

THE DEVELOPMENT AND IMPLICATIONS OF HERBICIDE RESISTANCE IN AQUATIC PLANT MANAGEMENT

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INTRODUCTION

Aquatic plants are among the most unique and potentially understudied plants in the world. Aquatic communities represent incredibly diverse ecosystems that perform many vital natural and anthropogenic functions – fisheries, navigation, water supply, recreation, and hydropower generation (Gettys *et al.*, 2009). Most often these anthropogenic requirements lead to manipulations of the aquatic environment, such as reservoir creation, changes in water fluctuation – intensity and frequency, removal of native freshwater vegetation and most importantly - alterations in water chemistry (Madsen, 2009).

These changes often lead to excessive aquatic plant growth, particularly from introduced exotic species. Problematic infestations generally require varying degrees of management. Managing plants in the aquatic environment is challenging and requires a multi-pronged approach, integrating biological, cultural, mechanical and chemical control methods. This paper will provide a brief overview of aquatic plants and management techniques, but chemical control through the use of aquatic herbicides, including the development and implications of herbicide resistance, will be the primary focus of this article.

Overview of Aquatic Plants and Management Techniques

Aquatic plants can be classified as macrophytic (visible to the naked eye) and microphytic (phytoplankton – microscopic). Phytoplanktons reside as free-floating suspensions throughout the water column while macrophytes require the water's surface or a soil/hydrosoil interface for growth. Emergent plants are rooted to the moist bank of a water body or in the shallow sediment and have leaves and stems that penetrate above or lie on the surface of the water. These plants are physiologically well adapted to changes in water levels and include such species as cattails (*Typha* spp.), pickerelweed (*Pontederia cordata*), the water lilies (*Lotus*; *Nuphar* and *Nelumbo* spp.) and many aquatic grasses including torpedograss (*Panicum repens*) and paragrass (*Urochloa* spp.).

Floating plants reside completely on the waters surface, with roots extending into the water column that absorb water and nutrients. The most common and problematic species of this

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classification is water hyacinth (*Eichhornia crassipes*), but also include the duckweeds (*Lemna* spp.), alligatorweed (*Alternanthera philoxeroides*), water lettuce (*Pistia stratioides*) and salvinia (*Salvinia* spp.). Submersed aquatic macrophytes live completely within the water column. They are rooted to the hydrosol and grow through the water column to the surface. Light is the major limiting factor for the growth and establishment of these species which include hydrilla (*Hydrilla verticillata*), elodea (*Egeria/Lagarosiphon* spp.), milfoils (*Myriophyllum* spp.), pondweeds (*Potamogeton* spp.), and several others.

Several methods can be utilized to provide control of nuisance aquatic vegetation. The first and most effective technique is prevention. While natural spread is common within adjoining water bodies, long-distance dispersal, particularly of problematic weeds, is through humans. Dredging, mechanical harvesting, boat trailers and the incredible increase in internet trade of exotic water plants all contribute to weed spread into vulnerable areas (Richardson, 2008). Educational awareness, coupled with monitoring and rapid response has been effective for certain species in the U.S. (giant salvinia - *Salvinia molesta*) thus far, but the decrease in governmental funding in this area is likely to result in increased infestations.

Mechanical control methods include various types of cutters, shredders, dredgers, suction hoses, and harvesters. The underlying premise of these devices is to physically remove or cut up the plant to provide a measure of control. While effective for certain areas and situations, mechanical control is often expensive and generally results in only short-term control. Moreover, mechanical control may actually increase infestations through fragmentation. It may, however, allow the reduced use of herbicides or biological control agents, such as the triploid grass carp (*Ctenopharyngodon idella*).

The sterile grass carp is the most widely used biological control and is very effective on soft tissue plants, particularly hydrilla. However, stocking rates as a function of infestation levels or plant community type is still questionable in areas where some submersed aquatic vegetation is desired. Other insect bio-control agents have been very effective, but wide-spread control across large geographic ranges is limited for many species.

Benthic barriers, drawdowns, shading, limiting nutrient influx or nutrient inactivation are considered physical methods of control and can be very effective, but not applicable or possible for all aquatic situations. Finally, chemical control through the use of approved aquatic herbicides is utilized by many aquatic plant managers, but this approach also has its challenges and pitfalls.

