

J. Aquat. Plant Manage. 42: 103-108

Carfentrazone-ethyl Pond Dissipation and Efficacy on Floating Plants¹

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ABSTRACT

Carfentrazone-ethyl (CE) is a reduced risk herbicide that is currently being evaluated for the control of aquatic weeds. Greenhouse trials were conducted to determine efficacy of CE on water hyacinth (*Eichhornia crassipes* (Mart.) Solms-Laub.), water lettuce (*Pistia stratiotes* L.), salvinia (*Salvinia minima* Baker) and landoltia (*Landoltia punctata* (G. Mey.) Les & D. J. Crawford). CE controlled water lettuce, water hyacinth and salvinia at rates less than the maximum proposed use rate of 224 g ha⁻¹. Water lettuce was the most susceptible to CE with an EC₉₀ of 26.9 and 33.0 g ha⁻¹ in two separate trials. Water hyacinth EC₉₀ values were calculated to be 86.2 to 116.3 g ha⁻¹, and salvinia had a similar susceptibility to water hyacinth with an EC₉₀ of 79.1 g ha⁻¹. Landoltia was not adequately controlled at the rates evaluated. In addition, CE was applied to one-half of a 0.08 ha pond located in North Central, Florida to determine dissipation rates in water and hydrosoil when applied at an equivalent rate of 224 g ha⁻¹. The half-life of CE plus the primary metabolite, CE-chloropropionic acid, was calculated to be 83.0 h from the whole pond, and no residues were detected in water above the limit of quantification (5 µg L⁻¹) 168 h after treatment. CE dissipated rapidly from the water column, did not occur in the sediment above the levels of quantification, and in greenhouse studies effectively controlled three species of aquatic weeds at relatively low rates.

Keywords: *Salvinia minima*, *Landoltia punctata*, *Pistia stratiotes*, *Eichhornia crassipes*, protox inhibitor, half-life.

INTRODUCTION

Carfentrazone-ethyl (α ,2-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]-4-fluorobenzenepropanoic acid, ethyl ester) (hereinafter referred to as CE) is a phenyl triazolinone herbicide that inhibits protoporphyrinogen oxidase (protox inhibitor) in the chlorophyll biosynthesis pathway causing lipid peroxidation and membrane disruption (Dayan et al. 1997, FMC Agricultural Products Group 2000). CE is registered by the U.S. Environmental Protection Agency as a post-emergent herbicide in wheat, barley, rye, oats, corn, soybeans, rice, and for use in turf and ornamental sites. Typical use rates are considered extremely low (as low as 9 g a.i. ha⁻¹), and CE is a selective, contact herbicide with activity on a variety of broadleaf weeds and sedges. The primary metabolite, CE-chloropropionic acid (CE acid), also has herbicidal activity with differential tolerance between species likely influenced by further metabolism of the free acid to unknown metabolites (Dayan et al. 1997, Thompson and Nissen 2000).

CE is converted rapidly to the CE acid in water primarily by pH dependent hydrolysis, while CE acid is relatively more stable and undergoes further breakdown through reductive dechlorination, dehydrochlorination, and oxidation (Elmarkby et al. 2001, Ngim and Crosby 2001). First order half-lives for CE have been reported in the range of 6.5 to 11.1 h in flooded rice fields, and from 3.4 h at pH 9 to 131 h at pH 7 for CE acid in laboratory hydrolysis studies (Ngim and Crosby 2001). Both CE and the major metabolite are relatively more stable to photolysis and are not likely to volatilize (Ngim and Crosby 2001).

EC₅₀ values reported for CE on the green algae *Selenestrum capricornutum* (Printz) and the blue-green algae *Anabaena flos-aquae* (Lyng.) Brebisson are 15 and 17 µg L⁻¹ respectively, while duckweed (*Lemna gibba* L.) had an EC₅₀ value of 5.7 µg L⁻¹ (FMC Agricultural Products Group 2000). CE's reduced risk classification, short half-life, and apparent acute toxicity on algae and duckweed at low concentrations have led to the further evaluation of CE as an aquatic herbicide. The

¹Published as Journal Series Number R-10256 of the Florida Agriculture Experiment Station. Received for publication April 29, 2004 and in revised form June 18, 2004.

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purpose of this study was to evaluate CE activity on common floating aquatic plants and characterize the dissipation of CE from a small pond applied at the proposed maximum use rate (224 g ha⁻¹).

