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RESTORING NATIVE VEGETATION IN A EURASIAN WATER MILFOIL-DOMINATED PLANT COMMUNITY USING THE HERBICIDE TRICLOPYR*

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ABSTRACT

In an effort to evaluate the selective control of the exotic weed Eurasian water milfoil (*Myriophyllum spicatum* L.) and to assess the recovery and restoration of the native submersed plant community, a 6-ha river and 4-ha cove plot were treated with the herbicide triclopyr at application rates of 2·5 and 1·75 mg/l, respectively, in the Pend Oreille River, WA, in August 1991. Water exchange half-lives within the plots were measured using rhodamine WT dye (river, $t_{1/2} = 20$ h; cove, $t_{1/2} = 52$ h), and triclopyr dissipation rates were also calculated (river, $t_{1/2} = 19$ h; cove, $t_{1/2} = 53$ h). Triclopyr concentrations were below the proposed potable water tolerance level (0·5 mg/l) within the river treatment plot by 3 days after treatment (< 0·01 to 0·41 mg/l), and 675 m downstream of that plot by 1 day after treatment (< 0·01 to 0·47 mg/l). Following the cove treatment, triclopyr residues ranged from 0·12 to 0·29 mg/l by 7 days after treatment, and from < 0·01 to 0·06 mg/l as close as 150 m downstream from the plot.

Eurasian water milfoil biomass was reduced by 99% in the treated plots at 4 weeks post-treatment, remained low one year later (river treatment, 28% of pretreat levels; cove treatment 1% of pre-treat levels) and was still at acceptable levels of control at two years post-treatment (river treatment, 47% of pre-treat levels; cove treatment, 24% of pre-treat levels). The four-week post-treatment efficacy results verified triclopyr concentration/exposure time relationships for controlling Eurasian water milfoil developed under laboratory conditions. Non-target native plant biomass increased 500–1000% by one year post-treatment, and remained significantly higher in the cove plot at two years after treatment. Native species diversity doubled following herbicide treatment, and the restoration of this robust community delayed the re-establishment and dominance of Eurasian water milfoil for three growing seasons. ©1997 by John Wiley & Sons, Ltd.

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KEY WORDS: aquatic plant control; aquatic weeds; Garlon® 3A; *Myriophyllum spicatum*; pesticide dissipation; rhodamine WT

INTRODUCTION

The submersed plant Eurasian water milfoil (*Myriophyllum spicatum* L.), hereafter called milfoil, has spread throughout many rivers and reservoirs since its introduction into the United States prior to the 1940s (Reed, 1977; Couch and Nelson, 1985). Once established, growth and physiological characteristics of milfoil enable it to form a surface canopy and develop into immense stands of weedy vegetation, outcompeting most submersed species and displacing the native plant community (Grace and Wetzel, 1978; Aiken *et al.*, 1979; Madsen *et al.*, 1988, 1991a; Smith and Barko, 1990). These surface mats can severely impair many of the functional aspects of regulated rivers such as maintenance of water quality for wildlife habitat and public health, water storage capacity, navigation and recreation (Hansen *et al.*, 1983; Newroth, 1985; Ross and Lembi, 1985; Nichols and

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Shaw, 1986). Furthermore, a milfoil-dominated submersed plant community can greatly reduce the biodiversity of an aquatic system (Smith and Barko, 1990; Madsen *et al.*, 1991b).

To develop methods for controlling the growth and spread of milfoil in public waters, our research group has been evaluating the herbicide triclopyr (3,5,6-trichloro-2-pyridinyl-oxyacetic acid) for restoring aquatic habitats dominated and degraded by this non-indigenous species. Triclopyr is a pyridine-based systemic compound registered since the mid-1970s in the US for control of broadleaf weeds and woody plants on rights-of-way, rangeland, industrial sites and other non-crop areas. Furthermore, in 1995 triclopyr received US registration for controlling weeds in rice grown for food production. Since the chemical has demonstrated potential for selectively controlling several aquatic weeds, including milfoil (Getsinger and Westerdahl, 1984; Langeland, 1986; Green *et al.*, 1989; Wujek, 1990), DowElanco Chemical Company is pursuing an aquatic registration for the triethylamine salt formulation of triclopyr (presently labelled as Garlon® 3A) under an experimental use permit (EUP) issued by the US Environmental Protection Agency (US EPA).

Previous aquatic testing has shown that triclopyr is susceptible to photolytic degradation and has a low toxicity to non-target organisms (Gersich *et al.*, 1984; Mayes *et al.*, 1984; McCall and Gavit, 1986; Dow Chemical Co., 1988; Woodburn *et al.*, 1993a,b). Field dissipation studies have indicated that triclopyr accumulation in sediment, shellfish and fish is negligible (Getsinger and Westerdahl, 1984; Woodburn *et al.*, 1993b). Laboratory studies have clearly shown that triclopyr efficacy is dependent upon the concentration and length of time milfoil remains exposed to the herbicide (Netherland and Getsinger, 1992). However, this compound can be subject to rapid dilution and dispersion from treatment areas through gravity flow, tides, thermal- and wind-induced water circulation patterns, etc. (Fox *et al.*, 1991a; Getsinger *et al.*, 1992). Although rapid dissipation may be environmentally desirable, this process can reduce the degree of plant control owing to insufficient herbicide exposure. Therefore, successful triclopyr treatment of milfoil in rivers and reservoirs requires knowledge of herbicide concentration and exposure time requirements for this species, as well as site-specific water exchange characteristics.

The Pend Oreille River, a regulated system located in north-eastern Washington, is a major tributary of the Columbia River and has been infested with milfoil for over a decade (Rawson, 1985, 1987; WATER Environmental Sciences, 1986, 1987). Milfoil control practices in the past have included herbicides such as 2,4-D (2,4-dichlorophenoxy acetic acid) and fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl)phenyl-4(1H)-pyridinone]}, and have been only moderately successful (Durando-Boehm, 1983; WATER Environmental Sciences, 1986, 1987). Recent water exchange studies conducted in selected sites on this river suggested that triclopyr contact times sufficient to provide acceptable levels of milfoil control could be achieved in these areas (Getsinger *et al.*, 1993). Moreover, the presence of a multispecies submersed plant community (albeit dominated by milfoil) provided the opportunity to assess the selective properties of this herbicide under field conditions. A large-scale study was conducted to evaluate triclopyr applications as a technique for restoring native submersed plant communities in a regulated river previously dominated by milfoil. In addition, dissipation rates of triclopyr from treated areas were determined and laboratory-derived triclopyr dosage rate relationships for controlling milfoil were verified.

MATERIALS AND METHODS

Study site and plot description

The study was conducted along a stretch of the Pend Oreille River (48° N, 117° W) between Albeni Falls and Box Canyon dams (Figure 1). River levels in this region are controlled by water inflowing from Albeni Falls Dam on Lake Pend Oreille, Idaho, and outflowing at Box Canyon and Boundary Dams in Washington, and at two dams in British Columbia, Canada. River discharge, measured at the Albeni Falls Dam, averages 565 cm per year, with a maximum of 1500 cm in May or June, and a minimum of 165 cm in January and February, or in August and September.

