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Aminocyclopyrachlor Absorption and Translocation in Three Aquatic Weeds

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Studies were conducted to evaluate ^{14}C-aminocyclopyrachlor absorption and translocation in alligatorweed, waterhyacinth, and waterlettuce. Alligatorweed plants were treated at the seven-node stage, waterhyacinth was treated at the five-leaf stage, and waterlettuce was treated at the eight-leaf stage. All plants were pretreated with nonlabeled aminocyclopyrachlor at 0.14 kg ai ha^{-1} with 1% (v/v) methylated seed oil (MSO). ^{14}C-aminocyclopyrachlor was then applied to a protected leaf, and plants were harvested at 1, 2, 4, 12, 24, and 96 h after treatment (HAT). Radioactivity was determined in the treated leaf, shoots above treated leaf, shoots below treated leaf, roots, and growing solution. Absorption was rapid in all species and reached a maximum of 73, 72, and 73% of applied radioactivity for alligatorweed, waterhyacinth, and waterlettuce, respectively. In alligatorweed at 96 HAT, 43% of absorbed carbon-14 (^{14}C) was translocated to shoots above the treated leaf and 17% was translocated to lower shoot tissue. In waterhyacinth at 96 HAT, 56% of absorbed ^{14}C remained in the treated leaf, whereas 14 and 13% were found in parts above and below the treated leaf, respectively. In waterlettuce at 96 HAT, 50 and 33% of absorbed radioactivity was located above the treated leaf and in the growing solution, respectively. The low recovery of aminocyclopyrachlor in alligatorweed roots and growing solution might explain regrowth potential after herbicide treatment. These results also indicate that the lack of waterlettuce control with aminocyclopyrachlor is not due to reduced absorption or translocation.

Nomenclature: Aminocyclopyrachlor; alligatorweed, *Alternanthera philoxeroides* (Mart.) Griseb. ALRPH; waterhyacinth, *Eichhornia crassipes* (Mart.) Solms EICCR; waterlettuce, *Pistia stratiotes* L. PIIST.

Key words: Herbicide translocation, herbicide uptake.

Aquatic herbicides provide low-risk and effective solutions for long-term control of invasive plants. Often, infestations are so thick that complete herbicide coverage is not possible and much of the plant biomass might be underwater. Therefore, systemic herbicides that translocate throughout the plant are critical for long-term control of invasive plants. Additionally, herbicides must be absorbed quickly before being washed off by rain or wave action. Herbicide activity in aquatic plants is not well-understood due to their unique physiology. In submersed portions of plants, phloem and xylem movement is limited, and many anatomical features such as cuticle and vascular tissues are vestigial or lacking (MacDonald 2012). Research has suggested that glyphosate and imazapyr translocation is limited in torpedograss (*Panicum repens* L.) stems that are submerged (MacDonald et al. 2005). Although numerous herbicides are available for use in terrestrial systems, only 14 active ingredients are registered for aquatic use across the United States, leading to limited herbicidal options in certain situations. Also, information regarding aquatic herbicide absorption and translocation is limited. Therefore, new active ingredients must be investigated in aquatic systems.

Aminocyclopyrachlor is a recently discovered synthetic auxin herbicide and has been registered for use on noncropland sites. It belongs to a new class of chemicals called pyrimidine carboxylic acids (Finkelstein et al. 2008). Aminocyclopyrachlor has a structure similar to the pyridine carboxylic acids; however, it contains an additional nitrogen atom in the aromatic ring and a cyclopropyl group. The herbicide interacts with 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

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low to facilitate long-distance phloem translocation. Limited movement of aminocyclopyrachlor would suggest reduced herbicide efficacy, but this can be overcome by greater activity at the site of action (Bukun et al. 2010). Aminocyclopyrachlor might be suited for use in aquatic environments due to its low use rates, rapid photodegradation in water, and high activity on target species (Finkelstein et al. 2008). Potential use patterns for the herbicide include foliar applications for emergent and floating weeds and in-water applications for submersed vegetation.

