The biology of biocontrol: fine-tuning the biological control of tamarisk to better serve riparian restoration and recovery.

Dan Bean[1], Alex Gaffke[2], Tom Dudley[3], Levi Jamison[4], Amanda Stahlke[5] and Zeynep Özsoy[6]

[1]Colorado Department of Agriculture, Palisade Insectary, Palisade, CO, USA; dan.bean@state.co.us
[2]USDA ARS, Gainesville, FL, USA; alexander.gaffke@gmail.com
[3]Marine Science Institute, University of California, Santa Barbara, CA, USA; tdudley@msi.ucsb.edu
[4]Moab, UT, USA; ljamisonresearch@gmail.com
[5]University of Idaho, Moscow, ID USA; amandastahlke@gmail.com
[6]Colorado Mesa University, Grand Junction, CO, USA; aozsoy@coloradomesa.edu

"Science is ahead of practice" Peter Skidmore 2-04-20

Riparian Restoration

Science of biocontrol

Questions (to complement restoration efforts)

1. Which beetles will be in the system?
   Bioinformatics
2. How often and when will they defoliate?
   Phenology and phenotype tracking
3. How can we manipulate populations?
   Semiochemicals to modify beetle behavior

For another day…

How will eco-evolutionary relationships impact dynamics of the tamarisk-*Diorhabda* interaction and riparian ecosystems?

Bioinformatics

1. Tracking *Diorhabda* populations
- Species
- Ecotypes
- Populations
- Genes of interest
- Alleles of interest

2. Genomics and bioinformatics used in understanding the phenology and evolution of *Diorhabda*

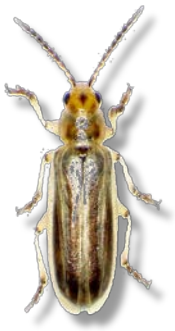
Stahlke AR, Özsoy AZ, Bean DW, Hohenlohe PA. (2019) Mitochondrial genome sequences of *Diorhabda carinata* and *Diorhabda carinulata*, two beetle species introduced to North America for biological control. Microbiology Resource Announcements 8:e00690-19. https://doi.org/10.1128/MRA.00690-19.

Diorhabda sublineata

Diorhabda elongata

Diorhabda carinata

Diorhabda carinulata



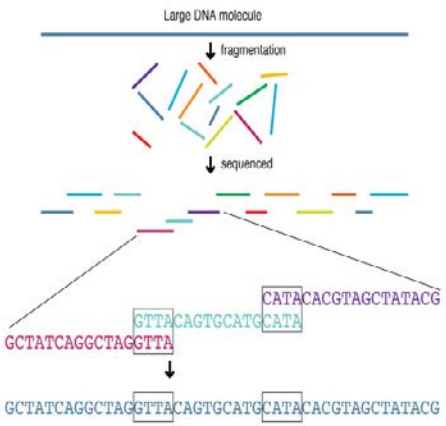
The **first** weed biocontrol agent genome assemblies

Cost	~$6,000
# contigs	16,291
Largest contig	8.74 Mbp

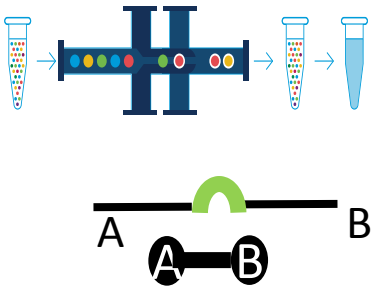
Pretty good for a non-model!

Collaboration	i5k, Earth BioGenome Project, USDA ARS
# contigs	179
Largest contig	43.31 Mbp

1 Shotgun (Illumina)

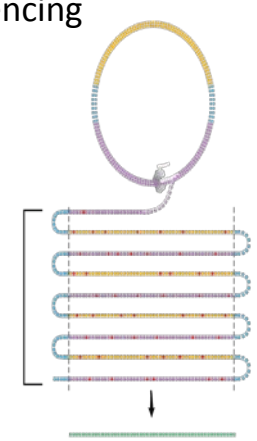


2 10X Linked reads (Illumina)



3 A gold standard

PacBio SMRT sequencing



http://i5k.github.io/ag100pest

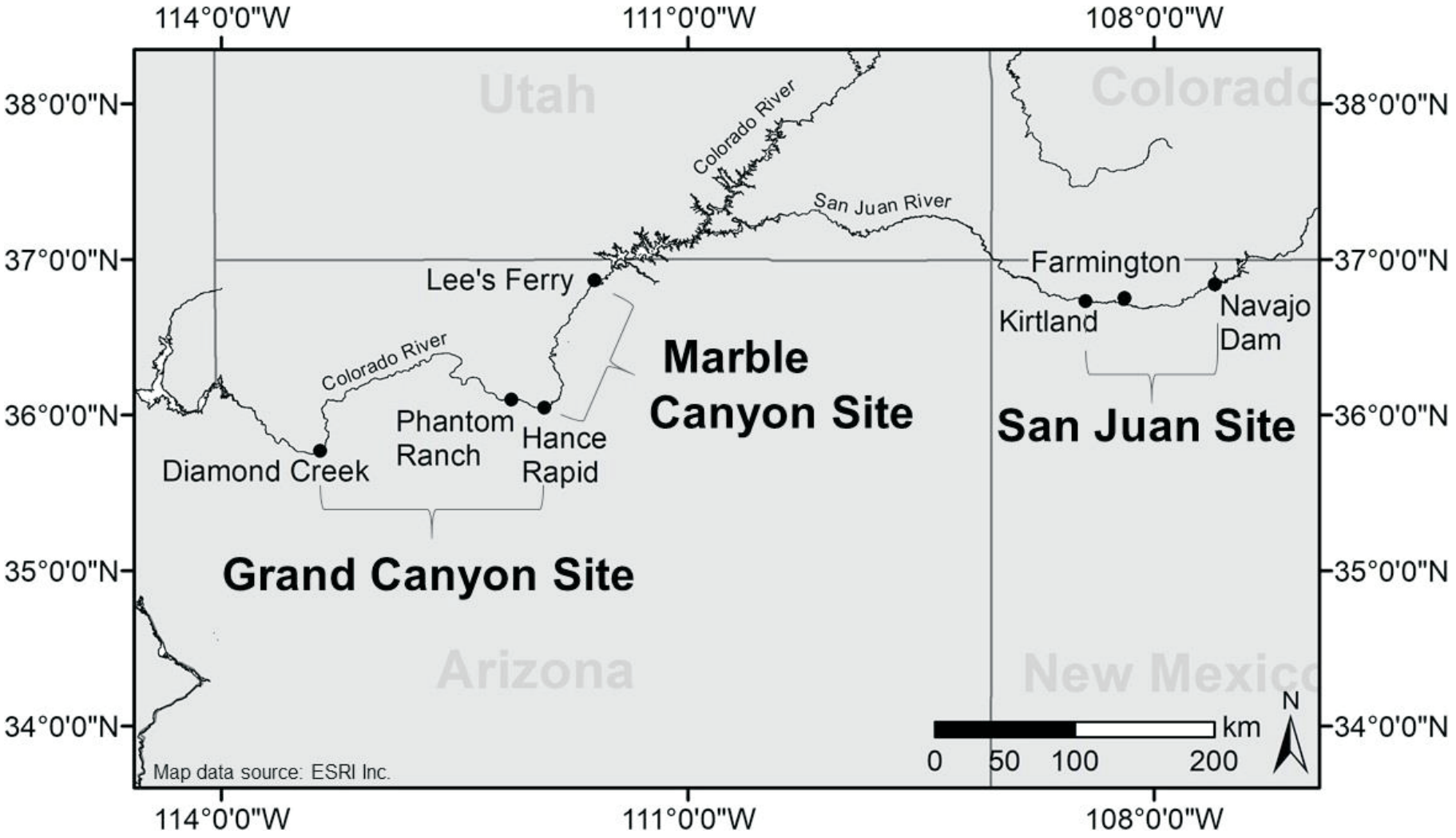
When and where will *Diorhabda* be present in defoliating numbers?

