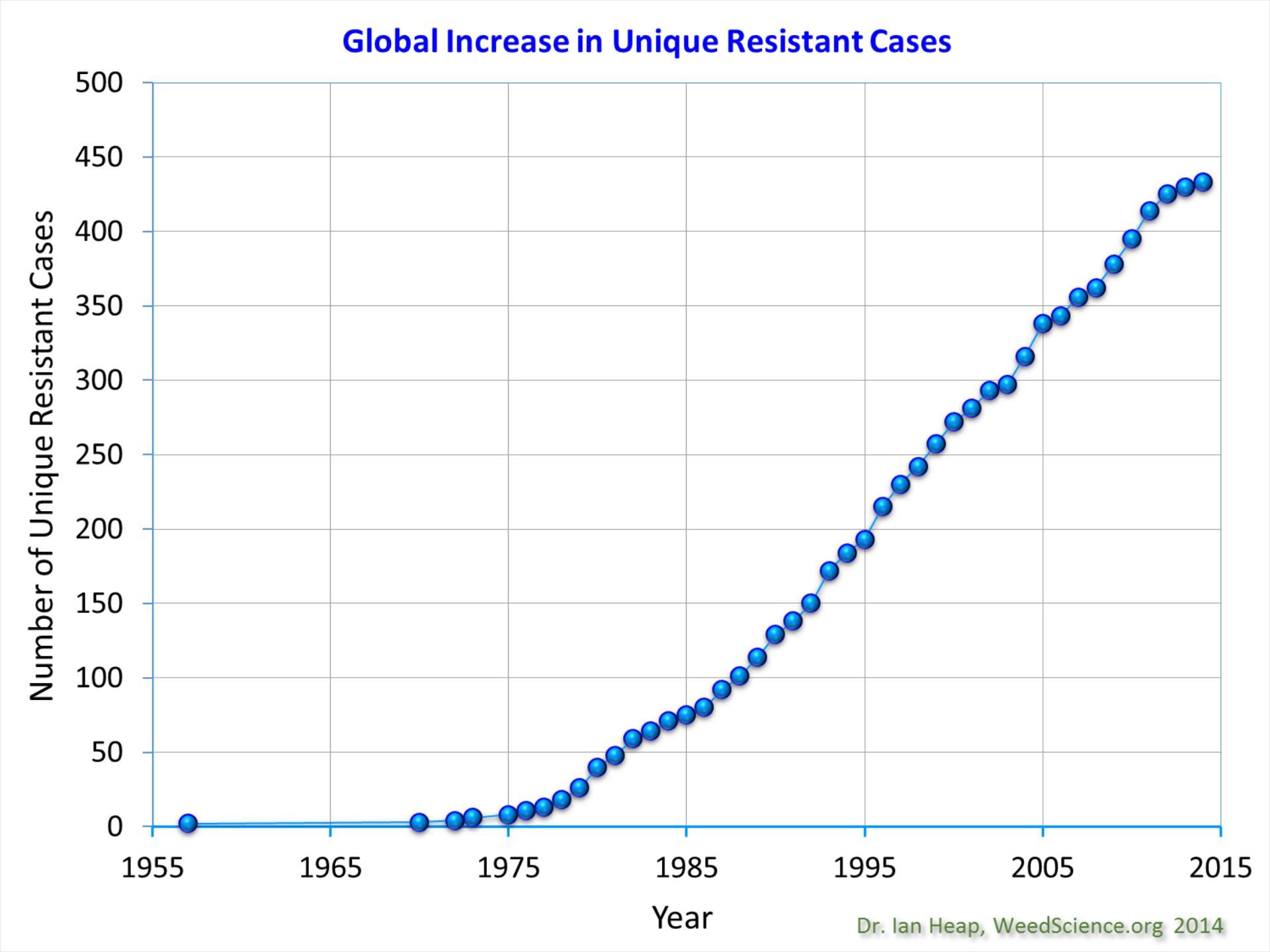
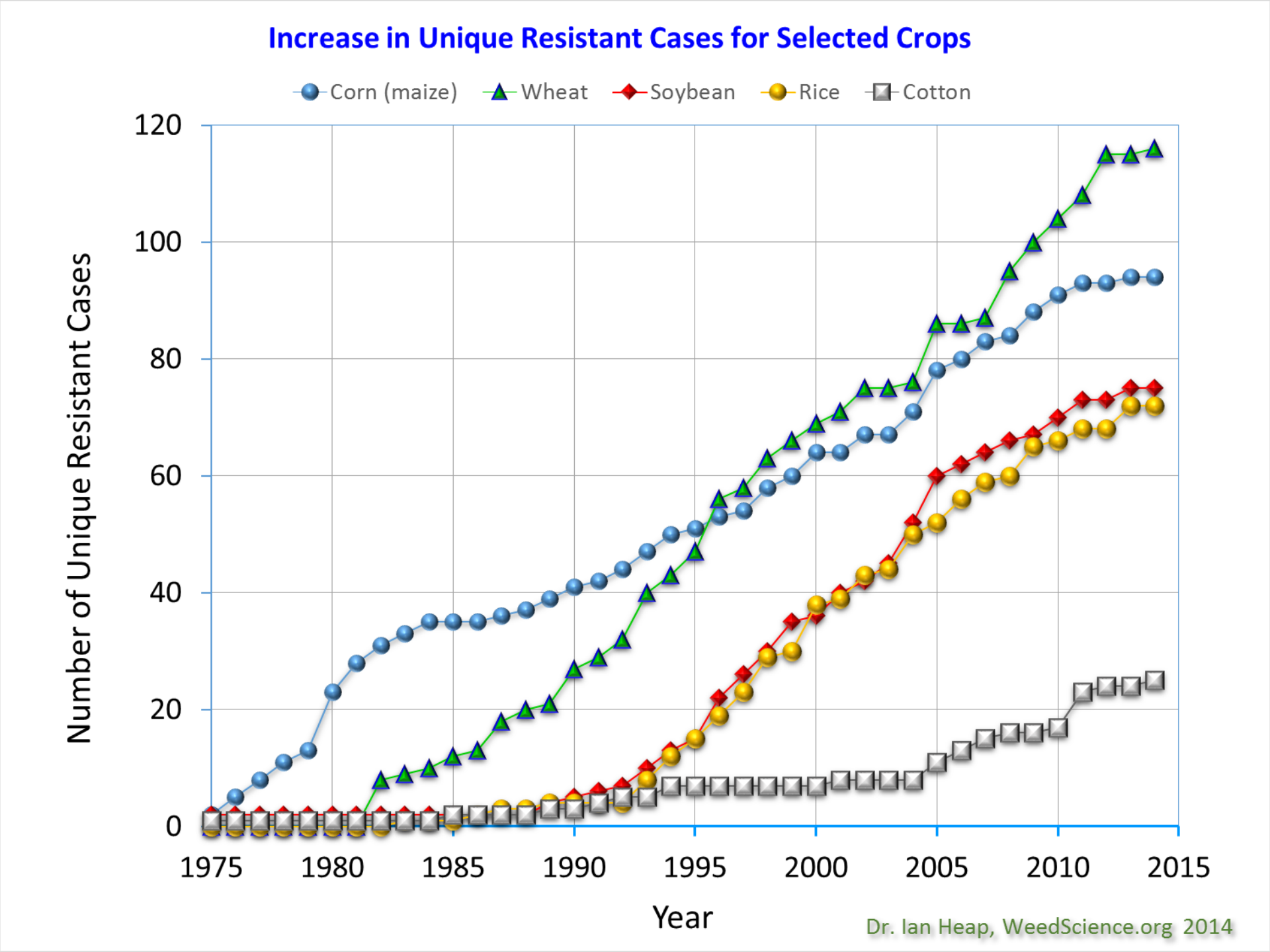
Herbicide resistance in *Hydrilla verticillata* and Other Aquatic Plants

