Using semiochemicals to manipulate populations of *Diorhabda* spp for wildlife management.
11/12/2020

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Semiochemicals,
Lovelock 2004

"Science is ahead of practice" Peter Skidmore, Walton Family Foundation Environment Program, REW Conference, 2-04-20

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

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Monitoring and manipulation of *Diorhabda* populations within southwestern willow flycatcher nesting territories

Alex Gaffke, Tom Dudley, Dan Bean

This proposed work will enable *Diorhabda* populations to be accurately monitored and future distributions predicted as they move into areas where southwestern willow flycatchers (SWFL) are known to nest in tamarisk. Information obtained through monitoring can inform efforts to manipulate spatial distribution of populations through the use of behaviorally active compounds (semiochemicals).

A more focused consideration of how the project can be applied in the upper Gila River watershed, and also our responses to questions from the US Fish & Wildlife Service, are included as appendices at the end of this document. The proposed work will be divided into three sections:

1) using semiochemicals to manipulate *Diorhabda* populations on a local spatial scale;
2) using developmental data to predict *Diorhabda* distribution and colonization events;
3) using molecular genetic data to identify *Diorhabda* populations, species and their hybrids.

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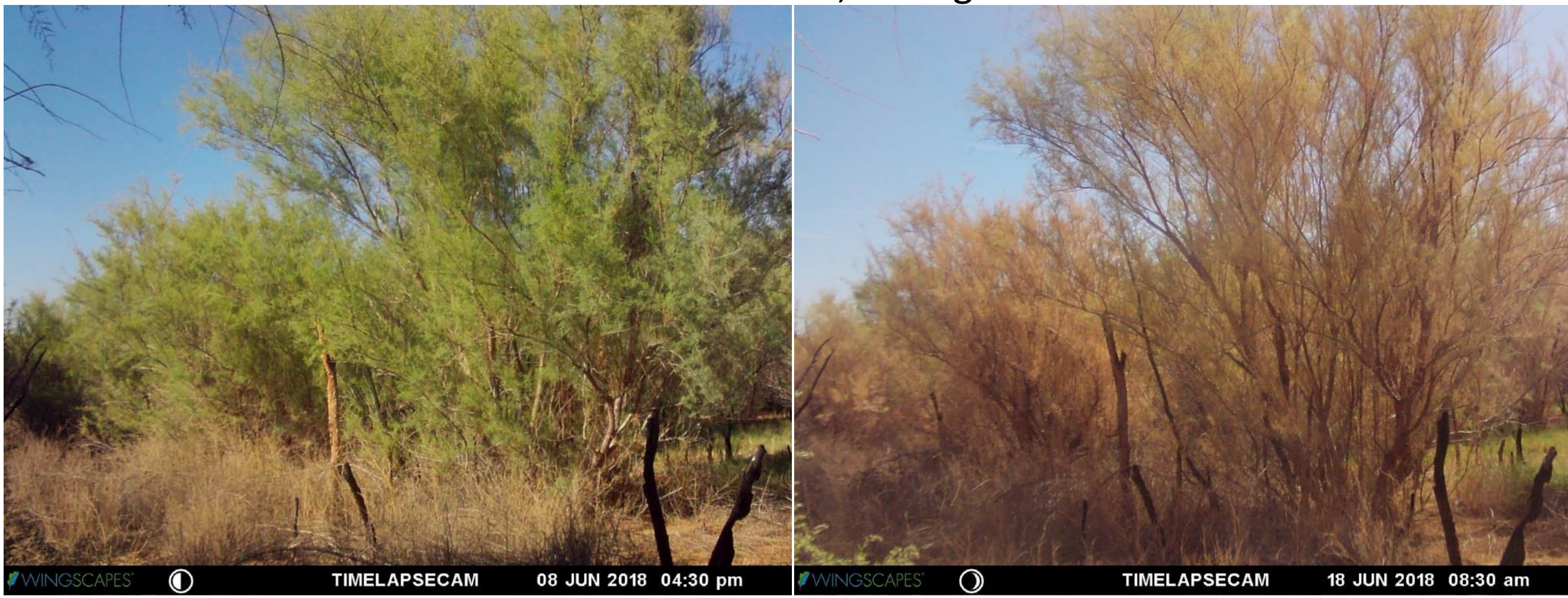
Beetles in the genus *Diorhabda* form aggregations as part of their behavioral strategy

Defoliation at the Cibola NWR, third generation larvae (2019)


Phenology camera at Cibola

1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees
3. They mate and lay eggs
4. Adults move on, larvae feed and defoliate
5. Mature larvae drop to the ground and pupate
6. The next generation adults emerge from the leaf litter

