Mesocosm Evaluation of the Species-Selective Potential of Fluridone

M. D. NETHERLAND[1], K. D. GETSINGER[1] AND J. D. SKOGERBOE[2]

ABSTRACT

Fluridone {1-methyl-3-phenyl-5-[3-(trifluoromethyl) phenyl]-4(1H)-pyridinone} was evaluated at rates of 0, 5, 10, and 20 µg/L in 6,700-L outdoor mesocosm tanks for selective control of the exotic species Eurasian watermilfoil (*Myriophyllum spicatum* L). Non-target species included elodea (*Elodea canadensis* Michaux), American pondweed (*Potamogeton nodosus* Poiret), sago pondweed (*Potamogeton pectinatus* L), and vallisneria (*Vallisneria americana* Michaux). Throughout the study, untreated mesocosms were dominated by Eurasian watermilfoil and elodea with limited growth of vallisneria and the two pondweed species. Fluridone treatments were conducted on April 23 and May 23 1995 to determine if plant growth stage affected species selectivity. Fluridone residues were monitored in water and target concentrations were achieved with a measured fluridone half-life of 33 days. The 5 µg/L treatments in April (90 days of exposure) and May (60 days of exposure) reduced biomass of Eurasian watermilfoil by > 90% in late July, while biomass of non-target species generally exceeded the levels of untreated reference plants. Treatments of 10 and 20 µg/L also reduced Eurasian watermilfoil biomass by > 90%; however, these application rates also reduced growth several non-target species by > 90%. Chara *(Chara spp.)* and southern naiad (*Najas guadalupensis* (Sprengel) Magnus) propagules sprouted and attained high levels of biomass in mesocosms treated at 10 and 20 µg/L. With the notable exception of vallisneria, plants exposed to 10 and 20 µg/L in April (90 day exposure) did not show potential for recovery when placed in untreated water for 40 days. In contrast, when transferred to untreated water all species (with the exception of sago pondweed) exposed to 5, 10, and 20 µg/L in May (60 day exposure) recovered significantly in comparison to plants that remained exposed to low concentrations of fluridone (1 to 4 µg/L). Results suggest that fluridone can selectively control Eurasian watermilfoil; however, initial treatment rate, length of exposure, and initial biomass of the plants are key factors. The range of fluridone concentrations that provide selective control of Eurasian watermilfoil in a mixed plant community may be quite narrow.

Key words: Eurasian watermilfoil, herbicide selectivity, Sonar, aquatic herbicide, weed control.

[1]US Army Engineer Waterways Experiment Station, 3909 Halls Ferry Rd., Vicksburg, MS 39180.
[2]US Army Engineer Lewisville Aquatic Ecosystem Research Facility, 1 Fish Hatchery Rd., Lewisville, TX 75057. Received for publication June 10, 1997 and in revised form on July 15, 1997.

INTRODUCTION

The use of aquatic herbicides for selective removal of a nuisance exotic species can be desirable for survival and growth of native vegetation as well as for recreational usage and aesthetics of a water body. Removal of a competitive, canopy-forming plant such as Eurasian watermilfoil (hereafter referred to as milfoil) opens new areas for colonization by native vegetation, and may increase native plant density and diversity within the system (Getsinger et al. 1997). Dense surface canopies of vegetation can dramatically alter water quality indices (dissolved oxygen, pH, temperature, light penetration) on a diurnal basis, creating a hostile environment for associated aquatic biota and the plants themselves (Bowes et al. 1979, Honnell et al. 1993). In addition, by limiting negative treatment impacts on native vegetation, important structure and habitat for invertebrate and fish populations are left intact (Dibble et al. 1996). Due to the often detrimental impacts of exotic species and benefits associated with native vegetation, removal of nuisance vegetation with minimal harm to non-target plants (i.e. selectivity) is a desirable goal for managing public and private waters.

The herbicide fluridone has been widely used for the past 10 years to control milfoil. Laboratory research indicates that fluridone can provide control of milfoil at initial treatment rates as low as 4 to 15 µg/L (150 µg/L is the maximum use rate), provided an adequate exposure duration is maintained (Netherland et al. 1993, Netherland and Getsinger 1995a, 1995b). As operational treatment rates have decreased, reports from northern tier states (e.g. Michigan, Minnesota) suggest fluridone effects have shifted from producing non-selective removal of most of the submersed vegetation within the treatment year, to allowing recovery of many non-target plant species within the year of treatment (Welling et al. 1997, Kenaga 1995).

While recent field observations indicate that fluridone can be used selectively, achieving predictable results has been difficult and quantification of water residues and subsequent plant response is often limited. Uncertainties in the aqueous concentrations achieved and the length of exposure following treatment, leaves the issue of defining optimal treatment rates for selective within-season plant control unresolved.

In order to quantify better the species-selective control potential for fluridone, a study was conducted in which fluridone was applied to mesocosms containing milfoil and the native species elodea, American pondweed (American pw), sago pondweed (sago pw), and vallisneria. The objectives of this study were to: (a) determine the effect of initial fluridone treatment rate and subsequent degradation on efficacy

against milfoil and selectivity against non-target species; and (b) determine the temporal effect of fluridone treatment on efficacy and species selectivity.

