

ABSTRACT

ISRAEL, TREVOR DAVIS. Aminocyclopyrachlor Efficacy and Behavior in Selected Aquatic Plants. (Under the direction of Dr. Robert J. Richardson).

Aminocyclopyrachlor is a recently discovered synthetic auxin herbicide and has potential utility in aquatic sites. Greenhouse trials were conducted to evaluate aminocyclopyrachlor-methyl ester (AMCP-me) and aminocyclopyrachlor acid (AMCP-a) efficacy on selected aquatic plants. AMCP-me at 140 g ai/ha controlled mosquitofern (*Azolla caroliniana* Willd.) 84% and reduced dry weights to 24% of nontreated controls at 4 weeks after treatment (WAT). Control of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] at 4 WAT was 94% with 280 g/ha AMCP-a. At 5 WAT, 280 g/ha AMCP-a controlled alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] 88% and reduced root weights to 28% of nontreated plants. Creeping water primrose [*Ludwigia grandiflora* (Michx.) Greuter & Burdet] and parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] control at 5 WAT with 35 g/ha AMCP-a was 85 and 95%, respectively. Parrotfeather and creeping water primrose root weights were less than 20% of nontreated controls at all rates tested. At 5 WAT, soft rush (*Juncus effusus* L.) injury was 5% or less at all rates tested, while pickerelweed (*Pontederia cordata* L.) injury with 35 g/ha AMCP-a was 83% and increased with rate. Lizard tail (*Saururus cernuus* L.) injury 5 WAT was 33 and 87% at 35 and 560 g/ha AMCP-a, respectively. AMCP-a was evaluated on alligatorweed planted at different water depths. Alligatorweed control at 4 WAT was 75% at 22 cm water depth. As depth increased, percent reduction in height decreased, but percent reduction in shoot weight increased. Field trials were conducted on alligatorweed, creeping water primrose, parrotfeather, and water hyacinth. At two sites, alligatorweed control was 82 and 87% at 8 WAT with 140 g/ha AMCP-a.

Combinations of 70 g/ha AMCP-a plus either 560 g ae/ha triclopyr, 1120 g ae/ha glyphosate, or 140 g ae/ha imazapyr controlled alligatorweed 80% at 8 WAT. Creeping water primrose was controlled 86% at 8 WAT with 210 g/ha AMCP-a. Combinations of 70 g/ha AMCP-a plus either 560 g ae/ha 2,4-D, 560 g/ha triclopyr, 1120 g/ha glyphosate, or 140 g/ha imazapyr, as well as glyphosate alone and imazapyr alone controlled creeping water primrose 81% or greater. At two sites, 200 µg ai/L AMCP-a controlled parrotfeather 65 and 99% and reduced biomass to 31 and 36% of pretreatment dry weights at 8 WAT. At two sites, water hyacinth control at 3 months after treatment (MAT) with 280 g/ha AMCP-a was 70 and 95%. Treatments of 280 g/ha AMCP-a, 560 g/ha 2,4-D, 560 g/ha triclopyr, 140 g/ha AMCP-a plus 560 g /ha 2,4-D, and 140 g/ha AMCP-a plus 560 g/ha triclopyr all resulted in significantly lower dry weights than untreated control plots at 3 MAT. Absorption and translocation of ¹⁴C-AMCP-a was evaluated in alligatorweed, water hyacinth and water lettuce with foliar applications. Absorption was 17 and 79% in alligatorweed at 1 and 96 HAT, respectively. Absorption was 59% or greater at all harvest times for water hyacinth and water lettuce. In alligatorweed at 96 HAT, 43% of absorbed ¹⁴C translocated to shoots above the treated leaf and 17% translocated to lower tissue. Water hyacinth parts above and below the treated leaf each contained 17% of absorbed ¹⁴C at 96 HAT. For water lettuce at 96 HAT, 53 and 36% of absorbed radioactivity was located above the treated leaf and in the growing solution, respectively.

Aminocyclopyrachlor Efficacy and Behavior in Selected Aquatic Plants

by
Trevor Davis Israel

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APPROVED BY:

Robert J. Richardson
Committee Chair

David L. Jordan

Michael D. Netherland

DEDICATION

I dedicate this body of work to the loving memory of my nephew, Ashton Trevor Bennett, cherished by his family and anyone who had the pleasure of knowing him. May we be forever reminded of life's fragility and uncertainty. Let us not take for granted any time we spend on this earth, any person we meet, or any opportunity we encounter. Let us behold the power of love and the strength of family and friends.

BIOGRAPHY

Trevor Davis Israel grew up in Candler, North Carolina and graduated from Enka High School. At a young age, he developed an appreciation for nature and realized the need for environmental stewardship. He received his B.S. degree in Environmental Sciences from North Carolina State University. He then began working as an Environmental Technician for the NC Department of Natural Resources, where he managed aquatic invasive weed populations across the state. During one project, Trevor met Dr. Rob Richardson and learned of graduate research opportunities available at NCSU in the area of aquatic plant management. Excited by the prospect of advancing his knowledge of invasive species, he then decided to pursue a M.S. in Crop Science. He has presented his research at annual meetings of the Aquatic Plant Management Society, South Carolina Aquatic Plant Management Society, Weed Science Society of America, Southern Weed Science Society, and the Weed Science Society of North Carolina. Trevor plans on pursuing a career in natural resource conservation or crop protection.

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Chapter 1

An Introduction to Aquatic Plant Management, Synthetic Auxins, and Aminocyclopyrachlor

ABSTRACT

Aquatic weeds can infest water bodies and cause a multitude of problems for resource managers and citizens. Invasive aquatic plants disrupt the ecological balance of ecosystems and crowd out native species, thereby decreasing biodiversity. In addition to environmental impacts, weeds cause a range of economic problems. They may cause flooding in agricultural lands by blocking drainage canals and restrict the function of hydropower and municipal water facilities by clogging intakes. Also, weeds can lower property values of waterfront real estate and impede recreational activities like swimming, boating, and fishing. The difficulties listed above have led to the comprehensive study of nuisance aquatic weeds by various institutions and government agencies and the development of efficient control methods. Chemical methods include the use of approved aquatic herbicides with different modes of action. Synthetic auxin herbicides have been utilized for several decades in successful aquatic weed management programs. Recently, a new auxin mimic, aminocyclopyrachlor, was discovered and efficacy trials of this herbicide are warranted for aquatic weeds. The following literature review will summarize some major species of aquatic weeds, current control strategies, synthetic auxin history, and aminocyclopyrachlor background.

AQUATIC PLANT MANAGEMENT

Background

Aquatic plants are necessary and important features in aquatic ecosystems. They provide oxygen to the system, habitat for fish and wildlife, habitat for food organisms, stabilization of sediments, and aesthetic appeal (Ross and Lembi 1999, McComas 2003, AERF 2009). Aquatic plants may be classified by where they are found in a body of water and are generally grouped into four categories: emergent, free-floating, rooted-floating, and submersed (Ross and Lembi 1999). Emergent plants, also referred to as shoreline or marginal plants, grow in shallow water, are rooted in the sediment, and have foliage extending above the water surface. Free-floating plants grow on the water surface, unanchored to the sediment. Rooted-floating plants are attached to the sediment with stems that rise to the surface to produce floating leaves. Submersed plants are usually rooted in the sediment with foliage growing completely under water, although flowers or flower parts can extend above the water surface. A certain level of aquatic vegetation is usually desired in aquatic systems, but excessive plant growth limits uses of the water body, threatens human health, and disrupts natural processes.

Nuisance aquatic plants restrict boating, fishing, and swimming in recreational lakes, and also interfere with hydropower and drinking water operations in industrial settings. In 1991, a floating mat of hydrilla [*Hydrilla verticillata* (L. f.) Royle] caused turbines to be shut down at the St. Stephen Hydroelectric Power Station in South Carolina, resulting in a \$4 million loss (Kirk and Henderson 2006). Agricultural endeavors can also be severely impacted. Aquatic weeds may cause flooding in fields, inhibit the use of water in irrigation

canals (Spencer and Coulson 1976, Parochetti et al. 2008), or directly invade flooded crops such as rice (Smith 1983). Nuisance plants pose threats to human safety and health.

Highways can become flooded after weeds block culverts and drainage canals (Ross and Lembi 1999). Dense vegetation from aquatic weeds causes large areas of water to become stagnant, providing mosquito breeding habitat (Spencer and Coulson 1976, Orr and Resh 1992). Environmental processes can also be affected by aquatic weeds. After senescence, the dense vegetation of exotic species leads to an accumulation of organic matter. The organic matter then decays, which lowers dissolved oxygen and increases nutrient loading into the water column (AERF 2009). Madsen et al. (1991) reported a decrease in the number of native species found in beds dominated by Eurasian watermilfoil (*Myriophyllum spicatum* L.). Monotypic stands of weeds not only decrease the diversity of plant species directly through competition, but also indirectly limit the types of fauna present, since diverse plant communities support more invertebrate species (Boyd 1971, McComas 2003).

The threats posed by certain plant species, including aquatic weeds, led to the establishment of a program to control the spread of noxious weeds under the Federal Noxious Weed Act of 1974, which was later superseded by the Plant Protection Act of 2000 (7 USC 7701 et seq.). The term “noxious weed” refers to “any plant or plant product that can directly or indirectly injure or cause damage to crops (including nursery stock or plant products), livestock, poultry, or other interests of agriculture, irrigation, navigation, the natural resources of the United States, the public health, or the environment.” In addition, an “invasive species” is one that is not native to a particular ecosystem and whose introduction does or is likely to cause economic or environmental harm or harm to human health

(Executive Order 13112). The USDA Animal and Plant Health Inspection Service (APHIS) maintains a list of currently designated noxious weeds, nineteen of which are aquatic and wetland weeds, in order to prevent their introduction into the United States or their dissemination within the United States (USDA APHIS 2011). Many state agencies also regulate additional aquatic species. Regardless of their regulatory status, aquatic plants can become problematic in certain situations. An understanding of the biology, morphology, and phenology of aquatic plants is essential for management programs. Though not an exhaustive collection, the weedy and desirable species described below are of particular concern for management in the southeastern United States. Many of the nuisance species are listed as some of most common or problematic aquatic weeds (Webster 2007).

Selected Species

Alligatorweed. Commonly found throughout the southeastern United States and California, alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.; Figure 1.1] is a mat-forming perennial aquatic weed that was introduced from South America in the late nineteenth century, probably as a contaminant of ship ballast (Zeiger 1967). It can be found canals, ditches, and slow-moving rivers and streams, and is tolerant of saline soils and waters (Holm et al. 1997). Alligatorweed is listed as noxious in Alabama, Arizona, Arkansas, California, Texas, and prohibited in Florida and South Carolina (USDA NRCS 2011).

Leaves are linear-elliptic in shape, up to 9 cm long, and have a prominent midvein (Godfrey and Wooten 1981). Young leaves have scattered hairs on either side and become glabrous with age (Godfrey and Wooten 1981). They are oppositely attached to the stem,

which becomes hollow in aquatic situations, facilitating rapid floating mat formation (Godfrey and Wooten 1981, Holm et al. 1997). The base of the leaf tapers to a petiole, which clasps the stem to form a narrow sheath (Godfrey and Wooten 1981). White, clover-like flowers are borne on 1 to 7 cm long peduncles, arising from the axils of upper leaves (Aulbach-Smith and de Kozlowski 1996). No viable seeds have been reported in the United States, and spread is almost entirely due to vegetative reproduction (Sculthorpe 1967, Spencer and Coulson 1976).

Creeping water primrose. Another emergent weed native to South America is creeping water primrose [*Ludwigia grandiflora* (Michx.) Greuter & Burdet]. It is now found throughout the southeastern United States and the west coast (Nesom and Kartesz 2000, USDA NRCS 2011). Creeping water primrose is considered invasive and is a noxious weed in North Carolina and Washington and is prohibited in South Carolina (USDA NRCS 2011). It occurs in marshes, swamps, ponds, ditches, canals, and around lake margins (Aulbach-Smith and de Kozlowski 1996, Okada et al. 2009).

Creeping water primrose stems are glabrous to sparsely pubescent, forming dense floating mats or erect growth up to 1 m tall (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Two distinct growth forms are present: the vegetative or rosette stage and the flowering stage (Aulbach-Smith and de Kozlowski 1996). The vegetative stage is characterized by light green, floating stems that produce alternate glabrous, shiny suborbicular to obovate leaves 1 to 4 cm long (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Vertical branches of horizontal stems produce opposite leaves with short internodes that resemble a rosette (Aulbach-Smith and de Kozlowski 1996). Nodes not

only produce wiry and branched roots, but also spongy, white aerenchymous roots (Aulbach-Smith and de Kozlowski 1996). Later in the flowering stage, the rosettes elongate and the stems become reddish, erect, and woody, bearing leaves that are somewhat larger than those below, lanceolate to elliptic in shape, and sparsely pubescent (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Solitary, showy bright yellow flowers are produced from late April to September on hairy pedicels 2 to 4 cm long from upper leaf axils (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Five or six narrow sepals surround 5 or 6 strongly-veined, 2 to 3 cm long obovate petals (Aulbach-Smith and de Kozlowski 1996). Fruit capsules are glabrous, cylindrical in shape, and contain many viable seeds (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Creeping water primrose spreads easily by both seeds and fragmentation (Okada et al. 2009).

Parrotfeather. Another perennial aquatic plant native to South America is parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.], which was probably introduced in the late 1800s or early 1900s (Sutton 1985). It is currently found in most of the United States, with the exception of upper New England and some Midwestern states (USDA NRCS 2011). Parrotfeather is listed as noxious, prohibited, or invasive in Alabama, Connecticut, Maine, Massachusetts, Vermont, and Washington (USDA NRCS 2011).

Parrotfeather grows either as a rooted submersed or as an emergent plant. The emergent stems grow up to 30 cm or more above the surface of moist areas and may extend several meters across the surface of the water (Sutton 1985). Submersed forms of parrotfeather may grow in shallow waters up to 1 m deep (Aulbach-Smith and de Kozlowski 1996). Whorls of 4 to 6 leaves surround the stem, with each leaf containing 20 or more

linear-filiform divisions (Sutton 1985, Godfrey and Wooten 1981). Emergent leaves are firm, 1.5 to 5 cm long, up to 1.5 cm wide, and pale green to grayish green in color (Aulbach-Smith and de Kozlowski 1996). Submersed leaves are usually larger and more finely segmented than emergent leaves (Aulbach-Smith and de Kozlowski 1996). Stems are elongated and relatively stout, often tinged red or orange, and contain many adventitious roots underwater (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Parrotfeather is rarely found in bloom, with inconspicuous white flowers borne in the axils of unreduced leaves (Godfrey and Wooten 1981). Although dioecious, only pistillate plants appear in the United States, and seed set does not occur (Aiken 1981). Thus, reproduction occurs almost exclusively through fragmentation.

Mosquitofern. Native to the eastern and southern United States, mosquitofern (*Azolla caroliniana* Willd.) is a free-floating aquatic leptosporangiate fern. It is currently found from Maine to Florida and west to Texas and South Dakota (USDA NRCS 2011). A similar species, *Azolla pinnata* R. Br., is classified as a federal noxious weed (USDA NRCS 2011).

The pinnately branched stems of mosquitofern contain small imbricate leaves, while the underside bears sparse adventitious roots. The leaves are alternately arranged and each one is divided into two parts. The upper, green to reddish floating lobe is 5 to 10 mm in diameter and is the portion of the leaf responsible for photosynthesis. The lower lobe of the leaf is submersed, nearly colorless, and is thought to absorb water and nutrients, thereby compensating for the poorly developed roots (Eames 1936, Demalsy 1953). Interestingly, *Azolla* spp. are able to obtain an important nutrient, nitrogen, through a unique symbiotic relationship. The cavity of each dorsal lobe contains filaments of the blue-green alga

Anabaena azollae, which reproduce there and fix atmospheric nitrogen (Sculthorpe 1967). The upper leaves of *A. caroliniana* contain fine bicellular hairs on the adaxial surface. The hairs trap a layer of air, keeping the leaves dry and preventing immersion by rain. The spore-containing organs of *Azolla* species appear in the axil of the dorsal aerial leaf-lobe (Sculthorpe 1967). Mature *Azolla* plants have poorly developed roots, a morphological reduction probably associated with the free-floating habit (Sculthorpe 1967). Fragmentation occurs markedly during senescence and axillary buds readily give rise to new individual plants (Sculthorpe 1967).

Giant salvinia. Another free-floating aquatic pteridophyte is giant salvinia (*Salvinia molesta* Mitchell), which is capable of forming dense mats on the surface of water. It is native to southeast Brazil and can be found in Alabama, Arizona, California, Florida, Georgia, Hawaii, Louisiana, Mississippi, North Carolina, South Carolina, and Texas (Forno 1983, USDA 2011). Giant salvinia can double in biomass in only a few days and is classified as a federal noxious weed (Cary and Weerts 1983, USDA NRCS 2011). Holm et al. (1977) classified it as one of the world's worst weeds.

