*J. Aquat. Plant Manage.* 56s: 17–25

# General guidelines for sound, small-scale herbicide efficacy research

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## INTRODUCTION

Invasive aquatic plant species can negatively impact aquatic ecosystems in a number of ways. Dense aquatic plant growth resists natural flow through aquatic ecosystems, leading to a reduction in the utility of drainage canals and hydroelectric power generation and an increase in siltation (Pitlo and Dawson 1993). Weedy species can reduce native fish and macroinvertebrate populations through reduced habitat complexity, hypoxia, and decreased food quality (Shultz and Dibble 2012), and reduce native plant diversity through competition and displacement. These infestations negatively impact the recreational utility of water bodies, reduce property values, and create habitat suitable for disease-carrying vectors, leading to an increase in the incidence of diseases such as avian vacuolar myelinopathy (AVM), malaria, yellow fever, encephalitis, and schistosomiasis, among others (Gangstad and Cardarelli 1993, Halstead et al. 2003, Wilde et al. 2005, Zhang and Boyle 2015).

One of the most commonly used methodologies for the management of invasive aquatic plants is the application of selective aquatic herbicides. Currently only 15 herbicides are registered for aquatic use. There is a need for the registration of more active ingredients and modes of action in order to respond to new threats or treatment scenarios, enhance selectivity, reduce use rates, and mitigate the potential development of herbicide resistance (Getsinger et al. 2008). Registration of additional herbicides will require the generation of consistent and scientifically sound efficacy screenings of candidate compounds for the species-selective control of target plants.

Efficacy of a compound can be impacted by the conditions under which screening occurs. Turbidity can impact the efficacy of herbicides that bind strongly to particles in the water column. For example, the highly polar and positive charge of diquat causes it to bind tightly to negatively charged clay particles in the water column, in some cases rendering it ineffective (Poovey and Getsinger 2002). High alkalinity can reduce the efficacy of copper-based algaecides, and high pH can lead to rapid hydrolysis and reduction of efficacy in carfentrazone and flumioxazin; changes in light regimes can impact the stability of compounds sensitive to photolysis (Mudge et al. 2010, Netherland 2014).

There are currently several independent laboratories in the United States with the capacity to conduct product efficacy screenings, including the more complex herbicide concentration and exposure time (CET) profiles on submersed plants. Because of the aforementioned potential differences in efficacy under varying conditions, there is a need for these laboratories to use a consistent and standardized methodology for conducting these small-scale studies, such that they may be repeated and comparable across studies and laboratories (Getsinger et al. 2008). In this article we will summarize commonly used methods and present a well-established and proven standard guideline for the evaluation of herbicide efficacy on aquatic plant species from laboratory glass flasks to small mesocosms maintained in greenhouses. Certainly there are other appropriate methods that can be used to modify the guidelines to suit specific conditions and species. Additional guidance for small-scale aquatic plant and algae toxicity testing can be found in the European-based Organisation for Economic Co-operation and Development (OECD) Guideline 201 (OECD 2006), American Society for Testing and Materials (Swanson et al. 1991), and the U.S. Environmental Protection Agency (USEPA) (Dobbins et al. 2010).

## SMALL-SCALE RESEARCH PHASES

Small-scale herbicide research is critical to the field of aquatic plant management. These research trials are important both for basic knowledge about herbicide properties as well as applied knowledge that is used to develop and refine field-use patterns. Basic trials can provide information on questions such as active-ingredient mode of action, degradation profiles, speed of activity, degree of translocation, relative sensitivity of multiple species, and other factors that outline how or why the herbicide may perform in field situations. Additional applied trials are necessary to address rate selection, efficacy of herbicide mixtures and adjuvants, CETs, timing of application, and many other considerations to refine or improve field-use patterns for maximum target control with minimal environmental impact. Research is generally approached with a series of experiments simultaneously scaling up in areal extent and/or volume of treatment and maturity of plants, as well as progressing from a controlled laboratory setting toward natural field conditions. We have broken this series into three phases that will be referred to in the general guidelines section below. The first two of these phases are only appropriate for evaluating herbicides against submersed plants, whereas the third phase can be applied to submersed, emergent, and floating vegetation. Additional guidelines have been provided in Table 1 and Appendix A.

