

Herbicide Concentration in Runoff and Sediment from Experimental Plots of Highland Cultivated Areas

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ABSTRACT

Bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon at the rates of 4 kg ai/ha, 4 kg ae/ha, 8 kg ae/ha, 8 kg ai/ha, 4 kg ai/ha, 2 kg ai/ha, and 2 kg ai/ha, respectively, were applied to cultivated soil of 8 to 10% and 25 to 28% slopes during the middle of rainy season at Royal Ang Khang Highland Agriculture Research Station, Fang district, Chiang Mai. At 8 to 10% slope the maximum concentration of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon detected in runoff were 29.1, 38.4, 809.7, 36.4, 110.7, 280.0 and 49.3 μ g/L, respectively. At 25 to 28% slope the maximum concentration of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon detected in runoff were 29.4, 31.3, 763.9, 6.9, 46.8, 65.3 and 10.7 μ g/L, respectively. The maximum concentrations of herbicides in runoff were observed within a few days after application and were drastically reduced which might be attributed to half life or persistence and leaching of herbicides in soil. The average amount of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon in water phase were 67.4, 56.2, 76.5, 32.1, 37.7, 64.2 and 69.2% of total amount of herbicides detected in both water and soil sediment phases, respectively. The maximum concentration of herbicides detected in runoff water were applied to sandy loam soil and leaves of test plants. No phytotoxicity of herbicides were observed except those of bromacil, linuron and oxadiazon when applied to leaves. Total losses of herbicides from both slopes were less than 2% of applied rates except 2, 4-D loss was less than 6%. All herbicides moved vertically to 7.5 to 15.0 cm depth. Except bromacil, the other herbicides moved laterally with runoff to surface of adjacent (non treated area) slope base. The persistences of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon were 117, 30, 30, 180, 180, 241 and 241 days respectively.

INTRODUCTION

The application of herbicides for weed control on the highland of Thailand which is the hill side is very useful during the rainy season. Nevertheless the levels of herbicides in runoff water must be recorded because they can be transported by water and might affect ecosystem.

The herbicides used in this study were bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon. Bromacil a pre- and postemergence herbicide is used for weed control mainly in pineapple and certain varieties of

orange. 2, 4-D and dalapon are postemergence herbicides. 2, 4-D is used for broadleaf weed control in grain crops such as rice, corn, and sugarcane. Dalapon is used for control of annual and perennial grasses in certain plantation crops and noncrop area. DCPA, diuron, linuron and oxadiazon are preemergence herbicides. DCPA is used mainly in vegetable and ornamental plants. Diuron is used in pineapple, cotton and certain plantation crops. Linuron is used in soybean and corn. Oxadiazon is used in rice, garlic and shallot. Paraquat and glyphosate are also applied on the highland.

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But both of them are strongly bound to soil sediment (Wauchope, 1978; Sprankle et al, 1975) and need specific extracting procedure. Furthermore they were reported that the maximum amount of these herbicides in runoff will be in sediment phase (Wauchope, 1978).

This study did not intend to compare the losses in runoff among the herbicides because of difference in their properties (Table 1). Furthermore the application rates and their formulation are also different (Table 1 and 3).

The objectives of this experiment are to determine : 1. the maximum concentration of herbicides in runoff, 2. the toxicity of herbicides

to sensitive crops when they were applied at the maximum concentration detected in runoff, 3. the total losses of herbicides from different slopes under natural rainfall condition, 4. the persistence of herbicides in soil, and 5. the lateral movement of herbicides to surface of slope base.

MATERIALS AND METHODS

Field experiment

Experiment was conducted at Royal Ang Khang Highland Agriculture Research Station, Fang district, Chiang Mai. Herbicides were applied on 8 to 10 and 25 to 28% slopes.

Table 1 Water solubility and formulation of herbicides and solvents used for extraction the herbicides from runoff water and eroded soil sediment.

Herbicides	Water solubility ²	Formulations	Solvent
	(mg/L)		
Bromacil	815	wettable powder	xylene
2, 4-D ¹	insoluble	emulsifiable concentrate	benzene
Dalapon	900	sodium salt	ethyl acetate
DCPA	0.5	wettable powder	benzene
Diuron	42	wettable powder	benzene
Linuron	75	wettable powder	benzene
Oxadiazon	0.7	emulsifiable concentrate	benzene

¹Butylester of 2, 4-D

²Anonymous, 1983

Table 2 Total volume of runoff water and amount of eroded soil sediment from 8 to 10% and 25 to 28% slopes.

Herbicides	8 to 10% slope			25 to 28% slope		
	Soil sediment	Water	Sediment : Water	Soil sediment	Water	Sediment : Water
	(kg/ha)	(L/ha)	(kg : L)	(kg/ha)	(g/ha)	(kg : L)
Bromacil	2,501.2	527,635	1 : 210	2,478.5	530,060	1 : 213
2, 4-D	2,456.7	359,120	1 : 146	1,474.3	221,875	1 : 150
Dalapon	1,968.4	262,620	1 : 133	2,646.2	282,500	1 : 106
DCPA	3,305.9	1,115,525	1 : 337	2,143.6	752,475	1 : 351
Diuron	3,368.4	990,490	1 : 294	2,134.7	666,990	1 : 312
Linuron	2,830.9	586,405	1 : 207	2,217.8	464,150	1 : 209
Oxadiazon	3,305.9	1,149,030	1 : 348	2,714.8	999,945	1 : 368

Table 3 Total amount of herbicides lost in runoff (water phase plus eroded soil sediment phase).