Research of aminocyclopyrachlor uptake and translocation has been reported in several terrestrial species, but not in aquatic plants. In research involving Canada thistle [*Cirsium arvense* (L.) Scop.], Bukun et al. (2010) reported that aminocyclopyrachlor absorption reached a maximum of 57% at 24 HAT. Additionally, the same researchers concluded that translocation out of the treated leaf reached a maximum of 47% of that absorbed and that the herbicide might have greater biological activity than other auxin herbicides. Aminocyclopyrachlor absorption and translocation has also been studied in prickly lettuce (*Latuca serriola* L.), rush skeletonweed (*Chondrilla juncea* L.), yellow starthistle (*Centaurea solstitialis* L.) (Bell et al. 2011), field bindweed (*Convolvulus arvensis* L.) (Lindenmayer et al. 2013), tall fescue [*Lolium arundinaceum* (Schreb.) S. J. Darbyshire] (Lewis et al. 2013b), leafy spurge (*Euphorbia esula* L.), and yellow toadflax (*Linaria vulgaris* P. Mill.) (Lym 2014). 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 aquatic species. Alligatorweed, waterhyacinth, and waterlettuce are common and problematic aquatic weeds that have different levels of susceptibility to aminocyclopyrachlor (Israel 2011).

Alligatorweed is an emergent aquatic plant that has been the target specimen in uptake and translocation experiments with many herbicides (Bowmer et al. 1993; Funderburk and Lawrence 1963; Tucker et al. 1994; Willingham et al. 2008). Effective long-term control of alligatorweed can be difficult with some herbicides due to little translocation to underground or underwater storage tissues (Kay 1992). In field studies, alligatorweed control with 0.14 kg ha^{-1} aminocyclopyrachlor ranged from 42 to 87% at 8 wk after treatment (WAT) and regrowth was evident (Israel 2011). Waterhyacinth is a floating aquatic weed and 95% control was observed at 3 mo after treatment in field experiments with 0.28 kg ha^{-1} aminocyclopyrachlor (Israel 2011). The weak-acid herbicides 2,4-D and glyphosate are also effective in controlling waterhyacinth; these two herbicides are quickly absorbed by leaves and translocate to meristematic regions of the plant (Singh and Muller 1979a,b; Tsai et al. 1986). Waterlettuce is a floating aquatic plant and has exhibited less susceptibility to aminocyclopyrachlor, with only 18% control observed at 4 WAT at the 0.14 kg ha^{-1} rate (Israel 2011). A review of the literature yielded no published data on the translocation of any herbicides in waterlettuce.

Research is needed to examine aminocyclopyrachlor activity in aquatic plants with different levels of susceptibility. Therefore, the objective of this study was to quantify the amount of ^{14}C-aminocyclopyrachlor absorbed and translocated in alligatorweed, waterhyacinth, and waterlettuce.

Materials and Methods

Plant Material. 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 nutrient solution (Hoagland's Modified Basal Salt Mixture, MP Biomedicals, LLC., Solon, OH 44139). Plants were allowed to grow for 4 wk until they had seven distinct leafy nodes above the roots.

Waterhyacinth and waterlettuce daughter plants were collected from existing greenhouse inventories (previously collected in North Carolina) and were placed in plastic cups filled with pond water and allowed to develop roots. Plants were then transferred to 450-ml glass jars (waterhyacinth) or 150-ml glass beakers (waterlettuce) containing 0.25-strength nutrient solution. Plants were grown for 4 wk until waterhyacinth reached five fully expanded leaves and waterlettuce reached eight to nine fully expanded leaves. Greenhouse temperatures were maintained at 29 C during the day and 24 C at night.