MATERIALS AND METHODS

Efficacy Trials. *Salvinia*, *landoltia*, water lettuce, and water hyacinth were each grown separately in dishpans (32 cm by 28 cm, 12 cm deep) containing 7 L of water in a greenhouse at the University of Florida, Center for Aquatic and Invasive Plants in Gainesville, FL. Plants were subjected to a 16-h photoperiod with maximum daytime temperatures of 33 ± 3C and minimum nighttime temperatures of 18 ± 3C. Plants were treated using a forced air CO₂-powered sprayer with different rates of CE, formulated as Aim™ EW (Stock# 10053332). An equivalent of 748 L ha⁻¹ diluent was delivered through a single TeeJet® 80-0067 nozzle at 10 psi with 3 replications per rate. Water was added to each dishpan to compensate for evaporation and nutrients were supplied using 5 g 15-9-12 slow release Osmocote® and 1 mL of Lesco® 12-0-0 chelated iron plus per dishpan. Dry weights for each species were determined following treatment by placing harvested plant material in a forced-air drying oven at 90C for 10 d.

Salvinia or *landoltia* each were placed into separate dishpans (50 g *salvinia* or 40 g *landoltia* fresh weight per dishpan) and sprayed with CE at rates of 0, 28, 56, 112, 168 and 224 g ha⁻¹. Methylated seed oil (Sun wet®) was added to the spray diluent at a concentration of 0.5% v/v. Plants were harvested 11 days after treatment (DAT) and total dry weights determined. During the *landoltia* harvest, a visual estimate of percent dead tissue for *landoltia* was reported for each treatment and all plant tissue (live and dead) harvested. The total dry weight of all tissue was multiplied by percent live tissue, based on the visual evaluations of percent control, to calculate the dry weight of *landoltia* not controlled by CE for each replication.

Six young water lettuce plants (8 to 15 cm diameter) or four young water hyacinth plants (4 to 6 leaf stage, 10 to 15 cm in height) were each placed into separate dishpans and sprayed with CE at rates of 0, 3, 19, 34, 191 and 336 g ha⁻¹. A silicone surfactant (Freeway®) at 0.25% v/v was used in the spray diluent. Live plants were counted and harvested 25 DAT and dry weights determined. This study was repeated using different concentrations of CE and a different surfactant on both water hyacinth and water lettuce. Plants were established in a similar manner, but treated with CE at rates of 0, 28, 56, 112 and 168 g ha⁻¹ on both species, with additional treatments of 84 g ha⁻¹ on water lettuce and 224 g ha⁻¹ on water hyacinth. A d,l-limonene surfactant (Cygnet Plus®) was utilized on water lettuce at 0.25% v/v, and methylated seed oil (Sun wet®) was used on water hyacinth at 0.5% v/v. Surviving plants were counted and harvested 29 DAT for water lettuce and 49 DAT for water hyacinth and dry weights determined.

Analysis of variance and non-linear regression were conducted using the SAS statistical package (SAS 1999). Non-linear regression was used to relate dry weights of surviving treated plants to rates of CE. These models were used to calculate effective concentrations causing a 90% reduction in dry weights (EC₉₀) compared to control plants.

Pond Dissipation. A small pond located at the Center for Aquatic and Invasive Plants in Gainesville, FL was used for the dissipation study. Grass carp had been previously stocked in the pond to control submersed plants, and only a small fringe of alligatorweed (*Alternanthera philoxeroides* (Mart.) Griseb.) grew around the pond edge to a water depth of approximately 0.5 m. The pond bank sloped sharply to a mean depth of 1 m, with a maximum depth of 1.4 m. The pond had an area of 0.08 ha and was oriented in a north/south direction (18 m by 46 m) with a volume of 828,000 L. The pond sediment consisted primarily of sand overlain by 15 to 20 cm flocculent organic layer, typical of most lakes and ponds in Florida.

CE at a rate of 224 g ha⁻¹ was applied to the northern half of the pond surface (0.04 ha) by mixing the appropriate amount of herbicide in an equivalent of 2025 L water ha⁻¹ in a 378.5 L spray tank with hydraulic agitation on July 9, 2003. Additional agitation was physically applied with a wooden paddle during the course of the application. A higher volume of water was used in the dissipation study (2025 L ha⁻¹) compared to our efficacy trials (748 L ha⁻¹) to ensure uniform coverage of the pond surface during field application. A methylated seed oil (Sun wet®) was added at a concentration of 0.1% v/v. The application was made by walking around the shoreline of the pond several times using a handheld spray gun with 30.5 m hose, with half of the pond covered from each side of the pond. The pump output was 15.5 L min⁻¹ and the tank was rinsed and flushed with 19 L of pond water twice after the primary application to ensure all CE was sprayed into the pond. The rinse water was applied to the pond in a manner similar to the primary treatment. Theoretical concentrations of CE in the pond, assuming uniform distribution throughout the volume of water, would be 20 µg L⁻¹ applied to half the pond, or a concentration of 12 µg L⁻¹ in the whole pond.

