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A FIELD TEST OF TETRAMINE TREATED DOUGLAS FIR SEED

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ABSTRACT

It has been found that the control of the small seed-eating mammals is an important requirement for the successful direct seeding of Douglas fir. Good initial control can be achieved by distributing a combination bait consisting of wheat treated with thallous sulfate and wheat treated with 1080 (sodium fluoroacetate). A mixture of equal parts of each bait are distributed simultaneously at a rate of one-half pound per acre prior to seeding. This practice has several disadvantages, many of which could be overcome by a chemical applied directly to the seed.

Tetramethylene-disulpho-tetramine (one of several candidates tested by Donald A. Spencer of U. S. Wild Life Research Laboratory in Denver, Colorado, in 1950) appeared to offer considerable promise when applied to Douglas fir seed. There was also some indication that slow feeding might build up an aversion to the treated seed.

To test the effect of field application of seed treated with "tetramine"* an exploratory project was undertaken in the Tillamook Burn area in the fall of 1951. The objectives of this preliminary study were to answer the following specific questions:

1. Will tetramine treatment prevent destruction of seed by small seed-eating mammals?
2. Is the viability impaired enough to prevent the establishment of a satisfactory stand of trees?
3. What are the effects of the treated seed on the resident seed-eating mammal population?

The project consisted of a 100-acre plot broadcast at the rate of one-half pound per acre with the treated Douglas fir seed. The plot was located in an area burned over by three Tillamook fires. The ground cover and soil condition was typical of the Tillamook Burn area. The exposure was generally southeast.

The study of the small mammals was begun in October prior to seeding and was continued until August of the following year. Sherman live traps and identifying eartags were used. A trapline was also maintained in the untreated area for a comparison.

The Douglas fir seed was treated with tetramine by soaking in a 1.0 per cent acetone solution for one hour. (Tetramine is only slightly soluble in water at low temperatures).

As a check on the persistence of tetramine's lethal characteristics, treated seed was exposed on soil all winter and recovered just prior to germination in the spring for tests with captive mice. For germination tests both treated and untreated controls were also exposed all winter under seed caps. Some of these were left to

* The term "tetramine" will be used to designate tetramethylene-disulpho-tetramine in the balance of this report.

germinate in place and others were picked up and germinated in the laboratory.

In June of 1952 stocking was found to be 41.2 per cent as evaluated from 160 one-milacre plots. In December, 1952 a more representative stocking survey was made. This survey indicated a stocking per cent of 50.2 by one-milacre plots. A stocking survey, representative of the entire 100-acre plot, made in October, 1953, indicated a stocking per cent of 25.0 by one-milacre plots, which is somewhat higher than the average results of present seeding practices.

The small mammal populations were greatly reduced during the winter on both the treated and the control area, although there were a few less found on the treated area. Since it is improbable that the baiting could have been responsible for reduction in population on the control area, such reduction was probably due to general conditions which caused similarly severe decreases in population on other areas sampled during the same winter.

The treated seed which had been weathered in the field all winter was tested with ten captive deer mice. An average of 9.75 seeds were ingested over a period of nine days before death occurred.

The laboratory germination tests of the untreated seed from the lot used indicated an approximate 60.0 per cent viability as compared with approximately 6.0 per cent immediately after treatment. After overwintering, however, germination was 59.9 per cent for the control and 31.5 per cent for the treated seed. Field germination was even better (considering only the comparison of treated seed with control seed) with 35.3 per cent for the control and 26.8 per cent for the treated seed.

Results of the experiment are encouraging. More seedlings were established on the seeded areas than are usually obtained with standard seeding practices. Data obtained on the effect on small mammals were not conclusive. There is considerable room for improvement in the application of the toxic material. Over-all indications are so favorable, however, that further tests should be made at the earliest possible date.

