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J. Aquat. Plant Manage. 27: 15-20

Integration of *Myrothecium roridum* and 2,4-D in Waterhyacinth Management

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ABSTRACT

Sub-lethal doses of the commonly used herbicide 2,4-D had no adverse effect on *in vitro* mycelial growth and *in planta* damage by *Myrothecium roridum* on waterhyacinth (*Eichhornia crassipes*). Efficacy of *M. roridum* as a biocontrol agent of waterhyacinth was augmented when used sequentially with low rates (5-10% recommended rate) of 2,4-D both under greenhouse and field conditions.

Key words: Sri Lanka, weed control, biocontrol, herbicide, pathogen.

INTRODUCTION

Success of biocontrol in one region of the world stimulates its adoption in other regions. On this basis the choice of a fungal pathogen for biocontrol of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms) in Sri Lanka would have been *Cercospora rodmanii*, the efficacy of which has been well established (1, 2, 3, 4). This fungus has not been encountered as a pathogen of waterhyacinth in Sri Lanka (5, 7). Considering local pathogens of waterhyacinth the choice fell on *Myrothecium roridum* which was found to be highly pathogenic to waterhyacinth and well distributed in Sri Lanka. However, lack of host specificity excluded it from being tried as a biocontrol agent in other countries (9, 10, 11).

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When growth of waterhyacinth was impaired, pathogens could invade and kill the plant faster (2). Such impairment of growth and weakening of waterhyacinth plants were observed on using 6.4% of the recommended rate of 2,4-D (2,4-dichlorophenoxy acetic acid) for waterhyacinth control (1). This paper reports on the potential for integration of *M. roridum* and sub-lethal doses of 2,4-D on the management of waterhyacinth.

MATERIALS AND METHODS

Myrothecium roridum Tode ex Fr. isolated from waterhyacinth leaf spots and maintained on Czapek-dox agar was used in all experiments.

The compatibility of *M. roridum* and 2,4-D (2,4-dichlorophenoxy acetic acid) was determined *in vitro* by measuring the radial growth of the fungus, inoculated as a 5mm diameter PDA disc, on potato dextrose agar (PDA) plates containing 20,10,5 and 3% of the recommended rate of 2,4-D for waterhyacinth control; the recommended rate being 2kg a.i. 1500 l^{-1} ha^{-1} (6).

In planta estimations were done by inoculating 2,4-D sprayed leaves with 5mm diameter discs containing the fungus (5) and measuring the length and width of lesions that developed.

Damage to waterhyacinth from the combined action of *M. roridum* and 2,4-D was assessed in greenhouse experiments using dry weight of plants as the parameter. Water was allowed to drain for 15 min. from small waterhyacinth plants with 5 leaves, which were then weighed and placed at the rate of 4 in each of the 20 plastic buckets (45cm height and 40cm diameter) containing tap water supplemented fortnightly with 0.5gl^{-1} Hyponex (J. L. Morison Son & Jones (Ceylon) Ltd, Sri Lanka). The plants were allowed to stabilize for 24h before treatments, which consisted of spraying with: (a) sterile distilled water (SDW), (b) a mycelial and spore suspension, (c) 2,4-D at 5% and 15% of the recommended rate, (d) 2,4-D followed immediately by the mycelial and spore suspension, (e) 2,4-D followed 3 days later by the mycelial and spore suspension.

In some experiments the sequence of application of 2,4-D and the mycelial and spore suspension in treatments d and e were reversed.

Preliminary field trials were conducted at Bellanwila in a waterway adjoining an abandoned paddy field infested with waterhyacinth. The experimental design consisted of randomized 2x2m areas demarcated by bamboo frames along the water edge, each filled with 2kg (fresh weight) of young waterhyacinth plants. Treatments in quadruplicate consisted of a, d and e as above. All treatments were repeated, twice, 30 days apart. The plants were harvested 18 days after the second treatment and dry weights determined.

The most effective treatment from the preliminary field trials was tested further in two larger field trials in a stream infested with waterhyacinth at Kelaniya. Treatment areas were 10x5m and 21x9m with equal areas demarcated as controls.

