SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 15, No. 4, pp. 441–451, 1996
Printed in the USA
0730-7268/96 $6.00 + .00

EFFECTS OF RODEO® AND GARLON® 3A ON NONTARGET WETLAND SPECIES IN CENTRAL WASHINGTON

SUSAN C. GARDNER and CHRISTIAN E. GRUE*
Washington Cooperative Fish and Wildlife Research Unit, School of Fisheries, University of Washington, Seattle WA 98195, USA

(*Received* 24 *February* 1995; *Accepted* 11 *August* 1995)

Abstract—Purple loosestrife (*Lythrum salicaria*) is an invasive wetland perennial that became established in northeastern North America in the early 1800s. Despite its designation as a noxious weed, its distribution has continued to expand. Treatment with herbicides is the most widely used means of controlling purple loosestrife. This study examined the nontarget effects of two herbicides, Rodeo® and Garlon® 3A, currently used or being considered for use in controlling purple loosestrife in Washington State, respectively. Growth and/or survival of duckweed, *Daphnia*, and rainbow trout were monitored for at least 24 h following an application of each herbicide. Free-living water column and benthic invertebrates were monitored 24 h and 7 d post-spray using activity traps and sediment cores. Neither chemical was associated with significant decreases in survival or growth of the bioassay organisms, with the exception that growth of duckweed was reduced 48 h after exposure to Rodeo. Nor were significant decreases in the abundance of free-living aquatic invertebrates detected following the herbicide applications. Results suggest that neither herbicide, at the application rates used, poses a hazard to aquatic invertebrates in wetlands in central Washington. However, Rodeo, because it is a broad-spectrum herbicide, may pose a greater hazard to nontarget aquatic vegetation.

Keywords—Wetlands Glyphosate Purple loosestrife Triclopyr Toxicity

INTRODUCTION

Purple loosestrife (*Lythrum salicaria*) is a wetland perennial that, although native to Eurasia, became established in estuaries of New England in the early 1800s and has now spread to wetlands throughout northern mid-/and western North America [1]. The effects of purple loosestrife on wetland ecosystems have not been well documented; however, once established, it frequently forms dense monospecific stands. The primary impacts of purple loosestrife appear to be the displacement of native plant species, invasion of mudflats, and loss of open water. Dense stands appear to provide poor habitat for wildlife [2,3].

Several methods have been used to control purple loosestrife, but all are expensive and require repeated applications over several years. For the next 5–10 years, herbicide treatment will undoubtedly be the primary control strategy [4]. Two herbicides, Rodeo® (active ingredient: glyphosate [*N*-(phosphonomethyl) glycine], Monsanto Agricultural Company, St. Louis, MO) and Garlon® 3A (active ingredient: triclopyr ([(3,5,6 trichloro-2-pyridinyl)oxy]acetic acid), DowElanco, Indianapolis, IN) show promise for purple loosestrife control [5–7] and their efficacy is currently being evaluated.

Despite the potential impacts of both purple loosestrife and its control on wetland ecosystems, little is known about their effects on water quality and wetland resources [4]. This information is essential in providing the ecological and economic justification for control and developing effective long-term chemical control strategies. In the present study, we examined the non-target effects of field applications of Rodeo and Garlon 3A in purple loosestrife-infested wetlands in central Washington using in situ bioassays (toxicity tests [8]) and aquatic community sampling.

*To whom correspondence may be addressed.

Presented at the Symposium on Methods for Monitoring Effects of Pesticides, 15th Annual Meeting, Society of Environmental Toxicology and Chemistry, Denver, Colorado, October 30–November 3, 1994.

MATERIALS AND METHODS

Study sites

Two wetlands (township 18N, range 26E, section 16) located adjacent to the Winchester Wasteway (0.75 km west of Dodson Road, 5.75 km south of I-90, and 29 km southwest of the Washington Department of Fish and Wildlife's Columbia Basin Fish Hatchery, Moses Lake, Washington) were chosen to represent wetlands in this area in late stages of purple loosestrife invasion 4 (Fig. 1A–C). Each wetland was a closed body of water in which the emergent vegetation was predominantly purple loosestrife (only a few scattered stands of cattail *Typha latifolia* and bulrush *Scirpus acutus* were present). The wetlands were 1.1 and 3.4 ha in size; preliminary measurements indicated similar physical and chemical water quality conditions (temperature = 18.2–18.6°C, conductivity = 0.40–0.45 mS/cm, pH 8.3–8.9, depth = 2.0–2.5 m).

Experimental design

We divided each wetland into four quadrants, corresponding to four randomly assigned treatments; a Garlon 3A-sprayed area, a Rodeo-sprayed area, and an untreated reference site for each herbicide treatment (Fig. 2). Four sites were sampled within each quadrant. Each site consisted of a 6 × 1.5-m homogeneous stand of purple loosestrife with an average height of 1.9 m (Figs. 1C, 2) Water depth at the edge of the purple loosestrife within the sites varied between 0.3 and 0.6 m. We subdivided each site into five stations within which sampling was randomly assigned and conducted (Figs. 1D, 2). A minimum of 10 m was maintained between any two sites of the same treatment type and a minimum of 30 m between the different treatments.