Overview of Herbicides Used for Aquatic Plant Management

Regardless of control tactic, an integrated management plan should be the first step in any aquatic plant control strategy. Site assessment, intended use or uses of the water body and long-term consequences of control are critical factors to be determined. Herbicide selection, including timing, rate and use pattern is also paramount. Currently there are 14 products registered for aquatic use in the United States. Table 1 reflects the herbicide active ingredient, mode-of-action, and general use pattern/plants controlled.

Table-1. Registered herbicides for use in aquatic plant management in the United States

Active Ingredient	Mode-of-Action	Usage Pattern & Plants Controlled
2,4-D	Synthetic auxin - systemic activity	Foliar for floating plants - hyacinth, and also submersed milfoil
Acrolein	Membrane disruption - contact activity	General submersed vegetation control in canal systems
Bispyribac-sodium	Inhibition of ALS enzyme - systemic activity	Submersed aquatic weeds
Carfentrazone-ethyl	Protox enzyme inhibition - contact activity	Foliar for floating plants and algae
Coppers (chelate, sulfate)	Membrane disruption - contact activity	Primarily algae control, some submersed plant control
Diquat	Photosystem electron diversion - contact activity	Foliar for floating plants - salvinia, duckweeds, also submersed control
Endothall	Membrane disruption - contact activity	Submersed plant control - hydrilla, milfoils
Flumioxazin	Protox enzyme inhibition - contact activity	Submersed plant control and algae - particularly effective on cabomba
Fluridone	Pigment synthesis inhibition - systemic	Submersed plant control - hydrilla and milfoils, entire water body treatments
Glyphosate	Inhibition of ESPS enzyme - systemic activity	Foliar plant control and emergent ditchbank weeds, no activity in water

Imazamox	Inhibition of ALS enzyme – systemic activity	Foliar plant control, particularly cattails and some submersed control
Imazapyr	Inhibition of ALS enzyme – systemic activity	Foliar and emergent ditchbank weeds, limited submersed activity
Penoxsulam	Inhibition of ALS enzyme – systemic activity	Primarily submersed plant control – hydrilla, others
Triclopyr	Synthetic auxin - systemic activity	Foliar for floating plants – hyacinth, and also submersed milfoil

Aquatic herbicides represent a very small number of chemicals compared to terrestrial agricultural situations. These materials fall into two primary categories, contact and systemic, which help to define herbicide movement and behaviour within aquatic plants. These terms are also used somewhat erroneously to describe herbicide persistence in the aquatic environment.

Contact time is the time needed for the herbicide to be in contact with the plant to cause the desired lethal effects. As a general rule, contact herbicides require a few hours to days, while systemic materials require several days to weeks for control.

Contact Herbicides

Contact herbicides are generally applied at use rates in parts per million (ppm) and provide fast-acting control of floating and submersed species. Contact herbicides, as their name suggests, provide control of those plant tissues that are contacted by the herbicide.

Once the herbicide is absorbed into the plant tissues, it is not moved or translocated to other parts of the plant. For this reason, contact herbicides must be applied in manner that ensures good coverage. They have limited activity on emergent and littoral plants.

These plants possess extensive below surface tissues such as rhizomes (water lilies, cattails) and will regrow rapidly because the herbicide is not translocated. Furthermore, contact herbicides dissipate quickly in the aquatic environment – therefore the regrowth of the target weed escapes the lethality of the once present herbicide.

Dense plant growth on the surface often requires more than one treatment because the herbicide is absorbed into the upper plants, killing those plants, but because of rapid dissipation, those plants below the initial surface are able to continue growing. This is most often observed with the duckweeds and salvinia, where several layers of plants can be matted together on the surface.

For submersed applications with contact-type herbicides, the herbicide must be in contact with the target plant in the range of hours to a few days to provide control. Plant uptake of these herbicides is very rapid, so applications must be close to the target plants to achieve good control. Contact herbicides for submersed plant control are generally used for site specific plant removal such boat ramps, shallow zones along shorelines, channels or other specific areas. Some whole lake treatments can be made with these types of herbicides, but the entire area must be treated. For this reason whole lake treatments are limited due to logistics and cost.

One of the oldest herbicides used for weed control is copper and copper salts. In the late 1800's, researchers found copper salts effective for selective broadleaf weed control in cereals. Selectivity was achieved primarily through differential uptake, whereby the copper salts rolled down and off the vertical leaves of the cereals and was retained on the horizontal surface of the broadleaf weeds.

