A BIOASSAY TECHNIQUE FOR MEASURING HERBICIDE RESIDUALS IN FOREST NURSERY SOILS [1]

HARRY W. ANDERSON, *Soils Forester*
Washington State Department of Natural Resources,
Olympia, Wash.

Persistence in soil of many herbicide types now in use, such as the triazines and the substituted ureas, has caused concern. Some herbicides may disappear completely in a month or so, but others may remain at a high level for up to a year or more.

The dissipation of a herbicide from any particular soil is accomplished by the following means: (a) Leaching, (b) adsorption on soil or organic particles, (c) volatilization, (d) chemical alteration, (e) plant uptake, (f) photo decomposition, and (g) microbial degradation (2). The rate that these factors act on a particular herbicide is regulated by the herbicide's chemical composition, the soil type, and the environmental conditions of the area.

The use of herbicides in forest nurseries has become an important cultural technique; however, the use of any one herbicide year after year, without some knowledge of its potential accumulation in the soil, could cause future problems. A nurseryman should know if a herbicide he uses might accumulate in the soil to the extent that tree seedlings are injured, or the soil made unfit for the more sensitive cover crop species usually rotated between tree crops.

A study was initiated at the L. T. "Mike" Webster Forest Nursery, near Olympia, Wash., during the summer of 1963, to develop a bioassay technique to estimate the amount of a particular herbicide in the soil (Tumwater fine sandy loam).

The basis for this study was an operational herbicide experiment established in 1962, during which the four herbicides shown below were tested in 1-acre plots to determine their effectiveness (1) .

Herbicide	Chemical composition	Application rate lbs. /acre active material
Dowpon	2-2 dichloroproprionic acid	10
simazine	2-chloro-4, 6-bis (ethylamino) s-triazine	4
Propazine	2-chloro-4, 6-bis (isopropyl amino)-s-triazine	4
Kloben	1-n-butyl-3-(3,4-dichloro phenyl)-1-methylurea	4

[1] Forest Land Management Division Contribution 99.

Methods and Procedures

The herbicides were applied with a conventional nursery spray rig on June 15, 1962, to 2-1 Douglasfir transplants. Soil samples were collected from each of the herbicide plots 6 months after application, and a greenhouse bioassay was run to determine residual levels of each herbicide.

Soil Sampling

Random soil samples were collected from each herbicide plot, with a soil sampling tube. Three soil layers were sampled (0-4 in., 4-8 in., 8-12 in.) for each herbicide. A total of 30 soil cores per herbicide were taken for each depth sampled.

Cores were taken as uniformly as possible, mixed together, air dried, and weighed. From these data, the weight (W) of the soil to the depth sampled, expressed as pounds per acre, was calculated by the following formula:

$$W = \frac{43{,}560 \text{ sq. ft./acre} \times G}{N \times CS \times 453.6 \text{ gm./lb.}}$$

where

G = air-dried weight of N number of cores in grams.

N = Number of cores

CS = Cross section of core in square feet.

Soil weights calculated for each soil depth were used as the basis for computing the herbicide standards (table 1). A sample calculation is shown as follows:

$$W = \frac{43{,}560 \text{ sq. ft./acre} \times G}{N \times CS \times 453.6 \text{ gm./lb.}}$$

where

G = 1,001.8 gm.

N = 30

CS = 0.0031 sq. ft.

$$W = \frac{43{,}560 \times 1{,}001.8}{30 \times 0.0031 \times 453.6}$$

W = 1,034,576 lb./acre for the 0–4 in. depth.

Bioassay technique

The procedure given is that described by Rahn and Baynard (5) and modified by the Dupont Company (3).

Stock mixtures were prepared with talc containing 1,000 p.p.m. of the active herbicide. Kloben required 0.20 gm. (50-percent wettable powder) per 100-gm. talc. Simazine and Propazine required 0.126 gm. (80-percent wettable powder) per 100 gm. talc while Dowpon required 0.118 gm. (85-percent wettable powder) per 100 gm. talc.

