March 1974

ALPHA-NAPHTHYLTHIOUREA AS A CONDITIONING REPELLENT FOR PROTECTING CONIFER SEED

Peter C. Passof
University of California, Ukiah, California

Rex E. Marsh
University of California, Davis, California

Walter E. Howard
University of California, Davis, California

ALPHA-NAPHTHYLTHIOUREA AS A CONDITIONING REPELLENT FOR PROTECTING CONIFER SEED

PETER C. PASSOF, Forest Advisor, Cooperative Extension, University of California, Ukiah, California
REX E. MARSH, Specialist in Vertebrate Ecology, Wildlife and Fisheries Biology, University of California, Davis, California
WALTER E. HOWARD, Professor of Wildlife Biology and Ecologist, Wildlife and Fisheries Biology, University of California, Davis, California

ABSTRACT: The object of this study was to find an alternative rodent repellent to take the place of endrin when direct seeding to regenerate coniferous forests. Compounds with aversive conditioning repellent attributes were screened. Even though still falling far short of endrin, which also acts as a lethal rodenticide, alpha-naphthylthiourea (ANTU) treated seed produced about twice as many seedlings as did the untreated seed. Both the laboratory and field evaluation procedures and results are discussed. It is hoped that with more research the efficacy of ANTU as a seed protectant can be further improved. The compound is much too promising to abandon as a candidate repellent for deer mice and possibly other rodent species.

INTRODUCTION

Deer mice (Peromyscus spp.) and other seed-eating rodents have long been considered a major problem when conifer-forest regeneration is by direct-seeding methods. The nature and severity of the problem and need for counter—measures have been well substantiated (Abbot, 1961; Hooven, 1958; Moore, 1949; Smith and Aldous, 1947; Spencer, 1954; Tevis, 1953 and 1956). Both deer mice and other rodent species consume much of the seed used in reestablishing vegetative cover following burns and in improving range or wildlife habitat. The literature on seed predation by animals has been very thoroughly reviewed by Janzen (1971)

Endrin, a persistent chlorinated hydrocarbon insecticide has been used as a rodent repellent on conifer seeds since 1956 (Fish and Wildlife Service, 1956). According to Radwan et al. (1970) endrin was introduced then by the U.S. Fish and Wildlife Service as an interim seed protectant until a better protectant was available. Early formulations used to protect conifer seeds contained both thiram (Arasan®) and endrin; however, in some regions of the western states thiram was eventually omitted because evidence was lacking that it benefited seedling production (Crouch and Radwan, 1972). Increasing concern over environmental contamination with chlorinated hydrocarbons has prompted studies to find an equally effective seed protectant that lacks the undesirable characteristics of endrin. Adverse effects on fish and other wildlife from endrin used as an insecticide have been well documented. The significance of isolated incidents of bird losses from endrin-treated tree seed has not been established (Hamrick, 1968).

Endrin as a conifer seed protectant seems, from the little evidence available, to possess no significant or potentially significant wildlife hazard. Endrin as an insecticide, however, is for the most part beyond the challenge as a hazard. The reason for the difference is that endrin as a seed protectant is used in relatively minute amounts and applied very infrequently. The difference is confirmed by research of Bollen and Tu (1971) and Marston et. al. (1969).

Because of current attitudes toward all persistent chlorinated hydrocarbons, however, it would appear that endrin will soon be drastically restricted if not eliminated.* Present constraints prevent certain governmental agencies from making use of endrin for any purpose. Hazards related to endrin-treated conifer seeds are currently being investigated in southeastern forests. Some foresters and scientists take the optimistic attitude that endrin as a seed protectant is a sound and environmentally acceptable practice to reduce rodent losses, as is evidenced by its use as a conifer seed protectant for 18 years. To discontinue use of endrin without the availability of suitable alternatives will certainly be challenged by many, because a substantial number of acres is seeded annually (Table 1), and endrin has been the most effective means of preventing rodent depredations.

* The authors' discussion of endrin in this article is for the purpose of providing background information and in no way is intended to constitute a recommendation or endorsement of the use of endrin.

Table 1. Forest direct-seeding in fiscal years 1967 to 1973 on federal lands, nonfederal public lands, and private property.*

Ownership	Acres Direct-Seeded 1967	1968	1969	1970	1971	1972	1973
Federal Lands	56,480	51,510	67,950	67,370	54,931	45,154	40,109
Nonfederal Public	4,555	8,153	9,600	8,647	8,778	4,115	8,628
Private Property	120,967	158,335	147,043	187,514	179,232	175,192	152,091
Total Reported	182,002	217,998	224,593	263,531	242,941	224,461	200,828

* Figures from U.S. Forest Service, Department of Agriculture, Annual Reports - Forest and Windbarrier Planting and Seeding in the United States.

It usually is not possible to establish tree stands of the density desired by direct seeding methods if an effective rodent repellent is not used. In some situations total failure can be expected. If populations of the depredating species of rodents are effectively reduced, much protection is provided to the untreated seed. The control of forest rodents was practiced prior to the introduction of endrin (Hooven, 1953), and in some situations rodent control with toxic bait is currently used in conjunction with endrin treated seed (Passof, 1974).

Many individuals recognized that foresters would inevitably be faced with the problem of losing endrin because of increasing opposition to persistent chlorinated hydrocarbon pesticides. We embarked upon preliminary rodent repellent studies in 1967, but did not initiate a systematic screening program in search of substitutes for endrin until early in 1969. This early research was supported by the California State Division of Forestry, and reported in the 1969-1970 Progress Report to that Division (Howard and Marsh, 1970). Unfortunately, an economy move forced the California Division of Forestry to terminate this support, but thanks to the efforts of concerned individuals at the Portland Center of the U.S. Bureau of Land Management, a contract was negotiated with BLM to continue our search for a substitute for endrin.

SELECTING CANDIDATE MATERIALS

We evaluated just those compounds that were known to us, based on our previous experiences in rodent behavior or on literature reports, to have repellent qualities. Where repellency appears to have some correlation with chemical structure, candidate compounds were then selected by chemical structure alone. In our selection of candidate compounds for evaluation purposes we took into consideration their availability, costs, suitability for registration, and their physical and pharmacological properties.