Leaf color of giant salvinia varies from bright green to brownish-yellow. Three growth stages have been distinguished: primary survival, secondary colonizing, and tertiary mat-forming stages (Sculthorpe 1967, Aulbach-Smith and de Kozlowski 1996). *Salvinia* in the survival stage exhibits leaves that are mostly rounded and small, about 1cm in diameter, and lie flat on the surface. The survival stage occurs where ample space is available or environmental stresses are present (Sculthorpe 1967, Aulbach-Smith and de Kozlowski 1996). Within a few weeks, plants develop into the colonizing stage, in which the floating

leaves are partly folded, 1 to 2.5 cm in diameter (Sculthorpe 1967, Aulbach-Smith and de Kozlowski 1996). The stems are branched and leaves are arranged in whorls of three, with the highly-modified lower leaf submersed and divided into segments resembling roots (Sculthorpe 1967, Aulbach-Smith and de Kozlowski 1996). The floating upper leaves are opposite and notched at the apex, with the upper surface covered with dense, upright white hairs arranged in rows (Aulbach-Smith and de Kozlowski 1996). Each hair is branched into four segments that fuse at their tips to form an "egg beater" structure (Aulbach-Smith and de Kozlowski 1996). Like *Azolla*, these hairs create buoyancy and shed water (Sculthorpe 1967). In the mat-forming stage, the acutely folded leaves are much wider than they are long, about 2.5cm by 3.5cm, and extend upward, facilitating water repellency down the midrib (Sculthorpe 1967). An identifying characteristic of giant salvinia is the linear alternating arrangement of small, brown sporangia on the submersed root-like leaves (Mitchell 1972, Aulbach-Smith and de Kozlowski 1996). However, giant salvinia is not known to produce viable spores due to anomalies at meiosis which prevent production of fertile haploid gametes (Oliver 1993). It spreads rapidly by vegetative reproduction, as the smallest part of the stem bearing an axillary bud readily gives rise to new individual plants (Sculthorpe 1967, Oliver 1993).

Water hyacinth. Native to Brazil, water hyacinth [*Eichhornia crassipes* (Mart.) Solms; Figure 1.2] is a free-floating plant and was first introduced to the United States in the mid-1800s (Penfound and Earle 1948). It is considered extremely invasive and is one of the world's worst aquatic weeds (Holm et al. 1977). Water hyacinth is found throughout the southeastern United States, as well as west coast states and a few New England States

(USDA NRCS 2011). It is listed as noxious in Alabama, Arizona, California, Texas, prohibited in Florida and South Carolina, and potentially invasive in Connecticut (CIPWG 2010, USDA NRCS 2011).

The monocotyledonous plant has a rosette habit, bearing aerial or surface-floating leaves from a crown-like stem (Sculthorpe 1967). In densely crowded conditions, leaves become upright and reach 1 m in length (Penfound and Earle 1948). Near the base of the plant, spongy, swollen petioles form as the lacunate mesophyll traps air during development, allowing the plant to float (Sculthorpe 1967). Subtending the petioles are waxy leaf blades, which are 1.5 to 20 cm long, elliptic or ovate in shape, and have parallel venation (Aulbach-Smith and de Kozlowski 1996). Roots may grow up to 1 m in length and are fibrous with a conspicuous root cap (Penfound and Earle 1948). They are purplish to black in color when exposed, but are white when in darkness or rooted in the substrate (Penfound and Earle 1948). Five to twenty showy, light-blue to lavender flowers are spirally-arranged along the spike form the inflorescence. Flowers are unspecialized and zygomorphic, with a bright yellow mark appearing on the upper petal, possibly serving as a guide to pollinators (Sculthorpe 1967). Although water hyacinth displays potential for rapid and abundant seed production, environmental factors may limit the extent of sexual reproduction (Barrett 1980). Asexual reproduction occurs by the formation of lateral stolons which produce terminal buds that develop into new rosettes (Sculthorpe 1967). Water hyacinth is susceptible to prolonged periods of freezing temperatures (Owens and Madsen 1995), and severe winters can limit its range (Luu and Getsinger 1990).

Water lettuce. Like water hyacinth, water lettuce (*Pistia stratiotes*) is a free-floating aquatic monocot with a rosette habit. The origins of the plant are unknown, although it was observed in Florida as early as 1765 (McCann et al. 1996). Water lettuce is found in most of the southeastern US, parts of New England, and several western states and is listed as noxious in Alabama, California, and Texas, potentially invasive in Connecticut, and prohibited in Florida and South Carolina (CIPWG 2010, USDA NRCS 2011). Common habitats include shallow marshes, swamps, and ponds and it is frequently mixed with water hyacinth.

Water lettuce leaves are grayish to light green, densely pubescent, ribbed, and up to 15 cm long (Sculthorpe 1967, Aulbach-Smith and de Kozlowski 1996). Mature leaves are ovate to obovate in shape with truncated apices, while younger leaves are rounded (Sculthorpe 1967). Older plants and those in crowded conditions are more erect, facilitating water runoff, whereas leaves of younger rosettes lie flat on the surface (Sculthorpe 1967). The root system is a yellow to brown cluster of primary roots up to 1 m in length extending from the underwater rhizome (Sculthorpe 1967, Weldon and Blackburn 1967). Secondary roots up to 4 cm in length are attached in rows to primary roots (Weldon and Blackburn 1967). Inconspicuous, dioecious flowers are borne on short stalks from the crown (Sculthorpe 1967), but viable seeds are typically not found in natural settings (Weldon and Blackburn 1967). The main method of reproduction in water lettuce is vegetative; offshoots from the crown of the mother plant extend to form new daughter rosettes (Sculthorpe 1967, Weldon and Blackburn 1967).

Three desirable native plants are discussed below in addition to the weedy species.

Soft rush. Frequently found along shorelines, marshes, and meadows, soft rush (*Juncus effusus* L.) is a perennial clump-forming monocot that is distributed throughout the United States (Godfrey and Wooten 1979, USDA NRCS 2011). The stems are stout, relatively soft, bright green, with minute striations and grow to 1.5 m in height (Godfrey and Wooten 1979, Silberhorn 1982). A single reddish-brown inflorescence forms laterally on the stem from June through August (Silberhorn 1982, Aulbach-Smith and de Kozlowski 1996). The perianth segments are equal in length to the many-seeded capsule (Godfrey and Wooten 1979, Aulbach-Smith and de Kozlowski 1996). Soft rush reproduces either by seeds or from rhizomatous roots (Godfrey and Wooten 1979, Aulbach-Smith and de Kozlowski 1996).

Lizard tail. Another commonly found emergent perennial is lizard tail (*Saururus cernuus* L.). The dicot is distributed from Texas to Florida and north to Quebec and listed as endangered in Connecticut and Rhode Island (USDA NRCS 2011). The somewhat pubescent stems grow to over 1 m in height and are unbranched below or with few ascending branches near the top of the plant (Godfrey and Wooten 1981). Leaves are alternate, heart-shaped, dark green above and light green below, and become glabrous with age (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). The inflorescence is a slender white raceme up to 20 cm long, borne on peduncles up to 8 cm in length, and is curved downward (Godfrey and Wooten 1981, Aulbach-Smith and de Kozlowski 1996). Flowers have no sepals or petals, with 3 to 6 pistils and stamens (Aulbach-Smith and de Kozlowski 1996). Lizard tail spreads primarily vegetatively, forming colonies via rhizomes (Aulbach-Smith and de Kozlowski 1996).

Pickerelweed. A member of the water hyacinth family, pickerelweed (*Pontederia cordata* L.) is a perennial monocot that is disseminated from Maine to Minnesota and south to Texas and Florida (USDA NRCS 2011). The plant can be found in ponds, lakes, and streams, frequently in water up to 75 cm deep. It grows to 1 m tall with basal leaves, either with lanceolate blades (*P. cordata* var. *lancifolia*) or with triangular-ovate to triangular-lanceolate blades (*P. cordata* var. *cordata*) (Aulbach-Smith and de Kozlowski 1996). Leaf blades are 15 to 30 cm long, leathery, entire, and parallel veined (Aulbach-Smith and de Kozlowski 1996). The inflorescence is a spike 5 to 15 cm in length, rising from a bladeless sheath (Aulbach-Smith and de Kozlowski 1996). Lavender or blue flowers are irregular in shape and the central upper lobe is marked with two yellow spots, similar to water hyacinth (Aulbach-Smith and de Kozlowski 1996). Pickerelweed spreads by seeds or vegetatively by rhizomes.

Control Methods

Prevention is the first step in an aquatic weed management plan. Therefore, steps should be taken to restrict the introduction of aquatic weeds to a water body. Lake users should be educated about how to identify nuisance species and how to prevent their transport to new areas. Frequent and thorough monitoring can identify new infestations and a rapid response to the problem will minimize potential environmental or economic harm. However, once aquatic weeds are established, controlling them involves mainly four methods: physical, mechanical, biological and chemical. Control measures, particularly chemical, are discussed for the above species.

Physical. These control techniques entail manual harvesting and habitat manipulation. One benefit of manual removal is that it allows for selective harvesting of nuisance weeds, permitting desirable species to continue growing. *Azolla* and *Salvinia* populations frequently infest rice paddies and are removed by raking (Sculthorpe 1967). However, manual removal of weeds is labor intensive and may not be feasible in large scale infestations of weeds. In addition, roots should be harvested as well, so that weeds do not regrow. Sometimes, follow up cuttings are also necessary to insure that recolonization of the invasive species does not occur (Boylen et al. 1996).

Habitat manipulation may involve drawdown or benthic barriers. The goal of a drawdown is to destroy seeds and reproductive structures through exposure to drying and/or freezing conditions and by altering their substrate (Cooke 1980). However, drawdown is typically effective only on submersed vegetation. Creeping water primrose (Hussner 2010) and parrotfeather (Moreira et al. 1999, Hussner et al. 2009) have been shown to be tolerant of varying water levels. Benthic barriers, such as polythene fabric, are installed on the bottom of lakes and shade all submersed species from sunlight. Several studies have reported control of Eurasian watermilfoil using benthic barriers (Boylen et al. 1996, Engel 1984, Perkins et al. 1980). However, Eurasian watermilfoil began to recolonize after barriers were removed or began growing in sediment above the barrier. Cooke and Gorman (1980) considered barriers to be most applicable to small-scale infestations.

Mechanical. Much literature is available describing mechanical control methods for harvesting invasive aquatic plants, particularly water hyacinth (Chikwenhere and Phiri 1999, Spencer et al 2006) and Eurasian water milfoil (Johnson and Bagwell 1979, Perkins and

Sytsma 1987). Mechanical harvesters are used in many areas for aquatic weed control, but they produce fragments of plants that can easily spread and infest new areas, since the majority of aquatic plants can propagate by vegetative means (Sculthorpe 1967).

Accordingly, Spencer et al. (2006) observed water hyacinth regrowth within one week from treatments with three different cutting machines. Perkins and Sytsma (1987) determined that summer mechanical harvesting, unless performed multiple times, would provide no more than immediate nuisance growth removal. Alligatorweed has also been reported to easily regenerate from chopped material (Hitchcock et al. 1950). Mechanical harvesting can also remove fish and other vertebrates (Booms 1999). Although mechanical harvesting is effective for immediate weed removal, issues of regrowth, spread, and impacts to aquatic fauna may limit its utility.

Biological. Fish, insects, and pathogens are the key species studied as potential biocontrol agents for aquatic weeds (Ross and Lembi 1999, Pípalová 2006, AERF 2009). Though research is ongoing with pathogens, fish and invertebrates have demonstrated success in managing certain aquatic weed populations (AERF 2009). The most popular biological agent utilized in aquatic plant management is triploid grass carp (*Ctenopharyngodon idella* Val.) for hydrilla control. Grass carp have been used in long-term management plans of hydrilla, although appropriate stocking densities, impacts to non-target species, and water quality changes are important factors to consider.

Although insect programs can provide long-term, selective control of host species, they often require considerable time and funding (AERF 2009). One species, *Euhrychiopsis lecontei* (Dietz) is a North American aquatic weevil that has been recognized as a potential

biocontrol agent for Eurasian watermilfoil (AERF 2009). Although some studies have associated milfoil population declines with the substantial weevil populations (Lillie 2000, Newman and Biesboer 2000), results are difficult to predict. The biological control agents alligatorweed flea beetle (*Agasicles hygrophila*), alligatorweed thrips (*Amynothrips andersoni*), and alligatorweed stemborer (*Vogtia malloi*) have been thoroughly investigated. Flea beetle programs have been successful in southern states (Maddox et al. 1971, Coulson 1977, Buckingham 1996), but studies in North Carolina have reported flea beetles to be ineffective for suppressing alligatorweed regrowth and surviving the cold winter months (Langeland 1986). For the most part, thrips are ineffective at controlling alligatorweed, especially because of predation by flower bugs and the inability to fly (Spencer and Coulson 1976). The stem-borer may be the most widespread of the insects (Spencer and Coulson 1976), but Langeland (1986) pointed out that, in North Carolina, damage to alligatorweed mats occurred too late in the year and they can regrow after the insect population diminished or moved.

Chemical. Herbicides can effectively control nuisance aquatic vegetation, provided that certain factors are taken into consideration. First, the uses of the water body may limit the choice of herbicide, because some herbicides have drinking, swimming, irrigation, or livestock watering restrictions. The species present also determine chemical control options. Emergent weeds are controlled using foliar applications, submersed weeds are controlled with in-water treatments, and free-floating plants may be controlled with both methods (AERF 2009). A broad spectrum herbicide can be used if total vegetation management is desired, whereas selective herbicides target specific vegetation. The concentrations and

exposure times of submersed applications also provide a degree of selectivity, because plants have different susceptibilities to herbicides. Other non-target species should also be considered, since large amounts of decaying aquatic vegetation consume dissolved oxygen, possibly suffocating fish. Also, broad spectrum control may leave little habitat for invertebrates. The persistence of herbicides depends on herbicide chemistry, water chemistry, and environmental conditions. Also, regulations often differ from state to state. Currently, fourteen active ingredients have been registered for full aquatic use in the United States.

SYNTHETIC AUXINS

History

In 1941, the phenoxyacetic acid compounds MCPA and 2,4-D (2,4-dichlorophenoxy acetic acid) were synthesized independently in the United Kingdom and the United States. During the wartime years, both investigations were carried out in secret, but were not used as chemical warfare agents at that time. The agricultural applications of the chemicals were realized and products were marketed in 1945 and 1946. Application of these herbicides resulted in plant reactions resembling natural auxin overdoses. Observations included stem elongation or twisting, tissue swelling, and leaf folding or cupping. In the many years that followed, many structural derivatives of phenoxy compounds were developed and investigated for herbicidal use. Auxin-like herbicidal activities were later discovered in other chemical families, including benzoic acids, aromatic carboxymethyl derivatives, pyridine derivatives, and quinoline carboxylic acids (Cobb and Reade 2010).

Mode of Action

Auxin herbicides mimic natural indol-3-yl-acetic acid (IAA), a natural plant hormone that helps regulate the division, differentiation, and elongation of plant cells (Cobb and Reade 2010). Flowering, fruit setting and ripening, tropic responses, leaf senescence and abscission, and apical dominance are all dependent on IAA (Cobb and Reade 2010). Early work on the mode of action of auxin herbicides or high levels of IAA involved the membrane-bound Auxin Binding Protein 1 (ABP1) and related proteins (Grossman 2009, Cobb and Reade 2010). It is speculated that rapid auxin-induced effects such as ion fluxes in the plasma membrane are mediated by ABP1 (Grossman 2009, Cobb and Reade 2010).

Grossman (2009) described a second pathway involving transport inhibitor response 1 protein (TIR1). First, synthetic auxin or IAA (Aux/IAA) binds to the transport inhibitor response 1 (TIR1) protein, which is the F-box recognition component of a Skp1-cullin-F-box protein (SCF) E3 ubiquitin ligase (SCF^{TIR1}). Aux/IAA binding stabilizes the interaction between the Aux/IAA transcriptional repressor and the receptor. The SCF^{TIR1} complex binds ubiquitin to the Aux/IAA repressor protein, marking it as a substrate for degradation via the ubiquitin-proteasome pathway. The loss of these repressor proteins leads to the upregulation of activator proteins called auxin response factors (ARFs). ARFs activate transcription of auxin-responsive genes, including 1-aminocyclopropane-1-carboxylic acid (ACC) synthase and 9-cis-epoxycarotenoid dioxygenase (NCED). Overexpression of ACC and NCED genes results in increased biosynthesis of ethylene and abscisic acid (ABA), respectively. Ethylene causes lateral cell expansion, leaf curvature (epinasty), stem swelling, and foliar senescence. The apparent cause of phytotoxicity is the overproduction of ABA. ABA mediates stomatal

closure, thus limiting transpiration and carbon assimilation, which eventually causes an overproduction of reactive oxygen species that degrade tissues.

Auxin herbicides have a major benefit of being selective (generally to monocots) in the weeds controlled. Selectivity usually depends on the amount of herbicide reaching the active site or by the sensitivity of the active site itself. Absorption, translocation, and metabolism differences determine the concentration of herbicide reaching the active site. The correlation between absorption, translocation, and selectivity remains unclear. In fact, higher rates of triclopyr absorption and have been documented in tolerant wheat (*Triticum aestivum* cv Norman) plants compared to susceptible chickweed (*Stellaria media*) plants (Lewer and Owen 1990). However, metabolism did play a role in the same study as chickweed converted triclopyr slower than wheat. It has also been suggested that the auxin receptor in monocots is less accessible than dicot receptors, but no direct evidence is available (Cobb and Reade 2010). Another theory is that since mature vascular tissues in monocots lack a layer of cambium, they may not possess cells capable of auxin reception (Cobb and Reade 2010).