MATERIALS AND METHODS

Pretreatment. This study was conducted from August 1994 to August 1995 at the US Army Engineer Lewisville Aquatic Ecosystem Research Facility, Lewisville, Texas. Details of the outdoor mesocosm system used for this evaluation have been described for previous herbicide selectivity studies (Getsinger et al. 1994, Smart et al. 1995). In late August 1994, 2000 plastic potting containers (pots), 4.7 L in size, were filled with topsoil amended with Osmocote 21-7-7 fertilizer at a rate of 15 g/kg of soil and flooded with pond water for several days. Following flooding, 1000 pots were planted with four apices of milfoil 15 to 20 cm in length, and 250 pots each were planted with either four apices of elodea 15 to 20 cm in length, or six tubers of American pw, sago pw, or vallisneria. The non-target species selected for this study are representative of common native species occurring in a milfoil-dominated community, and represent different morphological growth forms.

Planted pots were transferred to 6700-L mesocosm tanks (2.7 m in diameter and 1.4 m in depth) during the last week of March 1995. Each mesocosm tank received 30 pots containing milfoil and 32 pots containing native vegetation (8 pots of each species). Each container was marked with a plastic stake to allow for proper identification and for assessment of invasion by other species. The design for placement of the pots in the mesocosm tanks is similar to that described by Smart et al. (1995). Given that plant competition generally occurs over many years, this experimental design favored the dominance of milfoil and allowed it to form a vegetative canopy in the mesocosms by early to mid June.

A synthetic shade cloth was placed approximately 2 m above the tanks in April 1995. This canopy reduced ambient light by 30% and served to prevent the tanks from heating to levels unfavorable for plant growth. In addition, the reduction of light penetration into the generally clear water of the mesocosms favored growth of canopy forming plants.

On April 21, 1995, three replicate mesocosm tanks were assigned to ten treatments in a completely randomized design. Treatments included untreated reference tanks to be harvested on April 21, May 21, June 21, and July 21, and tanks to be treated with fluridone on April 23 and May 23.

Water Analysis. A stock solution of Sonar[R] AS formulation containing 100 mg fluridone/L was prepared, and 0.33, 0.67, and 1.34 L of this stock were applied evenly over the surface of the water in each tank to achieve nominal concentrations of 5, 10, and 20 µg/L for the April and May treatments. Air was bubbled through each tank to provide water circulation and aeration.

Water samples were collected in 500 ml amber polyethylene bottles at 2 and 12 hr posttreatment to verify initial treatment concentrations and to ensure that the herbicide was thoroughly mixed. Following these initial samples, residues were collected at 1, 2, 5, and 7 days after treatment (DAT), and weekly thereafter through 91 DAT. Samples were stored frozen until analyzed. Fluridone was analyzed using a High Performance Liquid Chromatography procedure. Mean per-cent recovery for blank and sample spikes was 95% with a minimum of 85% and a maximum of 103% (CV = 7.6%).

Growth of Untreated Plants. On April 21, May 19, and June 20, 1995, all pots were removed from untreated reference tanks and shoot biomass was sorted by species in each individual pot. Plants were placed in pre-weighed paper bags and dried to a constant weight at 70 C.

Fluridone Treatment Effects. On July 22, 1995, 10 pots containing milfoil and 6 pots each containing elodea, American pw, sago pw and vallisneria were harvested from all fluridone treated and untreated tanks. Shoot biomass was sorted according to species and then dried and weighed as described above.

April treatments had been exposed to fluridone for 90 days whereas May treatments were only exposed for 60 days. Therefore any direct comparison between these treatments should take into account the differences in initial biomass treated and length of exposure to fluridone.

Recovery Potential. On July 22 (90 DAT for April and 60 DAT for May), three pots containing each species were removed from treatments of 0, 5, 10, and 20 µg/L and transferred to tanks containing untreated water. These plants were compared for recovery potential against plants that remained in the fluridone treated tanks. Shoot biomass was harvested on August 31, at 40 days of recovery. Biomass of plants that remained exposed to low levels of fluridone for 130 days following the April treatments and 100 days following the May treatments was compared to that of plants placed in untreated tanks following 90 and 60 days of exposure respectively.

Statistical Analyses. Biomass data were subjected to analysis of variance and Dunnet's test (0.05 level of significance) to compare mean biomass values of fluridone treated plants to untreated references. In addition, t-tests (0.05) were used to compare biomass values for individual species among fluridone treatment rates.

RESULTS AND DISCUSSION

Water Residues. Residue analyses at 2, 12, and 24 hr posttreatment showed that initial target fluridone concentrations were achieved (Table 1). Subsequent analyses followed by calculation of half-lives ($t_{1/2}$) resulted in an average $t_{1/2}$ for fluridone in all mesocosm tanks of 33 ± 2 d (Table 1). Results indicate that active fluridone concentrations of 1 to 5 µg/L (based on laboratory results of Netherland and Getsinger 1995a) remained in all treatments throughout the sampling protocol.

Growth of Untreated Plants. April and May harvests of shoot biomass provided an estimate of pretreatment biomass of all species. Shoot biomass of each species generally doubled in untreated tanks from April to May (Figure 1). By June, milfoil biomass of untreated references had tripled, elodea doubled, and American pw increased by 6-fold. Sago pw and vallisneria increased only slightly (Figure 1). Milfoil biomass peaked in July, vallisneria and elodea increased slightly and a marked decrease was noted in American and Sago pw biomass. Vigorous lateral growth by elodea and fragmentation by milfoil resulted in their invasion of over 40% of the pots that had been planted with other species. Chara and southern naiad were rarely found in the untreated tanks.

