Employing a New Testing Method for Identifying Fluridone Resistance in Hydrilla

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BACKGROUND

Hydrilla & Fluridone

- *Hydrilla verticillata* is an invasive aquatic plant found throughout much of North America
- Hydrilla is ecologically and economically damaging due to waterway obstructions and forcing out native species
- Control of hydrilla has historically been tied to the use of fluridone, but resistance has developed over the past few decades in Florida waterbodies
- Resistance is conferred by multiple SNPs in the *PDS* gene associated with the production of phytoene desaturase
- These mutant populations are physically indistinguishable from the wildtype
- Existing strategies for identifying resistance are relatively slow, so there is always reason to look for faster and more cost-effective tools

DMAS-qPCR

- Double-mismatch allele-specific qPCR (DMAS-qPCR) is a modern approach that allows for the targeting of a SNP
- The process uses pairs of primers with a shared introduced mutation a few bases upstream of the target

Image courtesy of UF/IFAS

Objective

- Design a sets of DMAS primers for two mutant genotypes (CAT & AGT) and determine the viability of this method as a test for fluridone resistance in hydrilla

METHODS

Sample Selection

- Samples were collected in Florida by the United States Army Corps of Engineers and state resource managers
- Tested three samples per waterbody

Primer Design

- Sets of primers were designed with a forward primer corresponding to the wildtype, a forward primer corresponding to the target mutant SNP, and a shared reverse primer
- Primers (n = 18) were designed for all possible introduced mutant bases three pairs upstream, then tested against known mutant samples to select the most promising candidate for each pair

Testing

- Hydrilla samples (n=72) from 13 waterbodies have been tested in triplicate with the top candidate primer sets for wildtype, CAT mutants, and AGT mutants

Sample Range

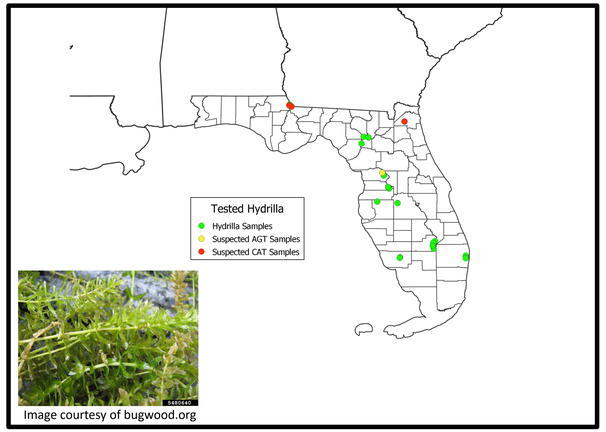
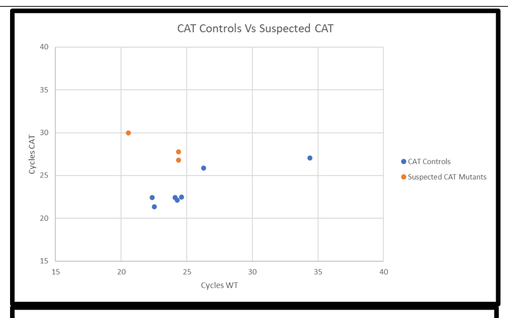
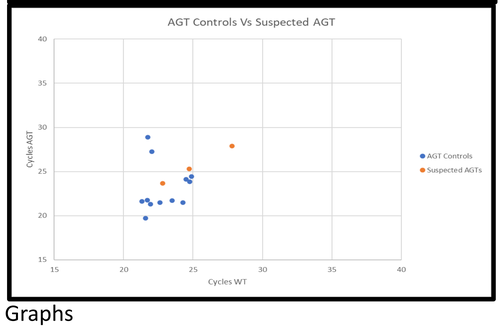


Image courtesy of bugwood.org





Interpreting the Graphs

Graphs were generated using Cq scores – a measure in cycles of the amount of time taken to reach a fluorescence threshold

The plotted scores are averages of the three replicates for each data point

The proximity of a point to each axis represents the strength of amplification corresponding to that primer within the set

Samples with low Cq scores (around 25 or less) for a mutant primer are the most likely at present to have resistance

Different DNA concentrations due to sample quality could account for variation in the data

RESULTS

Results

- Most locations (94%) associated more strongly with the wildtype primers, while some samples from Lake Hernando associated strongly with the AGT primers. Association with CAT primers across the board was weaker for the tested samples.

DISCUSSION

Conclusions

- DMAS-qPCR shows promise as a tool for identifying fluridone resistance in hydrilla
- Most locations had the wildtype and so should still be susceptible to fluridone. Several waterbodies appear to have mutants and these will be further tested with DNA sequencing of the PDS gene.
- Sequencing will be necessary to confirm results
- DMAS-qPCR could be implemented for other species that have known mutations for herbicide resistance

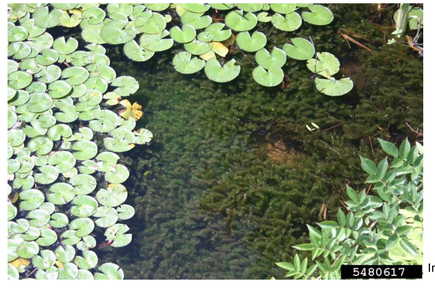


Image courtesy of bugwood.org

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