Herbicides	Rate	Total losses in runoff			
		8 to 10% slope		25 to 20% slope	
	(kg ai/ha)	(g/ha)	(%) ¹	(g/ha)	(%) ¹
Bromacil	4	6.3 ± 2.6	0.15	24.0 ± 11.8	0.60
2, 4-D	4 (ae)	6.4 ± 3.8	0.16	220.0 ± 25.1	5.50
Dalapon	8 (ae)	54.0 ± 8.7	0.68	94.0 ± 19.2	1.18
DCPA	8	23.0 ± 14.2	0.29	3.2 ± 2.8	0.04
Diuron	4	13.6 ± 1.6	0.33	28.0 ± 14.6	0.70
Linuron	2	35.0 ± 2.1	1.75	8.6 ± 0.6	0.43
Oxadiazon	2	34.0 ± 4.3	1.70	7.0 ± 1.3	0.35

¹ % of application rate

The plot size was 10 × 2 m and surrounded with 22.5 cm width corrugated roofing sheet to prevent the runoff from outside. The lower edge of corrugated roofing sheet was anchored into the soil, 5 cm depth. The 4.5 cm diameter pipe was connected between corrugated roofing sheet at slope base and 200 L metallic tank for runoff collecting. The tank was covered with corrugated roofing sheet to prevent the contamination from rain. Every day when runoff event was observed the water and sediment in collecting tank was sampled for extraction.

Herbicides (Table 1) were applied to soil surface by motor driven knapsac sprayer with a pressure of 2.27 kg/cm² on August 29, 1983. When the soil moisture was 23.09%. The main weed was *Imperata cylindrica*. Glyphosate at 3 kg ai/ha was applied for weed control at the middle of August. Weed stover residue was cleaned up and the soil surface was cultivated one day before herbicides application. Herbicides was applied on the area of 5 × 2 m which was the upper part of the plot. The treatments were replicated 3 times in randomized complete block design on each slope.

The volume of runoff water and eroded soil sediment in collecting tank was measured and thoroughly stirred before randomly collecting for 2.5 L per tank. After that the tank was cleaned up. Water and sediment at 50 ml was extracted 3 times by appropriate solvent (Table 1)

with a volume of 150 ml each. The other 50 ml of water and sediment was filtered by layers of Whatman No. 1 and 40 filter papers. The sediment was collected, dried and weighed to determine the amount of eroded soil. The filtrate was extracted 3 times by the same volume of above solvent. The preliminary study indicated that total amount of herbicides was completely extracted from soil and water by appropriate solvent. After partitioning the extracting solvent were pooled together and filtered through glass wool and sodium sulfate anhydrous for separation of sediment and water. After that it was evaporated by flash evaporator until the volume was reduced to 100 ml. The extracting solvent was transported to the laboratory for herbicides determination.

At 1, 11, 20, 30, 60, 117, 180 and 241 days after application the soil at 0 to 7.5 cm and 7.5 to 15.0 cm depth was 3 times randomly collected from treated and nontreated slope base area of each plot. The diameter of auger is 2.0 cm. The collected soil was kept in the glass bottle with exceed amount of appropriate solvent and transported to the laboratory.

Laboratory experiment

The volume of solvent from runoff extraction was reduced to 10 ml before injection into the gas chromatography.

For soil extraction after the solvent was evaporated the soil was ground and passed

through 20 mesh screen. The ground soil was extracted on the shaker (200 cycle/minute) for 30 minutes by 2 consecutive appropriate solvent. The volume of solvent : soil was 3 : 1. The preliminary study indicated that high amount of herbicide in soil was completely extracted on a shaker for 30 minutes by 2 consecutive appropriate solvent. The ground soil was dried and weighed to determine the weight of soil. The extracting solvent was combined together and the volume was reduced to 10 ml before injection into gas chromatography.

The tracer 560 gas chromatography with ^{63}Ni electron capture detector and the column of 1.5% ov-17 + 1.95% ov 202 on Chromosorb W WHP 80/100 mesh (glass column 2 m long and 0.62 cm width) was used in this study. The oven, detector and inlet temperatures were 200, 350 and 240 °C, respectively. The output and input attenuation were 8 and 10. N_2 flow and chart speed were 1.12 kg/cm² and 37.5 cm/h. The result was the average of 3 injections and compared to the known standard concentration of each herbicide.