Field surveys and models

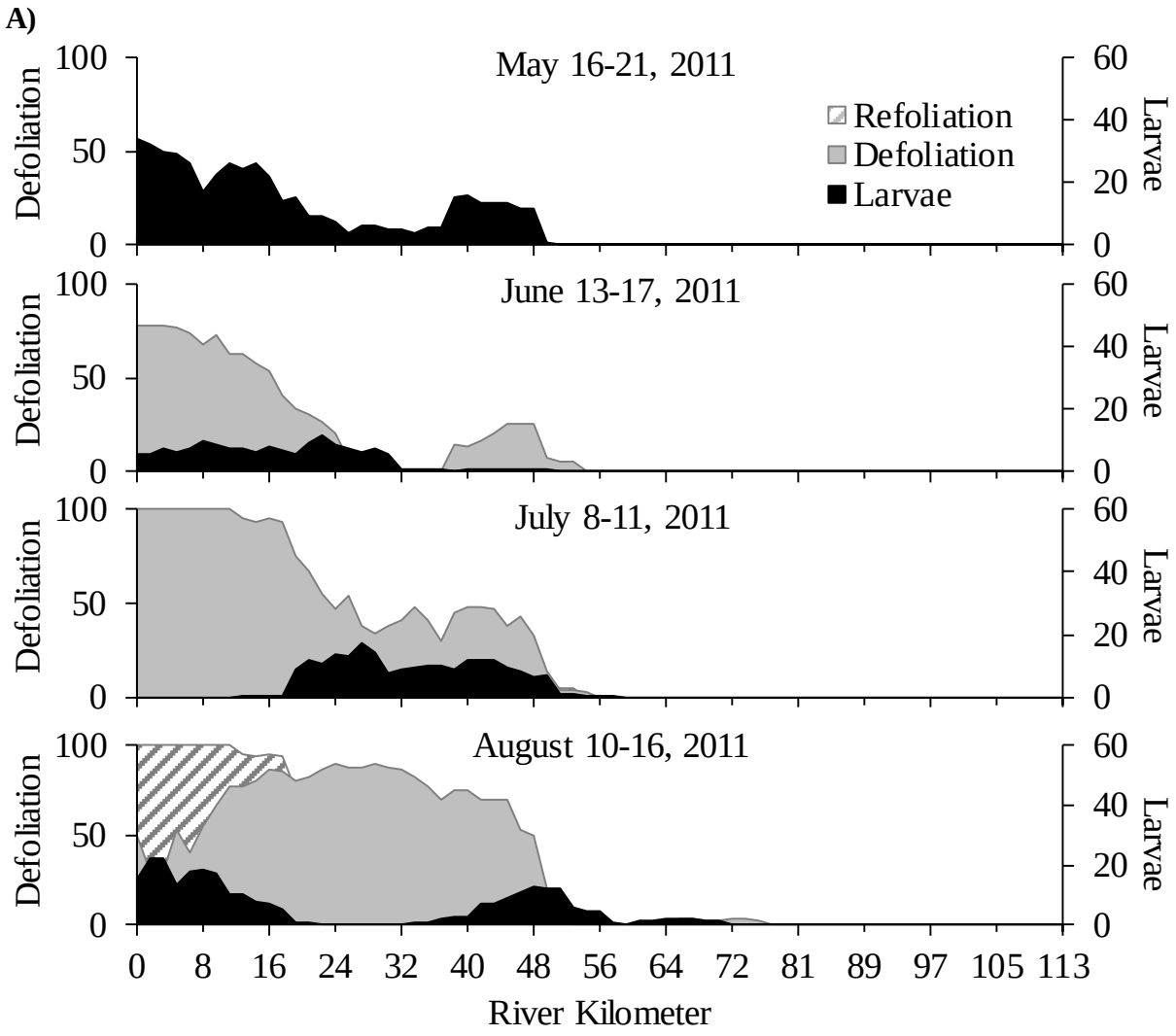
Levi Jamison

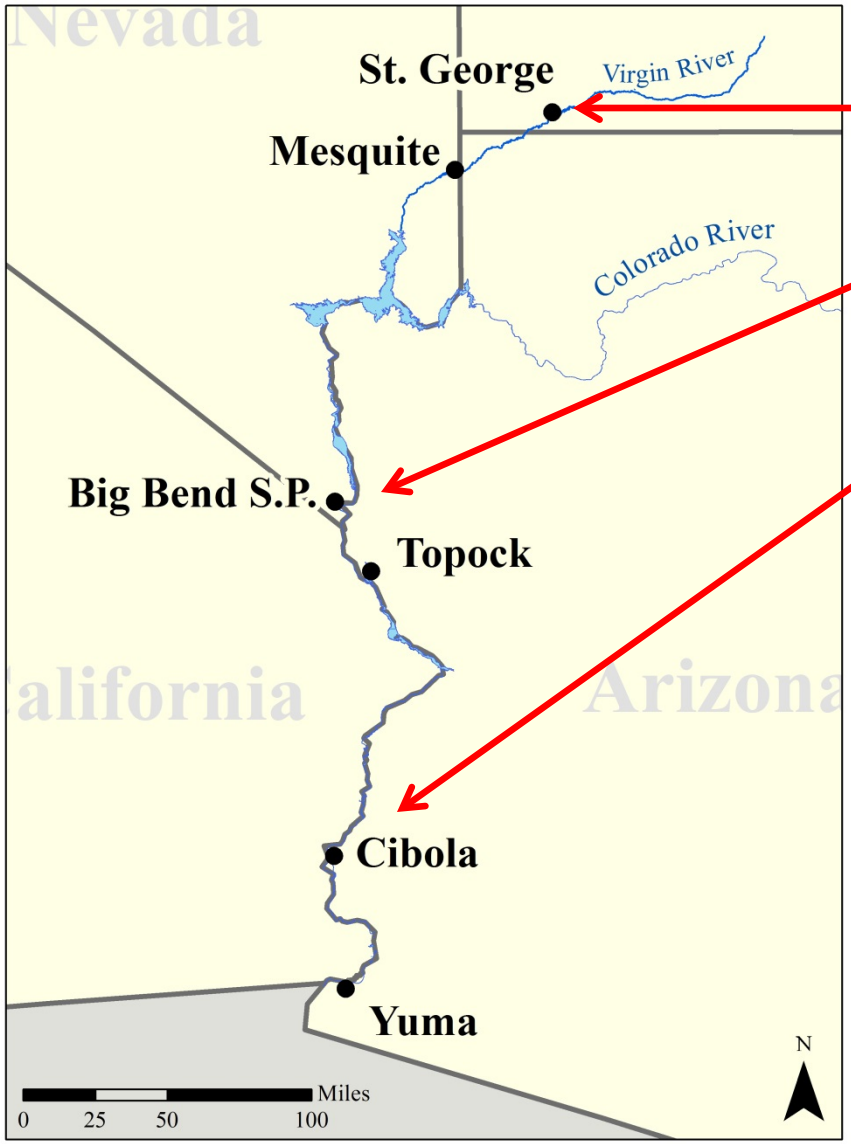
Tracking beetle movement and phenology on a landscape level

Jamison, L.R, Johnson, M.J., Bean, D.W. and C. van Riper III (2018) Phenology and abundance of northern tamarisk beetle, *Diorhabda carinulata*, affecting defoliation of *Tamarix*. Southwestern Entomologist 43: 571-584


114°0'0"W
111°0'0"W
108°0'0"W
38°0'0"N
37°0'0"N
36°0'0"N
35°0'0"N
34°0'0"N
Utah
Colorado
Arizona
New Mexico
Colorado River
San Juan River
Lee's Ferry
Farmington
Kirtland
Navajo Dam
Colorado River
Phantom Ranch
Hance Rapid
Diamond Creek
Marble Canyon Site
San Juan Site
Grand Canyon Site
Map data source: ESRI Inc.
km
0 50 100 200
N