The study took place over 5 weeks (12 July–13 August

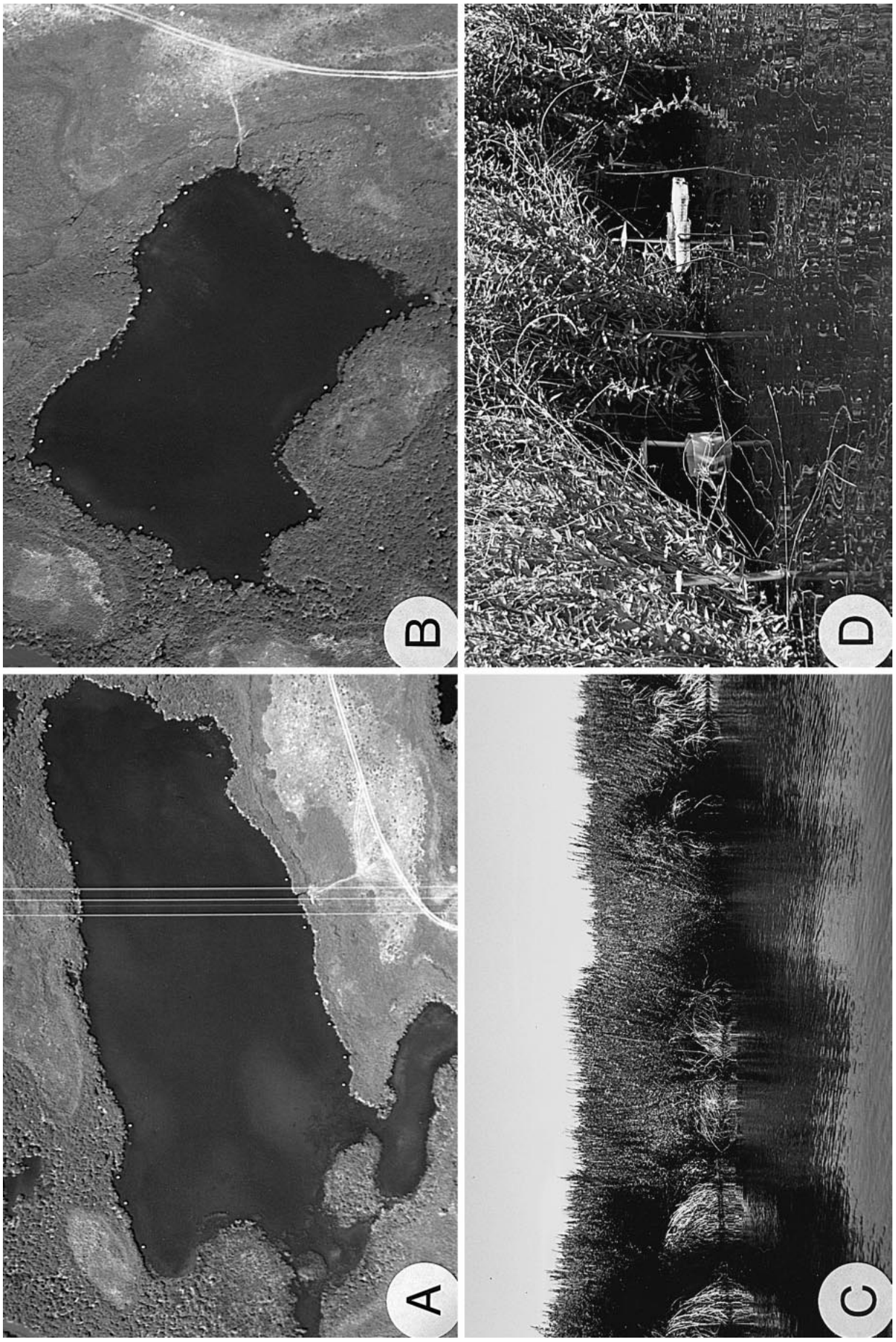

Fig. 1. Aerial view of study wetlands (**A**,**B**) located near the Winchester Wasteway in central Washington; white dots are study sites within purple loosestrife (**C**) in each wetland quadrant (Fig. 2). Sampling stations (**D**) were located along the edge of the loosestrife.

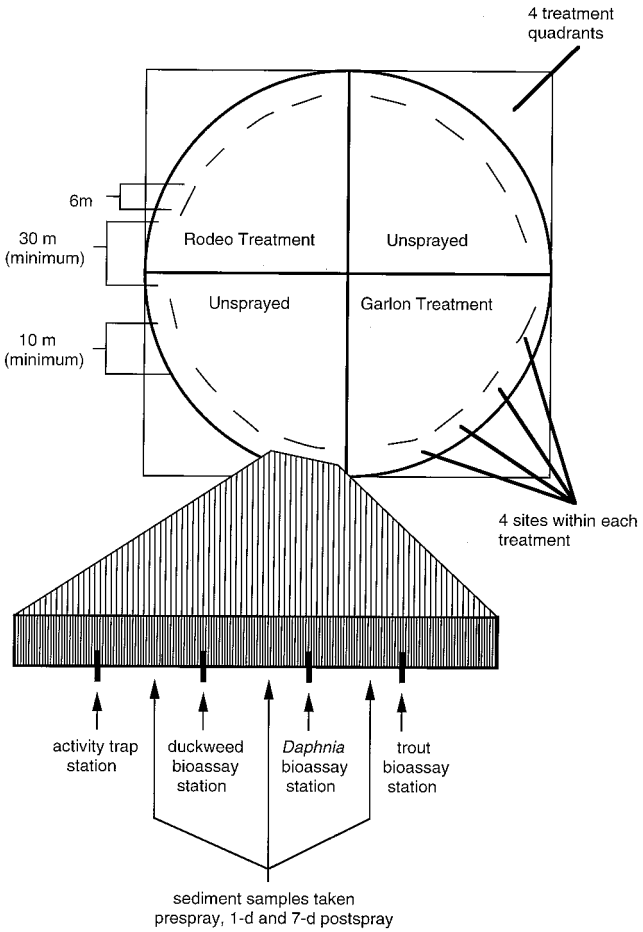

Fig. 2. Experimental design in which each wetland was divided into four quadrants corresponding to randomly assigned treatments. Within each quadrant, four sites were chosen for conducting the bioassays and sampling the aquatic community. At each site, we conducted three in situ bioassays (duckweed, *Daphnia*, and rainbow trout) and collected activity trap samples and sediment cores. The location of the bioassays and sampling stations within the sites were randomly selected and varied among sites. Sediment cores were collected at the base of the purple loosestrife plants in the area between the bioassays.

1993) with the effects Garlon 3A examined first, followed by the Rodeo applications.

Test organisms

Organisms for the in situ toxicity tests consisted of duckweed (*Lemna gibba*), *Daphnia* (*D. magna*), and rainbow trout (*Oncorhynchus mykiss*). The duckweed and *Daphnia* were purchased from Aquatic Research Organisms Co., Hampton, New Hampshire, USA, and maintained at the Columbia Basin Fish Hatchery. Duckweed plants were delivered 2 weeks prior to the first test and cultured in Hoagland's medium without EDTA or sucrose under continuous warm white fluorescent lighting and constant temperature (25 ± 2°C) according to the procedures described by the American Society for Testing and Materials [9].

Daphnia were delivered 2 d before each test and at the time of shipment were 500–700 μm in size and 1–3 d of age. They were maintained with gentle aeration in their shipment containers under constant temperature (21 ± 2°C) using a photoperiod of 16 h light : 8 h dark and fed daily with algae (*Selenastrum capricornutum*).

Rainbow trout were obtained from the Columbia Basin Fish Hatchery and averaged 2.8 g (SD = 0.7) in weight and 57.6 mm (SD = 6.3) in length during the Garlon 3A study and 2.6 g (SD = 0.9) in weight and 57.1 mm (SD = 4.8) in length during the Rodeo study. Prior to use in the toxicity tests, the fish were maintained in large in-ground tanks (60–80,000 fish/tank) with continuous water flow (14.5°C) and fed dry trout feed (2.2 kg Silver Cup [Nelson's Manufacturing, Murray, UT, USA]/45.5 kg fish/day).

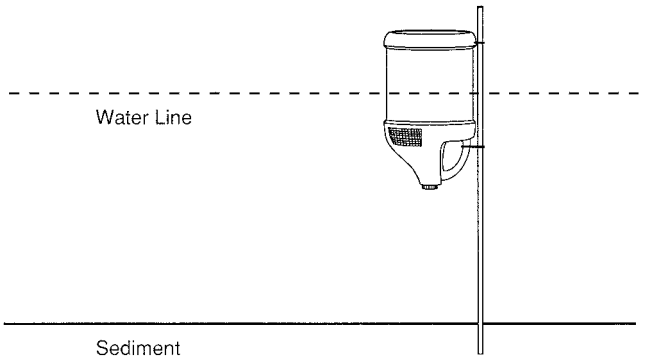

Fig. 3. Duckweed in situ bioassay chamber enabled the plants to float at the water surface while allowing water flow from the sides and bottom. These chambers were constructed out of inverted 3.8-L plastic jugs without caps with two side openings covered with 0.33 mm Nitex®. The bottoms of the jugs were removed to allow exposure of the duckweed to the herbicide spray.

In situ toxicity tests

At each site, we conducted in situ toxicity tests using duckweed, *Daphnia*, and trout. The organisms were placed at the sites on the morning of the spray (07:30–10:30). For the duckweed bioassay, healthy plants consisting of two uniform fronds of approximately the same size were removed from the stock culture and placed in glass vials. The vials were transported to the study wetlands in coolers at a constant temperature (20 ± 2°C). At each study site, 25 plants were placed in plastic flowthrough chambers that allowed the plants to float on the water surface (Fig. 3). The change in plant biomass at each treatment site was evaluated indirectly by determining the change in number of living fronds on the plants. Each frond that visibly projected beyond the edge of the parent frond was considered a separate frond and any frond that had lost more than 50% of its green pigment was not counted [9]. Duckweed tests were conducted for 48 h after each spray.