INTRODUCTION

Previous experimental seeding projects conducted by state, private and federal agencies have determined that successful seeding is not possible without control of the small seed-eating mammals. The deer mouse or white-footed mouse *(Peromyscus maniculatus var.)* appears to be the most numerous and destructive of tree seed in cut-over areas. Some other rodents commonly found are meadow mice *(Microtus spp.)*, jumping mice *(Zapus spp.)*, gray-diggers *(Citellus douglasii)*, and chipmunks *(Eutamias spp.)*. In addition to the rodents, shrews *(Sorex spp.)* may also be an important factor in the coastal fog-belt area.

Good initial control can be achieved by baiting prior to seeding with a combination bait. The materials most commonly used are thallous sulfate and sodium fluoroacetate (1080). Wheat has been found to be the most practical carrier. The thallous sulfate and the 1080 treated wheat are distributed simultaneously in equal quantities. The rate of application is usually ¼ pound of each bait per acre.

Although the initial effect of this practice in reducing the rodent population is good, it has the following outstanding disadvantages:

1. A buffer strip is required to prevent reinvasion.
2. A two-stage operation is required for seeding.
3. Small areas cannot be seeded economically because of the disproportionate cost of the one-fourth mile buffer strip.
4. Small mammals other than the tree seed eaters may be destroyed.
5. Baits deteriorate over winter and do not provide protection in the spring.

A relatively insoluble repellant or toxic chemical which could be applied to the seed without destroying the ability to germinate is a highly desirable solution to these small mammal control problems.

A cooperative study begun by Donald A. Spencer of the U. S. Wild Life Research Laboratory of Denver and the Oregon State Forestry Department was directed toward development of such a material.

One candidate material, tetramine, submitted to the State Forestry Department for tests with caged mice appeared quite promising despite the fact that it had been necessary to use acetone as a

solvent. Germination tests, appeared quite erratic, but laboratory tests conducted by both agencies indicated that the possibility of successful field application was good. Accordingly, the cooperative project was expanded to include other interested agencies.

The experiment described in this report was one of several cooperative plots seeded with tetramine-treated Douglas fir seed in 1951.

The other experiments were located in the State of Washington and will be described by the agencies involved.

PURPOSE OF EXPERIMENT

The results of laboratory tests in 1951 with tetramine-treated seed indicated that a pilot field test was warranted. The basic purpose of the project was exploratory. Answers to the following general questions were sought:

1. Will the tetramine treatment prevent destruction of the seed by small mammals under field conditions?
2. What are the effects of the treated seed on the normal mammal population?
3. What is the effect of the treatment on germination under field conditions ?

EXPERIMENTAL PROCEDURES

Description of the Experimental Plot

The plot chosen was 100 acres in size. It was located near the Wilson River Highway at an approximate elevation of 1500 feet (see Figures 1 and 2). The area had been burned over by the 1933, 1939 and 1945 Tillamook Fires. Salvage material had been removed from the area during several periods, but large amounts of debris still remained.

Woody plant cover consisted of salal *(Gaultheria shallon)* on the southwest portion and willow *(Salix spp.)* in clumps on the remainder. Bracken *(Pteridium aquilinum)* and trailing blackberry

