

Hilda Hatzell
Environmental Scientist
Water Quality Planning Branch
EPA Region 4
61 Forsyth St, SW
Atlanta, GA 30303

November 1, 2010

Re: Request for Modification to **Demonstration Project on Hydrilla and Hygrophila in the Upper Kissimmee Chain of Lakes, EPA Grant # X796433105-2**

Dear Ms. Hatzell,

On behalf of Osceola County, I am officially requesting to modify the work plan on the above mentioned project to revise **Element 2: Evaluation of Currently Registered Herbicides for Control of Hydrilla and Hygrophila.**

This revision includes an additional task, **Element 2, Task 4 Temporal and Spatial Sampling on the Kissimmee Chain of Lakes to Evaluate the Susceptibility of Hydrilla to Three Registered Herbicide Modes of Action.**

The purpose of this task is to perform a system-wide spatial and temporal sampling to determine:

- a) the current status of fluridone resistance six years after the last large-scale applications,
- b) comparative response of hydrilla to endothall, and
- c) comparative response of hydrilla to the acetolactate synthase (ALS) inhibitor penoxsulam.

The known development and widespread presence of fluridone resistant hydrilla in the KCOL, the heavy pressure of large-scale endothall use during the course of this project, the recent discovery of a strain of hydrilla in Orange County with elevated tolerance to the contact herbicide endothall, and the fact that hydrilla in the KCOL has now seen several exposures to penoxsulam (ALS mode of action), would suggest that a rigorous sampling effort be initiated.

This sampling effort will represent a baseline effort for the three separate herbicide modes of action and will allow us to detect any spatial differences in intra- and inter-lake susceptibility and any seasonal changes in susceptibility.

Element 2, Task 4 Work Plan Modification Request (page 2)

Sampling procedures and methodologies for determining susceptibility to the different herbicides (i.e. herbicide efficacy) are already addressed in the existing Quality Assurance Project Plan (QAPP) so we do not plan to submit any changes.

Work related to this revision would be completed within the approved no-cost extension and be completed prior to March 30, 2012. The total cost of this effort will be \$50,000 through a research agreement with University of Florida Center for Aquatic and Invasive Plants. The detailed budget justification for Element 2 Task 4 is included.

It will require a budget modification, but funds are available due to the discontinuation of **Element 3, Task 1: Demonstration of Hydrilla Control in Osceola County, Florida using *Mycoleptodiscus terrestris* (Mt), a New Contact Bioherbicide, for Aquatic Plant Management (Project ongoing).**

A budget modification will be submitted that reallocates unused funds from Element 3, Task 1 to cover this modification to Element 2, Task 4, as well as to provide funding for Osceola County personnel during the project extension through March 30, 2012.

Sincerely,



Eleanor Foerste, Osceola County Project Manager

Project Component- Element 2, Task 4 Temporal and Spatial Sampling on the Kissimmee Chain of Lakes to Evaluate the Susceptibility of Hydrilla to Three Registered Herbicide Modes of Action

Introduction:

One of the major factors that stimulated the need for the current EPA Demonstration Project on Hydrilla in the Upper Kissimmee Chain of Lakes (KCOL) was the widespread extent of fluridone resistant hydrilla throughout the KCOL. Fluridone acts by inhibiting the phytoene desaturase (PDS) enzyme and somatic point mutations in the PDS gene were ultimately found to be responsible for resistance development. Significant use of fluridone in Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee was initiated in the early to mid 1990's to address aggressive expansion of hydrilla. Early results using fluridone were encouraging and therefore use patterns were expanded. The increased use of fluridone selected for the more tolerant strains of hydrilla and widespread coverage of resistant hydrilla was documented by 2000. The perceived lack of large-scale alternatives to fluridone led to use of higher concentrations in 2001 through 2004 to try and overcome this tolerance. While treatment benefits were noted, the increased costs, non-target plant impacts, rapid recovery of the hydrilla, and conflicts between hydrilla control efforts and water management schedules led aquatic managers to the conclusion that fluridone had limited utility within the KCOL. For example, fluridone treatments in Lake Tohopekaliga averaged over 5500 acres treated per year in 2002-04, yet in the 6 years between 2005 and 2010, a total of <500 acres (< 100 acres per year) were treated with fluridone.