There are decided advantages of selecting compounds that are already on the market, especially those that are presently registered as pesticides. Expanding an existing registration to include the protection of forest seeds may take only a fraction of the time and expense required to register a new chemical. Also, seed protectants against rodents have such a limited marketing potential that few companies would be interested in anything other than one of their products already marketed.

The Fish and Wildlife Service Wildlife Research Center at Denver screened hundreds of compounds of relatively unknown characteristics for many years, testing for both toxic and repellent qualities (Kverno and Hood, I963). Since this procedure did not uncover a suitable and marketable substitute for endrin, we chose to make our selection of potential candidate repellents quite narrow, but to include compounds having aversive conditioning attributes.

Having more than a single research team involved in evaluating repellents has some decided advantages since different test approaches or procedures may select different compounds of merit as candidate materials. As an example, preceding our systematic screening program, the University's Statewide Extension Forester asked whether we had any suggestions for possible conifer seed protectants. Since research under way on rodent chemosteri1 ants had shown that estrogenic compounds such as diethylsti1besterol (DES) and mestranol in diets created strong aversions to those diets, they seemed logical candidates as rodent repellents. Subsequently, batches of Douglas-fir (Pseudotsuga menziesii), red fir (Abies magnifica), and ponderosa pine (<u>Pinus</u> <u>ponderosa</u>), seed were treated with DES, and other batches were treated with mestranol. A resulting series of Douglas-fir seed spot tests established in Mendocino County was discussed as part of an Animal Damage Field Trip held in the area in 1968 (Passof, 1968). Even though the field results were disappointing, M. A. Radwan of the U.S. Forest Service alertly observed possible reasons. Through his foresight and that of his colleagues, mestranol was further tested and demonstrated efficacy at levels of 2% on Douglas fir seed (Crouch and Radwan, 1971). This higher concentration of mestranol has given promising results in further tests in laboratory and field by the Forest-Animal Unit of the Bureau of Sport Fisheries and Wildlife, Olympia, Washington.

LABORATORY EVALUATION PROCEDURES

Our procedure for evaluating the laboratory efficacy of a candidate compound is designed for great sensitivity to avoid overlooking a compound which might produce a conditioned repellency. Conditioned repellency results from the ability of deer mice to associate an initially acceptable compound which produces a physiological change causing some discomfort or ill feeling in the animal. This previous experience causes the animal to reject or avoid the compound in future exposures. This learned avoidance or aversive conditioning is also called conditioned repellency. It could be considered loosely synonymous with "bait shyness", learned avoidance associated with sub-lethal toxicity. Conditioned repellency is believed to be quite different from simple taste or odor repellency, where a compound is rejected because of gustatory or olfactory stimulus without any deep-seated biological activity acting as reinforcement. After an initial exposure, both taste and odor can provide cues for future rejection. Olfactory acuity is highly developed in deer mice (Peromyscus maniculatus) and a major factor in their ability to locate conifer seed (Howard and Cole, 1967; Howard et al. 1968). Our previous studies suggest that conditioned repellency may take slightly longer to develop, but is more lasting. We have demonstrated that conditioned repellency produced in deer mice with sublethal levels of sodium f1uoroacetate (1080) lasted for at least eight months (unpublished).

In the initial screening tests, 50 treated sunflower seeds are offered to each of ten (sexes equal) individually caged mice not previously used on any test or previously exposed to sunflower seed. They were also provided with Purina laboratory chow to reduce any possibility of a test bias resulting from starvation. Laboratory chow is not a preferred food of deer mice. These tests were always conducted with a minimum of ten mice for six consecutive days, with results tabulated daily. The criteria used for determining the efficacy of a candidate compound in the laboratory was the number of treated seeds cracked open, regardless of whether the endosperm had been eaten. A corresponding number of mice were used for controls and received 50 untreated sunflower seeds. Since sunflower seeds are highly preferred by deer mice, they worked well in the initial screening tests. The most promising candidate compounds were later tested using 100 Douglas-fir seeds daily per test animal. Conifer seeds were not used in the initial screening tests because of their high cost and the quantity of seed required for the nutritional needs of deer mice to be satisfied even partially. We normally treat seeds in lots of, at least, 500 grams to minimize errors in preparation.

We believe that a test duration of six days is highly important and much more likely to reveal a conditioned repellency than the three-days as outlined by Kverno and Hood (1963). Other laboratories are now also using test periods longer than three days. It is highly possible that a ten-day period would turn up additional conditioning repellents, although losing so much seed initially under field situations could make the candidate material of low practical value.

LABORATORY EFFICACY

During *our* laboratory screening of selected candidate repellents we came up with several promising ones. This included several chlorinated hydrocarbons - dieldrin and chlordane, of which dieldrin held the greatest promise. Colchicine and Gophacide® were also effective aversive conditioners but, for other reasons were not pursued beyond the initial screening tests. Also waiting further evaluation is red squill, a plant extract containing "scilliroside", one of the glycosides which is quite biologically active on some rodent species. Of those compounds screened, thiourea and two derivatives, alpha-naphthylthiourea and 1-phenyl-2-thiourea, all ranked high in producing a conditioned repellency toward the treated seed. The influence on seed germination of these three compounds will be discussed later in detail, but phytotoxicity studies eliminated all except alpha-naphthylthiourea.

Alpha-naphthylthiourea (commercial rodenticide grade, active ingredient 92%) was evaluated in the laboratory as an aversive conditioner on P. maniculatus, P. truei, and laboratory mice (Mus musculus). Results were quite comparable for all three species.

A typical example of the ability of 4% alpha-naphthylthiourea (commonly referred to as ANTU) to induce an aversion to Douglas-fir seed is provided in Figure 1. The average consumption of Douglas-fir seed by P. maniculatus dropped from 60.2 to 1.2% by the third day and rose slightly to 6.2% on the sixth day. Reduction in seed consumption from the first day's exposure to the sixth day amounts to 89.7%. Seed consumption by P. truei on the sixth and final day of the test was 27.3% of that on day one (a 72.7% reduction).