In mid-August 1991, two milfoil-dominated submersed plant stands were selected for the study. The first was in the main stem of the river approximately 0·5 km upstream from river mile (RM) marker 62, and the second in a protected cove approximately 0·3 km downstream from RM marker 48. In shallow areas of these stands (< 1 m

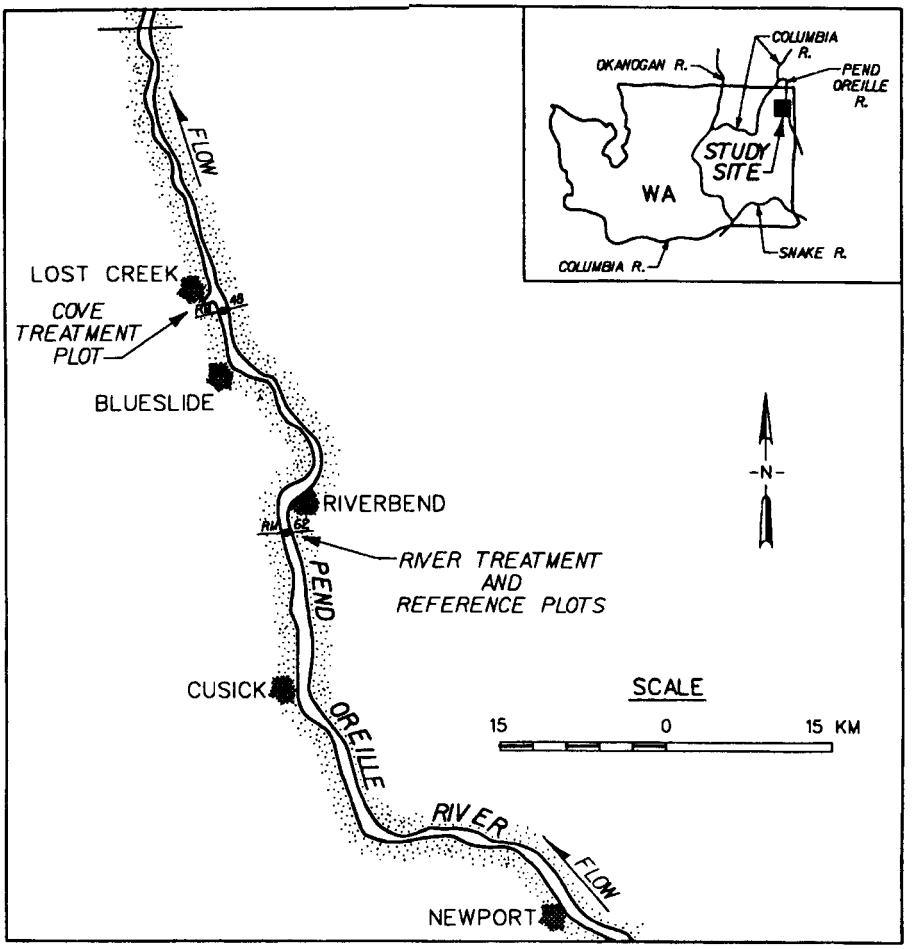

Figure 1. Location of study site for triclopyr herbicide treatment on the Pend Oreille River, WA

deep), entangled shoots of milfoil covered the surface of the water forming a dense mat. In deeper regions of the stands milfoil shoots formed a dense submersed canopy 15–20 cm below the surface of the water. Although milfoil was the dominant species in the plots, an understorey comprising 13 other submersed plants (one exotic and 12 natives) was encountered during the pretreatment evaluation (Table I). The other exotic plant was the monocotyledonous (monocot) species curlyleaf pondweed (*Potamogeton crispus* L.). Principal natives included the monocots elodea (*Elodea canadensis* L.), flatstem pondweed (*Potamogeton zosteriformis* Fernald) and water stargrass (*Heteranthera dubia* (Jacq.) MacM.), and the dicotyledonous (dicot) species coontail (*Ceratophyllum demersum* L.) and white water crowfoot (*Ranunculus longirostris* Godron).

The submersed plant communities selected for the study represented milfoil-dominated stands typical of those targeted for operational herbicide treatments. However, water-exchange characteristics of the two sites were dissimilar ($t_{1/2} < 20$ h in the river and > 50 h in the cove), thus providing the opportunity to compare the efficacy, selectivity and dissipation of triclopyr under different flow, concentration and exposure time conditions.

Two river plots were established in submersed plant stands in the River Bend area near RM 62. A 6-ha river treatment plot was located 250 m downstream from the 2-ha river reference plot (Figure 2). Both plots were situated in a parallel arm of the main river channel, bounded on the west by a narrow island, and bordered on the north, south and east by submersed plant stands or open water. These plots ranged in depth from 0·3 m (west side) to 2·5 m (east side), with a mean depth (±SE) of $1{\cdot}62 \pm 0{\cdot}07$ m ($n = 60$). Six water sampling stations (1–6) were established inside the RT (river treatment) plot representing three flow zones: Stations 1 and 2, upstream zone;

Table 1. Frequency of plant species in study plots in Pend Oreille River, WA (1991–1993), for all transects per plot and year: monocot (M), dicot (D), native (N), exotic (E).

		RR* Plot			RT† Plot			CT‡ Plot		
Species	Year	1991	92	93	91	92	93	91	92	93
coontail (DN)		2	5	10	9	28	28	20	59	61
Ceratophyllum demersum L.										
elodea (MN)		21	9	20	7	50	33	28	93	79
Elodea canadensis L.										
water stargrass (MN)		3	1	8	8	8	18	0	1	3
Heteranthera dubia (Jacq.) MacM.										
northern water milfoil (DN)		0	0	0	7	< 1	0	0	0	0
Myriophyllum sibiricum Komarov										
Eurasian water milfoil (DE)		100	98	95	94	56	78	89	25	59
M. spicatum L.										
whorled water milfoil (DN)		0	0	1	< 1	< 1	5	0	0	0
M. verticillatum L.										
curlyleaf pondweed (ME)		17	27	87	4	27	12	7	15	30
Potamogeton crispus L.										
American pondweed (MN)		8	5	5	< 1	< 1	0	0	0	0
P. nodosus Poiret										
blunt-leaf pondweed (MN)		0	0	< 1	0	39	0	6	7	< 1
P. obtusifolius Mert. & Koch										
sago pondweed (MN)		12	0	8	5	9	7	11	1	2
P. pectinatus L.										
redhead grass (MN)		2	0	< 1	2	6	3	< 1	1	1
P. perfoliatus L.										
whitestem pondweed (MN)		0	0	0	0	0	< 1	0	0	< 1
P. praelongus Wulfen										
small pondweed (MN)		0	0	< 1	0	0	32	0	0	1
P. pusillus L.										
Vasey's pondweed (MN)		0	0	0	10	0	< 1	8	1	0
P. vaseyii Robbins										
flatstem pondweed (MN)		15	11	16	28	64	77	40	36	53
P. zosteriformis Fernald										
white water crowfoot (DN)		5	8	21	12	50	16	3	19	1
Ranunculus longirostris Godron										

* River reference plot
† River treatment plot
‡ Cove treatment plot

Stations 3 and 4, mid stream zone; Stations 5 and 6, downstream zone. One water sampling station was established in the centre of the river reference plot.

A 4-ha cove treatment plot was established in the submersed plant stand in Lost Creek Cove, located on the west shore of the river (Figure 3), approximately 21 km downstream from the river plots. Water depth in this plot ranged from 0·75–2·8 m, with a mean depth of $1{\cdot}72 \pm 0{\cdot}04$ m ($n = 80$). Three water sampling stations were established inside the cove treatment plot, with Station 1 located in the southern half of the plot, Station 2 in the centre of the plot and Station 3 in the northern half of the plot.

In addition, several water sampling stations were established outside and downstream of the two treated plots. The locations of each of these stations were based on the presence and quantity of a fluorescent dye applied concurrently with the herbicide (described below). Downstream stations were used to monitor movement of triclopyr out of the treated plots. This dissipation information can be used to establish any label restrictions for potable water tolerance set-back distances in relation to triclopyr treatment sites and water intake structures. Potable water tolerance set-back distances ranging between 400 m (0·25 mi) and 800 m (0·50 mi) are currently being considered for the triclopyr aquatic label. In the river application, five water sampling stations were

