DETERMINING MINIMUM AMOUNTS OF TMTD RABBIT REPELLENT NEEDED TO PROTECT DOUGLAS-FIR PLANTING STOCK[1]

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Introduction

The feeding of wild mammals, particularly the Lagomorpha, can be highly detrimental to forest regeneration in the Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) region. Young plantations have been extensively damaged by hares and rabbits (4).

To protect newly planted seedlings from such damage, several nurseries now treat planting stock with TMTD repellent (1, 2). The repellent in a 10-percent formulation is sprayed on the seedlings shortly before lifting, and protection in the field is expected to last through the first dormant season. Nursery application of the repellent, however, has been based on very little research, and performance of treated seedlings in the field has not always been satisfactory.

[1] Active ingredient, tetramethylthiuram disulfide.

[2] The assistance of Dr. J. W. Duffield, former technical director of the Col. W. B. Greeley Forest Nursery; the Olympia. Wash., Station of the U.S. Bureau of Sport Fisheries and Wildlife; and the Washington State Department of Natural Resources is gratefully acknowledged.

The recent . evaluation of different concentrations of the TMTD formulation by the Denver Wildlife Research Center showed that minimum concentrations required to protect 2-0 Douglas-fir seedlings from hares and rabbits were 6 percent under field conditions and 3 percent in short-term pen tests. These results, while important, are of little help to nurserymen unless they are adapted to commercial nursery operations and conditions.

Objectives

We must determine (1) the minimum effective amount of TMTD that a 2-0 seedling must retain for protection after outplanting and (2) the minimum effective rate of spraying that will provide this protection. The experiments reported here dealt with the first objective. Investigation of the application rates is now in progress and will be reported later.

Determinations were made of (1) the amount of TMTD retained by 2-0 seedlings when diptreated with the minimum effective TMTD concentrations used in pen and field tests by the Denver Wildlife Research Center, (2) the amounts of TMTD lost from the seedlings due to weathering after planting and during the dormant season, and (3) the amounts of TMTD lost from the seedlings during standard nursery operations following repellent application.

Materials

The TMTD formulation used in all experiments contained 10 percent Rhoplex AC-33. However, TMTD concentration in the formulation varied--3 and 6 percent of TMTD were used in the first experiment, and 10 percent in the others.

[3] Dodge, Wendell E. Field testing and development of selected chemicals for preventing wildlife damage to forest crops in the Northwest. 20 pp. 1963. (Unpublished annual progress report January 1962-June 1963 on file at Wildlife Res. Work Unit, Denver Wildlife Res. Center, Denver, Colo.)

Experimental Procedures and Results

MINIMUM AMOUNTS OF TMTD PROTECTIVE TO SEEDLINGS

In this experiment determinations were made of the amount of TMTD retained by Douglas-fir seedlings when the plants were dipped in 3- and 6-percent suspensions, the minimum concentrations found effective in ,pen and field tests, respectively, by the Denver Wildlife Research Center.

Procedure.--Seedlings, selected for uniform size, were packed in bundles of 25 each. Roots in each bundle were surrounded by moist shingle tow and wrapped with protective paper. The foliage was then washed with tapwater, and the bundles were allowed to surface dry overnight at room temperature.

The seedlings in each bundle were treated with TMTD by dipping the foliage in repellent formulations of different concentrations. For each concentration, three bundles were treated and allowed to drip dry overnight at room temperature.

A five-seedling sample was taken from each bundle. The seedling roots were severed at the root collar and discarded. The tops of the seedlings were placed in glass containers and dried to a constant weight at 1050 .[4] The dry tissues were then weighed, ground to 60 mesh in a Wiley mill, and stored in airtight glass bottles.

Duplicate weighed portions of ground tissue were analyzed for TMTD according to Keppel's method (3). In each test, TMTD was extracted with chloroform, and aliquots of extracts were treated with cuprous iodide. The absorbance of the resulting solutions was then measured in a Beckman DU spectrophotometer at 440 millimicron, and the amount of TMTD was read from a previously constructed calibration curve.

[4] Experiments in our laboratory have shown that heat and Rhoplex AC-33, separately or in combination, do not affect the stability of TMTD.

Results.--When treated by the dip method with formulations of different TMTD, concentration, the seedlings held different amounts of TMTD (table 1). However, such amounts were not strictly proportional to concentration, possibly owing to differences in viscosities of the formulations. As TMTD concentration increased, the suspensions became thicker, and the dipped seedlings probably retained a larger proportion of the formulation.