Sampling stations were established in the deepest portions of the north end (treated station B) and the south end (untreated station A) of the pond. Sampling stations were centered east to west, but sampling station B was 11 m from the center of the pond and 12.2 m from the N shoreline. Sampling station A was 17.1 m from the center of the pond and 6.1 m from the south shoreline in order to sample in the deepest area of the pond. Duplicate water samples were collected 1, 3, 6, 12, 24, 48, 72, 96, 120, 168, and 336 hours after treatment (HAT). Samples were collected from the surface by submersing the sample bottle and filling with water from the top 1 cm of the pond, and at different depths (0.15, 0.3, 0.6, 0.9 and 1.2 m) by lowering a bilge pump to the appropriate depth. Water temperature was measured during each water-sampling event to monitor thermal stratification that may prevent uniform distribution of the herbicide, and pH measured from each sample. Sediment samples were collected on the same schedule as water, using a Ponar dredge (15 cm by 15 cm), by sub-sampling 3 randomly selected points around each sampling station A or B. The average depth of sediment sample collection was 7.9 cm. Each set of sediment samples was composited by sampling station for a single sediment sample during each sample time. Sediment and water samples were immediately chilled on ice and shipped overnight to FMC laboratory (Princeton, NJ) for analysis of CE and CE acid.

CE and CE acid were analyzed using methods developed by FMC Corporation. CE in water was analyzed using a gas chromatograph equipped with a mass selective detector (GC/MSD) after the water was partitioned with hexane. The remaining aqueous portion was filtered and analyzed for CE acid using liquid chromatography coupled to a triple quadrupole mass spectrometer (LC/MS/MS). The methodology for CE in sediment involved a solvent extraction with acetone/water followed by an evaporation step to remove acetone. The resulting aqueous extract was then partitioned with hexane. The hexane extract was passed through a silica gel solid phase extraction (SPE) cartridge and analyzed for CE using GC/MSD. The remaining aqueous portion was filtered and analyzed for CE acid using LC/MS/MS.

CE was quantitated using an Agilent 5890 gas chromatograph equipped with an Agilent 7673A autosampler and an Agilent 5972 mass selective detector. The column (15 m by 0.25 mm by 0.25 μm) was a DB-35MS (35% phenyl methyl silicone). Agilent Chemstation computer software was used for data collection and reporting. Instrument parameters were as follows: Injection port temperature, 250C; detector temperature, 280C; carrier gas (He) flow at $\sim 1 \text{ mL min}^{-1}$; column temperature program: initial 120C for 2 min, increased at 20C

min^{-1} to 280C and held for 5 min. The selected ions (m/z) for quantitation and confirmation were 312, 330 and 412 for CE.

CE acid was quantitated using an Agilent 1100 LC connected to a Micromass Quattro LC triple quadrupole mass spectrometer and MassLynx™ 3.5 software. An Agilent ZORBAX Eclipse XDB-C8 4.6 mm \times 250 mm LC column was used to analyze the CE acid. Mobile phase gradient was as follows: 30% acetonitrile (0.2% acetic acid) and 70% of 0.2% acetic acid increased to 95% acetonitrile (0.2% acetic acid) and 5% methanol in 3 min and held for 7 min and changed back to the initial composition in 2 min and held for 5 min. The mobile phase flow rate was 0.4 mL min^{-1} . The selected ions (m/z) for quantitation and confirmation were 382 (MS) and 346 (MS/MS) for CE acid.

The limit of quantitation (LOQ) was validated at $5 \mu\text{g L}^{-1}$ and the limit of detection (LOD) was set at $1 \mu\text{g L}^{-1}$ for both analytes in water and sediment. Method recoveries for the laboratory-fortified samples in water ranged from 82-112% and 93-126% and in sediment ranged 84-111% and 82-126% for CE and CE acid, respectively. The overall average method recoveries in water were $95 \pm 13\%$ (n = 6) for CE and $105 \pm 12\%$ (n = 6) for CE acid, and in sediment were $102 \pm 11\%$ (n = 5) and $98 \pm 20\%$ (n = 5) for CE and CE acid, respectively.