Defoliation at the Cibola NWR, third generation larvae



Overwintered spring adults

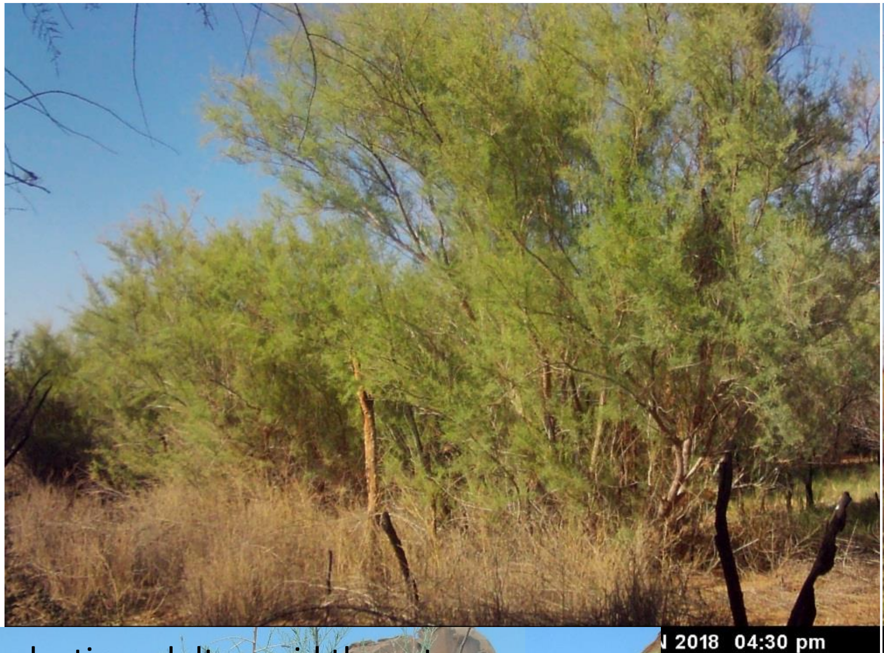
1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees
3. They mate and lay eggs
4. Adults move on, larvae feed and defoliate
5. Mature larvae drop to the ground and pupate
6. The next generation adults emerge from the leaf litter

Defoliation at the Cibola NWR, third generation larvae


Aggregating reproductive adults

1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees
3. They mate and lay eggs
4. Adults move on, larvae feed and defoliate
5. Mature larvae drop to the ground and pupate
6. The next generation adults emerge from the leaf litter

Defoliation at the Cibola NWR, third generation larvae



Defoliating late-stage larvae

1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees
3. They mate and lay eggs
4. Adults move on, larvae feed and defoliate
5. Mature larvae drop to the ground and pupate
6. The next generation adults emerge from the leaf litter

Pushed away from trees with heavy larval feeding

Pulled toward reproductive adult swarms

Nature's "push/pull" helps determine local beetle densities and the level and timing of defoliation

1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees ←
3. They mate and lay eggs
4. Adults move on, larvae feed and defoliate
5. Mature larvae drop to the ground and pupate
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Pushed away from trees with heavy larval feeding

Pulled toward reproductive adult swarms

What chemical signals mediate these behaviors?

Nature's "push/pull" helps determine local beetle densities and the level and timing of defoliation

1. Adults emerge from the leaf litter and feed
2. Adults aggregate on larva-free trees ⟵
3. They mate and lay eggs
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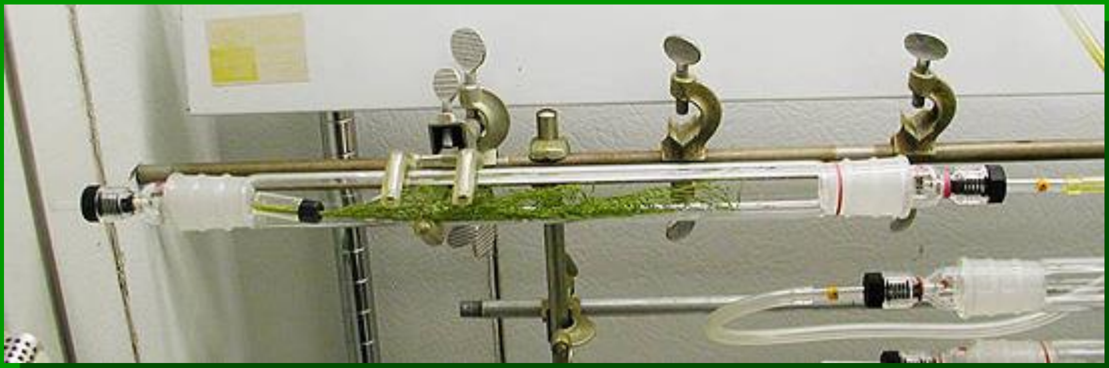
Allard Cossé checks field trials of attractants
Lovelock, NV, 2004

Bob Bartelt monitors
pheromone-baited trap,
Lovelock, 2003

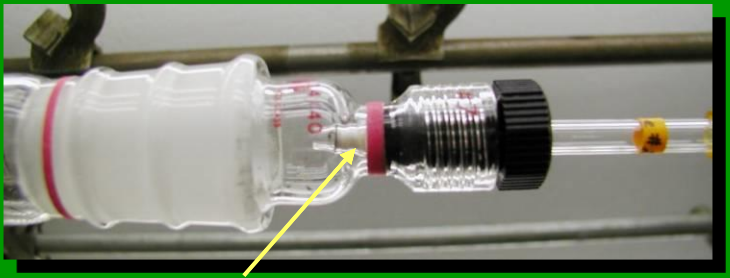
A pheromone blend produced by reproductive, feeding males mediates the formation of reproductive swarms of adult beetles, attracting females as well as other males