A series of standards were prepared by adding parts of the talc mixture to air-dried untreated Webster Nursery soil. The standards contained the equivalent of 0, 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0 pounds per acre (active) for simazine, Propazine, and Kloben, and 0, 0.2, ,0.4, 0.6, 0.8, 1.0, 1.5, 3.0, 4.0, and 5.0 pounds per acre (active) for Dowpon. Since the relative weight of the nursery soil per 4-inch layer was fairly consistent (table 1) , the average 0-4-in. depth was used as a basis for calculating the appropriate amount of talc mixture to add for each herbicide level. The amount of 1,000 p.p.m. talc mixture to add to each soil standard was calculated from the following formula:

$$T = \frac{1{,}000 \times S \times R}{W}$$

Where

T = weight of talc mixture, in grams

S = weight of soil used to make up standards in grams (in this formula, 1,000 gm.)

R = desired herbicide rate in lbs./acre/soil depth (0–4 in.)

W = weight of soil to depth sampled (soil, the average 0–4 in. depth—908,249 lbs.)

The amount of talc mixture added to each herbicide standard is shown in table 2. A sample calculation using the above formula follows:

$$T = \frac{1{,}000 \times S \times R}{W}$$

Where

S = 1,000 gm.

R = 5.0 lb.

W = 908,249 lb.

$$T = \frac{1{,}000 \times 1{,}000 \times 5.0}{908{,}249}$$

T = 5.50 gm.

TABLE 1.—*Weight of soil to depth sampled for each herbicide treatment, expressed as pounds per acre*

Herbicide treatment	Depth sampled	Weight of soil	
	In.	*Gm./sample*	*Lb./acre*
Simazine	0–4	924.0	954,230
	4–8	954.0	985,213
	8–12	1,064.1	1,098,914
Propazine	0–4	860.8	888,963
	4–8	1,119.0	1,155,610
	8–12	1,104.6	1,140,739
Kloben	0–4	824.9	851,888
	4–8	965.7	997,295
	8–12	1,001.8	1,034,576
Dowpon	0–4	908.2	937,914
	4–8	1,012.8	1,045,936
	8–12	1,035.5	1,069,378

Each herbicide standard was run in triplicate. The talc mixture was evenly distributed in 1,000 gm. of soil and placed in properly labeled 5-inch pots. A weak nutrient solution was also added to each pot.

TABLE 2.—*Talc mixture (1,000 ppm) added to 1,000 grams of untreated Webster Nursery soil (0–4-in. depth) to prepare a series of standards for four herbicides*

Herbicide		Herbicide rate/acre	Weight of talc mixture per pot
		Pound	*Gm.*
Simazine	All at the	0.0	None
Propazine	same rates		
Kloben		0.2	0.22
		0.4	0.44
		0.6	0.66
		0.8	0.88
		1.0	1.10
		1.5	1.65
		2.0	2.20
Dowpon		0.0	None
		0.2	0.22
		0.4	0.44
		0.6	0.66
		0.8	0.88
		1.0	1.10
		1.5	1.65
		2.0	2.20
		3.0	3.30
		4.0	4.40
		5.0	5.50

At the same time that the herbicide standards were being prepared, air-dried soil samples collected from each herbicide plot were also placed in pots and labeled. Fifteen oat seeds were planted in each pot. The oat seed had been dusted with Spergon to promote uniform seedling emergence.

The standards and the pots containing unknown amounts of herbicide were randomly arranged on a greenhouse bench. The pots were placed in flats containing wet peat. By keeping the peat wet, surface watering of the pots was necessary only every few days, and the possibility of leaching and overwatering young plants was reduced. A week after seeding, oat seedlings were thinned to a maximum of 10 plants per pot. Six weeks after seeding, all plants were harvested and green weights and ovendry weights of tops were determined for each pot.

