Effect of Pinolene (β-Pinene Polymer) on Carbaryl Foliar Residues[1]

Carlos H. Blazquez,[2] Akshay D. Vidyarthi,[3] Thomas D. Sheehan,[3] Michael J. Bennett,[3] and George T. McGrew[3]

The pesticidal life-extending properties of Pinolene, a β-pinene polymer, were determined. The results indicate that Pinolene increases the initial deposition of carbaryl and also decreases the rate of decay of carbaryl on tomato leaves. The combination of these two factors effectively lengthens (threefold increase) the period in which carbaryl residues remain on the foliage. The data were interpreted using the least squares method of linear regression. Also the concept of "residue control" is introduced and briefly discussed.

The use of spray adjuvants is a common agricultural practice in the application of pesticides on growing crops. Many experimental attempts have been made to show horticultural value of these spray additives (Ebeling, 1963; Furmedge, 1962; Hugolino, 1958; Pielou and Williams, 1962; Schenck, 1966). Both biological activities and chemical analyses have been used as criteria in these evaluations. In general, only limited success has been achieved (Ebeling, 1963; Pielou and Williams, 1962; Schenck, 1966). The purpose of this paper is to report that the use of Pinolene, a β-pinene polymer, will both increase the initial deposit of carbaryl on tomato leaves and reduce the rate of decay of carbaryl on the foliage.

The mechanism for the increased initial deposit has not been determined. It is thought to be intimately involved in both the dynamics of deposition and the retention ability of foliage for carbaryl. By the latter we mean the repulsion of carbaryl by the foliage. Pinolene may modify this force and increase the ability of the foliage to retain carbaryl.

The mechanism for the reduced decay rate is closely involved in the ability of a film of Pinolene to occlude a pesticide within its structure. When polymerized by sunlight, it protects the pesticide from rain and wind erosion, volatilization, photodecomposition, enzymatic degradation, and other natural degradation factors.

EXPERIMENTAL

Crops. Tests were carried out in Immokalee, Fla. Ten-foot single row plots of Homestead 24 tomato plants were established in a random block design with five plants per plot, with 2-ft spacing between plants. Plants were removed from the seedbed and were transplanted into the field plots on Sept. 5, 1968. Three fertilizer applications of 1000 lb per acre of 4-8-8 with 30% organic nitrogen were made on Aug. 29, Sept. 29, and Oct. 9, 1968. Dithane M-45 fungicide was applied as needed at the rate of 2 lb per 100 gal of water per acre with a standard 500 gal boom type sprayer drawn by a tractor. During the entire growing period, no infestations of cabbage loopers or armyworms were observed, so that it was not possible to obtain a biological activity comparison with the chemical activity of carbaryl with and without Pinolene. A severe wind storm on Dec. 3 damaged most of the vines and fruit, so that it was not possible to harvest the plots. Any possible differences in yields would have been confounded by the differences in damage to the plots by the storm.

Spray Details. Sevin 5 Aqua (an aqueous dispersion containing 5 lb of active carbaryl per gal of formulation) with and without Pinolene as a spray tank additive was sprayed on tomato plants at the rate of 2.5 pt per 100 gal of water per acre (1.56 lb of active carbaryl). The specific formulation of Pinolene was Nu-Film 17 and was used at three different rates —0.5, 1.0, and 1.5 gal per 100 gal of water per acre. The Nu-Film 17 and Sevin 5 Aqua were supplied by Miller Chemical & Fertilizer Corp., Hanover, Pa.

To be able to monitor the effect of Pinolene on the carbaryl residues as a function of time, 7-, 14-, and 21-day spray schedules were employed (Table III). The sampling dates are given in Table I and the spraying dates were Nov. 19, Nov. 26, and Dec. 4, 1968. The 7-day series was analyzed for the first 7 days. The results were not reported, as they were identical to the 14- and 21-day series.

Extraction of Leaves. Leaf samples were frozen soon after removal from the plants and kept frozen until the time of analysis. A complete and separate analysis was performed for each replicate.

[1] Florida Agricultural Experiment Stations, Journal Series No. 3557.