The *Daphnia* bioassay was conducted by placing 10 *Daphnia* (3–5 days of age) in each of three toxicity test chambers. The test chambers [10] were constructed of polyvinyl chloride compression couplers with open screw-on end caps (7 cm diameter by 17 cm length). The endcaps were covered with Nitex® screening (0.33 μm) glued on with nontoxic aquarium sealer. This allowed water to flow through the chamber but prevented the *Daphnia* from escaping. The chambers were paired and transported to the study sites in 3.8-L plastic bags (filled with water [22 ± 1°C] previously collected from the study wetlands) within insulated coolers. The chambers were placed from a canoe into floating cages [10] that maintained the organisms' positions at the water surface. Prespray to postspray survival was determined by counting the number of live *Daphnia* in each chamber 24 h postspray.

Trout were transported to the study site in a 760-L aerated tank mounted on a pickup truck. The tank was filled with water from the hatchery (spring water) the night before the trout were

to be transported to allow the water to reach a temperature (18 ± 1°C) more similar to that in the study wetlands. The trout toxicity tests were conducted using the same procedure for the *Daphnia* except that only four trout were placed in each chamber and the chambers were placed in an aerated insulated cooler (20 ± 2°C) for transportation to the study sites.

Four extra sets of chambers (*Daphnia* and trout) were taken into the field to determine the effect of transportation on the survival of the organisms. After the test chambers were placed at the study sites, the water temperature in the transportation tank and coolers was measured and the survival of travel controls determined.

Aquatic community sampling

Effects of herbicide application on the aquatic community were assessed by collecting samples of benthic and water-column invertebrates. Benthic invertebrates were collected by obtaining sediment samples at the base of the purple loosestrife plants using a cylinder coring device (10 cm diameter). Five core samples (≥8 cm deep) were collected and pooled at each site. The fine sediment was removed by screening the sample through a 0.5-mm screen and the remaining sample was flushed with 70% ethyl alcohol into a plastic sample bottle. The samples were collected in the morning at least 1 h prior to the placement of the toxicity test chambers.

To measure the invertebrates in the water column at each site, one funnel activity trap (15 cm diameter by 23 cm length) was placed vertically in the water column 10 cm above the sediment surface [11]. The traps were deployed for 24 h to collect organisms during their diel vertical migrations. After 24 h, the samples were removed by placing a rubber stopper in the funnel opening. The lid was removed at the rear of the trap and the contents were poured onto a 0.5-mm screen. The trap was rinsed with 70% ethyl alcohol to remove any clinging invertebrates and the screen contents were flushed into a plastic sample bottle.

The aquatic community samples were collected in the mornings just prior to each spray and then again 24 h and 7 d postspray. All specimens were preserved in 70% ethyl alcohol for identification at the end of the test period. The invertebrates were separated by taxa, and then the number of organisms within each group (order or class) was compared between treatments and through time.

Water quality measurements

Water quality conditions were monitored using submerged data recorders (Datasonde®, Hydrolab Corporation, Austin, TX, USA), which measured pH, dissolved oxygen, temperature, and conductivity every 15 min for 24 h. The Datasondes were randomly rotated among the sites within the wetlands so that throughout the study at least 24 h of data were collected at each sampling site.

Chemical applications

Tank mixes for the two herbicides conformed to label specifications and recommendations by the Washington Department of Ecology (Experimental Use Permit for Garlon 3A). The Garlon 3A tank mix consisted of 6% formulated product by volume (recommended rate for woody plant species), 0.5% LI 700® (a nonionic surfactant, Loveland Industries Inc., Greeley, CO, USA), and 93.5% water. The Rodeo tank mix consisted of 1% formulated product by volume, 0.5% LI 700, and 98.5% water.

Both herbicides were applied at the recommended application rate (1 L/ha for Rodeo and 5 L/ha for Garlon 3A) using a hand-held backpack sprayer (SUN® AP-20 [Fwu Tien Industry Co., Ltd., Tiawan]; R.E. Chapin [Batavia, NY, USA], 3-6000 variable nozzle [1.3–1.8 L/min at 30 psi]) spraying the plants until wet. Plants within a total area of 72 m^2 (9 m^2/site × four sites/wetland × two wetlands) were treated with each herbicide. The herbicides were applied to the water side of each site during the middle of the day (11:00–15:00 h) under favorable environmental conditions (Garlon 3A: 30 July; 21–29.5°C; wind [Dwyer® Wind Meter, Dwyer Instruments, Inc., Michigan City, IN, USA] >3–10 km/h; Rodeo: 4 August; 23–27°C; wind <3–8 km/h) on the intended dates.

Chemical analyses

Five 9-cm-diameter glass filter papers (Whatcom, VWR Scientific, San Francisco, CA, USA) were placed at each site (15 cm above the water surface) in order to quantify the amount of herbicide reaching the water. The cards were collected using acid-washed stainless steel tweezers and placed on ice in double-layered plastic bags until they could be stored in a freezer at −30°C.

Five water samples were collected at each of the herbicide-treated sites approximately 15–30 cm from the purple loosestrife plants, 5 cm below the water surface immediately following the sprays. The samples were stored in amber bottles (glass bottles were used for the Garlon 3A-treated sites; plastic bottles were used for the Rodeo-treated sites) to which two to three drops of 6 N HCl were added. The cap and bottle neck were wrapped in parafilm and the bottles buried in ice until they could be stored at −30°C. The water samples and spray deposit cards were shipped on dry ice to laboratories for chemical analysis (triclopyr: Quality Management and Analytical Services, Walhalla, ND, USA; glyphosate: A & S Environmental Testing, Inc., Reading, PA, USA).

The analysis of the water samples and filter papers for triclopyr was conducted using a 5890 Hewlett Packard gas chromatograph for quantification by electron capture detection. The detection limit was 10.0 ng/ml for the water samples and 1 ng/cm^2 for the filter papers.

Samples were analyzed for glyphosate and its primary metabolite, aminomethyl phosphonic acid (AMPA), using high-performance liquid chromotography following a procedure provided to the contract laboratory by the Monsanto Chemical Company. Water samples and filter papers were extracted and extracts were injected into a Kratos Spectroflow 980 Fluorescence Detector with a mobile phase of 0.005 M potassium dihydrogen phosphate in 4% methanol/deionized water at pH 2.0. The detection limit for glyphosate and AMPA in the water samples was 0.5 ng/ml and for the filter papers was 15 ng/cm^2.