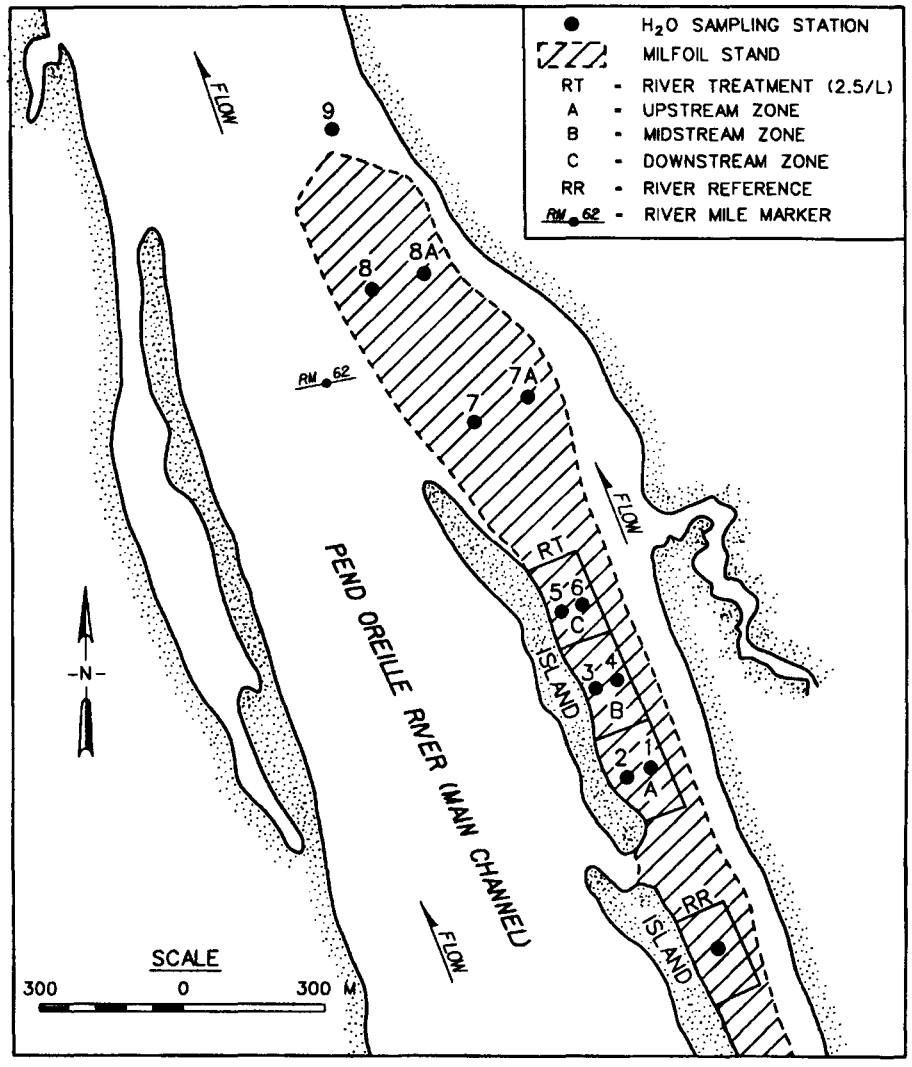

Figure 2. River treatment (RT) and river reference (RR) plots and water sampling stations on the Pend Oreille River, WA

established downstream of the northern edge of the plot (Figure 2): Stations 7 and 7a, 300 m downstream; Stations 8 and 8a, 675 m downstream; Station 9, 975 m downstream. In the cove application, two water sampling stations (4 and 5) were established at 150 m and 395 m, respectively, downstream of the plot (Figure 3).

Chemical applications and sampling regimes

On 21 and 22 August 1991, the river and cove treatment plots, respectively, were treated with a liquid formulation of the herbicide Garlon® 3A [31·8% triclopyr acid equivalent (ae)] using a conventional submersed application technique. The herbicide was injected 30–60 cm below the surface of the water using a pressurized diaphragm pump, fitted with a 208-litre (55 gallon) holding tank and a manifold with six hoses (60 cm length) attached at 30-cm intervals. Tee jet #6 nozzles affixed to the ends of the hoses provided an average nozzle output of 2·3 l/min at a pressure of 206 kPa (30 psi). The manifold was stern-mounted on an airboat, allowing the nozzles to penetrate the water column to a depth of 20–30 cm, and providing a 2·4-m application swath width.

The river treatment plot was treated as four subplots (1·5 ha each), with the application beginning in the downstream subplot (0800 hours) and, once completed, proceeding upstream until the entire 6-ha plot was treated

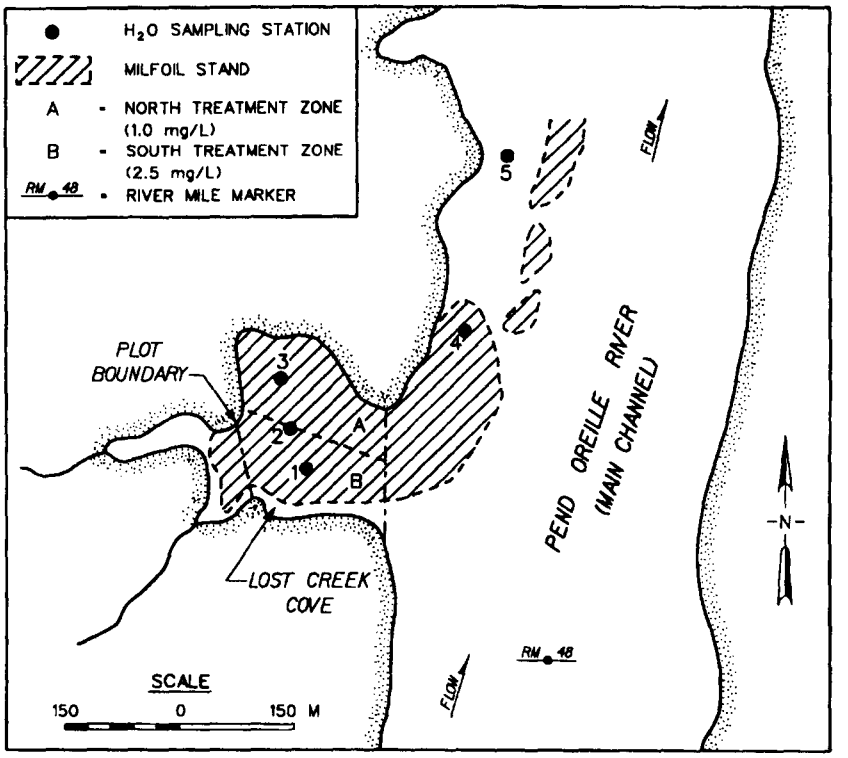

Figure 3. Cove treatment (CT) plot and water sampling stations on the Pend Oreille River, WA

(1130 hours). This subsurface application technique provided a nominal concentration of 2·5 mg/l triclopyr in the plot (the maximum EUP label concentration). At the time of treatment, skies were clear, water column temperature was essentially isothermal (25°C), and wind velocity was < 2 km/h from the east.

The cove treatment plot was treated as two subplots of 2 ha each, with the northern subplot treated first (0950–1020 hours), at a nominal triclopyr application rate of 1·0 mg/l, and the southern subplot receiving a nominal triclopyr application rate of 2·5 mg/l at 1035–1135 hours. The nominal triclopyr application rate for the entire plot was 1·75 mg/l. At treatment time, skies were partly cloudy, water column temperature was isothermal (24°C), and wind was south-east at approximately 10 km/h.

While treating each subplot, the airboat travelled at 5 km/h in an alternating east–west pattern that provided an even areal distribution of the herbicide throughout the plots. Application rates selected for both plots were based on results of previous water exchange studies in those sites, and on laboratory-derived triclopyr concentration and exposure time requirements (Netherland and Getsinger, 1992; Getsinger *et al.* 1993).

The inert fluorescent dye, rhodamine WT, was used to characterize water exchange and movement during the study, and to aid in the selection of water sampling stations outside the treated areas. This dye (US EPA approved for use in potable water at concentrations up to 100 μg/l) can be quantified *in situ* and is routinely used for water tracing and exchange studies (Johnson, 1984; Kilpatrick and Wilson, 1989). The dye has also been used to successfully simulate aqueous dissipation of several herbicides, including triclopyr, used for aquatic plant control (Fox *et al.*, 1991b, 1992, 1993; Turner *et al.*, 1994).

Rhodamine WT was applied immediately following the triclopyr treatment in the RT plot using identical application techniques to achieve a nominal aqueous concentration of 10 μg/l. In the cove treatment plot, the dye was tank mixed with the herbicide to achieve a nominal concentration of 4 and 10 μg/l in the north and south portions of the plot, respectively. The different initial dye concentrations in the cove treatment plot reflected the initial triclopyr application rates, and ensured that the empirical relationship between triclopyr and dye quantities would remain consistent throughout the plot. Dye concentrations were measured at 25-cm depth intervals at each

sampling station using Turner Designs Model 10-005 field fluorometers equipped with high-volume continuous flow cuvette systems. Water was circulated through the fluorometers with submersible pumps attached to the end of weighted opaque hoses. All dye values were temperature corrected according to Smart and Laidlaw (1977) using Cole–Parmer thermistors attached to the exhaust hoses of the fluorometers.