Mycelial and spore suspensions for spraying were prepared from 7-day-old potato dextrose broth cultures of the fungus. In greenhouse and preliminary field trials an atomiser was used for spraying and each leaf received 0.5ml of the mycelial and spore suspension containing 1.5-2.0x10^3 spores ml^{-1} (greenhouse), 5.0-6.5x10^4 spores ml^{-1} (field) and 0.5ml of 2,4-D at 5% and 10% the recommended rate.

In larger field trials, mycelial and spore suspension containing 6.0-7.0x10^5 spores ml^{-1} and 2,4-D at 10% the recommended rate was applied with a Knapsack sprayer. Treatments were repeated at intervals of 30-45 days depending on the condition of plants and weather.

Dry weight data obtained were subjected to analysis of variance (ANOVA) according to 1 and/or 2-way classification (8) and significant data were further subjected to Scheffe's test (12) to evaluate significance of treatment combinations.

RESULTS

There was a reduction in the radial growth of *M. roridum in vitro* (Table 1) at the highest sub-lethal concentration of 2,4-D tested; such effects were not apparent at lower concentrations. *In planta* damage (based on the lesion size, particularly lesion width) do not show such reductions at the highest sub-lethal concentration of 2,4-D tested (Table 1). Wider lesions produced points towards a possible stimulatory effect of 2,4-D at the sub-lethal concentrations tested on the growth and lesion induction by *M. roridum* on waterhyacinth leaves.

In 4 greenhouse experiments (Table 2) the combined treatments with 2,4-D and *M. roridum* (treatments d and e) were more effective in reducing growth of waterhyacinth than individual treatments with either 2,4-D (treatment c) or *M. roridum* (treatment b). In an analysis of variance based on a 2-way classification (Table 2), treatment combinations a b c d e and a b c e were significantly ($P = 0.05$) different from one another in all trials whereas treatment combinations a b c d were not significantly ($P = 0.01$) different in Trials 1 and 3. Integration of the fungus and 2,4-D (as in treatment d or e) was more effective than when used individually (as in treatment b or c). Further analysis by Scheffe's method (Table 3) confirmed the significance of treatments d and e, particularly treatment e over other treatments, and the absence of any significant difference between treatments d and e.

TABLE 1. EFFECT OF 2,4-D[1] ON THE RADIAL GROWTH OF *MYROTHECIUM RORIDUM*[2] ON PDA[3] PLATES AND THE DEVELOPMENT OF LESIONS ON WATERHYACINTH LEAVES.

2,4-D concentration as a % of the recommended rate of 2kg a.i. 1500 $l ha^{-1}$	Mean colony diameter after 9 days in cm ± SE	Leaf lesions after 5 days	
		length in cm ± SE	width in cm ± SE
0	2.5 ± 0.1	2.7 ± 0.5	1.0 ± 0.1
3.0	2.7 ± 0.0	2.2 ± 0.8	0.9 ± 0.1
5.0	2.8 ± 0.1	2.3 ± 0.3	1.2 ± 0.2
10.0	2.4 ± 0.4	2.3 ± 0.5	1.1 ± 0.2
20.0	2.0 ± 0.2	2.4 ± 0.5	1.4 ± 0.3

[1]Sprayed with an atomiser at the rate of 0.5ml $leaf^{-1}$
[2]5mm diameter PDA disc inoculum
[3]Potato dextrose agar

TABLE 2. DRY WEIGHT OF WATERHYACINTH PLANTS FROM 4 GREENHOUSE EXPERIMENTS HARVESTED 20 DAYS AFTER TREATMENT WITH 2,4-D AND *M. RORIDUM* AND ANOVA DATA.