[2] University of Florida, South Florida Field Laboratory, P.O. Box 937, Immokalee, Fla. 33934

[3] Miller Chemical & Fertilizer Corporation, P.O. Box 333, Hanover, Pa. 17331

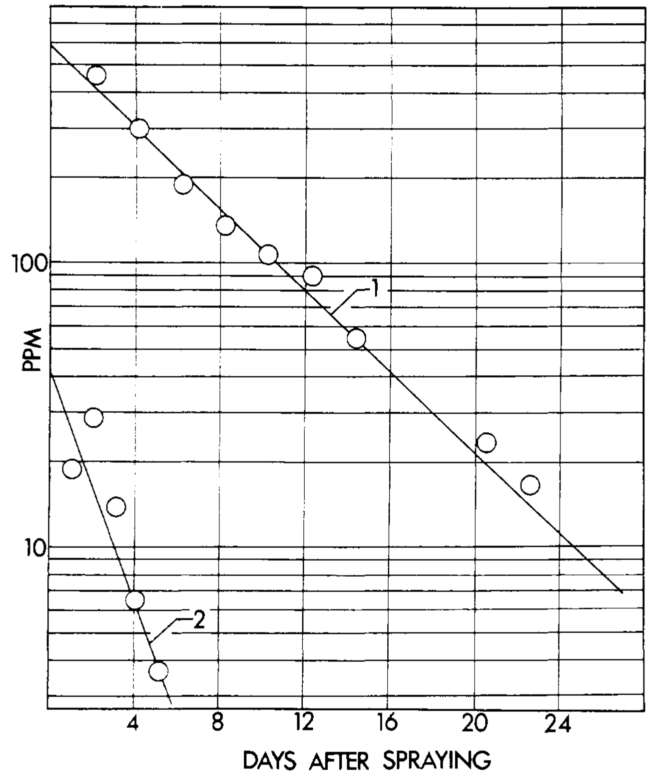

Figure 1. Amount of carbaryl as a function of days after spraying

21-Day spray schedule
1. 1.5 gal Pinolene
2. No Pinolene

Whole leaves were weighed and placed with 150 ml of benzene in a conical flask. The flask was clamped to a horizontal shaking apparatus for 2 hr to insure complete removal of carbaryl. Benzene was chosen as the solvent because Pinolene and carbaryl were soluble in it. The extract was then transferred to an evaporating dish, concentrated to 5 to 10 ml, and analyzed by thin-layer chromatography (tlc).

As a check, the leaves were re-extracted and concentrated to 1 ml. This second stage extract, when analyzed by tlc, confirmed the completeness of the original extraction.

Thin-Layer Chromatography. The tlc method for the detection of cholinesterase inhibiting pesticides (Mendoza *et al.*, 1968) was modified for better accuracy and sensitivity as follows. Plates were made 0.5 mm thick using a 1 to 1 combination of silica gel G and MN-Kieselgel G-HR (Brinkmann Instruments Co.). The ascending solvent was a mixture of *n*-hexane-acetone (120 to 30).

The developing reagents were made as follows. The liver homogenate was prepared by obtaining 1 to 2 lb of liver from a 1 to 2-yr-old steer immediately after slaughter. One-fourth of the liver was cut into small chunks and ground in a blender filled to two-thirds volume with ice cold distilled water. Immediately after obtaining a homogeneous mixture, it was poured into 30 ml bottles, capped, and frozen.

The substrate spray solution was prepared by making the following solutions, which were stored for future use and combined just before spraying. Solution A contained 30 mg of 5-bromoindoxyl acetate in 10 ml of ethanol. This solution was not stored. Solution B contained 80 ml of Tris buffer solution (pH 8.32) (Sigma Chemical Co.), 100 ml of 2*M* sodium chloride, 4 ml of 1*M* calcium chloride, and 76 ml of distilled water. Solution C was a mixture of 100 ml of 0.1*M* potassium ferrocyanide and 100 ml of 0.1*M* potassium ferricyanide. Ten milliliters of solution A were added to a mixture of 26 ml of solution B and 4 ml of solution C. This was used as the substrate spray solution. The carbaryl was visualized in the following manner. Thirty milliliters of the liver homogenate were added to 60 ml of Tris buffer solution (pH 8.32) and sprayed on the developed plate. The plate was air dried for 20 min and then sprayed with the substrate spray solution. Carbaryl was detected as white spots on a blue background. An R_f value of 0.3 was observed.

The spots developed by the tlc procedure were compared visually to known standards by spotting knowns and unknowns on the same plate.

Some measure of the quantitative accuracy (Table II) of this method was obtained as follows. Ten known samples of carbaryl, unknown to investigators, were spotted along with five known standards on a plate. This was done twice using different samples. A comparison of visual accuracy was then made by investigators familiar with tlc and reported in two ways.

The Overall Accuracy: The comparisons were either right or wrong and closeness didn't count.