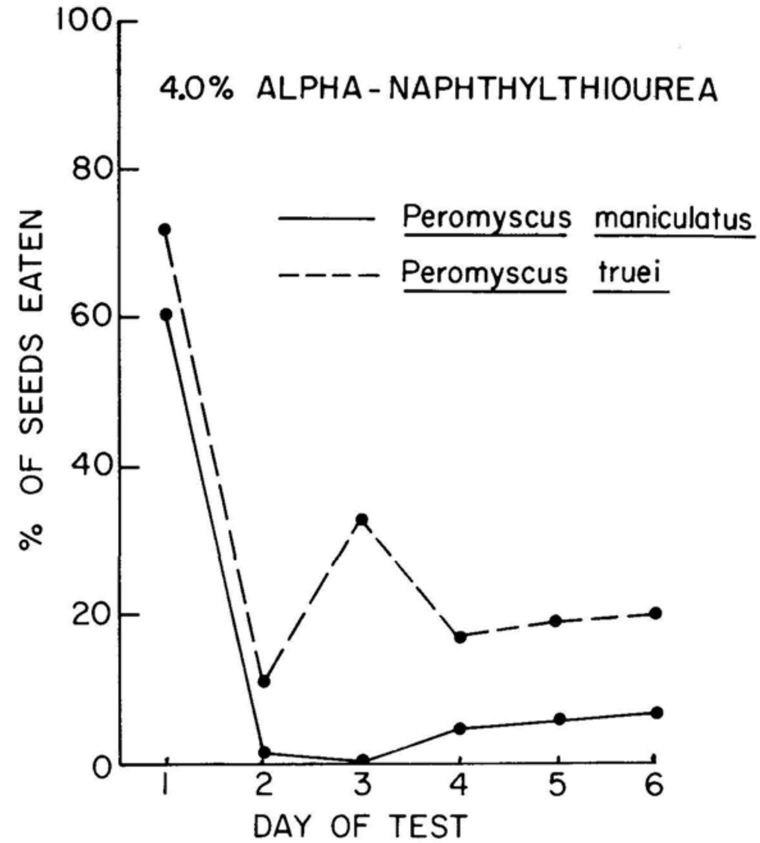

Figure 1. Aversive conditioning response when two species of wild captured Peromyscus were offered Douglas-fir seed treated with 4% ANTU for six consecutive days. Ten mice (sexes equal) made up each of the two groups, with each mouse offered 100 treated seeds daily plus laboratory chow.

P. maniculatus from our own breeding colony did not differ appreciably in results from P. maniculatus collected from the wild in Mendocino County (coastal region) and near Mount Shasta (inland region). Replicated tests indicate some variations between individual mice and between groups of mice, not surprising in view of the complexity of the cause and effect relation in aversive conditioning. The data suggest that aversion develops faster from a large initial (first night) exposure to the compound, though further study is needed.

While 1% ANTU protected sunflower seed from deer mice, 4% was necessary for the same protection of Douglas-fir seed. This is probably an expression of surface area, although hardness and texture of the seed coat and the ability of the mice to manipulate seed of certain shapes and sizes may also play an important role. The optimum percentage of active ingredient will have to be determined for each species of tree seed.

Adequate techniques for measuring simultaneously both the magnitude and frequency of reinforcement to maintain a meaningful aversion have not been developed for this compound or for any other potential conditioning repellent. From our particular studies of various aversive conditioners we are convinced that the aversion in deer mice is associated primarily with the chemical not with Douglas-fir seed. If that holds true in comprehensive studies, untreated seed will get very little protection from deer mice, if any, in blendings of treated and untreated Douglas-fir seed. This may not be the case with other rodents or with food items ranking lower in the animal's food preference.

Douglas-fir seed treated with 4% ANTU and offered in a free-choice situation under laboratory conditions is rarely fatal to deer mice.

CHARACTERISTICS OF ANTU

ANTU was initially developed in a rather unusual manner. During World War II a currently used rodenticide, red squill, was no longer available since it came mostly from the Mediterranean area, where shipping was curtailed. This prompted Dr. Curt Richter in early 1942 to investigate the possibilities of thiourea derivatives as a potential substitute for red squill. Phenyl thiourea (pheny1thiocarbamide or 1-phenyl-2-thiourea), used in numerous studies on taste thresholds and on the inheritance of taste ability in humans, was found to be highly toxic to laboratory rats (Richter and Clisby, 1941). This then led to a study of closely related compounds, including the parent compound, thiourea.

As the result of Richter's studies alpha-naphthylthiourea was selected and developed as a rodenticide for Norway rats (Rattus norvegicus). It was found not to be sufficiently toxic for other species. ANTU, ($C_{10}H_7$-$NHCSNH_2$) or ($C_{11}H_{10}N_2S$), is one of the single N-substituted thiourea derivatives. It contains the thioureido grouping ($NHCSNH_2$), in which only a single hydrogen of the thiourea molecule is replaced by a substitute (Dieke et al., 1947).

In our studies of suitable conifer seed protectants we evaluated only 14 of several hundred thiourea derivatives that are available. The thiourea derivatives are a fascinating group of compounds which should probably be scrutinized more closely for their potential in resolving vertebrate pest problems. Quite apart from the ANTU used as a rodenticide, other thiourea derivatives have proved to be interesting compounds in fields as varied as medicine, biochemistry, physiology, behavior, and genetics (Richter, 1945).

ANTU varies considerably in toxicity to different animal species, with Norway rats (Rattus norvegicus) being the most susceptible. In fact the closely related roof rat (R. rattus) and the house mouse (M. musculus) are not sufficiently susceptible for the compound to be useful as a rodenticide against them. Birds are considered highly immune to ANTU. Twelve Rhesus monkeys (Macaca mulatta) given ANTU by stomach tube in 10% acacia solution gave an LD_{50} of 4,250 mg/kg (Freeman, 1954). If toxicity to humans parallels that of primates, it means that the compound is a very safe material for man to use. In the absence of specific data on humans, however, ANTU should be handled with care to avoid accidental ingestion or unnecessary exposure.

The parent compound thiourea was shown in 1933 to prevent browning in cut fruit, and patents were obtained for that use. It was also found to prevent mold on wheat and to protect oranges against stem-rot, but since the compound depressed thyroid activity it was considered unsuitable for use in foods (Jacobs, 1958).

At present, although available and federally registered for Norway rat control only, ANTU is not used extensively as a rodenticide. Given most often for its lack of popularity are the following reasons:

1. It is effective only for Norway rats, not for other common commensal rodents such as roof rat and house mouse.

2. ANTU is not especially well accepted in baits, and, therefore, is effective only with highly preferred foods such as horse meat or fish. Prebaiting is necessary to insure maximum effectiveness.