Trial number	Start date	Harvest date	2,4-D concentration as a % of the recommended rate of 2kg a.i. 1500l ha^{-1}	Treatment[1]	Mean dry wt. in g ± SE	ANOVA data[3] Treatment combinations	F ratios: Treatments	F ratios: Replicates
1	Nov 28	Dec 28	10.0	a	15.5 ± 4.1	abcde	4.6*	2.4
				b	16.0 ± 3.8			
				c	10.5 ± 3.9	abcd	3.0	2.3
				d	11.5 ± 2.6			
				e	4.9 ± 3.6	abce	4.8*	2.2
2	Mar 01	Mar 28	10.0	a	32.0 ± 4.1	abcde	23.0**	1.7
				b	27.0 ± 9.9			
				c	28.0 ± 0.8	abcd	10.4**	1.6
				d	11.4 ± 5.2	abce	21.2**	0.9
				e	4.0 ± 1.2			
3	Mar 28	Apr 17	5.0	a	51.2 ± 18.3	abcde	17.3**	4.3*
				b	43.7 ± 11.8			
				c	38.2 ± 9.8	abcd	5.3*	5.5*
				d	18.6 ± 8.5	abce	21.7**	5.2*
				e	6.6 ± 3.6			
4[2]	Jun 06	Jun 16	5.0	a	27.9 ± 4.7	abcde	43.6**	1.9
				b	20.3 ± 3.8			
				c	16.1 ± 2.9	abcd	26.3**	2.4
				d	10.4 ± 0.7	abce	48.5**	2.4
				e	4.3 ± 1.1			

* = significant at P = 0.05, ** = significant at P = 0.01

[1]a = control, b = mycelial and spore suspension, c = 2,4-D, d = 2,4-D followed immediately by mycelial and spore suspension, e = 2,4-D followed 3 days later by mycelial and spore suspension

2,4-D sprayed at the rate of 0.5ml leaf^{-1} at 5% and 10% the recommended rate

Mycelial and spore suspension—sprayed at the rate of 0.5ml leaf^{-1} from a suspension containing 1.5-2.0x10^3 spore ml^{-1}

[2]For treatments d and e the sequence of application of 2,4-D and the mycelial and spore suspension reversed

[3]According to 2-way classification (8)

In the two field trials at Bellanwila, treatments d and e were both effective under field conditions (Table 4). The number and virility of plants in plots subjected to above treatments had decreased considerably, the decrease being more evident in plots subjected to treatment e (Figure 1A). The leaves exhibited slight wilting and had dark spots or typical *M. roridum* tear-drop shaped streaks. The mean height of randomly selected plants from plots subjected to treatment e was 19.0 ± 1.1cm as compared to control plants which were 40.0 ± 2.3cm. This is also reflected in dry weight of waterhyacinth plants subjected to treatments a and e (Table 4) at both concentrations of 2,4-D used. In spite of heavy rain during the 6th trial using 10% of the recommended rate of 2,4-D, treatment e was more effective than treatment d. This was not so in the 5th trial where only 5% of the recommended rate of 2,4-D was used (Table 4). Considering these data, treatment e with 10% recommended rate of 2,4-D was selected for further testing on large scale field trials.

The two field trials at Kelaniya, indicated the effectiveness of treatment e, and the treatment regime adopted (Table 5). In both trials stunting accompanied by browning and disintegration of treated plants become evident about 2 months after the first spraying (Figure 1C). Areas free of waterhyacinth became visible 2-3 months after the first treatment in both field trials (Figures 1B & D), but were more prominent in trial No. 8 where the plants were free floating. However, the relative stability of disease levels after initial infestation as shown by the parameters estimated (Table 5) indicate absence of recovery over the period of evaluation.

TABLE 3. DRY WEIGHT DATA FROM TABLE 2 SUBJECTED TO SCHEFFE'S TEST[1] FOR SIGNIFICANCE OF INDIVIDUAL TREATMENT COMBINATIONS.

Trial number	Concentration of 2,4-D as a % of the recommended rate (2kg a.i. 15001 ha^{-1})	Treatment combinations[2] ab	ac	ad	ae	bc	bd	be	cd	ce	ed
1	10.0	ns	ns	ns	*	ns	ns	*	ns	ns	ns
2	10.0	ns	ns	*	*	ns	*	*	*	*	ns
3	5.0	ns	ns	*	*	ns	ns	ns	*	*	ns
4	5.0	ns	ns	*	*	ns	*	*	ns	*	ns

[1]Reference 12

[2]ns = not significant, *significant at P = 0.05

DISCUSSION

M. roridum has not been considered earlier as a potential biocontrol agent due to lack of host specificity (9, 10, 11). However, its high virulence and wide local distribution (5, 7) heavily influenced its selection for the present work, rather than introducing well-tested species like *Cercospora rodmanii* which has not been recorded from Sri Lanka.

