3.7.2 Herbicide Resistance & Resistance Management of Aquatic Plants

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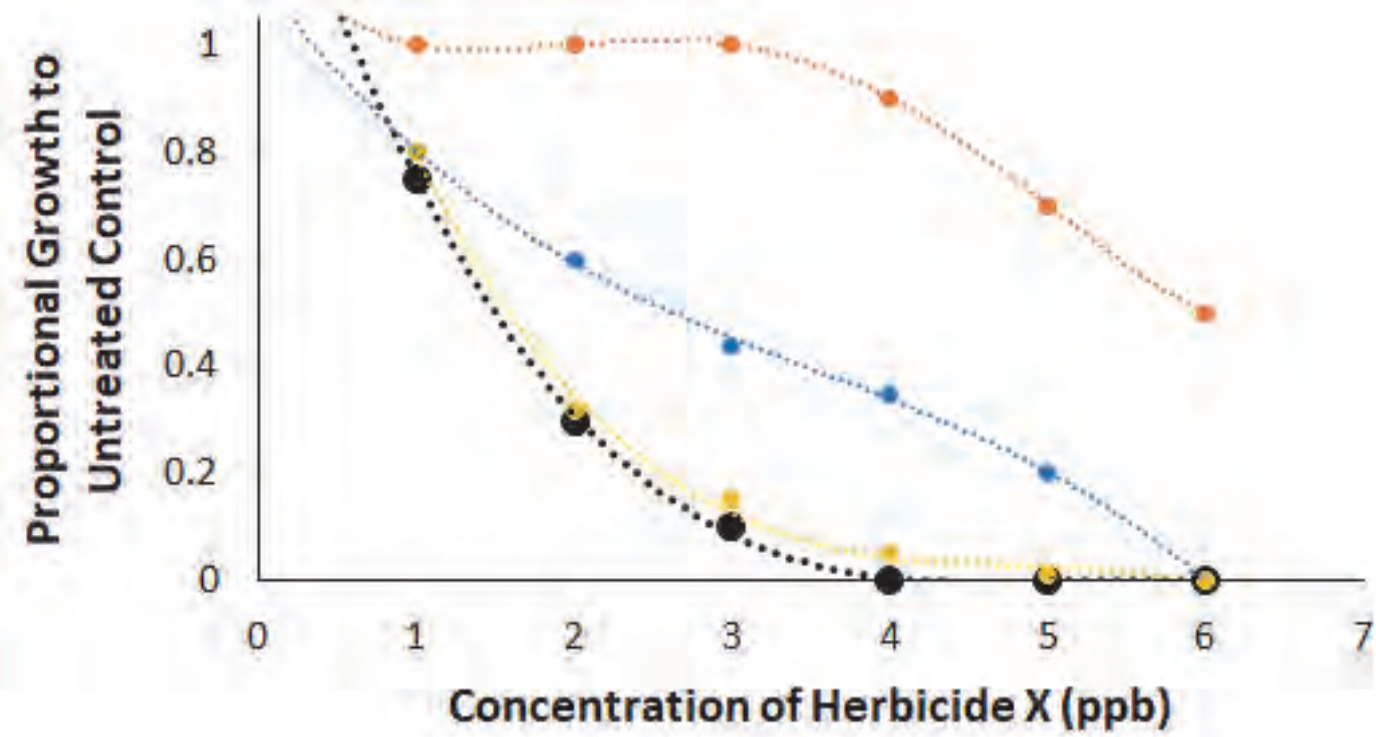
What is resistance?

The accepted definition of herbicide resistance is “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”. In the following paragraphs, we dissect this definition into its key terms.

The term “inherited” means the trait is passed down from parents to offspring, at least in part. This means that offspring tend to resemble their parents because they inherit their genes from their parents. More specifically, this means that variation among individuals for some phenotypic trait (e.g., how a plant responds to different doses of an herbicide) arises partly because those individuals possess different genes that influence the trait. This is in contrast to variation among individuals that arises solely from environmental factors, such as how much light or nutrients they receive, or whether or not they are under attack by natural enemies such as herbivores or pathogens.