3. Sublethal doses produce bait shyness, an aversion to the bait and/or the toxicant (Gaines and Hayes, 1952). Depending on the level of exposure this bait shyness in rats can last for at least four months. Hence, ANTU cannot be used on the same population of rats without a relatively long waiting period between poisoning programs.

4. An induced tolerance can develop in rats from small sublethal doses. This tolerance decreases control, though not nearly as significantly as does bait shyness.

The mode of action of this compound and factors affecting physiological activity have been studied in some detail (Bentley et al., 1955; Byerrum and DuBois, 1947; Dieke et al., 1947; DuBois et al., 1946a, 1946b; Dieke and Richter, 1945, 1946; Karel and Meyer, 1948 Kusano et al., 1972; Lillie, 1945; McClosky and Smith, 1945; Meyer and Karel, 1947, 1948; Rall and North, 1952).

DuBois et al . (1946b) found that ANTU markedly interferes with carbohydrate metabolism in rats. The prominent and consistent pathological changes are pleural effusion and pulmonary edema. An accumulation of fluid in the chest cavity literally drowns the animal. Norway rats are highly susceptible to ANTU, whereas dogs and pigs are moderately susceptible. Some species, such as dogs, can be protected by the emetic properties of the compound even if a fatal dose is ingested (Richter, 1945).

When ANTU is used for controlling Norway rats, there is little documented evidence of secondary poisoning occurring, and in some references secondary poisoning has been reported not to occur (Fish and Wildlife Service, 1959; National Pest Control Association, 1970). When rats poisoned with the compound were deliberately fed to pigs, no deaths resulted.

The various characteristics which make alpha-naphthylthiourea a poor rodenticide for most species are the reasons the compound was selected as a conditioning repellent to protect conifer seed from deer mice.

Any potential seed protectant must be tested for possible phytotoxicity to the seed and emerging seedlings. Inhibitory effects on germination were evaluated for the parent compound thiourea and two of its derivatives, 1-phenyl-2-thiourea and ANTU, all having repellent potentials. Each of three seed samples was treated with one of the test compounds at the concentration of 4% by weight. All treated seed and untreated controls were from a single lot of Douglas-fir seed. Seed germination tests for phytotoxicity were conducted by the California Department of Food and Agriculture Seed Laboratory prior to any field evaluations. Old seed which was unsuitable for commercial purposes was used, so germination percentages are lower than normal.

The results (Table 2) showed 1-phenyl-2-thiourea to be highly toxic to Douglas-fir seed. Thiourea is also considered toxic, although samples #10001 and #10005 had respective germinations of 36% and 25%. In any case, these two samples were classified as abnormal germinants by the seed analyst. These germination tests were read after 26 days and conducted without prechilling the seed at the seed laboratory.

Table 2. Germination tests of Douglas-fir seed treated with thiourea and two derivatives. All samples from the same seed lot (California Division of Forestry #23) and were treated on June 24 or July 8, 1971. After treatment, half the samples were stored at room temperature and half in the freezer until they were sent to the seed laboratory in mid-September, 1971.

Laboratory No.	Chemical Treatment (%)	Percent Germination	Posttreatment Storage
10004	1-phenyl-2-thiourea (4.0)	0	a
10000	1-phenyl-2-thiourea (4.0)	0	b
10005	thiourea (4.0)	25	a
10001	thiourea (4.0)	36	b
10006	alpha-naphthylthiourea (4.0)	40	a
10002	alpha-naphthylthiourea (4.0)	45	b
10003	control (untreated)	51	b

a = stored at room temperature until sent to seed laboratory.
b = stored in freezer until sent to seed laboratory.

Seed treated with ANTU and then stored at room temperature had 11% lower germination than the control. Seed stored in the freezer following treatment had only 6% lower germination than the control seed also stored in the freezer. Of the three compounds, ANTU had by far the least effect on germination.

These germination tests indicated that ANTU would not likely inhibit germination intolerably. A slight increase in seeding rate could be used to compensate for this loss, hence the first field tests were planned for and conducted in the winter of 1971.

In 1972-73 we encountered some problems of phytotoxicity from the seed treatment. To what extent it influenced our field studies we do not know, possibly very little, for the seed was applied very shortly after treatment. Many seed samples set aside for later germination studies had drastically reduced germination, but since one particular treated seed lot germinated at 89% and an untreated sample of the same lot germinated at 86%, we feel that the problem was caused or hastened by handling or storage conditions. Whether the ANTU itself or the chemical impurities present are responsible remains unknown. Impurities are under suspicion, and progress has already been made in finding sources of purer ANTU. Volatiles given off by ANTU or its impurities are apparently capable of inhibiting germination in untreated seed samples stored in the same container.

It is interesting that parent compound, thiourea, has been used to break dormancy in bitterbrush (Purshia tridentata) seed (Pearson, 1957). This permitted artificial seeding of bitterbrush in the spring, thereby reducing the much greater seed depredation by rodents that occurs when seeding is in the fall. Pearson might well have received some unknowing benefits from his dormancy-breaking seed treatment, for some repellency to rodents may have resulted if significant amounts of thiourea remained on the seed. The value of modifying seed germination with thiourea has been studied by others also (Deuber, 1932; Sanderson and Mclntosh, 1961; Thompson and Kosar, 1939; Tukey and Carlson, 1945). Johnson (1946) studied the effect of thiourea as a substitute for cold treatment on some forest tree seeds. A thorough review of research on thiourea and its value in breaking seed dormancy may provide a better insight on overcoming phytotoxicity.

FIELD TESTS

The most promising results in the 1971 — 1972 field evaluations were on a 20-acre rectangular parcel in Mendocino County. After division into two nearly square ten-acre plots of comparable physical characteristics, the west plot was seeded with 4% ANTU Douglas-fir seed, and the east plot with untreated seed, both seeded at 3/4 pound per acre with a cyclone seeder on December 8, 1971. Seeding counts were taken on 40 mil-acre plots in each ten-acre site on May 31, 1972. The ANTU plot averaged 1.42 seedlings per mil-acre samples, vs. 0.35 in the untreated plot (a 4:1 ratio).