Seedlings dipped in a 3-percent TMTD formulation held an average of 2.0 mg. TMTD, whereas those dipped in a 6-percent suspension produced seedlings retaining an average of 8.0 mg. Therefore, the minimum effective amounts of TMTD in short-term pen tests and in all-season field trials were 2.0 and 8.0 mg., respectively. In addition, the difference between the two TMTD requirements, as explained later in the discussion of weathering losses of TMTD, was probably because of the difference in duration of the two tests and the consequent differences in amounts of TMTD lost from seedlings owing to weather elements.

WEATHERING LOSSES OF TMTD

In this experiment the amount of TMTD lost from treated seedlings by weathering was determined and the biological stability of TMTD on the seedlings' surface was tested.

Procedure.--In November 1962, Douglas-fir seedlings in a seedbed at the Col. W. B. Greeley Forest Nursery were hand sprayed with TMTD. One week following treatment, the sprayed seedlings were lifted, graded for uniformity, and divided into two groups at random. The first group was transplanted in rows in a small outdoor bed at the nursery, and the second group was transplanted in plastic containers and transferred to a greenhouse, where the temperature was 70° F. for 16 hours of each daily cycle and 50° F. or higher for the remaining 8 hours. Treated seedlings from each group were sampled on the day after spraying and once a month thereafter until bud break. Two samples were taken each time from each treatment, and each sample consisted of three to five seedlings taken at random. Seedling roots were severed just above the root collar and discarded. Harvested seedling tops were processed and analyzed for TMTD in duplicate, following methods of the first experiment.

Results.--Data indicate that about 16 percent of the TMTD was lost in the greenhouse (table 2). This may be a result of the loss of some of the lower needles of the seedlings, which were noticed on top of the plant containers. TMTD, therefore, seems stable on surfaces of live seedlings and is not degraded by a biological factor.

In winter, however, seedlings lost about 69 percent of their TMTD content in 4 months. This is a significant loss and could account for the difference between the minimum requirements of pen and field tests. Accordingly, seedlings in field tests must have lost about 6 mg. of their original TMTD content (8.0 mg.) during the winter. This loss indicates agreement between field and pen tests (i.e., a TMTD requirement of 2.0 mg,) if weathering losses of TMTD are excluded. It also suggests a minimum requirement of 8.0 mg. TMTD per seedling for protection against hares and rabbits during the entire dormant season

TABLE 1.--TMTD retained by 2-0 Douglas-fir seedlings dipped in two formulations

Concentration of TMTD in formulation (percent)	TMTD per seedling[1]			
	Replication			Average[2]
	1	2	3	
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*
3.....	2.2	3.0	2.0	2.0
6.....	9.2	6.5	8.1	8.0

[1] Average ovendry weight per seedling was 2 to 3 grams. All TMTD values were adjusted to a 3.0-gram seedling to eliminate weight variations.
[2] Figures are rounded to the nearest milligram.

TABLE 2.--Effect of greenhouse and natural winter conditions on the persistence of TMTD Rhoplex film on 2-0 Douglas-fir seedlings

After treatment (months)	TMTD per seedling[1]		Monthly TMTD loss	
	Greenhouse	Natural conditions	Greenhouse	Natural conditions
	Milligrams	*Milligrams*	*Percent*	*Percent*
0.....	6.4	6.4	0	0
1.....	6.8	4.3	0	32.8
2.....	5.7	3.0	10.9	20.3
3.....	5.4	2.0	4.7	15.6
4.....	(2)	2.2	(2)	0
Total loss	--	--	15.6	68.7

[1] Averages of two replications.
[2] No sample taken because of bud break.

under weather conditions of western Washington.

Weathering losses also indicate that Rhoplex AC-33, the adhesive in TMTD formulations, did not provide sufficient binding of TMTD on seedlings. This adhesive proved unsuitable for outdoor use even when the TMTD formulations were applied at the ideal setting temperature of 70° F.[5] A search should be made to find another adhesive with better weathering properties. Until such an adhesive becomes available, spraying in the nursery should be done just before lifting to minimize the effect of weathering and conserve TMTD while the seedlings are in the nursery.

HANDLING LOSSES OF TMTD

Minimum amounts of TMTD applied in the nursery should be higher than those used in field tests where seedlings are outplanted immediately after treatment without further preparation and possible loss of TMTD. However, nursery-treated seedlings are subjected to several standard nursery operations before being outplanted. In this experiment, losses of TMTD owing to lifting and tying--the most important posttreatment nursery operations-were investigated.