Greenhouse experiment

Cucumber (local var.), sorghum (KU 439), and soybean (S.J. 5) were planted in sand and sandy loam soil in 300 ml cups. Pregerminated cucumber and soybean were planted at 10 plants per cup and sorghum was planted at 20 plants per cup. Five days after planting the cucumber and soybean, plants were thinned to 5 plants per cup and sorghum plants were thinned to 10 plants per cup. Hoagland solution and water were subirrigated to the cups every 2 days or when necessary to keep soil moisture at field capacity. At 10 days after planting, herbicides at various concentration (base on the maximum concentration detected in runoff) were applied to both sand and soil surfaces for root uptake study (Table 1). The same concentrations of herbicides were applied to leaves of test plants grown in sand culture. During application to leaves, the sand surface was covered with saw dust to prevent soil contamination from herbicides. For leaf uptake, herbicides were

applied every 2 days for 3 consecutive applications. Herbicides were applied by laboratory sprayer with a nozzle of 8004 E, a pressure of 2.04 kg/cm² and spray volume of 500 L/ha. Phytotoxicity of herbicides were observed and dry weight of plants were recorded at 14 days after first application of herbicides. Treatments were replicated 4 times in randomized complete block design.

RESULTS AND DISCUSSION

Maximum concentration

The maximum concentration of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon found in runoff water and eroded soil sediment were 36.5, 63.7, 993.1, 65.7, 171.0, 328.5 and 81.3 µg/L, respectively from 8 to 10% slope and 36.5, 65.7, 840.1, 32.7, 125.7, 112.9, and 15.9 µg/L, respectively from 25 to 28% slope (Table 4). It was at 2 to 4 days after application except those of oxadiazon which was at 8 days after application while high amount of water and soil were lost (Figures 1 to 8). After these periods the concentrations of herbicides in runoff were decreased. Since the concentration of herbicides in runoff were correlated with herbicides concentration at a soil surface (Leonard *et al*, 1979).

Phytotoxicity

2, 4-D, dalapon and DCPA at the concentration of 180.0, 310.0, and 180.0 µg/L (3 times of maximum concentration detected in runoff) did not reduce 10 days old cucumber and sorghum dryweight respectively (Table 5). Although 2, 4-D and dalapon can be absorbed into leaves of sensitive plants. Bromacil, diuron, linuron and oxadiazon at the concentration of 29.0, 170.0, 140.0 and 50.0 µg/L reduced 10 days old cucumber and soybean dry weight when the herbicides were applied to sand culture. But all herbicides at the same concentration did not affect plant dry weight when they were applied to sandy loam soil (Table 5). These results indicated that the herbicides might be adsorbed by clay particle and organic matter. However bromacil, linuron and oxadiazon at

Table 4 The maximum concentration of herbicides detected in water plus eroded soil sediment phases and in water phase.

Herbicides	Rate	8 to 10% slope		25 to 28% slope		Average herbicide in water phase
		Water + Soil sediment	water	Water + Soil sediment	Water	
	(kg ai/ha)	(μg/L)				(%)
Bromacil	4	36.5 ± 12.5	29.1 ± 11.0	36.7 ± 18.3	29.4 ± 18.4	79.9
2, 4-D	4 (ae)	63.7 ± 37.6	38.4 ± 21.3	65.7 ± 7.5	31.3 ± 15.1	53.9
Dalapon	8 (ae)	993.1 ± 161.1	809.7 ± 76.5	840.1 ± 172.3	763.9 ± 123.1	86.2
DCPA	8	65.7 ± 64.2	36.4 ± 28.7	32.7 ± 28.6	6.9 ± 8.5	38.2
Diuron	4	171.0 ± 21.4	110.7 ± 61.2	125.7 ± 65.5	46.8 ± 30.1	51.0
Linuron	2	328.5 ± 20.1	280.0 ± 7.5	112.9 ± 11.5	65.3 ± 14.6	71.5
Oxadiazon	2	81.3 ± 10.2	49.3 ± 8.5	15.9 ± 2.9	10.7 ± 2.3	64.0

the same concentration reduced dry weight of above plants when applied to leaves. Since these herbicides were applied at optimum concentration and absorbed by leaves of sensitive plant which were the properties of these herbicides (Anonymous, 1983).

Partitioning between water and sediment phase

According to different water solubility, herbicides of this study can be categorized into 4 groups : 1. bromacil and dalapon with high water solubilities of 815 and 900 mg/L respectively, 2. diuron and linuron with medium water solubilities of 42 and 75 mg/L 3. DCPA and oxadiazon with low water solubilities of 0.5 and 0.7 mg/L, and 4. 2, 4-D in butylester form with insolubility in water. The amount of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon detected in water phase were 79.7, 53.9, 86.2, 38.2, 51.0, 71.8 and 64.0% of total amount detected in runoff, respectively (Table 4). The amount of herbicides in water phase were relatively correlated with their water solubilities except oxadiazon. This might be the effect of emulsifier which mixed into oxadiazon formulation.

Total losses in runoff

The total amount of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron, and oxadiazon

in runoff from 8 to 10% slope were 6.3, 6.4, 54.0, 23.0, 13.0, 35.0 and 34.0 g/ha which equivalent to 0.15, 0.16, 0.68, 0.29, 0.33, 1.75 and 1.70% of applied rate, respectively and from 25 to 28% slope were 24.0, 220.0, 94.0, 3.2, 28.0, 8.6 and 7.0 g/ha which equivalent to 0.62, 5.57, 1.18, 0.04, 0.70 and 0.43% of applied rate, respectively (Table 3).