In the aquatic environment, differential uptake is also the key to selective control. This herbicide is primarily used for algae control; because algae have the greatest surface to volume ratio, more uptake occurs in algae compared to more complex macrophytes. Copper has also been used to increase the effectiveness of other herbicides such as diquat. Copper is a general toxin and is very rate dependent for activity. As the levels of copper increase, normal cell function such as co-factor mediated enzyme reactions and cell membrane integrity are compromised.

Copper is used as a chelated compound or as copper sulfate. The sulfate salt is considered to be more toxic due to increased uptake and is limited in areas devoid of desirable fish populations. This herbicide is rapidly absorbed into tissues and control is nearly immediate. As such, contact time for this herbicide ranges from a few hours to a day or so.

Acrolein herbicide is a general plant toxicant that is used for total vegetation control in irrigation canals. This herbicide is rapidly absorbed by submersed plant tissues and reacts with sulfhydryl groups in a variety of plant biochemical functions. Acrolein is not very effective on terrestrial plants due to limited uptake, but very little tolerance/selectivity is observed with submersed aquatic plants.

Diquat has been registered for aquatic weed control since the 1960's for foliar and submersed weed control. This herbicide is used for floating weeds such as water lettuce, salvinia, and duckweeds. Complete coverage is required for good control to ensure that all portions of the plant come in contact with the herbicide. In situations where the plants are crowded or layered, more than one application is generally needed. Submersed applications rely on the herbicide

dispersing quickly within the water column as diquat is rapidly absorbed by the plant tissues. In situations where there is dense plant growth, trailing hoses are used to place the herbicide several feet below the water surface.

Diquat is very effective on the milfoils, elodea, certain pondweeds and naiads (*Najas spp.*). Diquat is also effective on hydrilla but requires the addition of copper; the actual mechanism of this beneficial interaction on this species is unknown. Diquat interferes with the light reactions of photosynthesis, specifically by accepting electrons from photosystem (PS I) and passing this energy to oxygen – forming radical oxygen. This reaction is continuous, and radical oxygen reacts with lipids in the membranes, causing leakage and cell lysis and death.

Endothall is used primarily for submersed weed control and is formulated as a potassium salt or as an alkyamine salt. The latter is much more efficacious, providing filamentous algae control. However, concerns over fish toxicity result in the use of the potassium salt for most situations. Endothall is applied in a similar manner to diquat and is very effective on hydrilla, curly-leaf pondweed, milfoil and several other species. The mechanism of action of endothall is unknown, but appears to be membrane active, causing rapid cellular leakage.

Flumioxazin and carfentrazone-ethyl are two relatively new herbicides that have been introduced to the aquatic market in the United States. These herbicides cause a buildup of cytoplasmic protophorinogen which is rapidly converted into light accepting protoporphin IX. Because of the buildup in the cytoplasm, the energy cannot be utilized by the light reaction centers in the chloroplast. As such the energy is passed to oxygen creating singlet oxygen. This radical oxygen species then causes similar effects observed with diquat, including membrane perturbation and rapid cell death. Carfentrazone is labeled for floating plant control including water lettuce and salvinia. Flumioxazin is very effective for certain submersed species – especially cabomba, and filamentous algae.

Systemic Herbicides

Systemic herbicides, as the name suggests, translocate throughout treated plants. In aquatic habitats, these materials move within the phloem tissues and generally accumulate in areas of new growth. Systemic herbicides are used for floating, littoral/ditchbank and submersed plants. Herbicide translocation in aquatic plants is not well understood and will be discussed in more detail with respect to each individual herbicide.

Glyphosate is a non-selective herbicide used routinely for ditchbank weed management and provides good control of many grasses and other perennial species. It also provides good control of

most floating plants and emergent species such as lilies. Glyphosate has no soil activity and no activity in water. This material will translocate to growing regions of the plant, accumulating in the apical meristem. Recent research suggests glyphosate translocation is limited in stems that extend from the shoreline that dip below the water surface and then resurface some distance away (MacDonald *et al.*, 2005).

