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HERBICIDE TOXICITY IN MANGROVES

by

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ABSTRACT

The amine salts of 2,4-D and picloram were applied to the Florida species of mangroves: red mangrove (Rhizophora mangle), white mangrove (Laguncularia racemosa) and black mangrove (Avicennia germinans). Treatments were to soil or water, by aerial spray and to single leaves as droplets. The effects on radiochloride uptake and on localization of radiocarbon-labelled picloram after leaf application were studied in red mangrove.

"Lethal doses" for young seedlings were 2.7 kg/ha for white mangrove, 13 kg/ha for red and 13 kg/ha for black; for mature plants they were 2.7, 13 and >53 kg/ha respectively. "Tolerance doses" for young seedlings were 0.01, 5.3 and 5.3 kg/ha; for mature plants they were 0.5, 5.3 and 53 kg/ha. "No effect doses" for seedlings were <0.01 kg/ha for all species; for mature plants they were <0.1, 0.5 and 2.7 kg/ha.

Spray applications of 6.3 - 12.2 kg/ha of commercial mixture to the canopy of a mixed-species forest caused partial defoliation within three weeks. Within 16 months it killed all of the white, 78 - 100% of the mature red, but none of the mature black mangroves.

Radiocarbon-labelled picloram concentrated in dormant buds of red mangrove and it is concluded that the tree is killed by the mixture because of its effects on them.

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SECTION I

CONCLUSIONS

1. The Florida black mangrove is most resistant to herbicide, the red mangrove intermediate and the white mangrove most sensitive to Agent White by soil (water) or aerial spray application.

2. Species of the genus of the Florida black mangrove, Avicennia, are generally resistant to herbicide; species of the genus of the red mangrove, Rhizophora, are generally sensitive.

3. The Herbicide sensitivity of red mangrove is not due to root membrane alteration or effects on transpiration or translocation.

4. Sensitivity of red mangrove derives from the translocation of herbicide to and killing of all the tree's dormant buds.

5. Spray drift hazard to mangroves from amine salt forms of Agent White herbicide should be minimal.

6. Because of soil binding of herbicide, agricultural runoff from areas of Agent White use are unlikely to pose a hazard to mangroves

SECTION II

RECOMMENDATION

Although soil uptake and tidal washing minimize the likelihood of effects of herbicide on mangroves, it is recommended that the use of Agent White be restricted in areas where runoff might flow directly into a mangrove estuary or where spray drift might reach mangroves.

SECTION III

INTRODUCTION

Mangroves are trees or bushes that grow between the high water penetration of spring tides and mean low tide. They occur along tropical and subtropical shores where the wave energy is low and are found in the estuarine portions of rivers. The importance of coastal areas and estuaries has been recognized increasingly in recent years, for example in volumes edited by Thomas (1956), Lauf (1967), and Ketchum (1972). It has been estimated that as much as 98.5 percent of all commercial fish and shellfish species caught in the Gulf of Mexico off Florida spend part of their lives in estuarine environments (Glooschenko, 1968). Mangrove estuaries play an important role in the life cycles of shrimp in southern Florida (Idyll et al., 1968). Odum and Heald (1972) have made detailed studies of the role played by mangrove detritus in the food chains of estuaries in Everglades National Park in Florida. Mangrove estuaries clearly have great significance in food production of coastal areas.

Mangroves have been found to be very sensitive to auxin-type defoliants. Tschirley (1969) and Orians and Pfeiffer (1970) surveyed military defoliation sites in South Vietnam and reported that although only a modest kill of upland forest trees generally followed a single spraying, the great majority of mangroves were killed by the same treatment.

This sensitivity of mangroves to herbicides combined with the importance of mangrove estuaries in food webs pointed out the need to evaluate the hazard to mangroves from herbicide spray drift and herbicide-contaminated agricultural runoff water. Portions of the investigations reported here have been presented at an international symposium on mangroves and will be published in the proceedings (Teas and Kelly, 1975).

The defoliants that had proven so toxic to mangroves in South Vietnam were: Agent Orange (2,4,5-trichlorophenoxyacetic acid + 2,4-dichlorophenoxyacetic acid as n-butyl esters) and Agent White (2,4-dichlorophenoxyacetic acid + picloram, both as triisopropanolamine salts). All three of the active ingredients are absorbed by roots and tops and are translocated and concentrated in actively growing parts of plants (Weed Science Society of America, 1970). These three compounds function as defoliants and herbicides according to dosage, growing condition of the plants, and other factors. All three active compounds meet the stereochemical requirements for auxins (Eisinger and Morre, 1971).

Auxin herbicides are often selective with respect to tree species as shown, for example, by a study of picloram toxicity to red maple and white ash; red maple was killed by concentrations of picloram that only slightly injured white ash (Mitchell and Stephenson, 1973).

The United States military rate of application of Agent White in Vietnam was 3 gal/a which corresponds to 8.54 kg/ha of active ingredients (6.72 kg 2,4-D and 1.82 kg picloram) or to 7.62 lbs/a of active ingredients (6.0 lbs 2,4-D and 1.62 lbs picloram) (Lang, 1974).

In the present study the red mangrove, Rhizophora mangle; the black mangrove, Avicennia germinans; and the white mangrove, Laguncularia racemosa, were tested to determine "lethal", "tolerance" and "no effect" doses of Agent White. This military defoliant is identical to a commercial formulation of 2,4-D and picloram (Tordon [R]101) that is widely used as a brush killer. Because of the reported extreme toxicity of dioxins that are found in some samples of Agent Orange, no tests were conducted with this material.

Soil applications of Agent White were made to the three Florida mangrove species at three stages: 2 month old seedlings; 14 month old seedlings; and "mature" (estimated 4-10 year old) trees. Herbicide was applied to the water over soil in potted mangroves to simulate the effect of dissolved herbicide in runoff water.

Aerial spray applications were made to a mature mangrove forest in southern Florida to predict the effect of herbicide drift and in order to follow the effects of herbicides on mangroves, data that were not obtained in Vietnam (Lang, 1974). In addition, laboratory experiments were conducted with Agent White, with radioactive carbon-labelled picloram, and with radioactive chloride as part of an investigation of the basic mechanism of auxin-type herbicide toxicity to the red mangrove.