The total losses of bromacil, 2, 4-D, dalapon, and diuron from 25 to 28% slope were greater than losses from 8 to 10% slope and oxadiazon from 8 to 10% slope were greater than from 25 to 28% slope. Since the soil of 8 to 10% slope was clay loam which might be susceptible to erode than clay of 25 to 28% slope. Furthermore, total runoff water and eroded soil sediment from DCPA, linuron and oxadiazon treated plots of 8 to 10% slope were lost in greater amount than of 25 to 28% slope (Table 2). Many factors might affected herbicide losses in runoff including the type of soil and topography (Bailey *et al*, 1974).

Duration of losses in runoff and persistence

The last detectable value of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon were 36, 16, 12, 97, 61, 24 and 79 days after application, respectively (Figures 1 to 7). The duration of losses in runoff were relatively correlated with the persistences of herbicides in

Table 5 Effects of herbicides on dry weight of test plants.

Herbicides	Concentration	Applied to		Applied to leaves ¹
		sand	soil ²	
	($\mu\text{g/L}$)	(g/plant)		
Bromaci ^{3/}	0.0	0.32	0.22	0.19
	29.0 ⁶	0.29	0.18	0.15
	43.0	<u>0.28</u>	<u>0.20</u>	<u>0.15</u>
	LSD _{.05}	<u>0.02</u>	<u>NS</u>	<u>0.02</u>
2, 4-D ^{3/}	0.0	0.31	0.15	0.58
	60.0 ⁶	0.28	0.12	0.55
	180.0	<u>0.81</u>	<u>0.11</u>	<u>0.45</u>
	LSD _{.05}	<u>NS</u>	<u>NS</u>	<u>NS</u>
Dalapon ^{4/}	0.0	0.22	0.23	0.24
	770.0 ⁶	0.19	0.18	0.26
	2,310.0	<u>0.13</u>	<u>0.20</u>	<u>0.24</u>
	LSD _{.05}	<u>NS</u>	<u>NS</u>	<u>NS</u>
DCPA ^{4/}	0.0	0.38	0.25	0.52
	60.0 ⁶	0.34	0.20	0.39
	180.0	<u>0.31</u>	<u>0.23</u>	<u>0.29</u>
	LSD _{.05}	<u>NS</u>	<u>NS</u>	<u>NS</u>
Diuron ^{5/}	0.0	0.28	0.17	0.29
	80.0	0.20	0.14	0.28
	170.0 ⁶	<u>0.20</u>	<u>0.17</u>	<u>0.25</u>
	LSD _{.05}	<u>0.06</u>	<u>NS</u>	<u>NS</u>
Linuron ^{3/}	0.0	0.10	0.22	0.50
	140.0	0.07	0.17	0.40
	180.0 ⁶	<u>0.01</u>	<u>0.16</u>	<u>0.38</u>
	LSD _{.05}	<u>0.01</u>	<u>NS</u>	<u>0.08</u>
Oxadiazon ^{5/}	0.0	0.21	0.21	0.27
	50.0 ⁶	0.17	0.15	0.24
	70.0	<u>0.16</u>	<u>0.16</u>	<u>0.22</u>
	LSD _{.05}	<u>0.02</u>	<u>NS</u>	<u>0.02</u>

^{1/}Herbicides were applied to leaves of testing plants every 2 days, 3 times.

^{2/}Soil was sandy loam (67% sand, 22% silt, 11% clay and 0.77% organic matter)

^{3/}cucumber (local var.)

^{4/}sorghum (KU 439)

^{5/}soybean (S.J.5)

^{6/}The maximum concentration of herbicides detected in runoff water.

soil except linuron. This might be due to the leaching of linuron into subsoil. The persistences of bromacil, 2, 4-D, dalapon, DCPA, diuron, linuron and oxadiazon were

117, 30, 30, 180, 180, 241 and 241 days, respectively (Table 6). However the amounts of herbicides detected at these periods might not affect on the sensitive crops.

