Biocontrol & Threatened and Endangered Species

March 8, 2024

REW's 21st Biennial Riparian Restoration Conference: Restoration for the future

Grand Junction, CO

Integrating behavioral ecology and conservation science: Implications for southwestern T&E species

Satellite-based Habitat Models to Inform Restoration the Southwestern Willow Flycatcher and the Yellow-billed Cuckoo

Yellow-billed Cuckoo Breeding Phenology and General Nest Site Characteristics in Western Colorado

Using Tamarisk Beetle Pheromone Lures to Reduce Re-growth of Tamarisk Following Tree Cutting

State of the beetle:
Why monitoring *Diorhabda* is ***still*** crucial
& how we could do better

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March 8, 2024
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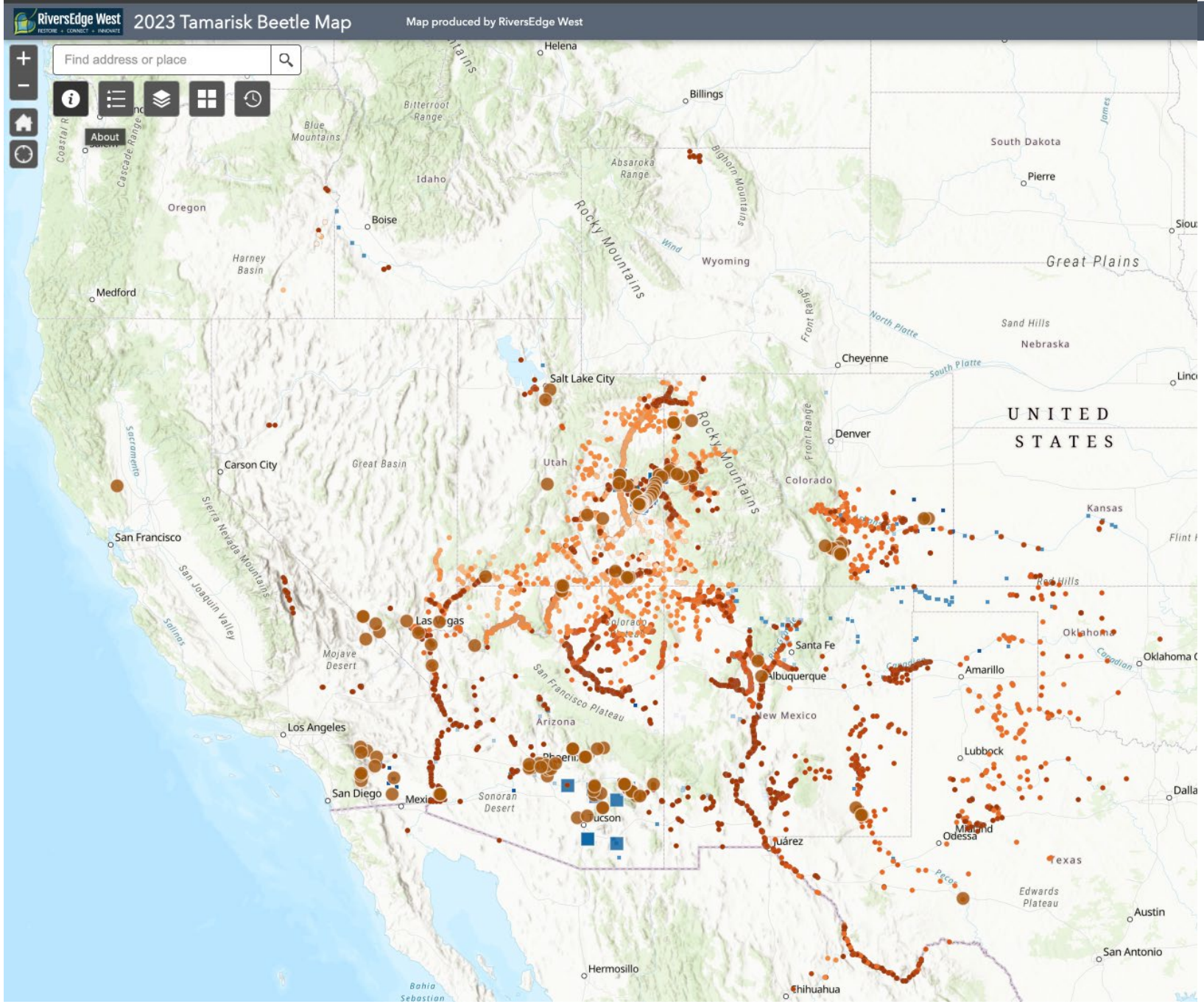