Specific Compounds in Aquatics

2,4-D was first registered for use in aquatic systems in 1959. It is primarily used for control of water hyacinth and Eurasian watermilfoil, but is also effective in controlling emergent and other submersed weeds (AERF 2009). Although 2,4-D may be preferred in some situations due to its selectivity, it can injure some native plants (AERF 2009).

Several investigations have reported the efficacy of 2,4-D. Thayer and Haller (1985)

reported no greater than 20% control of water lettuce and less than 10% control of common salvinia (*Salvinia rotundifolia* Willd.) fifteen days after treatment with 4.5 kg/ha 2,4-D. However, 2,4-D at rates of 0.75 to 2.25 kg/ha resulted in complete control of water hyacinth (Singh and Muller 1979). A North Carolina field study indicated 80% control of alligatorweed 2 weeks after treatment (WAT) with 0.46 kg ae/100L 2,4-D, but only 50% control 8 WAT (Langeland 1986). Results from a mesocosm study of parrotfeather showed 85% control 5 WAT from 2.1 kg ae/ha 2,4-D (Wersal and Madsen 2010). In a pasture study, soft rush was controlled 53% one month after treatment with 1.12 kg ae/ha 2,4-D (Rana and Sellers, 2009). Steenis (1950) reported good control of lizard tail after foliar application of 0.6% 2,4-D.

In 2002, the pyridine carboxylic acid triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) was registered for aquatic use. Use patterns for triclopyr are similar to 2,4-D, mainly selective control of water hyacinth and Eurasian watermilfoil (AERF 2009). However, it may be used in public waters in some states where 2,4-D is not permitted.

A review of recent literature indicates varied levels of control for different species. Triclopyr was determined to control water hyacinth at a rate of 3.3 kg/ha, but not water lettuce (Langeland and Smith 1993). Hofstra and Champion (2010) reported that although young alligatorweed plants treated with 6.5 kg ai/ha triclopyr had significantly reduced root biomass than controls, mature plants did not. Another study of alligatorweed indicated percent cover was reduced initially after April application of 1.7 to 5.2 kg ae/ha triclopyr, but began increasing 3 to 4 WAT (Allen et al. 2007). Champion et al. (2008) reported 100% control of primrose willow [*Ludwigia peploides* (Kunth) P.H. Raven] 3 WAT after triclopyr

was applied to runoff at a rate of 2 g ai/L. Parrotfeather percent cover was reduced in the field with foliar applications of 4 kg ai/ha (Hofstra et al. 2006). Wersal and Madsen (2010) reported 70% control 5 WAT from 6.7 kg ae/ha triclopyr.

Triclopyr and 2,4-D are currently the only auxin herbicides registered for use in aquatic systems (EPA 2010). These herbicides provide selective control of most herbaceous dicots, allowing resource managers to control invasive broadleaf species while maintaining populations of native monocots. However, 2,4-D and triclopyr do not effectively control some weedy species and may injure other desirables. Aminocyclopyrachlor may provide resource managers with another tool for effective and selective control of aquatic weeds. A review of aminocyclopyrachlor is given to familiarize the reader with this new herbicide.

AMINOCYCLOPYRACHLOR

Aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid) belongs to a new class of synthetic auxins called pyrimidine carboxylic acids (Finkelstein et al. 2008). It has a chemical structure similar to the pyridine carboxylic structure similar to the pyridine carboxylic acids; however, it consists of an additional nitrogen in the heterocyclic ring and a cyclopropyl side chain (Figure 1.3). The acid form and its methyl ester analog have been shown to control a number of broadleaf and brush species in turf, rangelands, pastures, industrial rights of way, and forestry site sites (Claus et al. 2008, Gannon et al. 2009, Meredith et al. 2011, Roten and Richardson 2011).

The herbicide is thought to target a family of auxin receptor complexes, where it disrupts the hormonal balance necessary for normal root and shoot development (Finkelstein et al. 2008). Additionally, aminocyclopyrachlor effects on the transcription process may be more intense than other compounds with a similar mode of action (Finkelstein et al. 2008). After application of aminocyclopyrachlor, broadleaf species exhibit symptoms typical of auxin mimic exposure, including leaf curling, epinasty, and tissue swelling (Bukun et al. 2010).

Aminocyclopyrachlor has potential utility in aquatic sites for several reasons. Foliar use rates of the herbicide are expected to be in the range of 0.07 to 0.32 kg ai/ha, which is lower than several commercial auxin herbicides (Finkelstein et al. 2008). The anticipated in-water rates are also low, in the range of 0.1 to 0.4 mg ae/L. The herbicide has a favorable environmental profile, as indicated by low mammalian toxicity and trout and daphnia toxicities comparable to that of 2,4-D and triclopyr (Senseman 2007, Finkelstein et al. 2008). Another benefit of aminocyclopyrachlor is that it undergoes rapid photodegradation (Finkelstein et al. 2008). The solubility of aminocyclopyrachlor is low (4.2 g/L) (Finkelstein et al. 2008). However, the anticipated low use rates may counteract the low solubility. Table 1.1 compares the properties of aminocyclopyrachlor to those of 2,4-D and triclopyr.

According to Finkelstein et al. (2008), aminocyclopyrachlor has a dissociation constant (pK_a) of 4.65, is rapidly absorbed by leaves and roots, and is translocated in both xylem and phloem to meristematic regions of the plant. Also, the log octanol-water partition coefficient ($\log K_{ow}$) of aminocyclopyrachlor is -2.48 and -1.12 at pH 7 and 4, respectively (Finkelstein et al. 2008). However, according to the model of phloem mobility proposed by

Hsu and Kleier (1996), the log K_{ow} of aminocyclopyrachlor is below the range for phloem mobility, despite having a pK_a similar to other phloem mobile herbicides. Limited basipetal movement of herbicides may result in reduced herbicide efficacy, as plants may regrow from roots and other underground storage tissues. Bukun et al. (2010) suggested that the additional nitrogen in the pyrimidine ring may give sufficient polarity to the molecule so that it remains hydrophilic. The researchers also postulated that it may have greater biological activity than other auxin herbicides, possibly due to more favorable binding kinetics at the site of action (Bukun et al. 2010).

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Figure 1.1. Floating alligatorweed mats in a pond in Sampson County, North Carolina.



Figure 1.2 Water hyacinth covering a pond in Carteret County, North Carolina

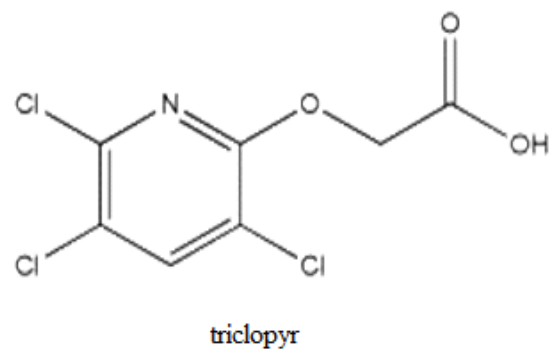
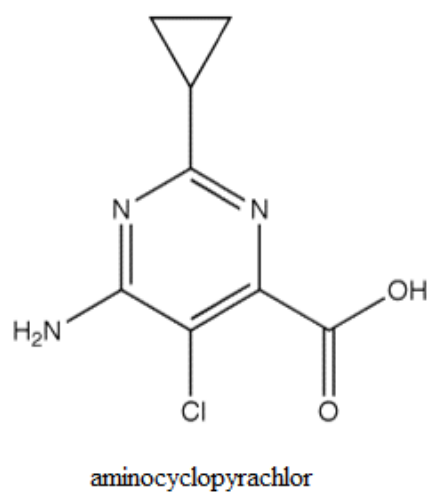


Figure 1.3. Chemical structures of aminocyclopyrachlor and the pyridine carboxylic acid herbicide triclopyr.

1 Table 1.1. Characteristics of aminocyclopyrachlor, triclopyr, and 2,4-D.^a

	Aminocyclopyrachlor acid	Triclopyr-TEA ^b	2,4-D-DMA
Soil half-life	37 to 128 days	10 to 46 days	7 to 28 days
K _{oc}	28 mL/g	20 mL/g	20 mL/g
Photodegradation	Rapid	Rapid	Minor
Solubility	4.2 g/L	2,100 g/L	796 g/L
Use rate (foliar)	0.07-0.32 kg ai/ha	1.68-6.73 kg ae/ha	2.24-4.49 kg ae/ha
Use rate (in-water)	0.1-0.4 mg ae/L	0.75-2.5 mg ae/L	1-4 mg ae/L
Trout toxicity	>122 mg ae/L	613 mg/L	250 mg/L
Daphnia toxicity	43 mg ae/L	775 mg/L	184 mg/L

2 ^aAdapted from Finkelstein et al. (2008) and Senseman (2007).3 ^bAbbreviations: TEA, triethylamine; DMA, dimethylamine.

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Chapter 2

Efficacy of Aminocyclopyrachlor Acid and Aminocyclopyrachlor-methyl Ester on Selected Aquatic Species in Greenhouse Experiments

ABSTRACT

Greenhouse studies were conducted to determine the response of several aquatic species to aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidine carboxylic acid, AMCP-a) and its methyl ester (AMCP-me). AMCP-me did not control giant salvinia (*Salvinia molesta* Mitchell) or water lettuce (*Pistia stratiotes* L.) at the anticipated use rates. AMCP-me at 140 g ai/ha controlled mosquitofern (*Azolla caroliniana* Willd.) 84% at 4 weeks after treatment (WAT). Control of water hyacinth [*Eichhornia crassipes* (Mart.) Solms] at 4 WAT was 94% with 280 g/ha AMCP-a. Alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.] control was 88% at 5 WAT with 280 g/ha. Creeping water primrose [*Ludwigia grandiflora* (Michx.) Greuter & Burdet] and parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] control at 5 WAT with 35 g/ha was 85% and 95%, respectively. At 5 WAT, soft rush (*Juncus effusus* L.) injury was 5% or less at all rates tested, while pickerelweed (*Pontederia cordata* L.) injury at 35 g/ha was 83% and increased with rate. Lizard tail (*Saururus cernuus* L.) injury 5 WAT was 33% and 87% at 35 and 560 g/ha, respectively. AMCP-a was also evaluated on alligatorweed planted at different water depths. The main effect of AMCP-a rate was not significant for any parameters measured, but the main effect of depth was significant on visual control, height reduction, and shoot weight reduction. Alligatorweed control at 4 WAT was 75% at 22 cm water depth. As depth

increased, percent reduction in height decreased, but percent reduction in shoot weight increased.

INTRODUCTION

Aquatic weeds cause a myriad of problems for farmers, resource managers, and outdoor enthusiasts. In the western United States, over 225,400 miles of canals and drains are susceptible to infestation by weeds, which directly impact the movement of water to agricultural systems (Parochetti et al. 2008). Aquatic weeds disrupt ecosystems by lowering dissolved oxygen levels and displacing native vegetation through direct competition (Madsen et al. 1991, Buckingham 1996, Villamagna and Murphy 2010). In addition, aquatic weeds can decrease real estate values and provide breeding habitat for mosquitoes (Holm et al. 1969, Orr and Resh 1992).

Numerous control strategies exist for aquatic weeds, including physical, biological, and chemical methods. Mechanical harvesters have long been used for the management of Eurasian watermilfoil (*Myriophyllum spicatum* L.) and water hyacinth, but studies have shown only short-term benefits because of the ability of these plants to regrow after cutting (Perkins and Sytsma 1987, Spencer et al. 2006). Biological control agents, such as fish and invertebrates, have shown promise in suppressing aquatic weed growth, but also have limitations. Grass carp (*Ctenopharyngodon idella* Val.) cannot be easily removed from a water body once a desired level of vegetation is reached and also migrate in open systems, thereby consuming non-target vegetation (Noble et al. 1986, AERF 2009). Classical insect biocontrol agents may not become established in colder climates and may only suppress

weed growth (Maddox et al. 1971, Langeland 1986). The threat of economic and environmental losses and the limitations of other control methods have led to the research and development of aquatic herbicides, because they provide control that can be cost-effective, selective, and site-specific. However, only fourteen compounds have been labeled for aquatic use, which limits herbicidal options in some situations.

Aminocyclopyrachlor is a recently discovered herbicide that belongs to a new class of synthetic auxins called pyrimidine carboxylic acids (Finkelstein et al. 2008). The acid formulation and the methyl ester have been shown to control a number of broadleaf and brush species in turf, rangelands, pastures, industrial rights of way, and forestry site sites (Claus et al. 2008, Gannon et al. 2009, Meredith et al. 2011, Roten and Richardson 2011). The herbicide targets a family of auxin receptor complexes and disrupts the hormonal balance necessary for normal shoot and root development (Finkelstein et al. 2008). After application of aminocyclopyrachlor, broadleaf species exhibit symptoms typical of auxin mimic exposure, including leaf curling, epinasty, and tissue swelling (Bukun et al. 2010, Israel et al. 2011).

Aminocyclopyrachlor has potential utility in aquatic sites for several reasons. Foliar use rates of the herbicide are most likely in the range of 0.07 to 0.32 kg/ha, which is lower than several commercial auxin herbicides (Finkelstein et al. 2008). The anticipated in-water rates are also low, in the range of 0.1 to 0.4 mg/L. The herbicide has a favorable environmental profile, as indicated by low mammalian toxicity and trout and daphnia toxicities comparable to that of 2,4-D (2,4-dichlorophenoxy acetic acid) and triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) (Senseman 2007, Finkelstein et al. 2008). Another

benefit of aminocyclopyrachlor is that it undergoes rapid photodegradation (Finkelstein et al. 2008). Table 1.1 compares the properties of aminocyclopyrachlor to those of 2,4-D and triclopyr, synthetic auxin herbicides currently registered for aquatic use.

No published research exists on the activity of aminocyclopyrachlor on aquatic plants. Therefore, the objective of this research was to evaluate the efficacy of aminocyclopyrachlor on seven aquatic weeds and three desirable aquatic plants. Characteristics of the species used in the following studies are presented in Table 2.1.

MATERIALS AND METHODS

Studies were conducted in greenhouses at North Carolina State University, Raleigh, NC.

Floating weeds. Mosquitofern, giant salvinia, water hyacinth, and water lettuce were propagated in 7 L black pails from existing greenhouse populations and allowed to grow for three weeks at 30 C. Plants received weekly liquid fertilizer¹ applications and were irrigated twice daily by an overhead sprinkler system to maintain water levels. In order to evenly distribute biomass, plants of uniform size were divided so that coverage in each pail was approximately 80%.

Pails were treated with AMCP-me² and AMCP-a³ using an indoor spray chamber with an overhead track sprayer⁴ calibrated to deliver 280 L/ha at 207 kPa. AMCP-me was applied at rates of 70, 140, 280, and 560 g/ha to mosquitofern, giant salvinia, and water lettuce. AMCP-a was applied at rates of 70, 140, 280, 420, and 560 g/ha to water hyacinth. Treatments included methylated spray oil⁵ (MSO) at 1% (v/v), as recommended (DuPont 2011).

The experimental design was a randomized complete block with four replications and was repeated. Visual estimates of weed control were determined on a weekly basis in comparison to the nontreated control. Phytotoxicity symptoms such as stem twisting, leaf curling, and necrosis were evaluated to assign weed control ratings. Weed control was assessed on a 0 to 100% scale where 0% indicates no plant response and 100% indicates complete plant death. Roots and shoots of plants could not easily be separated; therefore, at 4 WAT, the total biomass in each pail was removed, oven-dried at 50 C to constant moisture, and weighed.

Emergent weeds. Alligatorweed, creeping water primrose, and parrotfeather plants were propagated from clippings collected from greenhouse inventories. Two 10 cm plant clippings were planted in 10 cm square pots containing a commercial potting mix⁶ and 5 g slow release fertilizer⁷. Plants were irrigated under an overhead mist system four times daily for five minutes and received weekly liquid fertilizer additions after establishment. Plants were allowed to grow for one month until they reached approximately 40 cm.

Plants were then treated in an indoor spray chamber as described in study 1. Six herbicide treatments included AMCP-a at 35, 70, 140, 280, 420, and 560 g/ha. Each herbicide treatment included 1% v/v MSO. A pretreatment control and nontreated control were also included in the study. Four treatment replications were used, for a total of 32 pots of each species. Treatments were arranged in a randomized complete block design and repeated once.

Visual estimates of weed control were determined as described previously. At 5 WAT, the roots and shoots of each pot were separated and oven dried at 50 C to constant

moisture for dry weight determination. “Roots” refers to total below-ground biomass, and “shoots” refers to total above-ground biomass.

Desirable plants. Soft rush, lizard tail, and pickerelweed plants were propagated from NC field-collected populations. Soft rush plants were collected from Granville County, lizard tail plants came from Johnston County, while pickerelweed plants were collected from Halifax County. Plants were potted in 6 L pails filled with a mixture of 2:1 sterilized topsoil⁸ to peat⁹ and allowed to grow for one month. Plants were irrigated twice daily for three minutes by an overhead sprinkler system and received weekly liquid fertilizer additions. Experimental design and herbicide treatments were the same as study 2, with four replications and one trial repetition. Ratings were collected weekly for five weeks and were based on symptoms described above. At 5 WAT, roots and shoots were separated and rinsed, then processed as described above.