Lori K. Benoit, Ph.D.


Global Increase in Unique Resistant Cases
Number of Unique Resistant Cases
500
450
400
350
300
250
200
150
100
50
0
1955
1965
1975
1985
1995
2005
2015
Year
Dr. Ian Heap, WeedScience.org 2014


Increase in Unique Resistant Cases for Selected Crops
Corn (maize)
Wheat
Soybean
Rice
Cotton
Number of Unique Resistant Cases
120
100
80
60
40
20
0
1975
1980
1985
1990
1995
2000
2005
2010
2015
Year
Dr. Ian Heap, WeedScience.org 2014

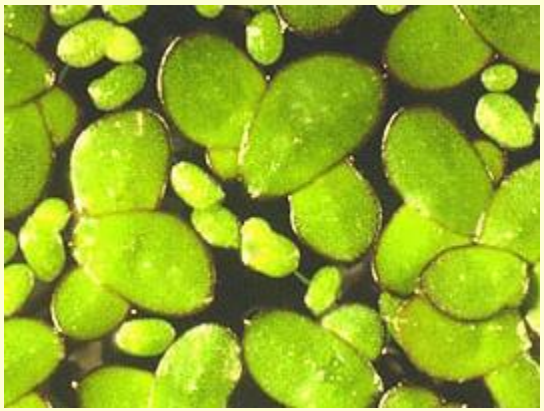
WHAT IS HERBICIDE RESISTANCE?

From the Weed Science Society of America:

“Herbicide resistance is the inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type.”

Where are resistant plants found?