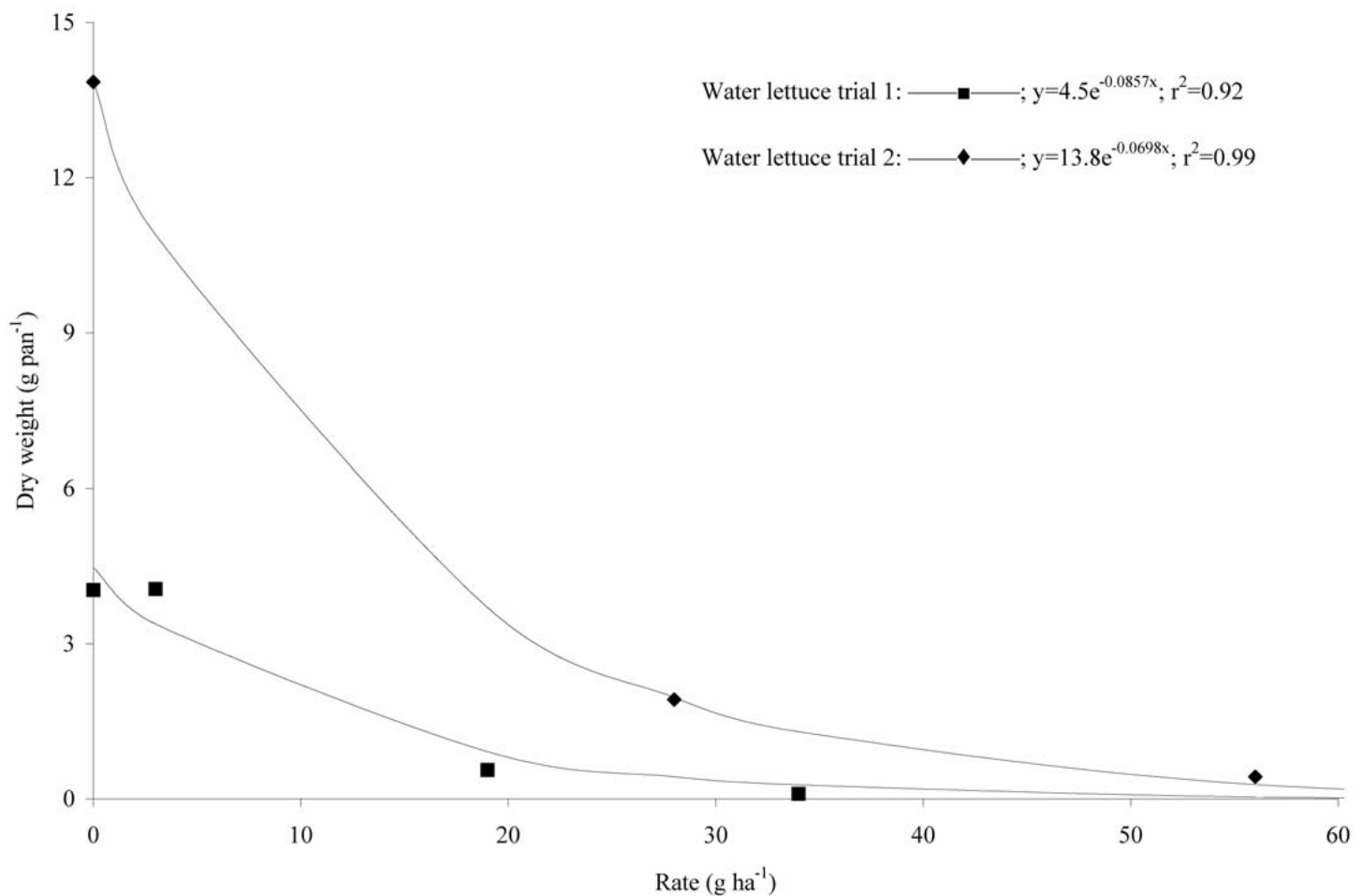


Figure 1. Carfentrazone-ethyl effects on dry weights of water lettuce from two independent trials (1 and 2). Trial 1 was conducted with spray diluent containing a silicone surfactant at 0.25% v/v, and trial 2 was conducted with spray diluent containing 0.25% v/v d,l-limonene surfactant.

The half-life was calculated by nonlinear regression analysis using the SAS statistical package (SAS 1999).

RESULTS AND DISCUSSION

Efficacy Trials. CE provided acceptable levels of control for all species, except landoltia. Initial injury symptoms in water lettuce appeared 2 to 5 d after application, but initial symptoms took longer to appear on water hyacinth (5 to 10 d). Bronzing of the leaves and limited necrosis were initially observed on water lettuce, and water hyacinth leaves initially exhibited blackening of younger leaves. Mature leaves were necrotic on water lettuce by 25 DAT, whereas mature leaves on water hyacinth showed few symptoms at all rates at this time. New leaves were produced from the meristematic region at sub-lethal concentrations for both water hyacinth and water lettuce. Symptoms and necrosis of leaf tissue were slower than reported by Thompson and Nissen (2000), which suggests the active primary metabolite (CE acid) may contribute to the herbicidal activity of CE on water lettuce and water hyacinth.