In concurrence with the initiation of the EPA Demonstration Project in 2006, new large scale use patterns for the registered contact herbicide endothall were planned and monitored under Element 2 of the project. Endothall use patterns on the KCOL have increased significantly and endothall now provides the primary means of hydrilla control. On Lake Tohopekaliga alone, endothall treatments of 7000+ acres and 4000+ acres were applied in December 2008 and 2009 respectively. Several aquatic resource managers have expressed concern that endothall use is following in the footsteps of fluridone and that current large-scale and consecutive use patterns could select for hydrilla with a greater tolerance to endothall. Endothall has been registered since 1958 and it has a complex mode of action that is thought to discourage development of resistance or enhanced tolerance. Nonetheless, recent findings suggest that hydrilla collected from two sites in Orange County show elevated tolerance to endothall compared to several other hydrilla accessions collected from around the state of Florida. It is important to note that large-scale operational treatments in these Orange County sites failed to provide adequate control. Given the scale and cost of large-scale endothall use on the KCOL, any change in hydrilla susceptibility would be of concern.

In addition to increased endothall use in the past several years, the herbicides penoxsulam and imazamox were registered in 2007 and 2008, and the herbicides bispyribac and bensulfuron are currently being evaluated under Experimental Use Permits (Element 1 of the Osceola Grant). To date, penoxsulam has seen the greatest use with both small (50 to 200 acres) and large-scale treatments (> 4000 acres) evaluated on the KCOL since 2008. All of the new herbicides mentioned above target the same single plant enzyme, acetolactate synthase (ALS), and based on the recent experience with fluridone are thus thought to be susceptible to resistance development by hydrilla. There is also concern that resistance to one of these products could result in cross resistance to all of the ALS herbicides. This could render all of the ALS inhibitors being developed for aquatic use (e.g penoxsulam, imazamox, bispyribac, and bensulfuron) ineffective. The fact that a wide range of ALS inhibitors have shown a history of rapid resistance development in terrestrial sites would also suggest that resistance development is a legitimate concern.

Brief Description:

Hydrilla will be collected at 65 sites throughout the KCOL (over 65,000 surface acres of water) at four different times of the year. Hydrilla coverage will dictate the initial sample site selection. Once collected, these plants will be transferred to the lab and assayed for their sensitivity to three different herbicide modes of action to determine if there are any intra-lake, inter-lake, or temporal differences in susceptibility of hydrilla to the herbicides. If any evidence of enhanced tolerance is detected, then more rigorous sampling will be initiated to determine the spatial extent of the tolerant strains. If no evidence of enhanced tolerance is detected during the course of this sampling, the information collected during these trials will serve to produce baseline susceptibility data that can be referenced in future years.

Task Objectives:

The objective of this work is to compare sensitivity of hydrilla throughout the KCOL to three different herbicide modes of action and determine if there are any spatial differences that would suggest elevated intra- or interlake tolerance or resistance to endothall or penoxsulam. In addition, this sampling effort will allow us to determine the relative distribution of fluridone resistant strains and fluridone susceptible strains of hydrilla approximately six years following the last large-scale fluridone application. Temporal sampling will be conducted to determine if sensitivity to any of these herbicides changes seasonally.

Justification:

Resistance to the herbicide fluridone has had significant impacts on hydrilla management in the KCOL as well as throughout the state of Florida. Researchers have been working diligently on developing alternate herbicide modes of action as well as new herbicide use patterns that are both cost-effective and selective. Therefore it is in the best interest of resource managers to avoid the fate of fluridone and detect increased tolerance or resistance at an early stage when alternatives to prevent rapid spread still exists. The lesson of fluridone resistance in the KCOL is to rotate modes of action when possible, pay attention to sites where herbicide performance is suspect, and develop methods that allow for early detection of resistance.

