STRYCHNINE RESIDUE STUDIES AND THEIR IMPLICATIONS IN RODENT CONTROL

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ABSTRACT: Applications of 0.29, 1.0, 2.63, and 5.26% strychnine-impregnated grain baits were applied below ground to 10' x 10' plots of alfalfa to determine translocation of strychnine from the soil to plants. Four replicates of each concentration were made and the alfalfa analyzed for strychnine residues. Four 10' x 10' plots were utilized as controls. Plant samples were taken on Day 1, Day 9, and Day 14. Strychnine was not detected in any of the samples above the limit of detectability (0.02ppm).

Apples from trees subjected to a normal, 1 x normal, and a 3 x normal gopher control strychnine bait application were sampled and analyzed for residues. Four replicates were used and samples were taken on Day 4, Day 14, and Day 28. Analytical methods were identical to those utilized for alfalfa with normal to 3 x normal application rates of strychnine baits. There was no detectable strychnine translocated from the soil to the fruit of the apple trees.

When using an authorized application rate of strychnine-impregnated bait utilized in a gopher control program, there is apparently no translocation of strychnine from the soil to the harvestable crops involved in this study.

Because of the strong sorption characteristics of strychnine, even on soils of low cation exchange capacity (6.8 meq/100g), mobility in soils was found to be minimal.

INTRODUCTION

The State of Nevada as well as many of the other western states utilize strychnine (Strychnos nux vomica), in both the sulfate and alkaloid forms, in their various rodent control programs. The strychnine is adhered to the surface of various agricultural products or by-products and then presented to pest rodents in many different manners, both above and below ground.

The agricultural enterprises of the State of Nevada are plagued by ground squirrels of the species Spermophilus beldingi and S. townsendi, and pocket gophers of the species Thomomys bottae, T. townsendi, T. talpoides and T. monticola. The ground squirrel control programs utilize above-ground applications of strychnine-cabbage and strychnine-grain while the pocket gopher programs utilize below-ground applications of strychnine-grains. Until such time as better compounds and/or methods are perfected, strychnine is absolutely essential to Nevada agriculture in holding these rodent pests populations to a tolerable level. It is estimated that the loss of strychnine for rodent control programs in the state would cost the alfalfa seed industry alone in excess of $1,000,000 per year.

The rebuttable presumption against registrations or reregistration (RPAR) notice for strychnine appeared in the Federal Register of December 1, 1976, (41 FR 52810). Of the more than 100 rebuttal responses received by the Environmental Protection Agency (EPA), the majority contained no scientific data. The data which were submitted were either insufficient or utilized inadequate test methods to support the positions taken. None of the data presented dealt with strychnine residues in plants nor with the movement of strychnine in the soil.

The fate of strychnine for rodent control programs appeared to be in great jeopardy due to gaps in the scientific data, especially in the area pertaining to its uptake by plants and its movement in the soil. Therefore, this study was undertaken to determine whether there was indeed uptake of strychnine by plants and whether or not movement in the soil occurred.