All regressions were significant ($p = 0.05$), and water lettuce was the most susceptible species to CE with a calculated EC_{90} value of 26.9 and 33.0 $g\ ha^{-1}$ for trial 1 and trial 2, respec-

tively (Figure 1). Water hyacinth (Figure 2) and salvinia (Figure 3) had similar susceptibilities to CE, and water hyacinth EC_{90} values were 86.2 $g\ ha^{-1}$ in trial 1 and 116.3 $g\ ha^{-1}$ in trial 2. The EC_{90} value for salvinia was 79.1 $g\ ha^{-1}$, and repeat applications would likely be necessary to control all salvinia due to incomplete coverage of all plants. Landoltia was the most tolerant species evaluated with an EC_{90} value of 772.7 $g\ ha^{-1}$ (Figure 3), which is in contrast to the apparent susceptibility of *Lemma gibba* as determined in laboratory studies (EC_{50} 5.7 $\mu g\ L^{-1}$) (FMC Agricultural Products Group 2000).

Pond Dissipation. Pond water pH ranged from 6.9 to 9.6 depending on the time of day, with higher pH occurring during the afternoon and evening and in the surface waters. The pond temperatures were 30.0C throughout the water column at the time of CE application, which permitted immediate vertical mixing of the herbicide. By 3 HAT vertical, thermal stratification was forming as surface temperatures (0.15 m below surface) rapidly increased to 31.8C while water at 1.2 m depth remained at 30.0C. Vertical temperature profiles each morning were essentially isothermal, and daily stratification occurred during the late afternoon, which permitted daily turnover in the pond and resulted in rapid mixing of CE. Therefore, concentrations for each sampling event are reported as the mean from all depths.