This and other plot data in Mendocino County gave considerable encouragement even though replicated one-acre test plots established on Bureau of Land Management property in Oregon that year were inconclusive, presumably because of their small size. Encouragement was sufficient to justify larger test blocks of 10-30 acres. Also, since seeding is normally by helicopter, subsequent field tests would be flown on for objective analysis of the compound at the operational level.

In the fall of 1972, we contacted four major forest landowners in California who were currently involved with operational programs of direct seeding. Permission was granted to use ANTU as an alternative to endrin on a portion of lands scheduled for seeding that year. Six additional locations were established in the BLM's Eugene District -- three on the interior Cascade Mountain Range and three on the coastal range.

Of the four California locations each had four treatment blocks: two ANTU, one endrin, one untreated control. The two Oregon locations each had three blocks: two ANTU and one untreated control. The total of 22 plots ranged from 10 to 50 acres (average 33 acres). Except for the McCloud River (California) location, seeded with a mix of 0.5 pound of Douglas-fir and 1.5 pounds of ponderosa pine seed per acre, all areas were seeded exclusively with Douglas-fir. The two Oregon locations were seeded at 1 pound per acre, Big River at 0.5 pound per acre, and the Navarro River and Klamath River sites at 0.75 pound per acre.

The ANTU seed used was prepared in a manner usable in normal operations not simply laboratory-size lots. The slurry was prepared with Rhoplex® AC-33 (Rohm and Haas Co.) diluted with water at the ratio of 1:7. Alkaline Fast Green 2-G dye (Allied Chemical Corporation, National Analine Division) was dissolved in this solution and ANTU was added and kept in suspension. This mixture was slowly poured over the conifer seed as it was tumbled in a cement mixer until a uniform coating had been achieved. The seed was spread in a thin layer on plastic sheeting and air-dried at least overnight at room temperature. The dried conifer seed contained 4% w/w ANTU (calculated on the basis that the chemical was 100% pure). The evaluation was strictly experimental since ANTU is not registered for use as a seed protectant. The endrin-treated seed (0.5% active ingredient) was obtained by private timber companies through their normal sources and seeded in accordance to their normal operations.

Rodent indices were established for all 22 plots. Transect-type trap lines were established, using on most plots 100 snap traps spaced approximately 30 feet apart, and run for three consecutive nights. Extremely inclement weather restricted trapping to two nights on three plots, and on very steep terrain the number of traps used was occasionally limited to 50 per night.

To evaluate the efficacy of the field treatments, a stocking survey was conducted on the four California locations by establishing 50 randomly located 1 mil-acre (0.001 acre) plots per block and counting all germinants. Used as an aid in the survey was a light wooden frame with an interior area of 1/4 mil-acre. Plots were established only on bare mineral soil, purposely ignoring areas with undisturbed vegetation. Since all of the California locations had been logged with crawler-type tractors, at least 50% of the ground was excellent seed bed.

Even though most of the Oregon plots were logged by cable yarders and in some cases there was not as much bare mineral ground, the same stocking survey procedures were used whenever possible. On two plots where little or no burning had been conducted, there was great difficulty in locating readable plots, so 80 plots of 1/4 mil-acre were established there.

RESULTS

Deer mice were by far the principal small mammal captured in the pretreatment censusing, and their numbers were sufficient to warrant normal seed-protection procedures (Table 3)-Also caught were pinyon mice (P. truei) (6), shrews (Sorex spp.) (11), voles (Microtus spp.) (23), and chipmunks (Eutamias spp) (6) , for a total of 46 additional mammals in 5,400 trap nights. Small rodents (other than P. maniculatus) and shrews represented only 7.3% of 630 animals captured in the plots.

Table 3. Deer mice (P. maniculatus) captures per 100 trap nights by location and plot treatment designation. All trapping was prior to seeding. Except where specified other-ise, plots were trapped for three consecutive nights.

Location (Dates of Trapping)	Number of Deer Mice Captured Per 100 Trap Nights			
	Alpha-naphthylthiourea Plot A	Alpha-naphthylthiourea Plot B	Control Plot	Endrin Plot
CALIFORNIA				
Navarro River (Nov. 28-30, 1972)	7.3	4.3	3.3	3.0
Big River (Nov. 19-21, 1972)	4.7	4.0	3.6	3.0
Klamath River (Jan. 4-6, 1973)	27.7	36.5*	35.3	19.0*
McCloud River (Nov. 7-9, 1972)	6.3	6.3	21.0	11.0*
OREGON				
Cascade Mt. Range (Nov. 28-30, 1972)	6.3**	6.6†	6.6†	-
Coast Mt. Range (Dec. 19-21, 1973)	1.3	9.3	10.6	-

* Weather permitted only two nights of trapping.
** Trapped November 29 through December 1, 1972.
† The same trapping data are used for the adjacent plots since the single trapline ran through both.

Figures 2 and 3 show the number of germinants per acre and percent of plots stocked with one or more seedlings. A series of analyses of variance were calculated to determine any significant differences in numbers of seedlings per acre and stocking among the various locations or treatments. Since the two Oregon plots had no plots with endrin treatment, the analyses examined differences within the California locations using endrin, and then compared the California and Oregon locations with the four endrin plots omitted.