SECTION IV

MATERIALS AND METHODS

HERBICIDE

The herbicide was Tordon 101 from the Dow Chemical Company. Herbicide or dilutions were applied to water over the soil with stirring. The experimental dosages used are shown in Table 1.

Picloram with carboxyl carbon-14 label, specific activity 1 mCi/mM, was obtained from the Dow Chemical Company. The triisopropanolamine salt of the labelled picloram was prepared and diluted approximately 9 to 1 with non-radioactive herbicide before application so that the 2,4-D and picloram constituents were present in almost the normal ratio.

SOIL APPLICATIONS

Mangrove plants were collected in southern Florida and planted in plastic dishpans, buckets or wading pools containing estuarine mud. Water levels were maintained about 5-10 cm above the soil. The water salinity was adjusted weekly to the range of one third to two thirds that of seawater during the first nine months of the experiments and less often thereafter. The plants were maintained at the Miami U. S. Plant Introduction Station under natural partial shade.

All three Florida mangrove species produce seed or propagules in the fall. "Two month" old seedlings were from the fall crop of 1973. They were planted directly from the field into plastic pans or buckets and treated with herbicide in January 1974.

Five to ten plants were used per pan and pans replicated 3 times for the white and black mangroves and 6 times for the red mangroves. "Fourteen month" old plants were from the fall crop of 1972. They were individually planted in 12-cm diameter plastic pots which were placed in plastic buckets to maintain the water level above the soil and were treated in January or February 1974. Treatments consisted of three plants per plastic bucket and three replicates for the red and white mangroves; two plants per bucket and three replicates for the black mangroves. Salinity was adjusted as noted above.

TABLE 1. HERBICIDE DOSAGES TESTED BY SOIL APPLICATION (WATER OVER SOIL).

Age Plant	Mangrove Type	Active Ingredients Dosage, kg/ha									Observation Period (wks)
		0.01	0.02	0.11	.52	2.63	5.26	13.2	26.3	52.6	
	White	X	X	X	X	X	X	X	X		40
2 mo.	Red	X	X	X	X	X	X	X	X		59
	Black	X			X	X	X	X	X	X	55
	White	X	X	X	X	X	X	X	X		55
14 mo.	Red	X	X	X	X	X	X	X	X		55
	Black	X	X	X	X	X	X	X	X		55
	White			X	X	X	X				59
Mature (estimated ages 4-10 yrs.)	Red				X	X	X	X	X		59
	Black					X		X	X	X	59

"Mature" plants were transplanted from the field into individual 65-cm diameter plastic childrens' wading pools. These plants had the mature form and were typically 1.5-2 m high. The red mangroves had 5 or more prop roots; the black mangroves had well developed pneumatophores; the white mangroves had basal trunk diameters of 2-5 cm. Salinity was adjusted as noted above. The ages of these mature trees were estimated to be 4-10 years. Space availability limited the numbers of these large plants. Four red mangrove plants were used per treatment, three plants per treatment in the case of black and white mangroves. Plants were examined weekly for six weeks and then less often for 40 to 59 weeks. Plants of all age classes were scored for leaf characteristics, that is, leaf size, leaf curl, browning, drooping, abscission and chlorosis; for growth rate and number of leaves and branches; for tumorous growths; for stem tip viability, and for overall plant viability.

The lowest herbicide concentration that killed the majority of plants within the 40-59 week observation period was considered to be the "lethal" dose. The maximum dosage from which 80% or more of the plants recovered, i.e. were still alive after 40-59 weeks, although not necessarily completely normal, was considered the "tolerance" dose. The highest dosage that gave rise to no detectable symptoms was considered the "no effect" dose.

SPRAY APPLICATIONS

Experimental spray plots were located in Collier County near Marco Island, Florida, on land of the Deltona Corporation. The plots were in a forest that is a well developed mixed mature stand of red, black and white mangroves about 8-13 m tall. Nine plots were laid out, each 20 by 40 m, 0.08 ha (0.2 acres).

Plots were treated with Tordon 101. Spraying was carried out in the calm weather of early morning by helicopter at a height of about 3 m above the forest canopy. Spray was applied from a 9-m wide boom in two passes per plot. Three plots were treated on 15 December 1972 and three additional plots were sprayed on 19 January 1973. Actual field herbicide dosage was determined from samples collected on Whatman No. 3 MM filter paper backed with aluminum foil. Collection papers were positioned above the canopy and others were located 0.5 m above the ground to assess the undercanopy dosage. Sample workup and quantitative estimations of picloram and 2,4-D by gas chromatography were carried out as the Perrine Primate Research Laboratory of the Environmental Protection Agency by procedures developed at the Gulf Breeze Environmental Research Laboratory, U. S. Environmental Protection Agency. Because of an accident, many of the filter paper collectors were lost; however, dosages had been determined for three herbicide sprayed plots.

Effects of herbicides were assayed by degree of defoliation and tree survival. Visual estimates of defoliation and leaf litter pan collections were made. Determination of whether the trees were "living" or "dead" was based on machete cuts. When a cut showed that the bark and wood were normally light colored and moist, the tree was classified as "living"; if the bark and wood were brown and dry, it was classified as "dead". Observations were carried out over a period of approximately 16 months following spraying.

SINGLE LEAF APPLICATIONS

Single-leaf applications of herbicide were made to individual leaves of a few red, black and white mature plants in plastic pools. Viability, appearance of leaves, branches, prop roots and pneumatophores were recorded.

Radiocarbon-labelled herbicide was applied to 2 year old red mangroves brought to the laboratory. Leaves nearest the apical bud were treated. Labelled herbicide was applied in the form of 20 or more micro drops per leaf at the rate of 2.63 kg/ha. Experiments were terminated at 15 hrs after application of radioactive herbicide. Plants were harvested and the leaves, apical buds, sections of stem, and dormant buds were dissected, and finely cut. The minced samples were extracted with four successive 5-ml aliquots of acetone, the acetone evaporated and the radioactivity counted using a Tracerlab liquid scintillation counter with standard scintillation fluor in toluene.

