Determination of the Effects of Contaminant Mixtures on Aquatic Macrophytes

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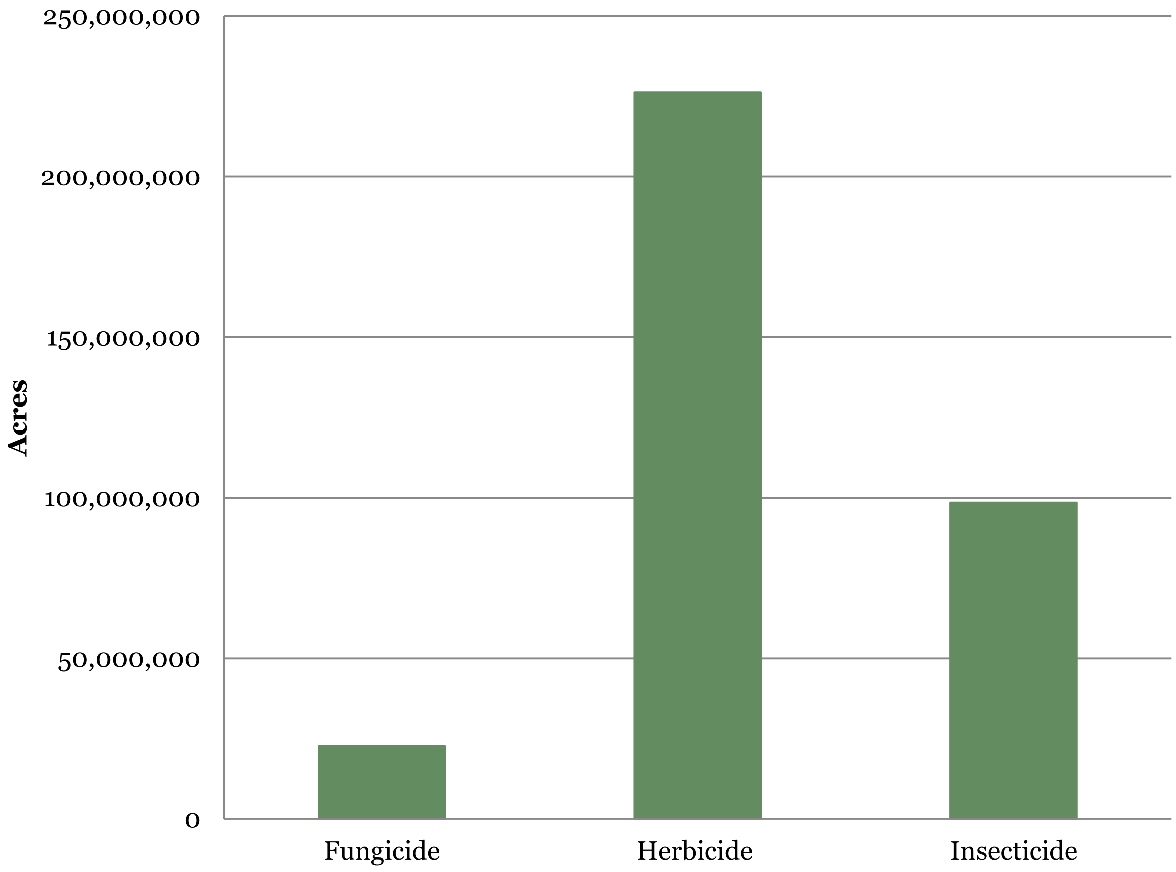
Introduction

More than 600 million pounds of pesticide were used in both 2006 & 2007.

Class	Millions Pounds a.i.	% of Total
2006		
Herbicides/PGR	407	63
Insecticides/Miticides	69	11
Fungicides	46	7
Nematicides/Fumigants	96	15
Other	25	4
Total	643	100
2007		
Herbicides/PGR	442	65
Insecticides/Miticides	65	9
Fungicides	44	6
Nematicides/Fumigants	108	16
Other	25	4
Total	684	100

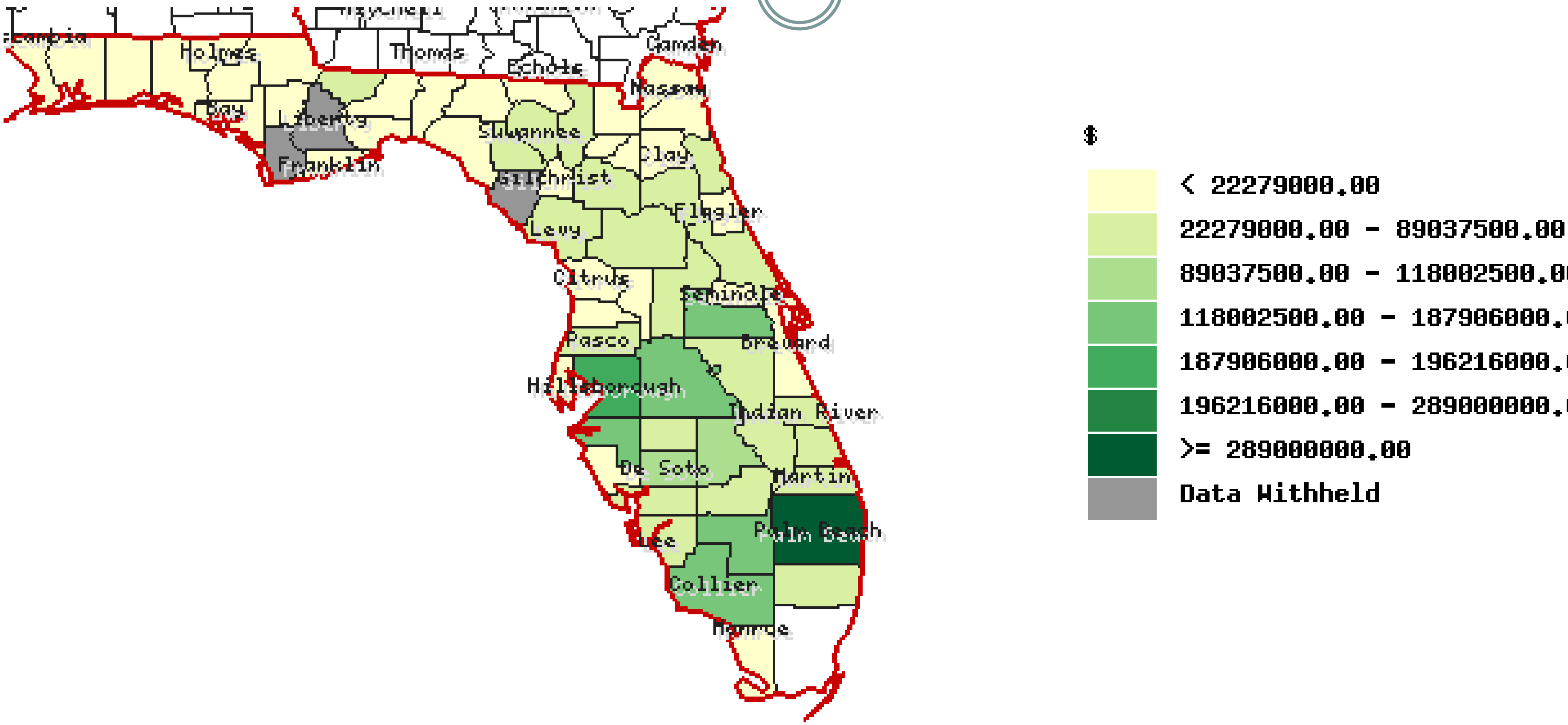
U.S. agricultural pesticide use by class – 2006 and 2007. (Fishel 2007)

Total pesticide application on US farms.

