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Efficacy of Fluridone, Penoxsulam, and Bispyribac-sodium on Variable-leaf Milfoil

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INTRODUCTION

Variable-leaf milfoil (*Myriophyllum heterophyllum* Michx.) is a native perennial submersed plant ranging from southwestern Quebec and Ontario to North Dakota and southward to New Mexico and Florida (Godfrey and Wooten 1981). It is classified as a species of concern in Kentucky and is endangered in Ohio and Pennsylvania (USDA 2007). In the northeastern United States, however, variable-leaf milfoil is not native and is considered an invasive and weedy species. Variable-leaf milfoil is listed as invasive in states such as Connecticut and Maine, prohibited in Massachusetts and New Hampshire, and is a class A noxious weed in Vermont (NH-DES 2007, USDA 2007). As an invasive milfoil, it causes many of the same problems as Eurasian watermilfoil (*Myriophyllum spicatum* L.), including shading out native submersed vegetation and interfering with recreational activities and water supplies (NH-DES 2002, Halstead et al. 2003). Variable-leaf milfoil has been estimated to reduce lakefront property values by as much as 20 to 40 percent in New Hampshire (Halstead et al. 2003). Variable-leaf milfoil has been described as an aggressive invader that can grow up to one inch per day under optimal nutrient, temperature, and light conditions and spreads mainly via fragmentation (NH-DES 2002).

To date, the auxin-type herbicides are considered the most effective at controlling variable-leaf milfoil. Research by Getsinger et al. (2003) found that triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) was effective at controlling variable-leaf milfoil over a wide range of rates and exposure times. Bugbee et al. (2003) reported good control of variable-leaf milfoil treated with 2,4-D ester [(2,4-dichlorophenoxy)acetic acid] in the field. Although effective, both triclopyr and 2,4-D have use restrictions for drinking water and therefore may not be a viable option for treatment around potable water intakes. Possible alternatives for controlling variable-leaf milfoil in large-scale situations where triclopyr and 2,4-D cannot be used include the herbicide fluridone and the new acetolactate synthesis (ALS) inhibitors bispyribac-sodium and penoxsulam. The use and impacts of fluridone for whole-lake management of Eurasian watermilfoil has been addressed by several researchers (Smith and Pullman 1997, Madsen et al. 2002, Bremigan et al. 2005); however, information is limited regarding the efficacy of fluridone on variable-leaf milfoil. Moreover, aside from laboratory scale studies with the ALS inhibitor bensulfuron methyl (Nelson et al. 1993) on Eurasian watermilfoil, there is no published laboratory or field information on ALS inhibitors for either Eurasian or variable-leaf milfoil.

Fluridone has been registered by the U.S. Environmental Protection Agency (USEPA) for aquatic use for more than 30 years, while penoxsulam was registered by the USEPA for aquatic use in 2007. Bispyribac-sodium is still being evaluated for aquatic use under an Experimental Use Permit (EUP). Like fluridone, the ALS inhibitors target a plant-specific enzyme; thus, at proposed use rates they have very low toxicity to mammals, fish, and invertebrates (WSSA 2007). As such, these favorable toxicology profiles will likely preclude consumptive restrictions on water use.

Fluridone inhibits the plant enzyme phytoene desaturase (PDS) in the carotenoid biosynthetic pathway. Carotenoids play an important role in preventing photooxidative damage by quenching chlorophyll triplets that would lead to oxygen singlets (Bartley and Scolnik 1995). Without carotenoids, new plant tissue becomes bleached due to photodestruction of chlorophyll (Bartels and Watson 1978). Bispyribac-sodium and penoxsulam inhibit the plant enzyme acetolactate synthase (ALS), which is involved in biosynthesis of the branched-chain amino acids. The ALS compounds inhibit the production of the amino acids valine, leucine, and isoleucine in plants by binding to the ALS enzyme (Tranel and Wright 2002). Without these amino acids, protein synthesis and growth are inhibited, ultimately causing plant death (WSSA 2007). The impact of these slow acting enzyme inhibitors on plants such as hydrilla (*Hydrilla verticillata* [L.f.] Royle) and Eurasian watermilfoil is most notable on the actively growing shoot meristems, and extended exposures (60 to 120 days) to both PDS and ALS inhibitors are likely required to achieve plant control (Langeland 1993, Nelson et al. 1993, Netherland et al. 1993, 1997, Netherland and Getsinger 1995). Our objective was to determine the efficacy of fluridone, bispyribac-sodium, and penoxsulam on variable-leaf milfoil.