Geneticists measure inheritance (also referred to as heritability) in different ways (see Thum 2018 listed in the additional information section of this handbook for more information). Since many aquatic plants can reproduce clonally, heritability can be demonstrated and estimated as differences among clones (genotypes) in a trait of interest (i.e., phenotype) under common conditions. For example, hybrid watermilfoil genotypes have heritable differences in their response to 2,4-D. Similarly, a hybrid watermilfoil genotype from Townline Lake in Michigan has been vegetatively propagated and has repeatedly shown a reduced response to fluridone compared to other genotypes, so we can confidently conclude that the Townline Lake fluridone response is inherited. Heritability can also be demonstrated if specific DNA sequences can be shown to determine particular traits of interest, since organisms pass their DNA to their offspring. An example of this is the mutations in the phytoene desaturase gene that confer different levels of fluridone resistance in hydrilla (Section 2.2). However, since the genes that determine most phenotypes are unknown, this method is uncommon and it is important to recognize that inheritance can be demonstrated without knowing the specific gene(s) involved in determining a phenotype.

Next, let’s break down the phrase “dose of herbicide normally lethal to the wild type”. A “wild type” is the typical prevailing characteristics of a species under natural conditions. However, atypical or mutant types can be found in most species. For example, most of us think of the familiar gray squirrel as having a grayish-brown coat on top with a white coat on bottom. However, all-black or all-white squirrels of this same species are sometimes seen. In this case, the grayish-brown squirrels are the wild type and the black or white squirrels are mutants with respect to the wild type. For a given trait, as long as there is a reference for what constitutes a normal, expected response of a species to an herbicide, then any herbicide response that is elevated over that reference would be considered resistance. For example, assume that 4 parts per billion (ppb) of herbicide X is normally lethal to the wild type for a species; a specific genotype of that species that can survive and reproduce when exposed to 4 ppb of herbicide X would be considered resistant, so long as it can be demonstrated that the ability to survive and reproduce at four ppb of herbicide X can be passed down from parents to offspring.



Example of genetic variation for herbicide response. The wild type (large black circles) is normally killed by four parts per billion of herbicide X. However, other genotypes have different responses. In some cases, response to the herbicide may only be slightly elevated for a different genotype (small yellow circles). However, other genotypes may exhibit a high level of resistance at concentrations normally lethal to the wild type (small blue circles and small orange circles).

Resistance arises because mutations occur naturally and randomly when DNA replicates. In some cases, these mutations happen to provide an advantage in survival and/or growth of a plant in the presence of a certain amount of an herbicide. Thus, when a population is exposed to the herbicide, the individuals that have mutations conferring some level of resistance are more likely to survive and reproduce than the wild type individuals. Over time, the population can become dominated by the mutant genotype(s) and these genotypes could also spread to other lakes.

Herbicide tolerance

Herbicide tolerance is defined 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 difference between resistance and tolerance is that the latter is a characteristic "of a species". For example, many monocots are naturally tolerant to doses of 2,4-D that impact Eurasian watermilfoil (Section 2.3) and therefore repeated use of 2,4-D may result in increased abundances of these naturally tolerant species. Therefore, the repeated use of a given herbicide may result in a shift in species composition of an aquatic plant community towards species that are naturally tolerant to that herbicide. This phenomenon is not the same as resistance, because resistance arises from genetic variation among individuals within a species that has a susceptible wild type. Nevertheless, the impacts of selecting for tolerant species via herbicide use can be of management concern.

Examples of herbicide resistance in aquatic plants

As of this writing, there are currently 512 cases of documented weed resistance to 167 different herbicides (International Survey of Herbicide Resistant Weeds, http://www.weedscience.org; accessed 28 May 2020). Most documented cases of herbicide resistant weeds come from terrestrial agriculture but several aquatic plants are resistant to herbicides used in rice agriculture in Australia and Asia. For example, populations of starfruit (*Damasonium minus*), dirty dora (*Cyperus difformis*) and arrowhead (*Sagittaria montevidensis*) have evolved resistance to bensulfuron. Pervasive weed resistance in agricultural systems understandably fuels concerns regarding herbicide resistance in aquatic plant management of private and public waters.