Water samples were collected for triclopyr residues concurrently with dye measurements, using fluorometers and pump systems described above, from each station inside the plots at one-third total depth below the surface (upper sample) and one-third total depth above the bottom (lower sample). Water was collected at a depth of 1 m at the river treatment plot downstream stations, and at 0·5 and 0·75 m at the CT plot downstream stations. Water was pumped into 500-ml amber polyethylene bottles, stored on ice in the field, and frozen when returned to the field station, within 6 h. Dye levels were recorded and triclopyr water samples were collected from all river treatment plot stations at pretreatment, 1, 5, 8 and 12 hours after treatment, and at 1, 2, 3 and 7 days after treatment. Dye levels were recorded and triclopyr water samples were collected from all cove treatment plot stations at pretreatment, 1.5 and 8 hours after treatment, and at 1, 2, 3 and 7 days after treatment. Additional triclopyr water samples were collected from all stations at 14 and 21 days after treatment. In the untreated upstream river reference plot, triclopyr water samples were collected at mid-depth at pretreatment, and 8 and 24 hours after treatment. Dye measurements were recorded on the downstream edge and at selected locations in the river reference plot from 1 hour after treatment to 7 days after treatment.

Water samples were analysed for triclopyr residues (detection limit $< 0{\cdot}01$ mg/l) using a high performance liquid chromatography method (DOW Chemical Co., Midland, MI) by the Tennessee Valley Authority Water Chemistry Laboratory, Chattanooga, TN. Mean percentage recovery of all triclopyr-spiked samples ($n = 38$) was $98{\cdot}12 \pm 0{\cdot}69$ SE.

Dye and triclopyr data were subjected to statistical analysis to obtain dissipation curves using Statgraphics 3·0 (Statistical Graphics Corp). Mean dye and triclopyr values were regressed against time using the exponential model:

$$y = \exp(a + bt),$$

where:

y = chemical concentration at time t, a = intercept of regression line, b = slope of regression line (dilution factor).

Dissipation half-lives were then calculated according to:

$$t_{1/2} = \frac{\text{natural logarithm of } 0{\cdot}5}{\text{slope of regression line}}$$

River discharge and flow rates

River discharge, as measured from the Albeni Falls Dam, ranged from 360 to 405 cm on the triclopyr application dates. River discharge slowly declined to a level of 245 cm by 4 days after treatment, and stabilized to a level of 170 cm by 7 days after treatment. Flow rates were measured using a Montedora–Whitney electronic flow meter in the open channel adjacent to the plant stands, and ranged from 2 to 3 cm/s. Flow rates were generally below the detection limits of the meter ($< 0{\cdot}1$ cm/s) 1–2 m inside the plant stands·

Plant biomass and diversity

At each plot, four 100-m long transects were established at equally spaced intervals (40 m, river reference plot; 75 m, cove treatment plot; 120 m, river treatment plot) in an east to west direction to quantify the amount of submersed vegetation. At each transect, three biomass samples were collected by a scuba diver from stratified random locations using a $0{\cdot}1\ m^2$ quadrat (Madsen, 1993), for a total of 12 biomass samples per plot. Samples were sorted to species, separated into roots and shoots, and dried at 50°C. Biomass samples were collected pretreatment (18–20 August, 1991) and 4 weeks (18–20 September, 1991), 1 year (10–14 August, 1992), and 2 years (16–20 August, 1993) after treatment. Biomass levels between years at given plots were compared statistically using a one-way analysis of variance (ANOVA), with significant differences between means calculated using a Bonferroni test at the $p = 0{\cdot}05$ level.

Transects were also used to quantify the distribution and diversity of aquatic plants. Each 100-m transect was divided into 1-m intervals, and species present under each interval were recorded by a diver (Madsen *et al.*, 1994). Transects were examined concurrently with biomass collection at pretreatment, and one and two years after treatment. Frequency of species or community classes (i.e. native or exotic monocots or dicots) were compared for all transects at a given plot between years using χ^2 analyses of two-by-two comparisons between means of actual number of transect intervals with and without that species or community class. Average number of species or species classes per interval were compared for all transects at a given plot between years using a one-way ANOVA, with significant differences between means calculated using a Bonferroni test at the $p = 0{\cdot}05$ level. Voucher specimens of plants were collected and archived at the USAEWES Lewisville Aquatic Ecosystem Research Facility, Lewisville, TX.

RESULTS AND DISCUSSION

Triclopyr dissipation from river treatment plot

Inside river treatment plot. At 1 h after treatment, the whole-plot aqueous triclopyr residue (mean ± SE of all stations, all depths) was $4{\cdot}59 \pm 1{\cdot}46$ mg/l (Table II). This greater than predicted whole-plot triclopyr concentration was primarily caused by high residue levels found at station 2 (14 mg/l, discrete station data not shown) which was located in a shallow area ($z = 0{\cdot}5$ m) of the plot. Elevated herbicide residues are not uncommon in site-specific regions of a treatment area immediately following a submersed application, which typically occurs in the upper levels of the water column. In addition, water column mixing of herbicides can be inhibited by factors such as linear flow, thermal stratification and wind-driven circulation patterns (Fox *et al.*, 1991a; Getsinger *et al.*, 1992). Although measured triclopyr residues were initially greater than the nominal application rate, concentrations were well below acute and chronic toxicity levels established for non-target aquatic organisms, and were present for only short periods of time. Conversely, some locations within the treated area received below the intended dose of triclopyr in the first few hours following application. If data from the shallow sampling station are excluded, the whole-plot triclopyr concentration was $2{\cdot}71 \pm 0{\cdot}88$ mg/l, very close to the nominal application rate of 2·5 mg/l.

Whole-plot triclopyr concentrations remained $\geqslant 2$ mg/l through 12 h after treatment and were > 1 mg/l at 1 day after treatment. Based on laboratory-derived concentration and exposure time relationships, a triclopyr dose of $\geqslant 1$ mg/l for 24 h should provide up to 85% milfoil control, with some regrowth potential likely by five weeks posttreatment (Netherland and Getsinger, 1992). Although the whole-plot aqueous triclopyr value was still relatively high at 1 day after treatment ($1{\cdot}27 \pm 0{\cdot}43$ mg/l), residues were below the proposed potable water tolerance level of 0·5 mg/l by 2 days after treatment, when herbicide concentrations were measured at $0{\cdot}27 \pm 0{\cdot}13$ mg/l. By 3 days after treatment, triclopyr concentration in the plot was $0{\cdot}17 \pm 0{\cdot}1$ mg/l, and was near or below detection ($< 0{\cdot}01$ mg/l) in the upstream (Stations 1,2) and midstream (Stations 3,4) zones. Triclopyr concentrations were below detection in all sampling zones by 7 days after treatment.

Whole-plot aqueous half-life of triclopyr (Table III) was calculated to be 19·4 h ($r^2 = 93{\cdot}9$), which was very similar to the calculated half-life of the dye (20·1 h, $r^2 = 96{\cdot}5$). Correlation of dye and triclopyr concentrations was significant ($p < 0{\cdot}001$), with an r^2 value of 0·80 (Turner *et al.*, 1994). When analysed by flow zones, actual mean triclopyr concentrations and calculated half-lives (Tables II and III) showed that the minimum herbicide contact time occurred in the upstream zone ($t_{1/2} = 2{\cdot}7$ h, near detection limit by 1 day after treatment). While triclopyr exposure times in the midstream ($t_{1/2} = 15{\cdot}9$ h, near detection limit by 3 days after treatment) and downstream ($t_{1/2} = 24$ h, near detection limit by 7 days after treatment) zones were much longer. The relatively constant gravity flow in the river would be expected to produce this type of progressive herbicide dissipation pattern through the zones of the plot. Also, a small channel allowing water to flow from the main river channel into the south-west, upstream corner of the plot may have contributed to the accelerated dilution of the herbicide in the upstream zone. The extended triclopyr contact times in the mid- and downstream zones would be expected to provide a greater degree of milfoil control in those regions of the plot. Aqueous triclopyr dissipation varied between the upper ($t_{1/2} = 14{\cdot}9$ h) and lower ($t_{1/2} = 26{\cdot}4$ h) water sampling locations in the plot (Table III),

Table II. Mean triclopyr residues (mg/l ± SE) in water column inside treatment plots following Garlon® 3A applications, Pend Oreille River, WA, August 1991.