- Mostly terrestrial
- Associated with agriculture
- Wetland species associated with rice fields
- Very few aquatics in lakes



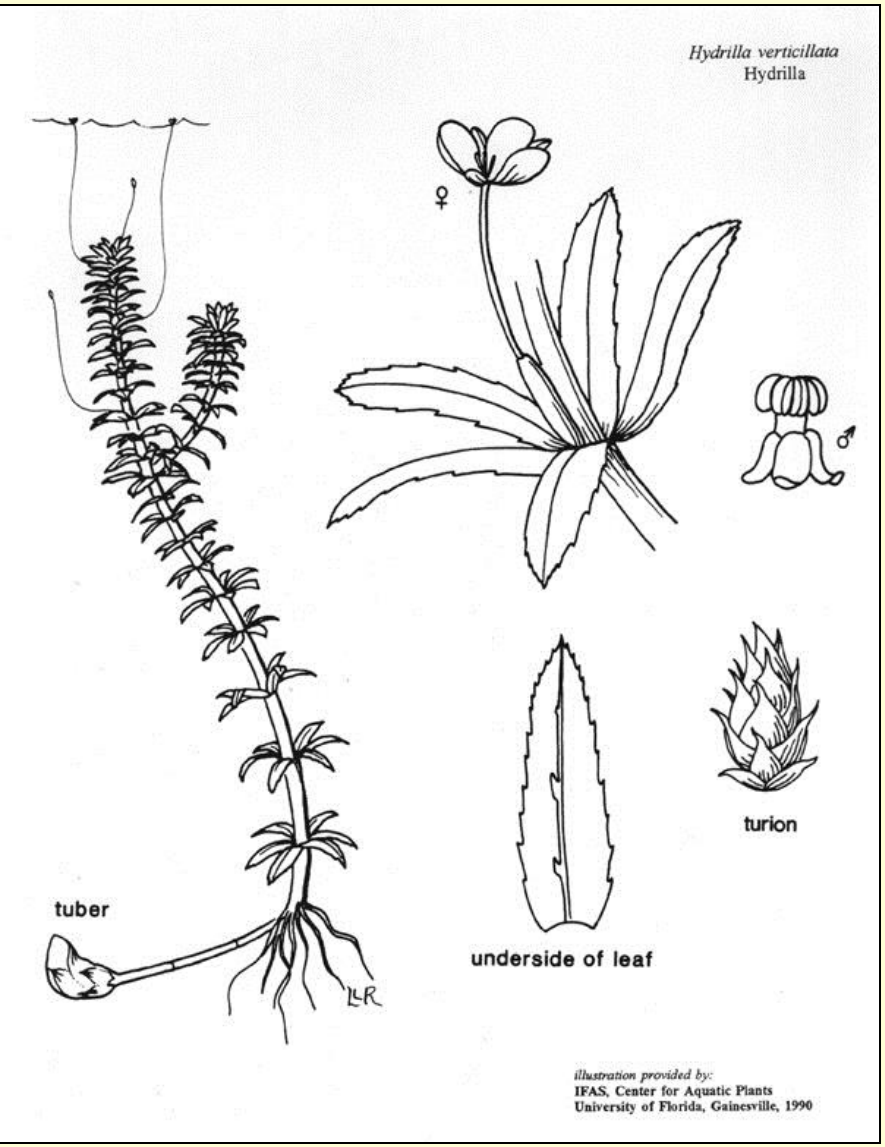
WHICH AQUATIC PLANTS IN THE U.S. HAVE DEVELOPED HERBICIDE RESISTANCE?

- A duckweed (*Landoltia punctata*) from Lake County, FL is resistant to diquat.

- Hydrilla (*Hydrilla verticillata*) in multiple locations throughout Florida. Resistant to Sonar (fluridone).

- Hybrid milfoil (*Myriophyllum spicatum* x *sibiricum*) in midwest lakes may have some resistance to fluridone.