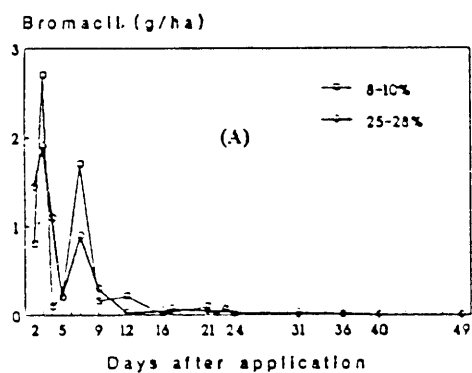
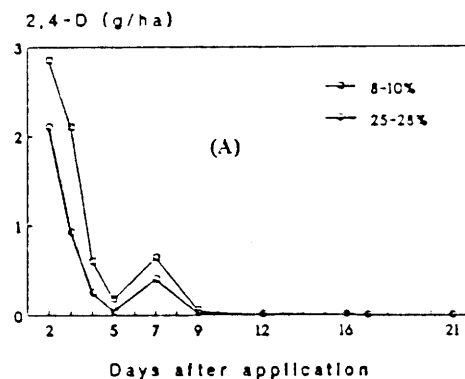
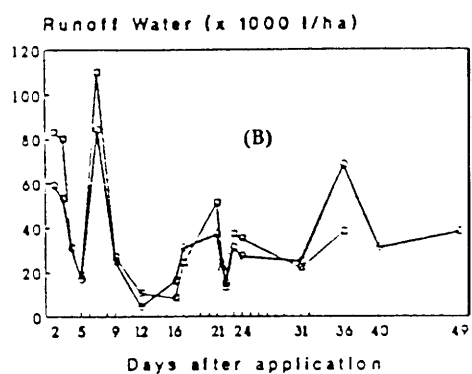
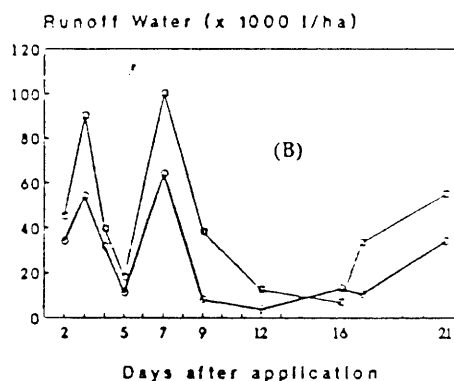
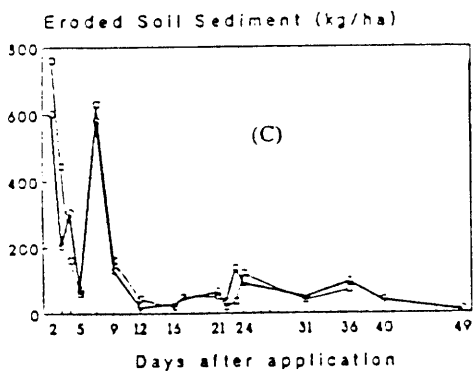
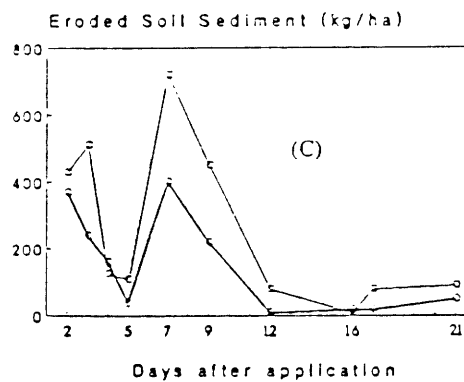


Figure 1 Amount of bromacil in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

Figure 2 Amount of 2, 4-D in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

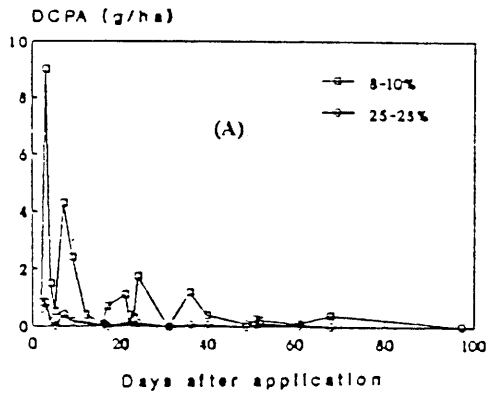
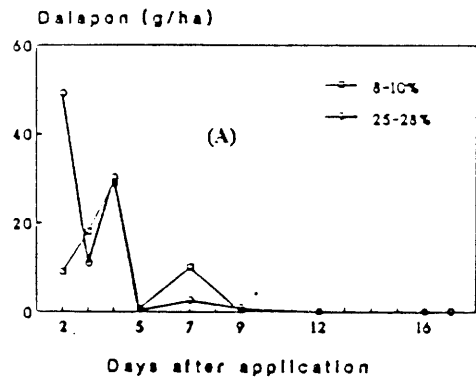
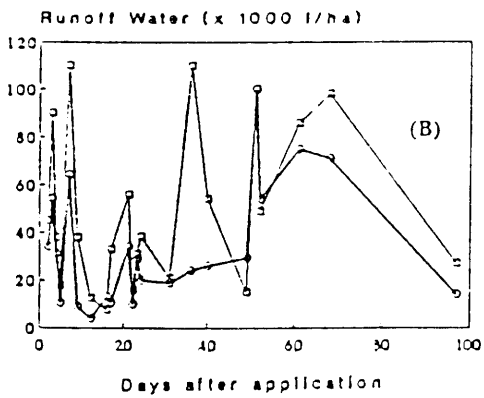
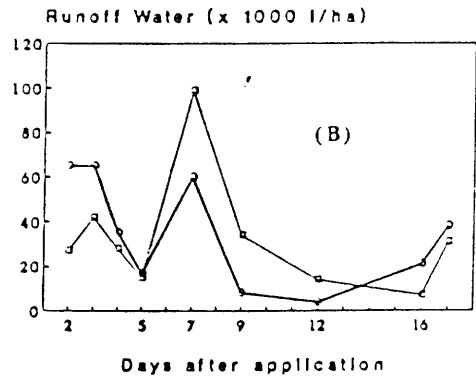
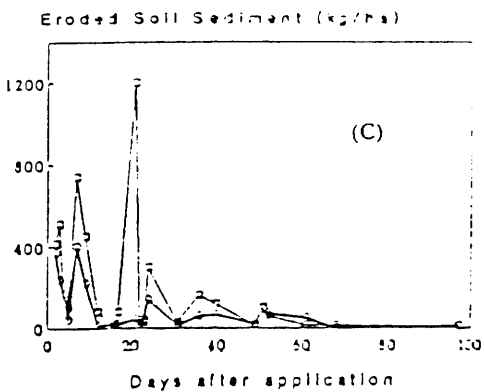
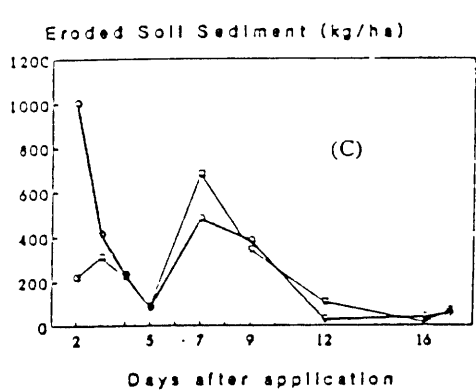


