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Confusion and ambiguity concerning the terms "resistance" and "tolerance" in aquatic plant management

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My view

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Introduction

The Weed Science Society of America (WSSA) defined the terms "resistance" and "tolerance" in 1998 to provide an official position so that terms are used accurately in all WSSA publications (WSSA 1998). The Aquatic Plant Management Society (APMS), an affiliate organization of WSSA, subsequently adopted the official WSSA definitions. However, distinguishing between tolerance and resistance has proven difficult for the aquatic weed management community; there has been disagreement over which term is appropriate in any given situation and a general default to using the word "tolerance" to describe most cases of reduced plant response to a herbicide.

In this paper, we argue that the terms "resistance" and "tolerance" as defined by the WSSA (1998) are generally appropriate for aquatic plants. However, we note potential sources of confusion and ambiguity as they relate to applying these terms in aquatic plant management versus the agricultural settings from which the original definitions emerged.

WSSA Definitions and Their Interpretation

The WSSA (1998) defines herbicide resistance as "the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type. In a plant, resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis."

The WSSA defines herbicide tolerance as "the inherent ability of a species to survive and reproduce after herbicide treatment. This implies that there was no selection or genetic manipulation to make the plant tolerant; it is naturally tolerant."

A key distinction between the two terms is that herbicide tolerance describes differences in herbicide response *between* species, whereas resistance refers to differences among genotypes (or lineages) *within* a species (Figure 1). So, one can ask the following simple question to determine which term is most appropriate: "Am I describing a species, or something nested within a species (populations, genotypes, strains, etc.)?" The term "resistance" should be used to describe variation in herbicide response that occurs within a species, such that some lineages are not killed (or controlled) to the same extent as we expect based on our experience with what is "typical" or "normal" (i.e., wild type). Further, in cases of resistance, the intraspecific variation for herbicide response has occurred—or otherwise been identified—after the introduction of a herbicide used to control the target species. In contrast, the term "tolerance" is reserved for entire species that are insensitive to a particular herbicide use pattern without any previous exposure to the specific herbicide, herbicides in the same family, or herbicides having the same mode of action.

Sources of Ambiguity and Confusion Regarding Resistance in Aquatic Plant Management

What Is the Wild Type?

A key facet of the WSSA's definition of resistance is whether the individual or population has a higher lethal dose than the wild type. The Oxford Languages' dictionary definition of wild type is "a prevailing characteristic of individuals in natural conditions, as distinct from an atypical mutant type." For example, our mental image of an Eastern gray squirrel (*Sciurus carolinensis*) is a gray-brown body with a white underside. However, there are also melanistic (black) or albino (white) squirrels in particular places, and these are considered mutants, because they are