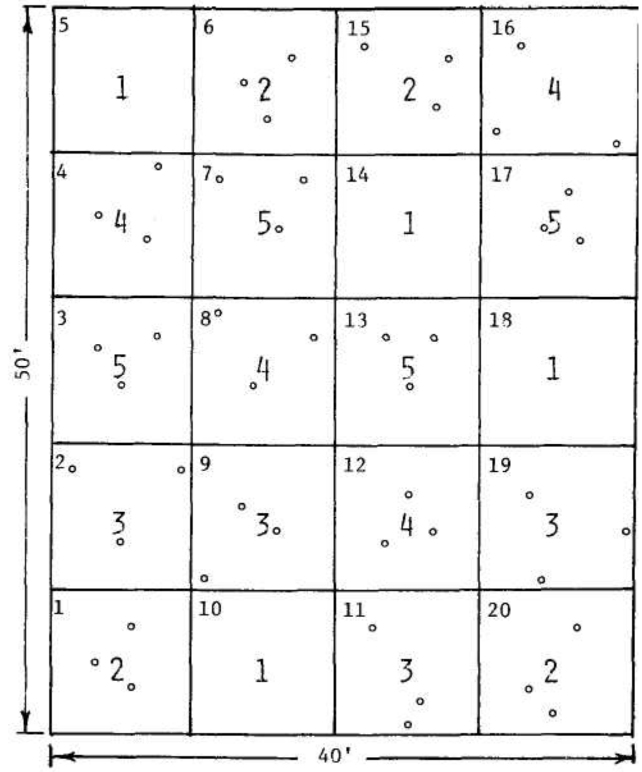
PROCEDURES

Alfalfa Studies

On July 22, 1980, test plots for strychnine residue analysis were initiated on the Capurro Ranch, Palomino Valley, Nevada, in a 5 year old, sprinkler-irrigated stand of alfalfa. The test area consisted of a 40' x 50' section subdivided into twenty 10' x 10' subplots (Fig. 1). Twenty preapplication samples were taken. Applications of strychnine-milo (bait most commonly used in Nevada gopher control programs) were made to a depth of 6" (average depth of gopher burrows in the Capurro field) utilizing an Elston Gopher Getter Jr. Assuming 3 gopher mounds per plot, 3 probes and bait deposits were made at random in each of the subplots. Subplots 5, 10, 14, and 18 were used as controls. Numbers 1, 6, 15, and 20 received 0.35% strychnine-milo; numbers 2, 9, 11, and 19 received 1.0% strychnine-milo; numbers 4, 8, 12, and 16 received 2.63% strychnine-milo; and numbers 3, 7, 13, and 17 received 5.26% strychnine-milo. The alfalfa at time of bait application was approximately 10" tall and in vigorous growth stage.

Samples weighing 10 lb were randomly taken from each of the subplots, placed in plastic bags, and frozen on July 13 and July 31, 1980 (1 and 9 Day samples). The alfalfa samples were then analyzed for strychnine residues utilizing a new gas chromatographic method developed by Dr. Glenn C. Miller which will appear in the July 1982 issue of JOAC. (Appendix 1)

Fig. 1. Strychnine test plots and controls. Probe and bait deposit locations are indicated by "o". The designated treatments are as follows: 1) Control (no bait placement), 2) 0.35% strychnine-milo, 3) 1.00% strychnine-milo, 4) 2.63% strychnine-milo, 5) 5.26% strychnine-milo.



Apples

Samples of apples were analyzed from a study performed by the Cooperative Extension Service of Washington State University. In their study, they utilized sliced carrot baits containing 0.015 oz of 5% strychnine per slice (135 slices mixed with 2 oz of 5% strychnine formulation). As gophers are solitary animals with territorial behavior, more than one bait placement per tree is seldom necessary. A normal bait placement consists of 2 slices of strychnine-carrots per placement. Assuming this, WSU used the following protocol in their treatment and sampling. (Fig. 2)