Absorption and Translocation. One day before treatment, plants of each species were transferred to corresponding vessels containing fresh growing solution at volumes of 125, 450, and 150 ml for alligatorweed, waterhyacinth, and waterlettuce, 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 waterhyacinth plant and the third fully expanded leaf of each waterlettuce plant were also marked and wrapped in aluminum foil. All plants were treated with nonradiolabeled aminocyclopyrachlor (Aminocyclopyrachlor acid, E. I. du Pont de Nemours and Company, Wilmington, DE 19898) applied at 140 g ha^{-1} with 1% v/v methylated seed oil[3] (MSO, E. I. du Pont de Nemours and Company) using a single nozzle overhead track sprayer calibrated to 280 L ha^{-1} output and 207 kPa pressure.

The radiolabeled treatment solution for alligatorweed and the first waterhyacinth experiment was prepared by diluting ^{14}C-aminocylcopyrachlor (pyrimidine-2-^{14}C, specific activity 40.2 µCi mg^{-1}, E. I. du Pont de Nemours and Company) in methanol and 1% v/v MSO to yield an activity of 14 µCi ml^{-1}. The spotting solution for waterlettuce and the second waterhyacinth experiment was prepared by diluting ^{14}C-aminocylcopyrachlor with 49.5% methanol, 49.5% deionized water, and 1% MSO to yield an activity of 7 µCi ml^{-1}. Following overhead application, plants were immediately returned to the greenhouse for radiolabeled herbicide application. Eight 1-µl drops of ^{14}C-aminocyclopyrachlor solution were added to the marked leaf of each alligatorweed plant and the first waterhyacinth experiment, and sixteen drops were added to each marked waterlettuce leaf and the second waterhyacinth experiment. Approximately 250,000 disintegrations per minute (DPM) or 0.11 µCi were applied to the marked leaves of plants. All plants were subirrigated with growing solution as needed to compensate for water loss.

Plants were harvested 1, 2, 4, 12, 24 and 96 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. Waterhyacinth and waterlettuce 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 cocktail (ScintiVerse® BD cocktail, Fisher Scientific, Fair Lawn, NJ 07410) and radioactivity was determined by liquid scintillation spectrometry (Packard Tri-Carb 2100TR Liquid Scintillation Spectrometer, Packard Instrument Co., Downers Grove, IL 60515). 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 50-mg subsamples were combusted in a biological oxidizer (Model OX-500 Biological Material Oxidizer, R. J. Harvey Instrument Co., Tappan, NY 10983). Radioactivity from oxidations was trapped using 15 ml of ^{14}C trapping cocktail (OX-161 Trapping Cocktail, R. J. Harvey Instrument Co.) and then quantified by liquid scintillation spectrometry. The total amount of ^{14}C absorbed was calculated as the amount recovered in the plant parts and growing solution. The percentage of ^{14}C absorbed was calculated as the amount absorbed divided by the amount applied. The amount of ^{14}C recovered per plant part was divided by the total amount of ^{14}C absorbed to give the percentage of ^{14}C translocated.

Absorption and translocation studies were arranged as a randomized complete block design with four replications and were repeated. Data were subjected to ANOVA using mixed-model methodology (SAS® v. 9.3, SAS Institute, Inc., Cary, NC 27513). Data were pooled over runs because no run by harvest time interaction was observed. Absorption data were analyzed using the asymptotic regression model proposed by Kniss et al. (2011) in commercial software (SigmaPlot v. 12, Systat Software, Inc., San Jose, CA 95110). Translocation means were analyzed using nonlinear regression models in the same software.

Results and Discussion

Aminocyclopyrachlor Absorption. Maximum absorption of ^{14}C-aminocyclopyrachlor was similar in all species, reaching 73% in alligatorweed and waterlettuce and 72% in waterhyacinth (Figure 1). The time at which 90% absorption occurred was calculated to be 11.8, 1.6, and 5.1 HAT for alligatorweed, waterhyacinth, and waterlettuce, respectively. Bukun et al. (2010) reported 57% absorption of total applied aminocyclopyrachlor 24 HAT in Canada thistle. Another study reported varying levels of aminocyclopyrachlor absorption in prickly lettuce (5%), rush skeletonweed (64%), and yellow starthistle (11%) at 24 HAT (Bell et al. 2011). Lym (2014) reported an average of 72% absorption at 48 HAT in both leafy spurge and yellow toadflax, similar to the rates observed in our research.