Herbicide resistance in aquatic plants is rare compared to the large number of cases documented in terrestrial agriculture. The most well-known case of herbicide resistance in aquatic plants is fluridone resistance in dioecious hydrilla, but a population of duckweed (*Landoltia punctata*; see Section 3.14) in a Florida canal was highly resistant to diquat and paraquat compared to wild type duckweed. Also, as mentioned earlier, hybrid watermilfoil from Townline Lake in Michigan has developed resistance to fluridone that is not seen in other Eurasian and hybrid watermilfoil genotypes.

The dearth of documented cases of herbicide resistance in aquatic plants in the US begs the question: is herbicide resistance in aquatic plants truly rare or is it present but not tested for or reported enough? It is possible that factors promoting herbicide resistance (see below) are commonly lacking in aquatic plant management, leading to very low occurrences of resistance evolution. However, it is also possible that reduced efficacy in some populations goes undetected because of a lack of quantitative pre- and post-treatment monitoring or because explanations for reduced efficacy do not consider the possibility of resistance (e.g., are explained by environmental factors that may have limited the dosage of herbicide). Thus, it is important to quantitatively monitor herbicide efficacy and to consider herbicide resistance when efficacy is lower than expected. Conclusive testing for resistance would then come from laboratory studies comparing suspected resistant types to known wild type (susceptible) genotypes.

Factors that may influence the development of herbicide resistance

It is important to recognize that there are different physiological and genetic mechanisms for herbicide resistance. **Target-site resistance** refers to mutations that lead to changes in the molecule(s) where the herbicide typically binds, which leading to less effective binding and thus less impact on the weed. For example, hydrilla that is resistant to fluridone has amino acid changes in the phytoene desaturase gene. It is generally thought that herbicides with a single site of action will be more prone to the evolution of resistance, since mutations occurring at the target enzyme can directly confer resistance. It is therefore important to note that many of the herbicides registered for aquatic plant control target a single enzyme. In contrast, **non-target site resistance** refers to genetic changes that occur not at the herbicide's site of action, but at other genes that are related to herbicide uptake, translocation, sequestering, detoxification or metabolism. Whether herbicide resistance results from target versus non-target site mutations should be contingent upon the supply of mutations available within a population when a herbicide is applied.

Genetic mechanisms for herbicide resistance can also be broadly classified into **single-gene** versus **polygenic** (multiple genes). In single-gene resistance, the level of resistance observed is conferred by mutation at a single gene, which would most likely occur at the target site. However, resistance can occur via changes at multiple genes, each of which confers some fraction of the overall resistance observed. Thus, a given level of resistance could reflect mutations at a single gene that have a large effect on resistance or by mutations at many genes that each have a small effect on resistance.

In general, high rates of herbicides should select for mutations in a single gene that have a large effect and low rates of herbicides should select for polygenic resistance [because at low rates and in genetically diverse populations there may be alleles (different forms of a trait) at different genes that can each allow higher survivorship and reproduction at low rates]. For example, some individuals may have genes that allow them to metabolize small amounts of a particular herbicide, so they can continue to grow in the presence of low doses of herbicide, while other individuals may have genes that help them to sequester small amounts of that herbicide. The population of individuals that survive treatment with that particular herbicide are then enriched for these two different genes, both of which confer a small degree of herbicide resistance (e.g., survival and continued growth at low doses). Subsequent intercrossing of individuals with these two different genes can allow "gene stacking" that confers resistance to a higher dose of herbicide to offspring than either parent can tolerate because the new genotypes can both metabolize and sequester the herbicide.

Examples of this phenomenon can be found in the terrestrial agricultural literature but it is unknown whether or how commonly this occurs in aquatic plants. Certainly, the exposure of plants to low rates of herbicides is inevitable in many aquatic plant management projects that utilize spot treatments in large water bodies where the herbicide will rapidly dissipate. Gene stacking of low-level resistance alleles will depend on the extent of genetic variation within and among populations and the extent to which sexual reproduction occurs. Thus, different control tactics (e.g., whole lake applications where long contact times are maintained vs. spot applications that likely dissipate to sublethal doses) may interact with plant life histories (e.g., asexual, sexual or mixed reproductive strategies) to influence the probability and magnitude of herbicide resistance. Strong academic research on this issue could be helpful in the future for building better models of when, where and how resistance could occur, as well as developing efficient tools to detect it.

