Douglas-fir seeds; however, there was an interaction between them for subalpine fir and lodgepole pine seeds. With subalpine fir seed, the reason for the interaction was the ineffectiveness of the slurry treatment in the unsterilized O-horizons to improve germination and its very highly significant effect in the autoclaved O-horizons. An opposite result was the basis for the interaction with lodgepole pine seed, as the slurry treatment had a very highly significant effect on germination in the unsterilized O-horizons but only a significant effect after the O-horizons had been autoclaved.

The very highly significant effect of the substrates on the germination of Engelmann spruce, Douglas-fir and lodgepole pine seeds was primarily because unsterilized Engelmann

TABLE 1. Average germination percentages of all stratification[a] treatments in first experiment

Stratification substrates	Unsterilized: No captan	Unsterilized: Slurry	Autoclaved: No captan	Autoclaved: Slurry
Engelmann spruce seed				
Engelmann spruce O-horizon	3.5	3.4	84.2	85.0
Subalpine fir O-horizon	73.6	78.4	83.0	86.2
Douglas-fir O-horizon	78.6	81.0	85.0	86.0
Lodgepole pine O-horizon	84.6	87.2	81.8	85.8
Subsoil	72.0	85.6	—	—
Sand	—	—	84.4	85.6
Subalpine fir seed				
Engelmann spruce O-horizon	0.3	0.5	0.6	2.7
Subalpine fir O-horizon	0.0	0.4	0.4	2.5
Douglas-fir O-horizon	0.3	0.6	0.7	2.5
Lodgepole pine O-horizon	0.6	0.6	0.5	1.0
Subsoil	0.7	1.3	—	—
Sand	—	—	2.0	4.6
Douglas-fir seed				
Engelmann spruce O-horizon	5.8	13.4	83.4	90.2
Subalpine fir O-horizon	61.7	77.7	79.8	81.4
Douglas-fir O-horizon	63.5	83.9	66.8	81.5
Lodgepole pine O-horizon	84.9	86.2	78.0	85.0
Subsoil	86.4	83.2	—	—
Sand	—	—	85.5	84.4
Lodgepole pine seed				
Engelmann spruce O-horizon	6.0	10.0	92.0	93.3
Subalpine fir O-horizon	81.7	90.6	90.6	90.8
Douglas-fir O-horizon	77.0	91.8	91.0	94.6
Lodgepole pine O-horizon	85.3	91.4	87.5	92.3
Subsoil	88.2	94.5	—	—
Sand	—	—	85.0	89.1

[a]Seeds started stratification Oct. 20, 1968.

TABLE 2. Analysis of variance for the effects of substrates and seed treatments for each species

Species of seed		Engelmann spruce		Subalpine fir		Douglas-fir		Lodgepole pine	
Sources of variation	*df*	*F*	sign.[a]	*F*	sign.	*F*	sign.	*F*	sign.
1. Fungicide treatments	1	6.62	*	54.9	***	8.05	**	65.1	***
2. Substrates	4[b]	142	***	13.1	***	29.6	***	259	***
3. Sterilization of substrates	1	230	***	82.1	***	49.3	***	432	***
4. Interaction of 1 × 2	4	0.70	n.s.	2.56	*	1.72	n.s.	1.40	n.s.
5. Interaction of 1 × 3	1	0.50	n.s.	27.0	***	0.21	n.s.	12.6	***
6. Interaction of 2 × 3	4	141	***	4.50	**	40.4	***	312	***
7. Interaction of 1 × 2 × 3	4	0.73	n.s.	1.42	n.s.	0.28	n.s.	1.29	n.s.
8. Experimental error									
Each species	80	110.3[c]		17.6		330.9		56.0	
9. Sampling error									
Each species	400	26.0		16.9		25.4		31.3	

[a]Significant difference $P = 5\%$ (*) (significant), $P = 1\%$ (**) (highly significant) and $P = 0.1\%$ (***) (very highly significant).
[b]Subsoil and sand are paired, one unsterilized and the other sterilized.
[c]Mean squares of the experimental errors and sampling errors.