MATERIALS AND METHODS

Study 1

This study was initiated on March 9, 2006, in the greenhouse at the U.S. Army Engineer Research and Development Center's Lewisville Aquatic Ecosystem Research Facility (LAERF) in Lewisville, Texas. Plastic pots (750 ml) were filled with LAERF pond sediment amended with 3 g L^{-1} Os-

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mocote (16-8-12). Each pot was planted with two 15-cm apical tips of variable-leaf milfoil. Pots were topped with a 1-cm layer of play sand, and two pots were placed in each aquarium. Aquaria were filled with alum-treated Lake Lewisville water. Eight aquaria were situated into each of eight 1000-L fiberglass tanks filled with water. Water temperatures were maintained at 22 to 24 C. Carbon dioxide was bubbled into each aquarium once a day to reduce pH (6.5 to 7.0) for improved plant growth.

Ten days after plants were established, aquaria were treated with one of the following herbicides: bispyribac-sodium, fluridone, or penoxsulam. Treatment rates included 5, 10, and 20 µg ai L^{-1}. The treatment rates evaluated were chosen based on current proposed use rates for the submersed use of ALS inhibitors and current recommended use rates for fluridone. After treatment, herbicides were left in the aquaria for a static exposure.

At 42 days after treatment (DAT) all viable shoot biomass was harvested, dried at 65 C, and weighed. Dry weight values for bispyribac-sodium, fluridone, and penoxsulam were transformed by squaring the data to meet the assumptions of normality and equal variance. Data were subjected to one-way analysis of variance (ANOVA) and means were compared via the Student-Newman-Keuls Method (SNK; $P \leq 0.001$).

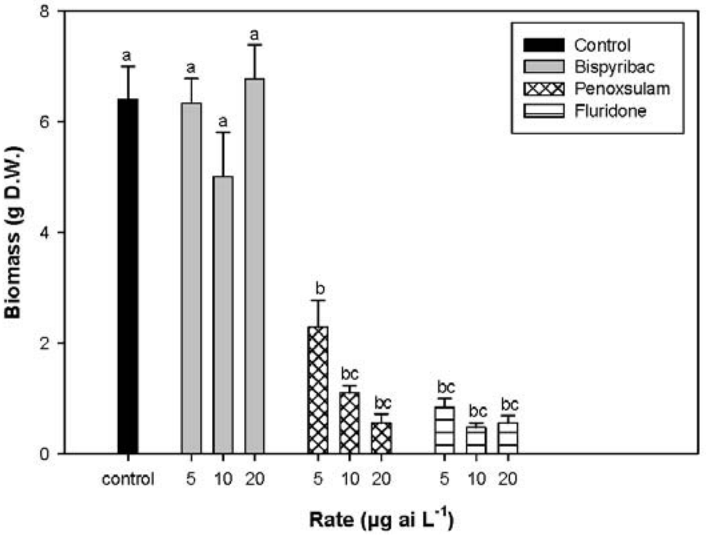

Figure 1. Mean (±SE) dry weight biomass (g) of variable-leaf milfoil biomass 42 days after treatment with bispyribac-sodium, fluridone, and penoxsulam in study 1. Each bar represents the average of four replicate treatments. Bars sharing the same letter do not significantly differ from each other. Data was subjected to a one-way analysis of variance, and means were separated using the Student-Newman-Keuls Method (SNK; $P \leq 0.001$).