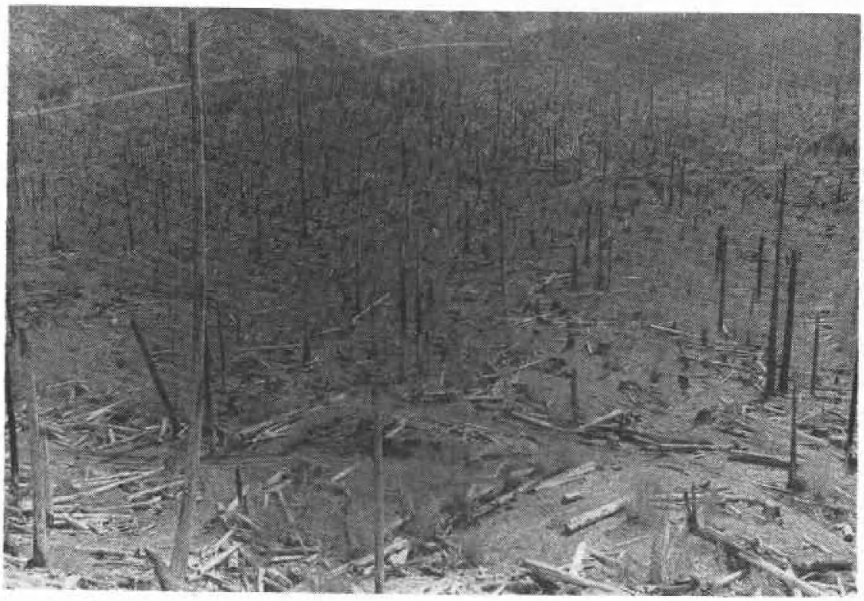

Figure 1. Panoramic View of Project Area

(Rubus vitifolius) were common over the entire area. There was also present some deer vetch *(Lotus spp.)*, fire weed *(Epilobium angustifolium)*, lupine *(Lupinus revularis)*, and pearly everlasting *(Anaphalis margaritacea var. occidentalis)*.

There was no Douglas fir seed produced within several miles of the area in 1951.

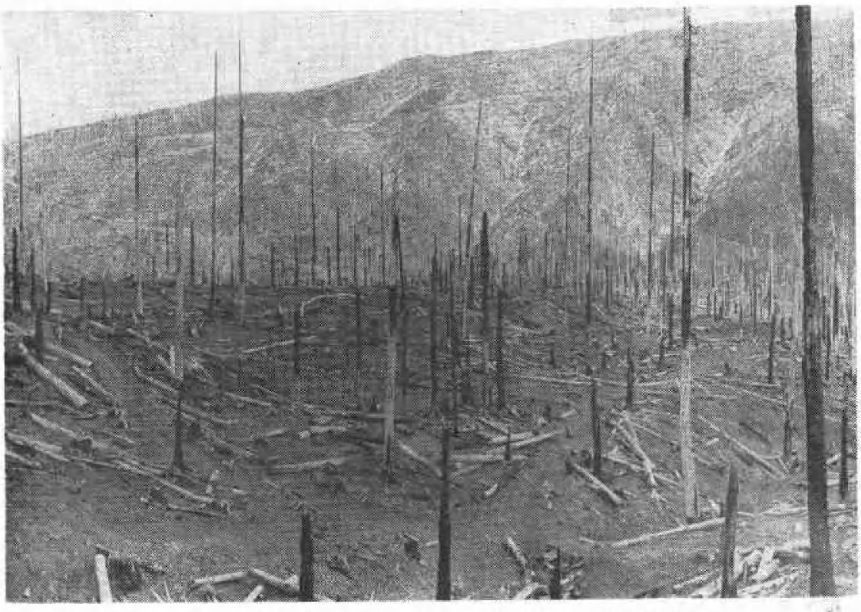

Figure 2. Close Up—Typical of Area Seeded

Seed Distribution

Fifty pounds of Douglas fir seed *(Pseudotsuga taxifolia, Poir)* treated with tetramine was distributed over the area by helicopter on December 18, 1951. Distribution, as determined by 100 two-foot by one-foot seed traps, was uniform.

Small Mammal Census

The small mammal population was observed by trapping, marking and releasing all mice taken alive. Trapping was begun October 30, 1951 and was continued until August of the following year. Both the seeded area and the nearby control area were trapped as regularly as the snow would permit (See Table 1).