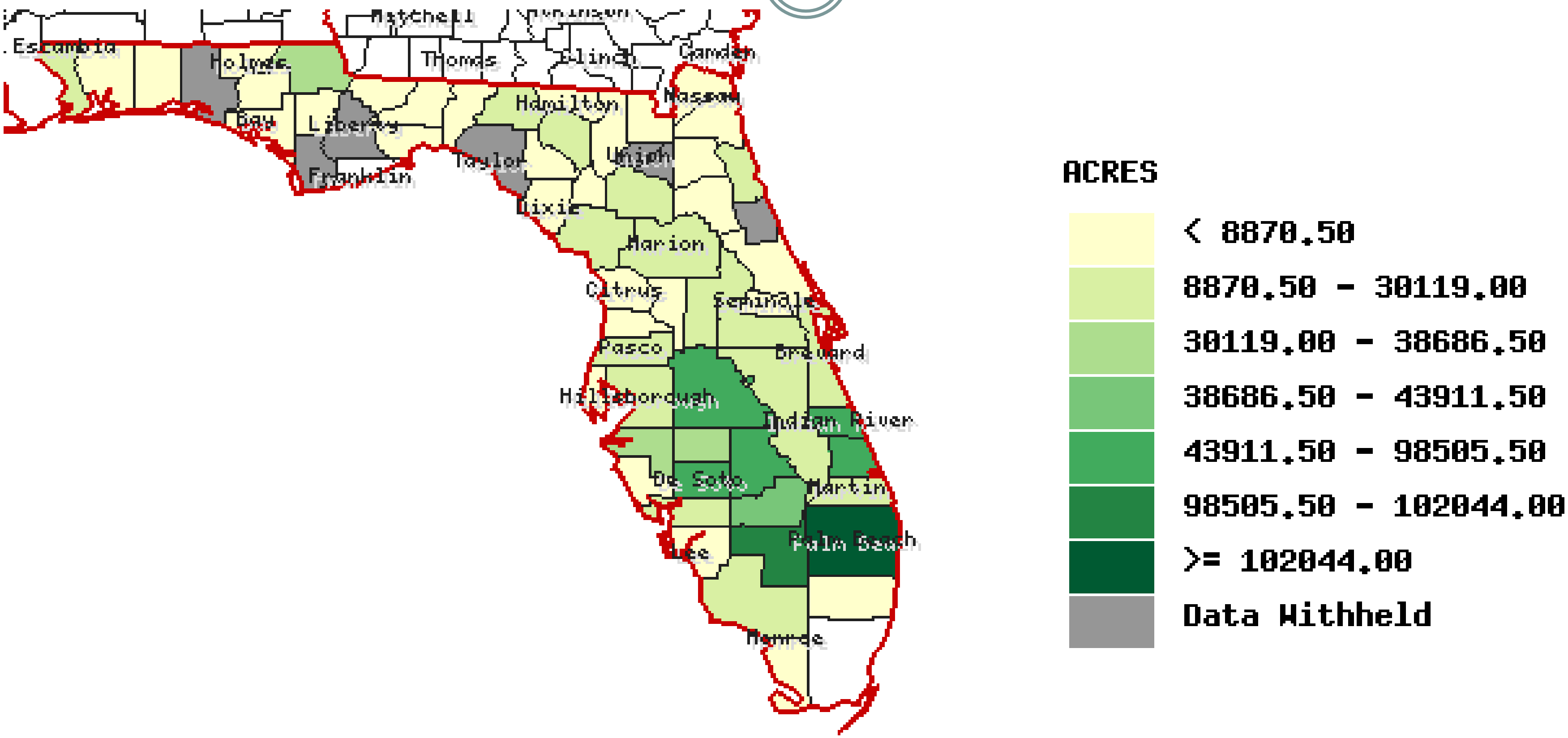


USDA/NASS estimates of pesticide application on U.S. Farms in 2007

Agriculture is a valuable industry in Florida



Florida Agriculture and Pesticide Application



Florida has to balance delicate ecosystems, agriculture and urban areas

Pesticides are commonly found in surface water samples nationwide

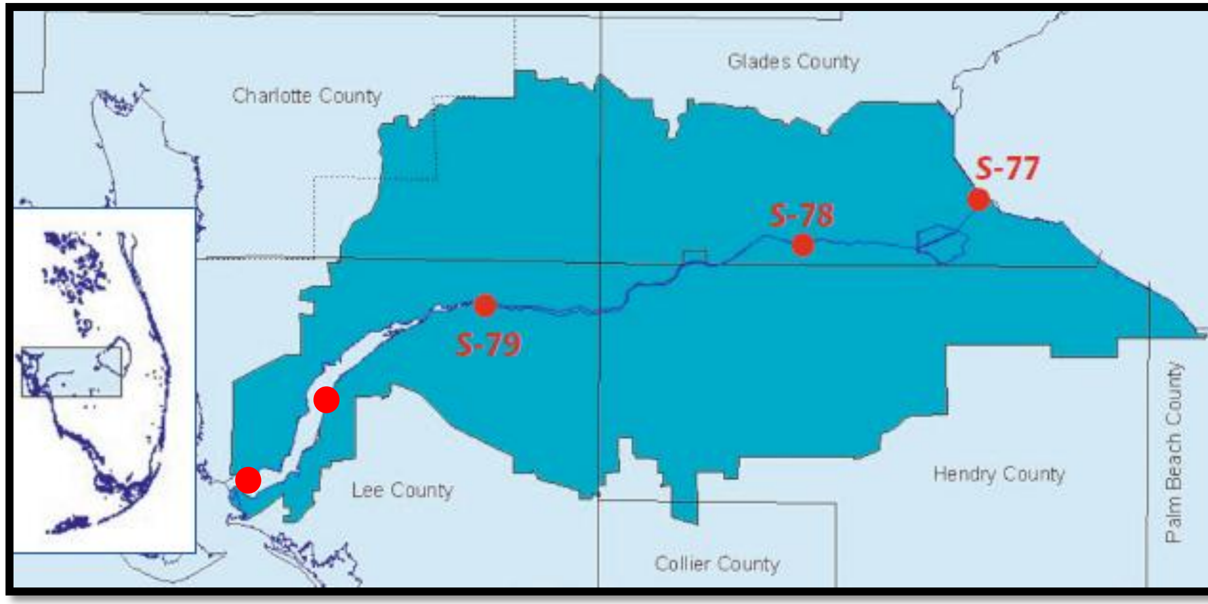
Atrazine

- Nationally: 85% of 1382 samples from 65 mixed-use streams (USGS).
- Max. concentration: 41.3 ug/L
- 3.6% > than 1 ug/L

Metolachlor

- Nationally: 68.13% of 1386 samples from 65 mixed-use streams (USGS).
- Max. concentration: 16.4 ug/L
- 1.4% >1 ug/L