Study 2

This study was set-up in the same manner as study 1; however, four pots of variable-leaf milfoil were planted in each aquarium on 13 July 2006. Ten days after planting, aquaria were treated with one of the following herbicides: bispyribac-sodium, fluridone, or penoxsulam. Rates of fluridone and penoxsulam were 2.5, 5, 10, 20, and 50 µg ai L^{-1}; bispyribac-sodium rates were 20 and 50 µg ai L^{-1}. Treatments were randomly assigned and replicated 4 times.

At 50 DAT all viable shoot biomass was harvested, dried at 65 C and weighed. Bispyribac-sodium data was square root transformed to meet the assumptions of normality and equal variance. The data were subjected to analysis of variance (ANOVA). Means were compared using the Student-Newman-Keuls Method (SNK; $P = 0.169$). Based on evidence of a dose response, fluridone, and penoxsulam data were subjected to regression analysis.

RESULTS AND DISCUSSION

Both fluridone and penoxsulam were active on variable-leaf milfoil, while bispyribac-sodium treatments showed limited activity at the use rates tested (Figure 1). Plants treated at 50 µg ai L^{-1} bispyribac-sodium in study 2 (data not shown) did not reach the water surface and showed increased production of lateral meristems and slight curling of apical tips at the time of harvest. A static exposure to bispyribac-sodium at 50 µg ai L^{-1} resulted in no biomass reduction compared to control, and effects were described as growth regulating.

Penoxsulam and fluridone were much more active against variable-leaf milfoil (Figures 1 and 2). The effect of penoxsulam and fluridone on variable-leaf milfoil (Figure 2A and 2B) can be described as exponentially curved. The lowest rate of each herbicide (2.5 µg ai L^{-1}) reduced biomass by about 27%. Fluridone at 5 µg ai L^{-1} reduced variable-leaf milfoil biomass by 75 and 87% in both studies; rates higher than 5 µg ai L^{-1} did not improve control. Fluridone symptoms appeared sooner in plants treated at the higher use rates, but by the time of harvest all treatments were similar. This lack of an improved response to increasing use rates of fluridone has also been described for Eurasian watermilfoil and hydrilla (Netherland and Getsinger 1995).

Penoxsulam controlled variable-leaf milfoil by 27 to 91% in both studies, and control increased as use rates increased up to 20 µg ai L^{-1}. There was no difference noted in the efficacy of the 20 and 50 µg ai L^{-1} treatments. Variable-leaf milfoil treated at 2.5 µg ai L^{-1} had grown to the water surface, whereas plants treated at 5 µg ai L^{-1} were vibrant and six inches below the water surface at the time of harvest. Plants treated at 10 and 20 µg ai L^{-1} had collapsed in the water column one week prior to harvest and started to decompose. Plants treated at 50 µg ai L^{-1} penoxsulam had little to no new growth after treatment and were starting to decompose at the time of harvest.

Of the herbicides tested only fluridone and penoxsulam showed potential for controlling variable-leaf milfoil at concentrations currently labeled or proposed for EUP aquatic labels. While these laboratory results suggest that variable-leaf milfoil is quite sensitive to low use rates of fluridone, reports from aquatic plant managers suggest that fluridone has provided inconsistent operational control of variable-leaf milfoil in the field. Prior fluridone studies in outdoor mesocosms have demonstrated that treatment timing is crucial for some fluridone-sensitive species such as elodea (*Elodea canadensis* Michx.), while treatment timing had much less impact on Eurasian watermilfoil (Netherland et al. 1997). Our laboratory studies suggest that plants like variable-leaf milfoil, elodea,

