# Effects of Biocatalysts on Cattail Treatments

Naturalake Biosciences' CattZilla & AquaSticker Testing 2022



#### **Gray Turnage**

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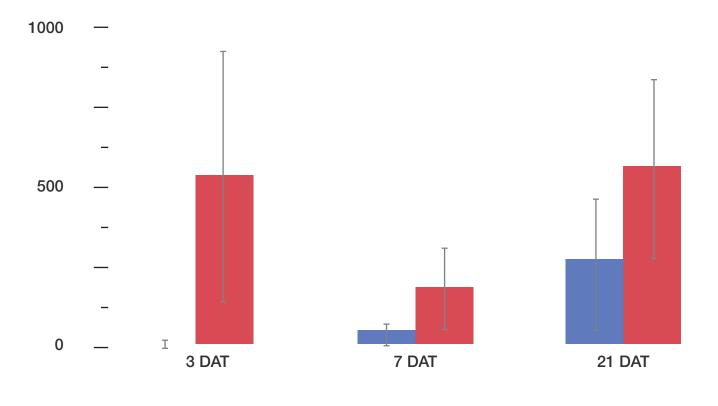
# **Study Highlights**

- HPLC analysis: CattZilla increased glyphosate penetration into cattail tissues
- Microscopy analysis: significantly more chlorosis (lack of chlorophyll or damage) in the glyphosate + CattZilla + AquaSticker treatment
- Increased cattail mortality observed at 21 and 28 days in comparison to glyphosate control (about 97% mortality vs 90% mortality)
- Significantly lower live leaf height for cattails treated with CattZilla and CattZilla + AquaSticker. This indicates cattails beginning to fall over at a faster rate (21-35 days vs 42 days for all cattail living leaves to be knocked down)



#### Glyphosate Concentration (ppb) in Cattail Root Over Time

Glyphosate Alone Glyphosate + CattZilla



#### Background

After treatment with herbicides, decaying plant material in U.S. water bodies can take weeks to fully degrade. In an effort to speed up this process, some aquatic herbicide applicators will add adjuvants containing compounds that aid in the breakdown process to spray solutions. However, little peer review data exists concerning these adjuvant products.

Purpose: To investigate the use of adjuvants (CattZilla and AquaSticker) as tank additives in foliar herbicide applications to speed up the breakdown of decaying plant material.

#### **Material and Methods**

This study was conducted in 30, 378 L (100 gal) mesocosms. Each mesocosm was filled with water to a depth of 20 inches. Cattail was established in 3.8 L pots (1 gal) filled with sand amended with a slow release fertilizer. Four pots were placed in each mesocosm. Plants were given 1.5 months to establish prior to herbicide application.

Biomass: Plants in 5 of the mesocosms were harvested prior to herbicide applications to determine pre-treatment growth levels. Pre-treatment specimens were separated into above and belowground biomass, dried, and then weighed. The remaining 25 mesocosms were used as treatment mesocosms. Treatments consisted of a nontreated reference, one herbicide only treatment, and 3 herbicide + adjuvant treatments for a total of 5 treatments (Table 1). Each treatment was replicated five times. The maximum label rate of glyphosate was applied alone or in combination with adjuvants as a foliar spray over the top of each mesocosm. At 7, 14, 21, 28, 35, and 42 days after treatment (DAT), maximum height of living and dead biomass, percent mortality (%-mortality), and the presence of new growth was recorded for each mesocosm. At 6 weeks after treatment (WAT), above and belowground biomass was harvested, dried, and weighed in the same manner as pretreatment specimens.

A one-way Analysis of Variance (ANOVA) was used to test for statistical differences in height, %-mortality, and mean biomass after the 6 WAT harvest event. Any differences detected were further separated using a Fishers Least Significant Difference test. Herbicide Residue: The uptake and translocation of glyphosate in cattail was determined using highperformance liquid chromatography (HPLC). Glyphosate was quantified in three different parts of the cattail plant: upper leaves (above water), lower leaves (below water), and roots/rhizomes at 3, 7, and 21 days after treatment (DAT). At each sampling event, one pot per mesocosm was selected for sample analysis; leaf and rhizome tissue was removed from each pot and separated into biomass components for HPLC analysis.