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Treatment	Days After Treatment									
	0.3	1	7	28	42	56	70	84	91	$t^{1/2}$ days
Target = 5 µg/L										
April	5.5	5.1	4.4	3.2	2.6	1.8	1.4	1.1	1.0	33
May	5.1	4.9	4.2	2.9	2.2	1.4	1.1	0.6	NS	31
Target = 10 µg/L										
April	10.3	9.9	8.5	5.5	4.6	4.2	2.9	2.0	1.4	35
May	10.8	10.7	8.7	5.3	4.1	3.3	2.4	1.6	NS	32
Target = 20 µg/L										
April	20.3	19.9	17.1	12.8	10.5	8.1	5.5	4.1	3.3	36
May	20.4	20.3	17.5	11.3	9.2	6.9	5.1	3.8	NS	31

Fluridone Treatment Effects at 10 and 20 µg/L. April fluridone treatments of both 10 and 20 µg/L resulted in notable early injury symptoms in all plants. Elodea tips were chlorotic within 7 d, while the lower stems of the milfoil defoliated and the tips eventually became necrotic by 30 DAT. American pw leaves became chlorotic and were 50 to 75% smaller than those of untreated plants by 30 DAT (data not shown), while vallisneria leaves ceased growth and eventually died back to the crown by 42 DAT.

Biomass harvests following the April treatments (90 days of exposure) at 10 and 20 µg/L indicate that fluridone was not selective at these rates (Figures 2 and 3). All species declined > 90% by the July harvest compared to untreated tanks. Chara and southern naiad propagules sprouted in late June and were dominant in the treated tanks through July (Figures 2 and 3). These results are similar to those of laboratory studies in which American and sago pw, and vallisneria biomass were all significantly reduced 75 to 95% following exposure to fluridone at rates of 10 and 25 µg/L for 60 days of exposures (Sprecher 1995).
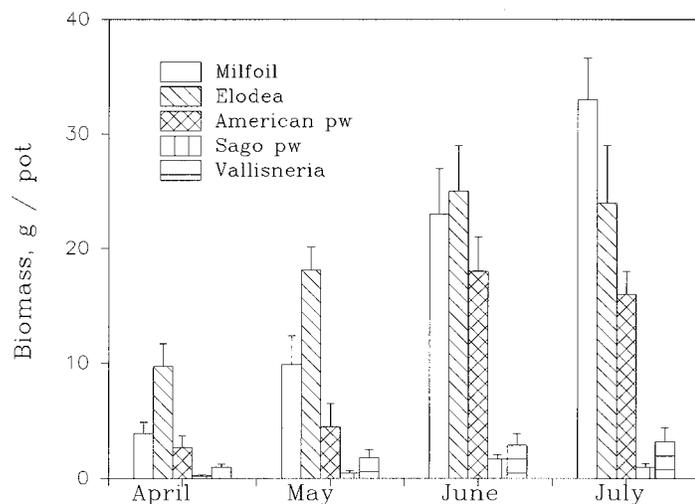
Figure 1. Dry weight biomass of untreated plants harvested over a 4 month period. Bars represent the average and standard error of three replicate mesocosm tanks. April and May data provide estimates of pretreatment biomass values.

May treatments (60 days of exposure) of 10 and 20 µg/L resulted in some notable differences in species response compared to the earlier April treatments (Figures 2 and 3). Milfoil biomass was greatly reduced; however, a significant number of stems and intact rootcrowns remained at the July harvest. At 10 µg/L, the floating leaves of American pw were 50 to 70% smaller than untreated leaves and biomass was reduced by 50% (Figure 2). As treatment rates increased to 20 µg/L, American pw biomass was reduced by >95% and no leaves were floating at the surface (Figure 3). American pw was the only species where a difference between the 10 and 20 µg/L treatments was detected. Although elodea was reduced by 37-45% compared to untreated tanks, injury symptoms (chlorotic apices) were absent during the July harvest. Both elodea and vallisneria biomass remained similar to pretreatment levels suggesting that fluridone was acting as a growth regulator. Sago pw was essentially eliminated following these treatments. Chara and southern naiad rapidly colonized pots in which vegetation was greatly reduced (Figures 2 and 3).

Both the 10 and 20 µg/L treatments were fairly non-selective and reduced community biomass by approximately 41% (Figure 4). If the pioneer species Chara and southern naiad are excluded, community biomass was reduced by > 97% for the April and 83% for the May treatments.

This study was designed to determine within-season selective potential of fluridone and therefore prediction of species that would likely recover the following growing season is not possible. Field applications in Minnesota in which fluridone residues exceeded 10 µg/L significantly reduced the within-season frequency of non-target species, followed by an increased frequency of certain species (e.g. sago pw, *P. crispus, Heteranthera dubia,* Chara and vallisneria) in subsequent years (Welling et al. 1997). Nevertheless the authors reported that many species (e.g. elodea, *P. amplifolius, P. zosteriformis, Ceratophyllum demersum, Myriophyllum* spp.) were reduced in frequency or eliminated for up to four years following fluridone treatment.