Collection of volatiles from tamarisk and feeding beetles



Collector tube with foliage and beetles

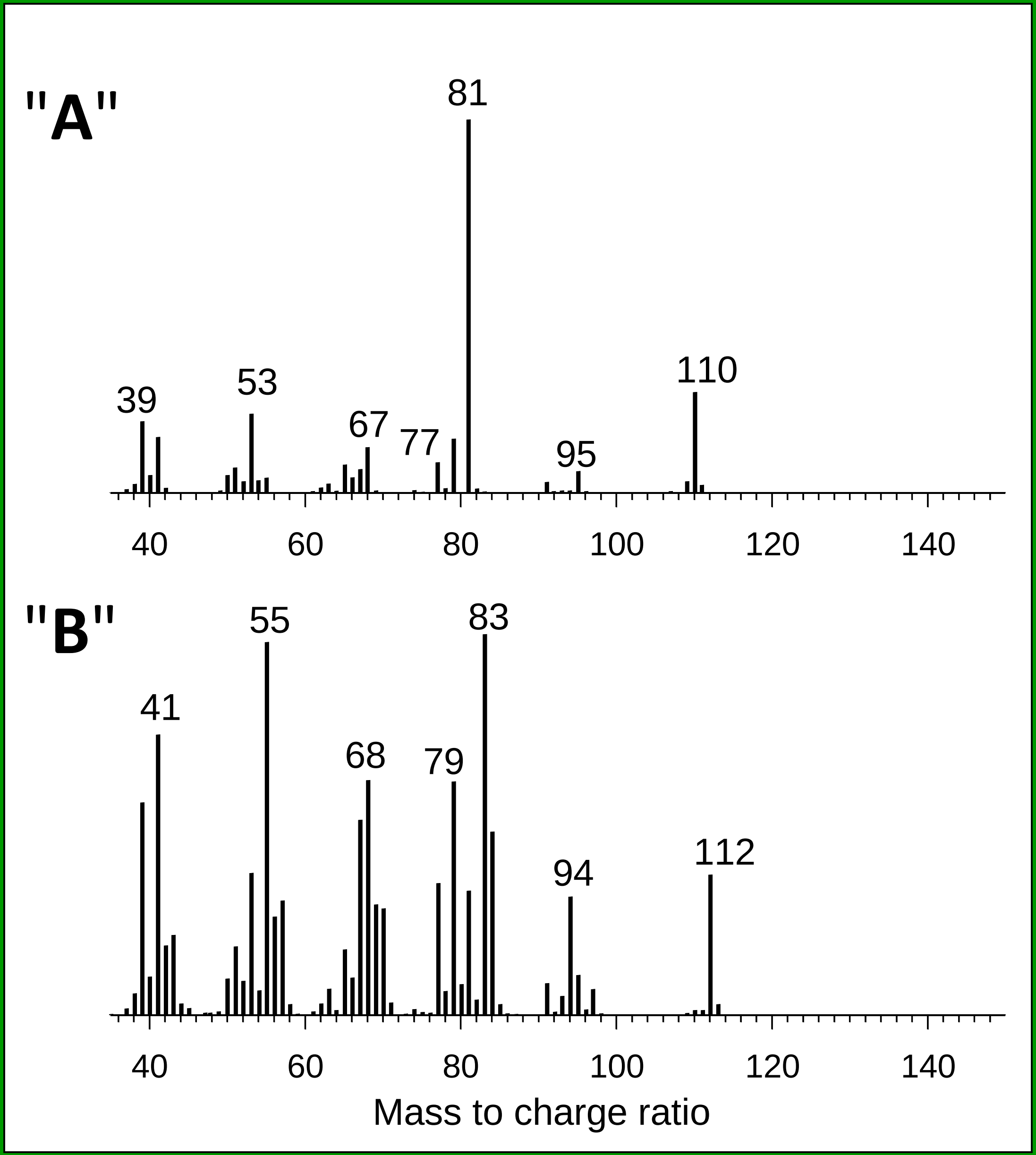
Beetles on foliage

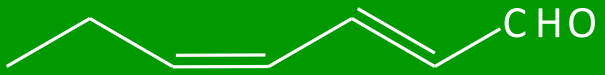


Close up of Super-Q filter

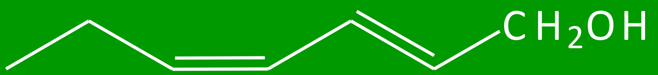
- Draw volatiles emitted from feeding beetles into filter of porous polymer ("Super-Q") with gentle vacuum; later on, rinse filter with solvent.
- On the plus side: Beetles + food is a "natural" situation; good chance of pheromone emission.
- On the minus side: plant compounds will also be collected.

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.

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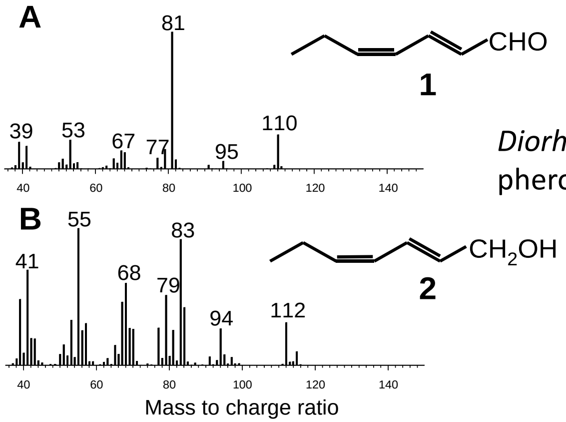
Discovery of the male-emitted aggregation-pheromone blend and plant-based attractants fueled efforts to use semiochemicals to:

1. Monitor *Diorhabda* populations at low densities
2. Assist in establishment of *Diorhabda* by keeping the adults in one place
3. Manipulate beetle populations with wildlife management as a goal ←

Alex Gaffke, Montana State University
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.