	Hours after treatment					Days after Treatment					
Station	1	1·5	5	8	12	1	2	3	7	14	21
RT*											
1–6	4·59 ± 1·46	NS†	2·72 ± 0·92	2·00 ± 0·48	2·23 ± 0·52	1·27 ± 0·43	0·27 ± 0·13	0·17 ± 0·10	BD‡	NS	NS
1–2	8·15 ± 3·44	NS	4·69 ± 2·43	2·53 ± 0·75	1·98 ± 1·19	0·02 ± 0·01	BD	BD	BD	NS	NS
3–4	1·86 ± 0·92	NS	1·18 ± 0·48	1·21 ± 0·32	2·08 ± 0·55	1·66 ± 0·57	0·06 ± 0·02	BD	BD	NS	NS
5–6	3·75 ± 1·97	NS	2·31 ± 0·98	2·27 ± 124	2·63 ± 1·09	2·14 ± 0·96	0·81 ± 0·22	0·41 ± 028	BD	NS	NS
CT§											
1–3	NS	2·32 ± 0·56	NS	2·03 ± 0·41	NS	0·78 ± 0·22	0·68 ± 0·23	0·47 ± 0·16	0·22 ± 0·03	BD	BD
1	NS	1·95 ± 0·05	NS	2·55 ± 0·05	NS	0·12 ± 0·09	0·07 ± 0·06	0·06 ± 0·05	0·12 ± 0·06	BD	BD
2	NS	3·55 ± 0·25	NS	2·75 ± 0·05	NS	1·03 ± 0·17	1·25 ± 0·25	0·45 ± 0·16	0·29 ± 0·02	BD	BD
3	NS	0·90 ± 0·30	NS	0·80 ± 0·50	NS	1·20 ± 0·0	0·72 ± 0·25	0·89 ± 0·08	0·25 ± 0·01	BD	BD

* River treatment; nominal triclopyr concentration = 2·5 mg/l
† No sample collected
‡ Below detection (< 0·01mg/l)
§ Cove treatment; nominal triclopyr concentration = 1·75 mg/l

Table III. Half-lives and regression equations for dissipation of triclopyr and dye for plots treated with Garlon® 3A and rhodamine WT, Pend Oreille River, WA, 1991. Unless noted, regression correlations (r^2) are significant at $p \leq 0.01$

Station	Depth	Regression equation $y = \exp(a + bt)$*	r^2	Half-life (h)
		River Plot		
1–6	all	[triclopyr] = exp(8·1335 − 0.0357t)	93·9	19·4
		[dye] = exp(2·3845 − 0·0344t)	96·5	20.1
1 + 2	all	[triclopyr] = exp(9·7465 − 0·2514t)	96·3	2·7
		[dye] = exp(4·8482 − 0·4429t)	88·6†	1·6
3 + 4	all	[triclopyr] = exp(7·6267 − 0·0434t)	68·6‡	15·9
		[dye] = exp(2·4227 − 0·0518t)	82·4	13·4
5 + 6	all	[triclopyr] = exp(8·1225 − 0·0288t)	95·4	24·0
		[dye] = [dye] = exp(2·0113 − 0·0206t)	52·3§	34·2
1–6	upper	[triclopyr] = exp(8·4471 − 0·0478t)	98·4	14·9
		[dye] = exp(2·7603 − 0·0466t)	99·5	14·5
1–6	lower	[triclopyr] = exp(7·8012 − 0·0262t)	84·7	26·4
		[dye] = exp(1·8864 − 0·0222t)	77·1	31·3
		Cove Plot		
1–3	all	[triclopyr] = exp(7·4469 − 0·0131t)	87·6	52·7
		[dye] = exp(1·9417 − 0·0133t)	87·4	52·0
1–3	all	[triclopyr] = exp(7·5279 − 0·0144t)	87·6	52·7
		[dye] = exp(2·0490 − 0·0148t)	87·4	52·0
1–3	all	[triclopyr] = exp(7·3881 − 0·0121t)	89·1	57·3
		[dye] = exp(1·8391 − 0·0120t)	88·1	57·7

* Chemical concentration (μg/l) at time (t) = exp ((intercept − slope(t))
† $p = 0.017$
‡ $p = 0.021$
§ $p = 0.066$

suggesting that laminar flow patterns (and perhaps triclopyr degradation rates) were dissimilar in these different layers of the water column.

Downstream river treatment plot. Aqueous triclopyr residues peaked at Stations 7 and 7a, located 300 m downstream from the northern edge of the river treatment plot, at 1·20 mg/l (1 day after treatment) and 0·42 mg/l (8 h after treatment), respectively (Table IV). Based on these residues, some off-target injury and/or milfoil control was expected downstream of the river treatment plot. At Stations 8 and 8a, located 675 m downstream from the plot, triclopyr residues peaked at 0·47 mg/l (1 day after treatment) and 0·12 mg/l (8 h after treatment), respectively. Residues at the 975 m downstream station (Station 9), were near or below detection throughout the post-treatment sampling regime. These low downstream triclopyr concentrations indicate that the potable water tolerance level (0·5 mg/l) set-back distances of 400–800 m (0·25–0·50 mile) being considered for the triclopyr aquatic label are appropriate for applications made along shorelines of slow-flowing rivers.

Triclopyr dissipation from cove treatment plot

Inside cove treatment plot. At 1·5 h after treatment, the whole-plot aqueous triclopyr residue (mean ± SE, all stations, all depths) was 2·32 ± 0·56 mg/l (Table II), somewhat greater than the nominal application rate of 1·75 mg/l. However, triclopyr concentration in the plot was 2·03 ± 0·41 mg/l at 8 h after treatment, and by 1 day after treatment a level of 0·78 ± 0·22 mg/l was measured. Triclopyr concentrations were below the proposed potable water tolerance level of 0·5 mg/l by 3 days after treatment, when triclopyr was measured at 0·47 ± 0·16 mg/l. By 7 days after treatment, the mean triclopyr concentration in the plot was 0·22 ± 0·03 mg/l, and was below detection at all stations and all depths by 14 days after treatment. Based on laboratory-derived concentration and exposure time requirements, a triclopyr dose of > 0·25 mg/l for ≥ 72 hours should provide excellent milfoil control with little or no regrowth (Netherland and Getsinger, 1992).

Table IV. Triclopyr residues in water downstream from treatment plots following Garlon® 3A application, Pend Oreille River, WA, August 1991

	Hours after treatment					Days after treatment					
Station	1	1·5	5	8	12	1	2	3	7	14	21
RT*											
7 300 m†	BD‡	NS§	0·23	0·55	0·97	1·20	0·57	0·57	0·06	NS	NS
7a 300 m	0·10	NS	0·21	0·42	0·03	0·02	0·02	BD	BD	NS	NS
8 675 m	NS	NS	BD	0·07	0·13	0·47	0·02	0·15	BD	NS	NS
8a 675 m	NS	NS	BD	0·12	0·09	BD	BD	BD	BD	NS	NS
9 975 m	NS	NS	BD	0·02	BD	BD	BD	BD	BD	NS	NS
CT¶											
4 150 m	NS	0·30	NS	0·28	NS	0·02	BD	BD	BD	BD	BD
5 395 m	NS	0·09	NS	0·32	NS	0·04	BD	BD	BD	BD	BD

* River treatment, samples collected at 1 m depth
† Distance downstream from plot
‡ Below detection
§ No sample collected
¶ Cove treatment, samples collected at 0·5 m (station 4) and 0·75 m (station 5) depths

Whole-plot aqueous half-life of triclopyr in the cove treatment plot (Table III) was calculated to be 52·7 h ($r^2 = 87·6$) which was nearly identical to the calculated half-life of the dye (52 h, $r^2 = 87·4$). Correlation of dye and triclopyr concentrations was significant ($p < 0·001$), with an r^2 value of 0·95 (Turner *et al.*, 1994). This high correlation coefficient indicates that a tank mix, rather than sequential (river treatment plot, $r^2 = 0·80$), application of triclopyr and rhodamine WT can improve the herbicide simulation characteristics of the dye.

When analysed by individual sampling stations, mean triclopyr concentrations were near target levels for both north and south subplots up to 8 hours after treatment (Table II). Residue levels declined most quickly at Station 1 in the higher water exchange subplot, diminishing to levels of approximately 0·10 mg/l or less by 1 day after treatment. The proximity of this southern portion of the plot to the main river channel and a tributary stream undoubtedly increased the degree of water exchange in that region of the plot. In contrast, triclopyr water residues at Stations 2 (mid-plot) and 3 (low water-exchange, northern subplot) remained at levels $\geqslant 0·25$ mg/l up to 7 days after treatment. These data suggested that optimum milfoil control could be expected in the mid and northern sections of the plot. Triclopyr dissipation half-lives in the upper ($t_{1/2} = 47·9$ h) and lower ($t_{1/2} = 57·3$ h) portions of the water column were more comparable in the cove treatment plot (Table III) than in the river treatment plot. Consequently, laminar flow was probably not a key component in the dissipation of triclopyr in the cove treatment.