These authors also demonstrated low levels of herbicide leakage with glyphosate into the surrounding water. It has been speculated that glyphosate applied to littoral vegetation 'leaks' out of the plant tissues once applied, and this is the reason for poor control in some cases. However, research has shown that glyphosate and imazapyr do not leak from plant tissues, suggesting poor translocation or initial uptake is the reason for poor control. Mechanistically, glyphosate blocks the formation of essential aromatic amino acids. This leads to a cessation of growth and eventual plant death.

Imazapyr, imazamox, bispyribac and penoxsulam are four recent herbicides labeled for use in the aquatic sector in the United States. These herbicides act in a similar manner on the same target enzyme (acetolactate synthase); as such these materials are commonly referred to as ALS herbicides.

Imazapyr and imazamox are used primarily for littoral weed control, and both compounds are particularly effective on Typha. Imazapyr is generally used for emergent plant control, especially littoral zone and wetland grasses such as torpedograss, phragmites and giant reed (*Arundo donax*). It is considered to be non-selective and has considerable soil activity and persistence. Imazapyr has limited activity in the water for submersed plant control. Imazomox is also used for emergent plant control, and is more selective compared to imazapyr. However, it can be used for submersed control of hydrilla where it provides suppression for several months.

Penoxsulam is primarily used for submersed plant control and is very effective on several species including hydrilla. It also appears to have some floating plant control from in-water applications, suggesting this herbicide is taken up by the roots of floating plants.

Bispyribac is the most recent registered herbicide for submersed plant control. Due to the recent registration of these herbicides, much is unknown regarding selectivity and effective use patterns for all aquatic situations.

2,4-D and triclopyr are growth regulator herbicides that mimic the plant growth hormone auxin. Effected plants undergo uncontrolled growth and eventually die. These materials are used for broadleaf and woody littoral zone and wetland species. Both products are also very effective for control of the floating species water hyacinth and the

submersed Eurasian water milfoil. Both of these materials are rapidly absorbed by leaf tissues from foliar applications and moved throughout the plant. In submersed applications, the herbicide is absorbed directly in shoot and leaf tissue, but the extent of translocation is unknown.

Fluridone is one of the most widely used herbicides for submersed control of hydrilla and milfoils. This herbicide blocks the formation of carotenoids, leading to the degradation of chlorophyll and white/bleached tissues. Unlike the other systemic herbicide labeled in aquatics, fluridone does not translocate in phloem tissues. Many submersed species lack functional xylem, so translocation of fluridone is not possible – thus leading to the conundrum of this herbicide being designated as systemic. It is xylem mobile from root uptake in terrestrial situations, and is often absorbed by littoral species, causing temporary bleaching. Fluridone does provide control of certain floating species, but it is unclear as to how the herbicide is being moved/absorbed into these plants.

Submersed aquatic species are the most difficult to control with either contact or systemic herbicides. In either classification, the concept of concentration exposure time (CET) is important to understand. Contact herbicides directly affect all plant tissues, so the key issue is having the herbicide in a lethal concentration for a long enough time to be absorbed. A CET of hours to a few days is long enough to affect control. However, with systemic herbicides, CET is much more critical. These herbicides do not equally affect all tissues of the plant, but directly affect new growth.

More importantly these herbicides do not impact existing plant tissues. The herbicide must be present in a sufficient dose and for a long enough time to prevent new growth. If CET is compromised through a loss of a lethal rate, the plant will be able to recover. Generally exposure time cannot be overcome since with higher rates; once the critical rate is met, additional herbicide is not needed. Higher rates do extend exposure time, but most plant managers prefer to add herbicide (bump treatments) when needed. Another interesting quandary concerning systemic materials, is whether the herbicide is concentrated within plant tissues, or simply equilibrates with the surrounding water.

Resistance and Tolerance in Aquatic Plants

There has been a great deal of confusion with respect to herbicide tolerance and resistance. Resistance is defined by the Weed Science Society of America as a plant population that has changed to resist a once lethal dose of an herbicide. Tolerance is defined as a plant population that has always resisted or 'tolerated' an herbicide when used at labeled rates. The development of resistance in

terrestrial cropping systems is characterized by a shift in the rate required to provide a comparable level of control.

In years past a 10-fold shift in the rate needed to provide control was considered to be true resistance. However more recent definitions state a shift in rate that no longer allows the herbicide to be effectively and safely used in the labeled situation. In some cases the herbicide is no longer effective at reasonable rates; in others the rate that will provide control will not allow for crop tolerance.