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Table 1. Summary of specific constraints for various small-scale experimental designs.

| | Jar Tests (Phase 1) | Growth Chamber (Phase 2) | Greenhouse (Phase 3) | Outdoor Tank (Phase 3) |
|---|---|---|---|---|
| Environment | Specifically controlled | Specifically controlled | Min and max settings | Ambient |
| Container size (L) | 250 ml | 2 | 2–100 | 100–250 ml |
| Initial plant size (cm) | 2.5–4 | 6 | 10–20 | 15–30 |
| Roots at start | No | Yes or no | Yes or no | Generally yes |
| Establishment period (d) | N/A | 10 | 10–14 | 14+ |
| Water media | 10% Hoagland's solution amended with sodium bicarbonate | Smart and Barko | Smart and Barko or other | Other |
| Minimum replicates | 4–6 | 4–6 | 4 | 3 |
| Repeat in time | Yes | Yes | Yes | Yes, if possible |
| Test duration (d) | 10–28 | 14 | 14–28 | 28+ |
| Assessment schedule (DAT) | 0, 4, 7, 14 | 0, 4, 7, 14 | 0, 7, 14, 28 | 0, 7, 14, 28 |

### Phase 1

Glass jar tests were among some of the first methodologies utilized for testing herbicides for the control of submersed aquatic weeds (Oborn 1954, Lawrence 1961, Blackburn 1963). Although methods have been refined since these initial studies, this remains a productive technique to screen herbicide efficacy rapidly with the use of small (250 ml) beakers and unrooted plant tissue. Jar/beaker tests are generally conducted in a laboratory setting where light regimes, temperatures, and water-quality characteristics can be manipulated and held constant. Because of the rigor of environmental control, this methodology produces toxicity screening results with the lowest level of variability in endpoints, as compared to more complex test systems (Mohr et al. 2013). Other benefits of this initial phase include limited space requirements, which allows for the low-cost testing of several species at a time at multiple application rates.

### Phase 2

For the next phase in the progression toward field conditions a protocol similar to the OECD guidelines is recommended for evaluating submersed plants. These guidelines increase the aqueous media treatment volume from 250 ml to 2 L. Additionally, shoot apices are planted in lake sediment and allowed to mature until root growth is observed (typically 2 wk). Plants are grown in Smart and Barko liquid medium (Smart and Barko 1985), which provides a balance of micronutrients for optimal submersed plant growth. The length of herbicide exposure recommended by the OECD is 2 wk and the test subject recommended is Eurasian watermilfoil (*Myriophyllum spicatum* L.), which is a common submersed plant that is native to Europe. However, this plant is a major invasive weed in other parts of the world, particularly in North America, and may not be the most suitable candidate for product evaluations as a nontarget species under many circumstances—as it is routinely targeted for eradication/control with herbicides. The OECD protocol outlines strict guidelines for this procedure, which we will not fully cover in this article, but should be adhered to with a few exceptions. Because of the weediness of Eurasian watermilfoil, it is recommended that a broader range of species be screened and that the herbicide exposure times be increased to 4 wk (Netherland and Richardson 2016).

### Phase 3

Compounds that provide successful control of nuisance species under laboratory conditions in Phases 1 and 2 should be tested in small-scale studies located in greenhouses, shallow outdoor mesocosms, and small ponds. These middle-scale systems are suitable for evaluations of submersed, as well as emergent and floating plants. The added complexity of these test systems will add variability to the endpoints (Mohr et al. 2013); however, this complexity more closely represents the variability expected in the field, while still maintaining some level of control over temperature, day length, and water chemistry.

## GENERAL GUIDELINES FOR SOUND HERBICIDE EFFICACY RESEARCH

Many scientists have recently expressed concern about the quality of ecotoxicology research (Harris et al. 2014). Additionally, many studies have been considered "not reliable" or "unacceptable" when analyzed through quality assessment criteria (Agerstrand et al. 2011). The following are general considerations for design, implementation, and reporting of herbicide trials. These points have been modified from principles proposed by Harris et al. (2014) for ecotoxicology trials in order to be more specifically relevant to small-scale aquatic herbicide trials.