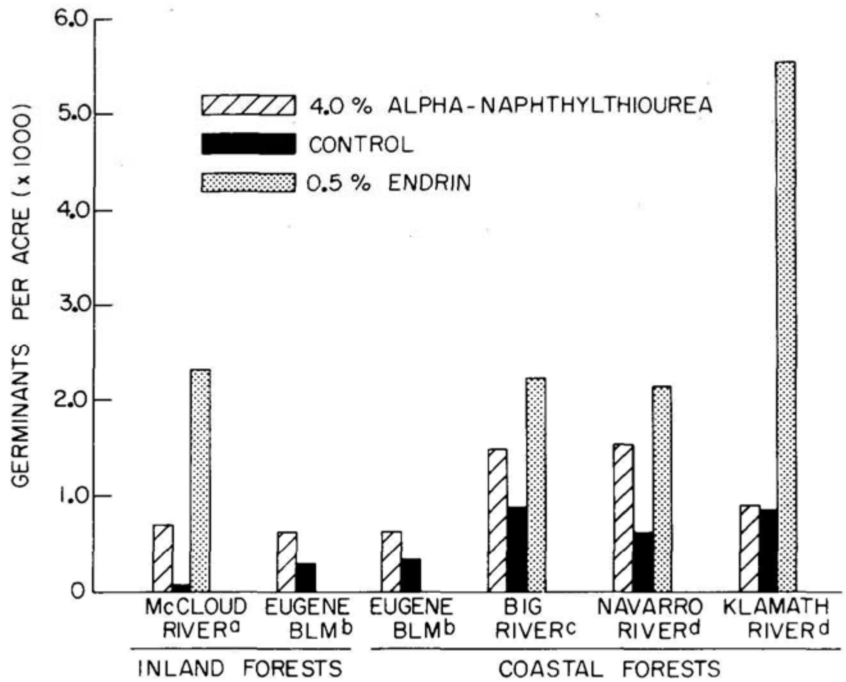

Figure 2. The average number of conifer germinants per acre resulting in each of six locations seeded with alpha-naphthylthiourea-treated seed, compared with areas seeded with untreated seed and (in four of the six locations) endrin-treated seed. All areas were direct-seeded by helicopter at the following rates: (a) 0.5 lb Douglas-fir and 1.5 lb ponderosa pine per acre; (b) 1.0 lb Douglas-fir per acre; (c) 0.5 lb Douglas-fir per acre; and (d) 0.75 lb Douglas-fir per acre.

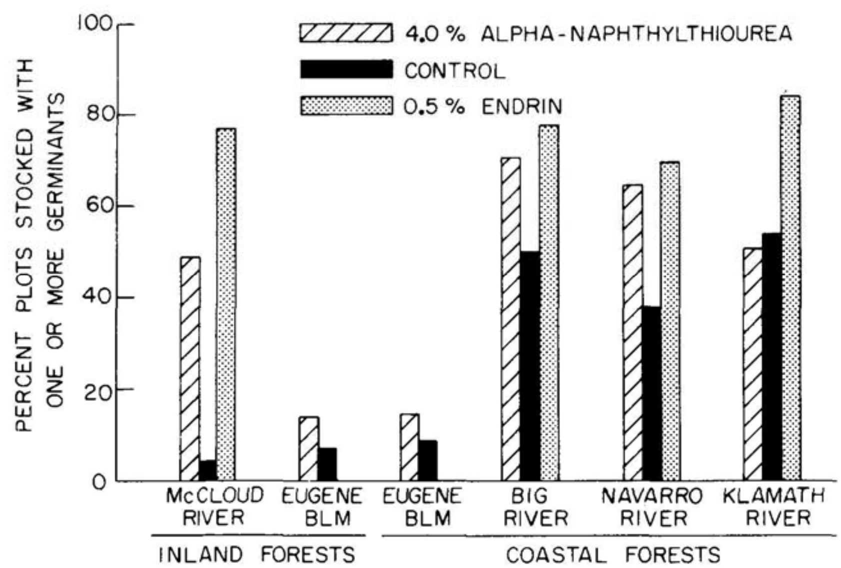

Figure 3. The percent of mil-acre plots stocked with one or more conifer germinants in each of six locations seeded with alpha-naphthylthiourea-treated seed, compared with areas seeded with untreated seed and (in four of six locations)endrin-treated seed. All areas were direct-seeded by helicopter at the rates indicated in Figure 2.

Where ponderosa pine was also used, approximately equal proportions of pine and Douglas-fir germinants were noted in the ANTU and endrin treated plots. It was established prior to seeding that 47% of the conifer seed mix (by count) was ponderosa pine, however, 58% of the germinated seedlings were pine. Since no germinate test was available for the pine the variation may be due to seed viability differences between the two species.

Tables 4 and 5 lead to the conclusion that endrin was best in giving adequate protection in the California tests. Excluding endrin from the analysis and examining the six locations in Oregon and California, however, indicates that ANTU seed did produce significantly more seedlings than untreated seed. This experimental seed protectant definitely shows excellent promise even though less effective than endrin.

Table 4. Statistically significant differences when analyzing numbers of seedlings per acre for California locations only.

Treatment	Average Number Seedlings Per Acre
Control Plots	595 a*
Alpha-naphthylthiourea Plots	1160 a
Endrin Plots	3055 b

* Means followed by the same letter are not statistically significant from one another at the 95% probability level. Least significant difference for control vs. endrin plots = 1917. LSD for alpha-naphthylthiourea vs. endrin or control = 1660.

Table 5. Statistically significant differences when analyzing numbers of seedlings per acre for all locations excluding endrin treatment.

Treatment	Average Number Seedlings Per Acre
Control Plots	505
Alpha-naphthylthiourea Plots	977*

* The mean is statistically significant from the control at the 95% probability level. Least significant difference (LSD) = 349.

Differences in the number of germinants between the endrin treated seed and ANTU treatment were greatest in the Klamath River site where deer mouse populations were extremely high. One possible explanation for this difference is that endrin is highly toxic to deer mice and, hence, both reductional control of the mouse population and seed repellency can be accomplished with the use of endrin treated seed. This can have the tendency to exaggerate the efficacy of endrin. Endrin's lethal effects make rather inappropriate a comparison with nontoxic repellents.

DISCUSSION AND CONCLUSIONS

Laboratory studies have shown ANTU to be an aversive conditioning repellent that can give Douglas-fir seed a high degree of protection from P. maniculatus and P. truei. The efficacy is not limited to the genus Peromyscus, as studies on laboratory mice strongly indicate. Since ANTU is effective on sunflower, Douglas-fir, and ponderosa pine seed, it may hold promise for a wide variety of seed.

Since ANTU seed produced about twice as many germinants per acre as did untreated seed, under a wide range of difficult operational field conditions, the compound merits consideration as a potentially useful conditioning repellent for protecting conifer seed.

Still, endrin treated seed yielded significantly more germinants than either ANTU seed or untreated seed. The reason may be the differing characteristics of ANTU and endrin (Marsh et al., in press). Birds, known predators of Douglas-fir seed (Hagar, 1960), might have consumed ANTU seed with no aversion; and insects, suspected in some instances of causing high field losses of seed (Laurence and Rediske, 1962), may be largely unaffected by ANTU.