Resistance management

Traditionally, the idea behind resistance management is to prevent or delay the evolution of resistance. Some commonly used methods of resistance management are listed below.

Rotations and/or combinations of herbicides with different modes of action can limit resistance evolution. Individuals are less likely to be resistant to all of the herbicides in the mixture or rotation because resistance to different herbicides will likely require independent mutations in different target genes. However, rotations and combinations do not guarantee that resistance will not evolve. If populations are large enough, it is possible (although unlikely) that an individual will have multiple mutations conferring resistance to multiple herbicides. Similarly, individuals that harbor different mutations at different target genes can intercross and produce offspring with mutations at all of the different target genes. Further, non-target site resistance mutations that influence uptake, translocation, metabolism or detoxification may confer resistance to multiple herbicides (see http://www.weedscience.org).

Another resistance management strategy is to allow a sufficient amount of time in between applications of the same herbicide. This idea is based on the premise that resistant genotypes that survive treatment may exhibit a trade-off whereby they are less competitive in the absence of the herbicide (i.e., there is a “cost” to resistance), so allowing time to pass between treatments should cause the number of resistant individuals to go down if there is a cost to being resistant in the untreated environment. However, it is important to recognize that the success of this strategy depends on sufficient costs of resistance, which isn’t necessarily true; also, it isn’t clear how long in between treatments is sufficient. Therefore, this strategy relies on having detailed information on the level of resistance, costs, and dynamics of competition over time.

One method that is used to reduce resistance evolution in agricultural systems is to maintain untreated populations adjacent to treated populations, with the hope that interbreeding with an untreated population where there is no selection for resistance will keep resistance genes from becoming dominant in the treated population. This strategy has not been intentionally implemented in aquatic plant management to our knowledge. However, it is possible that this strategy is implemented de facto because across the landscape there is a mosaic of treated and untreated lakes. Furthermore, spot treatments are common within many lakes, which may ultimately have this same effect. However, it is generally thought that sexual reproduction plays a small role in the overall reproduction of most managed aquatic plants, so it is unclear whether the intentional implementation of this strategy would work, unless resistant genotypes are frequently replaced by wild type genotypes due to a cost of resistance as described above.

In addition to resistance management strategies, it is important that managers implement best management practices to maximize the impact of any control implementation. First, early detection and rapid response methods are recommended so that populations are treated when they are small and before they become a problem. The probability that a population harbors a resistance mutation will be proportional to its size, and therefore small populations are less likely to harbor a resistance mutation unless the population was initiated by a resistant genotype to begin with (e.g., colonized from a nearby source that is resistant). Second, it is generally recommended to treat with the maximum allowable herbicide rate to minimize the number of surviving plants. Finally, management should include quantitative monitoring efforts so that surviving plants are identified and targeted for appropriate follow-up management and so that changes in efficacy over time can be identified (see below).

Limitations to implementing resistance management in aquatic plant control operations

Most stakeholders in aquatic plant management recognize the potential for herbicide resistance and therefore the benefits of practices that prevent or delay the evolution of resistance. However, it is also important to recognize that there are logistical realities that limit the implementation of resistance management practices in many locations. The most challenging conditions under which to practice resistance management are likely to occur in large, public, multiple use water bodies where numerous factors influence the control options that are available. In these water bodies, the choice of herbicide, dose and timing is influenced by a variety of factors, including balancing different uses of the water body, cost of management, selectivity and efficacy and hydrology. Given the limited number of herbicides available for aquatic use, resistance management practices that involve rotations or mixtures of different products may be infeasible in certain water bodies and options may be limited to non-herbicidal techniques.