Alligatorweed depth study. Alligatorweed plants were emersed in different water depths to determine possible effects on AMCP-a efficacy. The study was a three by two factorial design with three levels of water depth and two rates of AMCP-a. Two 10 cm plant clippings were planted in 15 cm round pots containing a commercial potting mix.¹⁰ Pots were capped with a 2 cm layer of sand to prevent suspension of soil in the water column. Plants were allowed to grow for 2 weeks, received weekly liquid fertilizer doses, and irrigated four times daily by an overhead sprinkler system. Upon establishment, pots were placed at the bottom of 15 L buckets containing pond water and plants were allowed to grow for four weeks. Three water levels were maintained: 2 cm above the bottom of the bucket for 100% shoot exposure to air, 22 cm above the bottom of the bucket for 67% shoot exposure to air, and 33

cm above the bottom of the bucket for 33% shoot exposure to air, hereafter referred to as water depths of 0, 11, and 22 cm, respectively.

AMCP-a was applied at 140 and 280 g/ha using a CO₂-pressurized backpack sprayer equipped with two nozzles¹¹ attached to a boom. The sprayer was calibrated to deliver 280 L/ha at 310 kPa. Herbicide treatments included 1% v/v MSO. Nontreated control plants were maintained at all three water levels. The study contained four replications and was repeated once. Alligatorweed plants were rated weekly for four weeks. At 4 WAT, heights of the longest green, viable stem were measured. Also, total plant material above the soil (shoots) and total plant material below the soil (roots) were separated and processed as described above.

Data Analyses. Visual ratings, floating weed dry weights, emergent weed and desirable plant root dry weights, and alligatorweed depth study plant heights, root dry weights, and shoot dry weights were subjected to analysis of variance (ANOVA). Root weights for emergent weeds and desirable plants were selected as a metric in order to estimate long term herbicide efficacy. Means from the floating weed, emergent weed, and desirable plant studies were separated using pairwise comparisons of individual treatments ($P \leq 0.05$) in commercial software¹². Percent reductions for the alligatorweed depth study were based on nontreated plants at each depth. Means from the alligatorweed depth study were separated using Fisher's Protected LSD ($P \leq 0.05$). Non-treated controls were not included in the analysis of visual ratings, but were included in dry weight analyses. For ANOVA, all data were combined as a treatment by trial repetition interaction was not observed. Regression analyses were performed in commercial software¹³ using the logistic equation $y = a/[1+(x/x_0)^b]$ for

alligatorweed, creeping water primrose, lizard tail, mosquitofern, and water hyacinth visual ratings, mosquitofern and water hyacinth plant dry weights, and alligatorweed, parrotfeather, and pickerelweed root dry weights. The exponential equation $y = a(1 - e^{-bx})$ was used for parrotfeather and pickerelweed visual ratings, while the logistic equation $y = y_0 + a/[1 + (x/x_0)^b]$ was used for creeping water primrose root dry weights, and the linear equation $y = a + bx$ was used for lizard tail root dry weights and water lettuce visual ratings and plant dry weights. Regression models were then used to calculate effective concentrations which reduced dry weight by 70% of nontreated controls (EC_{70}) for mosquitofern plant dry weights and alligatorweed, creeping water primrose, and parrotfeather root dry weights.

RESULTS AND DISCUSSION

Floating weeds. Mosquitofern was more susceptible to AMCP-me than either giant salvinia or water lettuce. At 4 WAT, mosquitofern was controlled 62 and 97% at rates of 70 and 560 g/ha, respectively (Figure 2.1). Dry weights of mosquitofern were 41 and 24% of nontreated controls with 70 and 140 g/ha AMCP-me, respectively (Figure 2.2). Calculated EC_{70} for mosquitofern dry weights was 121 g/ha. At 4 WAT, giant salvinia control was not affected by AMCP-me rate and averaged 10% over herbicide treatments (data not presented). Giant salvinia dry weights were not significantly different and averaged 3.7 g (data not presented). AMCP-me at 560 g/ha controlled water lettuce 48% at 4 WAT (Figure 2.1) and reduced dry weight to 70% of nontreated (Figure 2.2). Water hyacinth control at 4 WAT was 76% with 140 g/ha AMCP-a and 94% or greater at all higher rates (Figure 2.3). Dry weights were reduced to 58 and 38% of nontreated plants with 70 and 560 g/ha AMCP-a, respectively

(Figure 2.4). The discrepancy between the high levels of visual control and somewhat high dry weight measurements for water hyacinth may be explained by the fact that total plant biomass was collected and that plant parts had not fully decomposed by 4 WAT in the anaerobic environment.

The above findings are consistent with previous research of synthetic auxin efficacy on floating species. Thayer and Haller (1985) reported no more than 20% control of water lettuce and less than 10% control of common salvinia (*Salvinia rotundifolia* Willd.) fifteen days after treatment with 4.5 kg/ha 2,4-D. The above data showing susceptibility of *A. caroliniana* to high rates of AMCP-me are in accordance with effects reported by Holst et al. (1982), where 10 ppm 2,4-D killed all plants within 10 days. Langeland and Smith (1993) reported no control of water lettuce with triclopyr at rates of 0.83 and 3.3 kg/ha. Triclopyr and 2,4-D have been shown to provide acceptable control of water hyacinth, however. 2,4-D at rates of 0.75 to 2.25 kg/ha resulted in complete control of water hyacinth (Singh and Muller 1979). Triclopyr was shown to control water hyacinth at a rate of 3.3 kg/ha (Langeland and Smith 1993). Since control was 94% with 280 g/ha AMCP-a, water hyacinth may be controlled with considerably less active ingredient applied to the environment.

Emergent weeds. At 2 WAT, alligatorweed control was 82 and 95% with 70 and 280 g/ha AMCP-a, respectively (data not presented). At 5 WAT, alligatorweed regrowth was evident and control was 59 and 88% with the same rates (Figure 2.3). New shoots were present on plants at lower rates as early as 3 WAT; shoot dry weights at harvest were 32% of nontreated at 35 g/ha compared to 16% at 420 g/ha (data not presented). Alligatorweed root dry weights

declined gradually, as they were reduced to 49 and 28% of the nontreated at 35 and 280 g/ha AMCP-a, respectively.

Creeping water primrose control at 2 WAT was 74% with 35 g/ha AMCP-a and increased to 94% with 140 g/ha AMCP-a (data not presented). At 5 WAT, creeping water primrose control was 85 and 98% at the same rates (Figure 2.3). Creeping water primrose shoot dry weight was 21% or less of nontreated plants at all rates tested (data not presented). Root dry weight was 17% or less of nontreated plants at all rates tested (Figure 2.4).

Parrotfeather was also susceptible to all rates of AMCP-a, with 100% control 5 WAT at 70g/ha and above (Figure 2.3). Shoot dry weights were 14% or less of nontreated plants at all rates tested (data not presented). Parrotfeather root dry weights were 19% of nontreated plants at 35 g/ha AMCP-a and less than 7% at all other rates (Figure 2.4). Calculated EC_{70} values for root dry weights of alligatorweed, creeping water primrose, and parrotfeather were 186, 14, and 14 g/ha AMCP-a, respectively.

Results of this study are in agreement with previous research on triclopyr and 2,4-D. Hofstra and Champion (2010) not only reported significantly reduced root biomass in young alligatorweed plants treated with 3.2 kg/ha triclopyr, but also signs of plant recovery. A North Carolina field study indicated 80% control of alligatorweed 2 WAT with 0.46 kg ae/100L 2,4-D, but only 50% control 8 WAT (Langeland 1986). Champion and others (2008) reported 100% control of primrose willow [*Ludwigia peploides* (Kunth) P.H. Raven] 3 WAT after triclopyr was applied to runoff at a rate of 2 g/L. Results from a mesocosm study of parrotfeather showed 85% and 70% control 5 WAT from 2.1 kg ae/ha 2,4-D and 6.7 kg ae/ha triclopyr, respectively (Wersal and Madsen 2010).

Desirable plants. At the rates tested, soft rush was least sensitive to AMCP-a, while pickerelweed was the most sensitive. At 5 WAT, injury to soft rush was 0 and 5% with 70 and 560 g/ha AMCP-a, respectively, while root and shoot dry weights were not affected by AMCP-a rate (data not presented).

Lizard tail exhibited a more prominent rate response to AMCP-a than either soft rush or pickerelweed. At 5 WAT, lizard tail injury increased from 33 to 86% with 35 to 560 g/ha AMCP-a. Lizard tail root dry weights were 69% and 52% of nontreated controls at 280 and 560 g/ha AMCP-a, respectively. Shoot dry weights were 67 and 49% of the nontreated at the same rates (data not presented).

Pickerelweed injury at 5 WAT was 91% or greater with 70 to 560 g/ha AMCP-a (Figure 2.5). Pickerelweed root dry weights were 40 to 59% of nontreated controls at all rates of AMCP-a tested (Figure 2.4). Pickerelweed shoot dry weights were 41 to 63% of nontreated at all rates tested (data not presented).

In a study of Florida pastures, 1.12 kg/ha 2,4-D amine controlled nonmowed soft rush 53% one month after treatment (Rana and Sellers, 2009). In the same study, neither triclopyr plus fluroxypyr nor aminopyralid effectively controlled soft rush. Steenis (1950) reported good control of lizard tail after foliar application of 0.6% 2,4-D. Pickerelweed is listed as susceptible to triclopyr, with recommended use rates of 1.68 to 6.73 kg ae/ha (SePRO 2010). Aminocyclopyrachlor may provide control of nuisance aquatic weeds where protection of soft rush is desired, while injury to lizard tail and pickerelweed appears to be unacceptable for broadcast applications.

Alligatorweed depth study. For visual ratings, height reduction, shoot weight reduction, and root weight reduction, the main effect of AMCP-a rate was not significant (P values ranged from 0.0605 to 0.7148). The main effect of depth was significant for visual control at 4 WAT ($P \leq 0.0001$), height reduction ($P \leq 0.0001$), and shoot weight reduction ($P = 0.0003$). Alligatorweed control at 4 WAT was 100% at 0 cm depth and 75% at 22 cm depth (Table 2.2). Percent reduction of alligatorweed height decreased with increasing depth, from 100% at 0 cm to 45% at 22 cm. Reduction in root weight ranged from 46 to 56%. Interestingly, shoot weight percent reduction was 37% at 0 cm depth, but increased to 56% at 22 cm depth. The higher shoot weight reduction at greater depths is in disagreement with the other parameters measured. However, the morphology of alligatorweed in different water regimes may explain this discrepancy. Compared to terrestrial alligatorweed, aquatic plants have larger leaves and thicker stems (Holm et al. 1997). Geng et al. (2006) reported that alligatorweed plants under wet conditions had greater total biomass and leaf fraction than those under drought conditions. In our study, shoots from nontreated plants at 0 cm depth averaged 4.5 g, while those at 22 cm depth averaged 7.2 g (data not shown). The greater reduction in shoot weight at 22 cm depth could be partially explained by flooded plants having greater initial shoot biomass. Further research into aquatic plant response to herbicides under different water regimes is needed.

Alligatorweed control with systemic herbicides may be limited due to restricted translocation to underground and underwater biomass (Weldon 1960, Anonymous 1968, Julien and Broadbent, 1980). The reduction in root biomass of treated emerged plants in our study may be due to the top kill of above-water parts, leading to less carbohydrate transport

to roots. Results from this study indicate that alligatorweed plants treated with otherwise lethal rates of AMCP-a may regrow if significant portions of the plant are underwater.

AMCP-me effectively controlled mosquitofern and AMCP-a effectively controlled alligatorweed, creeping water primrose, parrotfeather, and water hyacinth at the anticipated use rates of 70 to 280 g/ha. AMCP-a appears to be selective to soft rush at the rates tested, but considerable injury to lizard tail and pickerelweed may inhibit the use of AMCP-a in broadcast applications where the latter two species are present. Field studies are needed to confirm or refute our greenhouse evaluations, especially because herbicide efficacy can be affected by a multitude of environmental conditions. The synthetic auxin herbicides 2,4-D and triclopyr are effective on many weeds, can be applied directly to water, and also provide selectivity to certain species. Therefore, future greenhouse and field research should also evaluate AMCP-a utility for different plant communities and in-water treatment scenarios.

SOURCES OF MATERIALS

¹Champion[®] 21-8-18, The Scotts Company, LLC, Marysville, OH 43041.

²Aminocyclopyrachlor-methyl ester, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

³Aminocyclopyrachlor acid, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

⁴Teejet[®] 8002E nozzle, Spraying Systems Co., Wheaton, IL 60187.

⁵Methylated spray oil, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

⁶4P Mix, Conrad Fafard, Inc., Agawam, MA 01001.

⁷Osmocote[®] Classic 19-6-12, The Scotts Company, LLC, Marysville, OH 43041.

⁸Collected from Sanford, NC, steam sterilized at North Carolina State University

⁹Pro-Moss[®], Premier Horticulture, Inc., Quakertown, PA 18951.

¹⁰2 Mix, Conrad Fafard, Inc., Agawam, MA 01001.

¹¹Teejet[®] XR 11003 nozzle, Spraying Systems Co., Wheaton, IL 60187.

¹²SAS[®] v. 9.2, SAS Institute, Inc., Cary, NC 27513.

¹³SigmaPlot[®] v. 11, Systat Software, Inc., San Jose, CA 95110.

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Table 2.1. Characteristics of aquatic species studied.

Common name	Botanical name	Growth habit	Weediness
Mosquitofern	<i>Azolla caroliniana</i> Willd.	Free-floating fern, spreads vegetatively or by spores	Native, may become problematic in certain situations
Giant salvinia	<i>Salvinia molesta</i> Mitchell	Free-floating fern, spreads vegetatively or by spores	Federal noxious weed
Water hyacinth	<i>Eichhornia crassipes</i> (Mart.) Solms	Free-floating, perennial in warmer climates, stoloniferous	Invasive; noxious in AL, AZ, CA, TX; prohibited in FL and SC
Water lettuce	<i>Pistia stratiotes</i> L.	Free-floating, perennial in warmer climates, stoloniferous	Invasive, noxious in AL, CA, TX; potentially invasive in CT, prohibited in FL and SC
Alligatorweed	<i>Alternanthera philoxeroides</i> (Mart.) Griseb.	Emergent, mat-forming, perennial, spreads by fragmentation	Invasive; noxious in AL, AZ, AK, CA, TX; prohibited in FL and SC
Creeping water primrose	<i>Ludwigia grandiflora</i> (Michx.) Greuter & Burdet	Emergent, perennial, forms mats in deeper water, spreads by seeds and fragmentation	Invasive; noxious in NC and WA, prohibited in SC
Parrotfeather	<i>Myriophyllum aquaticum</i> (Vell.) Verdc.	Emergent or submersed, perennial, spreads by fragmentation	Invasive; noxious in AL, VT, WA; prohibited in CT, ME, MA
Lizard tail	<i>Saururus cernuus</i> L.	Emergent, upright, perennial, spreads mainly by rhizomes	Native, usually desirable
Pickerelweed	<i>Pontederia cordata</i> L.	Emergent, upright, perennial, spreads by rhizomes or seeds	Native, usually desirable
Soft rush	<i>Juncus effusus</i> L.	Emergent, clump-forming, perennial, spreads mainly by seeds	Native, usually desirable

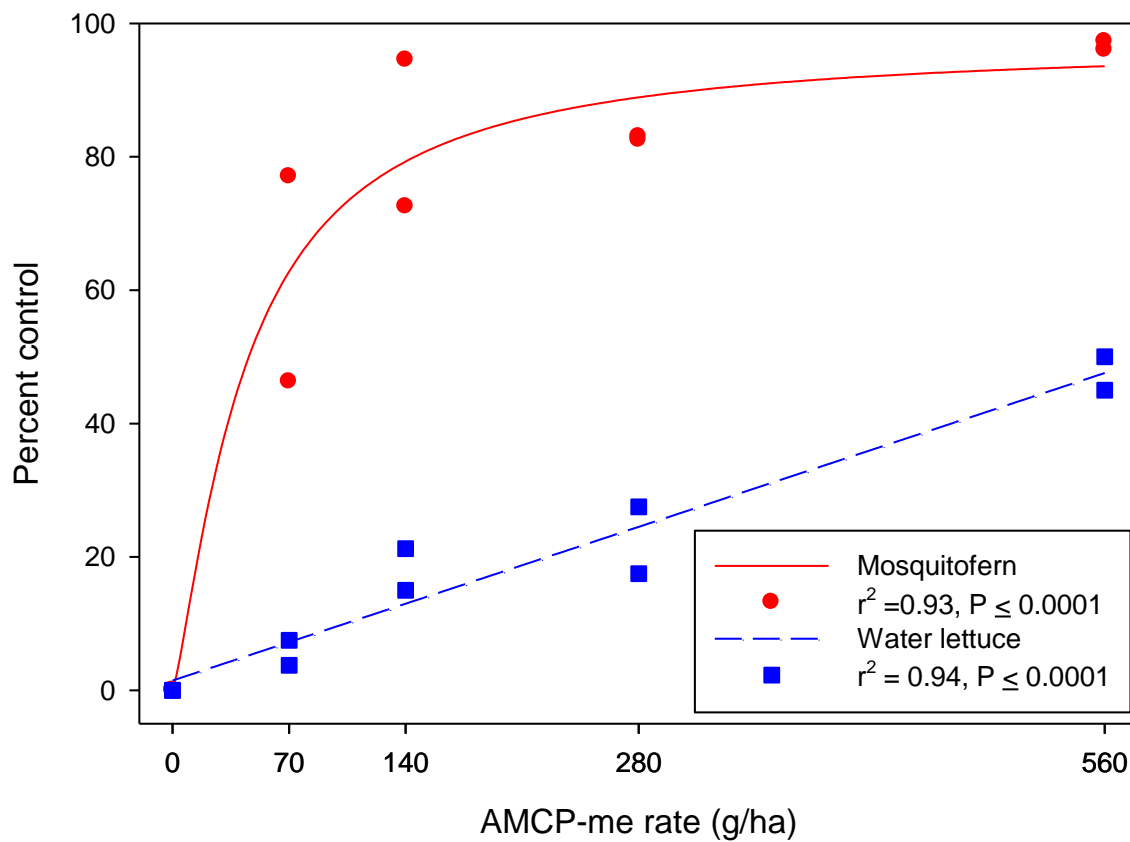


Figure 2.1. Visual control of mosquitofern and water lettuce at four weeks after treatment with increasing rates of aminocyclopyrachlor-methyl ester (AMCP-me). Mosquitofern: $y = 97.14/[1+(x/43.76)^{-1.28}]$. Water lettuce: $y = 1.45+0.08x$.