The Percent Error: The comparisons were rated to the degree of closeness and whether the comparison was higher or lower than the correct concentration. The results were expressed as + or − a percentage of the correct concentration.

Table I. Sampling Dates, Rainfall, and Temperature Ranges During Experiment

Sampling Dates	Temperature °F	
	Max	Min
Nov. 19, 1968	66	60
20	62	43
21	65	37
22	72	43
23	75	49
24	77	49
25	70	51
26	75	48
27	78	55
28	82	60
29	81	59
30[a]	83	62
Dec. 1	84	63
2	84	62
3[b]	83	61
6	71	40
9	68	41
10	70	47
11	66	41

[a] 0.05 in. rainfall in 1/2 hr. [b] 0.20 in. rainfall in 1/4 hr with severe wind.

Table II. Overall Accuracy and Percent Error of Four Investigators in Determinations of Carbaryl by the Cholinesterase Tlc Detection Method

Investigator	Overall Accuracy (%)	Percent Error (%)	
		High Comparisons	Low Comparisons
1	90	+0.0	−10.0
2	80	+2.5	− 3.3
3	80	+5.8	− 0.0
4	100	+0.0	− 0.0
Mean	88	+2.1	− 3.3

Table III. Pinolene–Carbaryl Residue Analysis

All Residues are an Average of Three Replicates

Gal Pinolene per 100 gal	ppm Carbaryl on X Day of Spraying																		
	X = 0[a]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	17	20	21	22
	14-Day Spray Schedule																		
1.5	625	600	470	350	308	220	197	175	140	123	112	105	95						
0	30	20	30	15	7	4	1												
1.0	500	500	470	350	306	220	197	175	140	123	105	98	95						
0	15	6	5	7	2	2													
0.5	405	470	410	308	260	220	197	175	140	123	105	96	92						
0	100	6	4	3	2	1													
	21-Day Spray Schedule																		
1.5	625	600	470	350	306	220	197	175	140	123	112	105	95	63	58	38	25	21	17
0	30	20	30	15	7	4	1												
1.0	500	600	470	350	306	220	197	175	140	123	105	98	95	61	56	31	23	21	15
0	15	6	5	7	2	2													
0.5	405	470	410	308	260	220	197	175	140	105	98	87	91	60	56	29	21	17	12
0	100	6	4	4	2	1													

[a] Sampled 6 hr after spraying.

Table IV. Least Squares as Applied to Carbaryl Analysis

Variable Values are Expressed in Logarithmic Terms

Gal Pinolene/ 100 Gal H_2O	Equation of Line	Y-Intercept	Slope of the Line	Standard Error of Estimate
	14-Day Spray Schedule			
1.5	Y = 2.7687–0.07209X	2.7687	−0.07209	0.0538
0	Y = 1.8459–0.2702X	1.8459	−0.2702	0.1800
1.0	Y = 2.7742–0.07368X	2.7742	−0.07368	0.0556
0	Y = 0.9901–0.1351X	0.9901	−0.1351	0.1396
0.5	Y = 2.7043–0.06642X	2.7043	−0.06642	0.0330
0	Y = 1.0134–0.1856X	1.0134	−0.1856	0.0830
	21-Day Spray Schedule			
1.5	Y = 2.7183–0.06614X	2.7183	−0.06614	0.1352
0	Y = 1.8459–0.2702X	1.8459	−0.2702	0.1800
1.0	Y = 2.7523–0.07089X	2.7523	−0.07089	0.0728
0	Y = 0.9901–0.1351X	0.9901	−0.1351	0.1396
0.5	Y = 2.7259–0.07202X	2.7259	−0.07202	0.0447
0	Y = 1.0134–0.1856X	1.0134	−0.1856	0.0830

RESULTS AND DISCUSSION

The foliar degradation of pesticides is assumed to be of first order reaction kinetics (Ebeling, 1963). The analytical results of carbaryl residues (Figure 1) suggest that the degradation of carbaryl closely approaches first order reaction kinetics.

To further analyze the significance of the data from Table III, the method of least squares was used to determine the correlation between the amount of residue and the days after spraying (Table IV). The very small values of the standard error of estimate (Table IV) clearly show that the semilogarithmic plot is linear.