Hydrilla verticillata

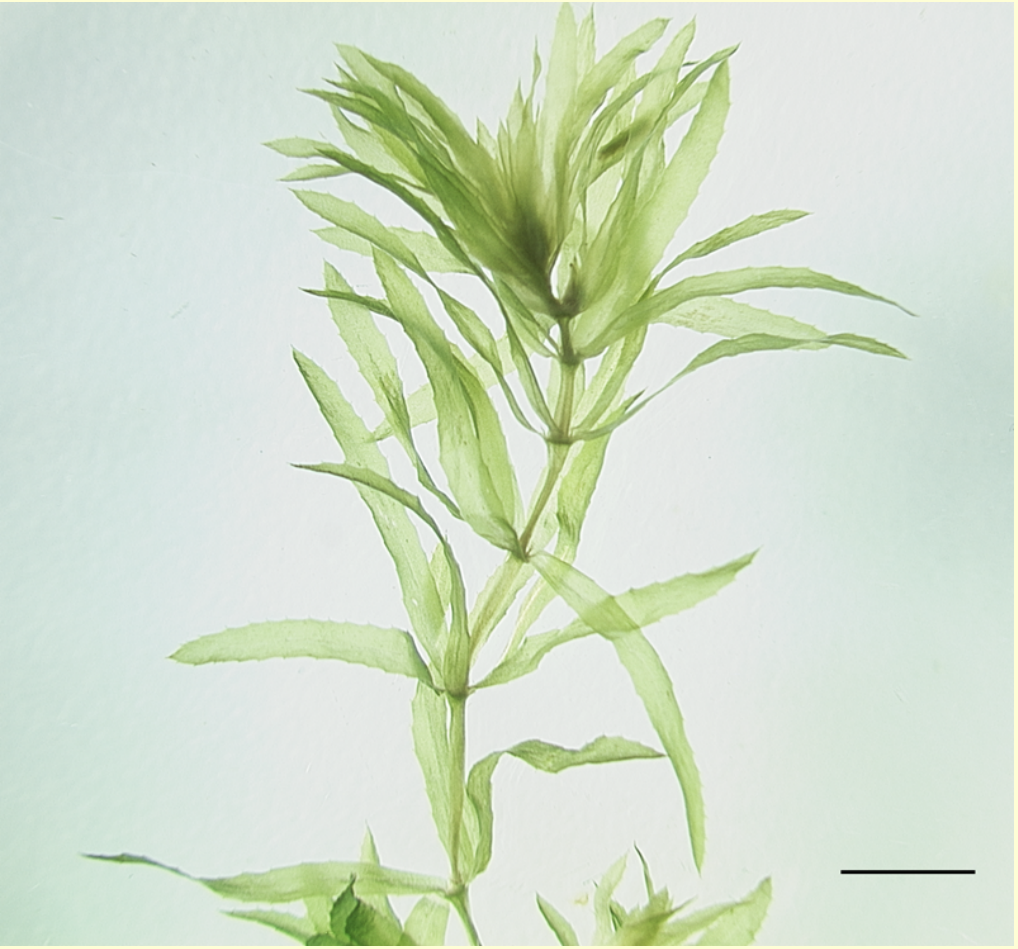


- Aquatic submersed rooted perennial, with sessile, toothed leaves in whorls of 5 (3-8)
- Two ‘strains’ or biotypes recognized: monoecious and dioecious
- High frequency of triploids 3x=24, (diploid 2x=16)
- Vegetative reproduction
- Nonindigenous and invasive in U.S.

Rake with Hydrilla tubers
Photo by W.T. Haller
Center for Aquatic and Invasive Plants

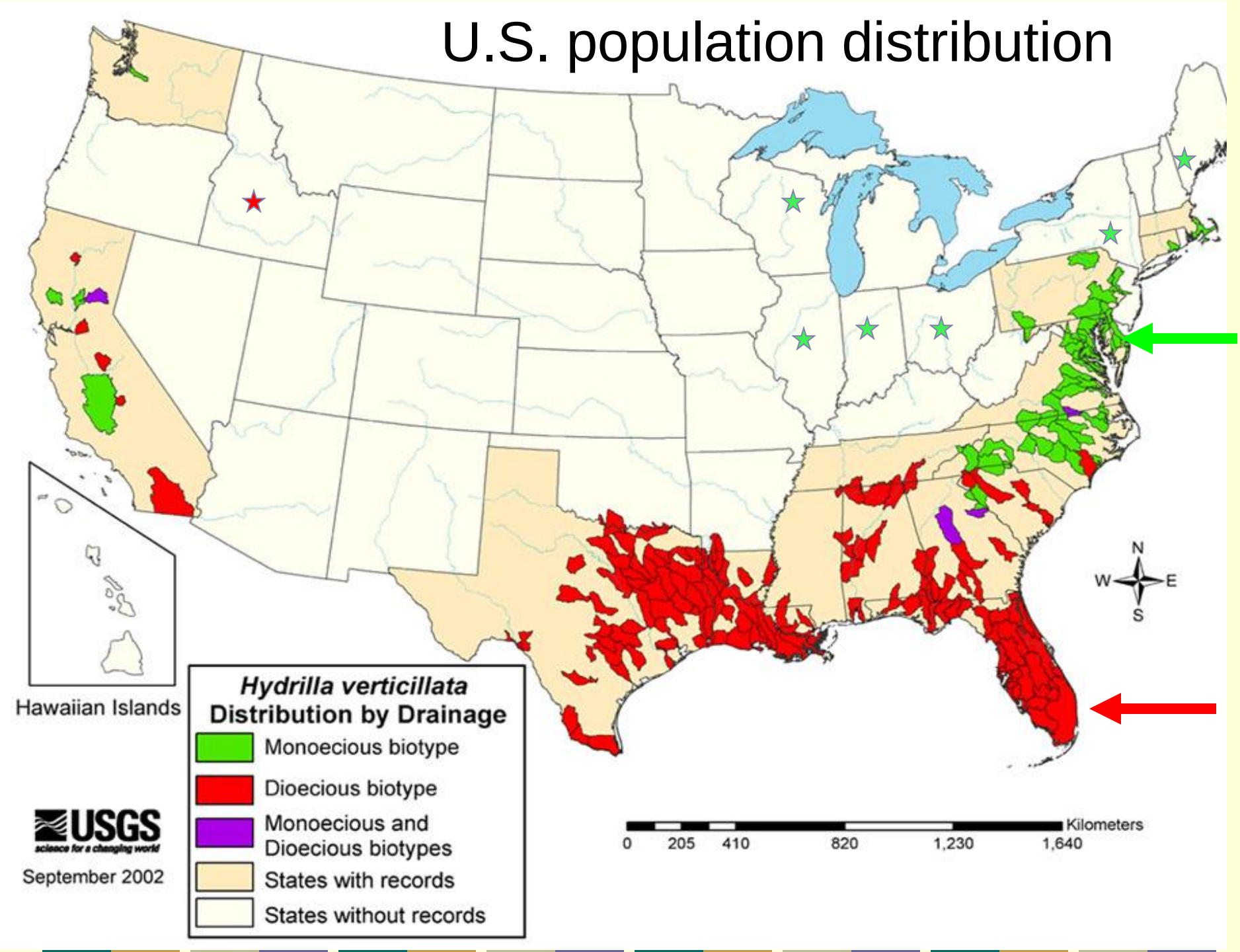
Hydrilla
Hydrilla verticillata
Photo by Vic Ramey