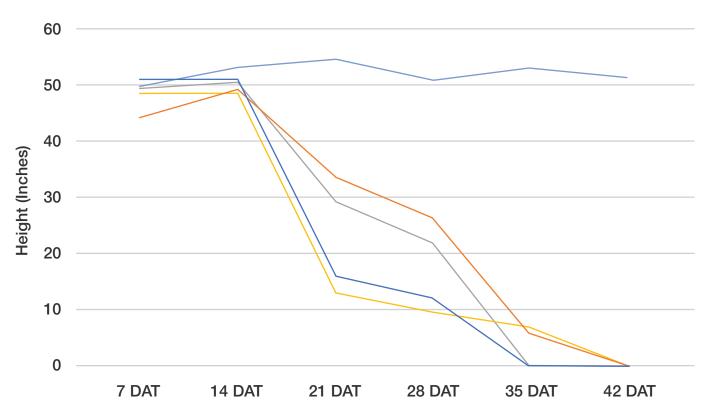
A one-way Analysis of Variance (ANOVA) was used to test for statistical differences in glyphosate residues in plant tissues at each sampling event. Any differences detected were further separated using a Fishers Least Significant Difference test.

Fluorescence Microscopy: A fluorescence microscope (BX51, Olympus, Japan) equipped with a cube consisting of D360/40×, 400dclp, and ET560lp filters was used to measure and visualize glyphosate injury in the upper and lower leaves and root/rhizome tissue. Thin sections of leaves were observed and the percentage of necrotic and dead regions on the leaf were determined using the ImageJ software (National Institutes of Health, Bethesda, MD).

## **Results and Discussion (by Michael Frett)**

Leaf height of living tissue did not differ among treatments at 7 DAT and 14 DAT (p=0.4096 and 0.9367, respectively). At 21 DAT, live leaf height of plants treated with CattZilla was reduced compared to reference and plants treated with glyphosate alone. All CattZilla treated plants observed lower live leaf heights than plants treated with Glyphosate alone or reference plants until 35 DAT. At 42 DAT, all live leaf heights of treated plants were similar.