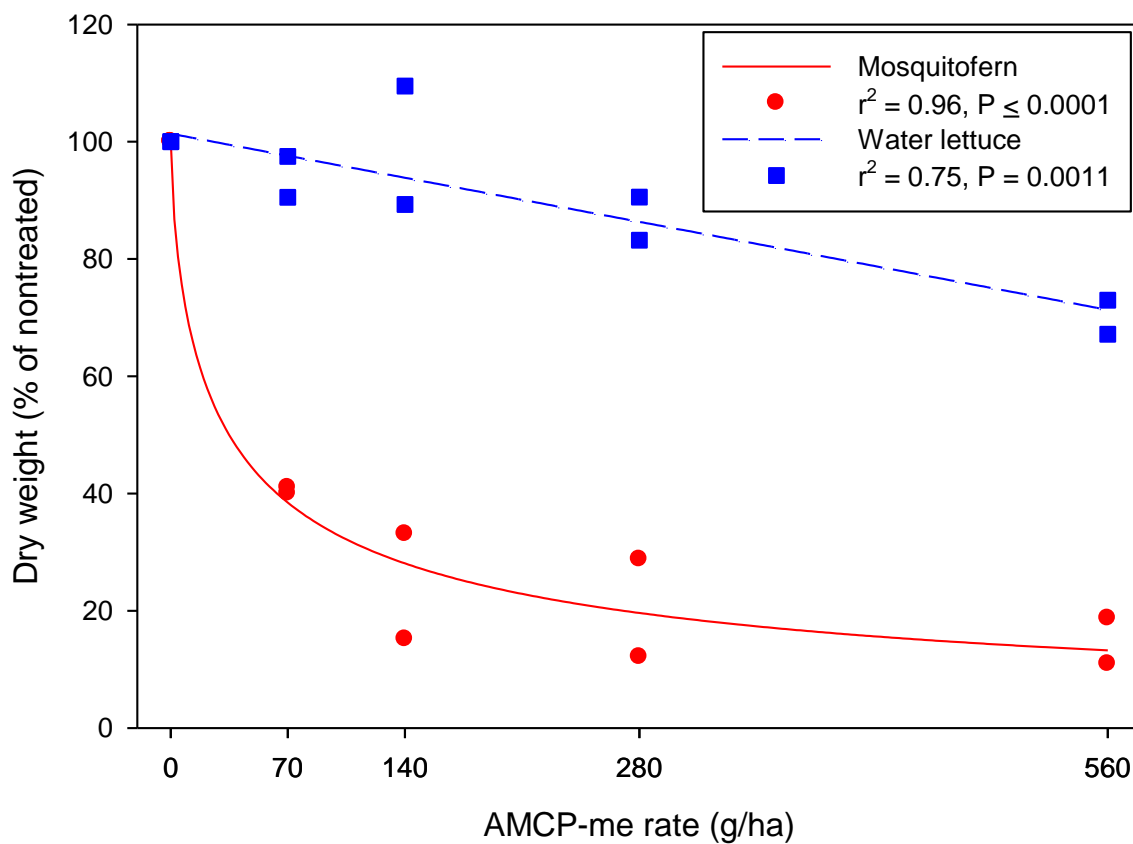


Figure 2.2. Response of mosquitofern and water lettuce dry weights to increasing rates of aminocyclopyrachlor-methyl ester (AMCP-me). Mosquitofern: $y = 100.05/[1+(x/34.87)^{0.68}]$.

Water lettuce: $y = 101.34 - 0.05x$.

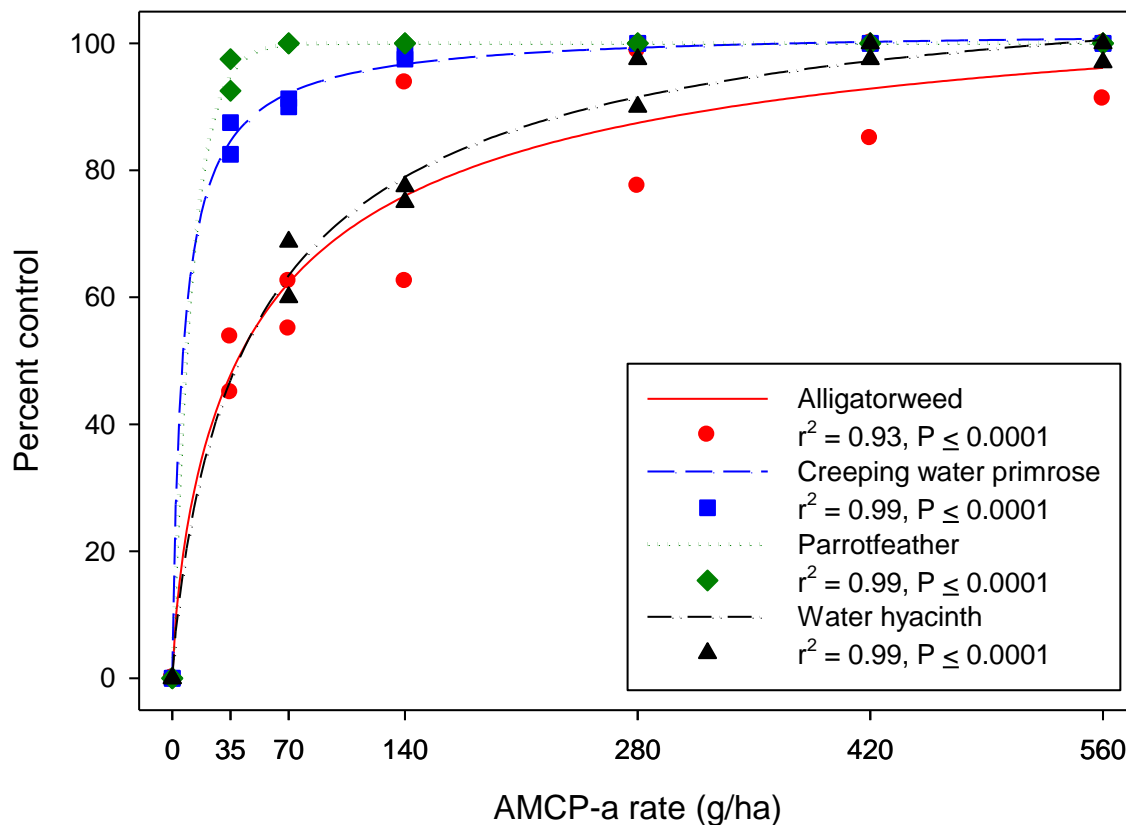


Figure 2.3. Visual control of alligatorweed, creeping water primrose, parrotfeather, and water hyacinth with increasing rates of aminocyclopyrachlor acid (AMCP-a). Data collected at five weeks after treatment for alligatorweed, creeping water primrose, and parrotfeather. Water hyacinth data collected at four weeks after treatment. Alligatorweed: $y =$

$112.62/[1+(x/52.69)^{-0.75}]$. Creeping water primrose: $y = 102.33/[1+(x/6.50)^{-0.93}]$.

Parrotfeather: $y = 100.05(1-e^{-0.09x})$. Water hyacinth: $y = 114.87/[1+(x/54.53)^{-0.84}]$.

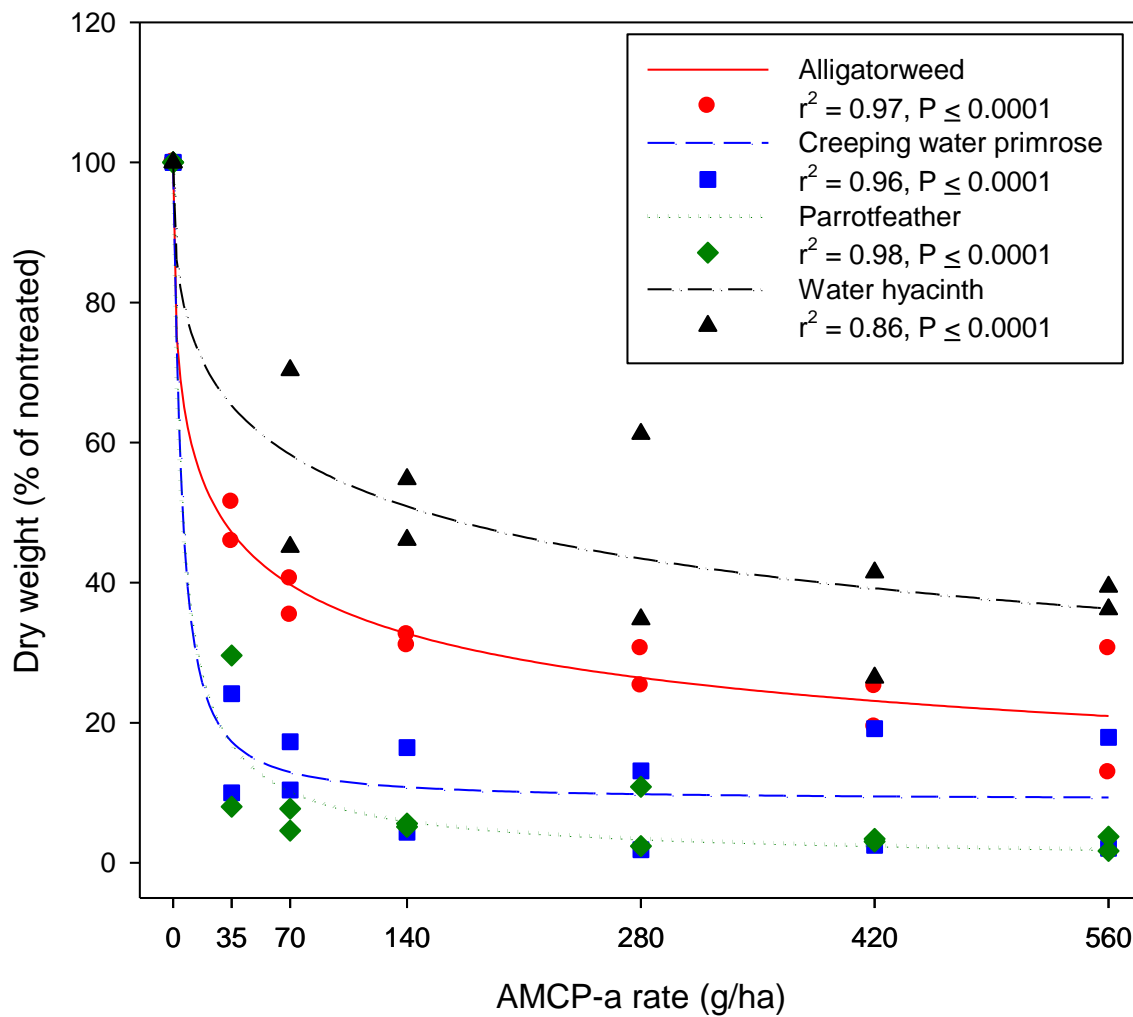


Figure 2.4. Response of alligatorweed, creeping water primrose, and parrotfeather root dry weights and water hyacinth whole plant dry weight to increasing rates of aminocyclopyrachlor acid (AMCP-a). Alligatorweed: $y = 100.04/[1+(x/27.06)^{0.44}]$. Creeping water primrose: $y = 8.98+91.02/[1+(x/4.84)^{1.16}]$. Parrotfeather: $100.01/[1+(x/5.20)^{0.84}]$. Water hyacinth: $y = 99.97/[1+(x/151.98)^{0.43}]$.

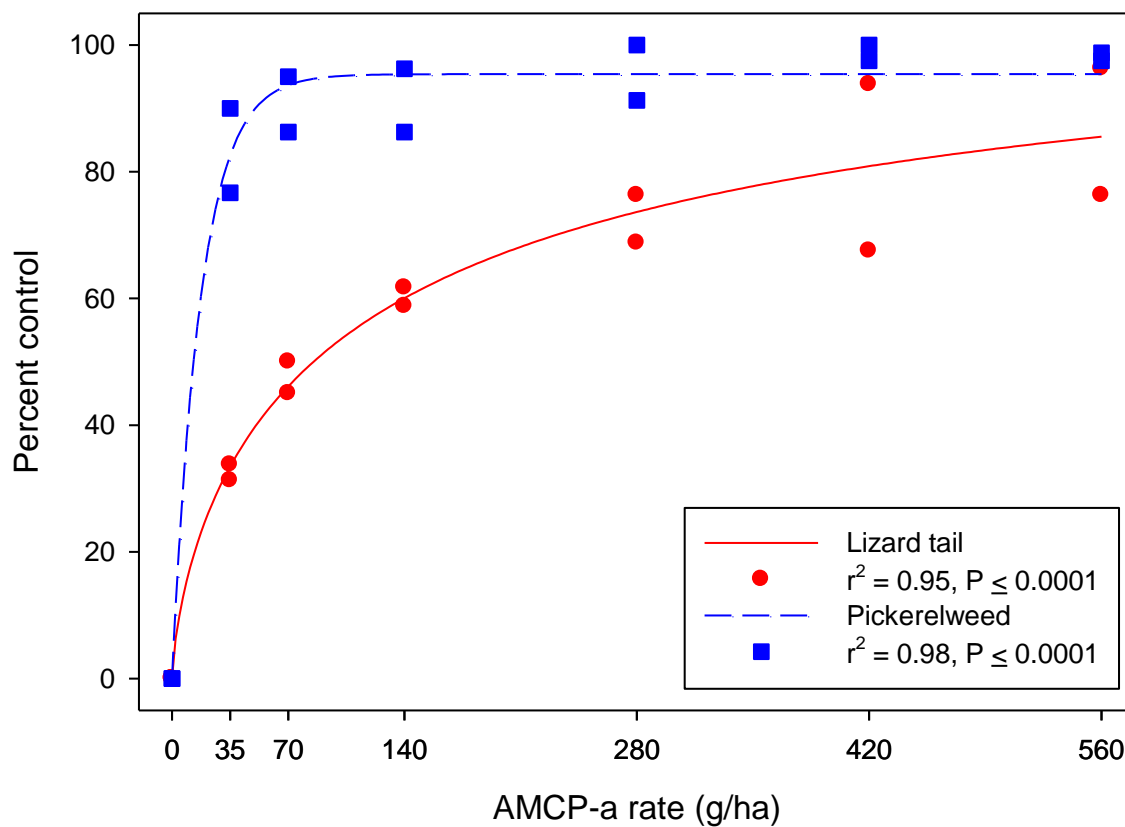


Figure 2.5. Visual injury to lizard tail and pickerelweed with increasing rates of aminocyclopyrachlor acid (AMCP-a). All data were collected at five weeks after treatment.

Lizard tail: $y = 114.84/[1+(x/123.06)^{-0.71}]$. Pickerelweed: $y = 95.39(1-e^{-0.06x})$.

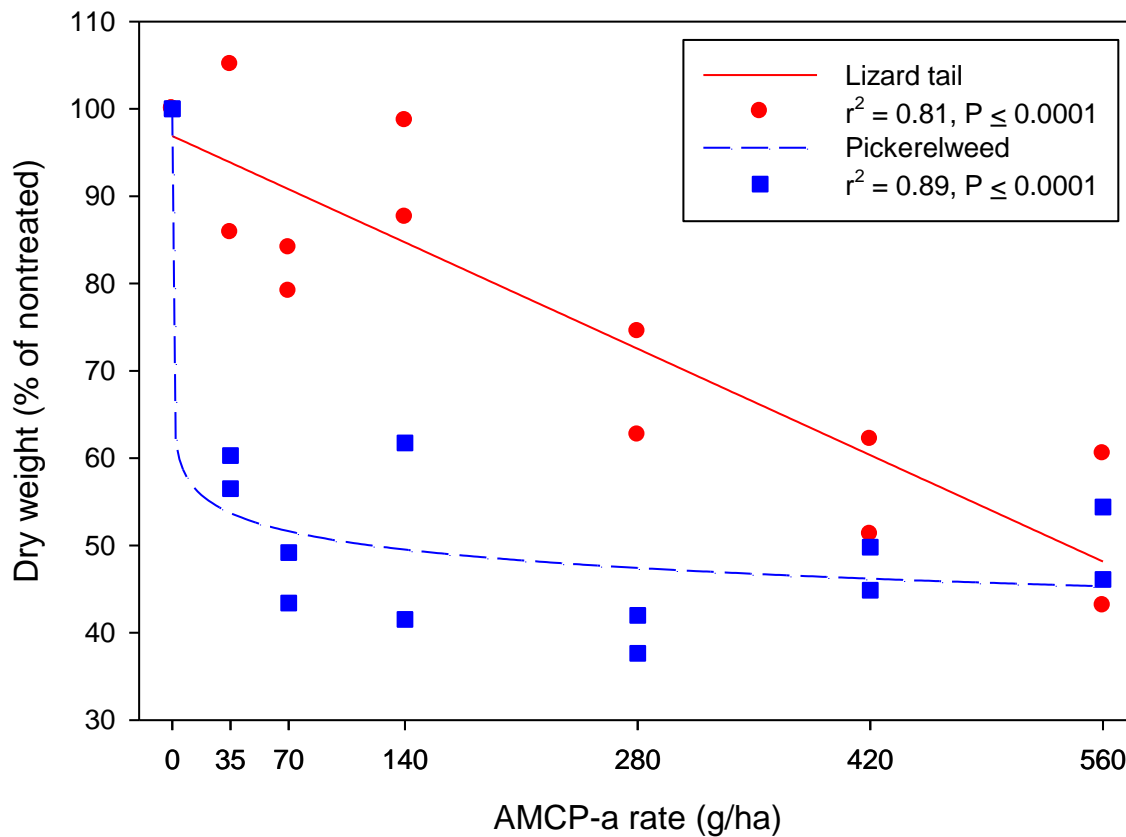


Figure 2.6. Response of lizard tail and pickerelweed root dry weights to increasing rates of aminocyclopyrachlor acid (AMCP-a). Lizard tail: $y = 96.89 - 0.09x$. Pickerelweed: $y = 100.02/[1+(x/118.33)^{0.12}]$.