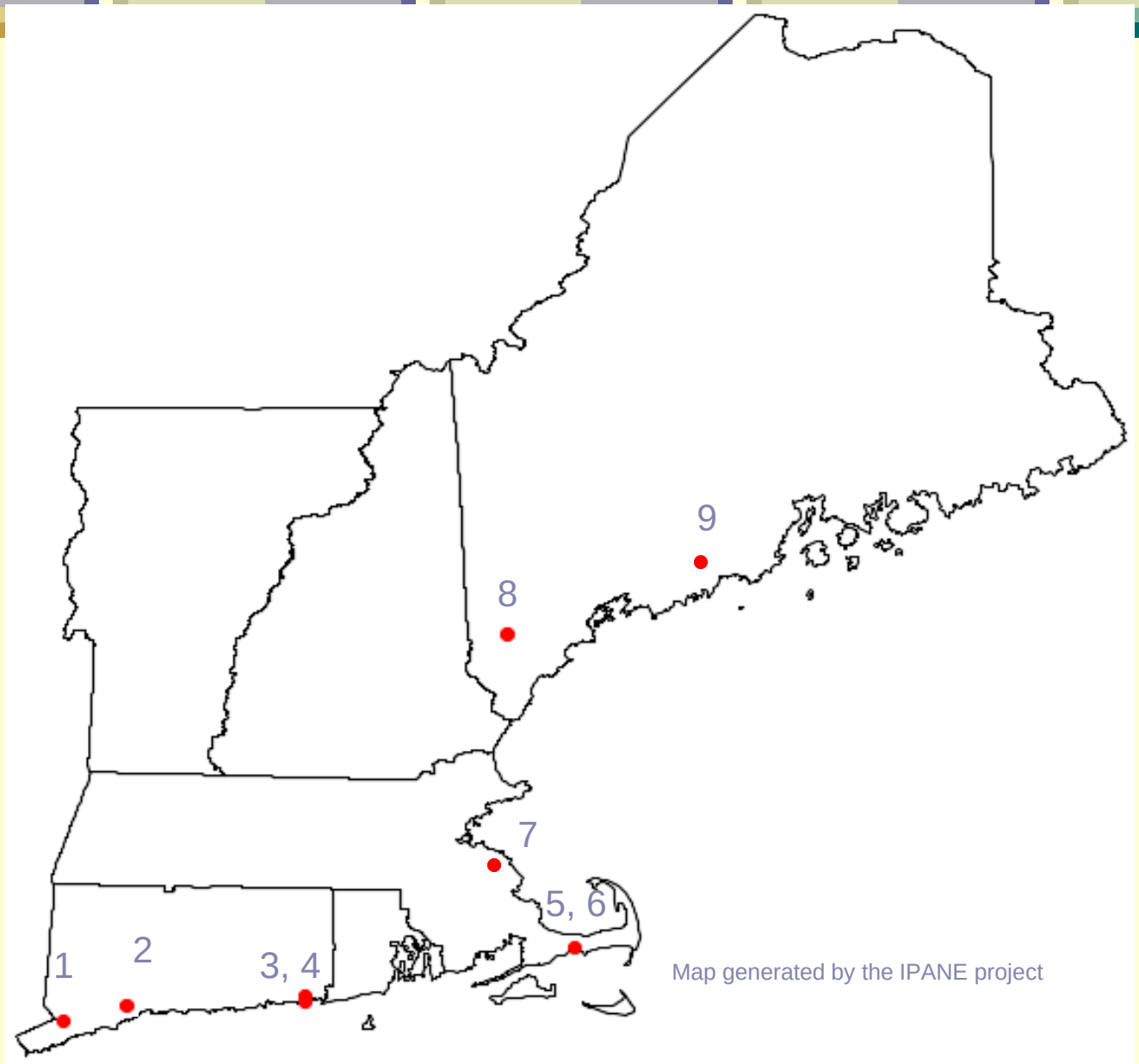
Identification of local monoecious *Hydrilla*



- Longer, narrower, limper leaves compared to dioecious
- No mid-rib spines
- Finer teeth along leaf edges
- Leaves in whorls of 3, as well as 5.