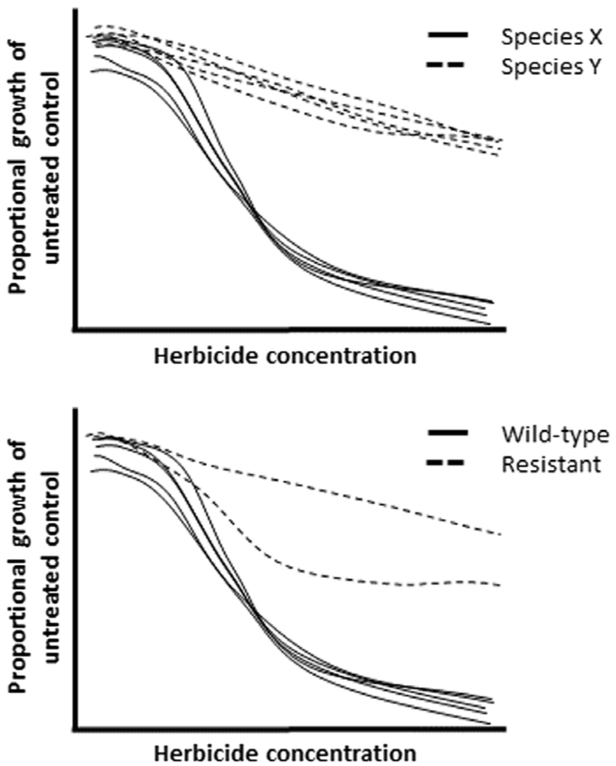

Figure 1. (Top) Hypothetical dose–response curves for several individuals, families, or populations for each of two different species. Species X is considered normally susceptible, whereas Species Y is considered tolerant. Note that in this case, the variation in response is primarily at the between-species level. (Bottom) Hypothetical dose–response curves for several different genetic individuals (i.e., clones), families, or populations within a single species (e.g., Species X). The species is considered to have a susceptible wild type (solid lines), but upon detailed examination of many genetic lineages, some are found to exhibit resistance (dashed lines). Note that in this case, the variation in response is among genotypes within the species.

unusual in their phenotype relative to what we consider "normal" (the wild type) for a gray squirrel. An important point here is that melanistic and albino squirrels are not recognized as different species; they are recognized as variants *within* the same species.

Research and development studies have specifically focused on application rate and timing interactions to achieve "consistency" of herbicide performance across landscape-sized application areas. However, it is important to note that studies to test the efficacy of herbicides on invasive aquatic plant species have not historically considered intraspecific genetic variation for herbicide response. Therefore, these studies have not had any clear understanding of the natural, quantitative variation in herbicide response in order to calibrate an expected wild-type response that is broadly efficacious across preexisting, natural variation. Therefore, we recommend that future dose–response studies for aquatic herbicide use pattern development be conducted on a random sample of populations (or clones, or families, etc.).

Despite this caveat, a number of aquatic herbicide use patterns have been developed for target aquatic plant species (e.g., dose–response studies). As a practical matter, we should consider the wild type for these species to be susceptible to the herbicides that have frequently been used to control it. For example, in Michigan, fluridone is used at 6 μg L^{-1} to control Eurasian watermilfoil (*Myriophyllum spicatum* L.), because early studies concluded that dose as one that should normally be lethal (Getsinger et al. 2001; MESB 1999). Therefore, a particular genotype (or strain) of *M. spicatum* that can survive and grow when exposed to 6 μg L^{-1} under the same environmental conditions and exposure time would be considered a resistant mutant.

Defining the wild type could have some ambiguity, depending on how taxa or groups are recognized. For example, there has been confusion as to whether tolerance or resistance is the appropriate terminology for interspecific hybrids between invasive aquatic *M. spicatum* and native northern watermilfoil (*Myriophyllum sibiricum* Kom.). Many aquatic plant management scientists and practitioners have preferred to use the term "tolerance" to describe hybrid watermilfoil (*Myriophyllum spicatum* L. × *Myriophyllum sibiricum* Kom.) under the assertion that hybrid *Myriophyllum* generally exhibit reduced responses to commonly used herbicides compared with pure *M. spicatum*; that is, the reduced response to herbicide is a property of hybridity. However, a synthesis of the scientific literature available demonstrates that many hybrid genotypes are impacted similarly to pure Eurasian genotypes and that only specific genotypes exhibit reduced susceptibility (Berger et al. 2012, 2015; Chorak and Thum 2020; Hoff and Thum 2022; Netherland and Willey 2017; Poovey et al. 2007; Slade et al. 2007; Thum et al. 2012). In other words, reduced herbicide response does not appear to be a general property of hybridity, per se. Therefore, the term "resistance" is more appropriate to describe the specific hybrid genotypes that exhibit reduced responses to certain herbicides.

The confusion and ambiguity over whether tolerance or resistance is more appropriate to describe hybrids further illustrates the importance of explicitly considering genetic variation in dose–response studies for aquatic herbicide use pattern development. Such studies would better capture the quantitative variation in herbicide response for a taxon or group, and therefore clarify whether resistance is more appropriate to describe variation in herbicide response among lineages within the taxon/group versus whether the taxon/group as a whole is tolerant relative to other groups/taxa.

The other two well-documented cases of resistance in aquatic plants—fluridone resistance in hydrilla [*Hydrilla verticillata* (L. f.) Royle] (Michel et al. 2004) and diquat resistance in dotted duckweed [*Landoltia punctata* (G. Mey.) Les & D.J. Crawford] (Koschnick et al. 2006)—have not confused the terms "tolerance" and "resistance," because there have been no questions regarding the taxonomic status of the less-susceptible lineages; they are clearly part of the same taxonomic group for which we consider susceptibility to these herbicides to be the wild type.