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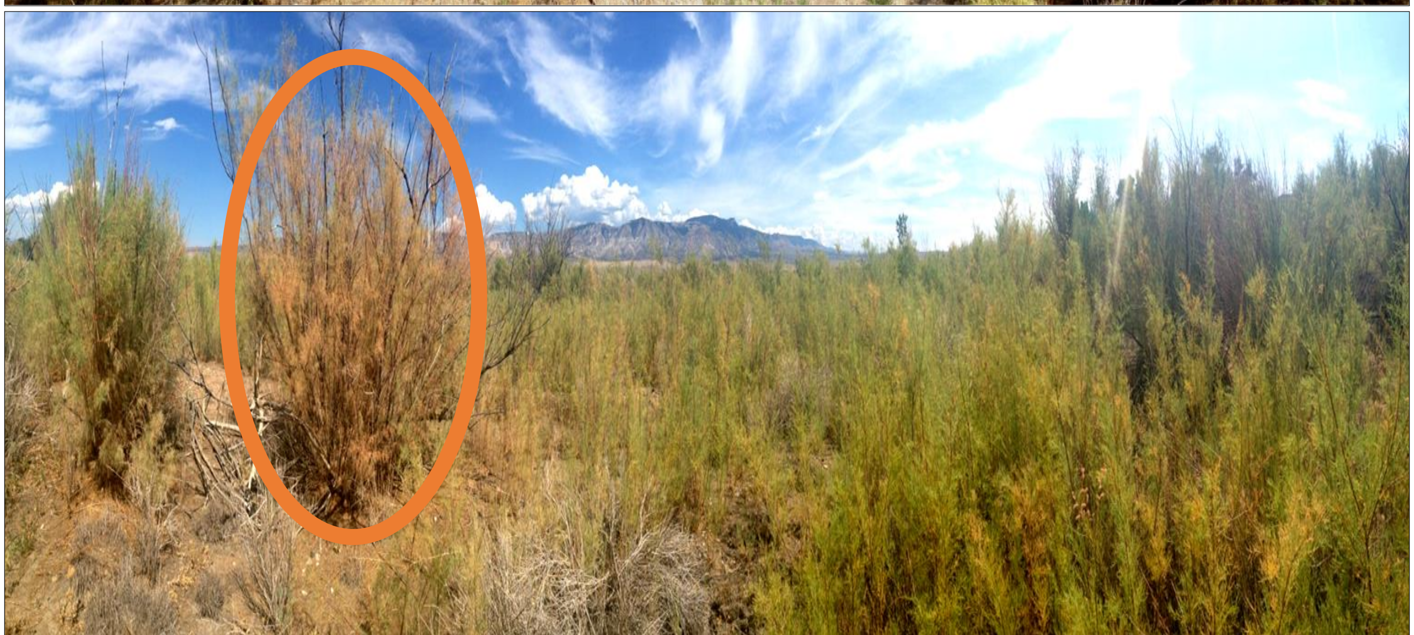
Gaffke AM, Sing SE, Dudley TL, Bean DW, Russak JA, Mafra-Neto A, Peterson RKD, Weaver DK (2020). Establishing *Diorhabda carinulata*: Impact of release disturbances on pheromone emission and influence of pheromone lures on establishment. Journal of Chemical Ecology 46: 378-386



Pheromone treatment 2013

Untreated Plants with no impacts from Diorhabda

Impacts of pheromone treatment: high levels of dieback

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

Discovery of compounds repellent to Diorhabda enable a “Push/Pull” strategy for spatial manipulation of populations

Gaffke AM, Sing SE, Miller JG, Dudley TL, Bean DW, Peterson RKD, Weaver DK (2020). An herbivore-induced plant volatile from saltcedar (*Tamarix* spp.) is repellent to *Diorhabda carinulata* (Coleoptera: Chrysomelidae). Environmental Entomology https://doi: 10.1093/ee/nvaa079