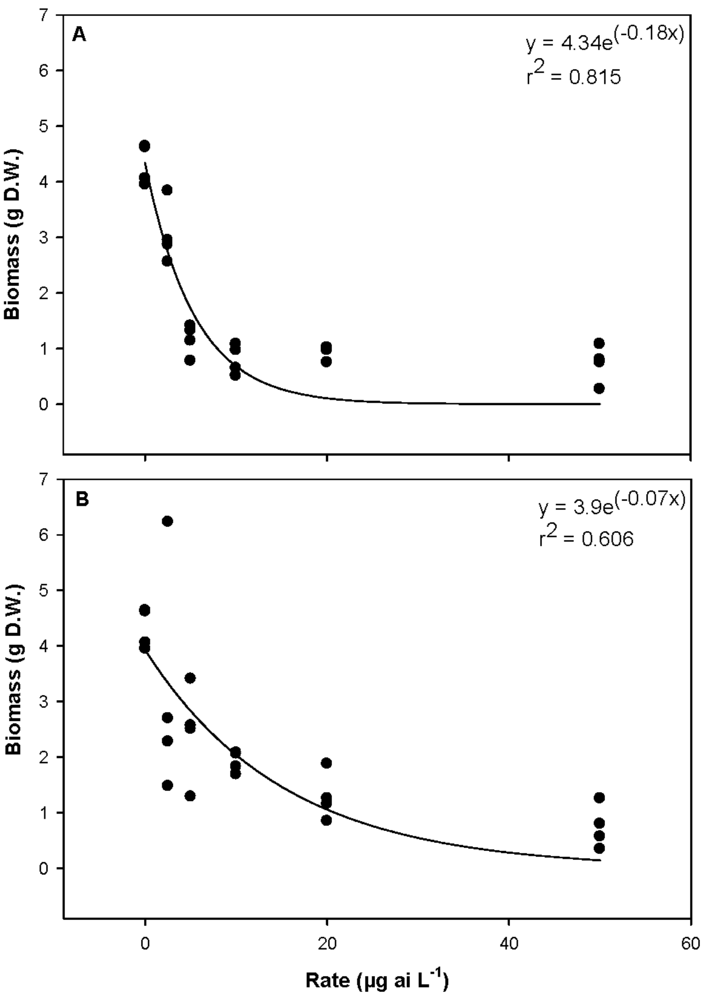

Figure 2. Dry weight biomass (g) of variable-leaf milfoil 50 days after treatment with (A) fluridone and (B) penoxsulam in study 2. Data were subjected to regression analysis.

Brazilian elodea (*Egeria densa* Planch.), and cabomba (*Cabomba caroliniana* A. Gray) can quickly grow to the water surface; however, once at the surface growth rates slow dramatically, and these plants do not form dense entangled canopies similar to Eurasian watermilfoil and hydrilla. Treatment with fluridone or ALS inhibitors when growth rates have decreased would result in reduced symptoms and less stress on the plant. The current laboratory data would suggest that early treatment of actively growing variable-leaf milfoil with fluridone or penoxsulam may allow for lower use rates than treatments conducted later in the season.

While penoxsulam was much more active than bispyribac-sodium, this difference in sensitivity to two different ALS inhibitors is not unexpected. Despite impacting a similar enzyme, ALS inhibitors show a surprising range of plant selectivity (WSSA 2007). Slight changes in the molecular structure of ALS-inhibiting herbicides greatly affect the potency and weed spectrum (Ladner 1991, Ren et al. 2000). The proliferation of ALS compounds in terrestrial agriculture has yielded several potential candidates for the aquatic market, and the differences in use patterns and selectivity in the terrestrial market suggest that we need to carefully evaluate the efficacy and selectivity of these ALS compounds. While variable milfoil is an aggressive target plant in the northeastern United States, it is often regarded as a valuable plant throughout its native range. In many cases, aquatic managers need information on both the proposed efficacy as well as the selectivity of a given compound (Koschnick et al. 2007). The information generated from these studies provides both efficacy and potential selectivity information for variable-leaf milfoil.

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