What Is a Dose Normally Lethal to the Wild Type?

For submersed aquatic plants, a herbicide dose is defined as a combination of the herbicide concentration and its exposure time.

It is important to recognize that the term "resistance" does not mean that a plant will survive *any* dose of a herbicide, because all plants will succumb to some dose of a herbicide. Rather, resistance is the ability of a lineage to survive a dose that is normally lethal to the wild type.

Our evaluation of what constitutes a dose that is "normally lethal" to the wild type is limited by the extent of sampling natural variation in target species. Thus, one area for improvement in developing herbicide use patterns for aquatic plants is to conduct dose–response studies on a random (or representative) sample of populations (or clones, or families, etc.) that cover a range of natural variation in order to calibrate an expected wild-type response. Such studies could also help clarify any possible confusion and debate about the terms “tolerance” and “resistance” as they relate to determining a dose that would normally be lethal to the wild type.

Defining a dose of herbicide that is normally lethal to the wild type has been a source of confusion or ambiguity—and thus debate—regarding the term “resistance” for aquatic plant managers. There is rarely a standard “field rate” of a herbicide to control a particular invasive aquatic plant species in different states; different states use different herbicides in different ways. Although aquatic herbicide labels carry maximum rates for target species, applying at maximum label rates is often inadvisable (or prohibited). This is particularly true in multi-use, public water systems that have a mixture of native and invasive species, and where selective control methods are desired to conserve or promote non-target, native plants. In fact, a great deal of agency funding has gone toward identifying the lowest herbicide rates to balance control of the target species against non-target effects and to minimize herbicide discharge into public waters (APMS n.d.). In short, different doses (concentrations and exposure times) are used in different places and for different reasons. This admittedly leaves some room for debate regarding whether allowable herbicide doses within a given state are sufficient, and therefore whether a lineage of aquatic plants that survives a particular herbicide application should be considered to be resistant.

The Herbicide Response Action Committee (HRAC) acknowledged a similar problem in terrestrial agricultural weeds—that different rates are used in different regions and for different crops. The HRAC defines herbicide resistance as “the evolved capacity of a previously herbicide-susceptible weed population to withstand a herbicide and complete its life cycle when the herbicide is used at its normal rate in an agricultural situation” (Heap and LeBaron 2001, p. 2). Thus, the HRAC’s resolution to the problem of different rates in different places is that a case of herbicide resistance must satisfy the criterion that there is a practical impact, in that the resistant population has caused a problem of control in the field when the herbicide is used at the recommended field rate. To go back to our example of fluridone resistance in *Myriophyllum* in Michigan, we argue that this criterion is met. First, the observed response of two fluridone-resistant strains are unusual in the sense that most strains appear to be sufficiently controlled at 6 μg L^{-1} fluridone. Second, because 6 μg L^{-1} is the recommended (enforced) use pattern in that state, survival of these strains to this use pattern has practical implications; namely, that the observed control is disproportionately low relative to the cost. In other words, 6 μg L^{-1} fluridone should not be used on these strains because it will not *consistently* manage plant populations with high proportions of these strains. We have observed a third *Myriophyllum* strain that exhibits an intermediate level of resistance based on laboratory dose–response curves (A Wolfe and RA Thum, unpublished data). However, it is unclear how this level of resistance translates into control efficacy (or lack thereof) in the field. Therefore, this constitutes an unconfirmed case of resistance, because it is not clear whether that level of resistance would have a practical impact in a 6 μg L^{-1} operational fluridone treatment.

To further complicate matters for aquatic plant managers, achieved herbicide doses are rarely measured during operational management. This is especially true for treatments where the herbicide is applied only in priority areas, as opposed to the waterbody as a whole (“spot treatments”). Such herbicide application patterns commonly result in dilution and dissipation of the herbicide that results in doses lower than the target dose (e.g., Nault et al. 2012). Therefore, in addition to uncertainty and debate about what doses would be lethal or sublethal, there is often uncertainty regarding what dose was actually achieved. In our experience, explanations for qualitatively insufficient efficacy often question herbicide dose before potential resistance.