Based on several post-treatment observations from the field, it can be generalized that species producing overwintering propagules such as tubers or turions (sago pw, vallisneria, *P. crispus)* may recover much more readily the season following fluridone treatments (especially at rates > 10 µg/L) than species that overwinter in an evergreen state (e.g. *Myriophyllum* spp., elodea, *Ceratophyllum demersum*).
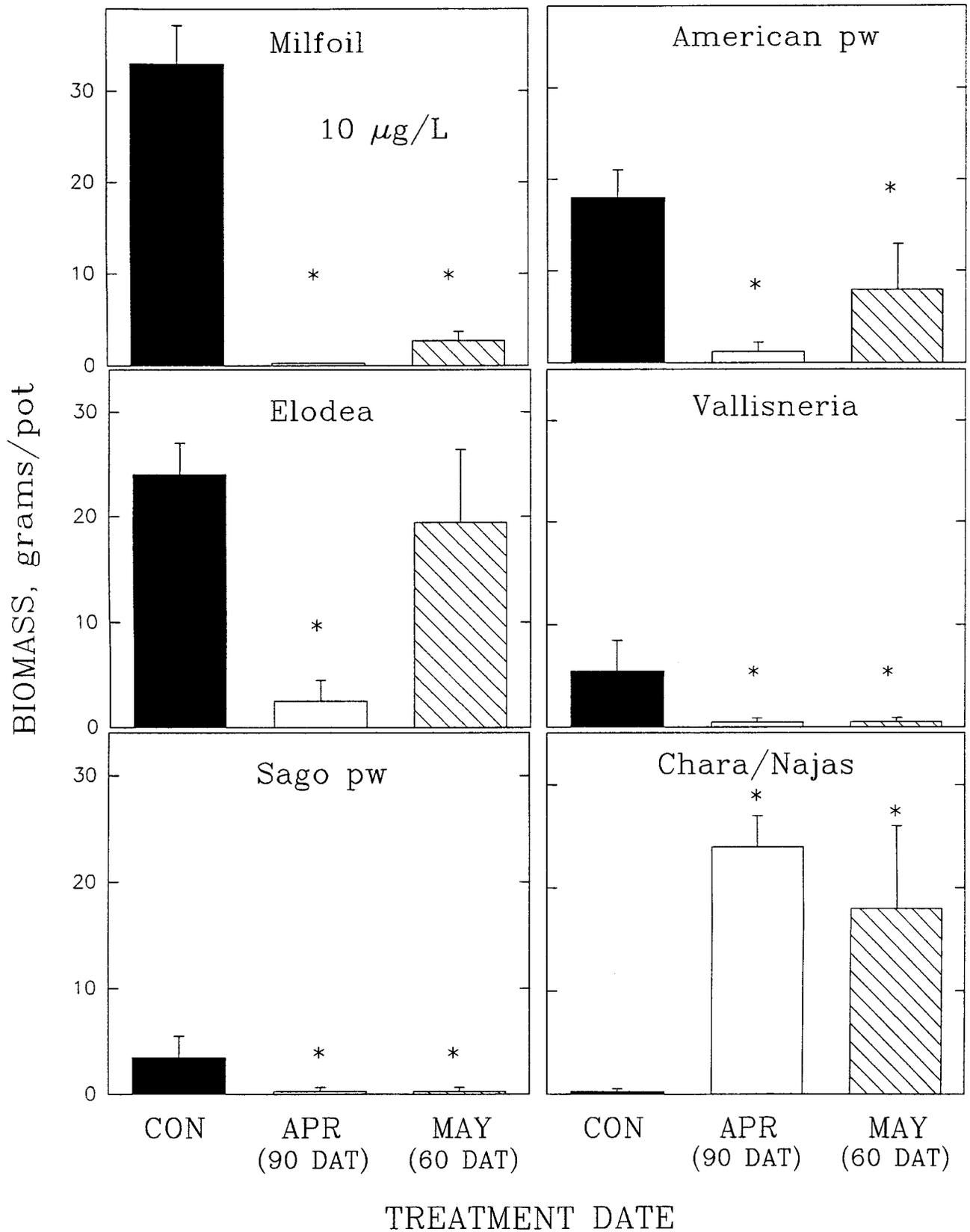
Figure 2. Dry weight biomass of untreated plants and plants treated with fluridone at 10 µg/L. Treatments were conducted on April 23 and May 23 and harvested on July 22 resulting in a 90 and 60 day exposure period respectively. Bars represent the average and standard error of three replicate mesocosm tanks. Asterisks indicate a significant biomass difference between untreated and fluridone treated plants at P ≤ 0.05 according to the Dunnet's test.
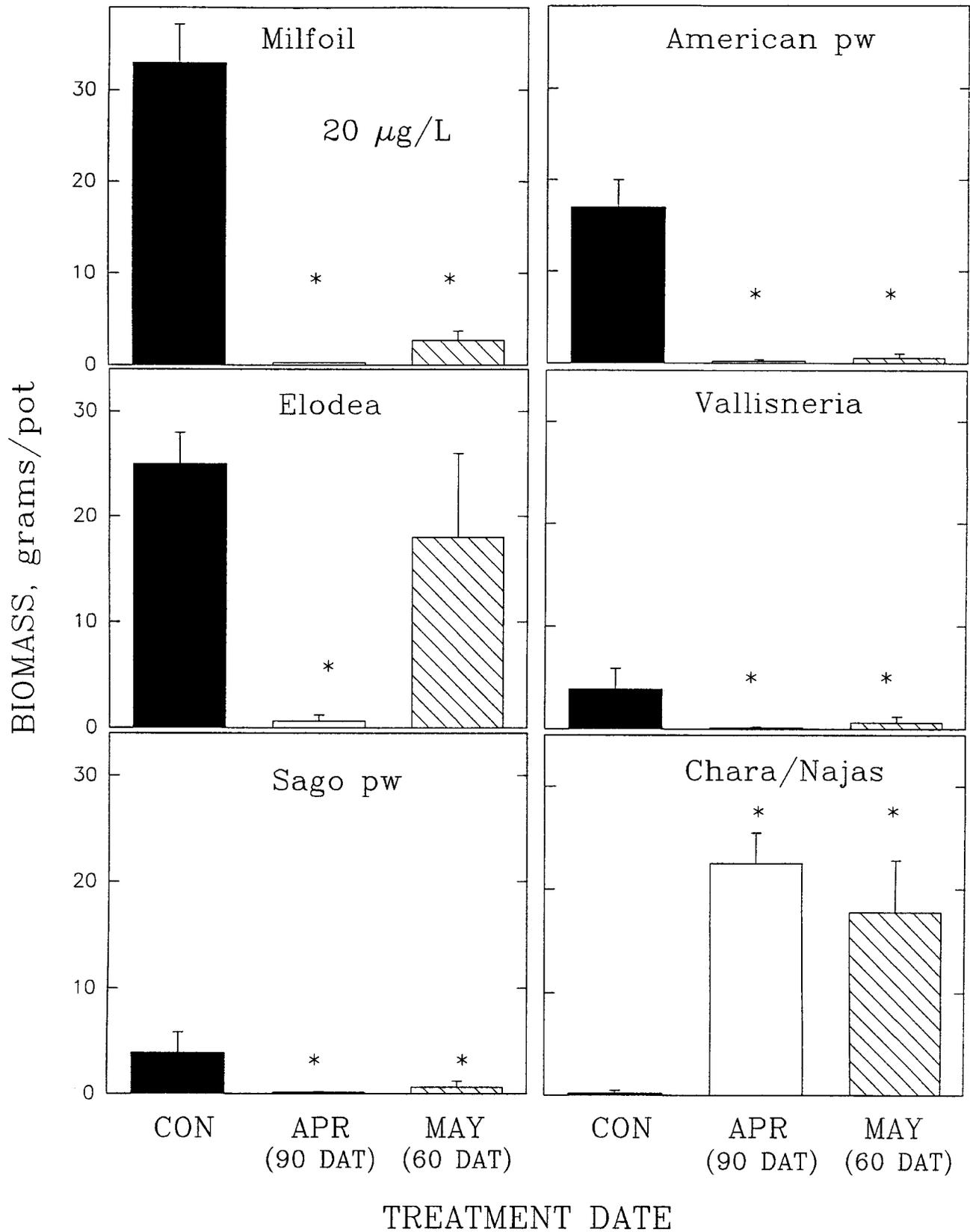