Results

The yield data obtained from each herbicide standard were plotted against active herbicide rate per acre. This was done for both green weight and ovendry weight determinations. Figures 1-8 show the growth response curves obtained for the four herbicides. Where a curvilinear relationship was evident, free hand curves were drawn. Where a linear relationship existed, linear regression was calculated. Correlation coefficients were also determined and are shown for each curve.

Simazine, Propazine, and Dowpon showed a good relation between oat yield and herbicide rate as evidenced by significant correlation coefficients. This was true for both green and ovendry weights.

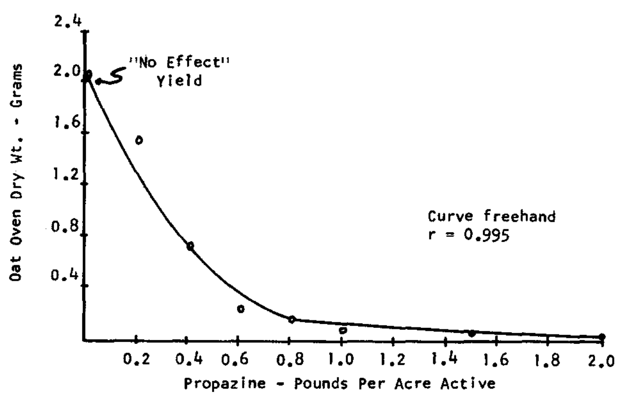

Figure 1.—Relation of dry weight yield of oats to level of Propazine herbicide in Webster Nursery soil.

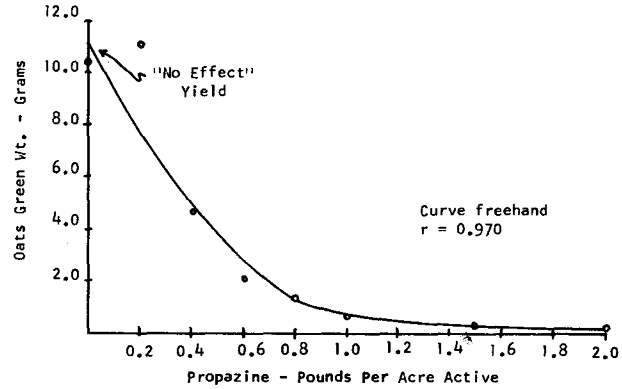

Figure 2.—Relation of green weight yield of oats to level of Propazine herbicide in Webster Nursery soil.

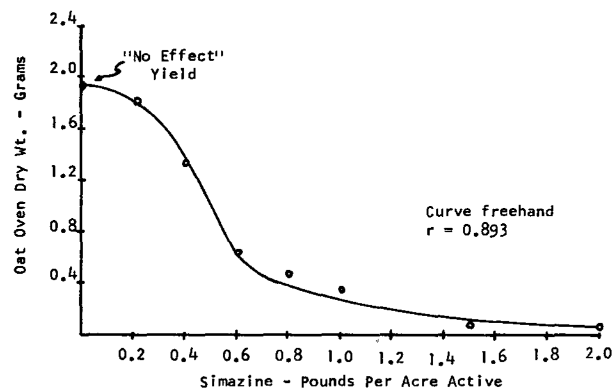

Figure 3.—Relation of dry weight yield of oats to level of simazine herbicide in Webster Nursery soil.

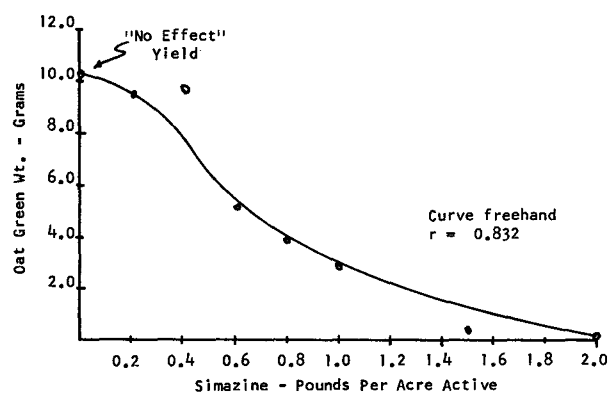

Figure 4.—Relation of green weight yield of oats to level of simazine herbicide in Webster Nursery soil.