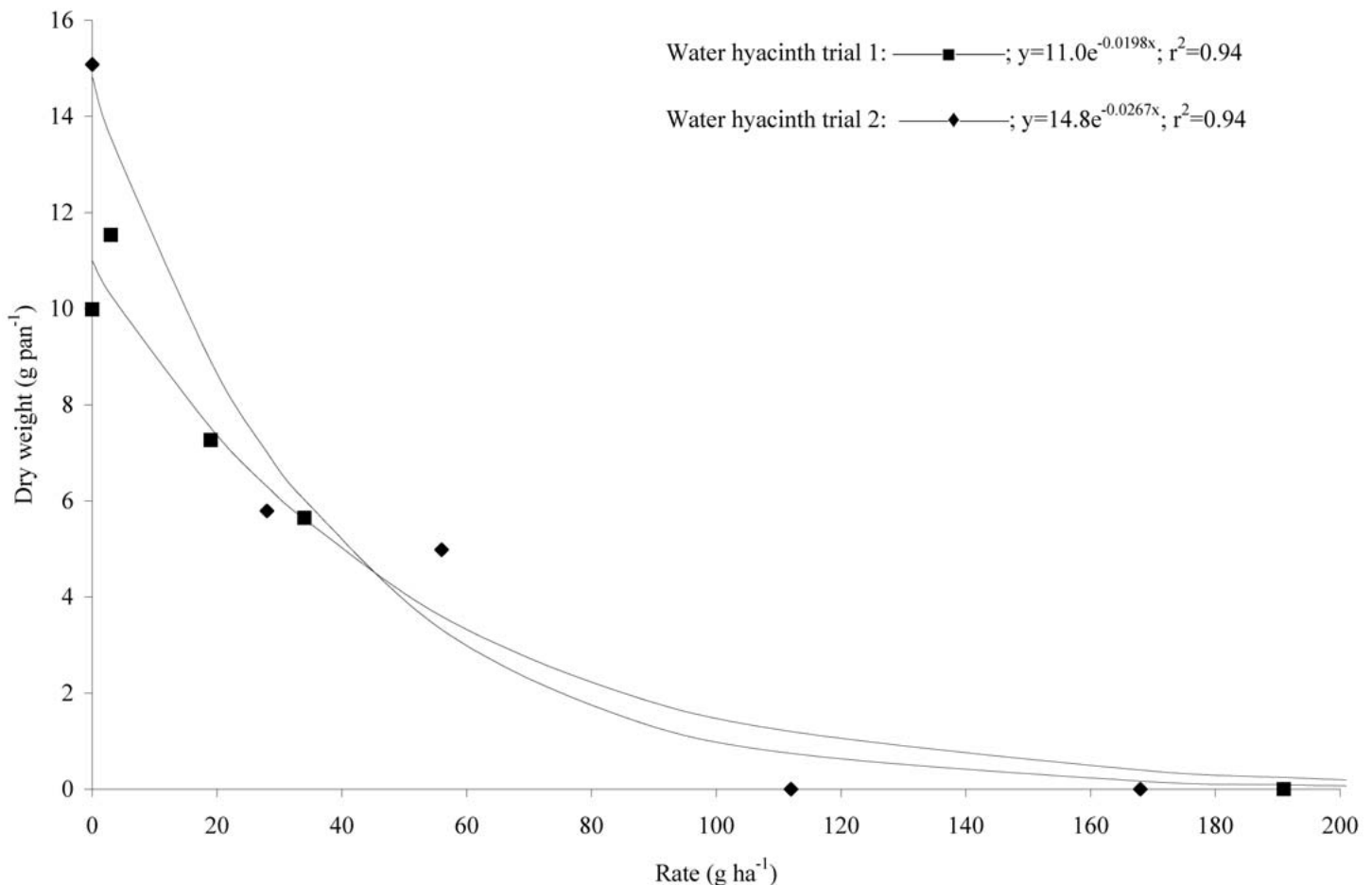


Figure 2. Carfentrazone-ethyl effects on dry weights of water hyacinth from two independent trials (1 and 2). Trial 1 was conducted with spray diluent containing a silicone surfactant at 0.25% v/v, and trial 2 was conducted with spray diluent containing 0.5% v/v methylated seed oil.