Efficacy

Efficacy of the herbicides was assessed by measuring stem density and plant height in 1993 (23 September) and 1994 (1 September). The 1993 measurements were taken approximately 6 weeks following the herbicide applications, and although effects from the herbicides were detectable, the difference between the 1992 and 1993 growth was still evident. Stem density was measured by counting the number of live and dead stems within a 30 × 60-cm frame. The stem density and height measurements were taken at three locations within

Table 1.Water quality parameters in two study wetlands in central Washington

		Wetland 1		Wetland 2	
		Prespray	Postspray	Prespray	Postspray
Garlon 3A	Temperature	18.1–23.3	18.5–21.7	20.8–25.0	18.8–21.8
	pH	7.31–8.59	7.65–8.94	7.87–8.60	7.36–8.66
	Conductance	0.38–0.40	0.36–0.39	0.38–0.41	0.38–0.42
	Dissolved oxygen	—	5.31–17.41	—	41.2–14.4
Rodeo	Temperature	20.8–24.7	22.0–26.1	22.3–26.0	23.2–26.8
	pH	7.53–8.85	7.75–8.72	8.20–8.89	8.34–8.76
	Conductance	0.38–0.44	0.38–0.44	0.39–0.41	0.39–0.42
	Dissolved oxygen	4.69–15.5	8.09–15.4	10.4–16.7	11.3–15.9

Temperature (°C), pH, specific conductance (mS/cm), and dissolved oxygen (mg/L) were recorded every 15 min over a 24-h period before and/or after the application of each of two herbicides, Garlon® 3A and Rodeo®. Values given represent the range for each parameter.

each site (at the center and 0.5 m from either end). Data from 1993 were compared to the plant growth in 1994.

Statistical analyses

The effect of herbicides on the in situ test organisms was assessed by comparing percent survival on control sites with that on the treated sites ($n = 4$ for each wetland). Data were analyzed using a one-tailed nonparametric Mann–Whitney test. For the *Daphnia* and trout tests, survival of each species within the three chambers at each site ($n = 4$) was averaged. Statistical tests were conducted for each wetland separately.

Changes in invertebrate abundance within taxa (postspray − prespray) were compared using a two-tailed nonparametric Wilcoxon paired-sample test. Tests were conducted for each taxa independently. Data from the two wetlands were pooled to increase the power of the test ($n = 8$).

Efficacy of the herbicide treatments (density of live and dead stems and height in 1993 and 1994) was compared using a two-tailed nonparametric Wilcoxon paired-sample test. The three measurements taken at each site were averaged by year and the data from the two wetlands were pooled ($n = 8$). In all tests, differences were considered significant if the probability associated with the test statistic was <0.05.

RESULTS

Water quality

Water quality 24 h pre- and postspray in each wetland is summarized in Table 1. Temperature varied greatly throughout the study (18.1– 25.0°C for Garlon 3A and 20.8–26.8°C for Rodeo). The greatest change in water temperatures occurred during the Garlon 3A study when the temperature varied >5°C during the 24-h postspray (18.1–23.3°C). Diel changes in dissolved oxygen were also greatest during the Garlon 3A study when values ranged from 5.3 to 17.4 mg/L (postspray Wetland 1). Conductivity remained fairly constant throughout the entire study and did not vary more then 0.08 mS/cm; pH varied by 1.6 units.

Chemical analyses

The results of the chemical analysis of the water samples and filter papers are given in Table 2. The concentrations of triclopyr detected in water ranged from 0.02 to 0.88 μg/ml. Triclopyr on the filter papers was between 1.42 and 9.72 μg/cm^2. For glyphosate, the concentrations ranged from 0.01 to 0.10 μg/ml and 2.63 to 12.01 μg/cm^2 for the water samples and filter papers, respectively. The AMPA concentrations in the water were either very low (0.0006–0.005 μg/ml) or not detectable. The average concentration of AMPA on the filter papers was 0.03 μg/cm^2 (SD = 17.2 ng/cm^2). No triclopyr, glyphosate, or AMPA was detected in the control samples. Percent recovery for triclopyr spiked samples ranged from 110 to 115% in water from the wetlands and 93 to 95% for the filter papers. Recovery of glyphosate and AMPA in spiked samples was 94.0–102.2% and 100.3–101.9% in wetland water samples and filter papers, respectively.

In situ toxicity tests

Twenty-four hours following the application of the herbicides, no statistically significant differences were detected between the average number of fronds at the control and treated sites for either Garlon 3A or Rodeo (Fig. 4). Forty-eight hours postspray, a decrease in the average number of fronds occurred

Table 2.Concentrations of triclopyr and glyphosate in water samples or glass filter papers following application to purple loosestrife in two wetlands in central Washington

		Triclopyr		Glyphosate	
		Wetland 1	Wetland 2	Wetland 1	Wetland 2
Water samples (μg/ml)	Control	<0.01	<0.01	<0.0005	<0.0005
	Treated	0.06	0.43	0.02	0.06
		(0.04)	(0.31)	(0.01)	(0.03)
Filter papers (μg/cm^2)	Control	<0.001	<0.001	<0.015	<0.015
	Treated	3.1	5.3	6.8	3.6
		(1.3)	(3.4)	(3.8)	(0.9)

Values given for the treated samples are averages of four sites (five samples or filter papers/site) within each wetland. Control water samples were taken from the nontreated sites within each wetland. Control filter papers were unused papers provided as blanks. Numbers in parentheses are standard deviations.

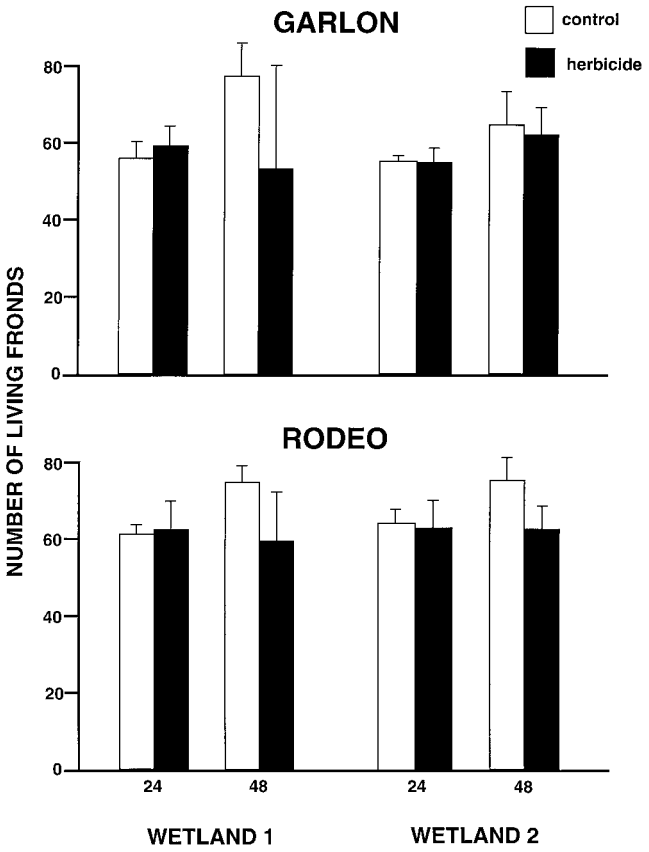

Fig. 4. Number of living duckweed fronds 1 and 7 d following the application of Garlon® 3A or Rodeo® in two wetlands in central Washington ($n = 4$ for each wetland). Vertical lines above bars are standard deviations. Differences between Rodeo-treated and control sites were statistically significant ($p < 0.05$) 48 h postspray.