Almost **20 years of monitoring data** as beetles spread have spread across the west

2006-2023: 13,067 unique presence or absence observations

Check out REW's interactive 2023 AGOL map

Alright, the beetles are basically everywhere now.

This is difficult to fund and maintain and doesn't directly contribute to recovery of SWFL.

Do we still need to monitor and maintain these data?

Why did we spend thousands (or hundreds of thousands) of dollars to track a beetle?

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Best case scenario assumptions:

- *Diorhabda* will have a large impact on tamarisk through defoliation events
- Major defoliation events will improve conditions for desirable plants to establish and propagate
- Ecosystem function will then improve and provide habitat for T & E
- What we learn through monitoring core populations can inform how we navigate the expanding range

Why did we spend thousands (or hundreds of thousands) of dollars to track a beetle?

Credit: USGS

D. carinulata (aka northern tamarisk beetle or Utah strain) populations expanded south unexpectedly.

COMPLAINT FOR INJUNCTIVE AND DECLARATORY RELIEF

1. This case challenges the Animal and Plant Health Inspection Service's ("APHIS") 2010 decision to terminate, without taking necessary remedial action, the agency's program authorizing wide-scale release of an invasive species known as the tamarisk leaf-eating beetle ("beetle") that is having, and will continue to have, devastating effects on the highly endangered Southwestern willow flycatcher ("flycatcher") and its habitat, including designated critical habitat. Not only did APHIS's failure to implement any mitigation measures to address the ongoing harm to the flycatcher directly caused by APHIS's beetle release program violate the Endangered Species Act ("ESA"), 16 U.S.C. §§ 1531-1544, in myriad ways – namely the ESA's affirmative duties (1) to avoid jeopardizing the continued existence of a listed species, and (2) to carry out programs for the conservation of the federally endangered flycatcher – but the agency's attempt to wash its hands of the ecological catastrophe that it caused also violates the National