Quality Assurance Project Plan (QAPP)

Sampling procedures and methodologies for determining susceptibility to the different herbicides (i.e. herbicide efficacy) are already addressed in the existing Quality Assurance Project Plan (QAPP) as modified and approved, Feb. 22, 2010 so we do not plan to submit any changes.

Methodology

Following lake-wide surveys, healthy apices of hydrilla will be collected from selected sites throughout the KCOL (25 sites in Lake Toho, 10 sites in Lake Cypress, 10 sites in Lake Hatchineha, and 20 sites in Lake Kissimmee) during fall, winter, spring, and summer. Site selection will be based on hydrilla presence, prior treatment history, and upcoming treatment plans. Due to space limitations in the growth chambers and the amount of work associated with collecting assay data, field sample efforts will likely be staggered over a 2 week period. Collection sites will be recorded via GPS and apical tips of hydrilla will be placed in labeled resealable bags and placed in coolers for immediate transport to the Center for Aquatic and Invasive Plants in Gainesville, Florida. Healthy apical shoots will be thoroughly cleaned, cut to 4 cm sections and placed in laboratory assays to determine response to fluridone, penoxsulam, and endothall.

Hydrilla from each collection site will be exposed to several concentrations of fluridone or penoxsulam and then placed in a Percival E36-L growth chamber for a 2-4 week incubation period. These samples will be compared to a known fluridone resistant and known susceptible strain of hydrilla. All studies will be run at 25 C and a 14:10 photoperiod. New growth that accumulates during this incubation will be harvested for pigment analysis or subjected to PAM fluorometry following fluridone exposures. The penoxsulam assay will include total increase in plant length and weight, and an analysis of comparative photosynthetic rates of new shoot growth at the end of the studies. New shoot growth will also be subjected to PAM fluorometry. For the endothall assay, hydrilla will be subjected to 3 concentrations for a 3 to 4 week incubation period and plant injury will be determined via changes in Net Photosynthetic Rates and PAM fluorometry. These samples will be compared to a known endothall tolerant strain of hydrilla. All data will be subjected to regression analysis and presented as means with 95% confidence intervals. Results from intra-lake, inter-lake, and temporal sampling efforts will ultimately be compared. If any notable differences in endothall or penoxsulam susceptibility are found, enhanced sampling efforts will be conducted in this zone

Element 5: Suggested Schedule

Oct 2010 - Initiate hydrilla sampling on Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee.

Oct-Nov 2010 - Conduct herbicide assays at UF Center for Aquatic and Invasive Plants

Feb 2011 – hydrilla sampling on Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee.

Feb-Mar 2011- Conduct herbicide assays at UF Center for Aquatic and Invasive Plants

May 2011 - Hydrilla sampling on Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee.

May-Jun 2011 - Conduct herbicide assays at UF Center for Aquatic and Invasive Plants

Aug 2011 - Hydrilla sampling on Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee.

Aug-Sep 2011 - Conduct herbicide assays at UF Center for Aquatic and Invasive Plants

Nov 2011 - - Hydrilla sampling on Lakes Tohopekaliga, Cypress, Hatchineha, and Kissimmee.

Nov-Dec 2011 - Conduct herbicide assays at UF Center for Aquatic and Invasive Plants

Jan –Mar 2012 – Preparation of report and submission of peer-review manuscript

Element 2, Task 4 Deliverables

Quarterly reports providing the data and results for each quarterly assay will be provided.

This data will be presented at the Annual Aquatic Plant Management Society and Florida Aquatic Plant Management Society meetings. In addition, a final report (submitted as a peer reviewed paper) will be prepared based on the overall sampling effort and submitted prior to March 30, 2012.