Tamarisk defoliation/refoliation Colorado River, Marble Canyon



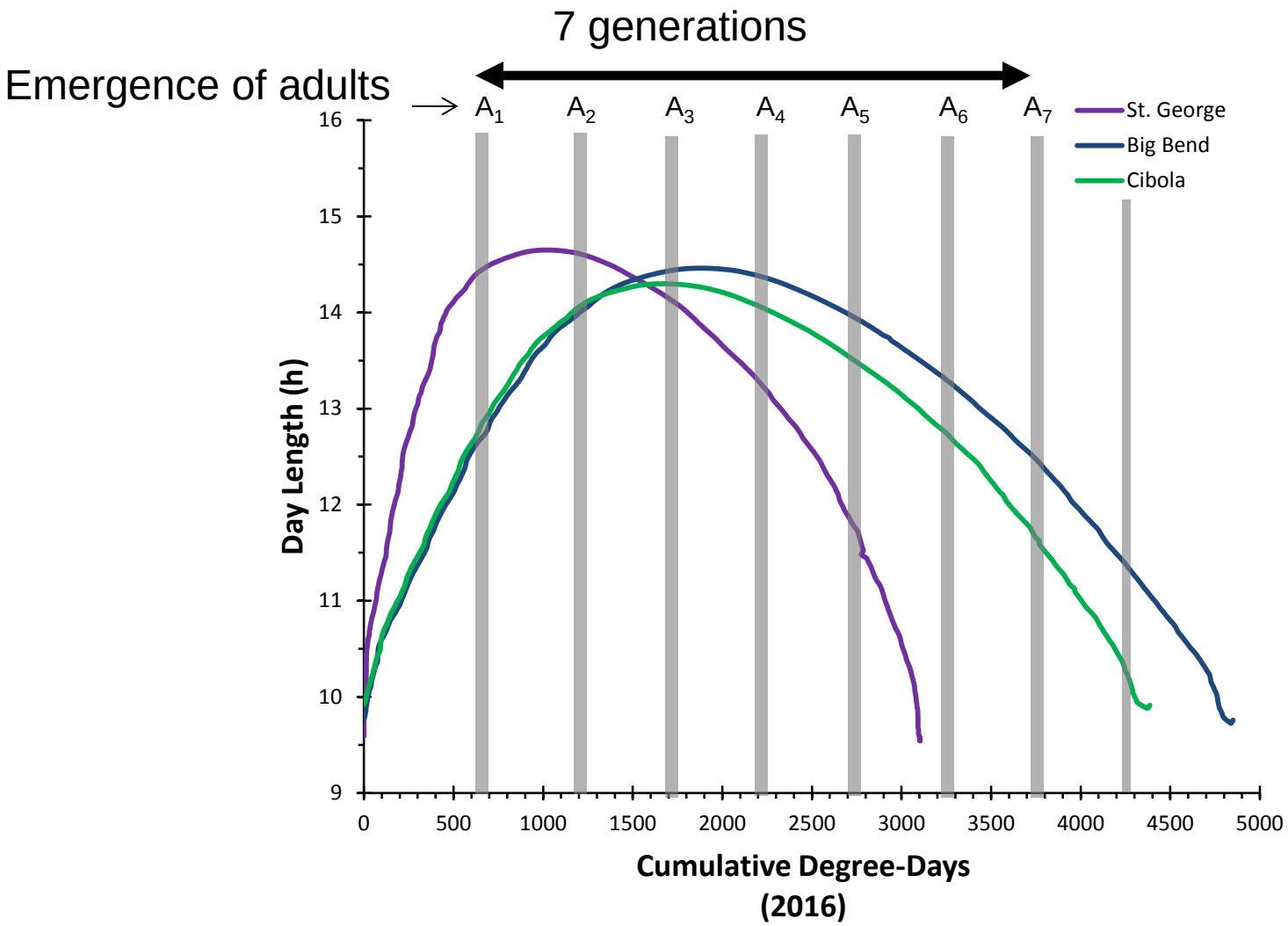


Lower Colorado River

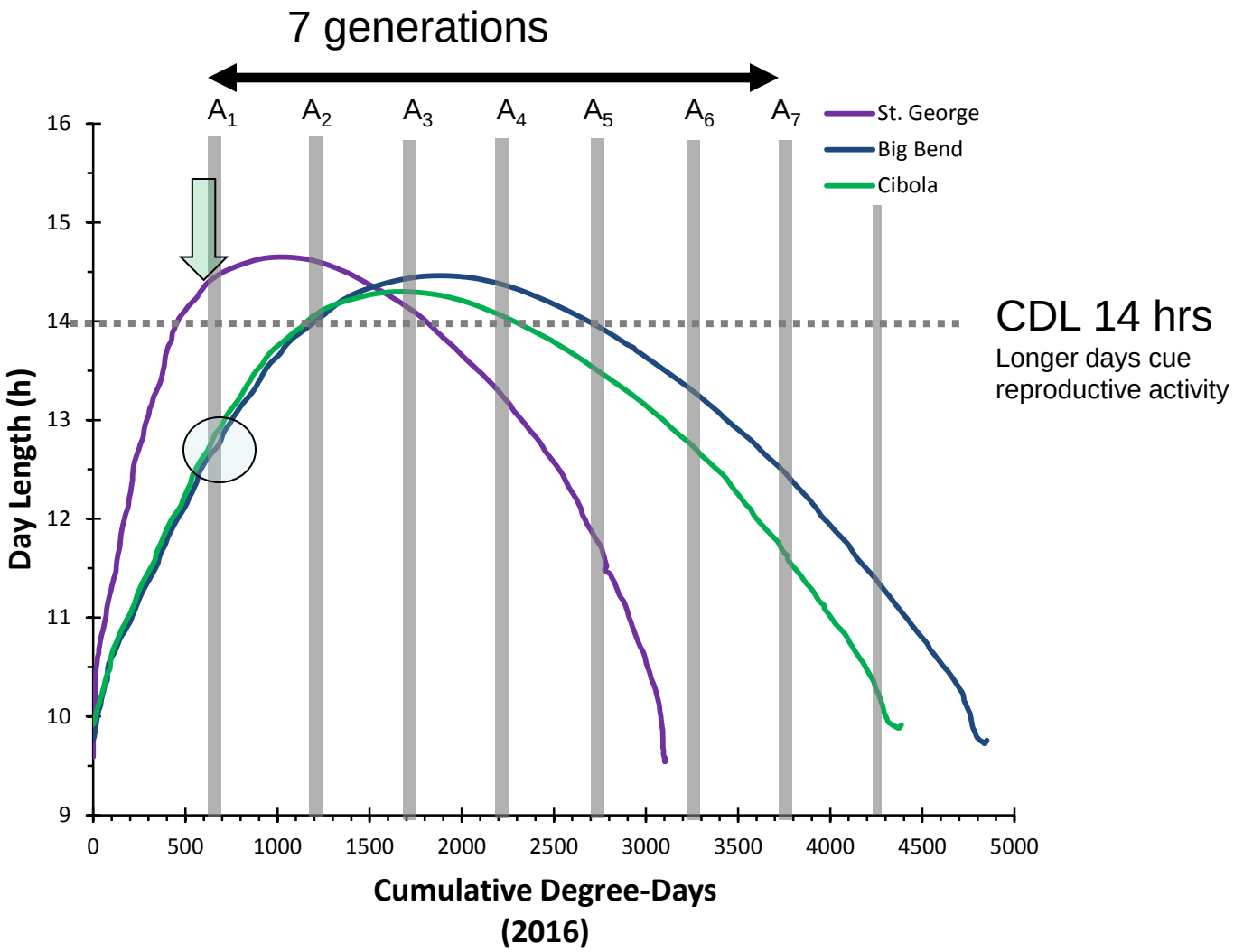
We have constructed a phenology model based on degree-days which incorporates photoperiodic cues into predicting developmental decisions.

Fritzi Grevstad

Len Coop



Diorhabda carinulata phenology model based on thermal currency (degree days) as well as developmental periodism (Critical Day Length or CDL)



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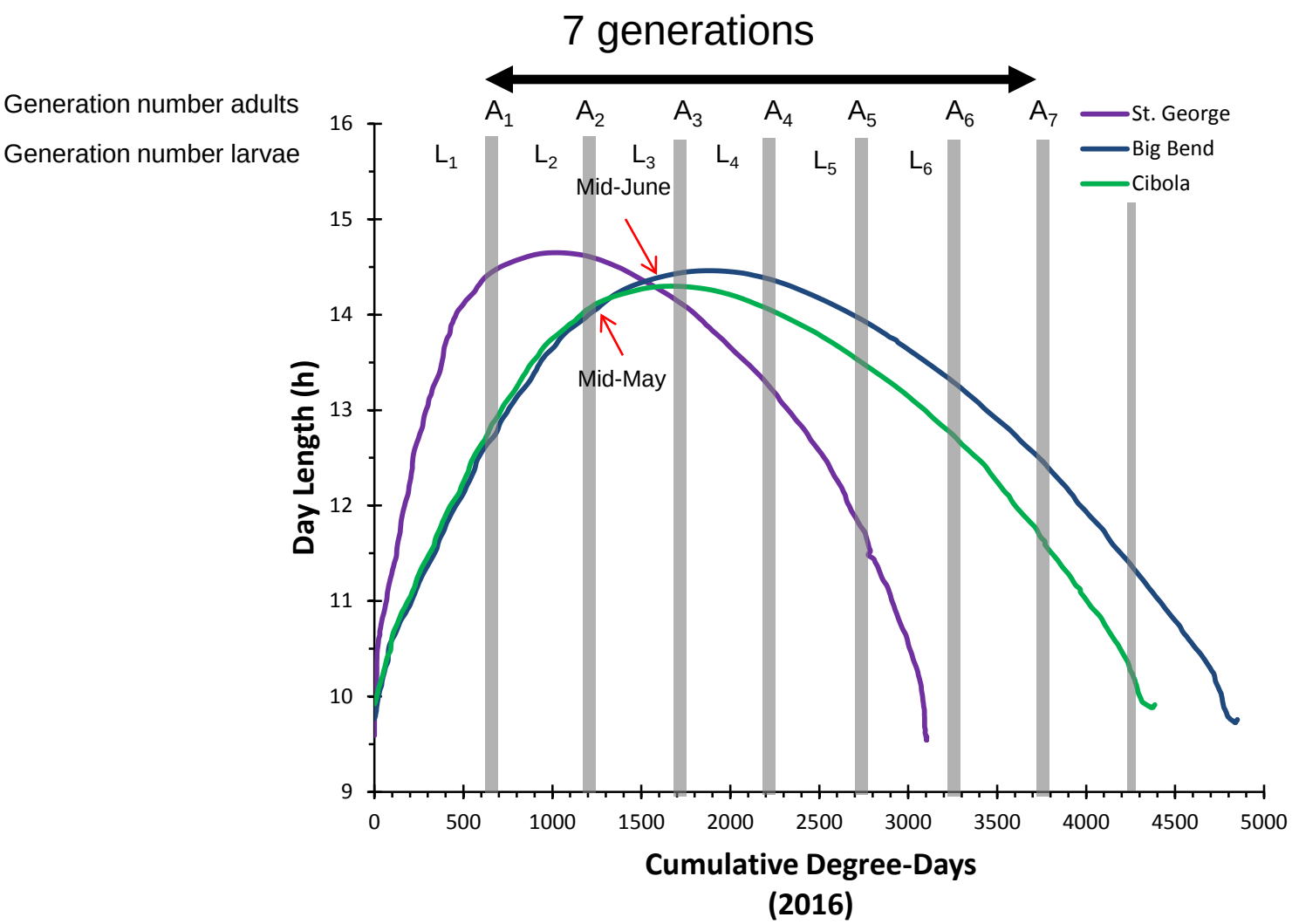


Diorhabda carinulata phenology model based on thermal currency (degree days) as well as developmental periodism (Critical Day Length or CDL)

Defoliation at the Cibola NWR, third generation larvae

Phenology camera at Cibola

The Toms (Dudley and Ryan) at Cibola site setup

Manipulating *Diorhabda* populations (i.e. beetle herding)