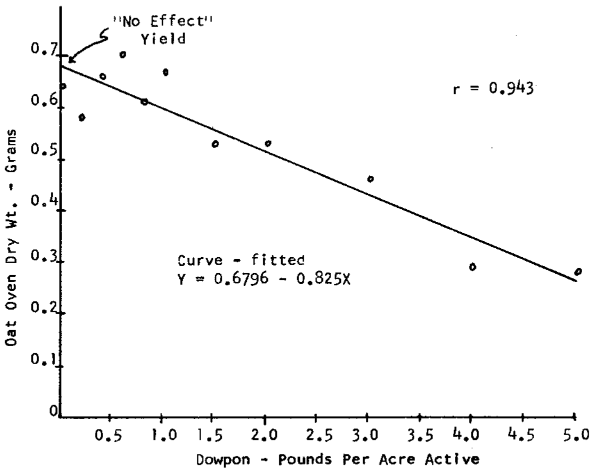

Figure 5.—Relation of dry weight yield of oats to level of Dowpon herbicide in Webster Nursery soil.

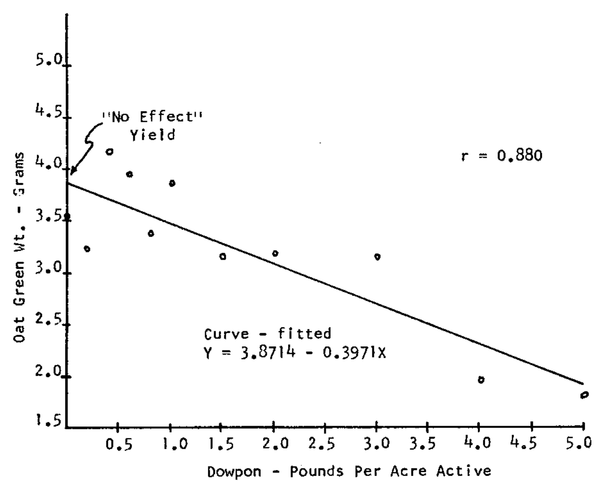

Figure 6.—Relation of green weight yield of oats to level of Dowpon herbicide in Webster Nursery soil.

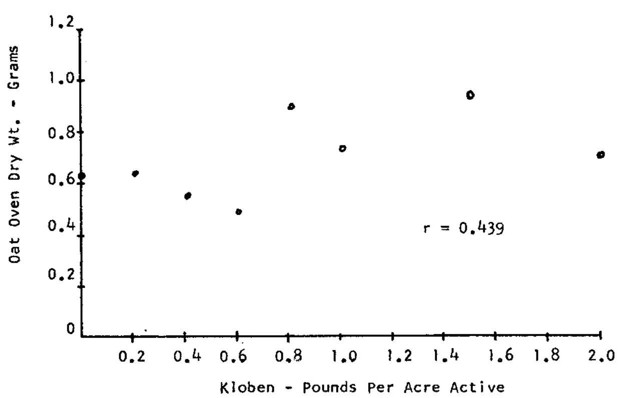

Figure 7.—Relation of dry weight yield of oats to level of Kloben herbicide in Webster Nursery soil.