Field stocking data are very difficult to analyze as to varying treatment effects. The number of seedlings reflects not only biological agents present but several physical factors such as abnormal weather, soil type, aspect, and time of seeding (Kverno and Hartwell, 1957; Strothman, 1971)

Further, what is an adequate number of seedlings per acre following artificial or natural seedfall? Guidelines vary with geography and landowner policy. Prior experience with anticipated mortality and competition dictate minimum acceptable stocking standards. Schubert and Adams (1970 suggested seeding rates adjusted to produce about 800 seedlings per acre. For Douglas-fir they recommend 0.5 to 0.75 pound of seed per acre (approximately 20,000 viable seeds per acre) to achieve the 800 per acre count. Minimum stocking as defined by the California Division of Forestry (1970) for the north coastal area has been established at 900 coniferous seedlings per acre at least one year old.

If the mediocre results with ANTU were due partly to reduced germination caused by poor storage conditions or, possibly, by chemical impurities, improvement of those conditions could give more than marginally acceptable stocking in future field studies.

Since ANTU at the rates used produced little or no mortality among deer mice, a large population such as found at Klamath might require preseeding applications of a toxic bait such as chlorophaci none (Marsh et al., in press; Passof, 1974).

Final judgment on ANTU must await further study. At present, however, endrin's performance as measured by germinants is superior. Its continued registration by the Environmental Protection Agency seems justified from a foresters viewpoint until comparable alternatives are well proven. More research is necessary to increase the efficacy of ANTU as a truly effective conifer seed protectant. It is, however, much too promising to abandon as a candidate repellent for deer mice and possibly other rodent species.

ACKNOWLEDGEMENTS

We acknowledge the basic financial support provided by the U.S. Bureau of Land Management, Portland, Oregon, Contract 53500-CT1-45(N) and the World Health Organization, Geneva, Switzerland. We are also grateful for the excellent cooperation received from timber companies who assisted in the project and generous assistance of personnel from the Eugene District of BLM. We are most appreciative of flying time donated by Western Helicopter Services, Newberg, Oregon. We thank technicians Nancy Bruss and Karen (Saul) Pearson, who spent many long hours conducting the laboratory tests, and the numerous other technicians who contributed to various phases of the project.

LITERATURE CITED

ABBOT, H. G. 1961. White pine seed consumption by small mammals. J. Forestry, 59:197-201.

BENTLEY, E. W., Y. LARTHE and A. TAYLOR. 1955. The effect of particle size on the toxicity of α-naphthyl thiourea (ANTU) to albino rats. J. Hyg. 53-328-334.

BOLLEN, W. B. and C. M. TU. 1971. Influence of endrin on soil microbial populations and their activity. USDA For. Serv. Res. Paper PNW-114. 4 pp.

BYERRUM, R. U. and K. P. DUBOIS. 1947. The influence of diet on the susceptibility of rats to alpha-naphthylthiourea. J. Pharm. 90:321-329

CALIFORNIA DIVISION OF FORESTRY. 1970. Forest practice rules for redwood forest district. The Resources Agency, Sacramento, Calif. 65 pp.

CROUCH, G. L. and M. A. RADWAN. 1971. Evaluation of R-55 and mestranol to protect Douglas-fir seed from deer mice. USDA For. Ser. Res. Note, PNW-170. 6 pp.

. and . 1972. Arasan in endrin treatments to protect Douglas-fir seed from deer mice. USDA For. Serv. Res. Paper, PNW-136. 7 pp.

DEUBER, C. G. 1932. Chemical treatments to shorten the rest period of red and black oak acorns. J. Forestry 30:674-679.

DIEKE, S. H. and C. P. RICHTER. 1945. Acute toxicity of thiourea to rats in relation to age, diet, strain and species variation. J. Pharm. and Exp. Therap. 83:195-202.

_____. and _____. 1946. Age and species variation in the acute toxicity of alpha-naphthyl thiourea. Proc. Soc. Exp. Biol. and Med. 62:22-25.

. , G. S. ALLEN and C. P. RICHTER. 1947. The acute toxicity of thioureas and related compounds to wild and domestic Norway rats. J. Pharm. 90:260-270.

DUBOIS, K. P., L. W. HOLM, and W. L. DOYLE. 1946a. Studies on the mechanism of action of thiourea and related compounds. J. Pharm. 87:53-62.

., ., and . 1946b. Biochemical changes following poisoning of rats by alpha-naphthylthiourea. Soc. Exp. Biol. and Med. Proc. 61:102-104.

EDGREN, J. W. I968. Potential damage to forest tree seed during processing, protective treatment, and dissemination. USDA For. Serv. Res. Note, PNW-89. 8 pp.

FISH AND WILDLIFE SERVICE. 1956. Formulation for the treatment of coniferous tree seed. U.S. Fish and Wildlife Serv., Wildl. Res. Lab, Denver. 2 pp. (Spec. Release).