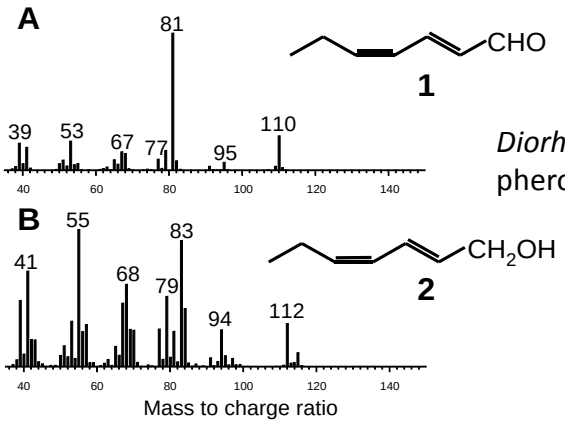
1. Attracting beetles to areas where control is a top priority
2. Keeping beetles away from sensitive areas (SWFL habitat)

Allard Cossé checks field trials of attractants
Lovelock, NV, 2004

Bob Bartelt monitors pheromone-baited trap

Alex Gaffke

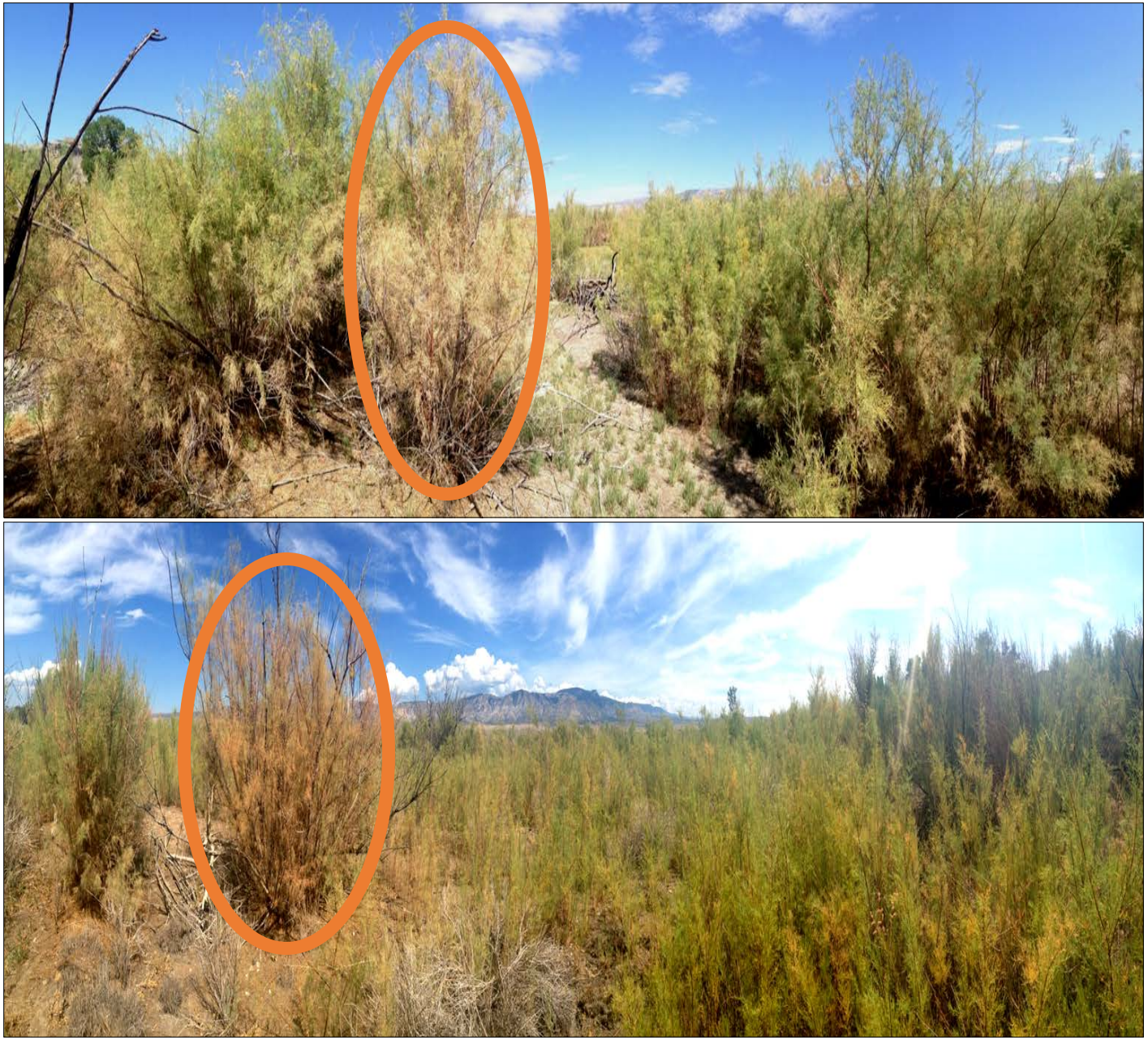
Beetle herding using behaviorally active compounds (semiochemicals) in *Diorhabda*



Diorhabda aggregation pheromone blend

Gaffke, A. M., S. E. Sing, T. L. Dudley, D. W. Bean, J. A. Russak, A. Mafra-Neto, P. A. Grieco, R. K. D. Peterson, and D. K. Weaver. 2018. Semiochemicals to enhance herbivory by *Diorhabda carinulata* aggregations in saltcedar (*Tamarix* spp.) infestations. Pest Management Science 74(6): 1494 -1503.

Gaffke, A. M., S. E. Sing, T. L. Dudley, D. W. Bean, J. A. Russak, A. Mafra-Neto, P. A. Grieco, R. K. D. Peterson, and D. K. Weaver. 2019. Field demonstration of a semiochemical treatment that enhances Diorhabda carinulata biological control of Tamarix spp. Scientific Reports 9: 1305 https://doi.org/10.1038/s41598-019-49459-5.(53)

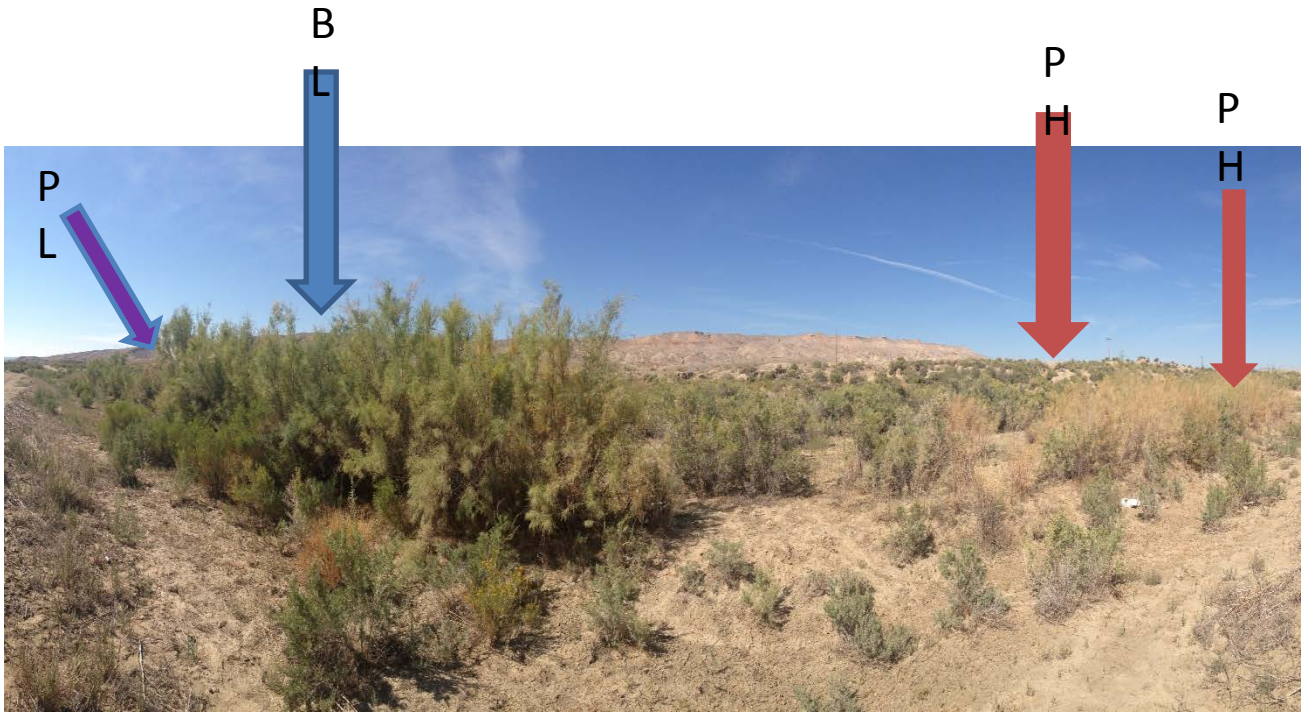


Pheromone treatment 2013

Untreated Plants with no impacts from Diorhabda

Impacts of pheromone treatment: high levels of dieback

Use “pull” to protect critical habitat



Push/ Pull chemical herding

Diorhabda spp. are aware of egg/larva densities and reproductive adults will bypass trees with large numbers of eggs/larvae