Florida Surface Waters: Caloosahatchee Study



Field sampling sites for related surface water pesticide sampling

Results of Caloosahatchee sampling December 2004-April 2006

Pesticide name	Use	Chemical type	MDL	n	Number of detections	Detection Frequency (%)	Highest conc. detected (ng/L)	Lowest conc. detected (ng/L)	Median Conc. (ng/L)	% RSD
ethoprop	insecticide	organophosphate		75	1	1.3	8.3	8.3	8.3	0.0
phorate	insecticide	organophosphate		75	0	0.0	nd	nd	nd	nd
CIAT	herbicide	triazine	7.2	75	61	81.3	181.6	3.9	21.4	106.0
CEAT	herbicide	triazine	8.4	75	56	74.7	93.8	3.8	17.1	74.4
atrazine	herbicide	triazine	8.4	75	74	98.7	2854.0	12.9	72.2	222.9
simazine	herbicide	triazine	8	75	45	60.0	121.6	2.7	9.4	139.5
acetochlor	herbicide	chloroacetanilide	5.2	75	0	0.0	nd	nd	nd	nd
alachlor	herbicide	chloroacetanilide	5.2	75	0	0.0	nd	nd	nd	nd
ametryn	herbicide	triazine		75	35	46.7	87.2	2.0	8.4	126.6
metolachlor	herbicide	chloroacetanilide	4.8	75	71	94.7	268.3	2.5	17.7	137.5
metribuzin	herbicide	triazine		75	5	6.7	46.8	9.0	12.5	77.9
p,p'-dicofol	insecticide	organochlorine		75	0	0.0	nd	nd	nd	nd
pendamethalin	herbicide	dinitroaniline	4.8	75	4	5.3	18.1	7.1	10.5	40.0
cyanazine	herbicide	triazine	6	75	0	0.0	nd	nd	nd	nd
fenamiphos	insecticide	organophosphate		75	0	0.0	nd	nd	nd	nd
ethion	insecticide	organophosphate		75	1	1.3	3.3	3.3	3.3	0.0
methoxychlor	insecticide	organochlorine		75	1	1.3	14.8	14.8	14.8	0.0
cis-permethrin	insecticide	pyrethroid		75	0	0.0	nd	nd	nd	nd
trans-permethrin	insecticide	pyrethroid		75	0	0.0	nd	nd	nd	nd
Trifluralin	herbicide	dinitroaniline	2.96	90	0	0.0	nd	nd	nd	nd
alpha-HCH	insecticide	organochlorine	8.8	90	0	0.0	nd	nd	nd	nd
diazinon	insecticide	organophosphate	33.2	90	9	10.0	17.3	12.7	15.4	12.1
gamma-HCH	insecticide	organochlorine	10.8	90	0	0.0	nd	nd	nd	nd
heptachlor	insecticide	organochlorine cyclodiene	3.8	90	2	2.2	6.2	5.2	5.7	12.8
chlorothalonil	fungicide	chloronitrile	6.4	90	10	11.1	11.3	2.5	4.0	55.3
aldrin	insecticide	organochlorine	1.64	90	1	1.1	3.1	3.1	3.1	0.0
chlorpyrifos	insecticide	organophosphate	6	90	0	0.0	nd	nd	nd	nd
malathion	insecticide	organophosphate	3.52	90	36	40.0	31.0	1.6	3.5	120.2
chlorpyrifos-oxon	insecticide	organophosphate	8.12	90	23	25.6	8.8	3.7	7.1	25.0
fipronil	insecticide	phenyl pyrazole	10	90	0	0.0	nd	nd	nd	nd
g-chlordane	insecticide	organochlorine	3.28	90	0	0.0	nd	nd	nd	nd
trans-nonachlor	insecticide	organochlorine	3.28	90	0	0.0	nd	nd	nd	nd
a-chlordane	insecticide	organochlorine	4.4	90	0	0.0	nd	nd	nd	nd
a-endosulfan	insecticide	chlorinated hydrocarbon	6.8	90	0	0.0	nd	nd	nd	nd
4,4'-DDE	insecticide	organochlorine	3.72	90	1	1.1	2.8	2.8	2.8	0.0
dieldrin	insecticide	organochlorine	0.48	90	15	16.7	1.5	0.2	0.6	54.5
cis-nonachlor	insecticide	organochlorine	2.8	90	0	0.0	nd	nd	nd	nd
4,4'-DDD	insecticide	organochlorine	18.4	90	1	1.1	26.5	26.5	26.5	0.0
b-endosulfan	insecticide	chlorinated hydrocarbon	10	90	0	0.0	nd	nd	nd	nd
4,4'-DDT	insecticide	organochlorine	0.8	90	0	0.0	nd	nd	nd	nd
endo-sulfate	insecticide	chlorinated hydrocarbon	8	90	1	1.1	3.2	3.2	3.2	0.0
Mirex	insecticide	organochlorine	0.4	90	1	1.1	0.9	0.9	0.9	0.0

Caloosahatchee River Data

Atrazine	Metolachlor
• Detection frequency: 98.7% • Maximum concentration detected: 2854 ng/L • Minimum concentration detected: 12.9 ng/L • Median concentration detected: 72.2 ng/L • %RSD: 222.9%	• Detection frequency: 94.7% • Maximum concentration detected: 268.3 ng/L • Minimum concentration detected: 2.5 ng/L • Median concentration detected: 17.7 ng/L • %RSD: 137.5%

It is rare to find only one pesticide in a surface water sample

When all 42 analytes were tested:

2-12 compounds were detected (n=75)

Average detections: 5.6

Median detections: 6

% relative standard deviation: 38.5%

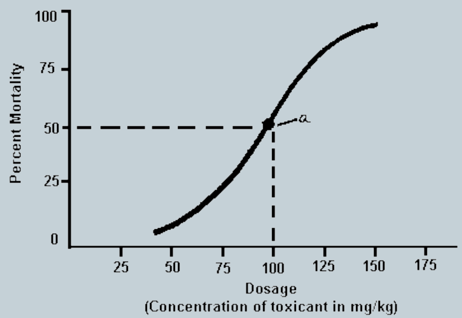
71 of 75 samples had BOTH atrazine & metolachlor present

Summary of Approach

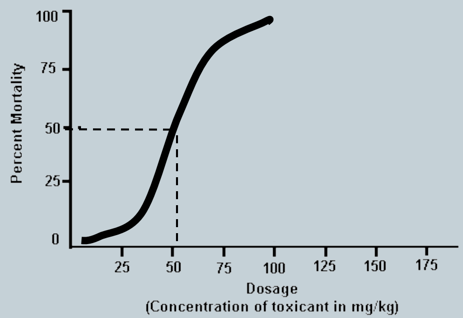
Toxic Unit Approach

- Concentrations of toxicants expressed in units of lethality or in units of effect (LD50, LC50 or EC50)

A



B



- Chemical A has LD50 of 100 µM. So 1 TU=100 µM
- Chemical B has a LD50 of 50 µM. So 1 TU=50 µM
- A study examining $0.5TU_A + 0.5\ TU_B = 1\ TU_{A+B}$
  - So expect 50% mortality (1 TU)with this mixture containing 50 µM A and 25 µM B

Toxic Units (TU)

Given a mixture of
0.5 TU A + 0.5 TU B,

The joint toxic effect of A and B is then defined as:

- Additive
  - if $EC50_{mix} = 1$ TU
- More than additive
  - if $EC50_{mix} < 1$ TU
- Less than additive
  - if $EC50_{mix} > 1$ TU

Toxic Units in a mixture study

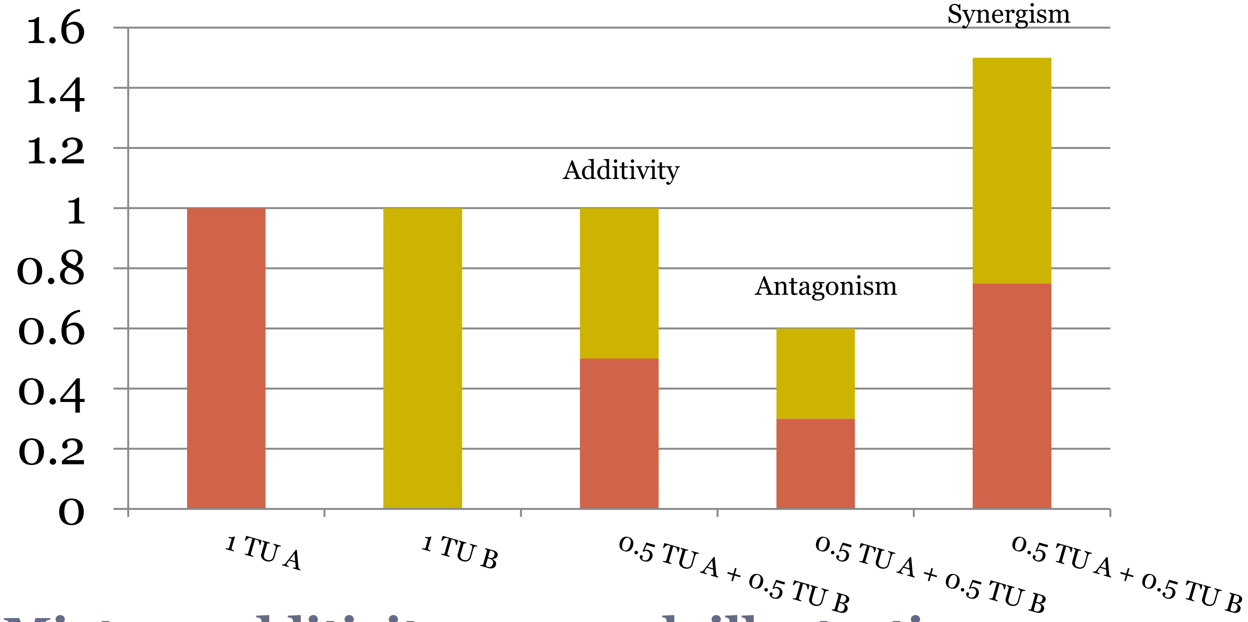
It is expected that

$0.5\ TU_A + 0.5\ TU_B = 1\ TU_{A+B}$

The mixture additivity approach uses the additive index (S) of Marking & Dawson (1975) :

$$S = A_m/A_i + B_m/B_i$$

Where A_m & B_m are the incipient EC50 of toxicants A & B when present in mixture, and A_i & B_i the toxicity of A & B when tested separately.