### 1. Proper study design is critical

There are several important criteria that must be included in study design in order to increase the strength of the research results and study conclusions, and to avoid the possibility of false findings. Clearly identifying the purpose of the trial will help to identify specific criteria that are most important to a successful outcome. Adequate planning must also be conducted to ensure that a proper research sequence (timeline of events) is followed. Needed measurements, such as pretreatment data, must be collected at the proper time or effort is wasted.

Key criteria include adequate replication, repetition in time and space, use of healthy organisms, test systems that will maintain healthy plants for the duration of the trial,

Table 2. Recommended sediment components from Organisation for Economic Co-operation and Development [OECD] (2014).

| Material | Target Concentration | Guidelines |
|---|---|---|
| Peat | 4–5% | Use powder form with pH 5.5 to 6.0 to obtain 2 ± 0.5% organic carbon |
| Kaolin clay | 20% | Kaolinite content >30% preferred |
| Quartz sand | 75–76% | At least 50% particle size of 50–200 μm |
| Aqueous nutrient medium | | Add as necessary to obtain "200 mg/kg dry sediment of both ammonium chloride and sodium phosphate and" obtain 30–50% moisture content. |
| Calcium carbonate | | Apply as necessary to pH to 7.0 ± 0.5 |

collection of data and observations, proper statistical analysis, and detailed reporting of methods and results that will lead to unambiguous and valid conclusions. Although each of these factors is important, methods may differ from trial to trial and still be suitable for peer-reviewed publication. Studies should be replicated a minimum of four times and repeated for a total of at least two runs separated in time. A complete block randomization perpendicular to any temperature or light gradients, if present, is recommended. A statistically strong study design needs to achieve all requirements above. If a lethal dose ($LD_{50}$) output is desired, then a minimum of five nonzero treatment rates should be used so that regression analysis can be properly performed. Herbicide rates should provide data means above and below the 50% control level, so that the regression model is valid. Recommended constraints of research design for planning purposes may be found in Table 1. OECD recommended soil components are included in Table 2.

## 2. Quantify the baseline when assessing endpoints

In many herbicide trials, untreated plants (controls) are compared to treated plants. Natural population variation should be considered and study design should be such that natural variation does not become mistaken for treatment effects. For instance, plants that are flowering may respond differently than plants that are not flowering. If plants are being propagated from shoot tips, then ideally all shoot tips would come from either flowering shoots or nonflowering shoots. If shoot tips must be collected from both, then they should be blocked by replication so that statistical analysis will take into account the natural variability. In addition, all shoot tips should be approximately the same length and taken from high-quality, healthy, and actively growing plants. Poor-quality plants may be under varying degrees of growth stress and can mask herbicide injury and treatment effects.

Long-term plant culture is another consideration. In many cases, continuous mesocosm culture of plants for long periods of time (e.g., 1 yr or longer) will result in a loss of characteristics typical of wild plants and development of characteristics more suited for mesocosms. Therefore, stock cultures should be replaced periodically or tested to ensure that the plants are maintaining characteristics demonstrated in the field.

## 3. Use controls that are appropriate for study design and test subjects

Without adequate untreated controls, herbicide effects cannot be adequately determined. The simplest control is a negative control for herbicide exposure. These are plants within the design that have no exposure and should demonstrate no response. Negative controls can be compromised by herbicide drift, volatility, tank residue, preexposure, algal growth, and likely many other confounding factors. Positive controls to provide reference numbers for expected effects may also be included. Often in herbicide trials, these represent "industry standard" treatments that are used commercially, and the treatment effects are well reported and considered predictable. Positive controls may also include treatments from previous research that properly documented the exposure effect or the symptomology of a specific mode of action. Another control that may be needed in herbicide trials would be an adjuvant control and/or herbicide with no adjuvant. Formulated products containing adjuvants may cause different responses from unformulated herbicides and proper untreated controls are required to predict what is caused by herbicide alone and what is caused by the full formulation. The same is true with formulated products containing mixtures of active ingredients. The individual herbicides should be applied at equivalent rates so that mixture effects (e.g., additive, synergistic) can be identified. As with plants in treated units, all untreated controls should be maintained to a uniform standard to ensure no treatment bias. This includes uniformity of plants (e.g., same size, same establishment period, same life stage, etc.), selection of containers (e.g., all new, all cleaned, all lined, etc.), randomization of study layout, management of algae, snails, or plant-feeding insects, and other aspects of the materials and methods.