2,4-D, an inexpensive biodegradable herbicide, has been effectively used in the chemical control of waterhyacinth (6). 2,4-D concentrations lower than 10% the recommended rate for waterhyacinth control has no signifi-

TABLE 4. DRY WEIGHT OF WATERHYACINTH PLANTS FROM 2 FIELD TRIALS CONDUCTED DURING 1986 AT BELLANWILA AFTER TREATMENT WITH 2,4-D AND *M. RORIDUM* AND ANOVA DATA.

Trial number	1st spray	2nd spray	Harvest date	2,4-D concentration as a % of the recommended rate of 2kg a.i. 1500l ha^{-1}	Treatment[1]	Dry wt in kg (mean of 4 replicates ± SE)	ANOVA data[2] Treatment combination	F ratios: Treatments 1 way	Treatments 2 way	Replicates
5	Jan 17	Feb 18	Mar 07	5.0	a	1.1 ± 0.5	ade	10.5**	10.8*	1.7^{ns}
					d	0.3 ± 0.1	ad		11.3*	2.7^{ns}
					e	0.3 ± 0.1	ae		13.5*	0.7^{ns}
6	Apr 08	May 10	May 27	10.0	a	1.0 ± 0.2	ade	40.7**	43.7**	1.2^{ns}
					d	0.5 ± 0.2	ad		20.4*	1.2^{ns}
					e	0.2 ± 0.0	ae		31.6**	1.0^{ns}

ns = not significant, * = significant at P = 0.05, ** = significant at P = 0.01

[1]a = control, d = 2,4-D followed immediately by mycelial and spore suspension, e = 2,4-D followed 3 days later by mycelial and spore suspension
2,4-D—sprayed at the rate of 0.5ml leaf^{-1} at 5% and 10% the recommended rate
Mycelial and spore suspension—sprayed at the rate of 0.5ml leaf^{-1} from a suspension containing 5.0-6.5x10^4 spores ml^{-1}

[2]According to 2-way classification (8)

cant effect on *in vitro* growth of *M. roridum* on PDA (Table 1). Similar observations were reported on *in vitro* compatibility of *C. rodmanii* with 2,4-D (1). Increased *in planta* damage as suggested by the development of larger leaf spots under greenhouse conditions (Table 1) might imply a weakening of host tissue and an increase in host susceptibility to *M. roridum* when exposed to 2,4-D.

In greenhouse experiments dry weight reduction by treatment c (2,4-D alone), although not sufficient for achieving control by itself, caused a substantial reduction in growth rate of waterhyacinth. Such reductions with low concentrations of herbicides have already been reported (2), and this type of growth reduction may enable the pathogen to invade and destroy the host tissues at a rate faster than the rate of production of new tissues. Hence, weakening of the plant can change the host-pathogen balance in the disease complex to one more favorable towards the pathogen, or, in other words, lower the host resistance. This is further confirmed by the higher efficacy of treatment e in the greenhouse trials where the application of 2,4-D and *M. roridum* was sequential in comparison with treatment d where the application was simultaneous. However, the sequence of application of 2,4-D and *M. roridum* do not show any significant change in the extent and pattern of control achieved in greenhouse trials.

Level of control achieved in field trials compared favor-

TABLE 5. EFFECT OF SEQUENTIAL TREATMENT OF WATERHYACINTH WITH 2,4-D AND *M. RORIDUM* IN TWO FIELD TRIALS CONDUCTED IN 1986 AT KELANIYA.

Trial number	1st spray	2nd spray	3rd spray	Sampling date	Number of days from 1st spray	Treatment[1]	Fresh wt in g	Dry wt in g ± SE	Height in cm ± SE
7[2]	Aug 12	Sep 12	Oct 22	Oct 16	65	Control	783	58 ± 12	94 ± 11
						Sprayed	338	24 ± 5	67 ± 19
						% of control	43	42	71
				Oct 30	79	Control	1138	66 ± 5	103 ± 11
						Sprayed	406	21 ± 8	51 ± 10
						% of control	36	31	50
				Nov 18	98	Control	850	53 ± 9	108 ± 15
						Sprayed	263	31 ± 11	56 ± 5
						% of control	31	58	52
				Nov 28	108	Control	1143	112 ± 17	112 ± 5
						Sprayed	513	50 ± 10	57 ± 15
						% of control	41	45	51
8[3]	Aug 22	Oct 06	—	Oct 21	60	Control	331	16 ± 5	75 ± 5
						Sprayed	125	9 ± 6	43 ± 5
						% of control	38	60	58
				Oct 30	69	Control	350	15 ± 3	75 ± 7
						Sprayed	83	7 ± 3	37 ± 3
						% of control	24	48	50

[1]treatment e—2,4-D followed 3 days later by mycelial and spore suspension

[2]muddy substrate with rooted sturdy plants

[3]free-floating less sturdy plants. Trial abandoned in early November due to disturbance of the site.