Mixture additivity approach illustration

Objective

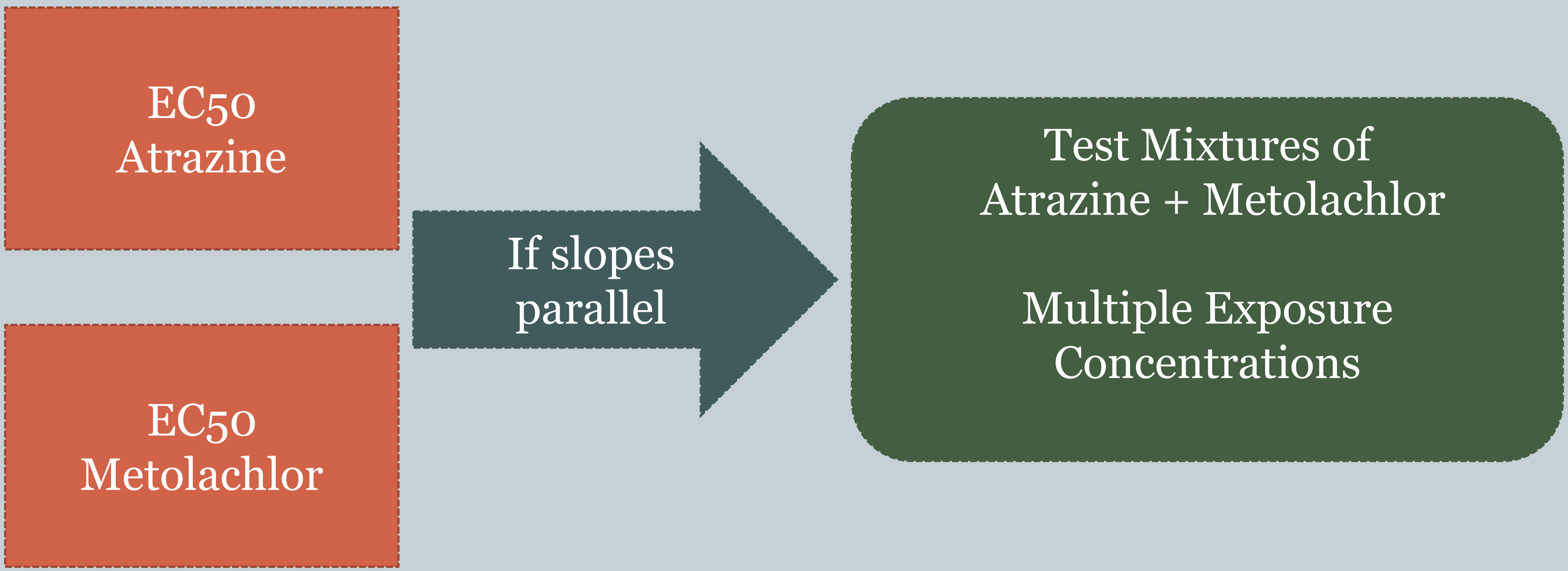
To determine the effects of atrazine and metolachlor on aquatic macrophyte growth, reproduction, and health

Methods

Duckweed was used as the test subject

Lemna minor **in culture**

Study Overview



Test conditions

- Glass vessels with lids
- 150 ml:
  - 20% stock Hoaglands media
  - 80% MHW
  - Pesticide dilutions added directly to vessel
- n = 4, 12 fronds per vessel
- Moved every other day
- 12 h light-12 h dark cycle
- Approximately 25° C
- 6 days exposure

Concentrations Confirmed by GC-TSD

- Percent recoveries in individual tests:
  - Atrazine: 88% - 113%
  - Metolachlor: 80% - 108
- Percent recoveries in mixture tests:
  - Atrazine: 100 - 107%
  - Metolachlor: 109 - 118%

Measured End Points

- Frond count
  (every 2 days)
- Root length
  (end of exposure only)
- Fresh weight
  (end of exposure only)
- Photosynthetic efficiency
  (Fv/Fm; end of exposure only)
- Chlorophyll & Carotenoid concentration
  (end of exposure only)

Culture dishes growing *Lemna minor*.

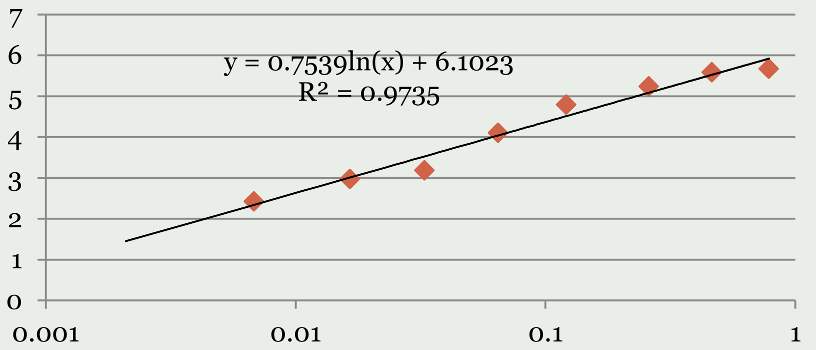
Results

Individual EC50 values based on frond count

Atrazine

- 0.232 ppm

Figure 9. Atrazine Day 6 Transformed logit

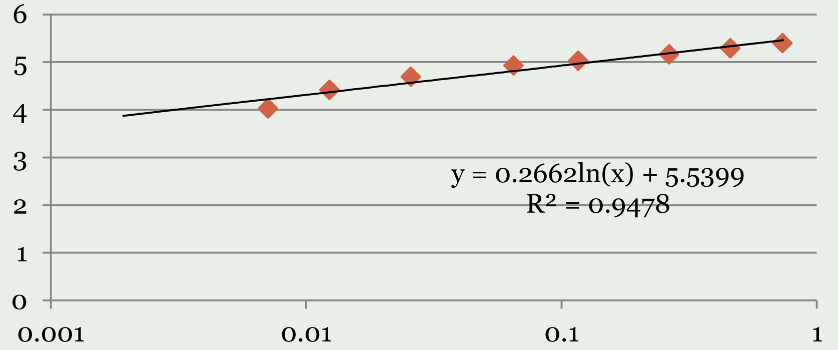


- 1 TU = 0.232 ppm

Metolachlor

- 0.132 ppm

Figure 10. Metolachlor Day 6 Transformed logit



- 1 TU = 0.132 ppm

Slope Analysis

Source of Variation	df	SS_Y	SP_{XY}	SS_X	$b_{Y \cdot X}$	$SS_{\hat{Y}}$	df	$SS_{Y \cdot X}$	$MS_{Y \cdot X}$
Atrazine	7	11.21238	1.982184	0.533019	3.718789	7.371322	6	3.841058	0.640176
Metolachlor	7	1.51306	0.667215	0.484618	1.376786	0.918612	6	0.594449	0.099075
Sum of Groups							12	4.435506	0.369626
Among b_i's							1	1.392271	1.392272
									Fs = 3.76671 ns
Pooled within	14	2.649398	2.649398	1.017636	2.603482	6.897662	13	5.827778	0.448291