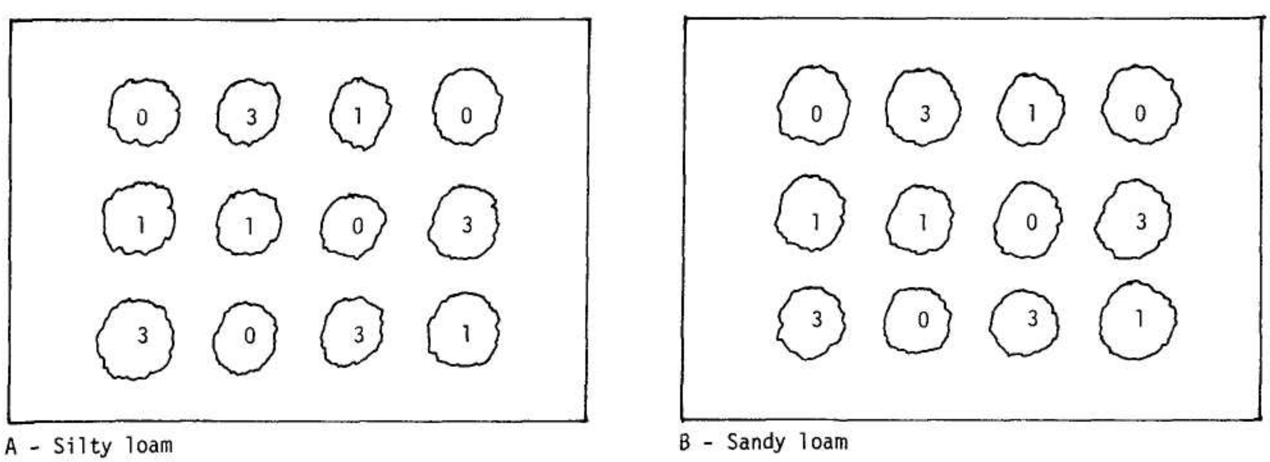


Fig. 2. Washington State University strychnine test plots, apple trees. Two soil types, silty loam and sandy loam. Legend: 0 = No bait placement - control, lx = 2 baits per tree, 3x = 6 baits per tree.

A. Three rates 0, lx, and 3x (0, 1, and 3 bait placements per tree).

B. One application (September 16, 1980).

C. Two locations (2 soil types, silt loam and sandy loam).

D. Four replicates (single tree reps).

E. Sample size - 12 apples (total of 72 samples collected) random selection.

F. Sampling - September 19 (3 days post-treatment), October 1 (15 days post-treatment, and October 14 (28 days post-treatment).

G. Each fruit sample was placed in a plastic bag, tied, and tagged to indicate plot, rep, treatment rate, and date harvested. Fruit samples were placed in cardboard apple boxes, stored in freezer locker, and then shipped to Reno for strychnine analysis.

The analysis procedure for strychnine was the same as that utilized in UNR's alfalfa study.

Soil Movement

Three Nevada soils were selected for determining sorption and movement characteristics of strychnine. Analysis of these soils is presented in Table 1.

Table 1. Analyses of soils.

Site	% Organic carbon	CEC me/100g	Soil texture	pH
Oppio	0.53%	13.5	Sandy loam	7.6
Kracaw	0.79%	6.8	Sandy loam	8.0
Main Station Farm	1.97%	16.2	Fine sandy loam	7.7

Strychnine (0.5, 1, and 2 mg) was applied to the top of 1 cm x 10 cm columns of each of the soils, and 55 ml of water was passed through each column, under unsaturated conditions, over a period of 2 to 3 hours. The columns were fractionated into 0.5 cm discs and strychnine was determined in each disc.

Analysis and procedure was that which was described by Miller and Letey (1975).

RESULTS AND DISCUSSION

The data derived from the samples from the alfalfa studies were obtained during the most vigorous growth stage of the plants (Table 2), a time when the maximum amount of nutrients, moisture, etc. was being translocated from the soil to the plants. Strychnine residues were not found in any of the samples, indicating that alfalfa plants do not take up strychnine from the soil.

Table 2. Strychnine analysis, alfalfa.

Date treated	Date sampled	Control	0.35%	% Strychnine-Grain Bait 1.0%	2.65%	5.26%	Strychnine residue
7-22-80	7-23-80	1	2	3	3	2	0
7-22-80	7-31-80	1	2	3	3	3	0
7-22-80	8-05-80	1	2	4	3	3	0

The application of strychnine-grain baits in this study simulated actual methods used in both California and Nevada. The 5.26% concentration of strychnine was twice the maximum (2.65%) authorized rate used by one of California's counties in its gopher control programs.

With the apple study, the Cooperative Extension Service of Washington State University did the treating and sampling while the University of Nevada-Reno did the analysis. As with the alfalfa study, an actual gopher control program was simulated. In the apple study, however, three times the normal rate was tested instead of twice the normal rate. The procedure used for analysis of strychnine in alfalfa was directly applicable to the apple samples without alteration. Strychnine was not detected in any of the 72 samples of apples analyzed (Table 3).

Movement in Soil

Vertical movement of strychnine in the soil was determined to be minimal. When 55 ml of water were passed through 1 cm x 10 cm columns of the 3 Nevada soils, for a period of 3 hours, strychnine was found in only the top 0.5 cm section. Studies of sorption/desorption characteristics, and their relationship to organic content, pH, CEC, and texture, were inconclusive and further work in these areas is being conducted.

Table 3. Strychnine analysis, apples.