RADIOCHLORIDE UPTAKE BY RED MANGROVE

Red mangrove seedlings at the 2-leaf stage were maintained at room temperature in the laboratory in individual 100 ml beakers of one-third strength seawater (10-12 ppt) containing radioactive chloride as ^{36}Cl. The roots were shaded by aluminum foil covers and opaque paper. The plants were lighted by a bank of flourescent lights programmed for

12-hr days. Two plants were treated with Agent White via the roots at 2.8 kg/ha, two at 28 kg/ha, and two served as controls. They were observed for six weeks, after which the leaves of each plant were thinly sliced and minced with a razor blade. They were then extracted with water, with intermittant swirling, for 12 hrs. Aliquots of the supernatant fluid were dried at 60 C in scintillation vials. The scintillation fluor contained Beckman BBS-3 solubilizer. and the samples were counted on a Tracerlab Corumatic counter.

CHLOROPHYLL CONTENT OF LEAVES

Leaves for chlorophyll determination were sampled by use of a sharp paper punch. Fresh weights of leaf samples were taken and the leaf discs ground in a porcelain mortar in 80% (v/v) acetone-water. The chlorophyll solution of acetone was decanted and leaf remains reextracted three times. The combined extracts were made to 15 ml volume in a glass-stoppered graduated centrifuge tube. Tubes were centrifuged and supernatant withdrawn for chlorophyll estimation. The chlorophyll solutions were diluted with 80% acetone where necessary to obtain appropriate optical densities. Estimation of chlorophyll content was carried out by use of a Spectronic 20 spectrophotometer from the general method of MacKinney (1941) and the equation developed by Arnon (1949):

$$C = \frac{D652 \times 1000}{34.5} \times \frac{V}{1000 \times W}$$

Where
C = Total chlorophyll, mg/g fresh weight
D652 = Optical density at 652 nm
V = Volume of extract
W = Weight of leaf sample, g

TRANSLOCATION OF SUCROSE

Translocation of photosynthate was measured by the use of uniformly labeled ^{14}C sucrose (1.135 mCi/mg) applied to punch holes in red mangrove leaves. Approximately 1 uCi was applied per leaf and samples were tested 18 hrs later. Leaf or stem material was thinly sliced using a single edged stainless steel razor blade, minced and extracted in water for 12 hrs with intermittent swirling. Sucrose from the application site was rinsed from each leaf with five aliquots of water. Aliquots of leaf or stem extract or of leaf rinsate were dried at 60 C in scintillation vials. The scintillation fluor contained Beckman BBS-3 solubilizer. Samples were counted in a Packard Tri-Carb scintillation counter.

TRANSPIRATION

Possible effects of herbicide treatment on transpiration were checked by a simple method. The plants were placed with their roots in glass jars. The tops extended through holes in the caps and the roots were in the dark. The jars had volume calibration marks on their sides and the amount of water transpired by the leaves was estimated by volume changes of the water in the jars.

ETHYLENE EVOLUTION FROM LEAVES

Ethylene evolution by herbicide-treated leaves was measured by chromatography of gas samples from chambers containing leaves. Four to six leaves were used per sample and leaf quarters or eighths incubated for 18 hrs. Samples of the atmosphere in which leaves had been incubated were chromatographed using an alumina column at ambient temperature. Ethylene measurement was by means of a Gow Mac hydrogen flame ionization unit and electrometer. An Esterline Angus recorder was used. Ethylene elution time had been checked against several other low M.W. gases with several types of columns. Sample values were calculated by comparison with a series of ethylene standards.

SECTION V

RESULTS

SOIL APPLICATIONS

The best estimates of lethal, tolerance and no effects doses for soil application are shown in Table 2. Because the experimental herbicide dosages were not numerous enough to accurately determine the lethal, tolerance and no effect levels in each case, the true lethality doses would be lower than those listed; the true doses for tolerance would be higher than those listed, and the true doses for no effect would be higher. The ranges of herbicide concentrations tested generally proved to be more suitable for assessing lethal doses than for tolerance and no effect levels.

It can be seen in Table 2 that the black mangrove is generally more resistant to soil applications of Agent White herbicide than is red mangrove, and both are generally more resistant than white mangrove. Mature plants are generally more resistant than are younger plants.

TABLE 2. "LETHAL", "TOLERANCE" and "NO-EFFECT" CONCENTRATIONS OF HERBICIDE APPLIED TO THE SOIL (WATER OVER SOIL).

Plant Age at Treatment	Mangrove Type	Dosage* (kg/ha)		
		Lethal	Tolerance	No-Effect
2 months	White	2.7	0.01	<0.01
	Red	13	5.3	<0.01
	Black	13	5.5	<0.01
14 months	White	2.7	0.5	0.01
	Red	2.7	0.5	0.01
	Black	27	2.4	0.10
Mature	White	2.7	0.5	<0.11
	Red	13	5.3	0.5
	Black	>53	53	2.7

*Active ingredients: 78.6% 2,4-D and 21.4% picloram

In only one case, in a mature red mangrove, was swelling of a stem tip noted after soil application of herbicide.

SPRAY APPLICATIONS

The herbicide dosages received by canopy top and at ground level are shown in Table 3. The visual estimates of defoliation are shown in Table 4.

TABLE 3. HERBICIDE DOSAGES RECEIVED AT CANOPY AND GROUND LEVELS (TEAS AND KELLY, 1975).

	Dose of Herbicide*			
	Canopy Top		Ground Level	
Treatment	2,4-D (kg/ha)	Picloram (kg/ha)	2,4-D (kg/ha)	Picloram (kg/ha)
Control	0	0	0	0
Herbicide treatment A	4.95	1.35	1.10	0.30
Herbicide treatment B	9.59	2.61	1.10	0.30
Herbicide treatment C	5.11	1.39	0.67	0.18

*Active ingredients

Leaves of herbicide-sprayed white mangroves began to fall in 2-3 weeks and the trees were completely defoliated by 5 weeks. Red mangrove leaves began to fall between 2 and 3 weeks after herbicide spraying and defoliation was approximately 85-90% by 16 weeks. Black mangrove leaf fall began in 3-4 weeks and reached an estimated 35-50% defoliation at the end of one year. By 16 months (66-71 weeks) the black mangroves were substantially refoliated.