U.S. population distribution





New England locations of Hydrilla

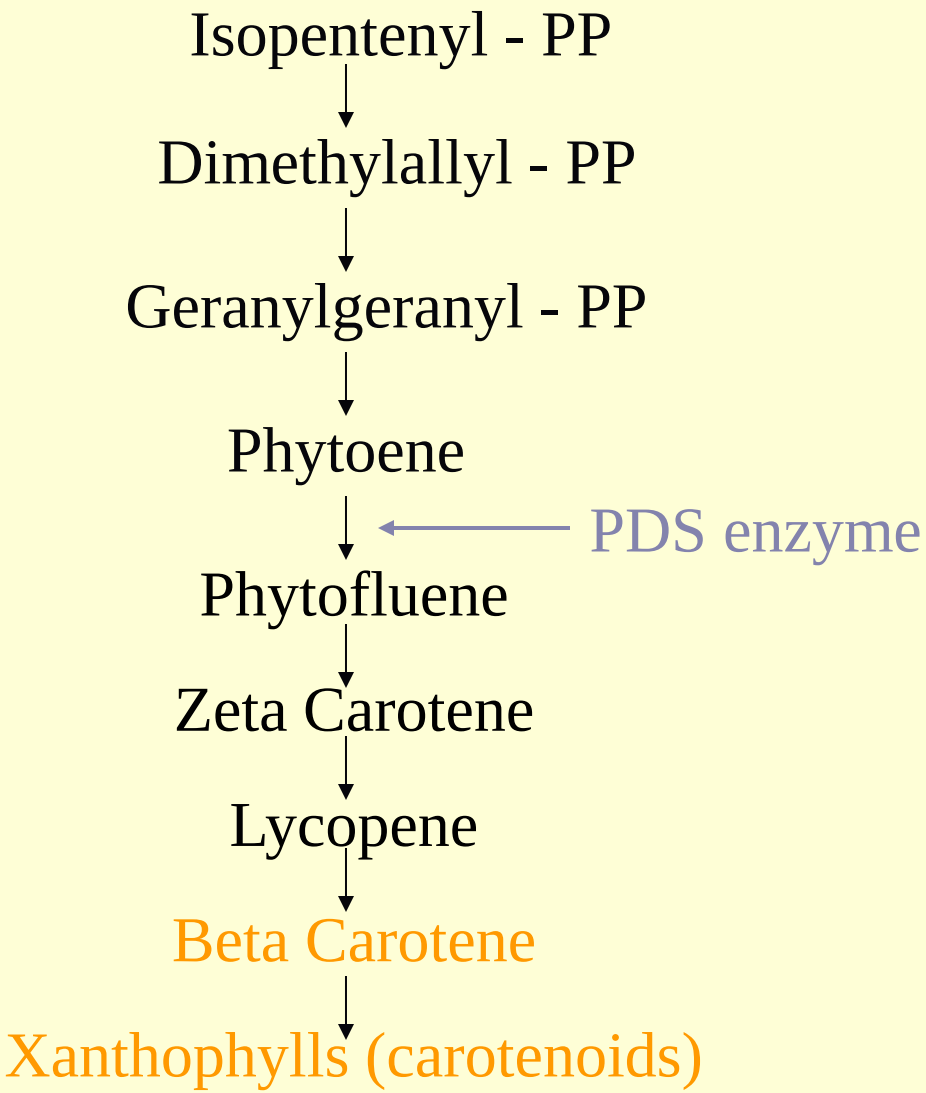
FLURIDONE: EFFECTIVE HERBICIDE FOR CONTROLLING HYDRILLA

Fluridone (Sonar©): an effective, systemic, slow-acting herbicide, with low toxicity to other organisms e.g. fish, birds, humans

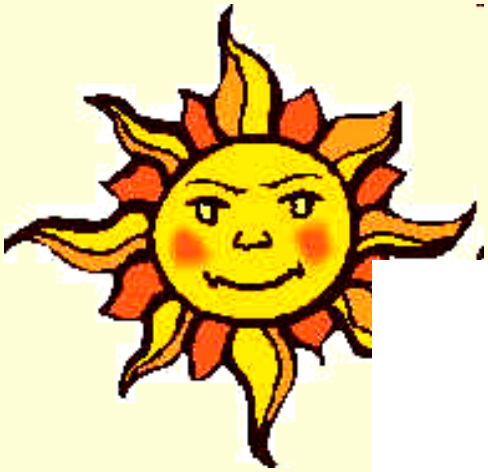
Evolution of resistant populations occurred in many Florida lakes following years of fluridone use

Hydrilla in Pond at Kenilworth Aquatic Gardens, Washington, D.C.