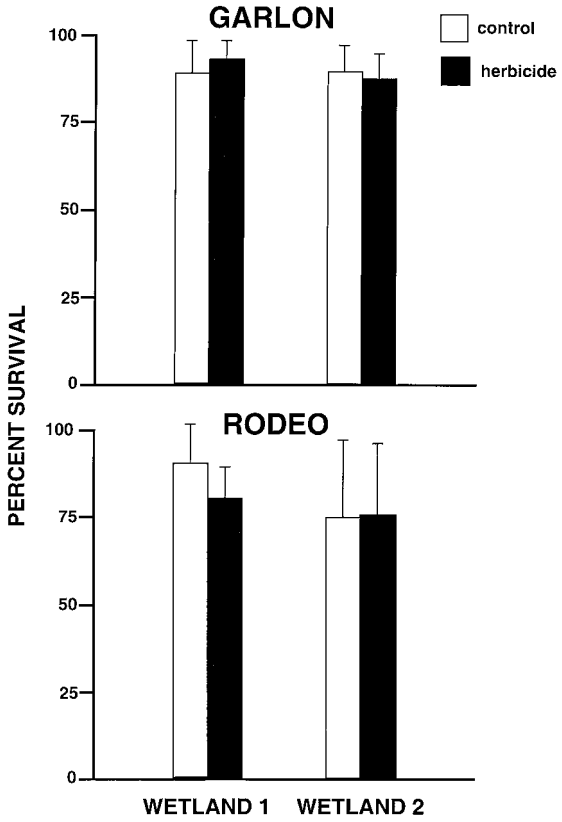

Fig. 5. Survival of *Daphnia* following the application of Garlon® 3A or Rodeo® in two wetlands in central Washington ($n = 4$ for each wetland). Vertical lines above bars are standard deviations. Differences between treated and control sites were not statistically significant ($p > 0.05$).

at the Garlon 3A-treated sites in Wetland 1 compared to the controls (52 and 77, respectively), but this difference was not statistically significant ($p > 0.05$). However, the average number of fronds alive after 48 h was significantly lower on the Rodeo-treated sites compared to controls on both wetlands.

The survival of *Daphnia* in the control and Garlon 3A-treated sites did not differ significantly in either wetland (Fig. 5). There were also no significant differences in survival following the Rodeo application.

Nor were there significant differences in the survival of trout between the the control and treated areas on either wetland following the Garlon 3A application (Fig. 6). The effects of Rodeo on trout survival could not be assessed due to the low survival of trout on the control and treated sites (0– 22%). The survival of all travel controls (*Daphnia* and trout) was high (100%).

Aquatic community samples—activity traps

The most frequently collected taxa in the activity traps were of the classes: Branchiopoda, Copepoda, Ostracoda, and Arachnida. The number of each of these taxa collected before the herbicide applications and the changes (Δ = postspray − prespray) in their abundance 1 and 7 d postspray are given in Table 3. Seven days after the Garlon 3A application the number of branchiopods and copepods (Δ = 4,429 and 310, respectively) captured at the treated sites increased significantly compared to the controls. Similar differences were not detected in the numbers of ostracods and arachnids, or among any of the other common taxa 1 d postspray. There were no significant differences in the numbers within the most common taxa at the corresponding control sites.

Following the Rodeo application, a significant increase in copepods was detected 7 d postspray (Δ = 53). No significant differences in the numbers of branchiopods, ostracods, or arachnids were observed 1 or 7 d postspray. However, a significant increase (Δ = 1,270) in the average number of branchiopods at the control sites occurred 7 d following the Rodeo

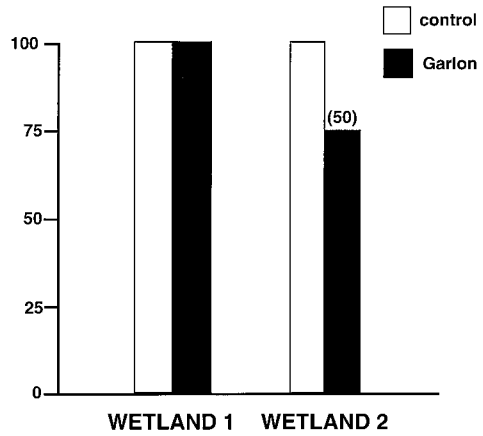

Fig. 6. Survival of rainbow trout following the application of Garlon® 3A in two wetlands in central Washington ($n = 4$ for each wetland). Number in parentheses is the standard deviation. Differences between treated and control sites were not statistically significant ($p > 0.05$).

Table 3.Average number of aquatic invertebrates (class) within 24-h activity trap samples before and after application of Garlon® 3A or Rodeo® to control purple loosestrife within two wetlands in central Washington in 1993

		Wetland 1			Wetland 2				
			Δ postspray			Δ postspray		Combined Δ postspray	
		Prespray	1 d	7 d	Prespray	1 d	7 d	1 d	7 d
Garlon									
	Arachnida	10	17	27	55	−28	−35	−6	4
		(7)	(9)	(36)	(69)	(80)	(74)		
	Branchiopoda	219	561	2,637	313	451	6,221	506	4,429*
		(187)	(939)	(3,873)	(182)	(460)	(5,888)		
	Copepoda	159	78	424	234	−58	203	10	310*
		(78)	(134)	(191)	(153)	(157)	(323)		
	Ostracoda	164	−46	145	68	47	75	1	110
		(189)	(106)	(193)	(32)	(90)	(113)		
Control	Arachnida	10	31	6	13	−19	40	30	17
		(12)	(67)	(15)	(9)	(10)	(57)		
	Branchiopoda	366	6,125	423	1,319	−825	−140	1,964	101
		(456)	(4,409)	(611)	(1,108)	(1,291)	(916)		
	Copepoda	80	31	234	233	29	92	53	205
		(3)	(69)	(181)	(147)	(187)	(419)		
	Ostracoda	257	−20	−145	25	101	66	−64	−37
		(388)	(105)	(388)	(8)	(56)	(54)		
Rodeo	Arachnida	1	8	3	4	5	−1	7	4
		(1)	(13)	(5)	(2)	(13)	(7)		
	Branchiopoda	65	309	872	292	82	525	196	690
		(49)	(421)	(1,146)	(165)	(221)	(513)		
	Copepoda	38	77	46	77	11	52	44	53*
		(22)	(60)	(77)	(27)	(57)	(65)		
	Ostracoda	22	150	−14	20	−13	−13	69	−11
		(24)	(216)	(35)	(10)	(11)	(12)		
Control	Arachnida	2	−2	1	1	5	3	2	2
		(2)	(2)	(1)	(0)	(6)	(6)		
	Branchiopoda	166	−86	1,528	201	156	1,417	30	1,270*
		(240)	(254)	(2,558)	(243)	(62)	(1,923)		
	Copepoda	56	45	102	34	62	23	52	52
		(62)	(114)	(100)	(26)	(61)	(27)		
	Ostracoda	6	23	1	13	−1	2	9	1
		(6)	(40)	(8)	(10)	(15)	(20)		

Averages for each wetland represent the means of four samples per treatment, combined = 8; Δ = postspray − prespray; standard deviations are given in parentheses. *Statistical significance ($p < 0.05$, Wilcoxon paired sample test).

application, suggesting that the herbicide may have depressed branchiopod populations on the treated sites.

Aquatic community samples—sediment cores

The most frequently collected taxa in the sediment cores were of the orders: Amphipoda, Diptera, and Odonata. Other taxa identified included Nematoda, Gastropoda, Ephemeroptera, and Oligochaeta. The number of organisms in each of the most common taxa collected prior to the herbicide applications are given in Table 4 as well as changes in the abundances (Δ = postspray − prespray) 1 and 7 d following the herbicide sprays. There were no significant differences in the the number of organisms collected before and after (1 d or 7 d) application of either Garlon 3A or Rodeo. Numbers of organisms within taxa also did not differ significantly through time on the control sites.

Efficacy

The response of purple loosestrife to a single application of Garlon 3A or Rodeo at the application rates used is shown in Table 5. The plants that were treated with Garlon 3A experienced a statistically significant increase in both the number of live stems (Δ = 410%) and height (Δ = 21%) in 1994 compared to 1993. There was no significant difference detected in the number of dead stems.