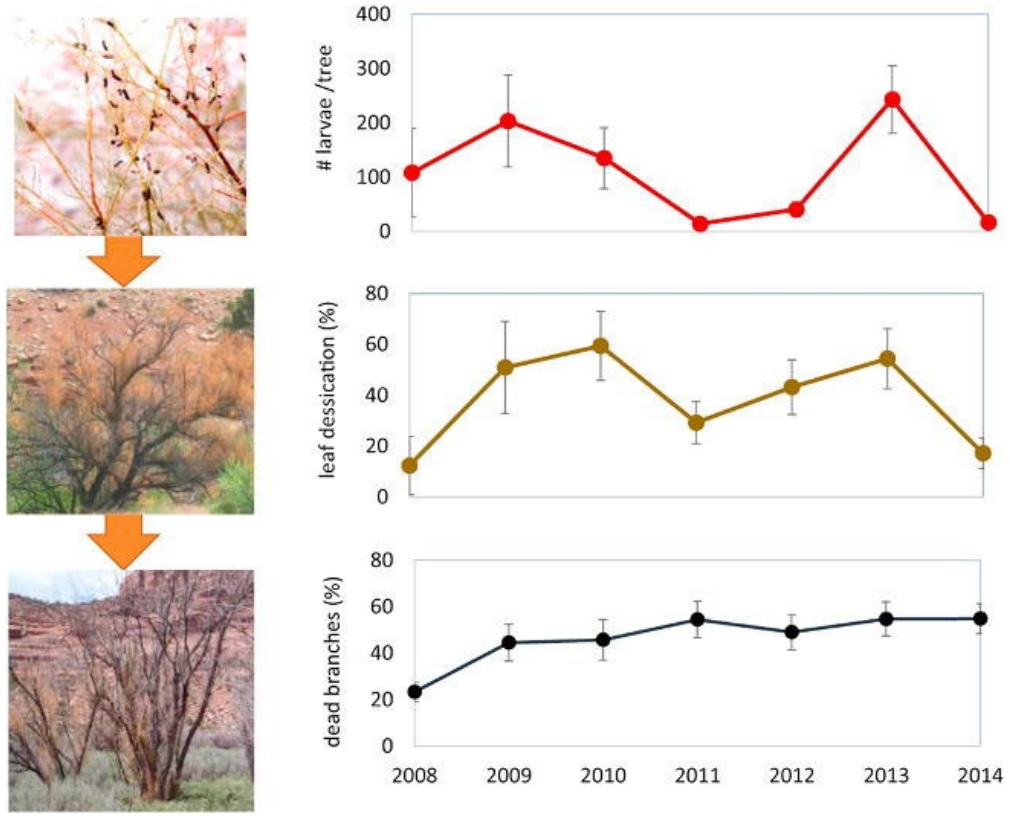
Center for Biological Diversity v. Vilsack, No. 2: 13-cv-01785-RFB-GWH (D. Nev. Aug. 1, 2017).

Restoration practitioners could use the beetle map to:

- Prepare for the onslaught
  - Better opportunities to plant
  - Divert precious resources elsewhere
  - Monitor and protect vulnerable wildlife to assess impact
- Diagnose need for intervention
  - Ramp up manual removal
  - Pheromones to attract, keep beetles in area longer, or deter

Our assumptions were tested

- *Diorhabda* have an *initial* large impact on tamarisk through defoliation events
  - *Sometimes* impact is predictably sustained
  - *Sometimes* impact is very ephemeral



Kennard, et al., 2016.

Outcomes of control and monitoring of a widespread riparian invader (*Tamarix* spp.): a comparison of synthesis approaches

Alexander R. B. Goetz[1,2], Eduardo González-Sargas[3,4], Mayra C. Vidal[5], Patrick B. Shafroth[4], Annie L. Henry[1], Anna A. Sher[1]