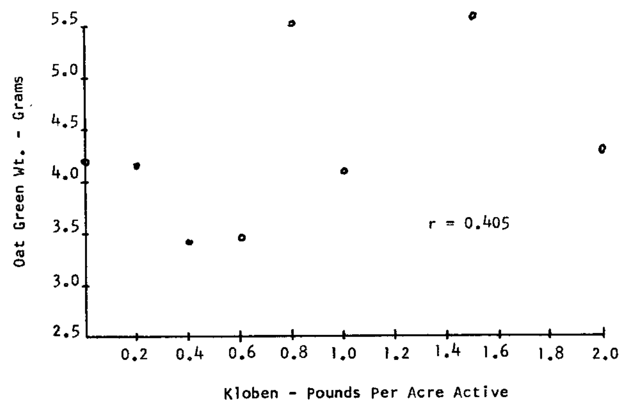

Figure 8.—Relation of green weight yield of oats to level of Kloben herbicide in Webster Nursery soil.

On the other hand, Kloben showed no relation between oat yield and herbicide rate as reflected in insignificant correlation coefficients of less than 0.5 for both the green and ovendry weight data.

The initial visual effect was a leaf-tip dieback beginning on the older leaves about 2 weeks after planting. This was followed by progressive chlorosis of leaves and retardation of growth, ending in the death of the plant. Simazine, Propazine, and to a lesser extent, Dowpon exhibited these typical symptoms, but Kloben showed no indication of herbicide activity.

The yield of oats from the unknowns collected from the herbicide plots was compared to the standard green and dry weight curves, and the estimated amount of remaining active herbicide in the Webster Nursery soil is shown in table 3. The amount of active herbicide remaining in the soil from both the green and dry weight data from simazine and Propazine was about equal. For Dowpon, green weight data indicated no active material remaining 6 months after application, and dry weight data showed a maximum of 1 pound active material at the 8- to 12-inch depth. Dry weight data appears to be a more sensitive measure of residual herbicide than green weight. No interpretation could be made for the Kloben data because no standard curves could be developed.

Approximately 85 percent of the original application of simazine was gone after 6 months, based on the dry weight data, and 90 percent of the Propazine was gone after the same period. Holly and Roberts (4) also noted that Propazine dis-

TABLE 3.—*Active level of four herbicides remaining in Webster Nursery soil 6 months after application, based on a greenhouse bioassay*

Herbicide	Original level	Soil depth	Oat yield green weight	Residual herbicide	Oat yield dry weight	Residual herbicide
	Lb./acre active	*In.*	*Gm.*	*Lb./acre active*	*Gm.*	*Lb./acre active*
Simazine	4	0–4	5.97	0.6	0.94	0.5
		4–8	3.87	0.8	0.64	0.6
		8–12	3.39	0.9	0.66	0.6
Propazine	4	0–4	4.58	0.4	0.73	0.4
		4–8	6.73	0.2	1.23	0.2
		8–12	4.52	0.4	0.78	0.4
Dowpon	10	0–4	4.07	0.0	0.64	0.75
		4–8	5.11	0.0	0.84	0.0
		8–12	4.05	0.0	0.60	1.0
Kloben	4	0–4	6.80	—	1.11	—
		4–8	6.74	—	1.15	—
		8–12	6.70	—	1.08	—

appeared more rapidly than simazine from three different soil types in England. They also found that both materials rapidly decreased in these soils during the first 8 weeks. This decrease was followed by a long, drawn-out period of reduced persistence, up to an additional year.

Small amounts of simazine, Propazine, and Dowpon appear to be residual for 6 months or longer. The value of a bioassay test is to determine the level of residual herbicide activity so that repeated applications can be adjusted according to the amount remaining in the soil.

Bioassay techniques for measuring soil persistence of a herbicide have an advantage over chemical methods in that they measure directly the residues affecting plant growth. With chemical methods, extraction techniques may remove residues, which do not affect plant growth. Also, such methods may not always differentiate the herbicide from possibly biologically inactive products of degradation. Although chemical techniques are much faster and can often determine smaller amounts with greater accuracy, the nurseryman is usually not able to run chemical tests for herbicide persistence; however, he is usually in a position to establish a bioassay analysis.

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