One year following the application of Rodeo, there was a significant decrease in the number of live stems (Δ = −74%) and a significant increase in the number of dead stems (Δ = 58%). However, from 1993 to 1994 there was a corresponding increase in the number of dead stems at the control sites (0 in 1993 to an average of 30.8 in 1994). There were no significant differences in the number of live stems or the height of the purple loosestrife plants on the control sites between 1993 and 1994.

DISCUSSION

Previous studies indicate that both herbicides persist in aquatic environments for relatively short periods of time. Following overwater application, triclopyr tends to reside in the water column where rapid photodegradation to 3,5,6-trichloro-2-pyridinol and CO_2 occurs under conditions of strong sunshine and low turbidity; McCall and Gavit [12] estimated the half-life of triclopyr acid under these conditions to be 2.1 h. Information on the persistence and movement of Garlon 3A is more limited. Solomon et al. [13] applied Garlon 4 (the ester form of triclopyr) to enclosures in a northern Ontario lake.

Table 4.Average number of aquatic invertebrates (order) within sediment cores collected before and after application of Garlon® 3A or Rodeo® to control purple loosestrife within two wetlands in central Washington in 1993

		Wetland 1			Wetland 2				
			Δ postspray			Δ postspray		Combined Δ postspray	
		Prespray	1 d	7 d	Prespray	1 d	7 d	1 d	7 d
Garlon	Amphipoda	1	14	9	1	6	−11	10	−1
		(1)	(18)	(8)	(1)	(16)	(16)		
	Diptera	11	−4	23	14	−3	1	−4	12
		(7)	(8)	(27)	(8)	(6)	(15)		
	Odonata	2	1	1	1	1	0	1	1
		(1)	(2)	(2)	(1)	(2)	(3)		
Control	Amphipoda	0	7	2	4	−1	−2	3	0
		(0)	(11)	(3)	(6)	(7)	(2)		
	Diptera	8	0	−1	16	−4	−5	−2	−3
		(13)	(7)	(18)	(13)	(19)	(18)		
	Odonata	1	2	1	1	0	1	1	1
		(1)	(2)	(1)	(1)	(1)	(2)		
Rodeo	Amphipoda	6	−3	−5	4	−10	1	−7	−3
		(10)	(11)	(10)	(3)	(4)	(5)		
	Diptera	30	−4	8	11	−5	8	−5	8
		(27)	(27)	(38)	(9)	(12)	(28)		
	Odonata	1	2	0	1	−1	3	1	1
		(1)	(4)	(2)	(1)	(1)	(3)		
Control	Amphipoda	9	13	−6	13	−7	−11	3	−9
		(6)	(12)	(11)	(11)	(14)	(11)		
	Diptera	18	−1	9	10	−3	17	−2	13
		(16)	(37)	(22)	(10)	(11)	(28)		
	Odonata	3	3	1	4	−3	−2	0	0
		(3)	(7)	(6)	(4)	(5)	(5)		

Averages for each wetland represent the means of four samples per five cores per treatment, combined = 8; Δ = postspray − prespray; standard deviations are given in parentheses.

Garlon 4 dissipated rapidly in the water and levels were less than 5% of the initial values within 15 d and could not be detected after day 42.

The half-life of glyphosate in deionized water under UV light is about 4 d. Lund-Høie and Friestad [14] have shown that in pure water (without sediments), glyphosate is photodegraded to its metabolite, AMPA by UV light. However, unlike triclopyr, glyphosate rapidly dissipates from surface waters and becomes bound to soil particles in the sediment or suspended in the water column [14–16]. Once bound to soil, glyphosate is readily biodegraded by soil microflora to AMPA and CO_2 [17]. The AMPA also undergoes rapid degradation

Table 5.Response of purple loosestrife following a single application of Garlon® 3A (5 L/ha) or Rodeo® (1 L/ha) in two wetlands in central Washington in 1993

		Wetland 1			Wetland 2			Combined
		93	94	Δ	93	94	Δ	Δ
Garlon	No. live stems	3.8	19.5	15.8	3.3	16.6	13.3	14.5*
		(4.0)	(9.3)	(8.8)	(2.0)	(3.1)	(4.6)	
	No. dead stems	42.5	38.5	−4.0	49.9	43.9	−6.0	−5.0
		(17.6)	(23.1)	(15.8)	(6.5)	(17.9)	(14.3)	
	Stem height	2.0	2.3	0.3	1.8	2.2	0.4	0.4*
		(0.1)	(0.3)	(0.3)	(0.1)	(0.2)	(0.1)	
Rodeo	No. live stems	3.1	0.3	−2.8	13.2	4.1	−9.1	−6.0*
		(2.0)	(0.3)	(2.2)	(3.7)	(4.3)	(6.6)	
	No. dead stems	37.8	65.7	27.9	42.2	61.2	18.8	23.3*
		(8.6)	(12.7)	(7.9)	(13.4)	(14.0)	(21.4)	
	Stem height	1.8	2.2	0.4	2.0	2.0	0	0.2
		(0.2)	(0.1)	(0.2)	(0.1)	(0.1)	(0.2)	
Control	No. live stems	33.3	32.8	−0.5	34.3	28.7	−5.6	−3.1
		(8.0)	(1.6)	(6.3)	(10.8)	(8.5)	(2.4)	
	No. dead stems	0	20.5	20.5	0	41.0	41.0	30.8*
		(0)	(6.4)	(6.4)	(0)	(2.8)	(2.8)	
	Stem height	1.8	2.2	0.4	2.1	2.0	−0.1	0.2
		(0.1)	(0.4)	(0.3)	(0.2)	(0.1)	(0.3)	

Values for herbicide-treated stands are means of three measurements (30 × 60 cm) within four sites per herbicide treatment in each wetland; Δ = 1994 − 1993; standard deviations are given in parentheses. Control values are means of three measurements within two sites per wetland. *Statistical significance ($p < 0.05$, Wilcoxon paired sample test).

in soil [17]. Using ^{14}C-labeled glyphosate, Rueppel et al. [17] determined that 50% of the labeled carbon had evolved as $^{14}CO_2$ within 28 d. Goldsborough and Beck [18] reported a half life for glyphosate of 1.5–3.5 d in small forest ponds.

The concentrations of triclopyr and glyphosate we detected in water immediately following hand application were a fraction of those known to be toxic to aquatic invertebrates and fish. For example, Gersich et al. [19]. calculated the 21-d LC50 of Garlon 3A to *D. magna* to be 1,140 mg/L. They determined that the maximum concentration of Garlon 3A that would be acceptable in the environment was 110 mg/L, which is considerably higher than the expected environmental concentration of Garlon 3A in water following aerial applications (0.1–10 mg/L) [19]. The highest concentration of triclopyr detected in the water during our study was 0.883 mg/L, which is only 0.8% of the environmentally acceptable concentration determined by Gersich and coworkers.

Wan et al. [20] evaluated the acute toxicity of triclopyr and its formulations to several species of juvenile salmonids. The 96-h LC50s of technical-grade triclopyr and Garlon 3A for juvenile rainbow trout (0.4–0.9 g) were 7.5 and 420 mg/L, respectively. Mayes et al. [21] calculated the 96-h LC50 of Garlon 3A to be 120 mg/L for 36-d-old fathead minnows (*Pimephales promelas*).