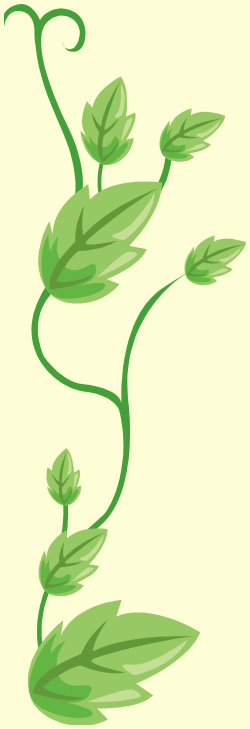
Hydrilla in pond, Mystic, CT

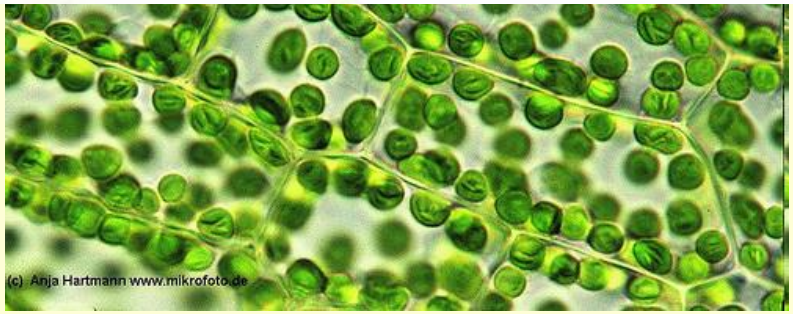


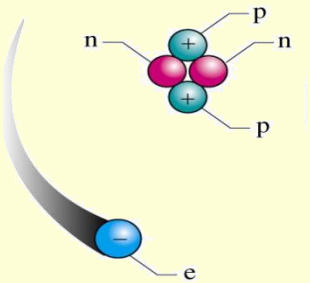
CAROTENOID BIOSYNTHESIS PATHWAY



Photosynthesis requires light capturing pigments: Chlorophyll (green), and







Detecting Herbicide Resistance

- Hydrilla resistant to fluridone has one of 3 mutations in *pds* gene at codon 304 (Michel et al. 2004)

Wild-type Arg(CGT) → Ser(AGT), Cys(TGT), His(CAT)

Fluridone can not bind effectively to a PDS enzyme when it has one of these mutations.

TESTING FOR PRESENCE OF HERBICIDE RESISTANCE

1)

Hydrilla growing in tanks at Sepro Corp.

- Collect plants from lake
- Apply herbicide fluridone (slow-acting, systemic)

Time: 3-4 weeks

Cost: $2500

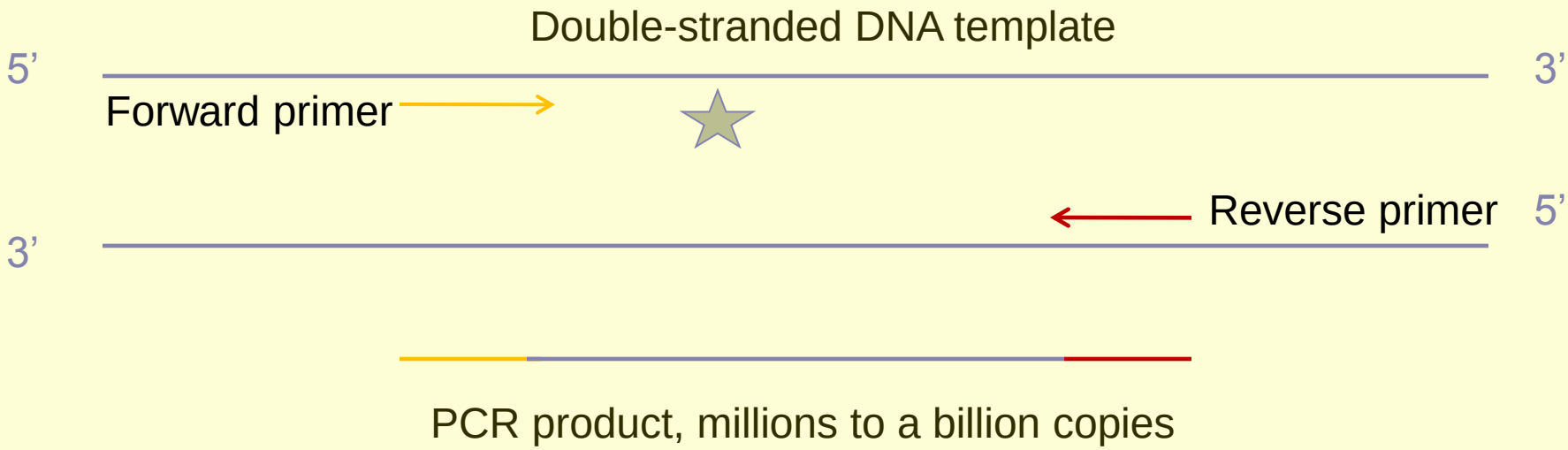
2) Determine the dose(s) of herbicide needed to control hydrilla

Characterization of resistance mutations

Codon 304 sequence		Fluridone resistance assessments 1	2
CGT	Wild type	none	0
AGT	mutation	low	2x-5x
TGT	mutation	medium	ND
CAT	mutation	high	7x