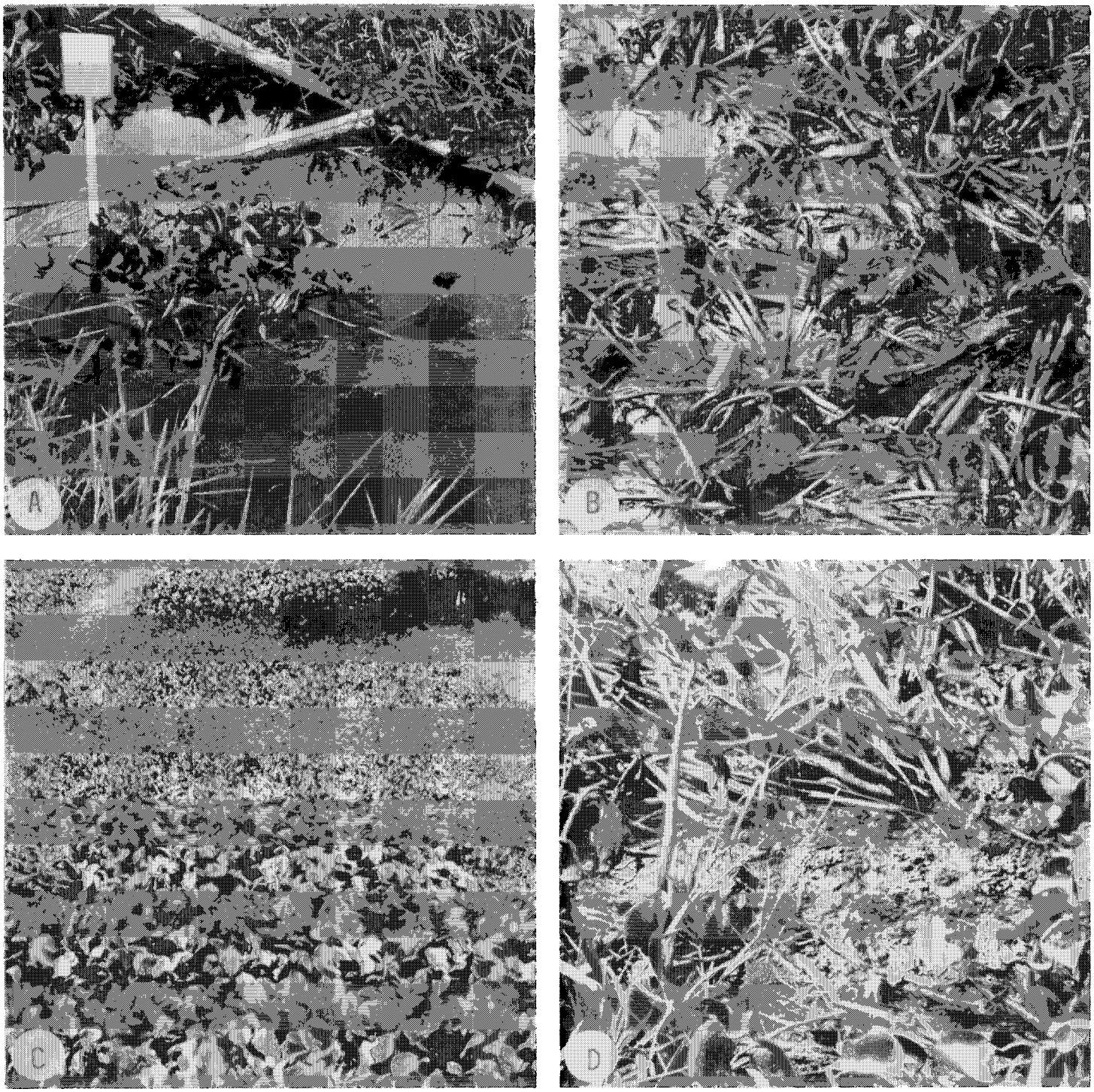

Figure 1. Field plots of waterhyacinth after spraying with 2,4-D and *Myrothecium roridum*. A = floating plants at Bellanwila 50 days after subjecting to treatment e (2,4-D followed 3 days later by the mycelial and spore suspension; B = floating plants from Trial No. 8 at Kelaniya 60 days after initial treatment e; C & D = rooted plants from Trial No. 7 at Kelaniya 98 days after initial treatment. Note stunting, browning and disintegtration of treated plants.

ably with that of greenhouse trials; but repeated applications were necessary to maintain consistently low levels of waterhyacinth infestation. The efficacy of integrated treatment was evidenced by reductions in dry weight (compared to controls) and accompaying reduction in plant height. Similar reductions in plant height have also been recorded in waterhyacinth plants treated with *C. rodmanii* and herbicides (2) and *C. rodmanii* and arthropods (1).

Successful biological or integrated biological control would not lead to complete eradication but to a reduction and maintenance of waterhyacinth population in dynamic equilibrium at an acceptable low level. Although our field

trial data show reduction of waterhyacinth stand by over 50% from control levels, the treatment regime necessary for maintenance of such levels over a long period is yet to be worked out. This can be expected to vary according to the type of waterhyacinth plant as evidenced by the higher effectiveness of the treatment regime on free floating plants when compared to rooted plants.

ACKNOWLEDGEMENTS

This work forms a part of an M. Phil project of University of Kelaniya (NPL) and was carried out under a research grant (RG/83/71) awarded by NARESA, Sir Lanka. The authors thank M/s Hayles Ltd, Sri Lanka for provoding the herbicide , and Mr. S. C. Kodagoda for technical assistance and photographs.

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J. Aquat. Plant Manage. 27: 20-23

Control of Common Cattail with Postemergence Herbicides[1]

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ABSTRACT