Summary:

Activity	Year 1 FY 2011 (Oct. 2010 – Sept. 2011)				Year 2 FY 2012 (Oct. 2011 – Mar. 2012)	
	Q1	Q2	Q3	Q4	Q1	Q2
Fall 2010 Sampling of KCOL (4 Lakes)	X					
Fall 2010 Lab Assays of Hydrilla	X					
Winter 2011 Sampling of KCOL		X				
Winter 2011 Lab Assays of Hydrilla		X				
Spring 2011 Sampling of KCOL			X			
Spring 2011 Lab Assays of Hydrilla			X			
Summer 2011 Sampling of KCOL				X		
Summer 2011 Lab Assays of Hydrilla				X		
Fall 2011 Sampling of KCOL					X	
Fall 2011 Lab Assays of Hydrilla					X	
Winter 2012 Preparation of Final Report						X

Budget Estimate

Work related to this revision would be completed within the approved no-cost extension and be completed prior to March 30, 2012. The total cost of this effort will be \$50,000 through a research agreement with University of Florida Center for Aquatic and Invasive Plants.

The information below represents a detailed breakdown of the expenses associated with the proposed new work effort under Element 2 Task 4. We will conduct 5 rounds of sample events. I anticipate needing approximately 1/3 of a year’s time for an OPS scientist in Gainesville to design, set up, collect and enter data, analyze data, and write reports. Due to the extensive time associated with set-up, tear-down, and clean-up associated with these trials, I will need two student workers to perform many of the routine tasks (e.g acid washing flasks, preparing culture media, etc). Each of the 5 trials will require preparation of large volumes of culture media and analysis of herbicide residuals for confirmation. This represents the bulk of the supply requirements.

In terms of equipment, we have most of the large expense items necessary (Percival growth chambers, spectrophotometers, and PAM fluorometers) for data collection, but will purchase a light meter for collection of PAR data in the growth chambers. Travel will include trips to assist Dean Jones with sample location and selected collections as well as travel to a meeting to present the results. The budget below reflects a close estimate of project costs.

1. PERSONNEL EXPENSES						
A. Salaries - (Name/Title/Position)						
	Hourly Cost		Hours			Totals
Research Sci./OPS	\$ 30.50	x	700	=	\$	21,350
OPS Student Workers	\$ 13.50	x	400	=	\$	5,400
	\$	x		=	\$	
	\$	x		=	\$	26,750
	Total Salaries					\$ 21,350
B. Fringe Benefits - (Rate % x Total salaries applicable)						
	(2.4 % x \$ 21,350)	Total Fringe Benefits			=	\$ 512.00
Total Personnel Expenses (A + B)					=	\$ 27,262.00
2. SUPPLIES						
	Description	Unit Cost		Quantity		Totals
	Culture media, solvents, glassware	\$	x		=	\$ 4362
	ELISA kits for immunoassay	\$ 650	x	6	=	\$ 3900
	New bulbs for growth chambers	\$	x		=	\$ 500
		\$	x		=	\$
	Total Supplies					\$ 8,762.00
3. EQUIPMENT						
	Description	Unit Cost		Quantity		Totals
	Li-Cor light Meter (underwater sensor)	\$	x	1	=	\$ 1676.00
		\$	x		=	\$
	Total Equipment					\$ 1,676.00
4. TRAVEL						
	Purpose/Destin.	Days	Per Diem	Fare/Rate	Mileage	Totals
	On-site sample/survey	[9 x	\$ 150]	+ [\$	(x)	= \$ 1350
	Meeting-report result	[3 x	\$ 150]	+ [\$	(x)	= \$ 450
		[x	\$	+ [\$	(x)	= \$
	*per diem estimated for hotels, meals, and driving					Total Travel \$ 1800.00
5. CONTRACTUAL						
	Name or Services	Fee/Rate		Hours		Totals
		\$	x		=	\$
		\$	x		=	\$
	Total Contractual					\$
6. MISCELLANEOUS						
	Description	Unit Cost		Quantity		Totals
	Overnight shipping of plants	\$	x	10 events	=	\$ 500
		\$	x		=	\$
		\$	x		=	\$
		\$	x		=	\$
	Total Miscellaneous					\$
SUBTOTAL (1.-6.)					=	\$ 40,000.00
OVERHEAD/INDIRECT Rate 25 % x Base \$ 40,000					=	\$ 10,000.00
Base:						
8. TOTAL BUDGET					=	\$ 50,000.00