One hundred sheet metal Sherman live-traps, 3 inches by 3 inches by 10 inches, were used for each trapline. Traps were located at permanent stations approximately 22 feet apart. Wherever possible traps were placed by logs, stumps, or other protective cover, and then left open for three successive nights. Early morning checks

TRAPLINE LOCATION

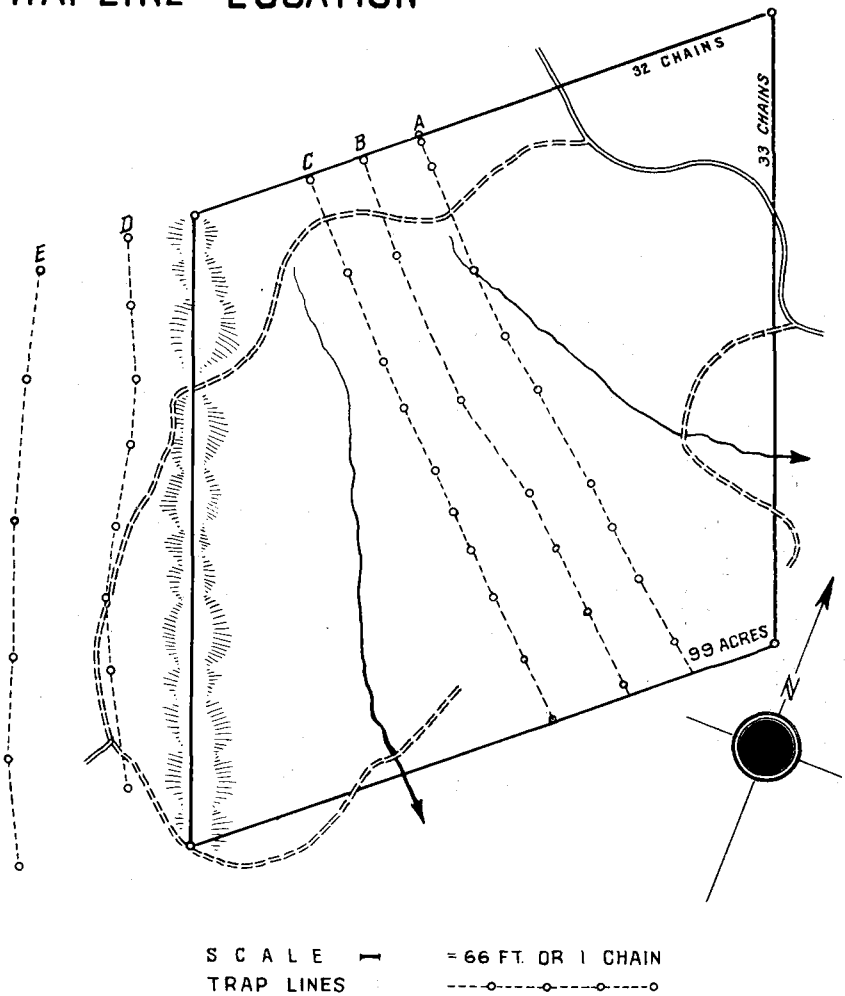

Figure 3. Location of Traplines

were made daily, traps were reset and all live mammals taken marked with a small numbered aluminum tag clipped to the ear and released. Date of capture, sex, approximate age, breeding condition, trap location and other pertinent data were recorded.

Traps were located on three different lines within the plots—"A", "B" and "C". (See Figure 3). In the control area outside the plot, two locations, "D" and "E", were used. After seeding only the "B" line of permanent trap stations was used in the seeded area and the "D" line in the control area.

Table I

TRAPPING RESULTS—BEFORE SEEDING
(100 traps exposed each night for three nights)

	INSIDE PLOT										OUTSIDE PLOT								
	Line A			Line B			Line C				Line D			Line E		
Date	Number caught for first time	Number of recatches	Total number of catches	Number caught for first time	Number of recatches	Total number of catches	Number caught for first time	Number of recatches	Total number of catches	Date	Number caught for first time	Number of recatches	Total number of catches	Number caught for first time	Number of recatches	Total number of catches
Oct. 30	12	....	12	12	....	12	14	....	14	Nov. 5	25	....	25	22	....	22
Oct. 31	10	10	20	7	11	18	6	11	17	Nov. 6	7	25	32	4	20	24
Nov. 1	4	13	17	2	20	22	8	15	23	Nov. 7	3	28	31	3	21	24
Total	26	23	49	21	31	52	28	26	54		35	53	88	29	41	70

Table includes only deer mice; numbers of other species were negligible.