A second limitation to resistance management practices is justification for implementing them if there is no evidence that repeated use of a herbicide in a water body is selecting for resistant genotypes. It remains to be seen whether the risk of herbicide resistance in aquatic plant management is high, and to date there are few documented examples of resistance. The evolution of resistance will be limited by the supply of mutations available to confer enhanced survival and reproduction after exposure to herbicides. Thus, herbicide resistance involves a "waiting time" for mutations, which may or may not occur over the life of a management project. Given the regulatory demands for demonstrating selectivity and efficacy, managers may be resistant to adopting resistance management strategies if they have no evidence that their favored strategy and tactics for a water body are deemed efficacious (Section 3.1), especially if alternatives employed in resistance management practices are more costly and/or have less public support for their use.

Finally, a geographic region may represent a mosaic of water bodies that do and do not implement resistance management practices. In this case, it would be reasonable for managers that voluntarily implement resistance management practices (or are required to do so) at additional costs to ask themselves why they are doing it if others are not. Furthermore, because many aquatic plants can spread vegetatively among water bodies, locations with resistance management practices could potentially be colonized by resistant genotypes from other areas that do not practice resistance management. Therefore, it is important to be on the lookout for resistance and understand its origins even when resistance management is practiced.

Recommendations for addressing the potential for herbicide resistance

As a practical matter then, how should aquatic plant managers address the issue of resistance? Managers should consider all available options to implement resistance management practices wherever and whenever possible. However, the likely reality is that in many situations, resistance management practices will be limited. An immediate priority should be to develop and implement methods that objectively and definitively identify whether control efficacy is lower than expected and the likelihood that reduced efficacy is due to the presence of resistant genotypes.

Lake management plans (Section 3.2) should have monitoring protocols that can objectively and definitively separate out factors influencing the variation in control efficacy. For example, temporal data on control efficacy when the same control tactic is repeatedly employed should be informative for identifying reduced efficacy over time within a water body. In such a case, laboratory determinations of dose-responses to the current practice and alternatives could be used to determine whether a change in management strategy is warranted. This kind of objective data could provide compelling evidence to warrant changes in management strategies or tactics that may otherwise be resisted due to increased costs, regulatory hurdles or unfavorable public opinion.

Genetic tools hold promise to increase the efficiency in which we are able to test for resistant genotypes and identify resistance evolution. Since resistance evolution occurs as the displacement of genotypes that have wild type sensitivity to herbicides by mutant genotypes that are resistant, genetic information could assist in the identification of resistance. More specifically, we expect to see shifts in the genetic composition of populations over time if resistance is developing within a water body. Moreover, since most aquatic plants reproduce primarily by asexual means (e.g., fragments, turions, etc.), we may expect lakes that have quantitative data demonstrating lower than expected control efficacy to be dominated by a single clone. Thus, these genetic signatures can be used to prompt laboratory dose-response studies of specific genotypes suspected of exhibiting herbicide resistance.

Genetic tools currently available for hydrilla and Eurasian watermilfoil illustrate the promise of these tools. For example, fluridone-resistant biotypes of hydrilla can be identified with genetic data, which can help managers determine the dose of fluridone required for efficacy given the genetic composition of the population or whether fluridone is a poor control option for that particular population. Similarly, a fluridone-resistant genotype has been identified in hybrid Eurasian watermilfoil; molecular markers can distinguish this genotype from other genotypes and have shown that this genotype has spread to several populations in Michigan. Similar to hydrilla, genetic surveys of hybrid Eurasian watermilfoil can be used to determine whether fluridone should be applied to particular lakes. Characterization of genotypes in other species could be employed in the same way.

Summary

Wild type (normal) plants are susceptible to a particular herbicide, while other genotypes of the same species may become resistant to that herbicide as a result of mutations or intercrossing. There are a number of tactics to reduce the likelihood of developing herbicide resistance in populations of aquatic weeds but options available to managers can be limited based on the situation. Rotating or combining herbicides with different modes of action may limit resistance, and genetic testing may be very useful to guide management decisions regarding herbicide selection.

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Page 179: Hydrilla bouquet; William Haller, University of Florida
Page 180: Genetic variation for herbicide response; Ryan Thum, Montana State University
Page 181: Landoltia duckweed; Ben Willis, SePRO Corporation
Page 183: Hydrilla tubers (white) and turions (green); Lyn Gettys, University of Florida