The cumulative leaf falls for the three experimental spray plots and control, measured by the use of litter pans, are shown in Table 5. In Florida, the annual natural leaf fall for red mangrove approximates the standing crop of leaves, which for a forest of this type is in the range of

TABLE 4. DEFOLIATION OF TREE MANGROVE SPECIES AFTER HERBICIDE SPRAYING (VISUAL RATINGS)*

		Weeks After Spray								
Treatment	Mangrove Species	2	3	4	5	6	12	16	49-54	66-71
Control	White	0	0	0	0	0	0	0	0	0
	Red	0	0	0	0	0	0	0	0	0
	Black	0	0	0	0	0	0	0	0	0
Herbicide Treatment A	White	0	10	100	100	100	100	100	100	100
	Red	0	10	50	75	90	90	90	99	99
	Black	0	10	25	25	25	25	25	50	10
" B	White	0	10	50	100	100	100	100	100	100
	Red	0	5	10	10	10	60	85	95	99
	Black	0	0	5	10	10	10	25	35	10
" C	White	0	75	100	100	100	100	100	100	100
	Red	0	10	25	50	90	90	90	99	99
	Black	0	10	10	10	10	20	25	50	10

* Data from Teas and Kelly (1975)

475-650 gm/m^2. The leaf fall data show that this primarily red mangrove forest suffered very extensive defoliation. The visual estimates of defoliation at 16 weeks were: 100% for white, 85-90% for red, and 25% for black.

TABLE 5. CUMULATIVE LEAF FALL IN EXPERIMENTAL PLOTS AFTER SPRAYING. (TEAS AND KELLY, 1975).

	Canopy Herbicide Dose*		
Herbicide Treatment	2,4-D (kg/ha)	Picloram (kg/ha)	Leaf Fall (gm/m^2 dry wt)
Control	0	0	164
A	4.95	1.35	497
B	9.59	2.61	348
C	5.11	1.39	561

*Active ingredients

The white mangroves began to die in 24 to 29 weeks. After white mangrove trees had been classified as dead by the machete test, several showed basal sprouting of malformed leaves and shoots, but these died within a few weeks. All white mangrove trees in the herbicide treated plots were killed.

Killing of red mangroves was first noted at 29-32 weeks after herbicide spraying (Table 6). By 49-54 weeks, 25-50% of randomly sampled trees were dead. With the killing of many canopy trees and the refoliation of some undercanopy red mangroves, it became apparent that larger trees, red, white and black, had protected undercanopy red mangroves from herbicide. The fraction of trees that had been killed at 66-71 weeks was determined for only tall, top-of-canopy trees that received the full herbicide dose. Herbicide plots A and C showed complete kill at that time; plot B had 11 living trees of 50 tested.

TABLE 6. KILLING OF RED MANGROVE AFTER HERBICIDE SPRAYING (TEAS AND KELLY, 1975).

Herbicide Treatment	Number of Weeks After Herbicide Spray (Trees Dead of Total Tested)					
	24	29	32	37	49-54	66-71*
Control	0/20	--	0/30	--	0/20	0/50
A	0/20	--	4/30	--	10/20	50/50
B	--	4/20	--	1/30	9/20	39/50
C	--	1/20	--	0/30	5/20	50/50

*Only the tall trees that reached the top of the canopy were scored.

Based on evidence from canopy and undercanopy trees, red mangroves appear to have been killed by a single spray application of Agent White at 6.3 kg/ha (2.2 gal/a), and to survive a treatment of 1.4 kg/ha (0.5 gal/a). These doses correspond to 4.95 kg/ha of 2,4-D and 1.35 kg/ha picloram in the first case and to 1.1 kg/ha 2,4-D and 0.3 kg/ha picloram in the second.

Several relatively small (less than about 5 cm diameter) black mangroves appeared to have been killed by the spray but no larger trees died. Some of the apparently dead small black mangrove trees in herbicide plots showed basal regrowth at 66-71 weeks.

Mature black mangrove (*Avicennia*) in Florida is clearly much more resistant to Agent White than red mangrove (*Rhizophora*). It is interesting that in the Saigon River delta, where military maps indicated that spraying had been with Agent White, *Avicennia* trees were often seen as the only survivors in areas that had formerly been predominantly *Rhizophora* forests (Teas, 1972).

Some of the stem growth patterns of red mangrove that developed after herbicide spraying showed apparent auxin effects. Five common patterns of red mangrove tip and bud swelling that were observed are shown in Fig. 1. The locations of stems showing these patterns generally suggest increasing dosage effects from I through V. Full canopy trees that received high dosages of herbicide and died early