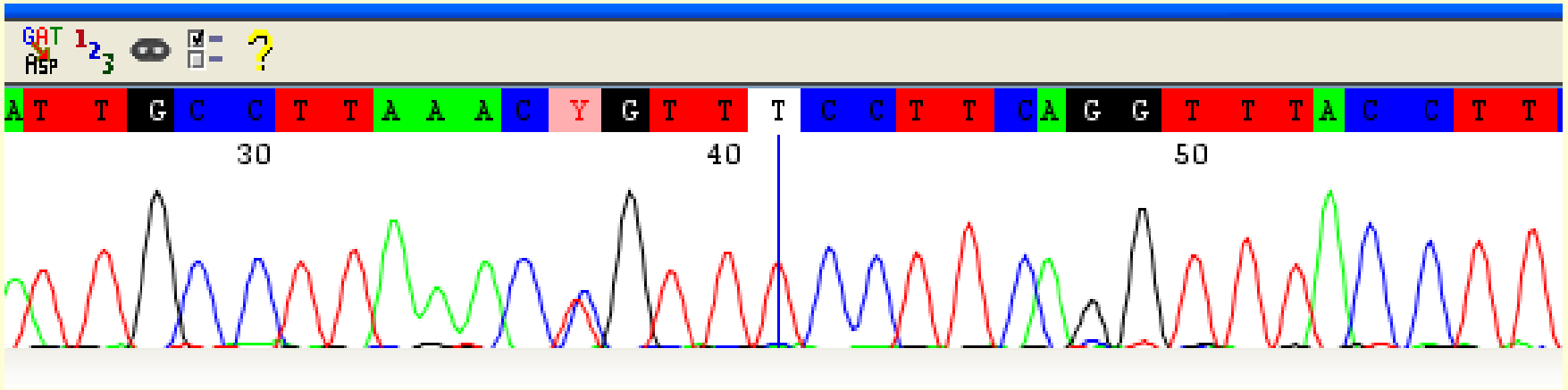
1) Michel et al. 2004, 2) Puri et al. 2006

Molecular genetic method to identify resistance conferring mutations: PCR and sequence the hydrilla *pds* gene region containing codon 304



- Using the published cDNA of hydrilla *pds* gene, designed forward and reverse primers that flanked codon 304. Amplify directly from extracted DNA.
- Then DNA sequence: determine the sequence of nucleotides in the piece of DNA.

DNA Sequences of *pds* gene region



Mutation appears as a double peak with wild type C nucleotide

DNA Sequences of *pds* gene region

Hydrilla in the U.S. is all triploid = three sets of chromosomes

Resistant Dioecious hydrilla:
Clone A = Mutation for herbicide resistance (AGT, TGT, CAT)
Clone B = always wild type (CGT)
Clone C = always wild type (CGT)

SCREEN FOR MUTATIONS AT CODON 304

SAMPLES	WATER BODIES	COUNTRIES	US DIO	US MONO
93	77	16	21	23

SIX LOCALITIES HAD HYDRILLA WITH FLURIDONE-RESISTANT MUTATIONS

FLORIDA: Five water bodies had hydrilla with mutations,
Three (3) AGT, One (1) TGT, One (1) CAT

GEORGIA: One (1) CAT

ALL MONOECIOUS WERE WILD-TYPE

SOME ANSWERS, AND MORE QUESTIONS

- All herbicide resistant hydrilla possess 2 genes with the wild-type (2 with CGT) and 1 with a mutation
- Mutations occur on the same copy of one of three chromosomes ("clone A")
- Is clone A more prone to mutation at codon 304 in *pds* gene?
- Monoecious (Northeast) hydrilla does not have this dioecious (Southeast) clone A sequence as one of its three chromosomes.
- Is monoecious hydrilla less prone to mutation at PDS codon 304, and therefore less likely to develop herbicide resistance?
- OR… has monoecious hydrilla been exposed to less fluridone?

COMPARISON OF TREATMENT HISTORY AND HERBICIDE RESISTANCE

Site	fluridone use (yrs)	Alternate herbicides used	Physical removal	Resistance	biotype
Lake Okahumpka, FL	8	0	No	Y	Dio.
West Lake Toho, FL	8	0	No	Y	Dio.
Wingates Landing, Lake Seminole, GA	10	0	No	Y	Dio.
Lake Gaston, NC & VA	6	?	grass carp	N	Mon.
Haven Lake, DE	2	1	mechanical harvester	N	Mon.
Concord Pond, DE	2	0	mechanical harvester	N	Mon.
Long Pond Barnstable, MA	8	0	No	N	Mon.

MANAGING HYDRILLA AND OTHER AQUATIC INVASIVE PLANTS TO PREVENT RESISTANCE

- Rotate chemical treatments with non-chemical methods e.g., physical removal by machine, hand or triploid grass carp
- Rotate types of herbicides used
- Very few herbicide approved for use in lakes. Others in development and testing stages. But… these are also single mechanism herbicides
- PREVENTION, Education, and Monitoring: Find early, treat early

Thank you

and

Questions?