## 4. Use appropriate herbicide exposure times and concentrations

Treatment rates to be evaluated may vary with the test species and compound tested but should cover a wide range of concentrations. Ideally in initial trials, the level of plant control obtained by the lowest concentrations tested should not significantly differ from untreated controls at the end of the exposure. Likewise, the highest concentrations tested should provide complete control (death) at completion of the study, if complete control can be achieved. Concentrations typically follow a geometric series. Clearly, determining the series of rates to test will require some prior knowledge of the compound and sensitivity of test species. Of course, resources are often limited and researchers must focus on a minimum number of rates necessary to obtain the desired output. It is even more imperative under restrictive conditions to plan the study design carefully to

ensure that the rates will provide the proper data for the desired output.

When appropriate, pilot studies may be conducted to help identify the appropriate range or series of treatments needed. Pilot studies may include three separate stages in order to elucidate fully the treatments that should be expected to provide the data required for trial success.

It should be noted that aqueous herbicide exposure periods may represent only hours of exposure when simulating treatments of submersed plants in waters that are impacted by rapid water-exchange patterns, such as in streams, rivers, or run-of-the-river reservoirs. This may also be the case when only a portion of a water body is treated with an herbicide (spot treatment)—even if the water body is essentially static. An understanding of how water-exchange processes impact herbicide dissipation patterns in various field situations can be used to select the appropriate range of herbicide exposure times in systems described for experimental phases 1–3.

*Phase 1*. The exposure time for water-only jar/beaker tests typically ranges from 10 to 28 d (Netherland and Lembi 1992, Glomski and Netherland 2011, Mohr et al. 2013, Berger et al. 2015). A minimum of 2 wk with intermittent measurement of control or efficacy is recommended.

*Phase 2*. The exposure period for the OECD-type protocols should be 14–28 d. As in the Phase 1 testing, a minimum of 2 wk with intermittent measurement of control or efficacy is recommended.

*Phase 3*. The exposure period in these more complex test systems can range from days to several weeks, or perhaps even months in large mesocosms (6,000 L) or small ponds, depending upon the compound and the ability to manipulate the test system. A minimum of 2 wk with intermittent measurement of control or efficacy is recommended.

*Static exposures*. Static exposures are frequently used in many ecotoxicology trials, but real-world environmental exposures are almost never static. This does not mean that static exposures should never be used, only that static exposures likely represent the maximum possible effect from a specific treatment and therefore may overpredict the effect that would be observed in field situations. Static-exposure trials are excellent for providing initial baseline results, quantifying the effect of a new herbicide, identifying the impact of adjuvants, quantifying the effect of an herbicide mixture, and for many other purposes. Static exposures are typically conducted during Phases 1 and 2 of small-scale testing.

In the field, aqueous herbicide concentrations are constantly reduced by environmental degradation and dissipation processes. Active ingredients are degraded by microbes, hydrolysis, photolysis, etc., and dissipation is driven by replacement of treated water with untreated water (dilution), adsorption to sediments and suspended particles, and uptake by aquatic organisms. The rates of herbicide degradation and dissipation influence herbicide exposure times (and herbicide concentrations) surrounding treated submersed plants. This CET relationship ultimately determines plant injury and treatment efficacy. Static exposures are useful for early-stage testing, comparison of sensitivities across species, or evaluation of other factors that may impact herbicide efficacy such as pH, light intensity, plant stage of growth, etc. Water replacement is another important consideration for static exposure trials. If water is lost through evaporation or transpiration, it is possible that the effective concentration actually increases during the trial period. In general, water should be added periodically to replace loss and hold levels constant.

If an $LD_{50}$ is desired, then a minimum of five nonzero herbicide rates should be used so that regression analysis can be properly performed. Rates should provide data means above and below the 50% control level so that the regression model is valid.