Downstream cove treatment plot. Aqueous triclopyr residues peaked at 1·5 h after treatment at Station 4 (150 m downstream) and at 8 h after treatment at Station 5 (395 downstream) at 0·30 mg/l and 0·32 mg/l, respectively (Table IV). Residues at both of these stations were near or below detection by 1 day after treatment. Based on these triclopyr levels, little off-target injury and/or milfoil control was expected. As shown in the river treatment, these low downstream triclopyr residues indicate that the proposed potable water tolerance level (0·5 mg/l) set-back distances of 400–800 m are appropriate for triclopyr applications in relatively quiescent coves of slow-flowing rivers.

River reference plot

No triclopyr residues were detected in the untreated, upstream river reference plot at pretreatment, 8 and 24 hours after treatment. In addition, dye was never detected at the downstream edge of the river reference plot, nor anywhere inside the plot during the seven-day post-treatment sampling period. These results showed that there was no upstream migration of the chemicals from the river treatment plot, and no milfoil injury and/or control was anticipated.

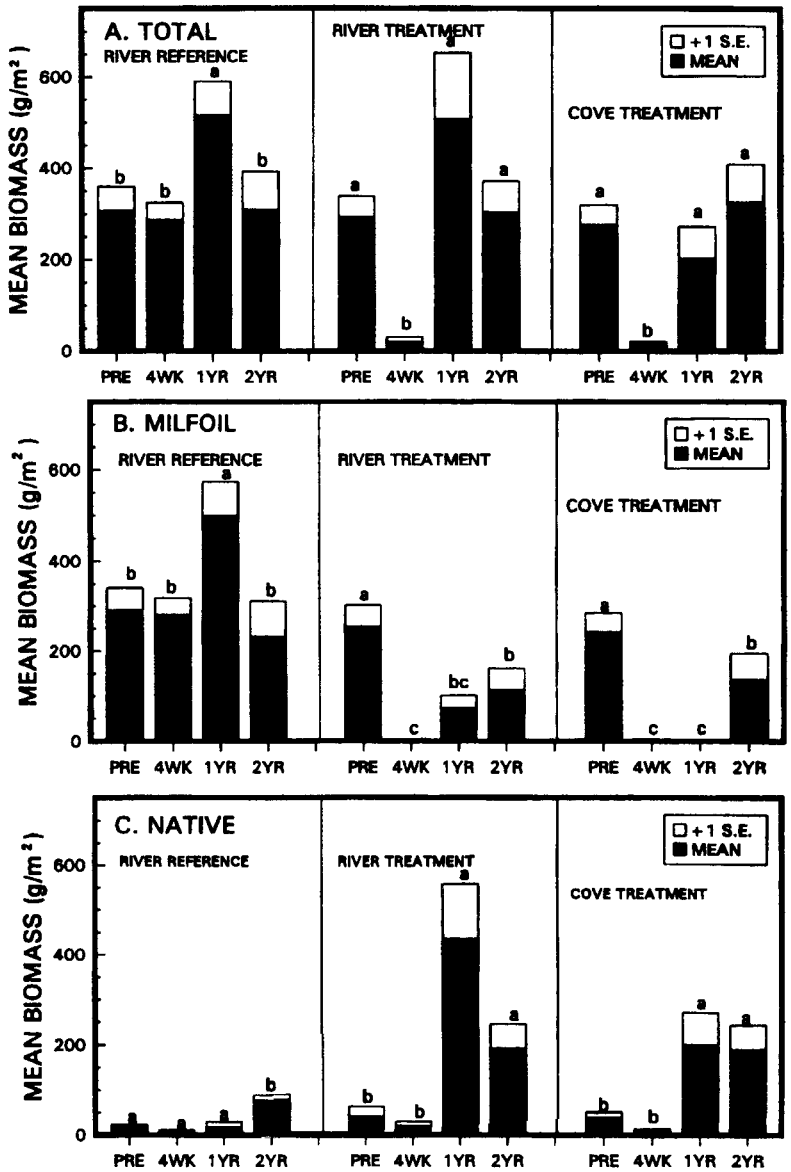

Figure 4. Plant community biomass at three study plots in the Pend Oreille River: (A) total community biomass, (B) Eurasian water milfoil biomass, (C) native community biomass. Letters indicate significant difference at the $p = 0{\cdot}05$ level using ANOVA Bonferroni LSD

Treatment efficacy: plant biomass

Total biomass. An examination of total biomass alone (Figure 4A) indicates that although the triclopyr treatment significantly reduced the amount of plants present in both plots four weeks after application, there was no effect on total community biomass one and two years post-treatment. In this respect, the triclopyr treatment had no long-term effect on plant productivity. However, closer inspection shows that the composition of biomass within the triclopyr-treated submersed plant community was significantly effected over the long term.

Milfoil biomass. Milfoil biomass in the untreated river reference plot maintained constant levels, with the exception of higher biomass during the first year after treatment (Figure 4B). In contrast, milfoil biomass was considerably reduced in both the river and cove treatment plots up to two years post-treatment. The amount of

milfoil at four weeks post-treatment was 1% of pretreatment levels in both treatment plots, indicating excellent triclopyr efficacy on the target plant. One year post-treatment, milfoil biomass in the RT plot was 28% of pretreatment and 1% of pretreatment in the cove treatment plot, and was still significantly lower (47–66%) in both plots two years post-treatment. Close examination of milfoil root crowns, an important source of new plant growth, revealed that most of these perennating structures were severely damaged or completely destroyed in both treated plots by four weeks post-treatment. These observations indicate that current-borne transport of healthy milfoil stem fragments, which is the species' primary reproductive strategy (Madsen *et al.*, 1988), from plants growing outside the treatment areas were primarily responsible for regrowth that occurred in the plots. Despite this reinvasion, duration of acceptable milfoil control at these sites using triclopyr was at least one year longer than reported from previous 2,4-D and fluridone applications in identical or similar locations in the river (Durando-Boehm, 1983; WATER Environmental Sciences, 1986, 1987).

Based on laboratory-derived concentration and exposure time relationships (Netherland and Getsinger, 1992), triclopyr levels in the river treatment plot should have at least 85% milfoil control, with some regrowth occurring by five weeks post-treatment; while milfoil control in the cove treatment plot should have been > 85%, with little to no regrowth occurring by five weeks post-treatment. In fact, field efficacy was better than the laboratory prediction, with triclopyr applications providing excellent control (99% milfoil biomass reduction) for the remainder of the growing season in both plots. Moreover, excellent (99% milfoil biomass reduction) and acceptable (72% milfoil biomass reduction) control were still being maintained in the cove and river treatment plots, respectively, at one year post-treatment. This enhanced field efficacy has been observed with other aquatic herbicides (Getsinger, 1993; Langeland, 1993; Netherland *et al.*, 1993; Nelson *et al.*, 1995) and may be related to levels of environmental stress (e.g. wave action, currents, water turbidity, microbes and pathogens, etc.) that are lacking or minimized in evaluations conducted under laboratory conditions.

Although water exchange and triclopyr half-lives in the river treatment plot suggested that milfoil control in the upstream zone might be less than that in the mid- and downstream zones, this was not the case. The four-week post-treatment efficacy evaluation showed excellent milfoil control throughout the plot, even along the upstream (southern) treatment boundary. High triclopyr concentrations (4·69 to 8·15 mg/l) measured in the upstream zone up to 5 h post-treatment, and concentrations in that zone of 2–2·5 mg/ through 12 h post-treatment, probably accounted for the good milfoil control in the upstream regions of the plot. Observations confirmed that milfoil was partially controlled at distances of up to 250 m directly downstream from the northern boundary of the river treatment plot, with more complete control occurring < 100 m downstream. This level of off-target control was not surprising, since triclopyr residues at Station 7 (300 m downstream) peaked at 1·2 mg/l at 1 day after treatment. As expected, no milfoil control was observed > 10 m upstream of the southern boundary or more than 10 to 20 m beyond the eastern boundary of the plot. Triclopyr injury symptoms were not observed on milfoil growing > 400 m downstream of the river treatment plot; this was expected from the low herbicide residues measured at those distances.