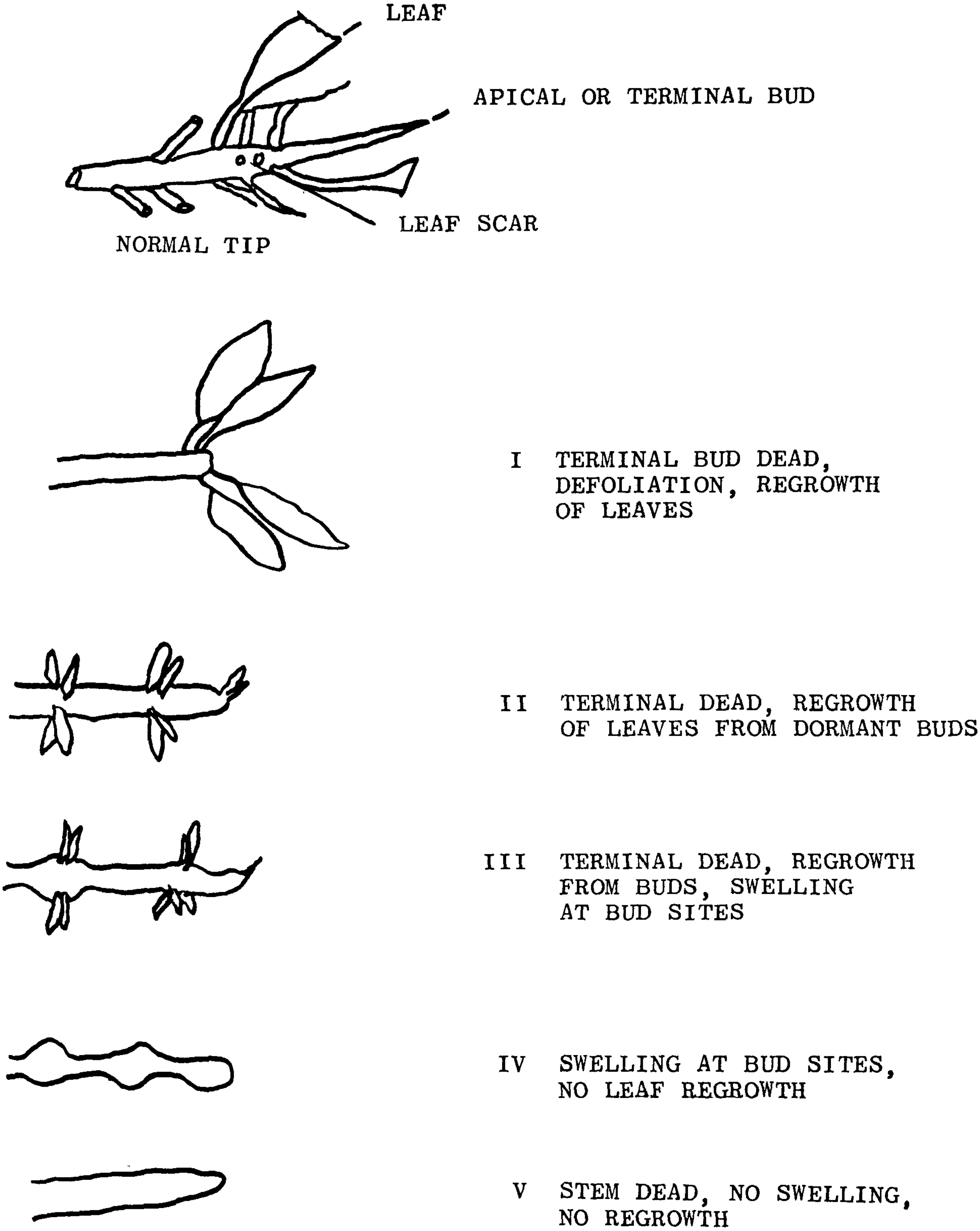

Figure 1. Forms of stem tips found in red mangrove after herbicide spraying (from Teas and Kelly, 1975).

had pattern V stems. The main canopy portion of some red mangrove trees within the spray plots showed pattern V stem tips, whereas the branches outside the spray plot had normal stems and leaves. Smaller trees, or branches of large trees, that were in positions where they would have been protected from the highest herbicide doses by the leaves and branches above them, often showed stems of patterns I-IV.

SINGLE LEAF APPLICATIONS

A small experiment was carried out in which herbicide was applied to individual leaves of mature plants in plastic pools. The effects after 6 weeks are shown in Table 7. In the white mangroves, all doses killed the leaves of the small branch, the small branch itself and one or more nearby branches. In the red mangroves, the two higher doses killed the leaves of the branch, the branch itself and one or more nearby branches. In the black mangroves the two higher doses killed the leaves of the branch, but not the branch or other nearby branches.

TABLE 7. SINGLE LEAF APPLICATIONS: RESULTS AFTER 6 WEEKS.

	Active Ingredients in Herbicide		Effects		
Mangrove Type	2,4-D (kg/ha)	Picloram (kg/ha)	Leaves	Branch	Nearby Lateral Branches
White	11.2	3.1	D**	D	D
	5.6	1.5	D	D	D
	1.12	0.31	D	D	D
Red	11.2	3.1	D	D	D
	5.6	1.5	D	D	D
	1.12	0.31	rolled leaves	ne**	ne
Black	11.2	3.1	D	ne	ne
	5.6	1.5	D	ne	ne
	1.12	0.31	rolled leaves	ne	ne

*Active ingredients applied per ha of leaf area. The area of each leaf was estimated from a series of leaf outlines

in which areas had been determined. The herbicide was applied with a microburet according to the leaf area.
**ne = no effect
D = dead

In two cases with 7.1 and 14.2 kg/ha doses to red mangroves, single leaf applications of herbicide resulted in swollen stem tips similar to patterns III and IV in Fig. 1. The active ingredient concentrations in these cases were 5.58 kg/ha 2,4-D and 1.52 kg/ha picloram in the first case and twice these doses in the second case.

The evidence for translocation of herbicide by adjacent branch kill of red mangrove, together with the very limited number of developable dormant buds, suggested that radiocarbon-labelled herbicide might prove useful in following local concentration points. Two experiments were carried out using carbon-14 labelled picloram. The principal distribution of label in leaves, apical bud, stem and dormant buds is shown in Table 8. Label was concentrated in the stem and in the dormant buds. The dormant buds in these plants were probably viable.

TABLE 8. TRANSLOCATION OF ^{14}C-LABELLED PICLORAM FROM LEAF APPLICATION IN RED MANGROVES.

	Radioactivity	
Tissue	Expt. 1 cpm/mg	Expt. 2 cpm/mg
Leaf	6.6	4.2
Leaf	5.8	6.8
Apical bud	7.8	1.7
Stem	14	18
Stem	--	9.9
Dormant bud	74	14
Dormant bud	102	50
Dormant bud	--	94

The labelled herbicide that could be rinsed from the leaves was measured after 15 and 24 hr. In both cases, approximately 75% of the label was recovered in the rinse, indicating that absorption occurred within the first few hours.

RADIOCHLORIDE UPTAKE

The possibility that the herbicides might kill red mangroves by altering root membranes so that they were no longer effective in excluding salt had been suggested by Walsh (1974). The uptakes of radiochloride by red mangrove seedlings at 0, 2.8 and 28 kg/ha Agent White are shown in Table 9. The active ingredient concentrations were 2.24 kg/ha of 2,4-D and 0.61 kg/ha of picloram in the lower dose and 10 times those concentrations in the higher dose. Leaves on herbicide treated plants showed signs of stress and appeared to be dying at 3-5 weeks. Leaves were harvested at 6 weeks. The results show that red mangroves take up some chloride, but that the levels of herbicide applied in these experiments, do not result in increased chloride uptake.