Figure 3. Dry weight biomass of untreated plants and plants treated with fluridone at 20 µg/L. Treatments were conducted on April 23 and May 23 and harvested on July 22 resulting in a 90 and 60 day exposure period respectively. Bars represent the average and standard error of three replicate mesocosm tanks. Asterisks indicate a significant biomass difference between untreated and fluridone treated plants at P ≤ 0.05 according to the Dunnet's test.
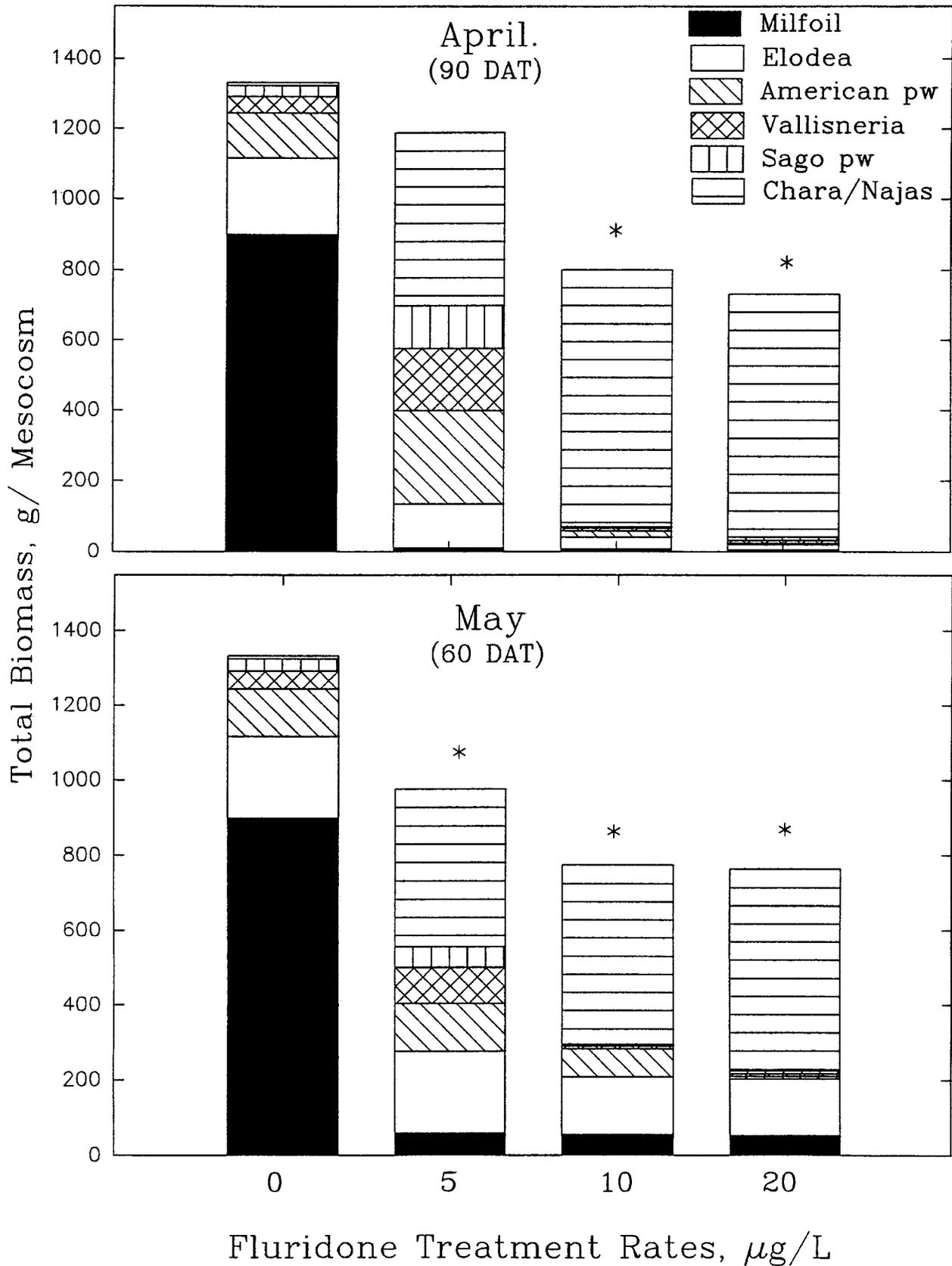
Figure 4. Total community dry weight biomass (separated by plant species) within untreated and fluridone treated (5, 10, and 20 µg/L) mesocosm tanks. Asterisks indicate a significant biomass difference between untreated and fluridone treated plants at P ≤ 0.05 according to the Dunnet's test.
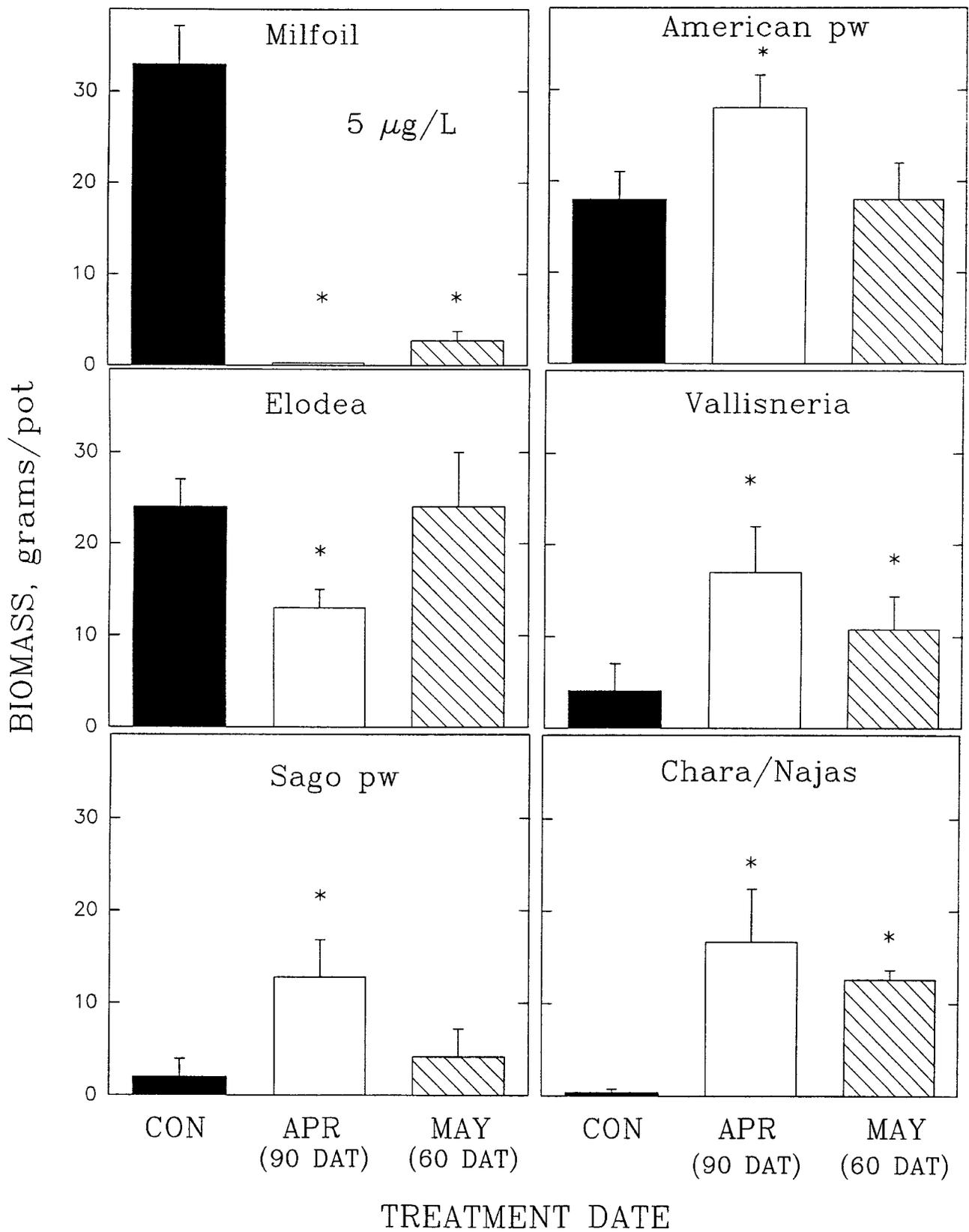
Figure 5. Dry weight biomass of untreated plants and plants treated with fluridone at 5 µg/L. Treatments were conducted on April 23 and May 23 and harvested on July 22 resulting in a 