Treated	Sampled	X - Normal Treatment Rate 0	1X	3X	Residue
9-16-80	9-19-80	8	8	8	0
9-16-80	10-01-80	8	8	8	0
9-16-80	10-14-80	8	8	8	0

From the University of Nevada-Reno's alfalfa study and Washington State University's apple study, it can be said that: "When pursuing a gopher control program, utilizing authorized rates of strychnine-grain or strychnine-carrots in a variety of soils, strychnine will not be taken up into the foliage or the fruit of the plants in the program area." Further, "Strychnine will move vertically a minimal distance in soils similar to the Nevada soils tested."

IMPLICATIONS IN RODENT CONTROL

The implications of the findings of this study to rodent control are simply this: Scientific data pertaining to strychnine, its uptake and movement, have been provided to the Environmental Protection Agency to satisfy data gaps pertaining to strychnine in these areas. As uses of strychnine have been severely criticized and the loss of the compound is a distinct possibility because of lack of data, possibly this study will be a deterrent to actions taken against it.

Until better methods and compounds are found, the retention of strychnine means that agriculture will be able to hold gophers and other rodent pest populations to a tolerable level. Our fields and orchards will not be ravaged to the extent that they are almost bare by creatures such as the pocket gopher, but will be lush, fruitful, and aesthetic.

LITERATURE CITED

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APPENDIX 1. ANALYSIS METHOD

A GAS CHROMATOGRAPH METHOD FOR DETERMINING STRYCHNINE RESIDUES IN ALFALFA*

Apparatus and Reagents

A. Gas Chromatograph - A Hewlett Packard model 5830 equipped with a nitrogen-phosphorous detector and 1.2m x 2.0mm i.d. glass column packed with 1.5% OV-17 - 1.95% OV-210 on 100-120 mesh Gas Chrom Q. Condition newly packed column 12 hr at 275° with helium flow. Operating conditions: temperatures (°C) - column 275° (isothermal), detector 275°, injector 275°; helium flow 30 ml/min; adjust hydrogen and air as recommended for detector by manufacturer.

B. Extracting Solvents - EtOAc (Mallinckrodt) and CH2CI2 (Mallinckrodt) were redistilled prior to use.

C. Standard Solution - Weigh lO.Omg strychnine alkaloid standard (Henry Interdonati, Inc., Great Neck, New York) into 100ml volumetric and dilute to volume with acetone. From this solution, accurately pipette 1.0ml into 100ml volumetric and dilute to volume with acetone.

Preparation of Samples

Chop frozen alfalfa in a Hobart chopper. Into 500ml Waring blender, add 50g chopped alfalfa, 20g granular anhydrous Na_2SO_4, 5g Na_2CO_3 and 200ml EtOAc. Blend 5 minutes and filter the entire contents under vacuum through #1 Whatman filter paper into a 500ml erlenmeyer flask. Transfer the filtrate to a 1 liter separatory funnel containing 100ml 0.1N H_2SO_4. Return the alfalfa and filter paper to the blender, and add an additional 200ml EtOAc, blend 3 minutes, and filter the contents as before. Blend the alfalfa residue and filter paper a third time with 200ml EtOAc, filter as before and combine with extracts in the liter separatory funnel. Extract the combined EtOAc with three 100ml portions of 0.1N H_2SO_4. To the aqueous extract, add 20g NaCl, 6ml 6N NaOH, and 2g Na_2CO_3 and adjust the pH to 915 with 6N NaOH. Return the aqueous solution to the 1 liter separatory funnel and carefully

*By G.C. Miller. To be published in July 1982 JOAC.

shake with three 100ml portions of CH_2Cl_2. Overly vigorous shaking will result in cumbersome emulsions. Pass the CH_2Cl_2 through granular anhydrous Na_2S0_4, and evaporate the CH_2Cl_2 to near dryness. Transfer the residue with acetone to a 5ml volumetric; use for analysis. Quantitate the strychnine within 30 minutes of final concentration to minimize degradation of the recovered strychnine.

Determination

Inject 3 µl aliquots of standard solution and compare average peak heights of samples (3 µl). Calculate amount injected as follows:

strychnine, ng = Hu/Hs x ng strychnine injected as std.

where Hu and Hs - average peak heights of sample and std., respectively.