Finally, in our opinion, the hesitancy to use the term “resistance” in aquatics reflects the perception that resistance factors identified in the small number of documented cases in aquatics are much smaller than resistance factors in a large number of documented cases in terrestrial agricultural weeds. While it is true that resistance factors in aquatics can be relatively low (approximately two to five for documented cases of *H. verticillata* fluridone resistance [Michel et al. 2004], *Myriophyllum* fluridone resistance [Berger et al. 2012; Thum et al. 2012], and *Myriophyllum* 2,4-D resistance [LaRue et al. 2013; HK Hoff, RM Newman, E Fieldseth, RA Thum, unpublished data]), resistance factors for *L. punctata* to diquat and paraquat were 50 and 29, respectively. And, although terrestrial herbicide resistance factors can be so high that several herbicides are no longer recommended for use for some terrestrial species, resistance factors exhibit a wide range in terrestrial species and can be as low as aquatics examples (e.g., 2,4-D resistance in a waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer] population had a resistance factor of ~3; Shergill et al. 2018). Nevertheless, it is important to recognize that low-level resistance in aquatics means that the herbicide can still be effective at higher rates that remain below maximum label rates. For example, many practitioners recognize that hybrid *Myriophyllum* commonly requires slightly higher rates of auxinic herbicides to have similar efficacy compared with pure *M. spicatum* and will therefore frequently use higher rates on known hybrid populations. In these cases, it is difficult for practitioners to refer to hybrids as resistant for fear that the auxinic herbicide(s) will not be permitted for control. We believe that the term “resistance” elicits fear for aquatic plant managers that low-level resistance will lead to a misperception that the herbicide is no longer effective at any practical rate. Therefore, the identification of resistance in aquatics should not necessarily be equated with the herbicide being completely unable to control a target species, but rather should be qualified with the magnitude of resistance and concomitant consideration of whether the herbicide can or cannot be used at a higher rate to accomplish site-specific management goals.

What Does “Naturally Occurring” Mean?

The word “naturally” appears in the definitions of both resistance and tolerance, and it is therefore important to comment on it here. We believe the addition of the word “naturally” in the definition of tolerance may cause some confusion among aquatic plant managers. As written, the term appears to juxtapose the idea of selection. However, the origin of a resistant mutant in a population is a natural process, as is the selection of a resistant mutant by herbicide application (i.e., natural selection). The contrast of the term “natural” with human manipulation is warranted, but the human application of herbicides and the natural selection that follows should not be confused. The key point about tolerance is that it is a property of a species that arose before the development and application of herbicides to control weeds; it is a property of its history.

It may also be confusing to use the term “naturally,” because it can be tempting to think of resistance as starting off as tolerance; a mutant that arose naturally within a population may be considered to be tolerant. Then, after a period of selection with a herbicide, the mutant allele increases in frequency, leading to population-level resistance. With this mental model, it is understandable to think of resistance in natural populations arising as a function of natural tolerance in the original mutant. However, this model is incorrect, because tolerance is a property of species, whereas resistance reflects variation within a species. Therefore, resistance genes arise via the natural process of mutation and then increase in frequency as a result of the natural process of selection. However, the initial mutant, even when it is at low frequency, would correctly be considered as resistant, and not tolerant.

To our knowledge, all cases of herbicide resistance in weeds have resulted from mutations that occurred without human intervention (i.e., without any human technology directing mutations; e.g., mutagenesis, CRISPR). It is important to recognize that the use of herbicides does not deterministically introduce herbicide-resistance mutations. Mutation is a natural consequence of errors in the DNA replication process, which are inevitable. Mutation is a random process that happens all of the time. When a random mutation that occurs in a weed population happens to increase the survival and subsequent reproduction of the individual harboring it in the presence of a herbicide, that mutation is expected to increase in frequency in that population over time. As that happens, there will be a concomitant decrease in the efficacy of the herbicide in that population.