90 and 60 day exposure period respectively. Bars represent the average and standard error of three replicate mesocosm tanks. Asterisks indicate a significant biomass difference between untreated and fluridone treated plants at $P \leq 0.05$ according to the Dunnet's test.
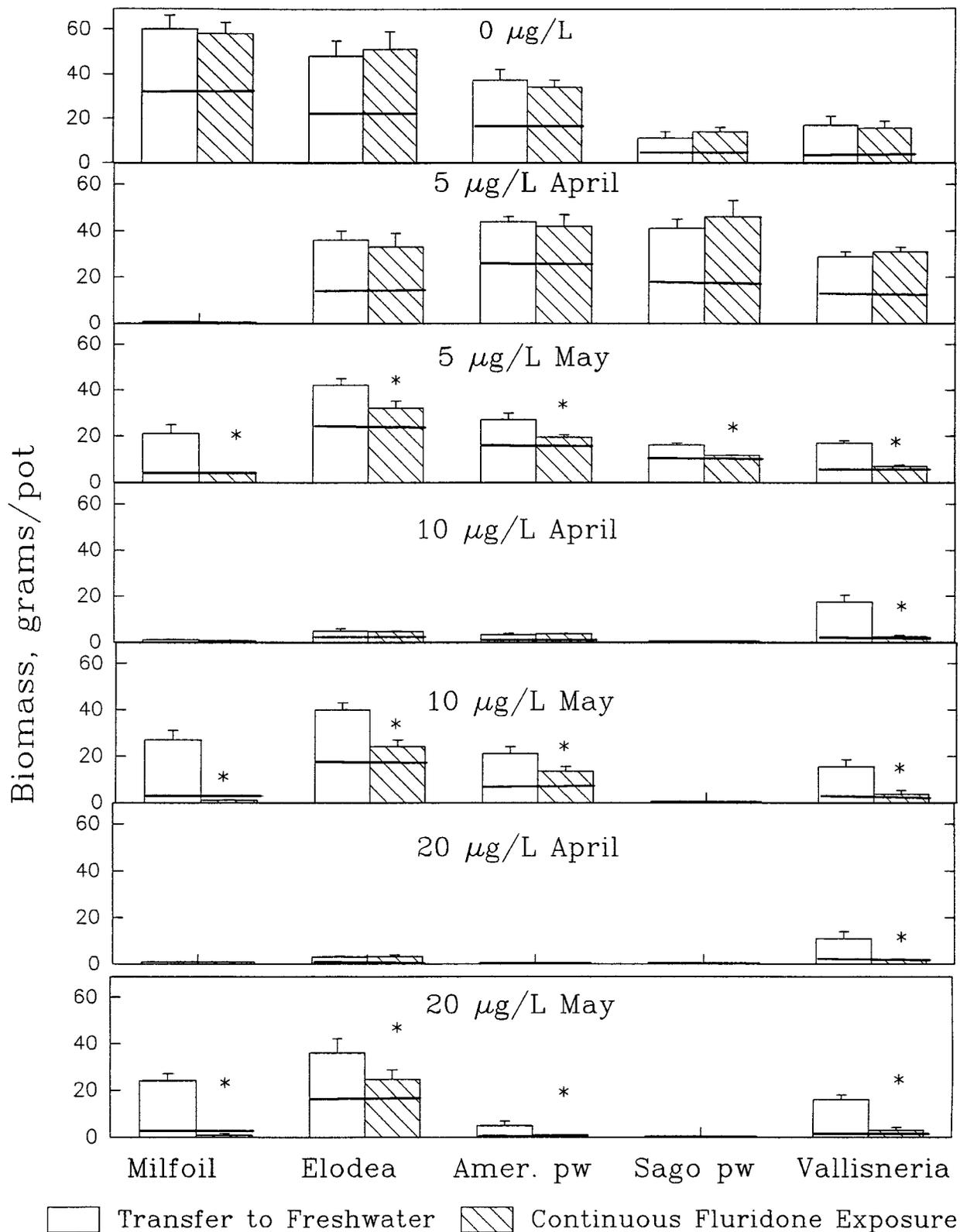
Figure 6. Comparison of dry weight biomass of plants transferred to untreated water following 90 d (April) and 60 d (May) exposure to fluridone versus plants that remained exposed to fluridone for an additional 40 days. Plants that remained in treated tanks were exposed to fluridone for 130 d (April) and 100 d (May) respectively. Untreated controls was placed in mesocosm tanks that had been treated at 10 µg/L in May. Bars represent the average and standard error of three pots. Horizontal lines through each bar represent biomass levels prior to the 40 day regrowth period. Asterisks indicate differences between treatments according to a t-test (P ≤ 0.05).