- Conclusion: Slopes are not statistically different; we can compare the individual compounds in a mixture study

Corrected Toxic Units for Mixtures

Combination	Measured Concentration	Measured Concentration	Total TU_{mix}
	Atrazine	Metolachlor	
0 + 0 TU (0 TU)	0 (0 TU)	0 (0 TU)	0 TU
0.25 + 0.25 TU (0.5 TU)	0.058 (0.25 TU)	0.036 (0.27 TU)	0.52 TU
0.5 + 0.5 TU (1 TU)	0.124 (0.53 TU)	0.072 (0.54 TU)	1.07 TU
0.75 + 0.75 TU (1.5 TU)	0.183 (0.79 TU)	0.11 (0.83 TU)	1.62 TU
1 + 1 TU (2 TU)	0.241 (1.04 TU)	0.156 (1.18 TU)	2.22 TU

Toxic Units and the mixture study

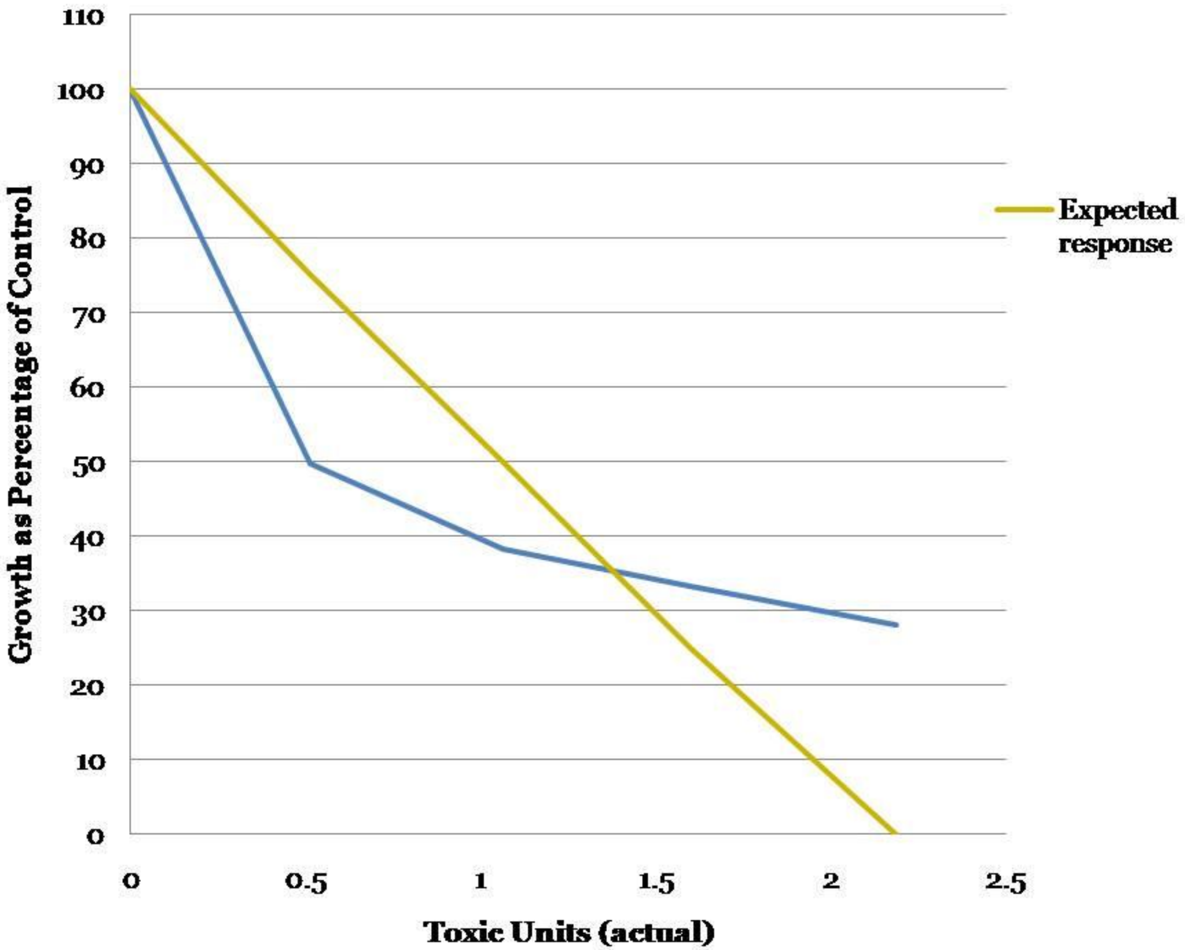
It is expected that

0.5 TU atrazine +

0.5 TU metolachlor

= 1 TU mixture

(50% growth rate of control)



Growth rate of *Lemna minor* as a percentage of controls in a mixture of atrazine and metolachlor.

Toxic Units and the mixture study

It is expected that

0.5 TU atrazine +

0.5 TU metolachlor

= 1 TU mixture

(50% growth of control)

S was calculated using the mixture additivity approach and the additive index of Marking & Dawson (1975)

$$S = A_m/A_i + B_m/B_i$$

Where A_m & B_m are the incipient LC50 of toxicants A & B when present in mixture, and A_i & B_i the toxicity of A & B when tested separately.

For atrazine and metolachlor mixture:

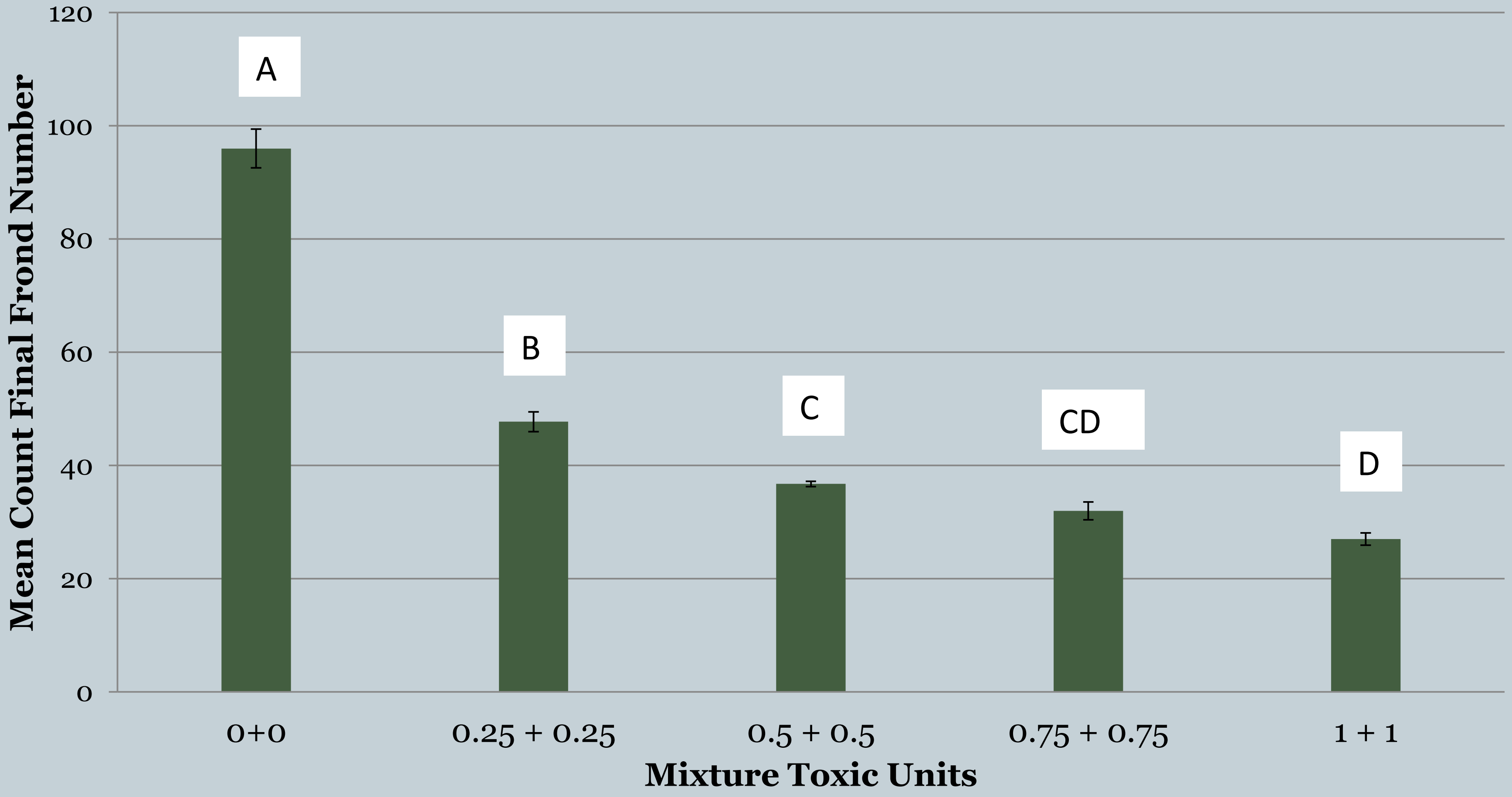
S= 1.05

indicating a

<u>synergistic relationship</u>

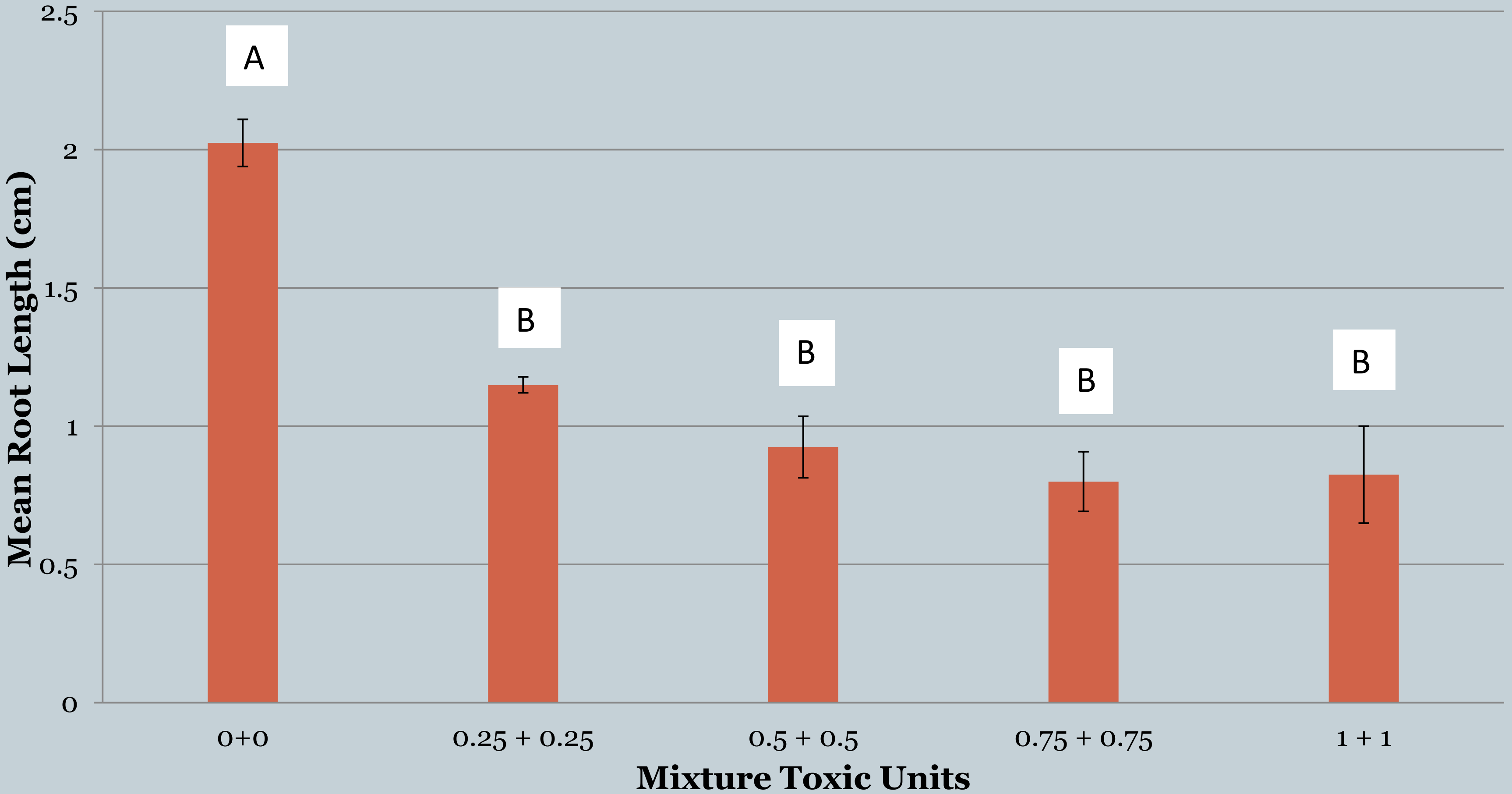
between the toxicants.

Frond Count (Day 6, n=4)



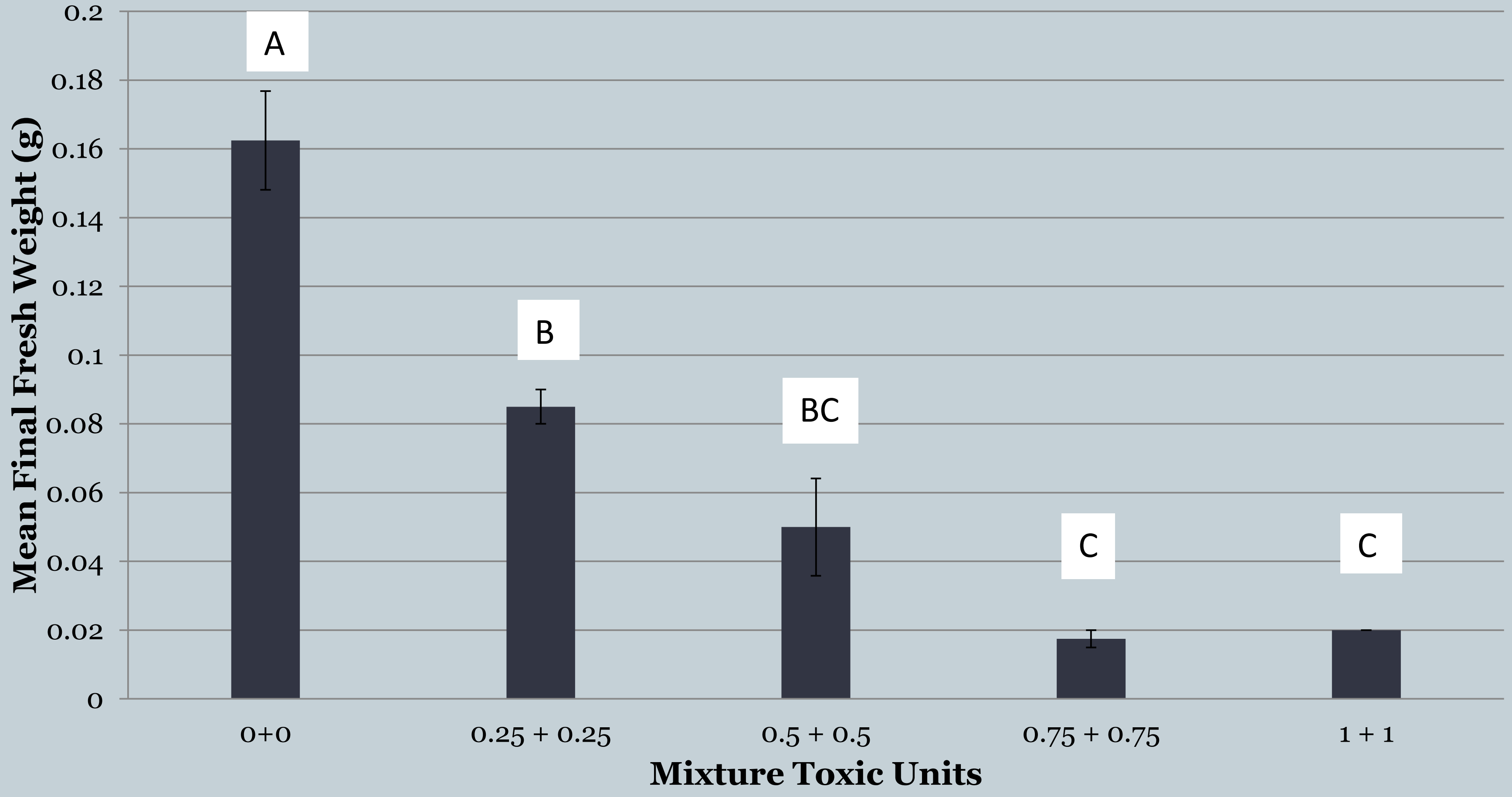
(α=0.05, P = <0.001, power=1.000)