Other studies have reported varying absorption levels of weak-acid herbicides in alligatorweed and

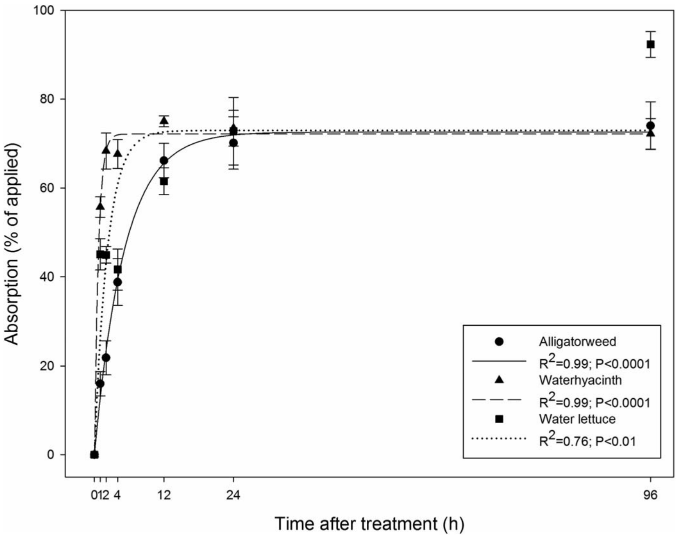

Figure 1. Foliar absorption of aminocyclopyrachlor in alligatorweed, waterhyacinth, and waterlettuce expressed as a percentage of applied radioactivity. Data points are means and standard errors. Alligatorweed: $y = 72.62[1 - e^{(\ln 0.1(x/11.84))}]$. Waterhyacinth: $y = 72.18[1 - e^{(\ln 0.1(x/1.57))}]$. Waterlettuce: $y = 72.97[1 - e^{(\ln 0.1(x/5.13))}]$.

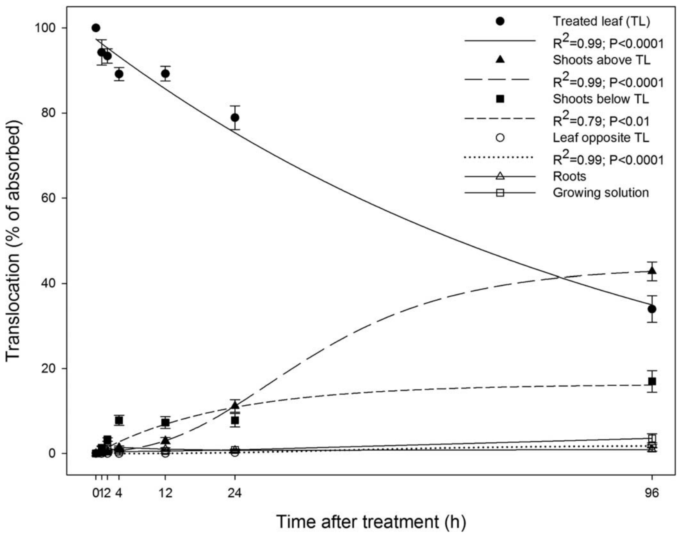

Figure 2. Translocation of ^{14}C-aminocyclopyrachlor in alligatorweed during a 96-h period. Data points are means and standard errors. Treated leaf (TL): $y = 97.35e^{-0.01x}$. Shoots above TL: $y = 43.84e^{(-e^{\wedge}(-(x - 29.54)/17.84))}$. Shoots below TL: $y = 16.29(1 - e^{-0.05x})$. Leaf opposite TL: $y = 1.90e^{(-e^{\wedge}(-(x - 40.29)/20.77))}$.