TABLE 3. Germination percentages of seeds stratified in unsterilized Engelmann spruce O-horizon with the multiple mean comparisons and the *F* values of the statistical analysis

	Fungicide Treatments			
Seed species	None	Slurry	Powder	*F* Values[a]
Engelmann spruce	3.5	7.3	77.3[b]	253
Subalpine fir	1.3	2.1	20.8	79
Douglas-fir	9.5	15.2	84.8	579
Lodgepole pine	11.9	16.3	85.7	393

[a]The *F* values are all significant at the $P = 0.1\%$ level with 2 *df* for fungicide treatments, 21 *df* for experimental error and 48 *df* for sampling error.
[b]Means not statistically different at $P = 5\%$ are underlined by the same line.

spruce O-horizon was so lethal to the seeds. For Engelmann spruce and lodgepole pine seed, the differences in germination among the other substrates were not significant. For Douglas-fir seed, the Engelmann spruce O-horizon had also a very highly significant difference in germination than the other substrate differences, but the effect of Douglas-fir and subalpine fir O-horizons on its germination was significantly different from the lodgepole pine O-horizon and the mineral substrates. Subalpine fir seed's germination gave a unique ordering of the substrates: the mineral substrates were very highly significantly different in germination from the other substrates while Douglas-fir and Engelmann spruce O-horizons gave significantly higher germination than subalpine fir and lodgepole pine O-horizons.

The very highly significant effect of autoclaving the substrates on germination of the seeds of the four species was largely the result

TABLE 4. Germination percentages of seeds with three treatments stratified[a] in unsterilized and autoclaved O-horizons of their own species with multiple mean comparisons within O-horizon treatments

Species of seed and O-horizon	Unsterilized O-horizon			Autoclaved O-horizon		
	No	Slurry	Powder	No	Slurry	Powder
Engelmann spruce	3.5	7.3	77.3[b]	68.7	75.4	76.8
Subalpine fir	0.3	0.8	23.8	4.5	3.5	11.8
Douglas-fir	77.9	85.2	80.6[c]	81.8	88.2	84.9[c]
Lodgepole pine	86.3	90.1	89.6	88.6	91.6	91.4

[a]Seeds started stratification on April 6, 1969.
[b]Means not statistically different at $P = 5\%$ level are underlined by the same line within O-horizon treatments.
[c]Value not significantly different from either of the two values.

of the increase in germination after sterilizing the Engelmann spruce O-horizon. In fact, there would have been no significant effect of autoclaving for Douglas-fir seed without the effect on its germination of sterilizing the Engelmann spruce O-horizon, and the very highly significant interaction between the substrates and autoclaving of the substrates with Douglas-fir seed has the same basis. The highly or very highly significant interaction between the substrates and autoclaving of the substrates for Engelmann spruce, subalpine fir and lodgepole pine seeds was caused by the failure of autoclaving to affect germination in the lodgepole pine O-horizon, whereas it caused significant to very highly significant effects in the other O-horizons.

The data of the second experiment (Table 3) demonstrates that powdered 75% Captan can protect any of the seeds in unsterilized Engelmann spruce and subalpine fir O-horizons. Thus the powdered Captan and autoclaving provided a similar degree of protection from the pathogens in the various O-horizons. The slurry treatment of the seeds of lodgepole pine and Douglas-fir was adequate to provide full germination; in fact, powdered 75% Captan appeared to reduce Douglas-fir germination. The data also confirm the first experiment's results that the effect of Engelmann spruce's unsterilized O-horizon is most severe on its seed. However, the unsterilized O-horizon of subalpine fir had apparently a more adverse effect on its seed than did Engelmann spruce's O-horizon on Engelmann spruce seed (Table 4). Douglas-fir's unsterilized O-horizon had a significant depressive effect on the germination of its seed while lodgepole's O-horizon had a significant but numerically small effect on its seed.