Figure 3 Amount of DCPA in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

Figure 4 Amount of dalapon in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

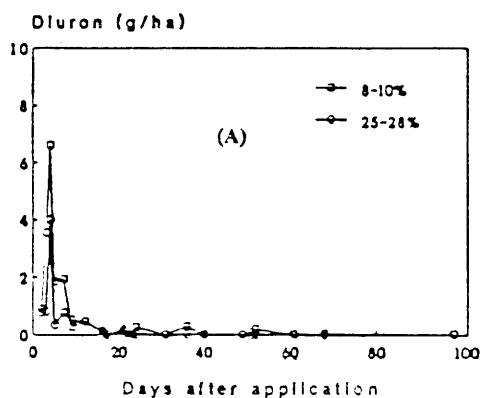
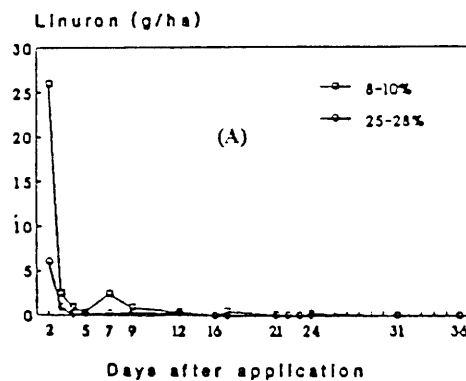
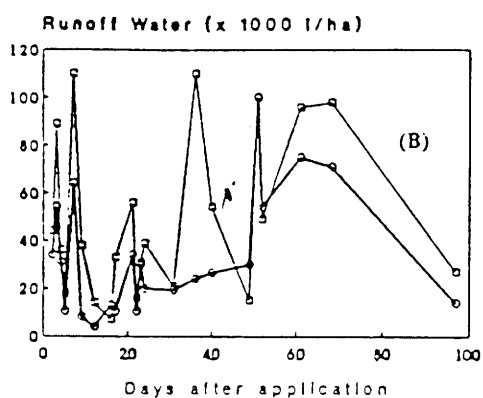
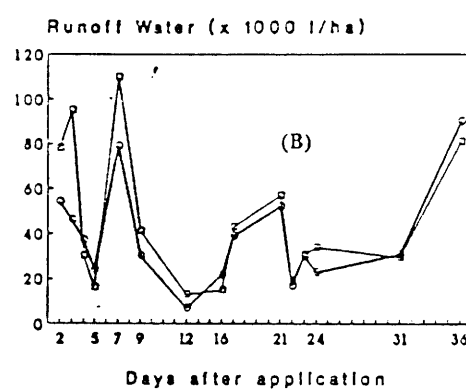
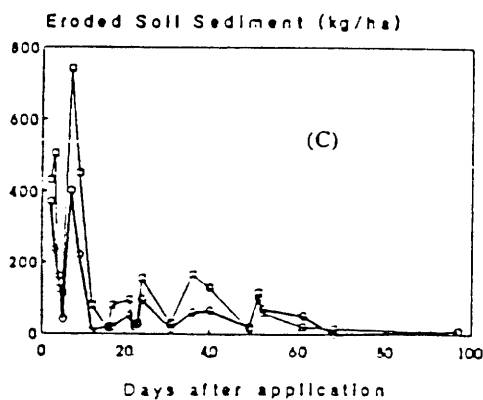
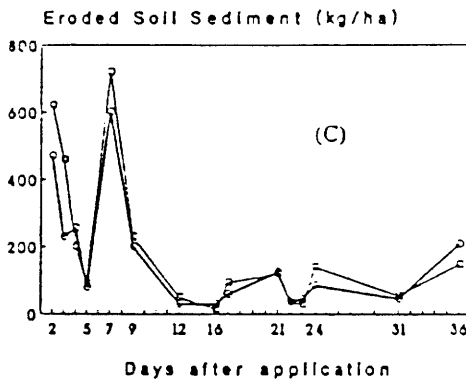


Figure 5 Amount of diuron in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

Figure 6 Amount of linuron in runoff water and sediment (A) and amount of runoff water (B) and eroded soil sediment (C) from 8 to 10% and 25 to 28% slopes.