waterhyacinth. Bowmer et al. (1993) reported approximately 48 and 61% of applied glyphosate at two different concentrations was absorbed into the plant 72 HAT. In another study, absorption rates at 96 HAT for glyphosate and imazapyr were 30 and 80%, respectively (Tucker et al. 1994). Absorption of penoxsulam was approximately 33% at 48 HAT (Willingham et al. 2008). In waterhyacinth, glyphosate absorption levels were reported to be 34 and 60% at 24 and 72 HAT, respectively (Tsai et al.1986). The absorption rates of aminocyclopyrachlor in alligatorweed and waterhyacinth appear to be on the higher end of the range of previously studied herbicides. Interestingly, aminocyclopyrachlor absorption in waterlettuce is rapid, but the plant is tolerant up to 0.56 kg ai ha^{-1} (Israel 2011). Therefore, the tolerance of waterlettuce is not due to reduced uptake.

Aminocyclopyrachlor Translocation. In alligatorweed, the majority of ^{14}C radioactivity was found in the treated leaf at all harvest times except 96 HAT, when 43% of absorbed radioactivity was found in plant parts above the treated leaf (Figure 2). At harvest times of 1, 2, 4, and 12 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.

Waterhyacinth plants retained the majority of radioactivity in the treated leaves at all harvest times (Figure 3). However, at 96 HAT, 14 and 13% of absorbed radioactivity was located in the above and below treated leaf parts, respectively. Although no more than 3% of radioactivity was present in roots at all harvest times, the growing solution contained 11% at 96 HAT, indicating possible translocation of aminocyclopyrachlor to roots with additional exudation from the roots to the growing solution.

In waterlettuce, translocation out of the treated leaf was steady over time, with only 11% of absorbed aminocyclopyrachlor remaining at 96 HAT (Figure 4). Like alligatorweed, radioactivity accumulated in plant portions above the treated leaf, reaching 50% at 96 HAT. Even though little radioactivity was found in the roots, as much as 33% was found in the growing solution. Although the growing solution did contain more radioactivity than roots, the mass concentration in roots was greater. When calculated on a mass basis, waterlettuce roots at 96 HAT contained 22 DPM mg^{-1}, whereas growing solution contained 0.57 DPM mg^{-1}, suggesting that radioactivity 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 aboveground parts than belowground parts at 48, 96, and 192 HAT (Bukun et al. 2010). Bell et al. (2011) observed the majority of aminocyclopyrachlor translocation to developing shoots in prickly

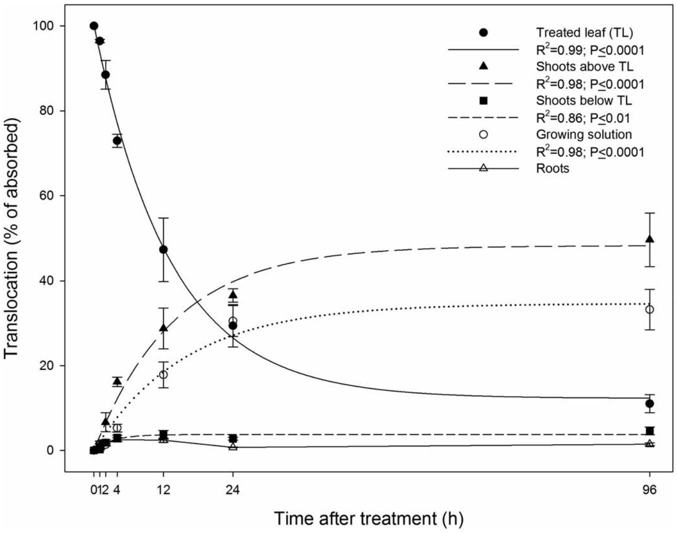

Figure 3. Translocation of ^{14}C-aminocyclopyrachlor in waterhyacinth during a 96-h period. Data points are means and standard errors. Treated leaf (TL): $y = 53.19e^{(5.80/(x + 8.84))}$. Shoots above TL: $y = 14.77e^{(-e\hat{}(-(x - 3.73)/2.13))}$. Shoots below TL: $y = 11.78(1 - e^{-0.14x})$. Growing solution: $y = 1.05 + 0.10x$.