TABLE 9. RADIOACTIVE CHLORIDE UPTAKE BY RED MANGROVE SEEDLINGS TREATED WITH HERBICIDE.

Herbicide dosage kg/ha	Radioactivity cpm/leaf pair
0	9,430
0	14,550
2.8	200
2.8	135
28	0
28	58

CHLOROPHYLL CONTENT OF LEAVES

Agent White herbicide was applied to the water in which approximately 2.5 year old red mangrove plants were growing in mud in plastic buckets. Leaves were sampled at 15, 20 and 28 days by the use of a hand paper punch. Separate samples from young and old leaves were collected on each date. By the last sampling date some of the leaves of plants in the highest doses were wilted. Leaves of plants that showed only epinasty were sampled for chlorophyll at 20 and 28 days, and only green, non-wilted leaves were used.

It is concluded that chlorophyll synthesis or degradation were not sensitive assays of herbicidal effect. Leaves abscized green, yellow or brown, making if difficult to rationalize a basic mechanism of action that involved chlorophyll. Results are given in Table 10.

TABLE 10. CHLOROPHYLL CONTENT OF LEAVES OF RED MANGROVE TREATED WITH 2,4-D AND PICLORAM.

Active ingredients in herbicide (kg/ha)				
2,4-D	picloram	Type leaf	Total chlorophyll (average) (mg/gm, fr. wt)	Notes
0	0	young	1.561	---
		old	1.993	---
11.2	3.1	young	1.878	young leaves showed some effect at 20 and 28 days
		old	1.949	
22.4	6.1	young	2.110	young leaves smaller and curled at 20 and 28 days
		old	1.924	

TRANSLOCATION OF SUCROSE

Translocation of C-14 sucrose from leaves of red mangrove was studied. The plants were approximately 2.5 yrs old and grew in mud in plastic buckets. The plants were treated with Agent White approximately 25 days previously. Leaves used were green and turgid but had petioles that showed epinastic bending. At the time of the translocation experiment, some plants in the plastic buckets had withered or abscised leaves. The counts, made 18 hrs after application and shown in Table 11, represent sucrose translocation from the leaf blades. It is concluded that inhibition of translocation was not a basic herbicide toxicity mechanism.

TABLE 11. TRANSLOCATION OF SUCROSE IN RED MANGROVES TREATED WITH 2,4-D AND PICLORAM.

Active Ingredients in Herbicide (kg/ha)		Total Radioactivity in Plant Sample (cmp)		Notes
2,4-D	picloram	petiole	stem	
0	0	482	107	-
0	0	233	217	-
11.2	3.1	210	310	-
22.4	6.1	85	161	Untreated leaves began to wither and die ca. 1 week after experiment

TRANSPIRATION

Red mangrove plants, approximately 2.5 years old, were matched in jars of 50% seawater. After treatment with Agent White they were observed during the time that the leaves began to show herbicidal effects and to wilt and/or fall. The data (Table 12) fail to show any obvious effects of herbicide except that transpiration rates decreased as leaves withered and/or abscised.

TABLE 12. TRANSPIRATION BY RED MANGROVE TREATED WITH 2,4-D AND PICLORAM.

Active Ingredients in Herbicide (kg/ha)		Transpiration Rate (average), ml/day	Notes
2,4-D	picloram		
0	0	3.24	-
0.22	0.06	4.32	-
2.24	0.61	3.60	-
11.2	3.1	3.12	lost some leaves
22.4	6.1	1.80	lost some leaves

ETHYLENE EVOLUTION FROM LEAVES

Ethylene evolution from Agent White herbicide-treated and control red mangrove leaves was measured. Leaves were taken 16 days after water treatment of 2.5 yrs old plants grown in mud in plastic buckets. Petioles and terminal stems exhibited epinasty at that time. That was also about the time when leaves began to wilt, but the leaves that were selected had not done so. Results are given in Table 13. No effect of herbicides on ethylene production was found.

TABLE 13. ETHYLENE EVOLVED FROM LEAVES OF RED MANGROVE TREATED WITH 2,4-D AND PICLORAM.

Active Ingredients in Herbicide (kg/ha)		Ethylene Evolved (µl/gm fresh wt/hr)
2,4-D	picloram	
0	0	4.2
0	0	2.2
11.2	3.1	4.5
22.4	6.1	5.4
22.4	6.1	3.3

SECTION VI

DISCUSSION

HERBICIDE TREATMENTS

The active herbicidal ingredients in Agent White are water soluble and are readily taken up (absorbed) by a variety of soils (Bovey et al., 1974; Lutz et al., 1973; Altom and Stritzke, 1973). Thus, it is likely that the herbicide applied to water over soil in the plastic containers in this study was effectively a soil application.

The persistences of 2,4-D and picloram in soil differ considerably. In one study using three soils it was found that the half-life of 2,4-D was 4 days, whereas that of picloram was more than 100 days (Altom and Stritzke, 1973). In other investigations it was found that soil temperature and moisture, but not soil type or organic content, were important factors in picloram disappearance from soil (Merkle et al., 1973). Both active ingredients in Agent White have been reported to be degraded in soils (Norris, 1970).

The mangroves in the present experiments were growing under somewhat different conditions than typical plants in their natural habitat. However, it should be noted that mangroves may continue to grow in the absence of tidal flow. Stoddart et al. (1973) reported on inland mangroves in Barbuda and there are examples in the Miami area where the three Florida species grow on dry land (Teas, 1974). The plants used in the present experiments, although not subjected to tides, appeared to be normal. Many of the larger ones developed prop roots or pneumatophores after transplanting from the field into plastic pans and some of them bloomed and set fruit that produced viable seed or propagules.

Tidal flushing would be expected to play a role in herbicide elution from soils. Blackman et al. (1974) carried out studies of picloram persistence from soil applications of Agent White in two tidally-washed mangrove forest areas in South Vietnam, one of which was clear-cut and the other relatively undisturbed. They found in the clear-cut plot that a marked loss of picloram from surface soil (top 13 cm) occured during the first day and in both cases there was a 90-99% reduction within about thirty days. They concluded that this loss was partly due to penetration of the picloram into the soil and partly due to tidal elution of herbicide. It is therefore possible and likely that the lethal, tolerance and no-effects concentrations for soil application which are reported in the present study (Table 2), in which no herbicide attenuation by deeper penetration into the soil or by tidal elution were possible, are conservative. Thus, under natural conditions of tidal washing, it is likely that higher doses of herbicide by soil (water) application would be required to produce the same effects as found here using plants growing in shallow undrained containers.