The slopes of the curves of carbaryl with Pinolene are less negative in magnitude than those without Pinolene, and are also alike in value. This is immediately apparent in Figure 2. The phrase "residue control" is used to describe this phenomenon. It is believed that the pesticidal residual extension by Pinolene is an occlusion and dissolution of the pesticide within the pliant film which Pinolene forms on the foliage. The pesticide is then protected within this film from rain and wind erosion, volatilization, photodecomposition, and other natural degradation factors. It is significant that higher residues of carbaryl were found on foliage 20 to 22 days after spraying with the Pinolene-carbaryl combinations than with carbaryl only. This residual extension property may explain in part the extension of the biological activity of combinations of fungicides and Pinolene tested previously (Blazquez, 1968).

If the initial deposits on the foliage were the same, the Pinolene treated plots would retain the carbaryl far longer than those untreated plots. This is inferred from the lower values of the negative slopes of the Pinolene treated plots.

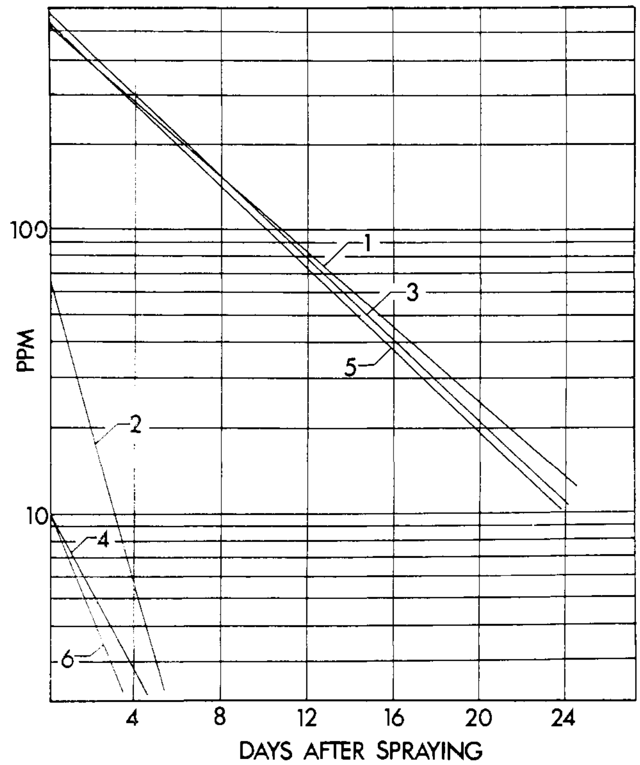

Figure 2. Amount of carbaryl as a function of days after spraying
Using least squares data 21-Day spray schedule
1. 1.5 gal Pinolene
3. 1.0 gal Pinolene
5. 0.5 gal Pinolene
2, 4, and 6. No Pinolene

The parallelism in the magnitude of the slopes of the three treatments with the three different concentrations of Pinolene (Figure 2), in addition, suggests that all three rates used were higher than necessary. Experiments are currently under way to determine the optimum rates to be used. Other workers (Frommer and Brown, 1968) have shown that Pinolene, when used with the volatile insecticide lindane, extended the biological activity significantly with as little as 1 oz of Pinolene per acre.

The higher initial deposit on the Pinolene treated foliage is a phenomenon which is not yet fully understood. It is believed that this increase may be due in part to the equipment type used, and pesticide chemical and physical properties. It has been reported (Hoskins, 1961) that there is an immediate rapid loss of pesticides from foliage following application due to natural degradation factors, and then a much slower loss. Pinolene prevents this initial rapid loss of carbaryl deposited. The present experiment averaged better than seven times more carbaryl initially on the foliage with Pinolene in the mixture. In the current investigations the increased initial deposit has been found to occur with several other pesticides: Captan (*N* - trichloromethyl - thio - 4 - cyclohexane - 1,2 - dicarboximide); Difolatan [*N*-(1,1,2,2-tetrachloroethylthio)-4-cyclohexane-1,2-dicarboximide]; and Dyrene [2,4-dichloro-6-(*o*-chloroanilino)-3-triazine]. Other workers have also reported on this phenomenon (Schenck, 1966).

The magnitude of the plant growth dilution effect on the slope of the decay curves is not known. If the plant growth dilution were by far the most significant factor in the decay of carbaryl, then Pinolene would have shown no trenchant effect. It is assumed that growth dilution is greater when plants are young but becomes less important near plant maturity. Work is presently under way that may in the future determine the importance of growth dilution to the overall modes of foliar pesticidal residue decay.

This work reports only on the chemical residue decay of carbaryl on tomato foliage. Investigations have been nearly completed correlating the chemical and biological activities of carbaryl residues with and without Pinolene, and will be included in a later publication.

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