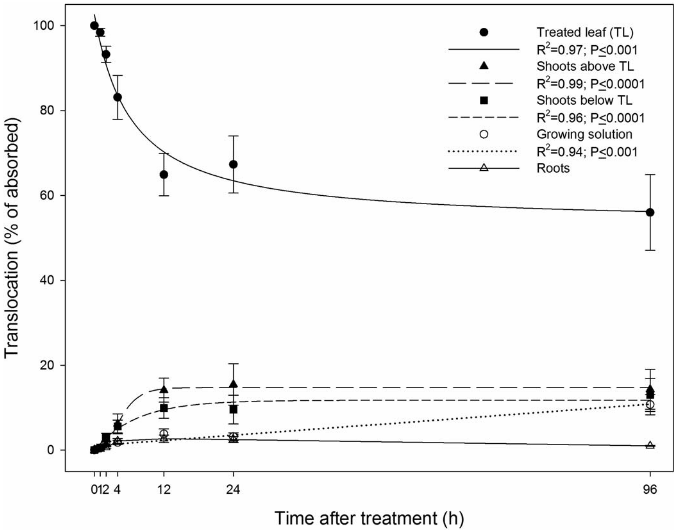

Figure 4. Translocation of ^{14}C-aminocyclopyrachlor in waterlettuce during a 96-h period. Data points are means and standard errors. Treated leaf (TL): $y = 12.30 + 87.89e^{-0.08x}$. Shoots above TL: $y = 48.28(1 - e^{-0.07x})$. Shoots below TL: $y = 3.77(1 - e^{-0.31x})$. Growing solution: $y = 34.63(1 - e^{-0.06x})$.

lettuce, rush skeletonweed, and yellow starthistle. In field bindweed, aminocyclopyrachlor translocation was evenly distributed, with 14% of applied radioactivity found in both above- and belowground plant portions (Lindenmayer et al. 2013).

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. Differences in absorption and translocation were attributed to the lipophilic nature of imazapyr. 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 2011; Lewis et al. 2013a). Based upon our data, absorption and translocation to above parts and little translocation to roots are responsible for the observed alligatorweed response to aminocyclopyrachlor.

Previous greenhouse research of waterhyacinth has indicated rapid necrosis from 0.28 kg ai ha^{-1} aminocyclopyrachlor (Israel 2011). The fact that the majority of radioactivity was found in the treated leaf at all harvest times suggests rapid deregulation of sink strength and therefore less translocation at longer harvest intervals. Similar results were reported for the auxinic herbicide 2,4-D, where 77 and 21% of recovered radioactivity was present in waterhyacinth treated leaves and newly formed leaves, respectively, at 144 HAT (Singh and Muller 1979b). The overall low levels of aminocyclopyrachlor absorption and translocation in susceptible prickly lettuce plants were also attributed to rapid growth inhibition and deregulation of sink strength (Bell et al. 2011).

Waterlettuce was not effectively controlled at any rate of aminocyclopyrachlor tested in previous greenhouse studies (Israel 2011). Although aminocyclopyrachlor was rapidly absorbed and translocated, waterlettuce plants might have reduced perception to auxin herbicides. For example, 2,4-D and triclopyr are also not effective at controlling waterlettuce (Langeland and Smith 1993; Weldon and Blackburn 1967). Other explanations for the lack of waterlettuce control with aminocyclopyrachlor are sequestration in newly developing tissue and exudation by roots, as evidenced by the large amounts of radioactivity recovered in the plant portion above the treated leaf and in the growing solution. Future research should compare metabolism rates and metabolites to determine the role of metabolism in the observed selectivity on these species.

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