Procedure.--In January 1963, Douglas-fir seedlings in several 4- by 330-foot nursery beds at the L. T. Webster Forest Nursery were treated with TMTD repellent. A formulation was applied at a rate of approximately 8 gallons per bed with an agricultural power sprayer mounted on a tractor.

Just before lifting and 2 weeks after spraying, two samples of 25 seedlings each, selected for uniform size, were taken from different locations in each of three randomly selected beds. Similar 25-seedling samples were taken from the beds after lifting and tying. The seedling-sample roots were severed at the root collar and discarded.

In the laboratory, a subsample of five seedlings was taken from each sample. All subsamples were processed and analyzed for TMTD as in the first experiment.

[5] Unpublished data on file at Pacific Northwest Forest and Range Expt. Sta., Portland, Oreg.

Results.--Both lifting and tying caused significant losses of TMTD (table 3). The two operations, however, did not contribute equally to the total loss, and losses varied widely among replications, 26 to 64 percent. In order to use the results of the second experiment (a TMTD minimum requirement of 8.0 mg. per seedling in field tests) for calculation of minimum amounts of TMTD for nursery operations, it was necessary to consider this range of losses. We determined that 11 to 22 mg. of TMTD (an average of 16 mg.) are required for protection of 2-0 seedlings when stock is treated in the nursery. All seedlings used in this experiment retained amounts of TMTD (8.6, 4.5, and 7.6 mg.) much below the minimum required for protection.

Furthermore, although beds for the three replications were sprayed the same day and under conditions presumed equal, amounts of TMTD per seedling varied greatly between seedlings of the different beds. Whether the reported variability is typical of all spraying jobs at the nursery is not known. However, if satisfactory protection of seedlings in the field is to be obtained, methods must be developed that will apply the minimum TMTD uniformly to stock in the nursery.

Discussion and Conclusions

In testing repellent formulations, the dip method is usually used to treat seedlings. This method is simple, fast, and apparently results in uniform application of repellent to test plants. In nursery applications, however, dipping is not practical. Nurseries in western Washington use power spraying equipment, and rates of application (amount of repellent per seedling) under these conditions differ from those obtained with the dip method, even when formulations of the same concentration are used.[5] Consequently, the results of testing experiments cannot be compared directly with nursery treatments. The results from such experiments can be used, however, to determine effective minimum amounts of TMTD needed for nursery application, which, in turn, can be utilized in determining minimum rates of application. The present study was concerned with the first part of this approach.

TABLE 3.--Effect of handling operations on loss of TMTD from nursery-treated 2-0 Douglas-fir seedlings

Handling operation	Residual TMTD per seedling following handling[1] by replication--			TMTD loss due to handling by replication--		
	1	2	3	1	2	3
	Milligrams	*Milligrams*	*Milligrams*	*Percent*	*Percent*	*Percent*
None.....	8.6	4.5	7.6	0	0	0
Lifting.....	7.1	4.0	3.9	17.4	4.8	48.6
Tying.....	6.6	1.5	4.0	8.1	59.5	0
Total loss....	--	--	--	25.5	64.3	48.6

[1] Average ovendry weight per seedling was 2 to 3 grams. All TMTD values were adjusted to a 3.0-gram seedling to eliminate weight variations.

Based on (1) concentrations of TMTD found effective in pen and field tests by the Denver Wildlife Research Center, (2) weathering losses of this repellent TMTD during the dormant season (69 percent), and (3) handling losses of TMTD in the nursery (26-64 percent), TMTD minimum requirements of Douglas-fir seedlings for western Washington conditions ranged from 11 to 22 mg. per seedling. Seedlings sprayed in the nursery contained amounts of TMTD much below the protective levels. Furthermore, the repellent was not applied uniformly to seedlings in the different seedbeds, indicating a need for additional research on application methods.

Significant losses of TMTD caused by weathering and nursery handling are probably due to the properties of Rhoplex AC-33, the adhesive now in use in TMTD formulations. This adhesive should be replaced by a compound with better weathering properties. Until such a compound is discovered, seedlings should be treated in the nursery as shortly before lifting as possible and handled with care.

Since this investigation was completed, field and laboratory studies have been initiated to determine minimum effective rates of spraying Douglas-fir in the nursery with 10 percent TMTD and to provide nurserymen with a simple method by which they can assess the accuracy and uniformity of spraying operations. Results of these studies will be reported later.

Literature Cited

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