______. 1959. Characteristics of common rodenticides. USDI, Wildlife Leaflet 337. 4 pp.

FREEMAN, R. B. 1954. Properties of the poisons used in rodent control. In Control of Rats and Mice (D. Chitty ed.), Vol. 1. pp. 122-124. Clarendon Press, Oxford. 305 pp.
GAINES, T. B. and W. J. HAYES, JR. 1952. Bait shyness to ANTU in wild Norway rats. Pub. Health Report 63 (3):306-311.
HAGAR, D. C. I960. The interrelationships of logging, birds, and timber regeneration in the Douglas-fir region of northwest California. Ecology. 41:116-125.
HAMRICK, W. J. I968. The effects of Arasan-endrin treated pine seed on bobwhite quail, gray squirrel and turkey. Proc. 22nd Annual Conf. Southeastern Assoc. of Game and Fish Commissioners. 9 pp.
HOOVEN, E. F. 1953. Some experiments in baiting forestland for the control of small seed-eating mammals. Oregon State Board of Forestry Res. Note, 8. 70 pp.
. 1958. Deer mouse and reforestation in the Tillamook burn. Oregon Forest Lands Res. Cent., Corvallis. Res. Note No. 37. 31 pp.
HOWARD, W. E. and R. E. COLE. I967. Olfaction in seed detection by deer mice. J. Mammal. 48:147-150.
. and R. E. MARSH. 1970. Rodent control in relation to forest regeneration - Progress Report 1969-7O. 21 pp.
., ., and R. E. COLE. 1968. Food detection by deer mice using olfactory rather than visual cues. Anim. Behav. 16:13-17.
JACOBS, M. B. 1958. Thiourea -- The chemical analysis of food and food products (third edition) pp. 173-174. D. Van Nostrand Co., Princeton, New Jersey. 970 pp.
JANZEN, D. H. 1971. Seed predation by animals. Annual Rev. Ecology and Systematics 2:465-492.
JOHNSON, L. P. V. 1946. Effect of chemical treatments on the germination of forest tree seeds. Forestry Chron. 22:17-24.
KAREL, L. and B. J. MEYER. 1948. The effect of various sulfur-containing compounds on alphanaphthylthiourea (ANTU) toxicity to rats. J. Pharm. 93:414-419.
KUSANO, T., Y. KASAHARA, and Y. KAWAMURA. 1972. Analysis of taste effectiveness of thiourea derivatives in rats. Appl. Ent. Zool. 7(1):17-26.
KVERNO, N. B. and H. D. HARTWELL. 1957- Progress report -- Pacific Northwest 1955-56 and 1956-57 experimental seeding studies. USDI, Bur. Sport Fish, and Wildlife. 50 pp.
_____ . and G. A. HOOD. 1963. Evaluation procedures and standards chemical screening and development for forest wildlife damage. U.S. Fish and Wildl. Serv., Wildl. Res. Center, Denver. 59 pp.
LAURENCE, W. H. and J. H. REDISKE. 1962. Fate of sown Douglas-fir seed. For. Science 8(3):21O-218.
LILLIE, R. D. 1945. Pathology of alphanaphthylthiourea (ANTU). Pub. Health Rept. 60(38):8-l3.
MARSH, R. E., P. C. PASSOF, and W. E. HOWARD. 1974. Anticoagulants and alphanaphthylthiourea to protect conifer seeds. In Proc. Symposium on Wildlife and Forest Management in the Pacific Northwest, Sept. 11-12, 1973, Corvallis, Oregon (in press).
MARSTON, R. B., R. M. TY0, and S. C. MIDDENDORFF. 1969. Endrin in water from treated Douglas-fir seed. Pesticides Monitoring J. 2 (4):167-171.
MCCLOSKY, W. T. and M. I. SMITH. 1945. Studies on the pharmacologic action and the pathology of alphanaphthylthiourea (ANTU). Pub. Health. Rep. 60(38):1101-1108.
MEYER, B. J. and L. KAREL. 1947. The effects of iodides, 1-thiosorbitol, and twenty-five other compounds on alphanaphthylthiourea (ANTU) toxicity in rats. J. Pharm. 92:15-31.
. and . 1948. The effect of environmental temperature on alpha-naphthy1thiourea (ANTU) toxicity to rats. J. Pharm. 93:420-422.
MOORE, A. W. 1949. Forest tree-seed-eaters and methods used to measure their populations in the Pacific Northwest Douglas-fir region. Univ. of Washington Forest Club Quarterly. 23(1):7.
NATIONAL PEST CONTROL ASSOCIATION. 1970. Good practice in the use of the rodenticide ANTU by the pest control operator. 2 pp.
PASSOF, P. C. 1968. Cooperative seed spotting trial using chemical sterilants and petroleum mulch as rodent repellents. Field Trip Leaflet. 1 p.
_____ .1974. Rodent control activities when direct seeding forest lands in northern California. In Proc. Sixth Vertebrate Pest Conf., Anaheim, Calif. (W. V. Johnson, ed) pp. 127-131.
PEARSON, B. 0. 1957. Bitterbrush seed dormancy broken with thiourea. J. Range Management 10(0:41-42.
RADWAN, M. A., G. L. CROUCH, and W. D. ELLIS. 1970. Impregnating and coating with endrin to protect Douglas-fir seed from rodents. U.S.D.A. For. Serv. Res. Paper, PNW-94. 17 PP.
RALL, D. P. and W. C. NORTH. 1952. Toxicity of ANTU for the rat and its lack of dependence upon body weight. Fed. Proc. 11 (1)383-384 .

RICHTER, C. P. **1945.** The development and use of alpha-naphthyl thiourea (ANTU) as a rat poison. J. Amer. Med. Assoc. 129(14):927-931.
_____ .1946. Biological factors involved in poisoning rats with alpha-naphthyl thiourea (ANTU). Soc. Exp. Biol. and Med. Proc. 63:364-372.
_____ . and K. H. CLISBY. 1941. Pheny1thiocarbamide taste thresholds of rats and human beings. Amer. J. Physiol. 134:157-164.
ROY, D. F. 1961. Seed spotting with endrin-treated Douglas-fir seed in northwestern California. U.S.D.A. For. Serv. Pacific Southwest Forest and Range Exp. Station Tech. Paper 61. 12 pp.
SANDERSON, R. and D. MCINTOSH. 1961. Effects of combined endrin-Arasan 75 and thiourea treatments on the germination of bitterbrush seed. U.S.D.A. For. Serv. Res. Note (PSW) No. 174. 7 pp.
SCHUBERT, G. H. and R. S. ADAMS. 1971. Reforestation practices for conifers in California. Calif. Div. of Forestry, Sacramento, Calif. 359 PP
SMITH, F. and E. ALDOUS. 1947. The influence of mammals and birds in retarding artificial and natural re-seeding of coniferous forest in the United States. J. Forestry 45:361-369.
SPENCER, D. A. 1954. Rodents and direct seeding. J. Forestry 52:824-826.
STROTHMANN, R. 0. 1971. Germination and survival of Douglas-fir in northern California ... effects of time of seeding, soil type, and aspect. U.S.D.A. For. Serv. Res. Note PSW-245. 6 pp.
TEVIS, L., JR. 1953. Effect of vertebrate animals on seed crops of sugar pine. J. Wildlife Management 17:125-131.
.1956. Responses of small mammal populations to logging of Douglas-fir. J. Mammalogy 37:189-196.
THOMPSON, R. C. and W. F. KOSAR. 1939. Stimulation of germination of dormant lettuce seed by sulfur compounds. Plant Physiol. 14:567-573.
TUKEY, H. B. and R. F. CARLSON. 1945. Breaking the dormancy of peach seed by treatment with thiourea. Plant Physiol. 20:505-5l6.

To simplify information, trade names of products have been used. No endorsement of named products is intended, nor is criticism implied of similar products which are not mentioned.