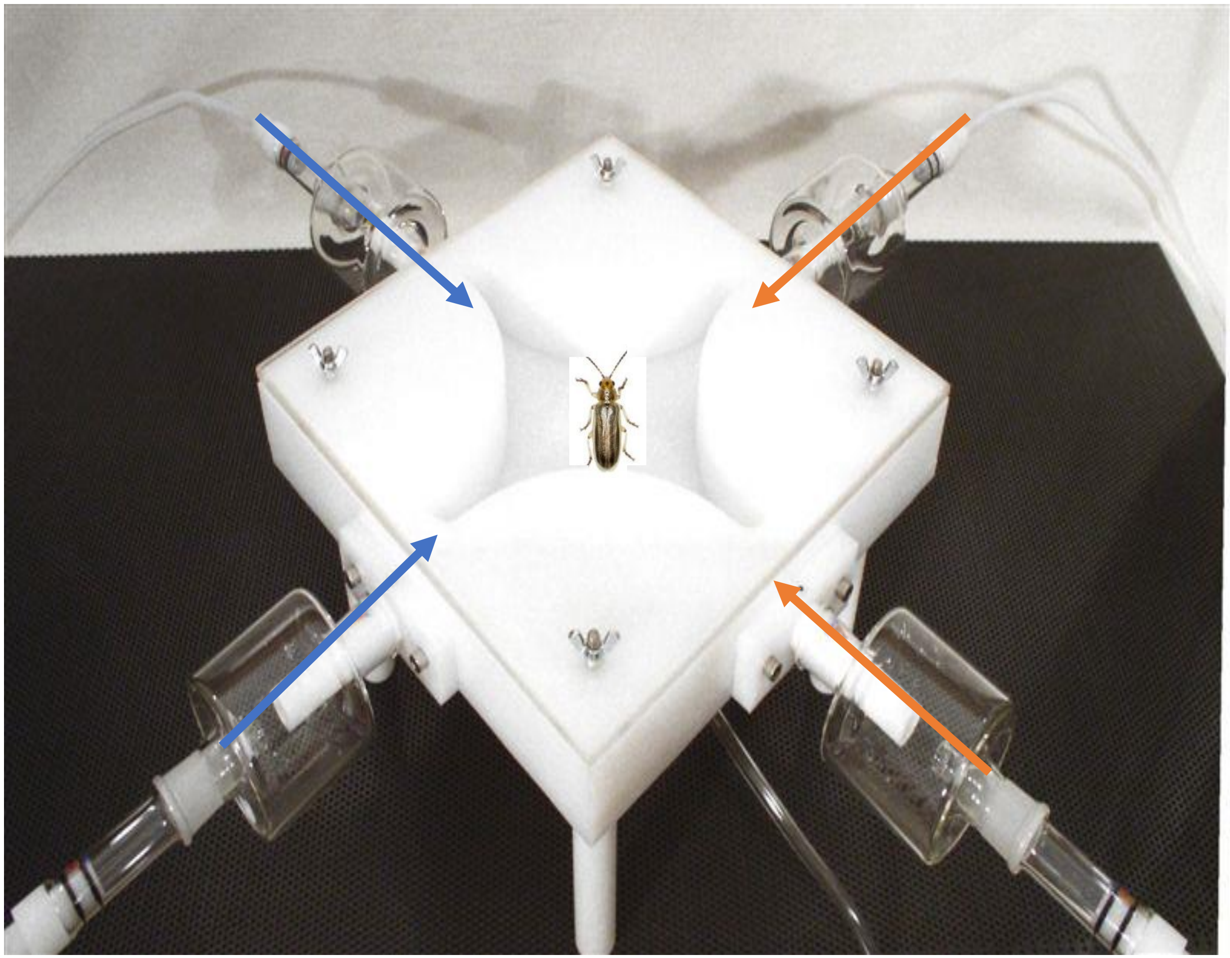
Push away from critical nesting habitat

Pull toward expendable tamarisk stands

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



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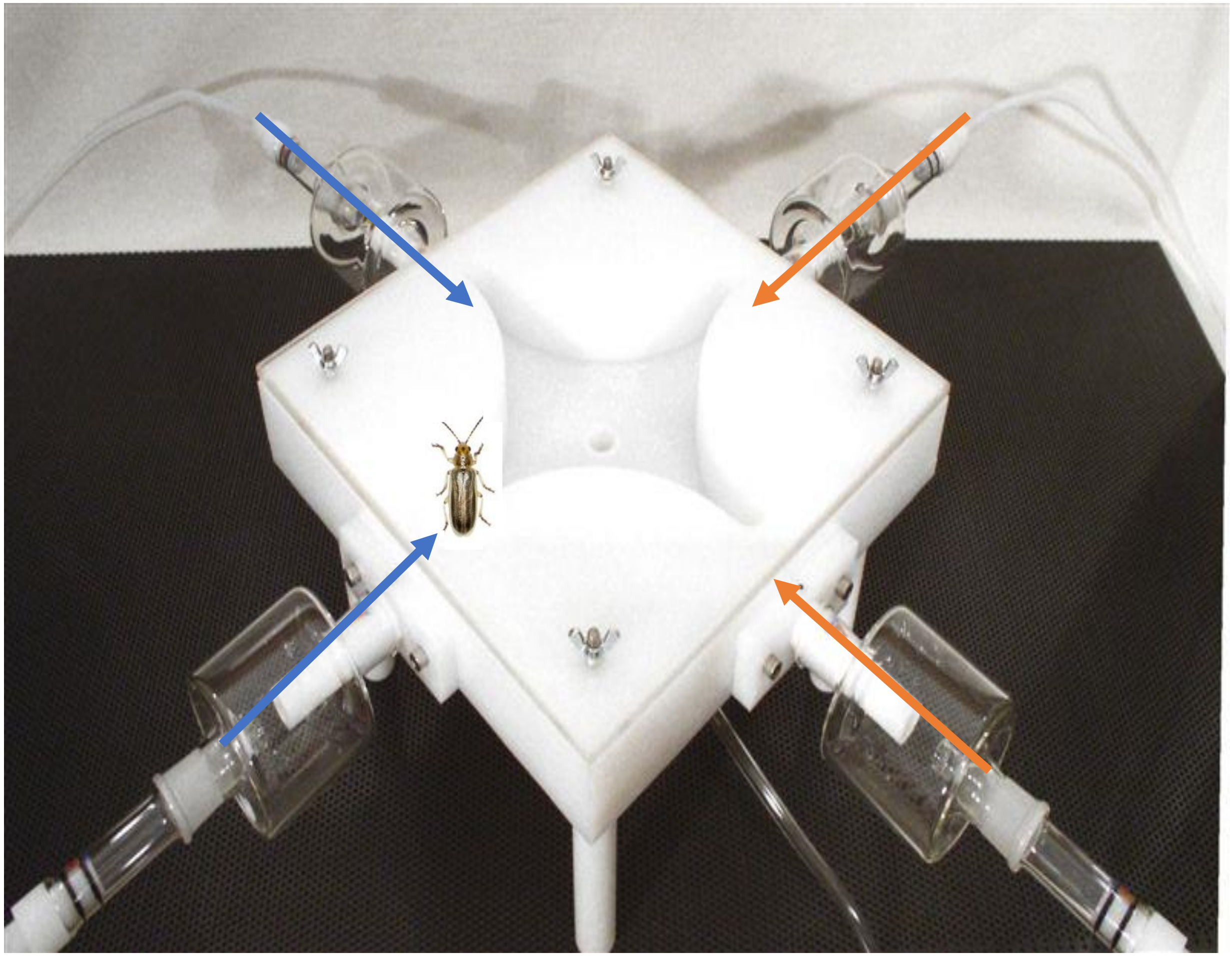
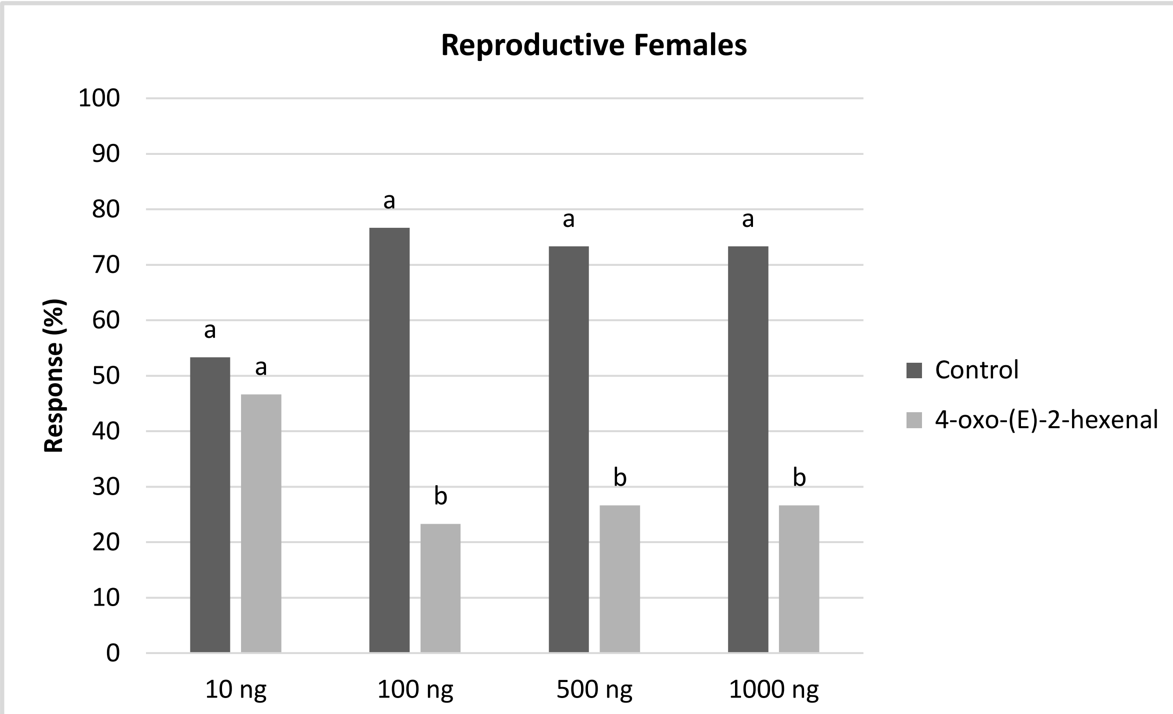