Field studies were conducted to determine the effect of herbicide rate, spray volume, and plant growth stage on the control of common cattail (*Typha latifolia* L.) with glyphosate. Control was best when the 3.3 kg ae/ha was applied to mature cattail about 1 week before the first autumn frost (96% stand reduction). Control of common cattail was equal when glyphosate at 3.3 kg/ha was applied in 280, 560, or 1120 L/ha (30, 60, 120 gpa) water plus 0.5% v/v surfactant. Treatments applied when common cattail was in mid to full bloom (early July) were much less effective than when applied later in the season (August-September). A single application of dalapon at 22 kg ae/ha was much less effective than glyphosate at 3.3 kg/ha, whereas control with amitrole plus ammonium thiocyanate at 8.8 + 7.3 kg ai/ha (92% stand reduction) was similar to glyphosate. The year following treatment, redtop (*Agrostis alba* L.) and horsetail rush (*Equisetum* spp.) dominated many plots that had been treated with amitrole plus ammonium thiocyanate and glyphosate, respectively.

Key words: Typha latifolia, glyphosate, dalapon, amitrole, plant-succession.

INTRODUCTION

Common cattail grows throughout most of the United States and northward to Alaska in drainage ditches, sluggish irrigation canals, shallow bays and marshes, and in the margins of ponds, lakes, and streams (6). Common cattail is the principal emersed species that interferes with the flow of water in irrigation canals and drainage ditches in the west (16). It is a nuisance in waterfowl management areas in many parts of the country, because stands of cattail encroach upon shallow water marshes and eliminate plants that provide food and cover for wildlife (1).

One goal of irrigation district personnel is to control all or most of the cattail in drainage and delivery channels to facilitate water movement, whereas wildlife managers desire to manage cattail populations at specific ratios to open

[1]Received for publication September 19, 1988, and in revised form January 23, 1989. Contribution of Agric. Res. Serv., U.S. Dep. Agric., in cooperation with the Washington State University Coll. of Agric. and Home Economics Res. Ctr., Agronomy and Soils Dept. No. 8802-17.

[2]Res. Agron. and Biol. Res. Tech., respectively, Agric. Res. Serv., U.S. Dep. Agric., Irrigated Agric. Res. and Ext. Ctr., Prosser, WA 99350.