Plants tolerate herbicides through a variety of mechanisms. These are most closely studied in crops, where tolerance is essential. Placement is used to limit absorption or uptake in desirable species, and is not a viable option for many aquatic applications. Limited uptake and or translocation are other mechanisms of tolerance where the herbicide is not present at the site of activity at a lethal concentration.

Metabolism of the herbicide includes breakdown or conjugation –sequestration which also provides a measure of tolerance. Finally, the active site – usually an enzyme, does not allow the herbicide to bind and cause inactivation. These mechanisms explain how certain plants – both crops and weeds are not affected by an herbicide. When resistance to an herbicide occurs, one or more of these mechanisms is responsible.

Selectivity of herbicides in the aquatic environment is not well understood, particularly as it relates to terrestrial systems. This is partially due to the difficulties with studying herbicide activity in aquatic plants but also the unique physiology of these plants. Phloem and xylem movement is limited and many traditional anatomical features such as cuticle and vascular tissues are vestigial or lacking.

There are specific examples where very similar plants react completely different to the same herbicide. The most notable example is hydrilla and elodea. Hydrilla is very susceptible to endothall herbicide and moderately effected by diquat, while the opposite occurs for elodea. Both plants are in the same plant family, grow in similar habitats and are almost indistinguishable. Endothall and diquat are non-selective herbicides in their mode of action, yet major differences result when applications are made to these two species. Since metabolism has not been reported with either herbicide, differential uptake appears to be the most plausible explanation.

Resistance to three herbicides has recently developed thus far in aquatics in the United States. The most notable was the development of fluridone resistance by the dioecious biotype of hydrilla in the late 1990's. This occurred in central Florida and has spread throughout much of the state. Research confirmed resistance is due to, in part, to an amino acid substitution in the gene coding for

phytoene desaturase (Puri *et al.*, 2007). However, resistance ranges from 3-fold to 7-fold with the same genetic change, indicating some other factor may be involved in resistance.

A similar situation has arisen with hybrid watermilfoil in the state of Michigan. Hybrid milfoils result from a cross between Eurasian and northern watermilfoils and have only recently been documented in the northern mid-western U.S. However, it is unclear whether resistance has developed to an existing population, or whether inherent tolerance is a result of the development of the hybrid. Studies to elucidate the level of tolerance and associated mechanisms are currently being conducted.

Resistance to diquat has developed in spotted duckweed (*Landoltia punctata*), also in central Florida (Koschnick *et al.*, 2006). Unlike fluridone, the level of resistance to diquat is greater than 50-fold. Interestingly, resistance can be overcome with the addition of copper. This led to the speculation that limited uptake was responsible for the resistance, although studies could not elucidate the exact mechanism. Hydrilla has also developed resistance to endothall herbicide, once again in central Florida (Berger *et al.*, 2011). The mechanism of resistance is still unclear and studies are currently being conducted on this phenomenon, and the level of resistance appears to be 3 to 5-fold.

In all cases of resistance in aquatics thus far, the underlying issue has been continuous use and /or long-term exposure to the herbicide. This has been the case for most resistance developments in terrestrial cropping systems and it is not surprising that this is same in the aquatic environment. The major difference is the development of resistance without genetic recombination, especially with dioecious hydrilla. This biotype only reproduces asexually in the U.S. so the development of resistance was thought to be minimal, given the clonal and theoretically identical plants. However, it was discovered that genetic variability exists in asexually propagated hydrilla and more than likely other predominantly vegetative species.

Resistance to herbicides in the aquatic environment poses severe implications to plant managers. There are very few herbicides registered for use in these areas and the loss of one herbicide can be extremely detrimental and result in the inability to control a particular species. An integrated approach utilizing non-chemical methods of control is always desirable and will prevent or substantially reduce the potential for resistance development.

The use of herbicides with different modes of action is also a proven method for deterring resistance. However, aquatic herbicide registration and labeling is very difficult so the option of readily available alternative herbicides is not always a feasible option.

An additional area of research is a more fundamental understanding of the activity and physiological mechanisms of herbicides within aquatic plants. Herbicide activity is closely linked with physiology and biochemistry, but aquatic plants are vastly different from typical terrestrial plants and cropping systems. The key to managing herbicide resistance in the aquatic plant environment is the same as terrestrial systems – rotating herbicide chemistries and alternative weed control strategies, and understanding the underlying mechanisms of herbicide activity.

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