In contrast to the presence of off-target triclopyr efficacy in the river application, no collateral damage was observed on milfoil growing a few metres past the eastern boundary of the cove application. Dye measurements taken during previous water exchange studies (Getsinger *et al.*, 1993) and during this treatment demonstrated that water exchange between the cove and river was relatively low; therefore, efficacious levels of triclopyr extending beyond the confines of the cove were unlikely. The quiescent nature of the cove waters would restrict rapid transport of triclopyr into the river, and would enhance the photolytic and microbial degradation of the herbicide. Lack of off-target injury symptoms and/or milfoil control observed at the CT (cove treatment) plot was supported by the low triclopyr residues measured at the downstream water sampling Stations 4 and 5.

In addition to verifying laboratory-derived dosage rates, the CT plot treatment demonstrated the value of matching herbicide application rates with site-specific water exchange information. Knowledge of the water exchange characteristics of Lost Creek Cove, allowed for 30% less herbicide to be used (1·75 mg/l, versus maximum rate of 2·5 mg/l) with a high degree of confidence to achieve excellent milfoil control. Most importantly, this technique of coupling herbicide dosage rate and water exchange data can aid in reducing the amount of herbicide used in operational treatments, lowering environmental loading of chemicals and costs associated with herbicide applications, without sacrificing efficacy. In regulated rivers, herbicide contact might be maximized by appropriately modifying discharge rates during and after chemical applications, or by scheduling

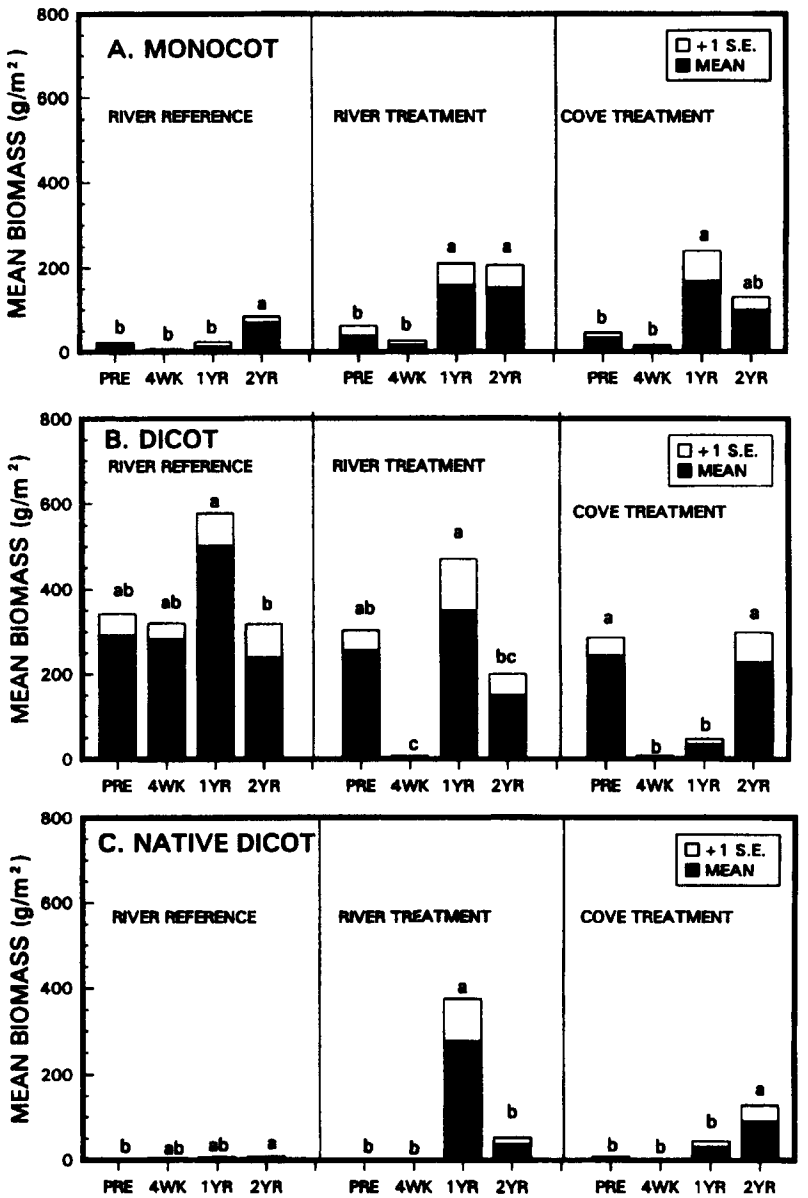

Figure 5. Submersed plant biomass (g/m^2 dry weight) categorized by taxonomic class (see Table I) at three study plots in the Pend Oreille River, (A) monocots, (B) dicots, (C) native dicots. Letters indicate significant difference at the $p = 0{\cdot}05$ level using ANOVA Bonferroni LSD

herbicide applications to take advantage of normal dam/spillway operations. While contact time is of primary importance, laboratory studies have shown that a relatively moderate increase in triclopyr exposure (i.e. from 12 to 24 hours) can provide acceptable control of milfoil at rates as low as 0·25 mg/l, 10 times below the maximum EUP label rate (Netherland and Getsinger, 1992).

Native plant biomass. Native plant biomass levels responded dramatically to the removal of milfoil (Figure 4C). At the untreated river reference plot, native plant biomass remained mostly unchanged, with a slight increase two years post-treatment. Although native plant biomass remained low four weeks after triclopyr application in the river and cove treatment plots, in part owing to the lateness of the growing season, it had increased dramatically (500–1000%) in both treatment plots one year post-treatment (Figure 4C). Native plant biomass remained significantly higher in both plots two years post-treatment. Thus, selective control of milfoil resulted in

higher abundance of native plants up to two years after treatment and suggests that a timely restoration of a diverse native plant community can delay the reinvasion and dominance of an aggressive and opportunistic weed. In fact, this reinfestation was delayed for at least two years in the treated plots, even though milfoil was selectively removed from only small areas (4–6 ha) surrounded by hundreds of untreated hectares infested with milfoil.

As expected from a product having an activity spectrum similar to 2,4-D and other auxin-type growth regulators that are non-toxic to most dicots, monocot species were not adversely affected by the triclopyr application. Rather, monocots significantly increased in abundance in post-treatment years one and two (Figure 5A). The dense milfoil canopy had apparently inhibited native monocot growth, and once this canopy was removed by triclopyr, monocots were able to flourish.

Response of dicots as a group to triclopyr includes the response of the target plant (Figure 5B), and although milfoil was significantly reduced, overall dicot biomass was not consistently different in the treated plots one and two years after treatment. Native dicots (Figure 5C) increased significantly in the river treatment plot one year after treatment, and in the cove treatment plot two years after treatment, largely owing to regrowth of white water crowfoot.

Treatment efficacy: Community diversity

Species frequency. A total of 17 submersed plant species were encountered during the one- and two-year post-treatment evaluations; two were non-native (exotic) species, 15 were native species, 12 were monocots and 5 were dicots (Table I). Transect data provided an assessment of the distribution of plants throughout each plot, and as such are a measure of eveness. Milfoil was observed in virtually all transect intervals in the untreated RR (river reference) plot in all three years (Figure 6A). Before triclopyr treatment, more than 90% of transect intervals had milfoil in both the river and cove treatment plots. These high pretreatment frequency values, coupled with biomass levels and observations by scuba divers, showed that mature milfoil plants were evenly distributed throughout the plots.