Application of Tetramine to Seed

The seed had been in storage since 1949 at 6° F. and removed October 9, 1951. It was treated approximately 18 hours later, after temporary storage at 35° F.

The treatment of the fifty pound seed lot was as follows:

1. The seed was washed for approximately ten minutes in six gallons of pure acetone to remove the resins in the seed coat.
2. It was then immersed for one hour in a 1.0 per cent by weight solution of tetramine and acetone.
3. It was spread on screens and dried for one-half hour.
4. The dry treated seed was placed in 35° F. storage until used.

Tests With Captive Mice

In addition to previous extensive tests made with captive mice, a rough check was made on the lethal qualities of the specific lot of seed prepared for the project. These tests consisted of feeding the seed to ten captive mice until death occurred (See Table 3).

Seed was also placed on the project area to weather naturally. These were for the purpose of testing the toxicity of the treated seed after overwintering under field conditions.

Laboratory Germination Tests

Laboratory germination tests were made (1) for the seed lot before treatment, (2) the freshly treated seed, (3) the untreated seed after overwintering under field conditions. These tests were made by the Oregon State College Seed Testing Laboratory, using its standard techniques for Douglas fir seed.

Field Germination Tests

On the day following seeding, 1000 treated seeds and 1000 untreated seeds were placed on the experimental plot to test the ability of treated seed to germinate under field conditions. The seeds were placed in lots of ten on mineral soil and covered with screen caps to prevent possible loss to seed-eaters. The screen covered seed spots were located in two parallel rows across the north portion of the plot. Distance between spots was 5 feet; between rows, 10 feet.

RESULTS

Small Mammal Census

Weather following seeding did not permit trapping since there were from two to four inches of soft snow on the ground almost continuously from the day following seeding until the latter part of March. When trapping was resumed in March, the mice caught per 100 trap-nights had declined 91 per cent on the treated area and 85 per cent on the control area. Trapping from March 25 to 27

Table 2

TRAPPING RESULTS—AFTER SEEDING
1951-1952
(100 traps exposed each night for three nights)

Date	Inside Plot: Caught for first time (No eartag)	Caught for first time this period (Ear-tagged previously)	Total number of individuals caught (not counting recatches)	Number of recatches	Total number of catches
Dec. 18	2	3	5	....	5
19	0	0	0	3	3
Totals	2	3	5	3	8
Feb. 5	1	6	7	....	7
6	0	0	0	7	7
7	0	0	0	7	7
Totals	1	6	7	14	21
Apr. 15	3	8	11	....	11
16	1	1	2	8	10
17	0	1	1	10	11
Totals	4	10	14	18	32
May 20	1	9	10	....	10
21	3	3	6	6	12
22	0	0	0	12	12
Totals	4	12	16	18	34
June 17	1	8	9	....	9
18	0	1	1	7	8
19	0	0	0	6	6
Totals	1	9	10	13	23
July 29	5	4	9	....	9
30	1	3	4	4	8
31	0	1	1	6	7
Totals	6	8	14	10	24
Aug. 27	3	5	8	....	8
28	0	2	2	3	5
Totals	3	7	10	3	13

Date	Outside Plot: Caught for first time (No eartag)	Caught for first time this period (Ear-tagged previously)	Total number of individuals caught (not counting recatches)	Number of recatches	Total number of catches
Mar. 11	0	1	1	....	1
12	1	1	2	....	2
Totals	1	2	3	....	3
Mar. 25	6	9	15	....	15
26	1	1	2	13	15
27	1	1	2	11	13
Totals	8	11	19	24	43
June 4	0	6	6	....	6
5	0	1	1	4	5
6	0	1	1	5	6
Totals	0	8	8	9	17
July 15	4	3	7	....	7
16	3	3	6	4	10
17	4	3	7	4	11
Totals	11	9	20	8	29