Repellent compounds are currently under investigation



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

Relevant points from Gaffke's work

1. A system was developed, in cooperation with a private company, for extended release of the male emitted pheromone (the system uses SPLAT, a proprietary slow-release formulation based on a waxy matrix that enables slow vaporization of the compounds).

2. Beetles can be herded to areas where they will defoliate tamarisk, while being distracted from other nearby trees. A compound was also found which repels female beetles and may prove useful as the push portion of a push-pull strategy

3. This work can be of immediate use to resource managers, providing a stop-gap measure to protect nesting birds from defoliation events while restoration is underway.

SPLAT pheromone release system

Implementation

1. Synthesize pheromone, develop strategic plan for the timing and spatial arrangement of lure placement
2. Work closely with those striving to restore the Gila watershed and increase ecosystem services (Gila Watershed Partnership and others)
3. Increase community education and involvement by providing the Gila Watershed Partnership with educational materials
4. Work closely with those involved in monitoring southwestern willow flycatcher populations and those involved in restoration of critical habitat
5. Coordinate with scientists working on various aspects of *Diorhabda* biology in order to better understand and predict life cycles and behavior of the beetle

 Catch and kill

Pull with attractants

Push with repellents

Early season use traps to catch and kill adults early in the log phase of population growth

 Catch and kill

Pull with attractants

Push with repellents

 Catch and kill

Pull with attractants

Push with repellents

Later in the season use attractants to distract beetles from nesting territories of the SWFL. Use repellents to discourage beetles from colonizing near SWFL nests.

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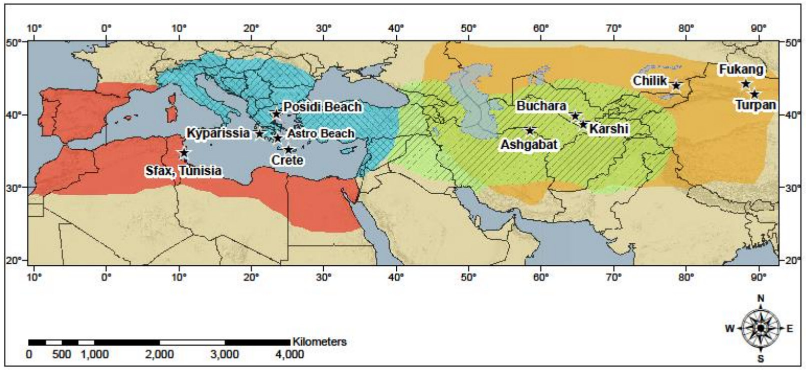
Which beetles are in the upper Gila drainage?

Diorhabda sublineata

Diorhabda elongata

Diorhabda carinata

Diorhabda carinulata



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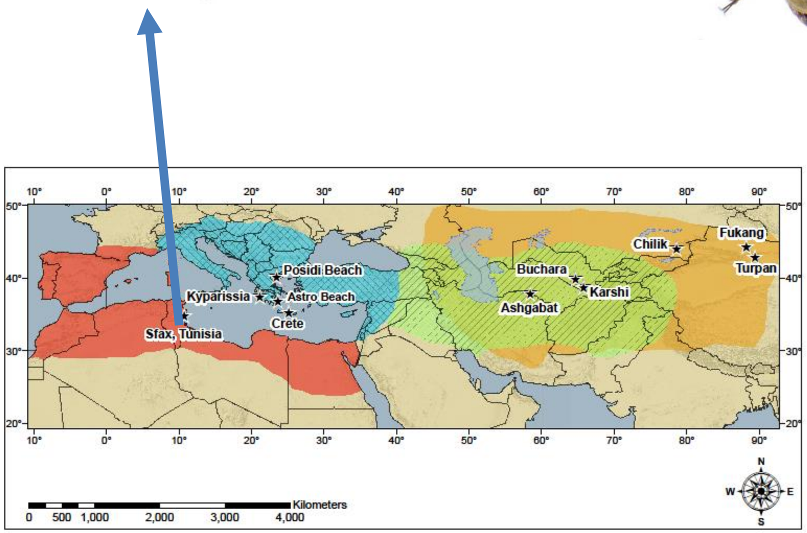