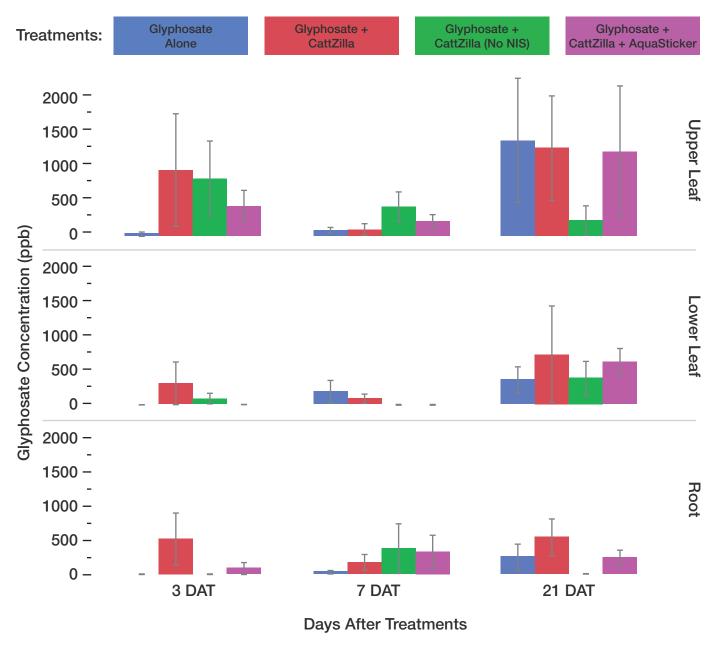
## Live Leaf Height



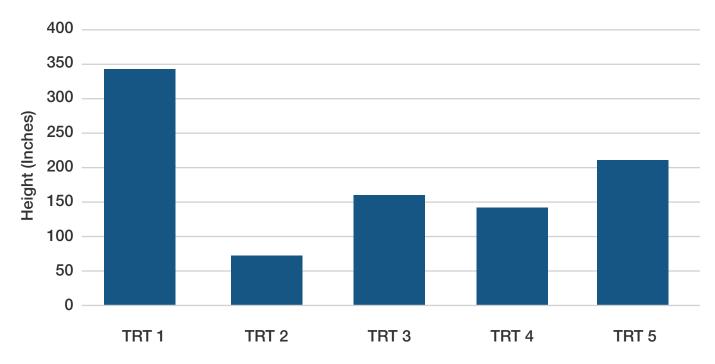
Height of dead leaves did not differ among any treatment at any sampling event; presence of dead leaves in reference treatments was likely due to normal senescence of a few leaves throughout the plants life cycle (p>0.05 for all sampling events). After treatments were administered, new growth was not observed in any treatment making statistical analysis impossible.

Herbicide Residue: Glyphosate residues among treatments at each sampling event had a high degree of variation. At 3 DAT, CattZilla treated plants showed more glyphosate penetration in the upper leaf, lower leaf, and root indicating more rapid response that glyphosate alone treated plants. CattZilla and CattZilla + AquaSticker treated plants had increased glyphosate penetration vs glyphosate alone treated plats at 3 DAT and 21 DAT.

## Mean (Glyphosate Concentration (ppb) 3) vs. Days After Treatments

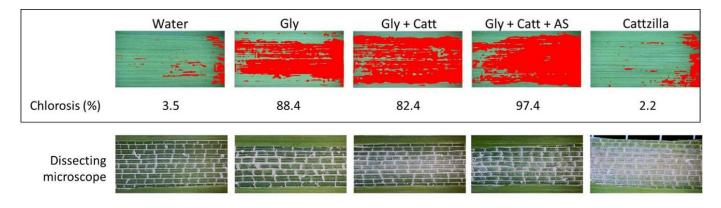


At 6 WAT, aboveground biomass was reduced (100% reduction) in herbicide treated plants compared to reference plants (p=0.0014; Figure 4); however, there was no difference among treated plants. At 6 WAT, belowground biomass did not differ among treatments (p=0.1667).



## **Belowground Biomass**

Fluorescence Microscopy: Fluorescence microscopy found that glyphosate and glyphosate + Catzilla had similar levels of chlorosis (88.4 and 82.4% chlorosis, respectively) but that the addition of Aquasticker increased chlorosis to 97.4% (Figure 7). All herbicide treatments had greater levels of chlorosis than reference plants (3.5% chlorosis) or plants treated with Catzilla alone (2.2% chlorosis).



## Study Summary (by Michael Frett)

In this study, the data suggests that additions of CattZilla and CattZilla + AquaSticker to glyphosate tank mixes are equivalent or better than glyphosate + NIS tank mixes. In the HPLC analysis, the addition of CattZilla increased glyphosate penetration into cattail tissues. In the Microscopy analysis, significantly more chlorosis was observed in the glyphosate + CattZilla + AquaSticker treatment.

Increased cattail mortality was observed at 21 and 28 days after treatment in comparison to the glyphosate control (about 97% mortality vs 90% mortality). Significantly lower live leaf height for cattails treated with CattZilla and CattZilla + AquaSticker was also observed. This indicates cattails beginning to fall over at a faster rate (21-35 days vs 42 days for all cattail living leaves to be knocked down. Overall, the data suggests the additions of CattZilla and CattZilla + AquaSticker may improve response times, increase glyphosate absorption, and breakdown the cattails faster.



# Notes

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Study crafted and completed by

# Naturalake Biosciences