We had intended to use larval fathead minnows instead of rainbow trout in our in situ toxicity tests. However, because fathead minnows are nonindigenous in central Washington and are not believed to be present there, we did not want to risk introducing them to the Winchester Wasteway area. Young rainbow trout represented a relevant alternative because they form the basis of a large recreational fishery in the area and are currently being stocked in nearby wetlands by the Washington Department of Fish and Wildlife.

Several studies have examined the toxic effects of technical-grade glyphosate and its formulations to aquatic invertebrates and fish. Most of these studies, however, have investigated the effects of Roundup, a terrestrial formulation of glyphosate; less information is available on Rodeo. Folmar et al. [22] reported the 48-h immobilization EC50 for glyphosate to be 55 mg/L in midge fly (*Chironomus plumosus*) larvae and the 96-h LC50 for rainbow trout to be 140 mg/L. The 96-h LC50 values for Rodeo in rainbow trout, chinook, and coho salmon ranged from 120 to 290 mg/L [23]. Immobilization EC50s for Roundup decreased with decreasing water temperature in both rainbow trout and bluegill [22]. Based on these results, we would not expect direct toxic effects to *Daphnia* or trout at the highest concentration of glyphosate detected (0.1 mg/L) in the water during our study.

Kreutzweiser et al. [24] investigated the response of stream invertebrates to aerial application of Roundup and found that movements of most invertebrates were not measurably affected. Solberg [25] also found no significant adverse effects on aquatic invertebrate abundance or diversity following aerial glyphosate treatment. Our aquatic community analyses concur with these results and indicate that although Garlon 3A would be expected to remain in the water column and Rodeo would partition to the sediment, under standard field application conditions, neither herbicide would be expected to persist in toxic concentrations. The biological significance of the increases we observed in the abundance of copepods and/or branchiopods is unclear. However, more detailed species identification may reveal changes in community structure such as those reported by others for different pesticides (e.g., Hurlbert et al. [26]).

Studies that have tested the toxicity of surfactants separately from technical-grade glyphosate have shown that the former are the primary toxic agents in both the Roundup and Rodeo formulations [22,23]. For our study, the surfactant LI 700 was chosen because of its lower toxicity to fish and aquatic invertebrates than other commonly used surfactants (R-11® Wilbur-Ellis Company and X-77®, Loveland Industries Inc., Greeley, CO). The results of our study may have been different had a more toxic surfactant been used.

Lockhart et al. [27] concluded that aerial application of glyphosate would have a much greater effect on emergent vegetation than absorption of glyphosate from the water. Glyphosate dissolved in the culture medium of duckweed had little effect on plant growth as compared to spray applications. The authors postulated that this was because glyphosate did not partition from the media to the plant. This hypothesis is supported by Sullivan et al. [28] who examined the effects of field applications of Roundup on diatoms in streams and ponds. Their study did not demonstrate significant effects on species composition or abundance. The phytotoxicity observed in our duckweed toxicity test 48 h following the application of Rodeo most likely resulted from a direct exposure to the herbicide spray rather than exposure occurring from the glyphosate in the water.

Because both Garlon 3A and Rodeo are systemic herbicides, the effects of herbicide applications on duckweed may not have been completely apparent in our 48-h test. However, both herbicides are rapidly absorbed and translocated within plant tissue (<12 h [29,30]). Netherland and Getsinger [30] reported overt effects of Garlon 3A on watermilfoil within 6–12 h posttreatment with efficacy directly related to concentration in the water and/or exposure time. Their findings, the rapid degradation of triclopyr in water [12], and the short doubling time for duckweed fronds (average = l.9 days [31]) suggest that the length of our test should have been adequate to detect herbicide effects.

As noted earlier, single control treatments are frequently not very effective in controlling purple loosestrife, particularly mature stands such as those on our study sites [32,33]. Although the Garlon 3A-treated plants were visibly affected within 7 d postspray and the Rodeo-treated plants were not, Rodeo appeared to be more effective in reducing new growth 1 year postspray. The number of live stems was reduced an average of 69 and 90% on the Rodeo-treated sites within the two wetlands; values comparable to efficacy reported elsewhere (41–90% reduction in canopy cover [33]). However, the single Rodeo application did not kill the plants. In contrast, the Garlon 3A treatment appeared to stimulate new growth (fourfold increase in number of live stems) 1 year later. Although studies suggest that Rodeo is more effective than Garlon 3A in killing mature purple loosestrife, and that the efficacy of Garlon 3A varies [33,34], stimulation of new growth on existing rootstocks has not been previously reported. We note, however, that the amount of Garlon 3A in our tank mix (6% formulated product by volume) was three times the current recommended percentage given in the recent Supplemental Labeling (EPA Section 18 Specific Exemption) for overwater use to control purple loosestrife in Minnesota [35], and three to six times the percentage found to be effective (Skinner et al. [33] and DowElanco, unpublished data). The rapid wilting and yellowing of the purple loosestrife we treated with Garlon 3A may have reduced the ability of the plants to translocate the her-

bicide to the root tissue, resulting in resprouting the following year (V. Carrithers, personal communication).

Hand application is currently the method by which herbicides are applied to purple loosestrife in Washington State. However, aerial application may be more effective in treating large homogeneous stands. Assuming that off-target deposition is minimized, herbicide concentrations may be greater in water among purple loosestrife stands treated by hand compared to aerial applications because in the former the applicator makes an effort to wet the entire plant. In addition, the dense canopies characteristic of mature stands would likely intercept a large portion of the herbicide when aerially applied.

Average herbicide concentrations in the water following hand applications in our study were low (0.02–0.4 μg/ml) and only a fraction of those known to be toxic to aquatic invertebrates and fish. However, non-target aquatic vegetation (e.g., *Lemna*, *Typha*, and *Scirpus* species) may be adversely affected by herbicides, particularly broad-spectrum compounds such as Rodeo. The rapid degradation of the two herbicides and their relatively low toxicity to aquatic invertebrates and fish should allow multiple applications with minimal risk to non-target aquatic organisms. However, to minimize the potential for non-target effects and maximize efficacy, control strategies that integrate a variety of control methods need to be developed.

Acknowledgement—We thank Maureen Beckstead, Robert Kent, Chuck Perry, Christine Bunck, Vanelle Carrithers, Chris Maynard, Steve Saunders, Kevin Kilbride, and Fred Paveglio whose reviews improved the study design and manuscript. We also thank Jonathan Dodge, James Martin Grassley, and Walter Major for assisting in the field and laboratory; Art Brown, Mike Holm, and Ken Westrope for providing lodging and technical assistance; and Heidi Perry for applying the herbicides. Funding was provided by the Washington Department of Fish and Wildlife (WDFW), University of Washington, and the Washington Cooperative Fish and Wildlife Research Unit (WCFWRU). Chemical analyses were conducted with support from DowElanco, and Monsanto Chemical Company. C.E. Grue is employed by the U.S. Department of the Interior, National Biological Service (NBS), and the WCFWRU is supported by NBS, the University of Washington, WDE, WDFW, the Washington Department of Natural Resources, and the Wildlife Management Institute.