Table 2.2. Alligatorweed response to aminocyclopyrachlor acid at three depths at four weeks after treatment^{a,b}.

Depth	Visual control ^c	Height reduction	Shoot weight reduction	Root weight reduction
cm	-----%-----			
0	100 a	100 a	37 a	56 a
11	92 b	64 b	49 b	46 a
22	75 c	45 c	56 b	49 a

^aWeed control rated on 0 to 100% scale; 0% = no plant response; 100% = complete death.

^bMethylated spray oil at 1% v/v included with all herbicide applications.

^cMeans within a column followed by the same letter are not significantly different according to Fisher's LSD ($P \leq 0.05$). Non-treated controls not included in statistical analysis of visual ratings.

Chapter 3

Aminocyclopyrachlor Efficacy on Selected Aquatic Species in the Field

ABSTRACT

Field trials were conducted to evaluate the response of four aquatic invasive weeds to aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidine carboxylic acid). Alligatorweed and creeping water primrose were treated with 70 to 350 g ai/ha aminocyclopyrachlor. In addition, 560 g ae/ha 2,4-D (2,4-dichlorophenoxy acetic acid), 560 g ae/ha triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid), 1120 g ae/ha glyphosate [*N*(phosphonomethyl)glycine], and 140 g ae/ha imazapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)5-oxo-1H-imazol-2-yl]-3-pyridinecarboxylic acid) were applied alone and in combination with 70 g/ha aminocyclopyrachlor. Water hyacinth was treated with 140 and 280 g/ha aminocyclopyrachlor. In addition, 560 g ae/ha 2,4-D and 560 g ae/ha triclopyr were applied alone and in combination with 140 g/ha aminocyclopyrachlor. Parrotfeather was treated in-water with 0.2 mg ai/L aminocyclopyrachlor. At two sites, alligatorweed control was 82 and 87% 8 WAT with 140 g/ha aminocyclopyrachlor. Combinations of aminocyclopyrachlor plus triclopyr, glyphosate, or imazapyr controlled alligatorweed 80% at 8 WAT. Creeping water primrose was controlled 86% with 210 g/ha aminocyclopyrachlor at 8 WAT. All combination treatments, as well as glyphosate alone and imazapyr alone controlled creeping water primrose 81% or greater. Water hyacinth control at two sites was 70 and 95% three months after treatment with 280 g/ha aminocyclopyrachlor. All herbicide treatments resulted in significantly lower dry weights than untreated control plots, with the

exception of 140 g/ha aminocyclopyrachlor at one site. Parrotfeather was controlled 99% at both sites four weeks after treatment. At eight weeks after treatment, control was 65 and 99% and biomass was reduced to 31 and 36% of pretreatment dry weights. Based on these results, the above species were sensitive to aminocyclopyrachlor in the field.

INTRODUCTION

Aminocyclopyrachlor (AMCP) is a recently discovered herbicide belonging to the pyrimidine carboxylic acid family (Finkelstein et al. 2008). The herbicide is rapidly absorbed by leaves and roots and is translocated in both xylem and phloem to meristematic regions of the plant (Finkelstein et al. 2008). After AMCP application, broadleaf species exhibit symptoms typical of auxin mimic exposure, including leaf curling, epinasty, and tissue swelling (Bukun 2010, Israel 2010). The herbicide is thought to target a family of auxin receptor complexes, where it disrupts the hormonal balance necessary for normal root and shoot development (Finkelstein et al. 2008). It has been shown to control many broadleaf and brush species in turf, rangelands, pastures, industrial rights of way, and forestry sites (Claus et al. 2008, Gannon et al. 2009, Meredith et al. 2011, Roten and Richardson 2011).

In addition to the uses listed above, AMCP has potential utility in the control of invasive aquatic weeds. One advantage is that foliar use rates of the herbicide are low, in the range of 0.07 to 0.32 kg/ha (Finkelstein et al. 2008). The anticipated in-water rates are also low, in the range of 0.1 to 0.4 mg/L. AMCP foliar and in-water use rates are lower than two commercial auxin mimic herbicides, 2,4-D and triclopyr (Table 1.1). AMCP has a favorable

environmental profile, as indicated by low mammalian toxicity and trout and daphnia toxicities comparable to that of triclopyr and 2,4-D (Senseman 2007, Finkelstein et al. 2008). The low solubility of AMCP (4.2 g/L) may be counteracted by the low use rates.

Numerous problems arise from aquatic weed infestations in water bodies in the southeastern United States. Negative impacts include navigation restriction, reduced water quality, blockage of drainage canals, displacement of native species, lowered property values, and increased breeding habitat for mosquitoes (Holm et al. 1969, Langeland 1986, Orr and Resh 1992, AERF 2009). Four of the most common and problematic aquatic weeds found in this region are alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb], creeping water primrose [*Ludwigia grandiflora* (Michx.) Greuter & Burdet], water hyacinth [*Eichornia crassipes* (Mart.) Solms], and parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] (Webster 2007).

Alligatorweed is an herbaceous perennial plant native to South America that can tolerate a range of conditions, from aquatic to dry terrestrial habitats. It may be rooted into the bank or substrate beneath shallow water or may form free-floating mats (Julien et al. 1995). Viable seed production by alligatorweed is limited in the United States; it spreads primarily by vegetative propagation (Sculthorpe 1967). Sustained alligatorweed control may be difficult, due in part to the fact that broken stems readily develop roots at nodes and float elsewhere to establish new populations (Spencer and Coulson 1976). It is currently found from Virginia south to Florida and west to Texas, as well as in Illinois and California. Alligatorweed is a noxious or state-prohibited weed in Alabama, Arizona, Arkansas, California, Florida, South Carolina, and Texas (USDA NRCS 2011).

Creeping water primrose, hereafter referred to as primrose, is an emergent herbaceous perennial native to Central and South America. When rooted in mud or shallow water, it grows upright to 1 m tall, but forms floating mats in deeper water. Primrose reproduces sexually and vegetatively, and both seeds and fragments are dispersed by water (Okada et al. 2009). It can tolerate varying water levels by significant changes to the root system (Hussner 2010). The current range of primrose is from New York to Florida and west to Texas, plus California, Oregon, and Washington. *L. grandiflora* ssp. *grandiflora* is listed as a plant pest in South Carolina, while *L. grandiflora* ssp. *hexapetala* is a plant pest in South Carolina, and a noxious weed in North Carolina and Washington (USDA NRCS 2011).

Water hyacinth is a free-floating monocot native to Brazil. Growing to nearly 1m in height, it is found in still or slow-moving waters and commonly forms dense, interlocking mats. Water hyacinth mats can deplete dissolved oxygen and create large quantities of detritus that eventually fill in a water body (Holm et al. 1969, Villamagna and Murphy 2010). Reproduction is primarily by stolons, although seed production can be important in temperate climates, where recolonization after freezing may be dependent on the seed bank (AERF 2009). Water hyacinth invasion success can be attributed to its rapid reproductive rate and the lack of biological control agents outside its native range (Villamagna and Murphy 2010). Hyacinth is widely distributed in the United States and is listed as a noxious or state-prohibited weed in Alabama, Arizona, California, South Carolina, Florida, and Texas (USDA NRCS 2011).

Parrotfeather is a perennial milfoil native to South America that grows emergent or submersed. The emergent stems grow up to 30 cm or more above the surface of moist areas

and may extend several meters across the surface of the water (Orchard 1981, Sutton 1985). Parrotfeather may grow submersed in shallow waters up to 1 m deep (Aulbach-Smith and de Kozlowski 1996). Parrotfeather reproduces by fragmentation, as only pistillate flowers occur in the United States (Aiken 1981). The plant can also tolerate various aquatic conditions and large fluctuations in water levels (Moreira et al. 1999, Hussner et al. 2009). Parrotfeather is disseminated throughout the United States and is listed as noxious or prohibited in Alabama, Connecticut, Maine, Massachusetts, Vermont, and Washington (USDA NRCS 2011).

To date, there have been no published data on the use of AMCP for control of aquatic weeds. The objective of this research was to evaluate the efficacy of AMCP on four nuisance aquatic species in field settings.

MATERIALS AND METHODS

AMCP field trials were conducted in North Carolina and all sites met the necessary conditions for the use of an experimental aquatic herbicide [40CFR Section 12.3]; therefore, small experimental areas were used. AMCP application rates for the following trials were based on anticipated use rates of the herbicide in aquatic environments. Low rates of other herbicides were applied alone and combined with AMCP, as synergism with some herbicides was anticipated. However, combination treatments were similar to AMCP applied alone and synergism effects were not investigated.

Alligatorweed. The first alligatorweed trial was initiated on September 3, 2009 in Northampton County, NC (Site A). Plots were 56 m² and contained 100% coverage of alligatorweed. The second trial was initiated on June 15, 2010 in Sampson County, NC (Site

B). Plots were 28 m² and contained 90% coverage of alligatorweed. The third trial was conducted on July 28, 2010 at a separate pond in Sampson County (Site C). Plots were 33 m² and contained 95% coverage of alligatorweed. All trials utilized a randomized complete block design and contained 13 treatments: 70, 140, 210, 280, and 350 g/ha AMCP¹, 560 g ae/ha 2,4-D², 560 g ae/ha triclopyr³, 1120 g ae/ha glyphosate⁴, 140 g ae/ha imazapyr⁵, 70 g/ha AMCP plus 560 g ae/ha 2,4-D, 70 g/ha AMCP plus 560 g ae/ha triclopyr, 70 g/ha AMCP plus 1120 g ae/ha glyphosate, and 70 g/ha AMCP plus 140 g ae/ha imazapyr. Herbicide applications were made with a CO₂-pressurized backpack sprayer⁶ equipped with a handgun. All treatments included 1% v/v methylated spray oil⁷ and were applied at a volume of 330 L/ha. Each trial included three treatment replications. Visual estimates of weed control were obtained at 1, 2, 4, and 8 weeks after treatment (WAT) in comparison to non-treated control plots. Ratings were based on a scale of 0 to 100%, where 0% equals no plant injury and 100% equals complete plant death within the plot. For AMCP, triclopyr, and 2,4-D, the presence and extent of typical auxin mimic symptomology were considered to determine control ratings. Initial symptoms of plants treated with auxin herbicides included stem swelling and elongation and leaf curling while later symptoms were chlorosis at growing points, growth inhibition, and necrosis. Glyphosate injury symptoms included foliar chlorosis and necrosis while imazapyr symptoms were similar, but developed more slowly.

Creeping water primrose. The first primrose trial was conducted on August 3, 2010 in a pond in Johnston County, NC. Plots were 19 m² and contained 90% coverage of primrose. The second trial was initiated on August 6, 2010 in a second pond also in Johnston County. Plots were 42 m² and contained 95% coverage of primrose. The treatments for the primrose

ponds were the same as for alligatorweed, except that the first pond had no 350 g/ha AMCP treatment, due to limited space. The experimental design was a randomized complete block with three replications. Herbicide applications and visual estimates of control were made in the same manner as described above.

Water hyacinth. The first water hyacinth trial was initiated on July 8, 2010 in a pond in Carteret County, NC (Site A). Plots were 14 m² and contained 100% coverage of water hyacinth. Squares constructed of PVC piping were placed in the center of each plot and held in place by a brick and string. Squares were sealed to maintain buoyancy and had a 1 m² internal area. The purpose of these squares was to prevent plants from moving into or out of the rated area as well as to provide a means of removing plants from the treated area in a uniform manner for dry weight determination. Treatments were arranged in a randomized complete block design and included 140 and 280 g/ha AMCP, 560 g ae/ha 2,4-D, 560 g ae/ha triclopyr, 140 g/ha AMCP plus 560 g ae/ha 2,4-D, and 140 g/ha AMCP plus 560 g ae/ha triclopyr. Herbicide applications were made using the same application method and surfactant amount described above. Visual estimates of weed control were obtained at 1, 2, and 3 months after treatment (MAT). At 3 MAT, all water hyacinth biomass contained within each PVC square was collected, dried for one week at 50C, and weighed.

The second water hyacinth trial was initiated on August 10, 2010 in a pond located in Granville County, NC (Site B). Greenhouse populations of water hyacinth were transported to the pond and equally distributed among PVC squares. Plants were allowed to grow for four weeks under natural conditions until herbicide treatment. Treatment applications were the same as the first water hyacinth trial, except the squares were sprayed using a 4 nozzle

spray boom attached to a handgun⁸. Rating dates and biomass collection methods were the same as described above.

Parrotfeather. Two parrotfeather trials were conducted in New Hanover (Site A) and Stanly (Site B) Counties, NC on August 27 and September 10, 2010, respectively. Pond volume estimates were made by implementing a perpendicular transect method to find the average depth and multiplying by the measured surface area. Depth measurements were obtained every 2 m along two central, perpendicular axes of the ponds while surface length and width measurements were taken with a surveyor's tape. AMCP was applied at a rate of 0.2 mg/L using the same backpack sprayer equipped with a handgun. The handgun was placed underwater and herbicide was released at several points within the ponds, to ensure even and adequate dispersion within the water column. Water temperature, pH, and dissolved oxygen were measured at treatment using a water quality probe.⁹ Coverage and percent control estimates were determined 2, 4, and 8 WAT, in addition to a pretreatment coverage estimate. Biomass samples were obtained pretreatment and 8 WAT with a weed rake at five points in each pond.

Data Analysis. All visual data for alligatorweed, primrose, and water hyacinth were subjected to analysis of variance and means were separated using Fisher's Protected LSD ($P \leq 0.05$) in commercial software¹⁰. Non-treated controls were not included in statistical analysis of visual ratings. Percent control of alligatorweed, primrose, and water hyacinth was arcsine square root transformed prior to analysis in order to maintain homogeneity of variances. The untransformed means are presented for clarity. Visual control ratings for primrose were combined across locations, as no treatment by location interaction was

observed; ratings for other species were not combined across locations. Means and standard errors were calculated for pretreatment and 8 WAT dry weights of parrotfeather.

RESULTS AND DISCUSSION

Alligatorweed. At site A, alligatorweed control 4 WAT was 82% with 70 g/ha AMCP and 97 to 98% with 210, 280, and 350 g/ha (Table 3.1). Combinations of 70 g/ha AMCP with 2,4-D, triclopyr, or glyphosate controlled alligatorweed 90, 97, and 98%, respectively. 2,4-D alone at 560 g ae/ha and imazapyr alone at 140 g ae/ha controlled alligatorweed 68 and 43%, respectively. At 8 WAT, all AMCP alone treatments controlled alligatorweed 87% or better, and combination treatments controlled alligatorweed at least 93%. In addition, alligatorweed control with combination treatments was significantly better than the respective non-AMCP treatments of the other herbicides alone. This was due to observed regrowth in plots treated with 2,4-D, triclopyr, and glyphosate alone.

At site B, alligatorweed control was 75% or better with all AMCP treatments 4 WAT, but regrowth was evident in most of the treatments by 8 WAT (Table 3.2). At 4 WAT, control with imazapyr alone was 95%, while control from 2,4-D, glyphosate, and triclopyr was 55, 78, and 52%, respectively. At 8 WAT, only AMCP plus imazapyr and imazapyr alone effectively controlled alligatorweed.

At site C at 4 WAT, all AMCP treatments controlled alligatorweed 100%, except AMCP plus glyphosate, which controlled alligatorweed 95% (Table 3.2). At 8 WAT, 80% or better control was observed with treatments of 140, 210, and 280 g/ha AMCP, AMCP plus triclopyr, AMCP plus glyphosate, AMCP plus imazapyr, and imazapyr alone.

One possible explanation for the poor control observed at site B compared to the other sites is the growth habit of alligatorweed. The pond at site B was more shallow, thus alligatorweed was rooted in sediment, and AMCP may have been degraded more rapidly by photolysis, whereas alligatorweed at the other two sites was mostly floating above the water. It is postulated that alligatorweed regrew more easily at site B, due to the underwater rooted stems. Another factor to consider in the observed visual control differences across sites is application timing. Ordering the sites by increasing levels of alligatorweed control at 8 WAT gives $B < C < A$, corresponding to the time of year when treatments were applied: June, July, September. Perennial plants transport carbohydrates and other nutrients to roots in autumn (Chapin III et al. 1990, Wyka 1999). Because herbicides are more likely to be transported with them into the roots, timing of AMCP application is likely a major factor in determining long-term control of alligatorweed.