Root Length (Day 6, n= 4)



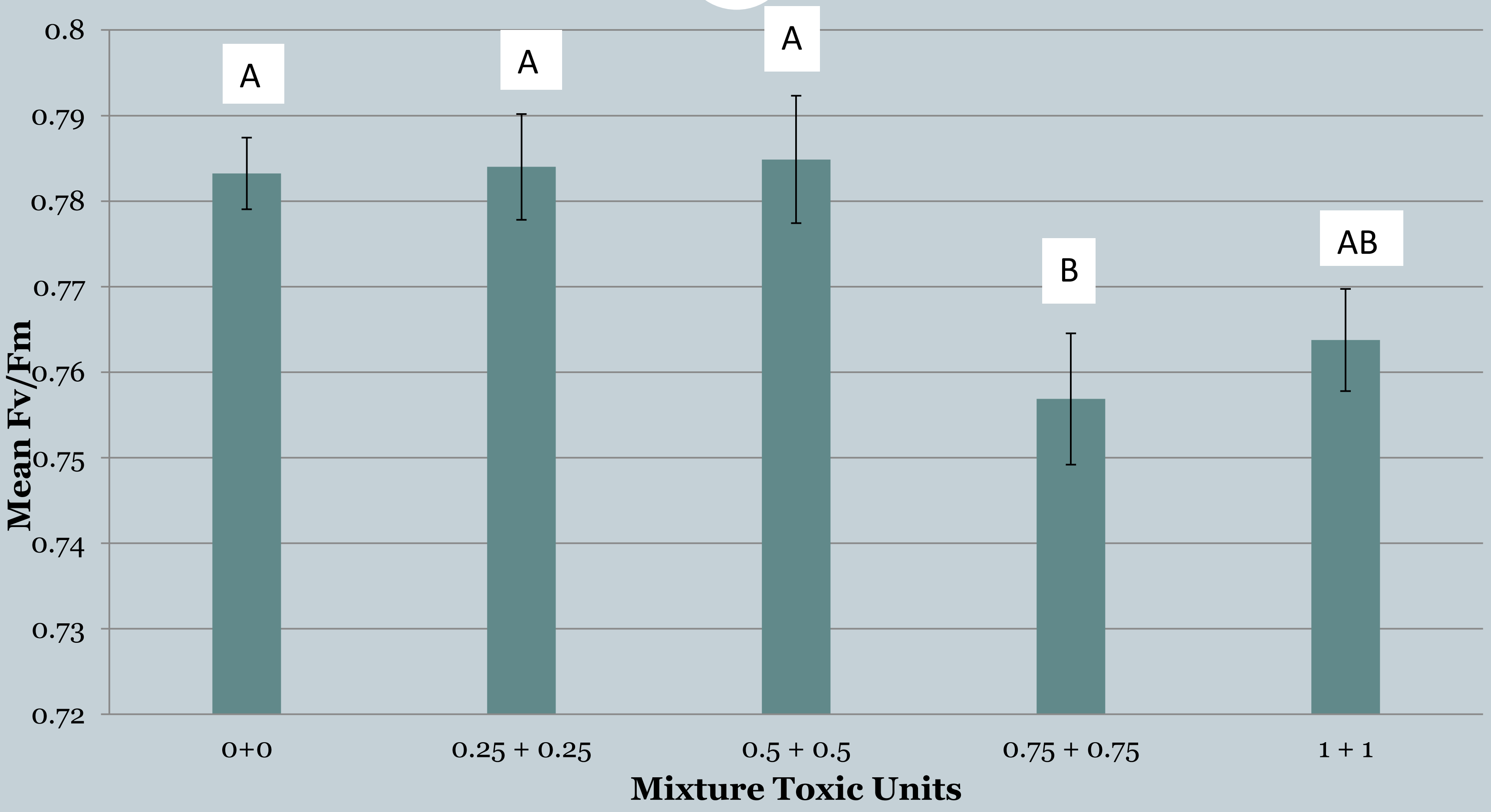
(α=0.05, P = <0.001, power=1.000)

Fresh Weight (Day 6, n=4)



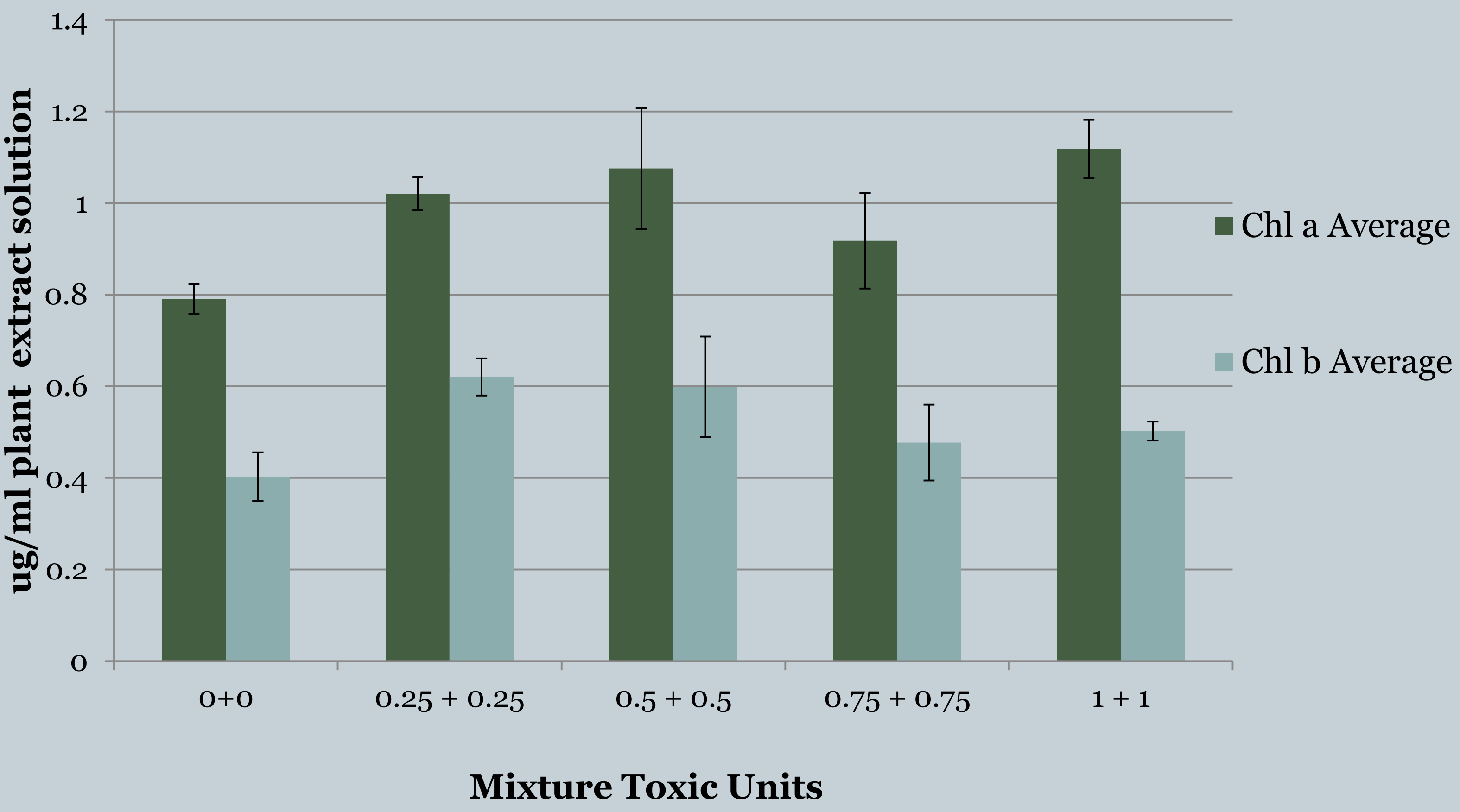
(α=0.05, P = <0.001, power=1.000)