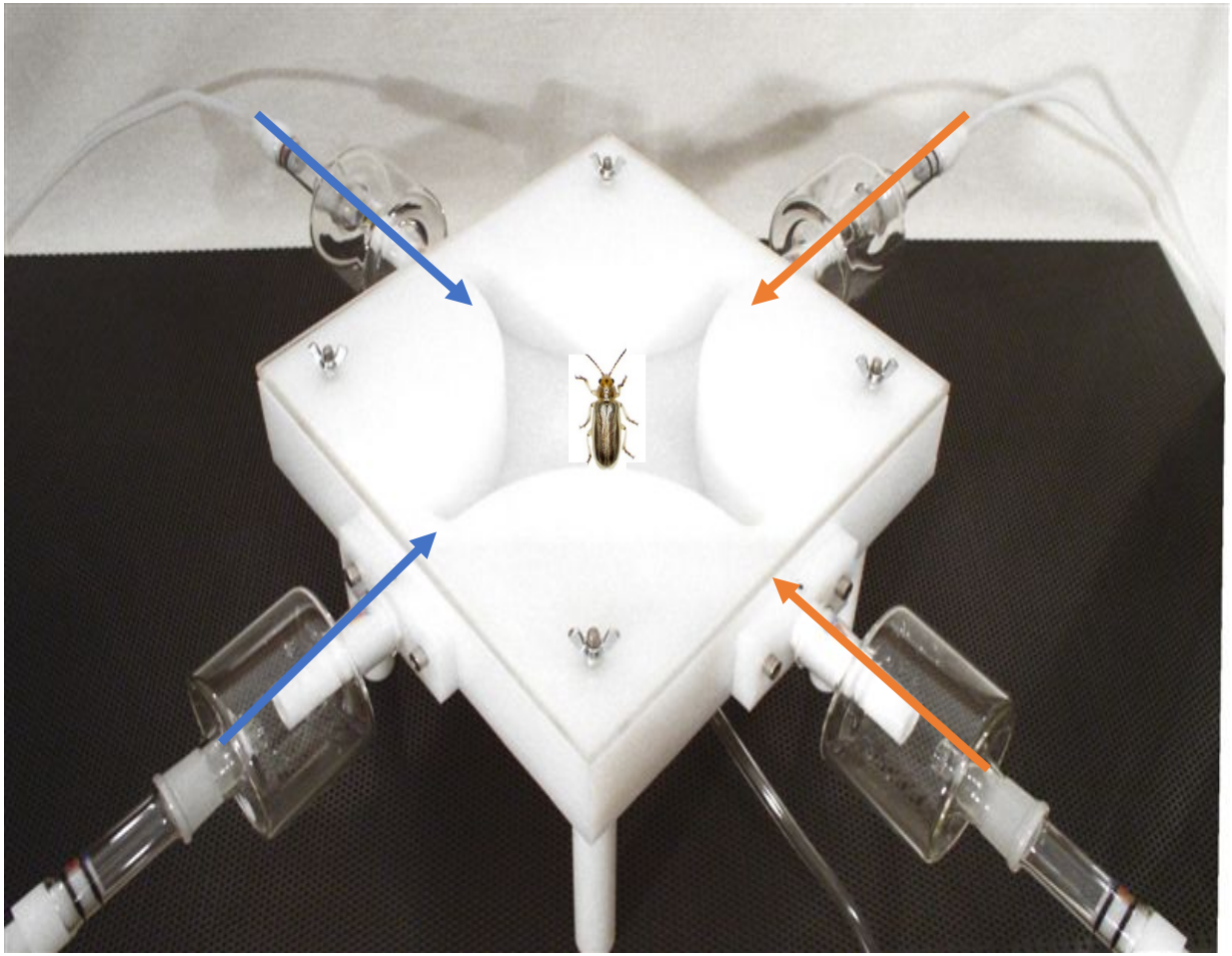
Volatile compounts may signal conspecific density, be repellent to reproductive adults

Develop chemical “push” to keep beetles out of critical habitat

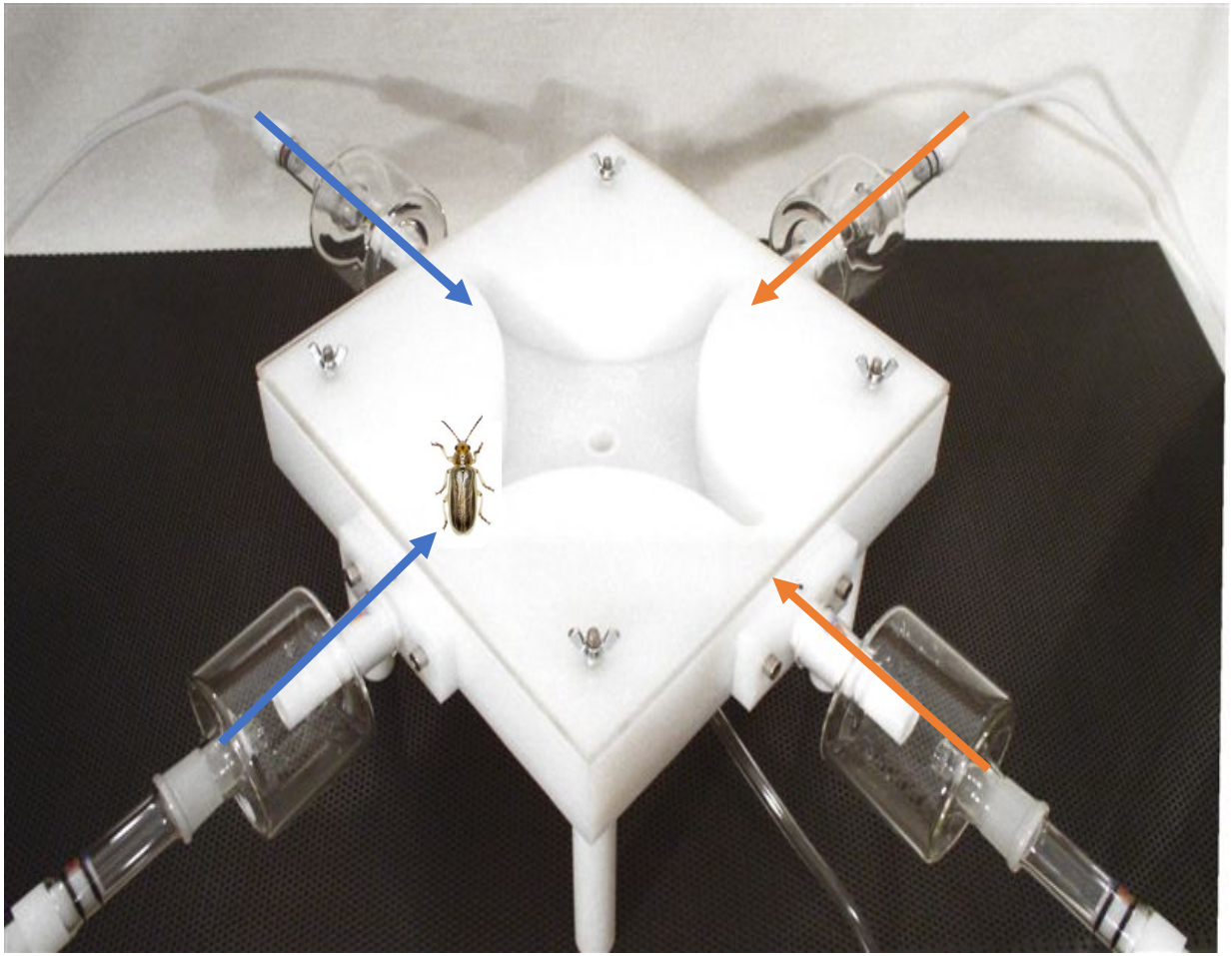
Quantities of **4-oxo-(E)-2-hexanal** released from *Tamarix* foliage plus adult D. carinulata, beetles alone and mechanically damaged foliage as controls

		Emission rate	
Component	Mean (ng/beetle/day)	Standard Error	*N*
Adult males	0.70	± 0.20	10
Adult females	2.63	± 1.10	10
Control foliage	0.0	± 0.0	4
Mechanically damaged foliage	0.0	± 0.0	4
Adults without foliage	0.0	± 0.0	4