Table includes only deer mice; numbers of other species were negligible.

on the control area (D-line) produced 17 deer mice, of which 9 had been caught and tagged prior to seeding (See Table 2). On the treated area (B-line), at approximately the same time (April 1 to 3),

9 deer mice were caught, of which 3 had been tagged prior to seeding.

From July 15 to 17, 15 deer mice were caught in the control area, of which 11 were untagged (not caught previously), whereas the treated area, from July 29 to 31, yielded 13 deer mice, of which 6 were untagged. The figures for July are listed irrespective of recatches, counting an animal the first time taken only. The July trapping period provided the first immature specimens. Ten weeks was the estimated age of the oldest of these.

Effects of Weathered Seed on Captive Mice

Samples of treated seed placed under protective screen caps were removed from the experimental area on March 27, 1952, after 109 days exposure to weathering on exposed soil. The local precipitation during the period was 30.21 inches.

Five seeds each were fed to ten captive deer mice. Two days later five additional seeds were offered to the survivors. Two days later seven seeds were offered and the number offered increased by two on alternate days until the death of all mice had occurred (See Table 4). Over a period of nine days, an average of 9.75 tetramine seeds were accepted before all died. No aversion to the treated seed was noticeable, although a reluctance to eat either wheat or seed, which was always readily available, become apparent shortly before death. Apparently the weathered seed had retained some of its toxic qualities.

The results of the tests were compared to a similar test made on 10 deer mice the previous October with freshly prepared seed (See Table 3). The mice were killed with an average of 5.9 freshly prepared seeds, whereas the weathered seed took 9.75 seeds to kill. Although not statistically significant, this difference did indicate that some toxicity is lost by over-wintering in the field. Further tests of this kind should be made to determine the extent of the loss and the actual effect on control of seed-eating mammals.

Germination Tests

Laboratory tests made immediately after treatment indicated a great reduction of the original viability of the seed. Two tests of the untreated seed samples produced a mean of 60.5 per cent, while viability tests of the treated seed immediately after treatment averaged but 6.0 per cent.

After over-wintering in the field, however, the laboratory germination per cent of treated seed showed great improvement The mean germination per cent had increased from 6.0 to 31.5 per cent.

The untreated seed remained approximately the same after weathering in the field. The germination tests averaged 60.0 per cent as compared to the 60.5 per cent of the unweathered seed.

Table 3

LABORATORY TESTS WITH CAPTIVE MICE—FRESHLY TREATED SEED OCTOBER, 1951

Date			Oct. 8				Oct. 9				Oct. 10				Oct. 11				Oct. 12, 13, 14			
Mouse No.	Sex	Weight (gms)	Offered	NT	Eaten	NT	Offered	T	Eaten	T	Offered	NT	Eaten	NT	Offered	T	Eaten	T	Offered	NT	Eaten	NT
			W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
1	♀	29.1	30	5	27	5	30	5	3	4	Dead Oct. 9											
2	♂	17.6	30	5	30	5	30	5	30	4	30	5	29	4	Dead Oct. 11							
3	♂	13.9	30	5	30	5	30	5	29	4	30	5	30	5	30	5	29	2	*	....	**	....
4	♂	11.7	30	5	30	5	30	5	8	4	30	5	29	5	30	5	29	5	*	....	***	....
5	♀	10.1	30	5	30	5	30	5	5	5	Dead Oct. 10											
6	♀	18.1	30	5	29	5	30	5	19	5	Dead Oct. 10											
7	♀	9.0	30	5	30	5	30	5	29	5	30	5	....	1	Dead Oct. 11							
8	♂	15.1	30	5	27	5	30	5	....	4	Dead Oct. 9											
9	♀	17.5	30	5	28	5	30	5	17	5	Dead Oct. 10											
10	♀	15.1	30	5	29	5	30	5	27	4	Dead Oct. 9											