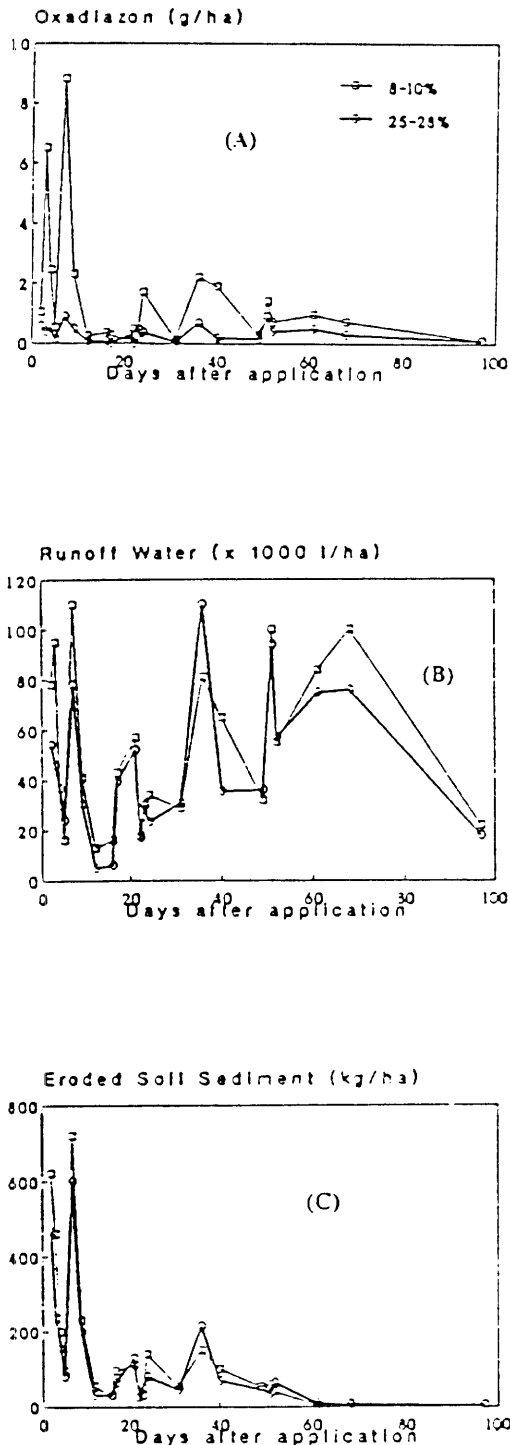


Figure 7 Amount of oxadiazon in runoff water and sediment
(A) and amount of runoff water
(B) and eroded soil sediment
(C) from 8 to 10% and 25 to 28% slopes.

Lateral and vertical movement

In contrast to bromacil the other herbicides moved laterally in the runoff to the adjacent (nontreated area) slope base (Table 7). This might be due to high rate of vertical movement of bromacil. All of herbicides did not persist in soil of the slope base longer than of those treated areas. The vertical movement of bromacil, linuron and oxadiazon was observed at 1 day after application, 2, 4-D, dalapon, and DCPA was observed at 10 days and diuron was observed at 20 days after application.

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Table 6 Persistence of herbicide in soil (of treated area).

Herbicides	Rate		8-10% slope			25-28% slope	
	(kg ai/ha) (days) ¹		(g/ha)	(%) ²	(days) ¹	(g/ha)	(%) ²
At the level of 0.0-7.5 cm.							
Bromacil	4	117	50.0 ± 45.0	1.25	117	40.0 ± 38.0	1.00
2, 4-D	4 (ae)	30	2.0 ± 18.0	0.05	30	1.0 ± 3.0	0.02
Dalapon	8 (ae)	30	10.0 ± 5.0	0.12	30	10.0 ± 5.0	0.12
DCPA	8	180	2.0 ± 1.0	0.02	180	1.0 ± 7.0	0.01
Diuron	4	180	40.0 ± 30.0	1.00	180	40.0 ± 13.0	1.00
Linuron	2	241	60.0 ± 36.0	3.00	241	50.0 ± 30.0	2.50
Oxadiazon	2	241	23.0 ± 50.0	1.5	241	30.0 ± 18.0	1.50
At the level of 7.5-15.0 cm.							
Bromacil	4	117	40.0 ± 36.0	1.00	117	20.0 ± 16.0	0.50
2, 4-D	4 (ae)	30	1.0 ± 0.5	0.02	20	30.0 ± 27.0	0.75
Dalapon	8 (ae)	30	8.0 ± 7.0	0.10	30	2.0 ± 1.8	0.02
DCPA	8	180	3.0 ± 2.8	0.04	180	1.0 ± 0.9	0.01
Diuron	4	180	10.0 ± 2.5	0.25	180	10.0 ± 9.0	0.25
Linuron	2	180	3.0 ± 1.5	0.15	241	10.0 ± 8.5	0.50
Oxadiazon	2	241	20.0 ± 10.0	0.85	241	10.0 ± 9.2	0.50