Their great great…great great grandmothers came from Tunisia

personal communication, Zeynep Özsoy, Colorado Mesa University, based on mitochondrial CO1 DNA sequence

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Diorhabda sublineata

X

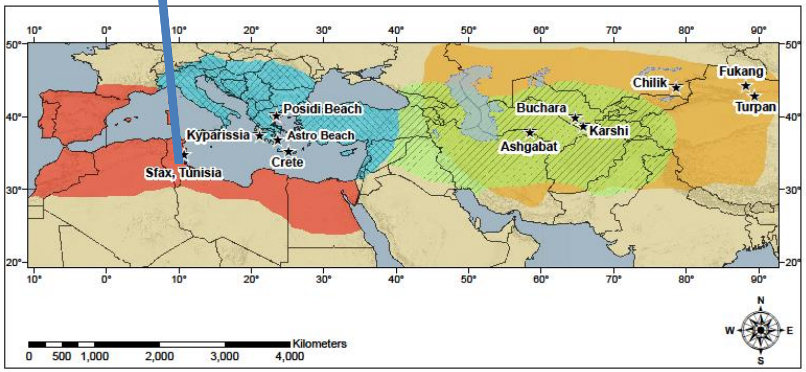
Diorhabda elongata

X

Diorhabda carinata

x

Diorhabda carinulata



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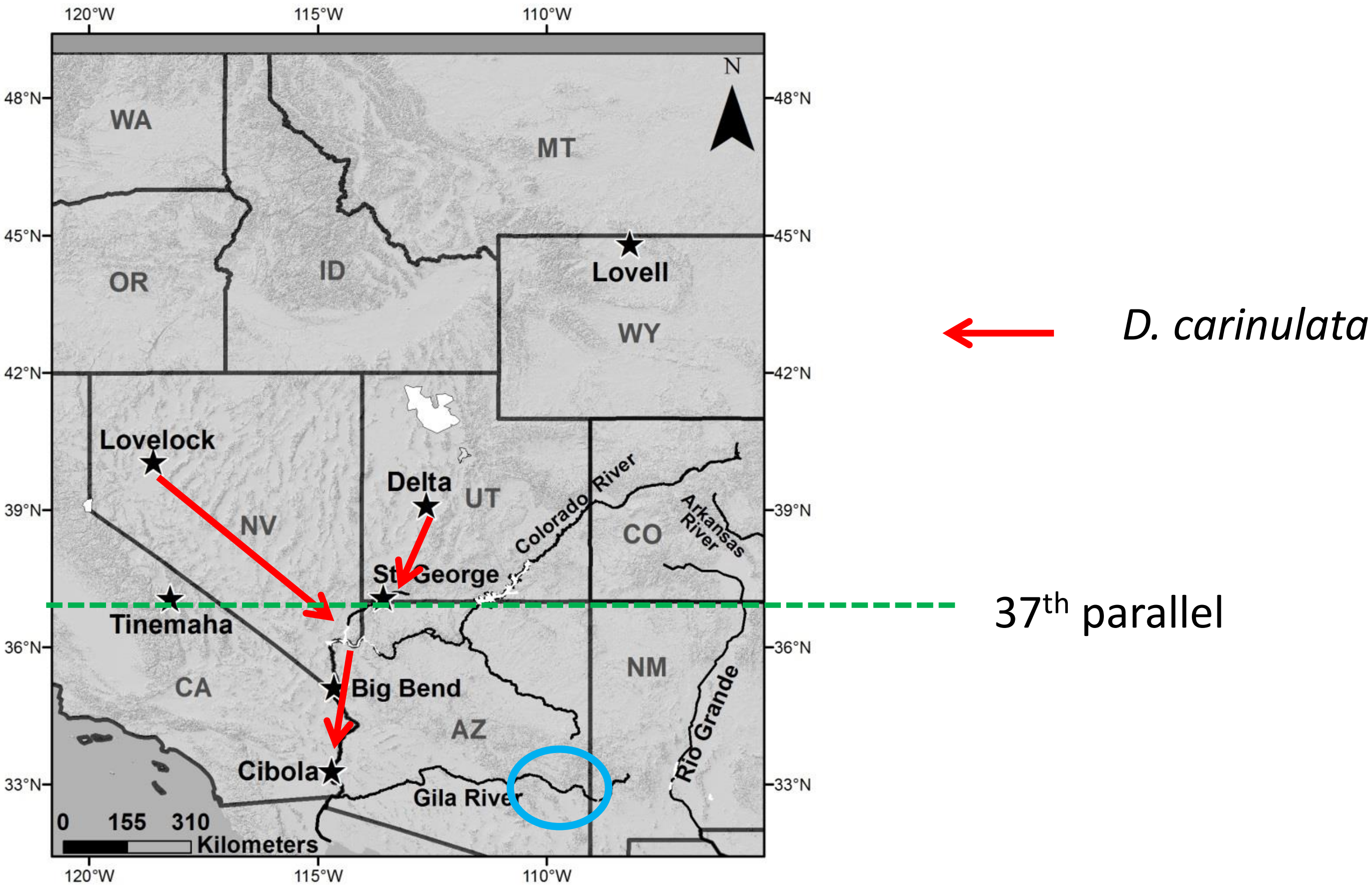
120°W
115°W
110°W
48°N
45°N
42°N
39°N
36°N
33°N
N
WA
MT
OR
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WY
Lovell
Lovelock
Delta
UT
NV
CO
Colorado River
Arkansas River
St. George
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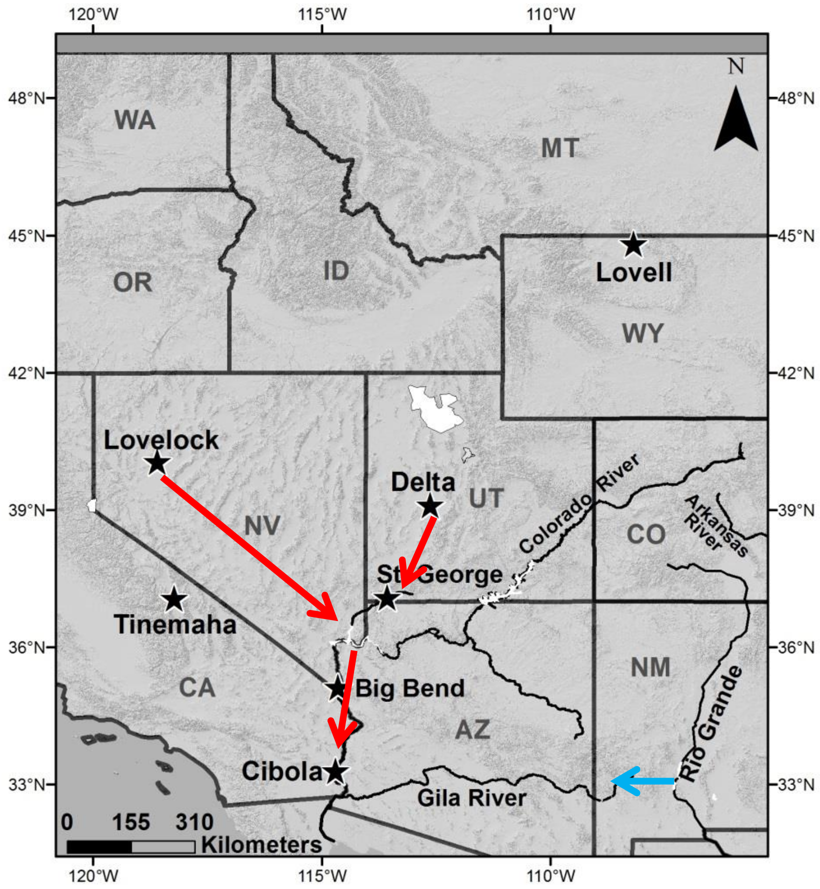