*Concentration and exposure-time (CET) trials*. Concentration and exposure-time trials are better designs for predicting real-world results that occur in the field and as such are generally conducted under Phase 3 conditions in greenhouses or mesocosms. All aquatic herbicides will degrade and/or dissipate to lower concentrations when applied directly to water in field situations (see above), and this reduction of aqueous herbicide levels typically occurs within the usual time period for small-scale herbicide trials (e.g., 2–6 wk). Therefore, multiple concentrations combined with various exposure times will better correlate to the herbicide exposures that will be present in field treatments of submersed plants. When foliage growing above the water surface is treated directly with an herbicide, aqueous CET relationships do not relate, because those applications are similar to terrestrial treatments where herbicide performance will be determined by factors such as rainfastness. Obviously, a level of common sense should come into play here as well. For example, herbicides designed for in-water use only (e.g., fluridone) should not be applied to plant foliage growing above the water surface; herbicides that rapidly bind in biologically active water (e.g., glyphosate) should not be applied as in-water treatments to control submersed plants, and herbicides should not be applied at a 10× rate when that specific exposure is expected to be used to predict a 1× field response.

## 5. Determine the field exposure

Specific goals of the study should dictate the number of exposures needed, the concentration of specific exposures, and whether static exposures are used or whether exposure times are varied. A minimum of five treatment rates should be used for any goal requiring regression analysis. If the study will require that treatment exposures be analytically quantified, then those exposures will have to be high enough to meet the specific criteria for the analytic test to be performed. Studies for regulatory purposes may need to determine a lowest-observed-effect concentration (LOEC), so additional exposures may be needed in the range where a LOEC is likely to be determined.

## 6. Understand test subjects

Specific plant species may respond differently under various water chemistries (e.g., Eurasian watermilfoil in alkaline vs. acidic water). A plant such as coontail would be a poor choice if the goal was to determine the impact of

herbicide applied to sediment because coontail does not develop true roots. Another good example is watermeal, in the duckweed family. Watermeal is relatively tolerant of herbicides under field conditions compared to other aquatic plants, and would generally be a poor choice if the goal were to make broad predictions of herbicide efficacy using that species alone. Likewise, Eurasian watermilfoil is very sensitive to auxin mimic herbicides and would also be a poor choice to make broad predictions (see OECD studies above), because the error is likely to be the exact opposite of a test subject such as watermeal.

When designing small-scale trials, species selection can be one of the most important decisions. Outside of targeted testing where the species is the primary factor, species selection should take several factors into consideration. Eurasian watermilfoil is an important species in European decision making; however, milfoils are very sensitive to auxin mimic herbicides and do not give a true indication of auxin sensitivity across aquatic macrophytes. Likewise, duckweed species are a popular choice historically, but duckweeds (especially watermeal) are relatively tolerant to aquatic herbicides in comparison to other aquatic macrophytes and may under represent toxicity. Ideally, research across species would include those likely to be tolerant, those likely to be very sensitive, and species that are likely to have an intermediate response.

Genetic variability of biotypes should also be considered when selecting test species. Recent work has shown that biotypes existing in nature, such as hybrid watermilfoils (Eurasian watermilfoil × northern watermilfoil) are exhibiting levels of herbicide sensitivity that differ from the parent biotypes (LaRue et al. 2012).

## 7. Use appropriate statistical analyses

If data are the bricks and boards used to build a house, then statistical analyses are the fasteners and mortar that hold everything together and provide the structure for the final product. Improper use of statistics will cause a structural failure just as quickly as bad data or poor study design. Proper understanding of, as well as proper use of, statistics are vitally important for sound conclusions. For instance, using a mean separation on structured data (e.g., multiple herbicide rates) may tell you that effects of rates A and B of a sequence A to E are not significantly different when a valid regression analysis says that each rate of the sequence created a significantly different result. Therefore, choosing the proper analysis to match the intent of the design is essential.

Utilization of a randomized complete block design is common and may be preferential depending on the research purpose. Blocking should take into account any gradients from temperature, light, air circulation, etc., so that variation from these factors will be properly accounted for in analysis of data. Although growth chamber studies should be gradient free, these conditions are more likely to be present in greenhouse or outdoor trials. Blocking can also be used to identify variability, such as what might occur from differential plant size. When in statistical doubt, consultants are available to assist researchers in trial design and analysis.

## 8. Evaluate the logic of results

In instances where results from herbicide trials are unexpected, the researchers must justify the findings or adjust the study design in order to obtain defensible results with another run of the trial. Two examples of nonstandard, but defensible results include low-dose growth stimulation by auxin-mimic herbicides or finding no differences at the trial endpoint between a contact herbicide and the control when the contact herbicide killed the plant shoot immediately after application (and the plants regrew). In the second example, an intermediate time period of data collection should show a response in between the initial high level observed and the end point with minimal response observed. It is always better to discard unneeded data rather than need an extra data set when reporting findings.