TOXICITY FROM SOIL APPLICATIONS

The present experiments show that the black mangrove is most resistant and the white mangrove least resistant to soil applications of herbicide. The killing of mature red mangroves by soil application required herbicide dosages somewhat higher than the lethal dosage by canopy spraying (approximately 13 kg/ha for lethal effect by soil application to plants in plastic pans versus 6.3 kg/ha for spray application to plants in the forest). The active ingredients for soil applications were 10.2 kg/ha of 2,4-D and 2.8 kg/ha of picloram. The active ingredients for spray application were 4.95 kg/ha of 2,4-D and 1.35 kg/ha of picloram. Thus spray application is not likely to have killed red mangroves by absorption of wash-through and drift-through herbicide taken up from the soil. Soil application resulted in only one case of a swollen stem tip, a herbicide response from spray or single leaf application. This suggests that translocation of Agent White to the sensitive dormant buds of the plant is more effective from leaf rather than from root application.

Blackman et. al (1974) found that _Rhizophora apiculata_ propagules were killed by the military dose levels of Agent White (8.5 kg/ha) applied to the soil surface before planting. Walsh et al. (1973), who treated _Rhizophora mangle_ propagules and young seedling plants in plastic pans with a 2,4-D + picloram herbicide (of different formulation than Agent White), obtained a complete kill from 11.2 kg/ha, but no kill from 1.12 kg/ha. The active ingredient concentrations were 4.4 kg/ha 2,4-D + 1.6 kg/ha picloram in the first case and 0.44 kg/ha 2,4-D and 0.16 kg/ha picloram in the second case. In the present investigation, 13 kg/ha of Agent White killed young _Rhizophora mangle_ seedlings. Blackman et al. (1974) reported that 3-4 weeks after field applications of Agent White at 8.5 kg/ha to soil which was subject to tidal inundation the herbicide no longer inhibited the growth of

Rhizophora propagules. This dose contained 6.72 kg/ha 2,4-D and 1.82 kg/ha picloram as the active ingredients.

Calculations from the present experimental values for the most sensitive stage of the most sensitive of the three mangrove species, i.e. young seedlings of the white mangrove, indicate that they are affected by, but recover from, doses of less than 10 ppb picloram (For this comparison only the picloram content of the Agent White is considered and a water depth of 5 cm is assumed.). It can be noted for comparison that Flater et al. (1974) reported the early growth of tomato (*Lycopersicon esculentum*), alfalfa (*Medicago sativa*), alsike clover (*Trifolium hybridum*), and potato (*Solanum tuberosum*) were affected by picloram at 100 ppb, but not by 1 ppb. The white mangrove seedling is thus a sensitive species and growth stage.

The red mangrove experiments with radioactive chloride showed that herbicide toxicity in this species does not result from loss of chloride-exclusion ability of roots: very little chloride was taken up by the herbicide-treated plants.

SPRAY AND LEAF APPLICATIONS

The black mangrove is clearly the most resistant of the Florida mangroves to spray applications of Agent White and the white mangrove most sensitive.

Leaf fall from the red mangrove after herbicide spraying did not follow the pattern of auxin-induced gradual yellowing (senescence) and abscission that has been reported (Hallaway and Osborne, 1969). The leaves that fell were a mixture of green leaves, yellow leaves and brown leaves, which indicated that mechanisms other than auxin-induced ethylene production and ethylene-induced abscission were involved.

Red mangroves have poor regenerative powers compared to black and white mangrove. This is evidenced by cases where Florida mangrove species have been topped, as in the clearing of survey lines, canalside trimming, or roadside mowing along causeways. The observed poor regenerating ability has as its basis the gradual loss of viability of older dormant lateral buds in the red mangrove (Gill and Tomlinson, 1971).

It is well established that auxin-type herbicides translocate and concentrate in growing points and buds (Weed Science Society of America, 1970; Hamill et al., 1972).

The finding in the red mangrove of many under-canopy trees, each with numerous swollen dormant buds following spray application, together with the concentration of carbon-14 labelled picloram in dormant buds, indicates that killing results from herbicide translocation to and concentration in the viable dormant buds. Here, the auxin-herbicide either kills the buds outright or stimulates such tissue proliferation that the dormant (suppressed) buds are overgrown and cannot give rise to shoots and leaves. Gill and Tomlinson (1971) reported that overgrowth by bark (without stem swelling) is the normal fate of these buds within a few years, which appears to be the anatomical basis for the poor coppicing ability of the red mangrove. When the buds of the red mangrove near the terminal leaf whorls have been killed or inactivated, the tree has no buds that can give rise to new leaves or shoots. This is undoubtedly the reason for the observation that some mature red mangrove trees, which had been completely defoliated and showed no leaf regrowth, were still alive by the machete test after several months. This survival of completely or partially defoliated mature red mangrove trees for a period of months indicates that the functioning of root membranes, transpiration and probably translocation are operational and therefore that inhibition of these functions is not the basis of lethality.

The pattern of mangrove kills in Vietnam provides evidence on the nature of herbicidal effects. The tree-kills along spray plane paths in the Saigon River delta often were straight and sharp, even to individual trees. They did not follow the irregular patterns that would be expected if killing were the result of herbicide uptake from the water (Teas, 1972). The South Vietnam mangrove herbicide spray kill patterns thus support a direct herbicidal action on tree canopies.

The Florida spray experiments also provide evidence for the direct action of herbicide on tree canopies. The spraying of the Marco Island Florida herbicide plots was carried out from the minimum height practical above the canopy and provided fairly precise spray patterns along the edges of flight lines. Observations at 49 and 54 weeks showed several cases where mature red mangrove trees near the edges of plots were defoliated except for portions of well-leafed branches that extended outside the plots. Thus, defoliation is specific not only to the tree, but to the individual branch. The existence of such trees further argues against spray killing of red mangroves by root absorption of herbicide.