Previous research has indicated similar results for the control of alligatorweed. After foliage was sprayed to wet with glyphosate at rates of 0.24 to 0.60 kg ai/100L, alligatorweed control was fair to good at 2 WAT, but decreased at 8 WAT (Langeland 1986). Reduced control was also observed in plots treated in June compared to August or September. In the same study, alligatorweed control was 80% at 2 WAT with 0.46 kg ae/100L 2,4-D, but decreased to 50% at 8 WAT. Sustained control was observed with 0.06 kg ae/100L imazapyr, 95 to 100% at 8WAT and 90 to 99% at 18 WAT. A second study of alligatorweed indicated percent cover was reduced initially after April application of 1.7 to 5.2 kg ae/ha triclopyr, but began increasing 3 to 4 WAT (Allen et al. 2007). April applications of 0.58 kg ae/ha imazapyr resulted in low alligatorweed coverage at 12 WAT. Alligatorweed coverage

remained low 12 WAT after July applications of both herbicides. In New Zealand, Hofstra and Champion (2010) reported that triclopyr reduced biomass and percent cover of young alligatorweed plants, but only repeat applications of imazapyr and metsulfuron reduced biomass of mature plants. Our data indicate that alligatorweed can regrow following applications of AMCP, 2,4-D, glyphosate, and triclopyr, and that application timing and environmental conditions may affect regrowth potential. Further studies are needed to determine times of year and environmental conditions best suited for AMCP application.

Primrose. At 4 WAT, 140, 210, and 280 g/ha AMCP controlled primrose 80, 81, and 87%, respectively (Table 3.2). The combination treatments, with the exception of AMCP plus imazapyr, were not significantly different from 70 g/ha AMCP. However, AMCP plus 2,4-D and AMCP plus triclopyr were significantly better than the respective treatments of 2,4-D and triclopyr alone. At 8 WAT, imazapyr alone controlled primrose 91%, but did not differ from 210 and 280 g/ha AMCP, AMCP plus glyphosate, or AMCP plus imazapyr. In a study on a similar species, *Ludwigia peploides* (Kunth) P.H. Raven, 100% control at a rate of 2 g ai/L triclopyr (sprayed to runoff) at 3 and 33 WAT was reported (Champion et al. 2008).

Water hyacinth. At site A, water hyacinth control 1 MAT was 60, 74, 68, and 65% with AMCP alone at 280 g/ha, AMCP plus 2,4-D, AMCP plus triclopyr, and 2,4-D alone, respectively (Table 3.3). At 3 MAT, no treatment differences were observed. However, dry weights from all treatments, except AMCP at 140 g/ha, were significantly different from the control treatment. AMCP plus 2,4-D and AMCP plus triclopyr decreased hyacinth biomass 67 and 73%, respectively.

In previous studies, 2,4-D at rates of 0.75 to 2.25 kg/ha resulted in complete control of water hyacinth (Singh and Muller 1979). Also, triclopyr was shown to control water hyacinth at a rate of 3.3 kg/ha (Langeland and Smith 1993). Further studies are warranted to investigate additional herbicide combinations to assess lower use rates for water hyacinth control, as recommended by Wersal and Madsen (2010a).

Parrotfeather. At both sites, parrotfeather control with 200ppb AMCP at 2 and 4 WAT, was 90 and 99%, respectively (Table 3.4). At site A, regrowth of parrotfeather was evident 8 WAT. Adventitious stems had formed, although leaves were stunted and not fully expanded. However, biomass was reduced to 31% of pretreatment weights. At site B, control of parrotfeather was sustained, still 99% at 8 WAT. Biomass was reduced to 36% of pretreatment weights.

Previous research has indicated that synthetic auxin herbicides applied directly to water or subsurface effectively control parrotfeather. A study in Portugal reported an 85% decrease at 8 WAT in fresh weight of parrotfeather plants treated with 6.5 kg ai/ha 2,4-D, corresponding to 640 ug ai/L in the water column immediately following application (Moreira et al. 1999). A static exposure of parrotfeather to 1 mg ae/L 2,4-D gave 100% control 3 WAT (Gray et al. 2007). However, Wersal and Madsen (2010b) reported only 20% control 3 WAT from 4 mg ae/L 2,4-D, where concentration time was 48 H. In the same study, 2.5 mg ae/L triclopyr controlled parrotfeather 80% 3 WAT. AMCP may provide control of parrotfeather with considerably less active ingredient, but further research into concentration and exposure times is needed.

SOURCES OF MATERIALS

- ¹Aminocyclopyrachlor acid, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.
- ²Weedar[®] 64, Nufarm, Inc., Burr Ridge, IL 60527.
- ³Renovate[®] 3, SePRO Corp., Carmel, IN 46032.
- ⁴Rodeo[®], Dow AgroSciences, LLC, Indianapolis, IN 46268.
- ⁵Habitat[®], BASF Corp., Research Triangle Park, NC 27709.
- ⁶Spraying Systems Co., Wheaton, IL 60187.
- ⁷Methylated spray oil, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.
- ⁸Spraying Systems Co., Wheaton, IL 60187.
- ⁹YSI Inc., Yellow Springs, OH 45387.
- ¹⁰SAS[®] v. 9.2, SAS Institute, Inc., Cary, NC 27513.

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Table 3.1. Control of alligatorweed in sites A, B, and C after foliar applications of aminocyclopyrachlor, glyphosate, imazapyr, triclopyr, and 2,4-D.^{a,b}

Herbicide	Rate	Site A		Site B		Site C	
		4 WAT ^{c,d}	8 WAT	4 WAT	8 WAT	4 WAT	8 WAT
	g/ha	-----%-----					
AMCP	70	82 bc	88 bcd	77 bc	32 b-f	100 a	73 bc
AMCP	140	83 bc	87 cd	80 ab	42 b-e	100 a	82 abc
AMCP	210	97 a	100 a	83 ab	27 def	100 a	87 ab
AMCP	280	98 a	100 a	88 ab	33 b-f	100 a	82 abc
AMCP	350	97 a	98 a	87 ab	40 b-e	100 a	75 bc
AMCP plus glyphosate	70 + 1120	98 a	100 a	75 bc	28 c-f	95 b	80 bc
AMCP plus imazapyr	70 + 140	82 bc	93 abc	93 a	78 a	100 a	88 ab
AMCP plus triclopyr	70 + 560	97 a	97 ab	83 ab	48 bc	100 a	80 bc
AMCP plus 2,4-D	70 + 560	90 ab	95 ab	82 ab	50 b	100 a	63 c
Glyphosate	1120	80 bc	60 e	78 bc	47 bcd	63 d	65 c

Table 3.1 Continued.

Imazapyr	140	43 d	80 d	95 a	92 a	100 a	95 a
Triclopyr	560	85 bc	77 de	52 d	23 ef	92 b	75 bc
2,4-D	560	68 c	58 e	55 cd	17 f	83 c	63 c

^aWeed control rated on 0 to 100% scale; 0% = no plant response; 100% = complete death.

^bMethylated spray oil at 1% v/v included with all herbicide applications.

^cAbbreviations: AMCP, aminocyclopyrachlor; WAT, weeks after treatment.

^dMeans within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Non-treated control not included in statistical analysis of visual ratings.

Table 3.2. Control of creeping water primrose at two field sites after foliar applications of aminocyclopyrachlor, glyphosate, imazapyr, triclopyr, and 2,4-D.^{a,b,c}

Herbicide	Rate g/ha	4 WAT ^{d,e}		8 WAT	
		-----%-----			
AMCP	70	71	cd	76	c
AMCP	140	80	bc	79	bc
AMCP	210	81	bc	86	ab
AMCP	280	87	ab	84	abc
AMCP + glyphosate	70 + 1120	78	bc	84	abc
AMCP + imazapyr	70 + 140	95	a	84	abc
AMCP + triclopyr	70 + 560	79	bc	81	bc
AMCP + 2,4-D	70 + 560	75	bc	83	bc
Glyphosate	1120	80	bc	88	ab
Imazapyr	140	94	a	91	a
Triclopyr	560	60	de	74	c
2,4-D	560	46	e	77	c

^aWeed control rated on 0 to 100% scale; 0% = no plant response; 100% = complete death.

^bMethylated spray oil at 1% v/v included with all herbicide applications.

^cData combined for both locations as no treatment by location interaction was observed.

^dAbbreviations: AMCP, aminocyclopyrachlor; WAT, weeks after treatment.

Table 3.2 Continued.

°Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$). Non-treated controls not included in statistical analysis of visual ratings.

Table 3.3. Control and dry weights of water hyacinth at sites A and B after foliar applications of aminocyclopyrachlor, 2,4-D, and triclopyr.^{a,b}

Herbicide	Rate	Site A			Site B		
		1 MAT ^{c,d}	3 MAT	Dry wt.	1 MAT	3 MAT	Dry wt.
	g/ha	-----%	-----	-----g-----	-----%	-----	---g/m ² ---
AMCP	140	35 b	41 a	1995 ab	79 c	75 a	139 b
AMCP	280	60 a	70 a	1239 bc	85 bc	95 a	109 b
AMCP + triclopyr	140 + 560	68 a	72 a	761 c	98 ab	97 a	7.6 b
AMCP + 2,4-D	140 + 560	74 a	63 a	936 c	99 a	98 a	2 b
Triclopyr	560	25 b	26 a	1655 bc	81 c	78 a	52 b
2,4-D	560	65 a	59 a	1351 bc	95 ab	79 a	25 b
none	--	-- --	-- --	2815 a	-- --	-- --	1357 a

^aWeed control rated on 0 to 100% scale; 0% = no plant response; 100% = complete death. Plants harvested for dry weight determination 3 MAT.

^bMethylated spray oil at 1% v/v included with all herbicide applications.

Table 3.3 Continued.

^cAbbreviations: AMCP, aminocyclopyrachlor; MAT, months after treatment.

^dMeans within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P \leq 0.05$).

Non-treated control not included in statistical analysis of visual ratings.

Table 3.4. Control and dry weights of parrotfeather in the field after in-water applications of aminocyclopyrachlor.^a

Location	AMCP ^c rate	Control			Dry wt. ^b	
		2 WAT	4 WAT	8 WAT	Pretrt.	8 WAT
	mg/L	-----%-----			-----g-----	
Site A	0.2	90	99	65	289 ± 89	90 ± 22
Site B	0.2	90	99	99	125 ± 15	45 ± 7

^aWeed control rated on 0 to 100% scale; 0% = no plant response; 100% = complete death.

^bStandard errors shown, N = 5.

^cAbbreviations: AMCP, aminocyclopyrachlor; WAT, weeks after treatment.

Chapter 4

Absorption and Translocation of ^{14}C -Aminocyclopyrachlor in Three Aquatic Plants

ABSTRACT

Greenhouse studies were conducted to evaluate ^{14}C -aminocyclopyrachlor absorption and translocation in alligatorweed [*Alternanthera philoxeroides* (Mart.) Griseb.], water hyacinth [*Eichhornia crassipes* (Mart.) Solms], and water lettuce (*Pistia stratiotes* L.). Alligatorweed plants were treated at the seven-node stage, water hyacinth was treated at the five-leaf stage, while water lettuce was treated at the eight-leaf stage. All plants were pretreated with non-labeled aminocyclopyrachlor at a rate of 0.14 kg ai/ha with methylated spray oil (MSO) at 1% (v/v). ^{14}C -aminocyclopyrachlor was then applied to a protected leaf, and plants were harvested at 1, 2, 4, 12, 24, and 96 hours after treatment (HAT). Radioactivity was determined in the treated leaf, shoots above treated leaf, shoots below treated leaf, roots, and growing solution. Absorption was 17 and 79% in alligatorweed at 1 and 96 HAT, respectively. Absorption was 59% or greater at all harvest times for water hyacinth and water lettuce. In alligatorweed at 96 HAT, 43% of absorbed ^{14}C translocated to shoots above the treated leaf and 17% translocated to lower tissue. In water hyacinth at 96 HAT, parts above and below the treated leaf each contained 17% of absorbed ^{14}C . In water lettuce at 96 HAT, 53 and 36% of absorbed radioactivity was located above the treated leaf and in the growing solution, respectively.

INTRODUCTION

Aquatic weeds cause significant problems for farmers and water resource managers, and may pose serious threats to human health. Weeds interfere with agriculture by clogging irrigation ditches and invading flooded crops (Parochetti et al. 2008, Smith 1983, Langeland 1986). Weeds can block navigation routes and impede recreational activities like swimming, boating, and fishing as well as clog intakes in water supply reservoirs and power plants. Also, nuisance plants can displace native species and decrease biodiversity (Madsen et al. 1991). Weed growth causes water bodies to become stagnant, thereby diminishing water quality and creating mosquito breeding habitat (AERF 2009, Orr and Resh 1992). Managers must address weed infestations on an individual basis and use appropriate methods to minimize deleterious effects. Aquatic herbicides provide safe and effective solutions for long-term control of invasive plants. However, only fourteen herbicides are registered for use in North Carolina, leading to limited herbicidal options in certain situations. Therefore, new active ingredients are needed in aquatic systems.

Aminocyclopyrachlor (6-amino-5-chloro-2-cyclopropyl-4-pyrimidinecarboxylic acid) is a recently discovered synthetic auxin herbicide and has been registered for use on turf and non-cropland sites. It belongs to a new class of chemistry called pyrimidine carboxylic acids (Finkelstein et al. 2008). Aminocyclopyrachlor has a structure similar to the pyridine carboxylic acids; however, it consists of an additional nitrogen in the heterocyclic ring and a cyclopropyl side chain (Figure 1.3). The herbicide targets a family of auxin receptor complexes and disrupts the hormonal balance necessary for normal shoot and root development (Finkelstein et al. 2008). The dissociation constant (pK_a) of

aminocyclopyrachlor acid is 4.65, which lies in the standard range of other phloem mobile herbicides (Hsu and Kleier 1996). The log octanol-water partition coefficient ($\log K_{ow}$) of aminocyclopyrachlor is -2.48 and -1.12 at pH 7 and 4, respectively (Finkelstein et al. 2008). According to the model of phloem mobility by Hsu and Kleier (1996), the $\log K_{ow}$ of aminocyclopyrachlor is too low to promote long distance phloem translocation. Limited movement of aminocyclopyrachlor would suggest reduced herbicide efficacy, but this may be overcome by greater activity at the site of action. Aminocyclopyrachlor effects on transcript response are more intense than other molecules that exhibit a similar mode of action (Finkelstein et al. 2008).

Little information has been reported about the uptake and translocation of aminocyclopyrachlor. In one study of Canada thistle [*Cirsium arvense* (L.) Scop.], Bukun et al. (2010) reported that the methyl ester was absorbed more than the free acid, resulting in greater above ground translocation. Additionally, the same researchers concluded that the herbicide translocated as the free acid and may have greater biological activity than other auxin herbicides. The potential application of this herbicide to aquatic environments and the lack of published information regarding its behavior in aquatic plants necessitate comprehensive research into its movement in different species.

Alligatorweed is an emergent aquatic plant that has been the target specimen in uptake and translocation experiments with many herbicides (Funderburk and Lawrence 1963, Bowmer et al. 1993, Tucker et al. 1994, Willingham et al. 2008). Effective long-term control of alligatorweed may be difficult with some herbicides, due to little translocation to underground or underwater storage tissues. A higher percentage of imazapyr (2-[4,5-

dihydro-4-methyl-4-(1-methylethyl)5-oxo-1H-imazol-2-yl]-3-pyridinecarboxylic acid) than glyphosate [*N*-(phosphonomethyl)glycine] was absorbed by leaves and translocated to roots, even though similar percentages translocated to apical meristems (Tucker et al. 1994).

Differences in absorption and translocation were attributed to the lipophilic nature of imazapyr. Water hyacinth is a floating aquatic weed with a waxy cuticle and has exhibited susceptibility to aminocyclopyrachlor (Israel et al. 2011). The weak acid herbicides 2,4-D (2,4-dichlorophenoxy acetic acid) and glyphosate are effective in controlling water hyacinth, are quickly absorbed by leaves, and translocate to meristematic regions of the plant (Singh and Muller 1979a,b; Tsai et al. 1986). Water lettuce is a floating aquatic plant with a pubescent leaf and has exhibited less susceptibility than water hyacinth at similar rates of aminocyclopyrachlor (Israel et al. 2010). Glyphosate is effective at controlling water lettuce, while 2,4-D is not (Martins et al. 2002). A review of the literature yielded no published data on the translocation of herbicides in water lettuce.

Research is needed to examine herbicide activity in plants with different characteristics, growth habits, and susceptibilities to herbicides. The objectives of this study were to quantify the amount of ¹⁴C-aminocyclopyrachlor acid absorbed and translocated in alligatorweed, water hyacinth, and water lettuce. These are three common and problematic aquatic weeds (Webster 2007) with different leaf structure.

MATERIALS AND METHODS

Alligatorweed terminal stem clippings from existing greenhouse plants (previously collected in North Carolina) were placed in 125 ml glass Erlenmeyer flasks containing pond

water and were allowed to develop roots. Once roots were established, plants were transferred to 125 ml flasks containing 0.25 strength modified Hoagland's solution¹ (Hoagland and Arnon 1950). Plants were allowed to grow for four weeks until they had seven distinct leafy nodes above the roots. Greenhouse temperatures were maintained at 29 C during the day and 24 C at night.