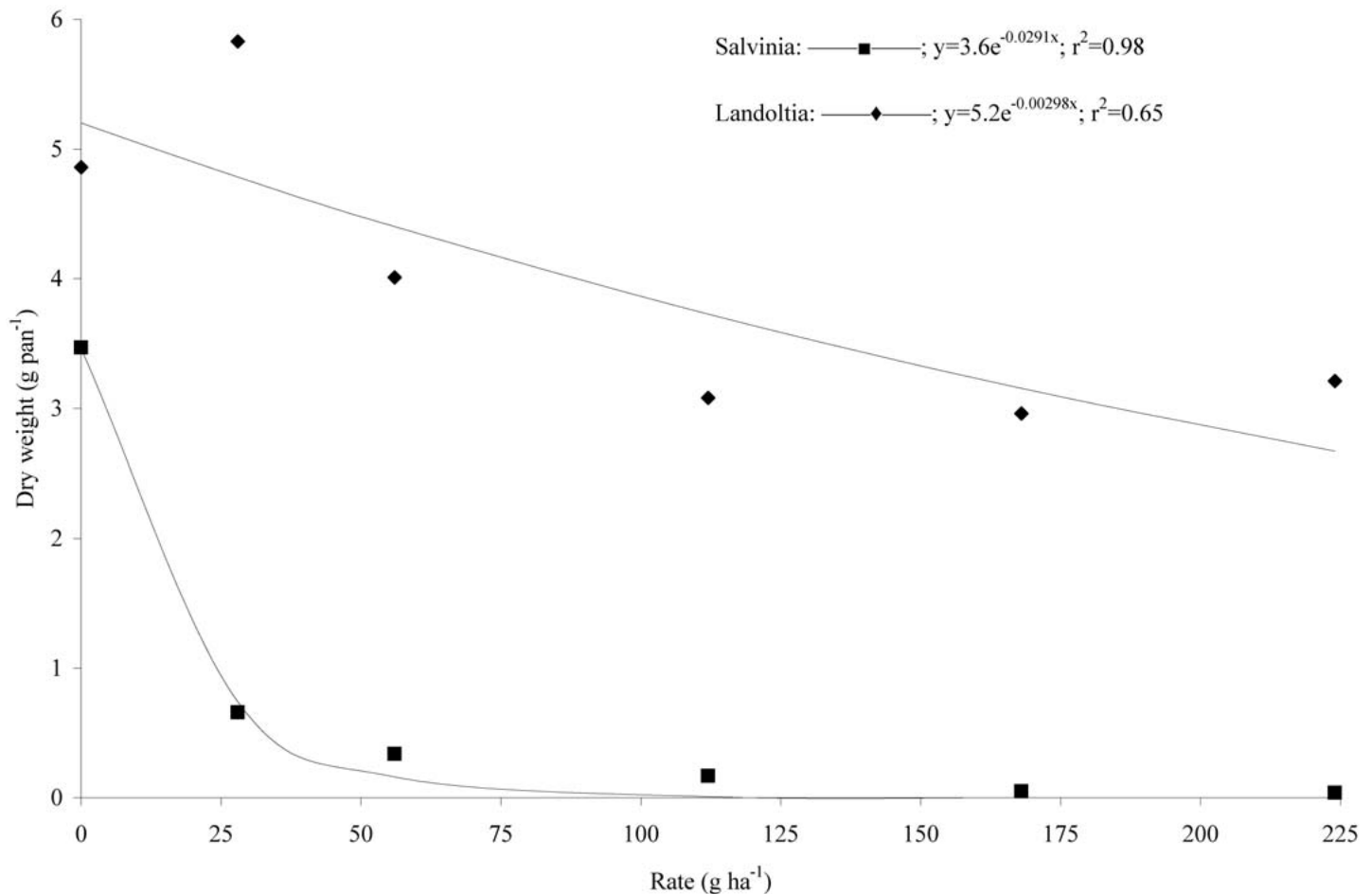


Figure 3. Carfentrazone-ethyl effects on dry weights of salvinia and landoltia. Methylated seed oil (Sun wet®) was added to the spray diluent at a concentration of 0.5% v/v.

Maximum concentrations of the parent molecule (CE) were measured 1 HAT ($8.2 \mu\text{g L}^{-1}$), and dissipated quickly from the pond as it was rapidly converted to CE acid and other breakdown products. No CE was detected on the treated portion of the pond 3 HAT, but was detected on the untreated portion below the LOQ ($5 \mu\text{g L}^{-1}$) at a depth of 15 cm. By 6 HAT, no CE was detected. Therefore, for the purpose of this study, the reported concentration of CE is the sum of both the parent molecule and CE acid unless otherwise noted.

Residues from only the treated portion of the pond are presented for the first 6 HAT, as CE had not uniformly mixed into the untreated portion of the pond at the 1, 3 and 6 HAT sampling events (Figure 4). The concentrations of CE from the untreated ($12.5 \pm 3.1 \mu\text{g L}^{-1}$) and treated half ($10.5 \pm 2.4 \mu\text{g L}^{-1}$) of the pond were similar 12 HAT, and are reported in Figure 4 as the mean from the entire pond (treated and untreated halves). Target concentrations of approximately $20 \mu\text{g L}^{-1}$ ($26.4 \pm 3.7 \mu\text{g L}^{-1}$) were initially detected 1 HAT, and during the first 24 HAT concentrations fluctuated throughout the pond as expected when treating only half of a small water body. Maximum mean concentrations of CE were detected 1 HAT on the treated half of the pond ($26.4 \mu\text{g L}^{-1}$), and on the untreated portion maximum mean residues were recorded 24 HAT ($16.8 \pm 2.9 \mu\text{g L}^{-1}$). The half-life of CE was calculated

to be 90.1 h with an r^2 value of 0.43 through 168 h after treatment. The high variation (low r^2) is due to incomplete mixing of the herbicide across the entire pond during the 1, 3, 6 and 12 h samples. Therefore, the half-life is reported in Figure 4 as 83.0 h (r^2 of 0.75) after regressing residues starting 24 h after application of the herbicide, which allowed for more complete mixing. Residues degraded below the limit of quantification (LOQ = $5 \mu\text{g L}^{-1}$) by 168 HAT, and residues below the LOQ were not used to calculate the half-life of CE.

Residues of the parent molecule were not detected in the sediment through 28 DAT, and CE acid was detected in the sediment initially 1 HAT, but did not exceed the LOQ (data not shown). CE and CE acid degraded rapidly from the aquatic environment and did not accumulate in the sediment. The degradation of CE is highly influenced by pH, and similar dissipation would be expected from water with similar pH ranges. Combined with the efficacy data, these results suggest that CE would be a good candidate herbicide for controlling several floating aquatic plants. Additional trials should be conducted to determine the selectivity of CE on additional species, and studies should evaluate the effects of surfactants on efficacy. Additional trials indicated that different surfactants might affect CE performance on less susceptible species (i.e., water hyacinth).