* 20 gms of wheat given as food for period Oct. 12-14
** 11.5 gms of wheat eaten during period Oct. 12-14
*** 12.5 gms of wheat eaten during period Oct. 12-14

W—Wheat grains
D—Douglas fir seed
NT—No tetramine
T—Tetramine

Note: Tetramine treated and untreated Douglas fir seed were given on alternate days to determine if the mice developed an adversion to Douglas fir seed after ingesting treated seed.

Table 4

LABORATORY TESTS WITH CAPTIVE MICE—WEATHERED SEED APRIL, 1952

Date			April 8				April 9				April 10				April 11				April 12			
Mouse No.	Sex	Wt. (gms)	Offered	T	Eaten	T	Offered	NT	Eaten	NT	Offered	T	Eaten	T	Offered	NT	Eaten	NT	Offered	T	Eaten	T
			W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D
1	♂	.....	30	5	30	5	30	....	30	....	30	5	....	....	Dead Apr. 11							
2	♀	12.6	30	5	20	5	30	....	29	....	30	5	30	5	30	....	14	....	30	7	10	6
3	♀	12.6	30	5	28	5	30	....	29	....	30	5	30	....	30	....	30	....	30	7	30	7
4	....	13.5	30	5	24	3	30	....	30	....	30	5	30	1	30	....	30	....	30	7	30	7
5	♂	16.1	30	5	25	5	30	....	24	....	30	5	15	5	30	....	8	....	Dead Apr. 12			
6	♂	19.1	30	5	1	4	Dead Apr. 9															
7	....	16.8	30	5	30	5	30	....	30	....	30	5	....	....	30	....	15	....	30	7	20	2
8	♀	14.5	30	5	19	5	30	....	13	....	30	5	30	5	30	....	2	....	Dead Apr. 12			
9	♀	24.2	30	5	3	5	Dead Apr. 9															
10	♂	17.9	30	5	5	5	30	....	30	....	30	5	5	3	30	....	30	....	30	7	28	4

W—Wheat grains
D—Douglas fir seed
NT—No tetramine
T—Tetramine

TABLE 3—Continued

LABORATORY TESTS WITH CAPTIVE MICE—FRESHLY TREATED SEED OCTOBER, 1951

Oct. 15				Oct. 16				Oct. 17					Total		Total		Total	
Offered	T	Eaten	T	Offered	NT	Eaten	NT	Offered	T	Eaten	T		Offered	Eaten	Offered NT	Eaten NT	Offered T	Eaten T
W	D	W	D	W	D	W	D	W	D	W	D		W	W	D	D	D	D
													60	30	5	5	5	4
													90	89	10	9	5	4
30	5	30	5	30	5	30	5	Dead Oct. 17					180	178	15	15	20	11
30	5	30	....	30	5	30	5	30	5	30	3	Dead Oct. 18	210	186	15	15	25	12
													60	35	5	5	5	5
													60	48	5	5	5	5
													90	59	10	5	5	5
													60	27	5	5	5	4
													60	35	5	5	5	5
													60	56	5	5	5	4

Table 4—Continued

LABORATORY TESTS WITH CAPTIVE MICE—WEATHERED SEED APRIL, 1952

April 13				April 14				April 15				April 16				Total		Total	
Offered	NT	Eaten	NT	Offered	T	Eaten	T	Offered	NT	Eaten	NT	Offered	T	Eaten	T	Offered	Eaten	Offered T	Eaten T
W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	D	W	W	D	D
																90	60	10	5
Dead Apr. 13																150	103	17	16
30	....	30	....	Dead Apr. 14												180	177	17	12
30	....	29	....	30	9	30	....	30	....	30	....	30	11	30	5	270	26	37	16
																120	72	10	10
																30	1	5	4
30	....	29	....	30	9	....	....	30	....	....	....	30	11	Dead		270	104	37	7
																120	62	10	10
																30	3	5	5
Dead Apr. 13																150	98	17	12

Germination of treated and untreated seed was quite similar under field conditions. Germination of the treated seed under protective screens was 26.8 per cent and of the control 35.3 (These test spots were protected from seed eaters by hardware cloth caps.) (See Table 5).