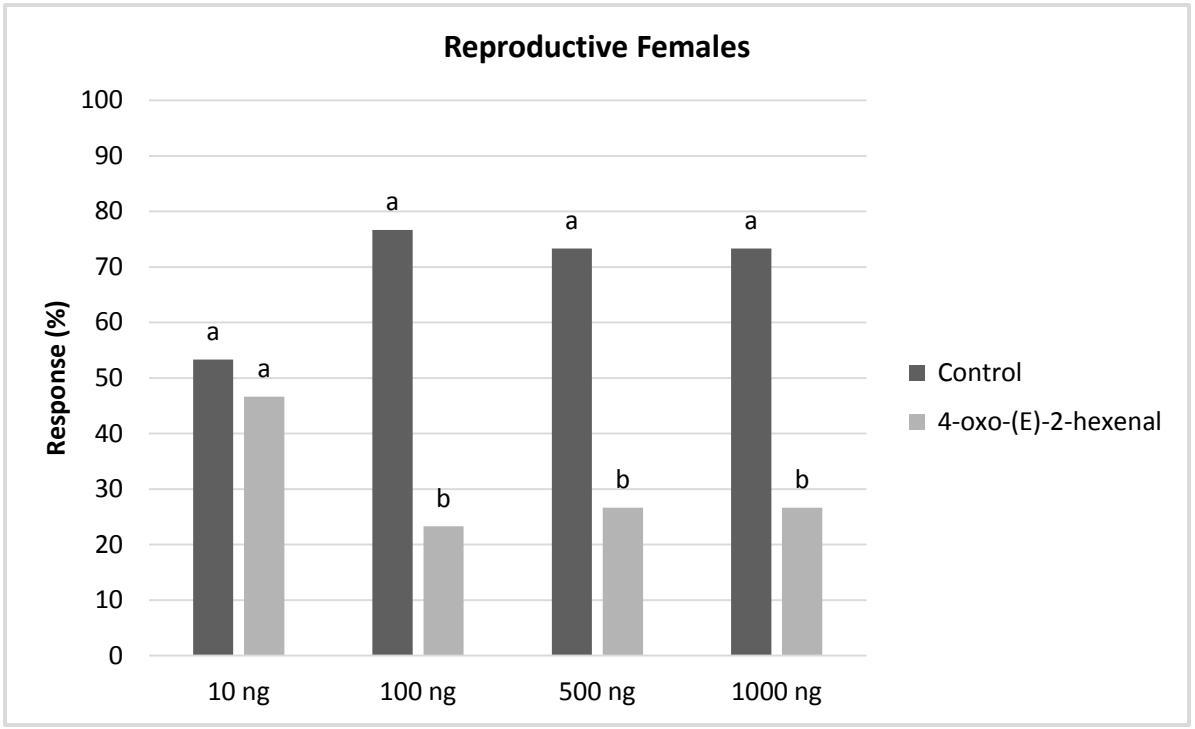
Behavioral bioassay



Behavioral bioassay



Repellent compounds are currently under investigation



Repellent Field Trials

Location	**Date**	**Mean Adult Capture ± SE**		
		Control	Treatment	*P*
Saltcreek, CO	07/14/2018	23.1 ± 8.5	9.6 ± 3.1	0.04
Rangely, CO	7/31/2018	0.36 ± 0.2	0.52 ± 0.2	0.5
Cheney, CO	08/04/2018	1.7 ± 0.6	1.0 ± 0.3	0.2
Blythe, CA	08/20/2018	5.0 ± 1.0	1.9 ± 0.6	0.04
Blythe, CA	08/27/2018	1.4 ± 1.1	1.6 ± 0.4	0.35

1. Sample beetles in the region. Determine type using sequence information
2. Perform developmental /physiological tests to confirm appropriate model parameters
3. Track with on-the-ground sampling
4. Predict timing of appearance near critical habitat

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Yuma Wash

A Push/Pull schematic

1. Determine which beetles are in the area
2. Predict and track phenology
3. Deploy push/pull strategy

Repellent

Pheromone

Critical Nesting Habitat

A Push/Pull schematic

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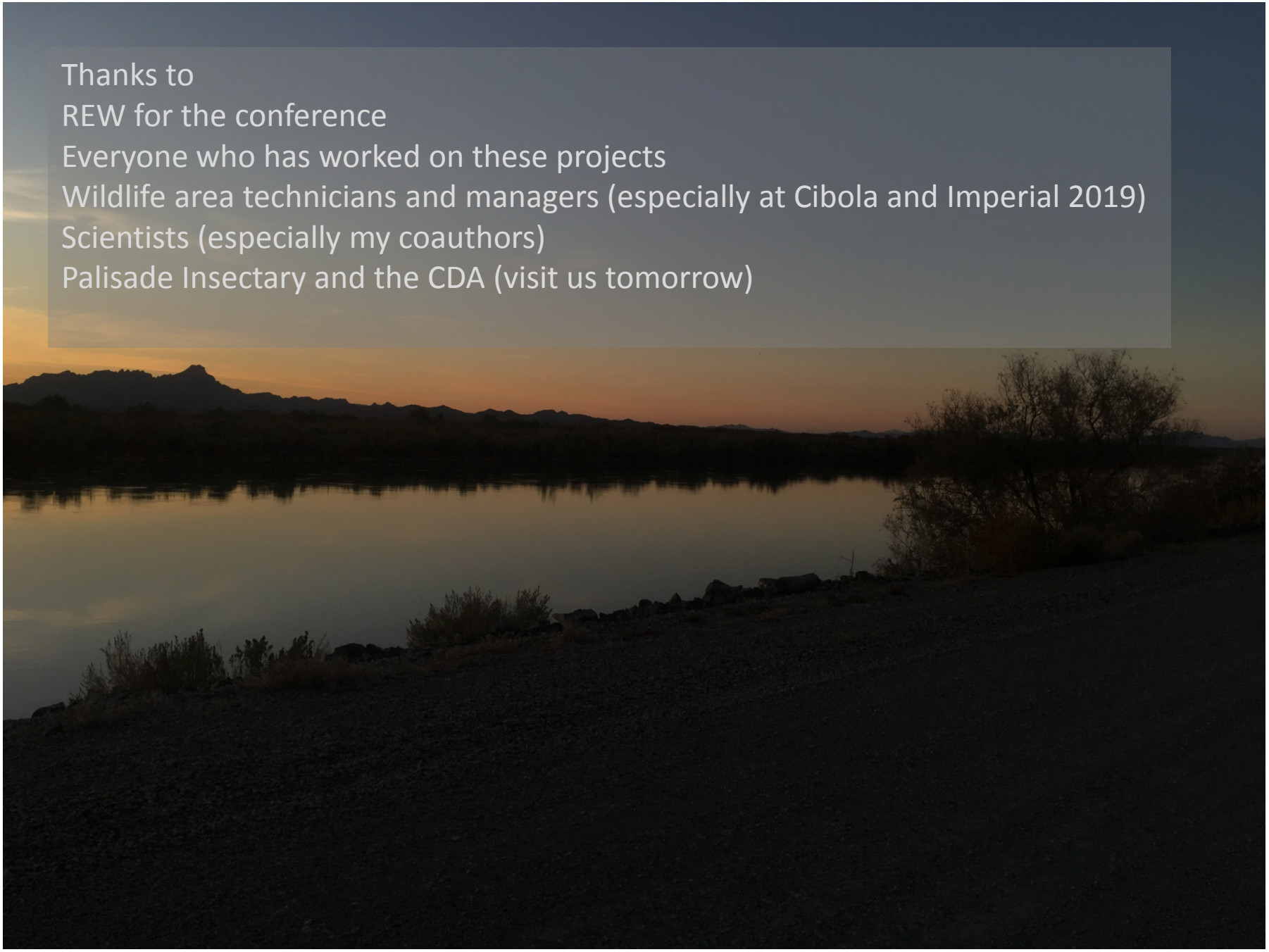
Critical Nesting Habitat

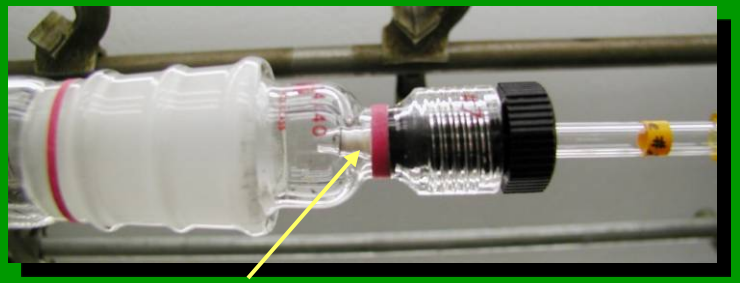
Thanks to
REW for the conference
Everyone who has worked on these projects
Wildlife area technicians and managers (especially at Cibola and Imperial 2019)
Scientists (especially my coauthors)
Palisade Insectary and the CDA (visit us tomorrow)