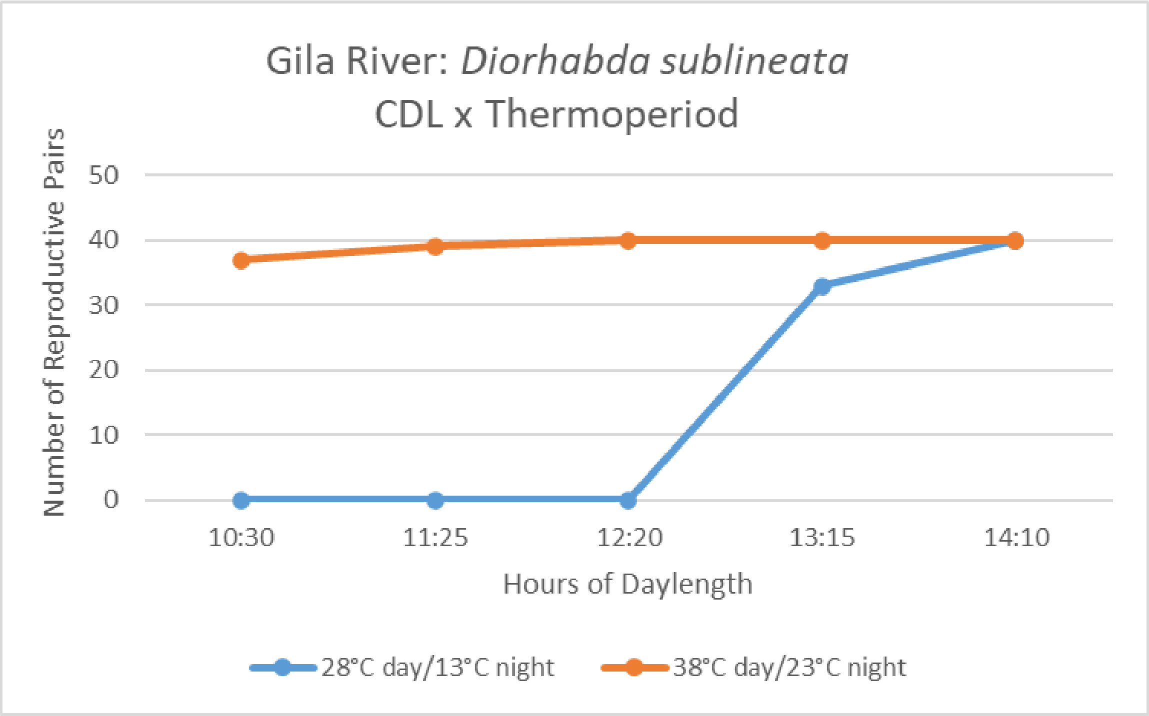
Big Bend
AZ
Rio Grande
Cibola
Gila River
0 155 310
Kilometers
D. carinulata
37th parallel



D. carinulata

D. sublineata and hybrids

The first experiment with Gila River beetles (subtropical tamarisk beetle) shows that photoperiodic diapause induction is highly sensitive to temperature, unlike with the northern tamarisk beetle. 40 pairs were tested per treatment



No diapause seen when daytime temperatures reach 38°C (100° F).

Thank you!

For questions please contact the authors. Alex Gaffke (alexander.gaffke@gmail.com), Tom Dudley (tdudley@msi.ucsb.edu) and Dan Bean (dan.bean@state.co.us)

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