Fluridone Treatment Effects at 5 μg/L. April fluridone treatment rates of 5 μg/L provided nearly 100% control of milfoil by July (90 days of exposure), and with the exception of elodea (40% reduction), biomass of non-target species was much greater than untreated reference tanks (Figure 5). The May treatments at 5 μg/L provided about 90% milfoil control by July (60 days of exposure), while elodea, American and sago pw and vallisneria were not different from untreated controls. Chara and southern naiad colonized the majority of pots that had previously contained milfoil.

Community biomass following the 5 μg/L treatment in April and May was reduced by 13% and 27% respectively compared to untreated tanks (Figure 4) However, it should be noted that most of this reduction was due to the lower comparative biomass values of *Chara* spp. and southern naiad in pots that had previously contained milfoil.

Milfoil was the most sensitive species to fluridone treatment in this study, nonetheless a narrow range of tolerance was noted for the other species. The 5 μg/L difference between 5 and 10 μg/L treatments produced dramatically different results as far as selectivity on non-target species was concerned. Recent treatment strategies for lakes in northern states are targeting initial fluridone concentrations as low as 5 μg/L. It is believed that these treatments may increase the species-selective potential of fluridone. Based on results of this study, reducing fluridone rates by just a few μg/L may have profound impacts on improving selectivity.