Collection of volatiles from tamarisk and feeding beetles

Collector tube with foliage and beetles

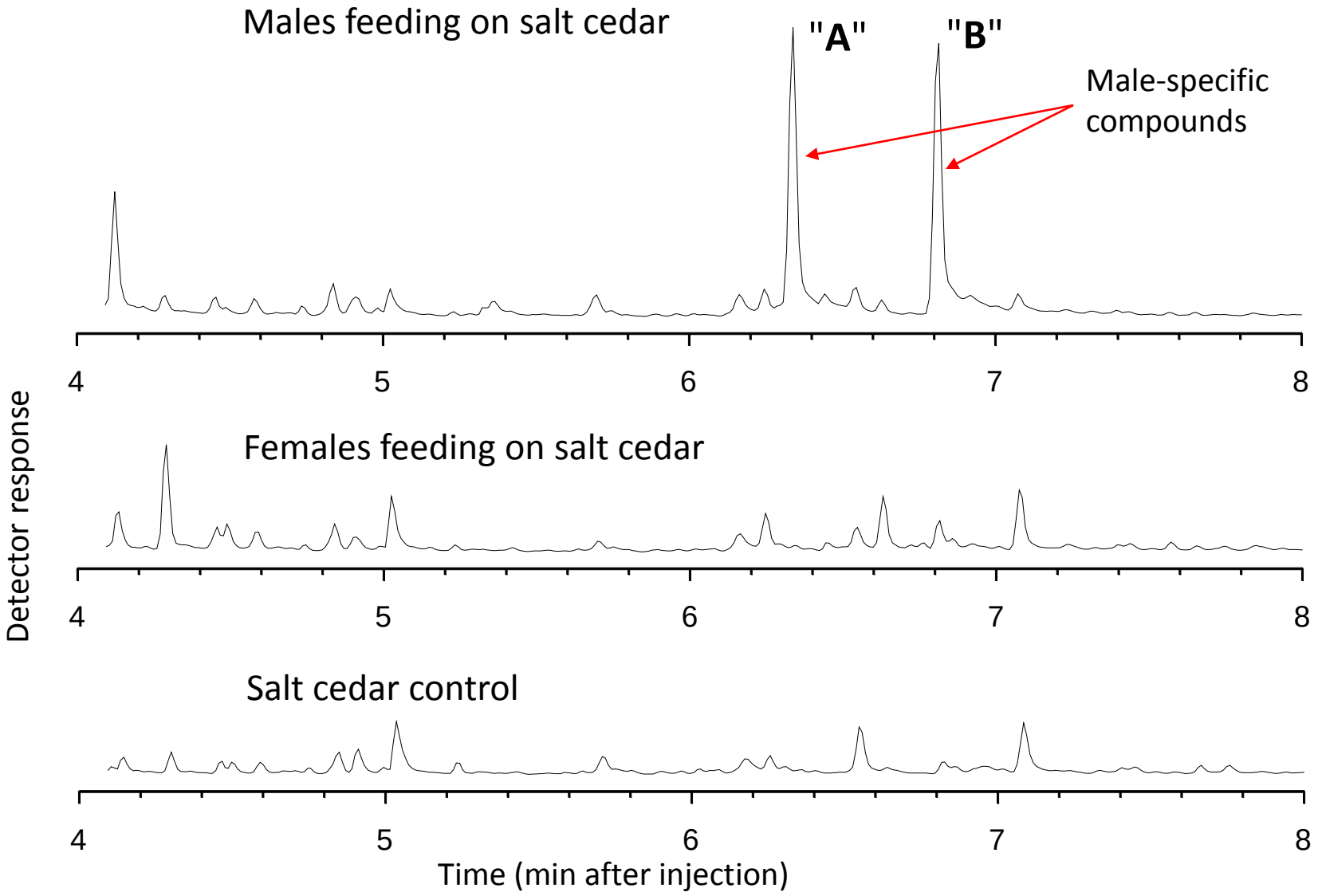
Beetles on foliage



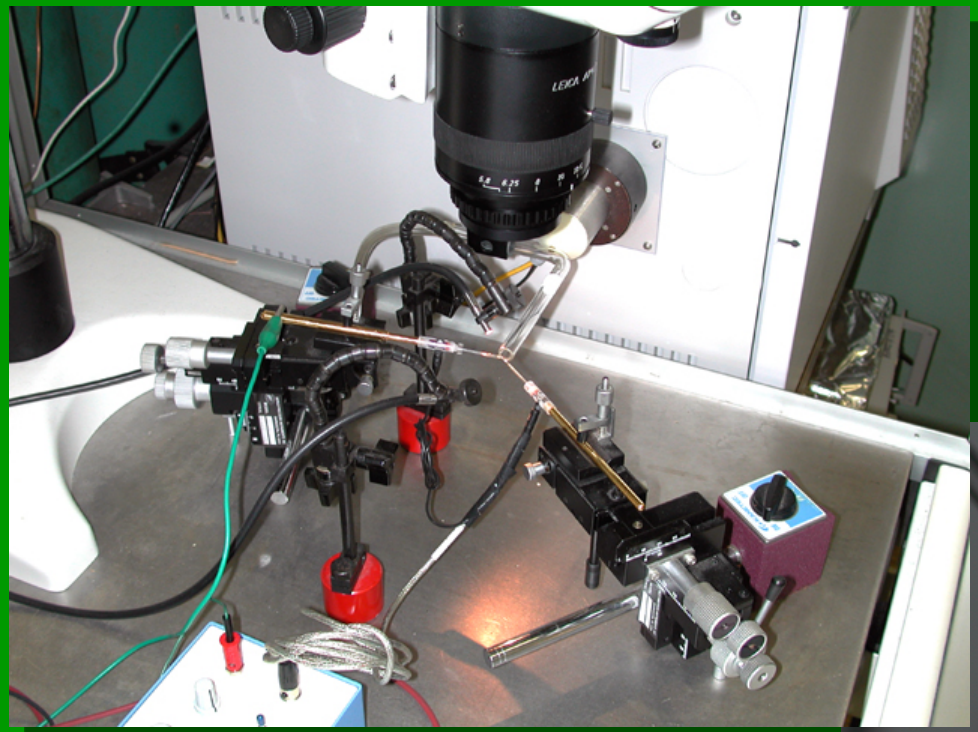
Close up of Super-Q filter

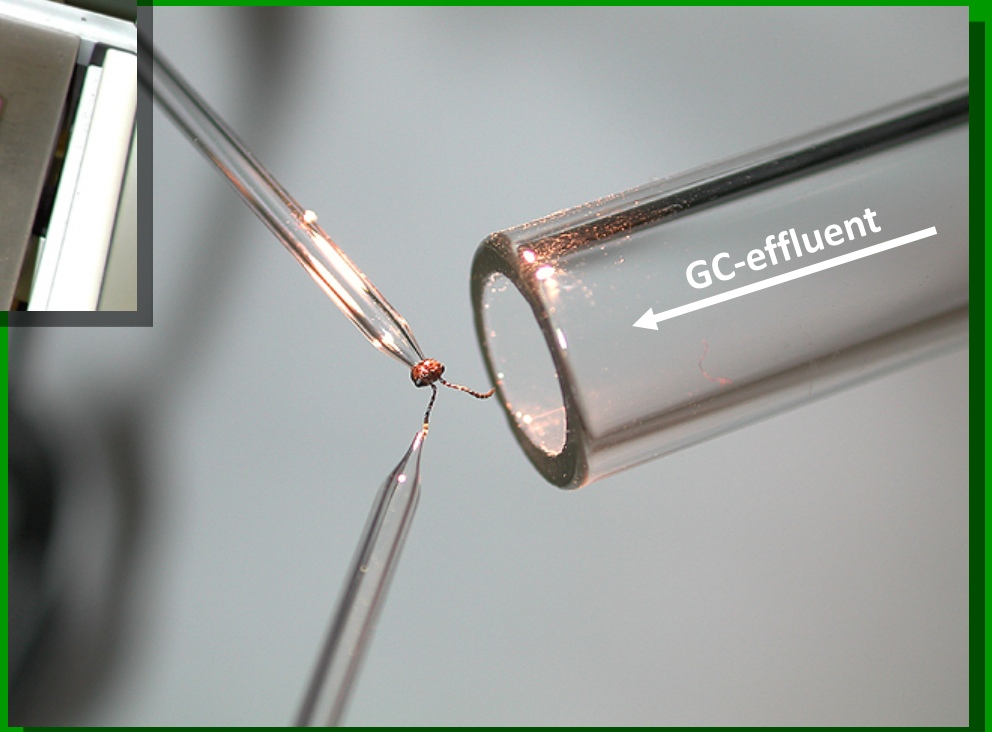
- Draw volatiles emitted from feeding beetles into filter of porous polymer ("Super-Q") with gentle vacuum; later on, rinse filter with solvent.
- On the plus side: Beetles + food is a "natural" situation; good chance of pheromone emission.
- On the minus side: plant compounds will also be collected.