ASSESSMENT OF HAZARD TO MANGROVES FROM AGRICULTURAL USE OF AGENT WHITE

Hazard to mangroves from herbicidal treatment of agricultural lands might occur from spray drift or from runoff water that reached estuaries.

Experience from aerial applications in Vietnam provides some evidence on spray drift. Spray plane missions were typically flown at 45 m above the canopy (Lang, 1974) whereas normal application under civilian conditions is usually from a much lower altitude. As noted above, the patterns of mangrove killing indicated that spray drift in Vietnam was often minimal. It can be concluded that with appropriate precautions only mangrove areas immediately adjacent to herbicidal spraying would be expected to show damage from the low volatility amine salt form of 2,4-D and picloram.

Herbicide concentration in agricultural runoff water that reached mangroves in estuaries would be determined by several factors, including: herbicide dosage applied, area treated, soil binding and elution, rainfall, and tidal and other dilution.

Recommended application rates for Tordon 101 (Agent White) vary from 1.4-11.3 kg/ha (Dow Chemical Company). Thus the initial herbicide dosages may differ by almost a factor of 10 according to the species of plant being controlled.

A number of workers have reported values for herbicide concentrations in runoff from herbicide treated land. Davis and Ingebo (1973) found concentrations of picloram as high as 350-370 ppb in chaparral runoff water. Bovey et al. (1974) reported that picloram concentrations were high (400-800 ppb) if heavy rainfall occurred immediately after treatment but low ($<$ 5 ppb) if no major storms occurred until one month or more after treatment. Norris (1969) treated small fractions of watersheds and found that in some cases little or no 2,4-D or picloram could be detected after even heavy rainstorms. In addition, he noted that early light rains favored infiltration and soil binding of herbicide. Bovey et al. (1974) reported no damage to cotton (*Gossypium hirsutum*) (a plant that is very sensitive to picloram) in fields adjacent to and below several herbicide treated watersheds, from either spray drift or from runoff water.

Herbicide eluted from small treated areas by rainfall apparently can be absorbed by untreated soil that it flows over. Rainfall amount and timing are important in determining herbicide concentration in runoff water, since light rain can bring about deeper soil penetration and therefore slower subsequent elution. Apparently heavy rainfall is more effective in eluting herbicide from soil.

If agricultural runoff water were to flow over extensive areas of untreated soil before reaching the mangroves, its concentration of herbicide would be reduced. If, in addition to herbicide loss by soil binding, the runoff water were diluted with other waters before reaching the estuary, very low concentrations of herbicide would be expected. Still further reductions by soil binding within the estuary and by tidal dilution would be expected.

If the herbicide applied to mangroves in plastic pans, pools and buckets is calculated as concentration on a volume basis, the tolerance doses of herbicide in water are: white mangrove seedlings 20 ppb, for red seedlings 10,600 ppb, and for black seedlings 10,600 ppb; for mature mangroves the respective values are: 1,000, 10,000, and 106,000 ppb.

No effect doses for young seedlings are: 20 ppb for all three species; for mature mangroves they are: 200, 1,000 and 5,400 ppb.

Data cited above show that picloram concentrations in immediate plot runoff can vary from <5 ppb to 800 ppb as a function of soil binding and rainfall elution. If high dosages of Agent White were applied to large areas of agricultural lands near mangroves, and there was heavy rain soon after application, it is possible that no effect or tolerance doses for one or more species of mangroves would be exceeded. However, because of the attenuating factors of soil absorption and dilution, and the typical separation of mangroves and agricultural land, it is not likely that such values would be found in estuaries. In order to assure that there is no hazard to mangroves, special attention should be given to herbicide applications near estuaries.

SECTION VII

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SECTION VIII

ABBREVIATIONS

a -- acre

cm -- centimeters

gal-- gallon

ha -- hectare (2.47 acres)

kg -- kilogram (2.2 pounds)

lbs-- pounds

ppb-- parts per billion

ppm-- parts per million

ppt-- parts per thousand (seawater is approximately 35 ppt salinity)

SECTION IX

GLOSSARY OF TERMS

Agent White -- a formulation of 2,4-D and picloram, as triisopropanolamine salts, used as a defoliant in Vietnam

coppicing ability -- capability of a plant to regrow after having been trimmed as a hedge

defoliant -- a substance or formulation that causes plant leaves to fall

herbicide -- a substance or formulation that kills plants

lethal dose -- minimum dosage for killing majority of plants

military application dose (for Agent White) -- 8.5 kg/ha (3 gal/a)

no effects dose -- maximum dosage at which no lethality, leaf symptoms, bud killing or other abnormal development were noted

tolerance dose -- maximum dosage from which 80% of plants recover, or do not die

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15. SUPPLEMENTARY NOTES

16. ABSTRACT The amine salts of 2,4-D and picloram were applied to the Florida species of mangroves: red mangrove (Rhizophora mangle), white mangrove (Laguncularia racemosa) and black mangrove (Avicennia germinans). Treatments were to soil or water, by aerial spray and to single leaves as droplets. The effects on radiochloride uptake and on localization of radiocarbon-labelled picloram after leaf application were studied in red mangrove. "Lethal doses" for young seedlings were 2.7 kg/ha for white mangrove, 13 kg/ha for red and 13 kg/ha for black; for mature plants they were 2.7, 13 and > 53 kg/ha respectively. "Tolerance doses" for young seedlings were 0.01, 5.3 and 5.3 kg/ha; for mature plants they were 0.5, 5.3 and 53 kg/ha. "No effect doses" for seedlings were < 0.01 kg/ha for all species; for mature plants they were < 0.1, 0.5 and 2.7 kg/ha. Spray applications of 6.3 - 12.2 kg/ha of commercial mixture to the canopy of a mixed-species forest caused partial defoliation within three weeks. Within 16 months it killed all of the white, 78 - 100% of the mature red, but none of the mature black mangroves. Radiocarbon-labelled picloram concentrated in dormant buds of red mangrove and it is concluded that the tree is killed by the mixture because of its effects on them.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Defoliants, Herbicides, Spraying, Chlorides, Transpiration, Ethylene, Chlorophylls	Florida, Mangroves, Agent White, Translocation	6C 6F

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