In contrast, many crops are resistant to one or more herbicides as the result of intentional human intervention. There are obvious incentives to introduce resistance to crops via a number of genetic tools so that the same herbicide can be used to control weeds without harming the crop. Such crops still fit the WSSA's definition of resistance, because the wild-type (ancestral) crop is killed by the herbicide. However, there is some movement to distinguish crops that have been engineered to withstand a herbicide as tolerant, in order to distinguish them from herbicide-resistant weeds. The debate about whether to refer to intentional manipulation (of crops) as tolerance versus resistance is currently irrelevant to aquatic plant managers, because there have been no attempts to genetically manipulate native/non-target aquatic plants, as far as we are aware.

Misconceptions about the Frequency and Intensity of Herbicide Use

It is commonly thought that repeated use of a herbicide is required for the evolution of herbicide resistance. This is certainly true in the sense that more frequent use of a herbicide means that there are more independent opportunities for herbicide resistance to develop. The probability that an individual population harbors a resistance mutation may be small, but the cumulative probability is much higher when integrated over a large number of populations. However, it should be clear that mutations are stochastic, and there is no threshold number of times a herbicide can be used before resistance will deterministically occur.

It is also important to recognize that once a resistance mutation establishes in a population, there is the potential for that mutation to spread to other populations through the ecological process of dispersal and the genetic process of gene flow. Therefore, it is possible for a local population to exhibit resistance even if that physical location has never been treated with that herbicide. For example, if a herbicide-resistant strain of *Myriophyllum* colonizes a lake that has never before had *Myriophyllum* management, that population will be resistant to the herbicide even before the herbicide has ever been applied to that lake. This is because the lineage that colonized the lake evolved resistance elsewhere. We have identified a fluridone-resistant strain of *Myriophyllum* in several lakes in Michigan, including lakes that have no management history.

Plant species that clonally propagate—like many aquatic weeds, in contrast to most terrestrial agricultural weeds—may evolve herbicide resistance levels much more quickly than sexually reproducing species. In a clonal population, individuals leave identical copies of themselves, and they do not need to pass on their genes through sexual reproduction. This means that the frequency of resistant and sensitive individuals can change more rapidly than in sexual populations, in which several generations may be required to drive resistance alleles to high frequency, especially when the genetic control of resistance is complex (dominance, epistasis, etc.). On the other hand, outcrossing species may transfer resistance alleles more quickly among populations through sexual reproduction compared with plants that reproduce primarily through clonal reproduction. Therefore, the relative importance of clonal propagation and outcrossing in the spread of resistance in aquatic plants warrants more attention.

Importance of Appropriate Use of Terminology

The term "resistance"—as defined by WSSA—should be used to describe variation in herbicide response that occurs within a species, such that some lineages are not killed (or controlled) to the same extent as we expect based on our experience with what is "normal," or wild type. So, one can ask the following simple question to determine which term is most appropriate: "Am I describing a species, or something nested within a species (populations, genotypes, strains, etc.)?" The term "tolerance" should not be applied to cases of resistance.

We recognize that the term "resistance" may be uncomfortable due to uncertainty regarding how public stakeholders will react, how herbicide use and sales may be affected, whether there might be additional regulatory burdens, and so on. For aquatic plant management, additional reluctance to use the term may stem from fear that the term will be misperceived as a total inefficacy of the herbicide at any concentration, whereas higher concentrations could provide efficacy for low resistance factors. However, these concerns should not cloud the use of precise scientific terms that have been adopted to facilitate understanding and communication. Therefore, although the term "resistance" as defined by the WSSA applies to aquatics, the identification of resistance should be qualified with the magnitude of resistance, and the evaluation of control options should include whether the herbicide can be used at a higher rate to accomplish site-specific management goals.

Although it may seem pedantic to some, it is important to recognize resistance when it occurs. Resistance requires the development of alternative management strategies, including new herbicide registrations, new use patterns, and so on, that may be less cost-effective, more restrictive, or may have little public support. Therefore, stewarding existing herbicide technologies is paramount to managing freshwater ecosystems and protecting them from invasive plants. Failure to do so will result in degradation of aquatic ecosystems and will require exponentially more funding (tax dollars) to develop new options.

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