Following triclopyr application, milfoil frequency in the river treatment plot dropped to 60% one year after treatment, and remained less than 80% at two years post-treatment. Cove treatment plot milfoil was more affected, with less than 30% frequency one year post-treatment, and 60% two years post-treatment. When these frequency values are coupled with corresponding biomass levels and observations by divers, a clear depiction of

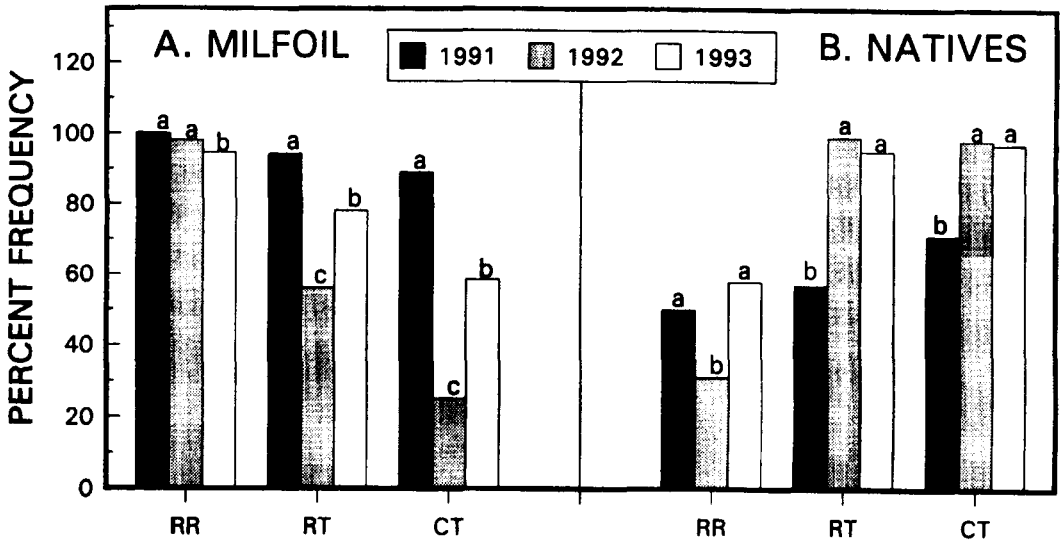

Figure 6. Frequency of plants along transects at three study plots in the Pend Oreille River over the three study years; (A) Eurasian water milfoil, (B) native plant species (all). RR, river reference; RT, river treatment; CT, cove treatment. Letters indicate significant difference at the $p = 0.05$ level using χ^2 analysis

triclopyr efficacy emerges: young shoots of milfoil (initiating from imported stem fragments) unevenly distributed within the treated plots, particularly at one year post-treatment.

Frequency of native species (non-milfoil, non-curlyleaf pondweed) was approximately 50–70% in the treatment plots before triclopyr treatment (Figure 6B). The untreated river reference plot had native plant frequency values from 40% to 60% (Figure 6B). Once treated however, natives increased to nearly 100% frequency two years after treatment. Thus, the seed/propagule bank was sufficient in these submersed plant communities to provide sources for re-establishing native plants; removal of the dense milfoil canopy was all that was required to restore the native plant community.

Species richness. The diversity measure used in this study was average number of species per transect interval, or average species richness. When all species are included, the three plots were at approximately two species per interval prior to triclopyr treatment (Figure 7A). Species richness remained low in the untreated river reference plot one year post-treatment, but increased to over 2·5 at two years post-treatment owing to the increased distribution of the exotic monocot, curlyleaf pondweed. Richness increased to over three species per interval in both treated plots two years post-treatment. When only native species are considered, all three plots were at

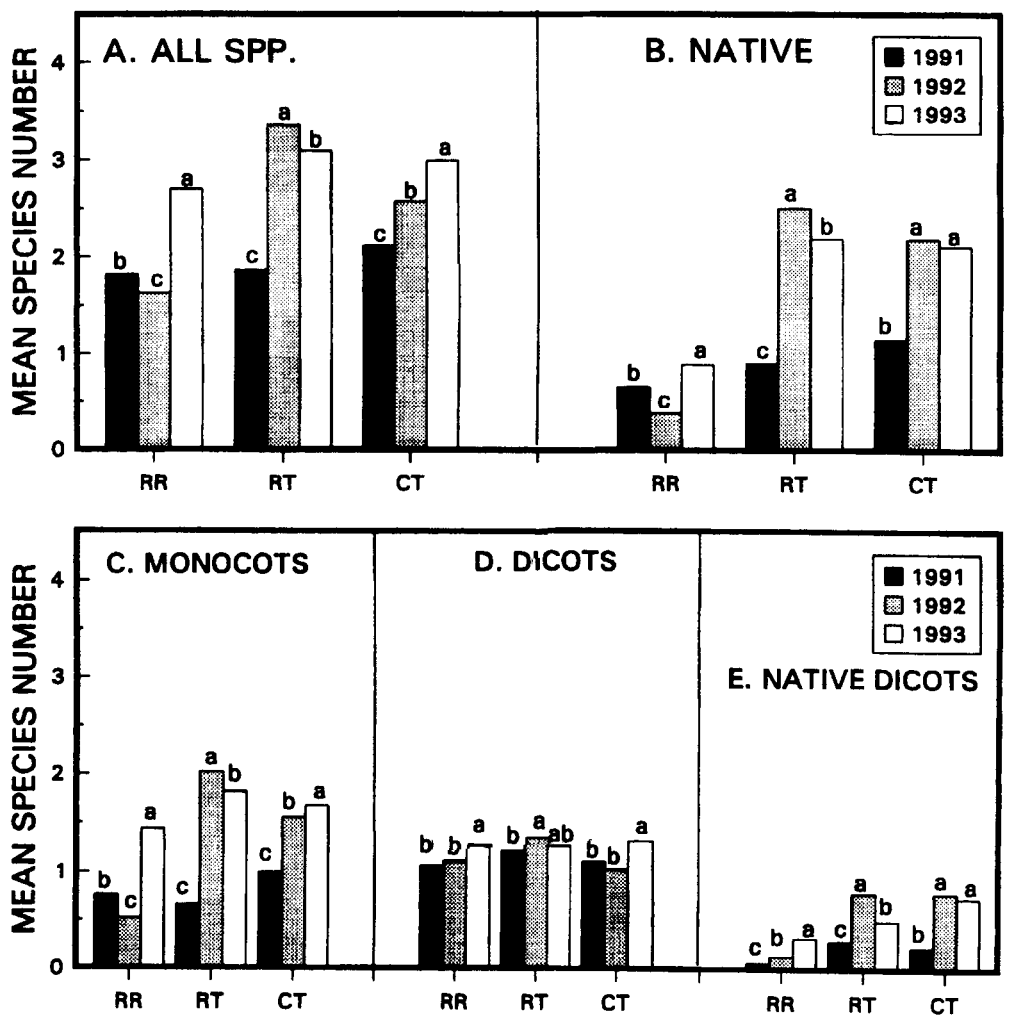

Figure 7. Average number of species per transect interval at three study plots in the Pend Oreille River over three study years; (A) all species; (B) native species only; (C) monocots; (D) all dicots; (E) native dicots only. RR, river reference; RT, river treatment; CT, cove treatment. Letters indicate significant difference at the $p = 0{\cdot}05$ level using ANOVA Bonferroni LSD

approximately one species per interval before treatment, and the untreated river reference plot remained near this level throughout the study (Figure 7B). Following herbicide treatment, richness of native species increased to over two species per interval, more than doubling the diversity of native species in both treatment plots. Higher plant diversity remained in both the river and cove treatment plots two years post-treatment.

The main component in this restoration of plant diversity was the monocot species, which more than doubled in average diversity along transects in the treated plots, both one and two years after treatment (Figure 7C). These were predominantly the native pondweeds (*Potamogeton* spp.). Dicot diversity as a whole was unchanged, owing to the substantial decrease in milfoil distribution (Figure 7D). As with the monocot community, native dicot diversity increased substantially in the rive and cove treatment plots, more than doubling after triclopyr treatment (Figure 7E). It is apparent that the triclopyr treatment did not have a prolonged negative affect on the native dicot community, and in fact allowed these dicots to flourish by removing the dense monoculture of milfoil that had been suppressing their growth.

CONCLUSIONS

This study has demonstrated that the herbicide triclopyr can be used to control selectively the exotic weed Eurasian water milfoil in coves and along shorelines in regulated rivers, while restoring diverse native submersed plant communities in these sites. Such native communities can delay the re-establishment of problematic levels of milfoil for up to three growing seasons. Within a similar areal scale and under comparable hydrodynamic and environmental conditions, triclopyr residues in treated water can be expected to dissipate and/or degrade to very low levels in a short period of time. In addition, this study shows that judicious planning and application can maintain triclopyr concentrations outside treated areas at levels that are extremely low or below detection, and that proposed potable water tolerance set-back distances of 400–800 m are adequate. Finally, we have seen that a knowledge of site-specific water exchange characteristics, coupled with well-established herbicide concentration and exposure time relationships, can be used to prescribe applications that will minimize herbicide dosage rates while maximizing effectiveness against a target plant.

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