## 9. Repeat the experimental trial

Although the general rule is that every trial should be repeated in time and space, this may not be practical for every trial, field of study, or situation. Results from well-designed but unrepeated studies may be acceptable for peer-reviewed publication. For instance, a Phase 3 large-tank trial evaluating the sensitivities of multiple species to an elaborate CET herbicide treatment list would be very time consuming and resource intensive. It may not be practical to repeat such a trial in time and space. That said, a justification for the exclusion of repetition should be made when reporting results. In contrast, a Phase 1 or Phase 2 benchtop small container trial with one floating species and a small number of herbicide treatments would require few resources to carry to completion. It would be much harder to justify not repeating this type of trial.

There also may be instances where a trial is repeated with a modified treatment structure. Perhaps an additional rate is needed to encompass a full response across the rate range or a herbicide combination is added for a specific reason. In these cases it is best to maintain the original treatment structure and add to it, rather than modify a previous treatment. If questionable results are obtained, then the trial should be repeated to verify or refute the previous findings.

## 10. Consider factors that could confound results

Any factors that may influence experimental results should be considered a confounding factor. A classic example in submersed macrophyte research would be abundant algal growth, either planktonic or epiphytic. Algae could absorb/adsorb herbicide, block sunlight, alter pH, compete for nutrients, or muddle other factors. Other confounding factors may be related to herbicide properties. An herbicide with affinity for binding to plastic may best be evaluated in glass containers. Combining two unformulated herbicides may provide a different result than the two herbicides in commercial formulation with adjuvants.

Table 3. Organisation for Economic Co-operation and Development (OECD) summary of minimum biological assessments for determining herbicide impacts on plant growth. Table adapted from OECD (2014).

| | Rooted Macrophyte | | | |
|---|---|---|---|---|
| Day After Treatment (DAT) | Shoot Length, Side Shoot Length, and Shoot Number | Visual Assessment of Shoots | Shoot Fresh and Dry Weight; Visual Assessment of Roots | pH and $O_2$ |
| 0 | Required | Required | Required | Required |
| 4 | Optional | Optional | Optional | Optional |
| 7 | Optional | Required | Optional | Required |
| 14 | Required | Required | Required | Required |

Consistent growth conditions across laboratories will help to reduce confounding factors when comparing studies. Appendix 1 reviews the commonly used and recommended test conditions for the three phases of small-scale studies discussed in this manuscript.

## 11. Consider the data to be collected and then the weight of results

Measures of efficacy will vary with the mode of action of a given compound and the complexity of the study being conducted. For bleaching herbicides, such as fluridone, it is common to use methods focused on pigment loss including nondestructive methods such as pulse-amplitude-modulation (PAM) fluorometer measurements and destructive methods such as tissue analysis for total chlorophyll and total carotenoids (Netherland and Lembi 1992, Glomski and Netherland 2011, Berger et al. 2015). Electrolyte or ion leakage from plant tissue can also be measured in a Phase 1 study to indicate the level of damage an herbicide is causing (MacDonald et al. 1993, Koschnick et al. 2006, Glomski and Netherland 2013).

For more complex, longer-running studies, we recommend visual ratings of efficacy be collected at the midpoint of the study and at the study conclusion. At the conclusion of the study plants should be separated into shoot and root tissues, dried (at least 48 h at 70 C), and weighed on an analytical balance to determine dry weight (biomass) for samples. Additional intermittent measures of efficacy that can be nondestructively measured include total shoot length and PAM fluorometry (OECD; Ralph et al. 1998). Dry biomass, percent control measures, and total shoot length can be used to generate environmental concentration $EC_{50}$ and $EC_{90}$ values based upon a percent inhibition of growth. The OECD assessment schedule includes minimum data collection recommendations (Table 3).

## 12. Report findings in a clear and unbiased manner

According to the scientific method, all research should be conducted in a manner such that another individual can repeat the study exactly as conducted in order to verify the findings. Apart from groundbreaking research, very little research today is repeated exactly as originally conducted. However, that is not adequate justification for researchers to omit details, or even worse, "spin" results in a different angle than where the data leads. All methods, including statistical analyses, should be clearly reported so that the trial could be repeated if needed.