Water hyacinth and water lettuce daughter plants from existing greenhouse inventories (previously collected in North Carolina) were placed in plastic cups filled with pond water and were allowed to develop roots. Plants were then transferred to 450 ml glass jars (water hyacinth) or 150 ml glass beakers (water lettuce) containing 0.25 strength Hoagland solution. Plants were allowed to grow for four weeks until water hyacinth reached five fully expanded leaves and water lettuce reached eight to nine fully expanded leaves. Greenhouse conditions were the same as described above.

One day before treatment, plants of each species were transferred to corresponding vessels containing fresh growing solution at volumes of 125 ml, 450 ml, and 150 ml for alligatorweed, water hyacinth, and water lettuce, respectively. For each alligatorweed plant, one mature leaf at the third leafy node above the roots was marked and wrapped in aluminum foil to protect it from overhead application. The third leaf from the bottom of each water hyacinth plant and the third fully expanded leaf of each water lettuce plant were also marked and wrapped in aluminum foil. All plants were treated with non-radiolabeled aminocyclopyrachlor² applied at 140 g/ha with 1% v/v methylated spray oil³ (MSO) using a single nozzle overhead track sprayer calibrated to 280 L/ha output and 207kPa pressure.

The radiolabeled treatment solution for alligatorweed and the first run of water hyacinth was prepared by diluting ^{14}C -aminocyclopyrachlor (pyrimidine-2- ^{14}C , specific activity $40.2 \mu\text{Ci}/\text{mg}$)⁴ in methanol and 1% v/v MSO to yield an activity of $14 \mu\text{Ci}/\text{ml}$. The spotting solution for water lettuce and the second run of water hyacinth was prepared by diluting ^{14}C -aminocyclopyrachlor with 49.5% methanol, 49.5% deionized water, and 1% MSO to yield an activity of $7 \mu\text{Ci}/\text{ml}$. Following overhead application, plants were allowed to dry for no longer than 20 minutes and eight $1 \mu\text{l}$ drops of ^{14}C -aminocyclopyrachlor solution were added to the marked leaf of each alligatorweed plant and the first run of water hyacinth, while sixteen drops were added to each marked water lettuce leaf and the second run of water hyacinth. Approximately 250,000 disintegrations per minute (DPM) or $0.11 \mu\text{Ci}$ were applied to the marked leaves of plants. All plants were sub-irrigated with growing solution as needed to compensate for water loss.

Plants were harvested 1, 2, 4, 12, 24 and 96 hours after treatment (HAT). Alligatorweed plants were separated into five sections: 1) treated leaf, 2) leaf opposite treated leaf, 3) tissue above treated leaf, 4) tissue below treated leaf, and 5) roots. Water hyacinth and water lettuce plants were separated into four sections: 1) treated leaf, 2) tissue above treated leaf, 3) tissue below treated leaf, and 4) roots. The treated leaf was washed in a solution of 50% methanol and 50% deionized water. Growing solution volumes were measured at harvest. A 1 ml aliquot each of leaf wash and growing solution was placed in a scintillation vial containing 15 ml of scintillation cocktail⁵ and radioactivity was determined

by liquid scintillation spectrometry (LSS).⁶ The plant sections were frozen and then oven dried at 60 C for 72 h.

After drying, plant sections were ground into a homogeneous powder and 50mg subsamples were combusted in a biological oxidizer⁷. Radioactivity from oxidations was trapped using 15 ml of ¹⁴C trapping cocktail⁸ and then quantified by LSS.

The total amount of ¹⁴C absorbed was calculated as the amount recovered in the plant parts and growing solution. The percentage of ¹⁴C absorbed was calculated as the amount absorbed divided by the amount absorbed plus the amount recovered in the leaf wash. The amount of ¹⁴C recovered per plant part was divided by the total amount of ¹⁴C absorbed to give the percentage of ¹⁴C translocated.

Absorption and translocation studies were arranged as a randomized complete block design with four replications and were repeated in time. Data were pooled over runs, as no run by harvest time interaction was observed. Data were then subjected to analysis of variance (ANOVA) and treatments were separated using Tukey's HSD test at P = 0.05 in commercial software.⁹

RESULTS AND DISCUSSION

Absorption of ¹⁴C-aminocyclopyrachlor in alligatorweed increased over time, from 17% at 1 HAT to 79% at 96 HAT (Table 4.1, Figure 4.2). In water hyacinth, absorption increased from 62% at 1 HAT to 82% at 96 HAT, similar to water lettuce at 59 and 95%, respectively. Absorption reached a maximum at 12 HAT in alligatorweed and water lettuce, compared to 2 HAT in water hyacinth.

Bukun et al. (2009) reported 57% \pm 14.6 absorption of total applied aminocyclopyrachlor 24 HAT in Canada thistle. In our research, absorption was greater at 24 HAT in water hyacinth and water lettuce, but alligatorweed was similar to the Bukun et al. (2009) results. Other studies have reported different absorption levels of weak acid herbicides in alligatorweed. Bowmer et al. (1993) reported approximately 48.2% and 61.3% of applied glyphosate at two different concentrations was absorbed into the plant 72 HAT. Based on total recovered radioactivity, absorption rates at 96 HAT for glyphosate and imazapyr were 29.5 and 80.0%, respectively (Tucker et al. 1994). Approximately 33.3% of recovered penoxsulam (2-(2,2-difluoroethoxy)-*N*-(5,8-dimethoxy[1,2,4]triazolo[1,5-*c*]pyrimidin-2-yl)-6(trifluoromethyl)benzenesulfonamide) was found in alligatorweed plants at 48 HAT (Willingham et al. 2008). In water hyacinth, glyphosate absorption levels were reported to be 34% and 60% at 24 and 72 HAT, respectively (Tsai et al.1990). Interestingly, water lettuce is tolerant to aminocyclopyrachlor up to 560 g ai/ha but water hyacinth is susceptible at 280 g ai/ha (Israel et al. 2010). The tolerance of water lettuce is not due to reduced uptake. Fast absorption rates of triclopyr, a similar synthetic auxin, have also been reported in moderately tolerant barley (*Hordeum vulgare* cv Igri) and tolerant wheat (*Triticum aestivum* cv Norman) (Lewer and Owen 1990).

In alligatorweed, the majority of ¹⁴C radioactivity was found in the treated leaf at all harvest times except 96 HAT, when 43% of recovered radioactivity was found in plant parts above the treated leaf (Table 4.2). At harvest times of 1, 2, and 4 HAT, more radioactivity was found in shoots below the treated leaf than shoots above the treated leaf. At 24 and 96

HAT, the reverse was true; a higher amount was located above the treated leaf than below the treated leaf.

Water hyacinth plants retained the majority of radioactivity in the treated leaves at all harvest times. However, 17% of recovered radioactivity was located in each of the above and below treated leaf parts 96 HAT. Although no more than 2% of radioactive compounds were present in roots at all harvest times, 10% was found in the growing solution 96 HAT, indicating possible translocation of aminocyclopyrachlor to roots and exudation.

In water lettuce, like alligatorweed, most of the radioactivity remained in the treated leaf, except at 96 HAT, where 5 and 53% was found in the treated leaf and parts above the treated leaf, respectively. Translocation of radiolabelled compounds was evident at 1 HAT, as 14% was located above the treated leaf. Even though little radioactivity was found in the roots, large amounts were found in the growing solution, reaching 36% at 96 HAT. While the growing solution did contain more radioactivity than roots, the mass concentration in roots was greater. When calculated on a mass basis, water lettuce roots at 96 HAT contained 25.73 dpm/mg, whereas growing solution contained 0.57 dpm/mg, suggesting that radioactive molecules diffused from roots to growing solution by mass flow (data not shown).

Similar translocation patterns of aminocyclopyrachlor have been reported in Canada thistle, where higher amounts of radioactivity were found in above ground parts than below ground parts at 48, 96 and 192 HAT (Bukun et al. 2010). Previous research of herbicide translocation in alligatorweed has indicated different behavior among herbicides. Tucker et al. (1994) reported 0.7 and 11.6% of extracted ^{14}C was found in roots 96 HAT for glyphosate

and imazapyr, respectively. Penoxsulam distribution in alligatorweed at 48 HAT was 1.3, 1.5, and 1.2% of total recovered in parts above treated leaf, below treated leaf, and roots, respectively (Willingham et al. 2008). Another study reported both upward and downward movement of 2,4-D in alligatorweed (Funderburk and Lawrence 1963). Previous research of aminocyclopyrachlor activity in alligatorweed has indicated auxin symptomology in new growth and regrowth from roots at low application rates (Israel et al. 2011, Lewis et al. 2011). Based upon our data, absorption and translocation to above parts and little translocation to roots are responsible for the observed alligatorweed response to aminocyclopyrachlor.

An investigation of 2,4-D translocation in water hyacinth at 144 HAT reported that 20.8% of recovered radioactivity was present in newly formed leaves while just 0.8 and 1.8% was present in other leaves and roots, respectively (Singh and Muller 1979b). Previous greenhouse research of water hyacinth has indicated rapid necrosis from 280 g ai/ha aminocyclopyrachlor (Israel et al. 2011). Although little radioactivity was found in roots, the efficacy of aminocyclopyrachlor may be attributed to the even distribution in other leaf parts. Water lettuce was not effectively controlled at any rate of aminocyclopyrachlor tested in previous greenhouse studies (Israel et al. 2010). Although ¹⁴C-aminocyclopyrachlor was rapidly absorbed, it was potentially metabolized to non-herbicidal compounds or exuded by roots, which would explain the lack of control. The rate of metabolism of triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) was considered a likely factor in the selectivity observed in wheat and barley (Lewer and Owen 1990).

Absorption and translocation patterns were different among alligatorweed, water hyacinth, and water lettuce. It was expected that waxy cuticles of water hyacinth plants and pubescent leaves of water lettuce plants would impact the amount of aminocyclopyrachlor absorbed. Considering the high amounts of radioactivity recovered in both water hyacinth and water lettuce, it appears that leaf surface properties did not affect absorption rates in these species. Aminocyclopyrachlor recovery in roots was low in each species, which may explain regrowth potential in alligatorweed. The higher recovery in water lettuce growing solution suggests that tolerance to aminocyclopyrachlor could be due to root exudation. Future research should compare metabolism rates and metabolites to determine the role of metabolism in the observed selectivity on these species.

SOURCES OF MATERIALS

¹ Hoagland's Modified Basal Salt Mixture, MP Biomedicals, LLC., Solon, OH 44139.

² Aminocyclopyrachlor acid, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

³ Methylated spray oil, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

⁴ ¹⁴C-aminocyclopyrachlor acid, E. I. du Pont de Nemours and Company, Wilmington, DE 19898.

⁵ ScintiVerse[®] BD cocktail, Fisher Scientific, Fair Lawn, NJ 07410.

⁶ Packard Tri-Carb 2100TR Liquid Scintillation Spectrometer, Packard Instrument Co., Downers Grove, IL 60515.

⁷ Model OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Co., Tappan, NY

10983.

⁸OX-161 Trapping Cocktail, R. J. Harvey Instrument Co., Tappan, NY 10983.

⁹SAS[®] v. 9.2, SAS Institute, Inc., Cary, NC 27513.

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temperature and propanil on penoxsulam efficacy, absorption, and translocation in alligatorweed (*Alternanthera philoxeroides*). *Weed Sci.* 56:780-784.

Table 4.1. Absorption of ^{14}C -aminocyclopyrachlor expressed as a percentage of total ^{14}C -aminocyclopyrachlor recovered.

Species ^b	Harvest timing ^a					
	1	2	4	12	24	96
	-----%-----					
Alligatorweed	17 a x	23 a x	44 a y	71 a z	72 a z	79 a z
Water hyacinth	62 b x	73 b xy	79 b xy	87 a y	81 a xy	82 a y
Water lettuce	59 b x	61 b x	60 ab x	82 a y	82 a y	95 a y

^aMeans within a column followed by the same letter (a-b) are not statistically different according to Tukey's HSD at $P \leq 0.05$.

^bMeans within a row followed by the same letter (x-z) are not statistically different according to Tukey's HSD at $P \leq 0.05$.

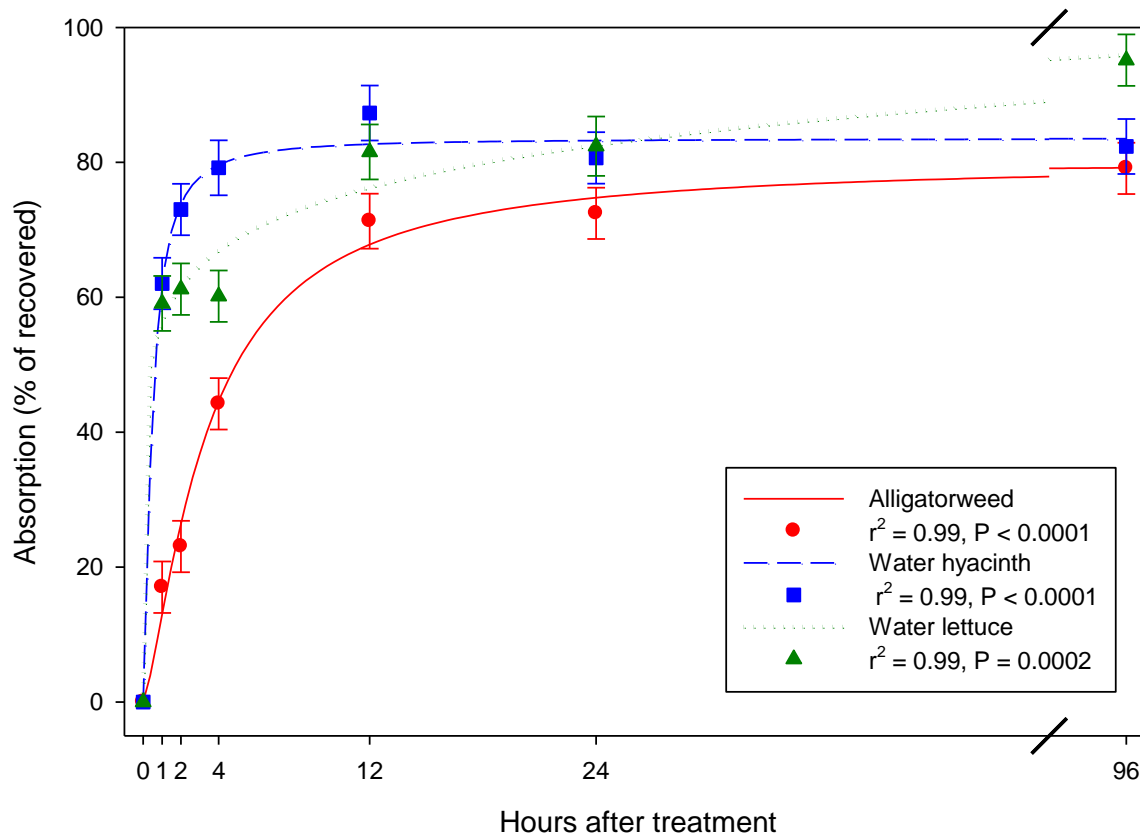


Figure 4.1. Foliar absorption of aminocyclopyrachlor acid in alligatorweed (AW), water hyacinth (WH), and water lettuce (WL). Data are based on the amount of radioactivity recovered in plant parts and leaf wash. Break shown from 48 to 90 HAT. AW: $y = 80.06/[1+(x/3.37)^{-1.35}]$. WH: $y = 83.56/[1+(x/0.48)^{-1.42}]$. WL: $y = 276.04/[1+(x/4952.64)^{-0.16}]$.

Table 4.2. Translocation of ^{14}C -aminocyclopyrachlor expressed as a percentage of total ^{14}C -aminocyclopyrachlor absorbed.

Species	Part ^b	Harvest timing ^a					
		1	2	4	12	24	96
		-----%-----					
Alligatorweed	TL	94 a	93 a	92 a	87 a	79 a	34 b
	ATL	<1 c	<1 c	1 c	5 b	10 b	43 a
	BTL	4 b	5 b	5 b	6 b	8 c	17 c
	R	1 c	1 c	1 c	1 c	1 d	1 d
	GS	1 c	1 c	1 c	1 c	2 d	3 d
	LOTL	<1 c	<1 c	<1 c	<1 c	<1 d	2 d
Water hyacinth	TL	85 a	84 a	84 a	81 a	77 a	54 a
	ATL	6 b	6 b	7 b	8 b	9 b	17 b
	BTL	4 b	5 b	5 b	6 b	8 b	17 b
	R	2 b	2 b	2 b	2 b	2 c	1 c
	GS	2 b	2 b	3 b	3 b	4 bc	10 bc

Table 4.2 Continued

Water lettuce	TL	74 a	74 a	72 a	66 a	58 a	5 c
	ATL	14 b	15 b	15 b	19 b	24 b	53 a
	BTL	2 c	2 c	2 c	3 d	3 d	5 c
	R	2 c	2 c	2 c	2 d	2 d	1 c
	GS	7 bc	8 bc	8 bc	11 c	14 c	36 b

^aMeans within a column of the same species followed by the same letter are not statistically different according to Tukey's HSD at $P \leq 0.05$.

^bAbbreviations: TL, treated leaf; ATL, tissue above treated leaf; BTL, tissue below treated leaf; R, roots; GS, growing solution; LOTL, leaf opposite treated leaf.