¹ Days after application of herbicide until the last day which herbicide can be detected.² % of applied rate.**Table 7 The maximum rate of herbicides found in soil of the adjacent (nontreated area) slope base.**

Herbicides	Rate		8-10% slope			25-28% slope	
	(kg ai/ha)(days) ¹		(g/ha)	(%) ²	(day) ²	(g/ha)	(%) ²
At the level of 0.0-7.5 cm.							
Bromacil	4	—	ND ³	ND	—	ND	ND
2, 4-D	4 (ae)	11	30.0 ± 25.0	0.75	11	40.0 ± 30.0	1.00
Dalapon	8 (ae)	20	50.0 ± 17.5	0.62	11	10.0 ± 8.0	0.12
DCPA	8	60	20.0 ± 15.0	0.12	20	20.0 ± 13.0	0.25
Diuron	4	30	170.0 ± 0.3	4.00	60	100.0 ± 60.0	3.75
Linuron	2	60	3.0 ± 2.0	0.15	60	1.0 ± 5.0	0.05
Oxadiazon	2	11	10.0 ± 8.0	0.50	180	40.0 ± 10.0	2.00
At the level of 7.5-15.0 cm.							
Bromacil	4	—	ND	ND	—	ND	ND
2, 4-D	4 (ae)	—	ND	ND	20	3.0 ± 1.5	0.08
Dalapon	8 (ae)	—	ND	ND	—	ND	ND
DCPA	8	60	30.0 ± 20.0	0.37	117	40.0 ± 30.0	0.50
Diuron	4	117	10.0 ± 5.0	0.25	180	10.0 ± 7.0	0.25
Linuron	2	—	ND	ND	11	20.0 ± 11.0	1.00
Oxadiazon	2	60	1.0 ± 0.7	0.05	117	10.0± 1.5	0.50

¹ Day after application² % of applied rate³Not detectable

Passage of Pollens through Proventriculus in Honey Sacs of European Bee (*Apis mellifera carnica* L.)

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ABSTRACT

During the transfer of pollen grains in sugar solution through the proventriculus, a selection of pollen grains took place according to their size and shape, which altered the relative proportions of the different pollen grains. After feeding, amounts of pollen grains in honey sac were remarkably reduced. Pollens of rape (*Brassica napus* L.) which are small (about 25 micron) and have smooth exine could not be completely filtrated by the filiform-hair of the proventriculus. One hundred thirty five minutes after feeding, they remained in the honey sac. Pollens of pine (*Pinus sylvestris* L.) which have two air-sacs (wing) floated on the upper side of honey sac. Pollens of aster (*Aster* sp.) and dandelion (*Taraxacum officinale* Web.) are relatively bigger and more spiny than rape pollens. They could be easily filtered from the fluid in honey sac by the filiform-hairs of the comb and passed into the midgut in 95 and 100 min., respectively.

INTRODUCTION

It is not yet possible to identify the botanical and geographical origin of honey by chemical uses. However, the microscopical examination of pollen in honey can be applied. Honey-Pollen analysis is one important method which was used for along time for this purpose (Louveaux *et al.*, 1978; Sawyer, 1981; Sturm, 1988). But difficulties are still to be faced because the appearance of the pollen in honey and in honey sac depends on not only kinds of plant which secretes nectar but also alimentary structure of the honeybees. Proventriculus is a sphincter-like valve of honey sac which regulates flow rate of food being passed into the midgut and prevents backflow of food from the midgut when digestion and absorption take place (Barker and Lehner, 1972; Snodgrass 1935). Although suggested function of the bee's proventriculus have long been reported, the question how pollen grains are filtered or reduced is still unclear. Whitcomb and Wilson (1929) and Todd and Vansell (1942)

found that there was a reduction process of the amount of pollen between in nectar and in honey sac. Bailey (1952) showed that some pollen grains were eliminated during the ripening process. They found that proventriculus trapped pollen grains from then crop and passed them from the crop into ventriculus (midgut or stomach)

This experiment was designed to study the passage of pollen grains of different sizes and forms through the proventriculus in honey sac of the European bees.

MATERIALS AND METHODS

5% pollen solution from rape (*Brassica napus* L.), dandelion (*Taraxacum officinale* Web.), pine (*Pinus sylvestris* L.) and Helianthus-form pollen (*Aster* sp.) collected from bee hives were prepared by using 50% sugar solution (w/v). The pollen solution were transferred in petridish and then were placed on the comb in honeybee hive. The bees took up the pollen solution. Until about 200 bees came to gather

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