A depressive effect on germination of stratification without seed treatment in its autoclaved O-horizon was significant for all species (Table 4). The common occurrence of seed coat pathogens could explain the effect inasmuch as an autoclaved O-horizon would provide an ideal environment for their development because it lacked competition for the nutrients. In subalpine fir, the depression of germination, even with the powder treatment, could indicate something more severe than seed coat pathogens, such as possible growth inhibitors; with eight replications of three 50-seed samples for each value, the results would be difficult to attribute to sampling error.

The pH determinations of all the substrates showed that there was no significant difference between unsterilized and autoclaved O-horizons. For the O-horizons of Engelmann spruce, subalpine fir and Douglas-fir, the pH of each averaged 6.7 in the first experiment. In the second collection of O-horizons, Douglas-fir was slightly more acid while Engelmann spruce had a pH of 6.4 and subalpine fir a pH of 6.0. Lodgepole pine had a constant pH of 5.6. The quartz sand had a pH of 7.5 and the subsoil was pH 5.8. These differences were very highly significant. The two substrates with the most neutral and uniform germination were the lodgepole pine O-horizon with the lowest pH and sand with the highest pH. It would seem unlikely that the differences in pH would account for the results of the treatment variations.

The moisture content of the O-horizons during stratification averaged 287% in the first experiment and 332% in the second. There were no significant differences in moisture content between any two O-horizons in either experiment, but the difference between experiments was very highly significant. The subsoil had 22.6% moisture and the sand 5.8%. The uniformity of the peak values in the germination of Douglas-fir and lodgepole pine seeds indicates that the differences in moisture had not biased the results.

An observation with an unknown degree of influence on the results is concerned with the age and degree of decomposition of the O-horizon. The oldest O-horizon occurred under the Engelmann spruce while lodgepole pine was definitely the youngest. The amount of fine particles (humus) was in descending order: Engelmann spruce, subalpine fir, Douglas-fir and lodgepole pine. The ordering parallels the results of this study, but the differences in the results are an order of magnitude greater than the differences in age or decomposition of the O-horizons. The failure to germinate under the different conditions can be attributed to the action of pathogens that can be controlled by autoclaving or the use of a fungicide. The unsterilized O-horizons after 90 days of stratification were masses of fungal hyphae of about four kinds (an identification and reaction study is in process) while the autoclaved O-horizons in the petri dishes had a few scattered round beads of fungal hyphae which probably developed from contaminations during handling. These observations, however, leave the question as to why only the Engelmann spruce O-horizon is so lethal to all the overwintering conifer seeds or why the subalpine fir O-horizon has such a differential in germination among the species in view of its elthal action on its own seeds.

The study confirms the repeated advice on the advantages of a mineral seedbed and adds one more reason why an O-horizon seedbed may make a poor one for some species. It illuminates the reason for the observation that Engelmann spruce and subalpine fir seedlings are commonly found on rotten logs and stumps and the exposed soil of overturned trees.

BALDWIN, H. I. 1942. Forest tree seed of the north temperate region with special reference to North America. Chronica Botanica Co., Waltham, Mass.

BARR, P. M. 1930. The effect of soil moisture on the establishment of spruce reproduction in British Columbia. Yale University School of Forestry, New Haven, Conn., Bull. No. 26.

DANIEL, T. W. and GLATZEL, G. 1966. Duff. A lethal seedbed for overwintering Engelmann spruce seeds. Proc. Sixth World Forestry Congr., Madrid. **2**, 1420–1424.

DENGLER, A. 1944. Waldbau auf oekologischer Grundlage. 3rd ed. Springer-Verlag, Berlin.

KOESTLER, J. 1950. Waldbau. Paul Parey Verlag, Berlin.

MOORE, B. 1926. Influence of certain soil and light conditions on the establishment of reproduction in eastern conifers. Ecology, **7**, 191–220.

ROE, A. L., and SCHMIDT, W. C. 1967. Factors affecting natural regeneration of spruce in the Intermountain Region. U.S. Forest Serv., Inter. For. and Range Exp. Sta. Unpublished.

SMITH, J. H. G. 1954. A cooperative study of Engelmann spruce – alpine fir silviculture and management. Northwest Sci. **28**, 157–165.