Recovery potential. All species removed from the untreated controls nearly doubled in biomass. This included untreated plants that were placed in fluridone treated water (fluridone residues in these tanks were 3 to 3.7 ppb). High initial biomass and low levels of fluridone resulted in no measurable effect on these plants in comparison to plants transferred to untreated water.

The April fluridone treatments at all rates prevented milfoil recovery, yet only the 5 μg/L treatment resulted in extensive recovery by all non-target species (Figure 6). Recovery of sago pw following the 5 μg/L treatment almost quadrupled regrowth compared to untreated controls. Vallisneria recovery exceeded that for untreated controls at treatment rates of 10 and 20 μg/L, while American pw recovery was limited following these treatments. The more robust recovery displayed by certain species treated at 5 μg/L compared to untreated plants is probably due to the larger initial size of these plants due to the lack of competition from other milfoil. In contrast, 10 and 20 μg/L treatments had severely injured the plants and reduced biomass to a level where recovery was not possible. Vallisneria was a notable exception as it recovered readily from the 10 and 20 μg/L treatments when placed in untreated water.

With the exception of sago pw, plants treated in May (60 days of exposure) recovered from all fluridone treatment rates when placed in untreated water (Figure 6). Although milfoil was in very poor condition in July, enough of the stems and rootcrown remained intact to produce rapid regrowth when placed in untreated water. Elodea and American pw that remained exposed to fluridone for 100 days (see concentrations in Table 1) continued to increase in biomass (with the exception of American pw at 20 μg/L), although this growth was reduced compared to plants transferred to untreated water (Figure 6). Vallisneria also showed potential for rapid recovery when no longer exposed to fluridone. None of the species that recovered displayed any residual fluridone symptoms.

Comparison of plants exposed for 90 DAT following the April application and 100 DAT following the May application strongly suggests that elodea and American pw were less sensitive to the May treatments at the 10 and 20 μg/L application rates. In contrast, milfoil was reduced by > 98% following 100 days of exposure. Reduced growth of plants such as elodea and American pw indicate that low rates of fluridone have a growth regulator-like effect on these species.

The transfer experiments support previous laboratory studies (Netherland and Getsinger 1995a and 1995b) which showed that duration of exposure to fluridone is critical in determining the recovery potential of plants. Therefore selectivity with fluridone appears to be related to both initial treatment rate and the duration of exposure to herbicidally active residues which are as low as 1 μg/L for some species. Results of this mesocosm study suggest that within the treatment season the order of most to least sensitive species to fluridone was milfoil > sago pondweed > elodea > American pondweed > vallisneria > Chara/Najas.

It should be noted that in laboratory studies if initial treatment rates of > 3 μg/L were not achieved, milfoil was not controlled regardless of the exposure period (Netherland and Getsinger 1995b). Therefore caution is suggested when applying low rates of fluridone in the field, as a fine line exists between rates that provide good and poor milfoil control. It is likely that many species respond in a manner similar to milfoil, requiring a critical concentration and exposure period, and that these differences between species form the basis for fluridone selectivity.

ACKNOWLEDGMENTS

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