GC profiles of collected volatiles from feeding *D. elongata*



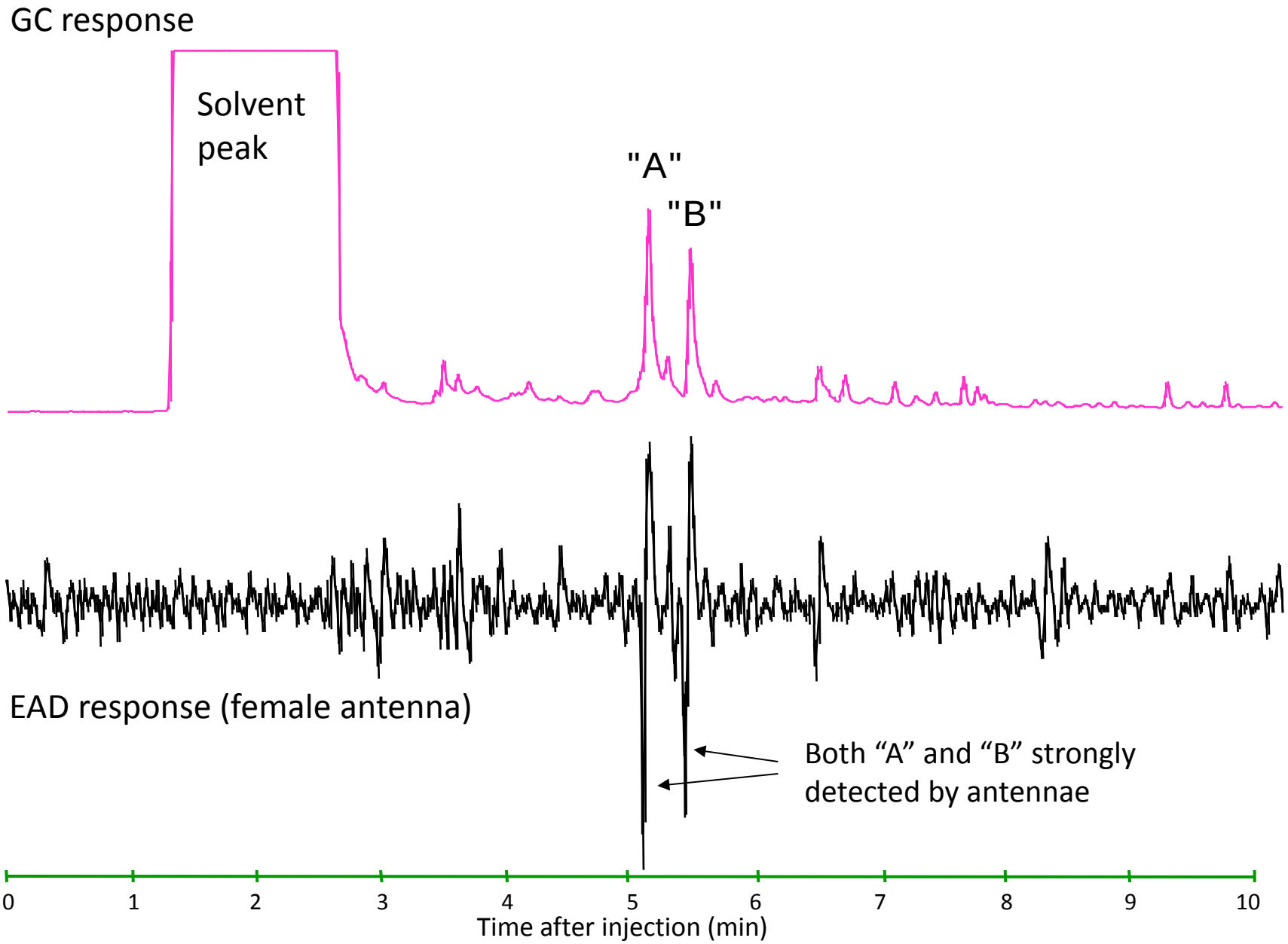
GC-coupled electroantennographic detection (GC-EAD)



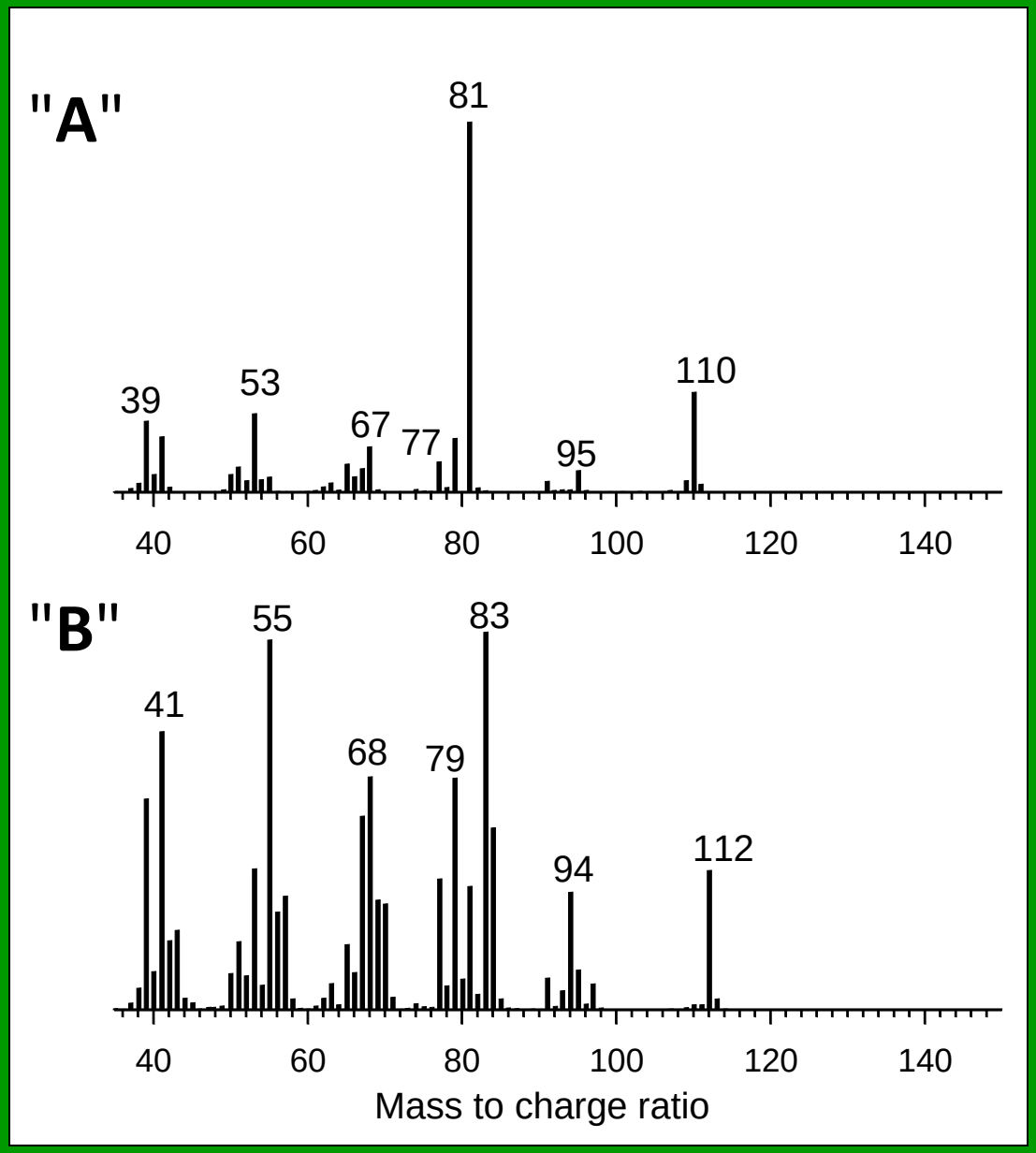


Electrophysiology setup (GC-EAD)

GC-EAD Response to Volatiles Collected from Feeding Male *D. elongata*



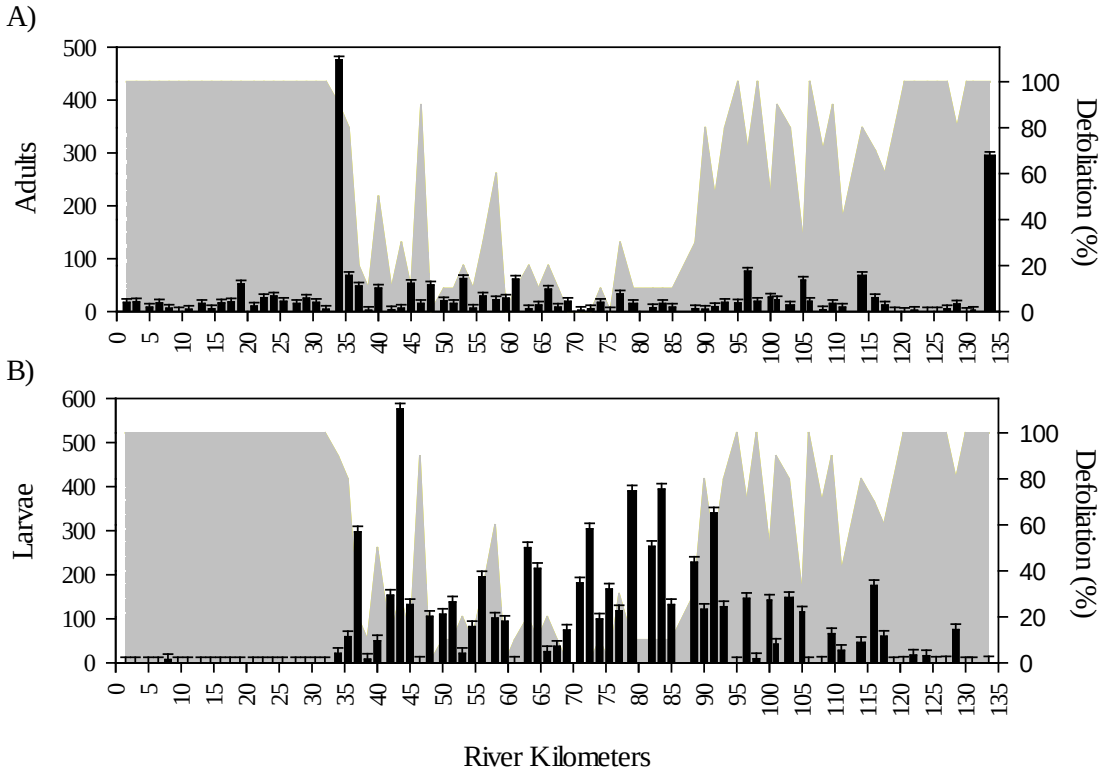
Mass spectra of male-specific compounds and ID's, based on MS library and analytical comparison with standards



(2*E*,4*Z*)-2,4-heptadienal
= "2*E*,4*Z*-7:Ald"

(2*E*,4*Z*)-2,4-heptadien-1-ol
= "2*E*,4*Z*-7:OH"

Cossé et al., 2005, J. Chem. Ecol.



Defoliation and beetle stage, San Juan, late July 2010