Results should also be reported in a clear manner and in ways that are substantiated. If one trial produces a significant difference between treatment A and B, but another trial shows no difference, the abstract should not simply state that treatment A provided greater control than treatment B. Likewise, use of words such as "control" (in context of herbicide did control the weed) should have uniform meaning across published studies and not mean 50% biomass reduction for one author, but 90% biomass reduction for others. It is important to state explicitly what is meant by terms such as "control."

In conclusion, small-scale studies are critically important for the development and refinement of herbicide use patterns. Following sound guidelines while implementing these trials will ensure that maximum benefits are obtained and results can be efficiently translated to field management programs.

## ACKNOWLEDGEMENTS

We thank Andrew Howell for his assistance in manuscript preparation, as well as Kurt Getsinger and Jay Ferrell for their review of this manuscript.

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## APPENDIX: TEST CONDITIONS

### Treatment vessels

*Phase 1*. Initial screenings of herbicides typically occur in small (~250 ml) glass treatment flasks or beakers—for submersed plant test species.

*Phases 2 and 3*. Treatment vessels should be made of inert materials and should be of sufficient depth to allow for submersed plant growth above the sediment level during course of the experiment (Smart and Barko 1985). Typically depths range from 12 to 18 in. Vessel material should take into account convenience, price, and ability of the given compound to bind to the material. Herbicides with a high affinity for glass, such as diquat, should be evaluated in plastic container alternatives (Connard and Criddle 1975). The use of liners where applicable to help mitigate the potential of previously used chemicals to leach into and impact future experiments performed in the same vessels is recommended. If unlined vessels are to be reused for additional trials, soaking and cleaning them with a dilute bleach and/or exposure to direct sunlight is recommended. These cleansing treatments will aid in breakdown of herbicide residues that might remain in the vessels.

### Greenhouse material

*Phase 3 only*. When working with light-sensitive compounds one should consider whether the study should be conducted in a glass greenhouse or a plastic greenhouse. In general glass greenhouses will block UV light to a higher degree than older plastic greenhouses. However, modern plastics also block some UV light. Likewise, the use of a shade cloth over plastic greenhouses will decrease UV light and help regulate temperature within the greenhouse. A good understanding of the material that greenhouses are made of and their potential to block UV light should be considered, if light sensitivity or photo-degradation is a concern.

### Soil

*Phase 1*. No soil is used in these small-scale jar tests.

*Phase 2*. The OECD guidelines recommend the use of sifted natural lake sediments for this scale of testing. Previous small-scale studies have incorporated 1–5 g/L of Osmocote® as a slow-release fertilizer amendment to ensure nutrient availability throughout the course of the efficacy study (Netherland et al. 1993; Netherland and Getsinger 1995; Glomski and Netherland 2007; Everitt et al. 2011; Vassios et al. 2011). In an effort to maintain consistent growth conditions, we would recommend the use of topsoil amended with 3 g of Osmocote per liter of soil.

*Phase 3*. Smart and Barko (1985) recommend the use of sifted natural lake sediments for the long-term culturing of aquatic plants. They state that commercially available potting soil is not suitable for plant growth because of limited nutrient availability and buoyancy of components. However, natural lake sediments are tedious to collect, sift, and settle. Natural lake sediments also contain a large seed bank that cannot be removed by sifting alone and in some cases even by steam sterilization (personal communication, Sara True-Meadows, U.S. Environmental Protection Agency). In addition to the high resource requirements, natural sediments can vary considerably across locations in terms of fertility and texture. Analogous of 2, in an effort to reduce the effects of sediment differences on plant growth, the use of topsoil mixed with Osmocote at a rate of 3 g/L of soil is recommended. Topsoil is not buoyant, and with the addition of Osmocote slow-release fertilizer, has been shown to be an effective substrate for the growth of aquatic plants at least in short-term (less than 3-mo) studies. A layer of washed sand should be placed at the sediment–water interface to hinder suspension of soil particles into the water column (Netherland et al. 1993).