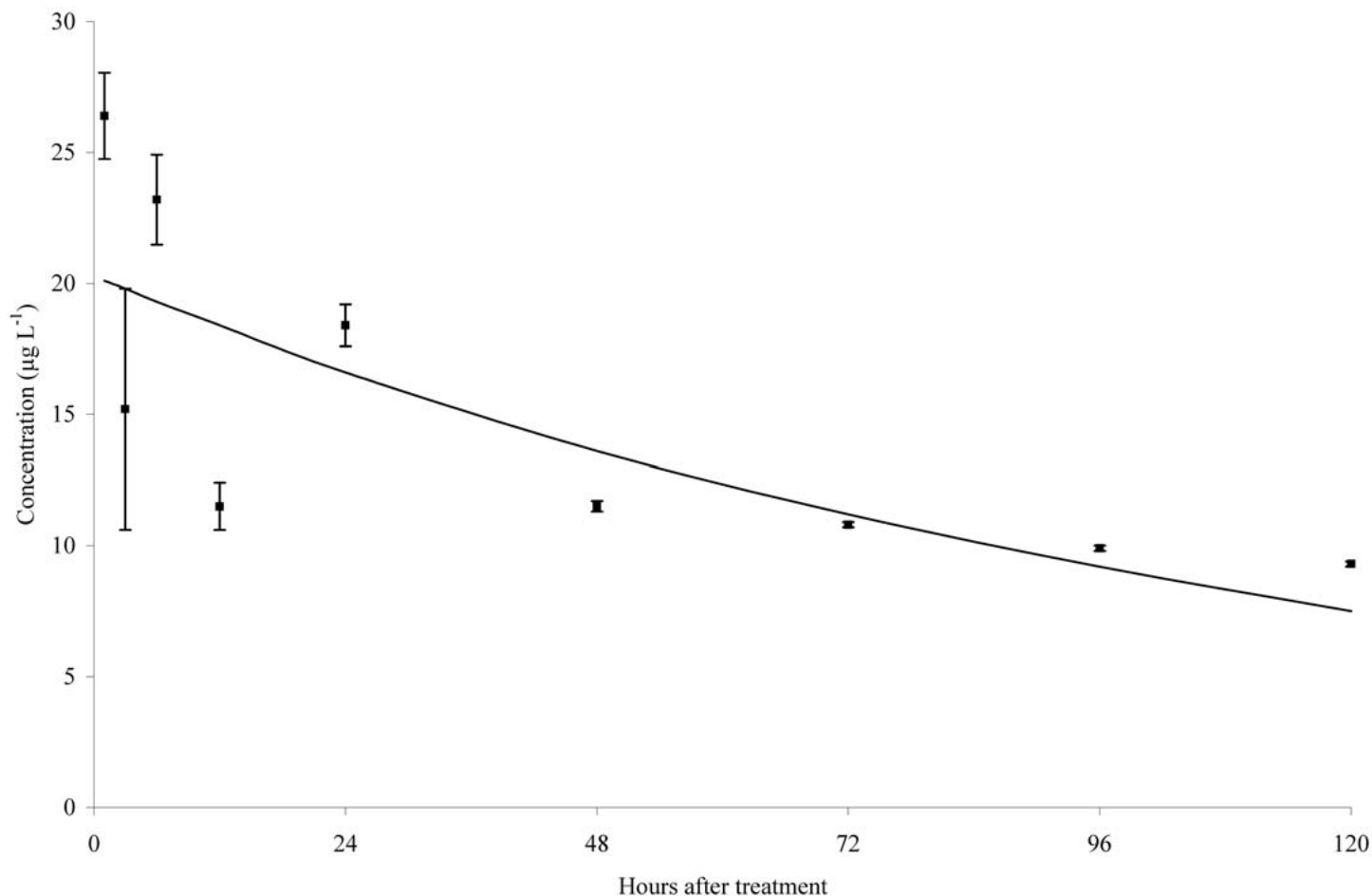


Figure 4. Dissipation of carfentrazone-ethyl (CE) + carfentrazone-ethyl chloropropionic acid (CE acid) from a small pond, where CE was applied to only half the pond at a rate of 224 g ha^{-1} ($x = 21.1e^{-0.00835t}$; $r^2 = 0.75$; half-life 83.0 h). CE (parent molecule) was only detected in the pond 1 hour after treatment (HAT) at a concentration of 8.2 µg L^{-1} . All residues after 1 HAT are CE acid, as no additional CE was detected above the limit of quantification. All residues are reported as the mean (\pm standard error) from the treated portion of the pond for the first 6 HAT, and as the mean from the entire pond thereafter. Due to incomplete mixing of the herbicide during the first 24 h after application, half-life based on regression starting 24 hours after application.

ACKNOWLEDGMENTS

The authors would like to thank FMC for providing the product, partial funding and technical support for this project. Additional thanks go to the Aquatic Ecosystem Restoration Foundation for providing additional funding for this research.

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