REFERENCES

1. **Thompson, D.Q., R.L. Stuckey** and **E.B. Thompson.** 1987. Spread, impact and control of purple loosestrife (*Lythrum salicaria*) in North American wetlands. *U.S. Fish Wildl. Serv. Fish Wildl. Res.* 2.
2. **Rawinski, T.J.** and **R.A. Malecki.** 1984. Ecological relationships among purple loosestrife, cattail and wildlife at the Montezuma National Wildlife Refuge. *N.Y. Fish Game J.* **31**:81–87.
3. **Balogh, G.R.** and **T.A. Bookhout.** 1989. Purple loosestrife (*Lythrum salicaria*) in Ohio's Lake Erie marshes. *Ohio J. Sci.* **89**:62–64.
4. **Arroll, S.G., C.E. Grue, C.J. Perry** and **G.L. Piper.** 1994. Potential impacts of purple loosestrife and its control on wetlands in Washington State: An evaluation of research needs. *Lake Reservoir Manage.* **9**:171–174.
5. **Malecki, R.A.** and **T.J. Rawinski.** 1985. New methods for controlling purple loosestrife. *N.Y. Fish Game J.* **32**:9–19.
6. **Notestein, A.** 1985. Experimental control of purple loosestrife (*Lythrum salicaria* L.) with herbicides at Horicon National Wildlife Refuge. *Estuaries* **8**:111A.
7. **Mal, T.K., J. Lovett-Doust, L. Lovett-Doust** and **G.A. Mulligan.** 1992. The biology of Canadian weeds. 100. *Lythrum salicaria. Can. J. Plant Sci.* **72**:1305–1330.
8. **Chapman, P.M.** 1989. A bioassay by any other name might not smell the same. *Environ. Toxicol. Chem.* **8**:551.
9. **American Society for Testing and Materials.** 1991. Standard guide for conducting static toxicity tests with *Lemna gibba* G3. E 1415. In *Annual Book of ASTM Standards*, Vol. 11.4. Philadelphia, PA, USA, pp. 1171–1180.
10. **Bennett, J.K.** 1994. Bioassessment of irrigation drainwater effects on aquatic resources in the Klamath Basin of California and Oregon. Ph.D. thesis. University of Washington, Seattle, WA, USA.
11. **Swanson, G.** 1978. Funnel trap for collecting littoral aquatic invertebrates. *Prog. Fish-Cult.* **40**:73.
12. **McCall, P.J.** and **P.D. Gavit.** 1986. Aqueous photolysis of triclopyr and its butoxyethyl ester and calculated environmental photodecomposition rates. *Environ. Toxicol. Chem.* **5**:879–885.
13. **Solomon, K.R., C.S. Bowhey, K. Liber** and **G.R. Stephenson.** 1988. Persistence of hexazinone (Velpar), triclopyr (Garlon), and 2,4-D in a northern Ontario aquatic environment. *J. Agric. Food Chem.* **36**:1314–1318.
14. **Lund-Høie, K.** and **H.O. Friestad.** 1986. Photodegradation of the herbicide glyphosate in water. *Bull. Environ. Contam. Toxicol.* **36**:723–729.
15. **Spankle, P., W.F. Meggitt** and **D. Penner.** 1975. Absorption, mobility, and microbial degradation of glyphosate in the soil. *Weed Sci.* **23**:229–234.
16. **Goldsborough, L.G.** and **D.J. Brown.** 1993. Dissipation of glyphosate and aminomethylphosphonic acid in water and sediments of boreal forest ponds. *Environ. Toxicol. Chem.* **12**:1139–1147.
17. **Rueppel, M.L., B.B. Brightwell, J. Schaefer** and **J.T. Marvel.** 1977. Metabolism and degradation of glyphosate in soil and water. *J. Agric. Food Chem.* **25**:517–528.
18. **Goldsborough, L.G.** and **A.E. Beck.** 1989. Rapid dissipation of glyphosate in small forest ponds. *Arch. Environ. Contam. Toxicol.* **18**:537–544.
19. **Gersich, F.M., C.G. Mendoza, D.L. Hopkins** and **K.M. Bodner.** 1984. Acute and chronic toxicity of triclopyr triethylamine salt to *Daphnia magna* Straus. *Bull. Environ. Contam. Toxicol.* **32**: 497–502.
20. **Wan, M.T., D.J. Moul** and **R.G. Watts.** 1987. Acute toxicity to juvenile Pacific salmonids of Garlon 3A, Garlon 4, triclopyr ester, and their transformation products: 3,5,6-Triclopyr-2-pyridinol and 2-methoxy- 3,5,6-trichloropyridine. *Bull. Environ. Contam. Toxicol.* **39**:721–728.
21. **Mayes, M.A., D.C. Dill, K.M. Bodner** and **C.G. Mendoza.** 1984. Triclopyr triethylamine salt toxicity to life stages of the fathead minnow (*Pimephales promelas* Rafinesque). *Bull. Environ. Contam. Toxicol.* **33**:339–347.
22. **Folmar, L.C., H.O. Sanders** and **A.M. Julin.** 1979. Toxicity of the herbicide glyphosate and several of its formulations to fish and aquatic invertebrates. *Arch. Environ. Contam. Toxicol.* **8**: 269–278.
23. **Mitchell, D.G., P.M. Chapman** and **T.J. Long.** 1987. Acute toxicity of Roundup and Rodeo herbicides to rainbow trout, chinook, and coho salmon. *Bull. Environ. Contam. Toxicol.* **39**: 1028–1035.
24. **Kreutzweiser, D.P., P.D. Kingsbury** and **J.C. Feng.** 1989. Drift response of invertebrates to aerial applications of glyphosate. *Bull. Environ. Contam. Toxicol.* **42**:331–338.
25. **Solberg, K.L.** 1989. Chemical treatment of monodominant cattail strands in semipermanent wetlands: Duck, invertebrate, and vegetation response. M.S. thesis. South Dakota State University, Brookings, SD, USA.
26. **Hurlbert, S.H., M.S. Mulla** and **H.R. Willson.** 1971. Effects of an organophosphorous insecticide on the phytoplankton, zooplankton, and insect populations of freshwater ponds. *Ecol. Monogr.* **42**:269– 299.
27. **Lockhart, W.L., B.N. Billeck** and **C.L. Baron.** 1989. Bioassays with a floating aquatic plant (*Lemna minor*) for effects of sprayed and dissolved glyphosate. *Hydrobiologia* **188/189**:353–359.
28. **Sullivan, D.S., T.P. Sullivan** and **T. Bisalputra.** 1981. Effects of Roundup herbicide on diatom populations in the aquatic environment of a coastal forest. *Bull. Environ. Contam. Toxicol.* **26**:91– 96.
29. **Spankle, P., W.F. Meggitt** and **D. Penner.** 1975. Absorption, action, and translocation of glyphosate. *Weed Sci.* **23**:235–240.
30. **Netherland, M.D.** and **K.D. Getsinger.** 1992. Efficacy of triclopyr on Eurasian watermilfoil: Concentration and exposure time effects. *J. Aquat. Plant Manage.* **30**:1–5.
31. **Wang, W.** 1990. Literature review on duckweed toxicity testing. *Environ. Res.* **52**:7–22.
32. **Welling, C.H.** and **R.L. Becker.** 1993. Reduction of purple loosestrife establishment in Minnesota wetlands. *Wildl. Soc. Bull.* **21**: 56–64.
33. **Skinner, L.C., W.J. Rendall** and **E.L. Fuge.** 1994. Minnesota's purple loosestrife program: History, findings, and management

recommendations. Special Publication 145. Minnesota Department of Natural Resources, St. Paul, MN, USA.
34. **Shane Gabor, T., T. Haagsma, H.R. Murkin** and **E. Armson.** 1995. Effects of triclopyr amine on purple loosestrife and non-target wetland plants in southeastern Ontario, Canada. *J. Aquat. Plant Manage.* **33**:48–51.
35. **DowElanco.** 1994. Supplemental labeling—Garlon® 3A. Indianapolis, IN, USA.