Final Fv/Fm values (Day 6, n=4)



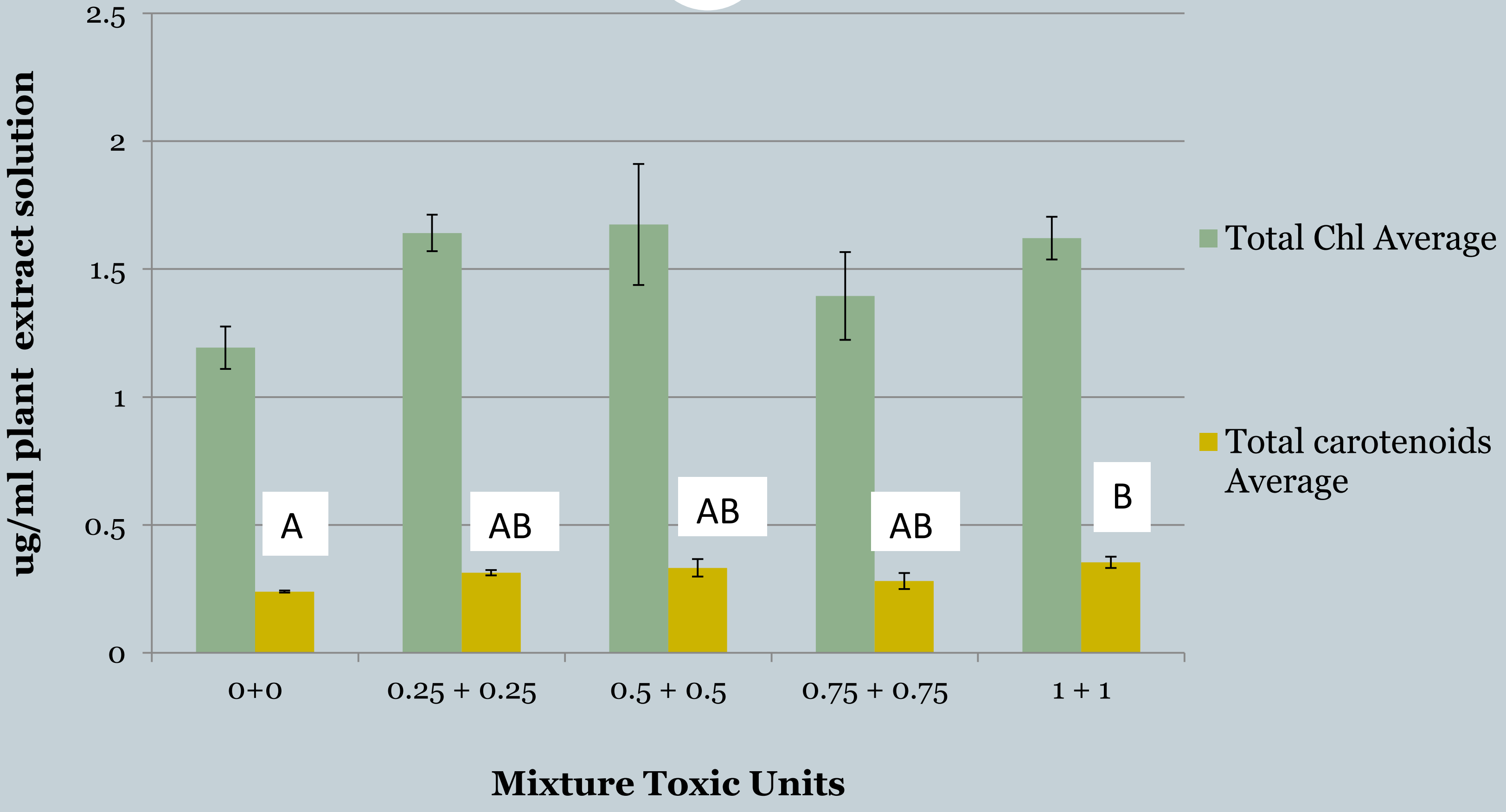
(α=0.05, P = 0.007, power=0.781)

Chlorophyll a and Chlorphyll b values (Day 6, n=4)

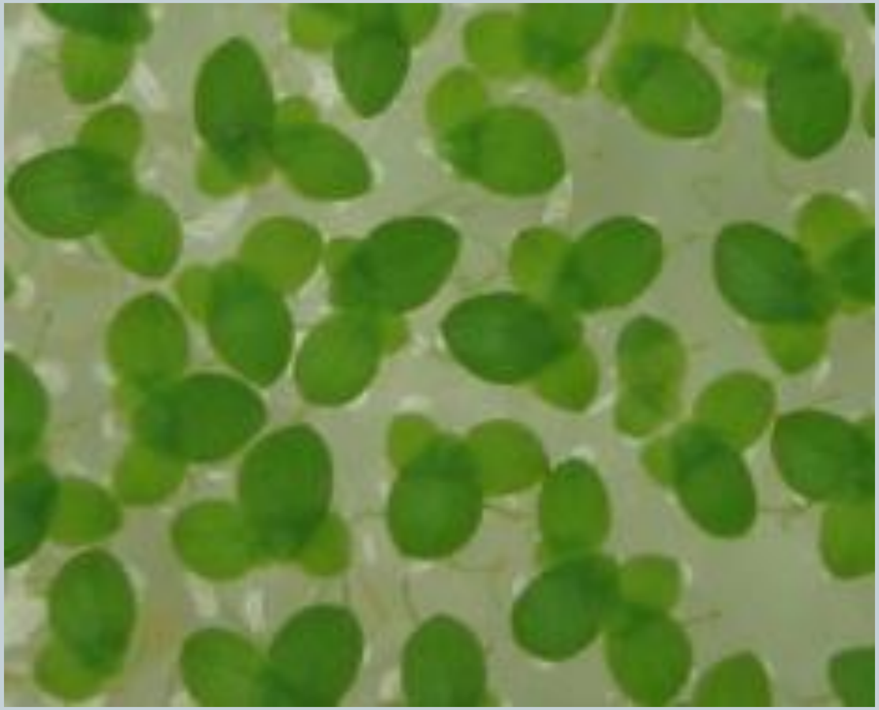


p= 0.086 and p=0.211

Total Chlorophyll and Total Carotenoid Content (Day 6, n=4)



Morphology effects

Control culture	In 0.75 ppm Metolachlor
	

Conclusions

Conclusions: Mixture effects of Atrazine & Metolachlor on *Lemna minor*

- Synergistic effects
  - Growth rate
- Significant effects
  - Frond count
  - Root length
  - Fresh weight
  - Carotenoid content
- No trend effects
  - Chlorophyll a & b content
  - Fv/Fm

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Any Questions?