## Water

*Phase 1*. Deionized (DI) water amended with a nutrient solution is recommended. Nutrient solutions for these studies vary including Gerlof's, M4 medium, sucrose, Andrew's Medium and Hoagland's solution (Netherland and Lembi 1992, Knauer et al. 2006, Mohr et al. 2013). All of these nutrient solutions are appropriate; however, we would suggest the use of a 10% Hoagland's solution amended with sodium bicarbonate.

*Phase 2*. The OECD guidelines recommend the aqueous medium for treatment be composed of DI water amended with Smart and Barko (1985) solution. It is also recommended that the pH not increase by 1.5 units over the course of the test.

*Phase 3*. Using conditioned tap water is recommended for Phase 3. An approximately neutral pH water is best, unless a different pH is required for activity of the herbicide, or optimal growth of the plant (Spence 1967, Hutchinson 1970, Hellquist 1980, Mudge et al. 2010). Herbicides that require low pH for activity, such as flumioxazin, should be applied early in the morning when pH is naturally lowest. If an additional drop in pH is necessary, aeration with $CO_2$ can be used to reduce pH without negatively impacting plant growth. To ensure uniform plant growth among emergent or floating species, the use of overhead mist irrigation at 0.635 cm to keep plantings saturated twice daily while rearing test subjects prior to treatment is suggested. If applicable, incorporating a water-exchange procedure in treatment vessels may be beneficial for reducing algae, maintaining temperature, and increasing water-column $CO_2$ prior to treatment.

## Species selection

A wide variety of genera including target and nontarget species, monocots and dicots, submersed, floating, and immersed, should be tested to achieve a full sense of the activity and species selectivity of the compound. Because of its relative ease and reduced resource requirements, Phase 1 should be utilized to test the largest number of species, and then activity on a subset of these species should be confirmed in Phases 2 and 3.

## Plant size

*Phase 1*. In previous studies, plant segments generally ranged in size from 4 to 7 cm, with 4 cm being more common (Netherland and Lembi 1992, Knauer et al. 2006, Glomski and Netherland 2011).

*Phase 2*. The OECD guidelines recommend the use of 6-cm (±1 cm) healthy apical tips. The tips are planted such that they are 3 cm are below the sediment surface. This planting technique will allow for adequate development of adventitious root growth in the sediment.

*Phase 3*. Healthy shoot fragments from submersed plants of equivalent size and maturity should be cultivated. Generally, fragments of submersed species for this scale range from 10 to 15 cm (Netherland et al. 1993; Everitt et al. 2011, Vassios et al. 2011), with 5 cm planted below the sediment (for adventitious root development) and 5–10 cm maintained in the water phase. Healthy floating plants of equivalent size are recommended for testing purposes. Tubers, rhizome fragments, or winter buds are recommended to cultivate emergent plants for testing.

## Light and temperature

*Phase 1*. Following treatment, flasks are maintained in indoor growth chambers for the duration of the study. Light and dark (L : D) regimes are typically either 16L : 8D or 14L : 10D (Knauer et al. 2006, Glomski and Netherland 2013). Selection of L : D regimes should be selected based on field day-length conditions to be simulated.

Temperatures are typically maintained between 20 and 26 C. Light intensity can exhibit the most variation between studies. A light intensity of 140 (±20) μE $m^{-2}$ $s^{-1}$ (OECD) is recommended. Full-spectrum or halogen lighting should be used, as many fluorescent lights do not provide the necessary radiant power of the appropriate wavelengths for photosynthesis (Lobban et al. 1985).

*Phase 2*. The OECD guidelines recommend the use of white fluorescent lighting with an irradiance in the range of about 140 (–20) μE $m^{-2}$ $s^{-1}$ (±15%) at the water surface and a L : D ratio of 16 : 8 h. For this phase it is also recommended that temperature be maintained at 20 ± 2 C. When testing light-dependent herbicides, laboratory

lighting may require supplemental ultraviolet light to reach levels found in natural sunlight.

*Phase 3*. Greenhouse trials will utilize natural light. Lighting should be supplemented to a minimum of 14 h of daylight utilizing overhead halogen bulbs to account. Often, ultraviolet radiation from daylight may be too intensive for young plants at shallow depths, and the use of a 30% shade cloth is recommended for those situations. Day and night temperatures should remain constant or be maintained to simulate field-appropriate diurnal responses.