Table 5

RESULTS OF GERMINATION TESTS

	Duration in days	Germination Per Cent: No Chill	2 weeks Chill	Mean
Laboratory Tests				
Tetramine treated Douglas fir (Petri-Towel)	35	10.0	7.0	8.5
Tetramine treated Douglas fir (Petri-Towel)	35	5.0	2.0	3.5
Mean		7.5	4.5	6.0
Control Douglas fir (Petri-Towel)	35	63.0	58.0	60.5
Tetramine treated Douglas fir				
Placed in field 12-8-51				
Removed 3-27-52				
(Sand)	35	27.0	37.0	32.0
(Vermiculite)	35	32.0	41.0	36.5
(Petri-Towel)	35	22.0	30.0	26.0
Mean		27.0	36.0	31.5
Control—Douglas fir over-wintered on forest soil				
Placed in field 12-8-51				
Removed 3-27-52				
(Sand)	35	61.0	57.0	59.0
(Vermiculite)	35	58.0	58.0	58.0
(Petri-Towel)	35	59.0	67.0	63.0
Mean		59.3	60.7	60.0

Field Tests

Field Germination of Seed Under Screen Caps, 1000 Seeds Each Sample

Treated	12-8-51 to 6-13-52	26.8
Untreated Control	12-8-51 to 6-13-52	35.3

Stocking

On June 16, 1952, as soon as germination was practically complete, a preliminary stocking survey was made. Forty four-milacre plots were located. These were further subdivided into four quadrants, thus providing a total of 160 one-milacre sample plots. Of these, 41.2 per cent were stocked. This represents from 525 to 775[1] trees per acre.

[1] From Oregon State Board of Forestry, Research Note No. 9 "The Relationship of Stocking Percent to Number of Trees Per Acre on Artificially Seeded Areas", by Dale N. Bever.

In December, 1952 a more extensive survey was made. The percentage of one-milacre plots found stocked was 50.2 or 750+ trees per acre.

In the fall of 1953, 400 mechanically distributed plots were examined. Of these 100 were stocked. Although this indicated a considerable loss of seedlings since June, 1952, the results compare very favorably with the results of project seeding on nearby areas in 1951. (The average stocking per acre for the 11,720 acres seeded on these projects was 16.6 per cent as compared to 25.0 for the experimental plot).

CONCLUSIONS

The results of the field test of tetramine-treated Douglas fir seed were encouraging. The initial germination and survival were somewhat better than the average that has been obtained from the standard baiting and seeding procedure.

Conclusive answers to some of the important questions, however, were not forthcoming from this experiment. Small seed eating mammal populations appeared subnormal throughout the trapping period outside as well as inside the plot.

Tests indicated that the tetramine treatment did reduce the viability of the seed, especially immediately after treatment. Results in terms of stocking were quite good for field seeding. This indicates that either the passage of time, weathering in the field, or a combination of these or other factors, may tend to lessen the adverse effect upon the viability.

Definite statements as to the effect of tetramine-treated seed on the normal populations of small seed-eating mammals are impossible. Trapping records were not conclusive due to the small populations, both inside and outside the plot, and to the fact that trapping was interrupted during extreme fire weather conditions.

Over-all indications were so favorable, however, that it is recommended that further tests be continued at the earliest possible date. If these results are substantiated by further experiments, then tetramine treatment could be recommended as a procedure superior to the baiting and seeding methods now in current use on artificial reforestation projects.