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Goetz et al., 2024

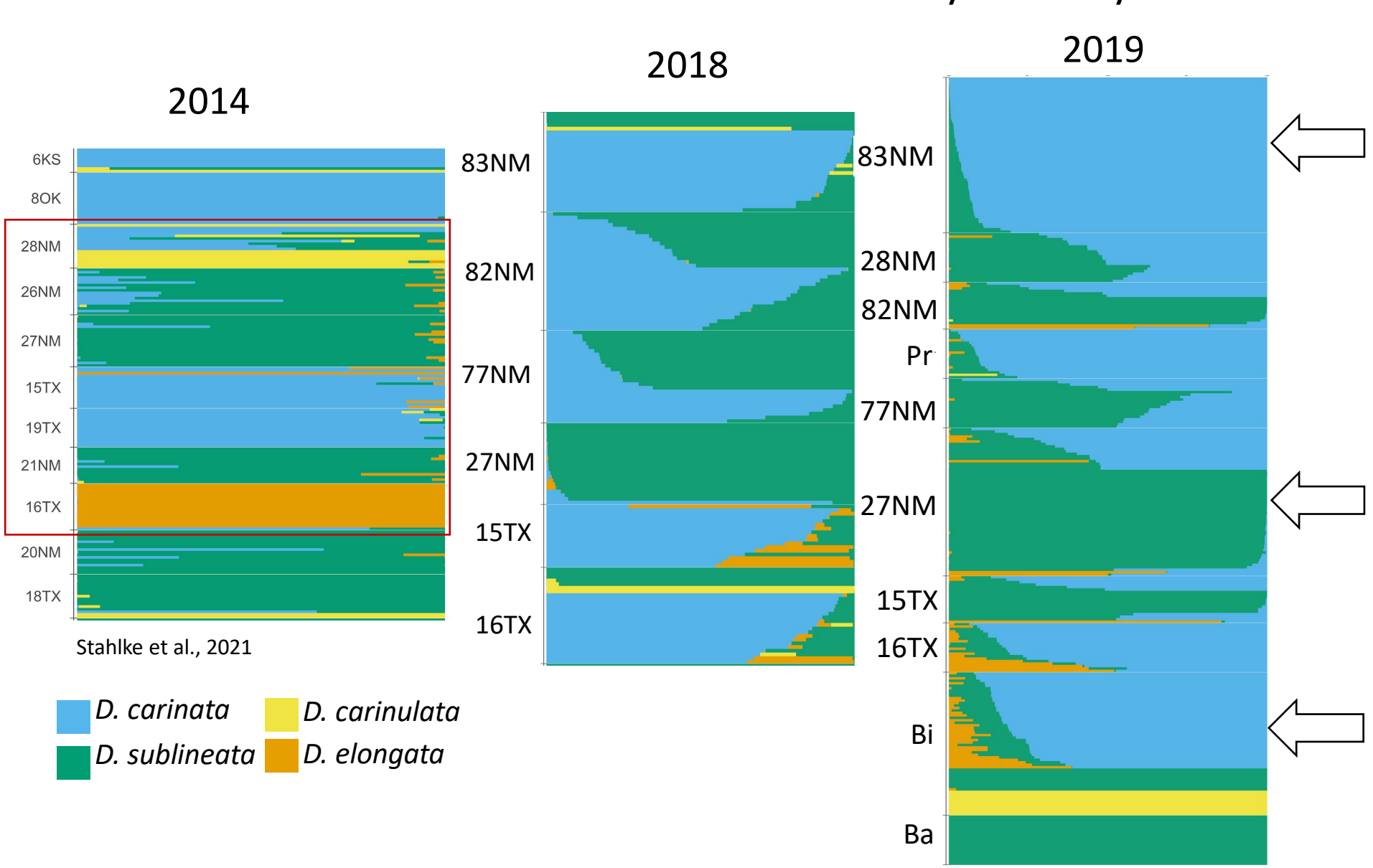
Our assumptions were tested

- *Diorhabda* have an *initial* large impact on tamarisk through defoliation events
  - *Sometimes* impact is predictably sustained
  - *Sometimes* impact is very ephemeral
- Major defoliation events *sometimes* improve conditions for desirable plants to establish and propagate
  - Without hydrological conditions or planting intervention, sometimes defoliation does more harm than good
- *Diorhabda* populations are vulnerable to eco-evolutionary processes
  - Interspecific interactions (hybridization and competitive exclusion)
  - Rapid dispersal, rapid evolution

https://riversedgewest.org/resource-center/tamarisk-beetle

Host-preference and performance was stable among hybrids

Time series data show a dynamic hybrid zone

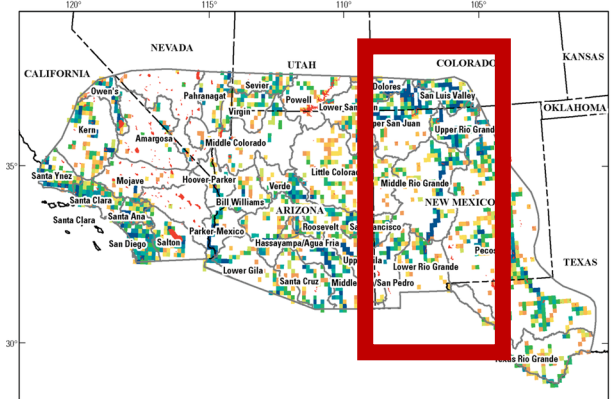


Stahlke et al., 2021

D. carinata *D. carinulata*

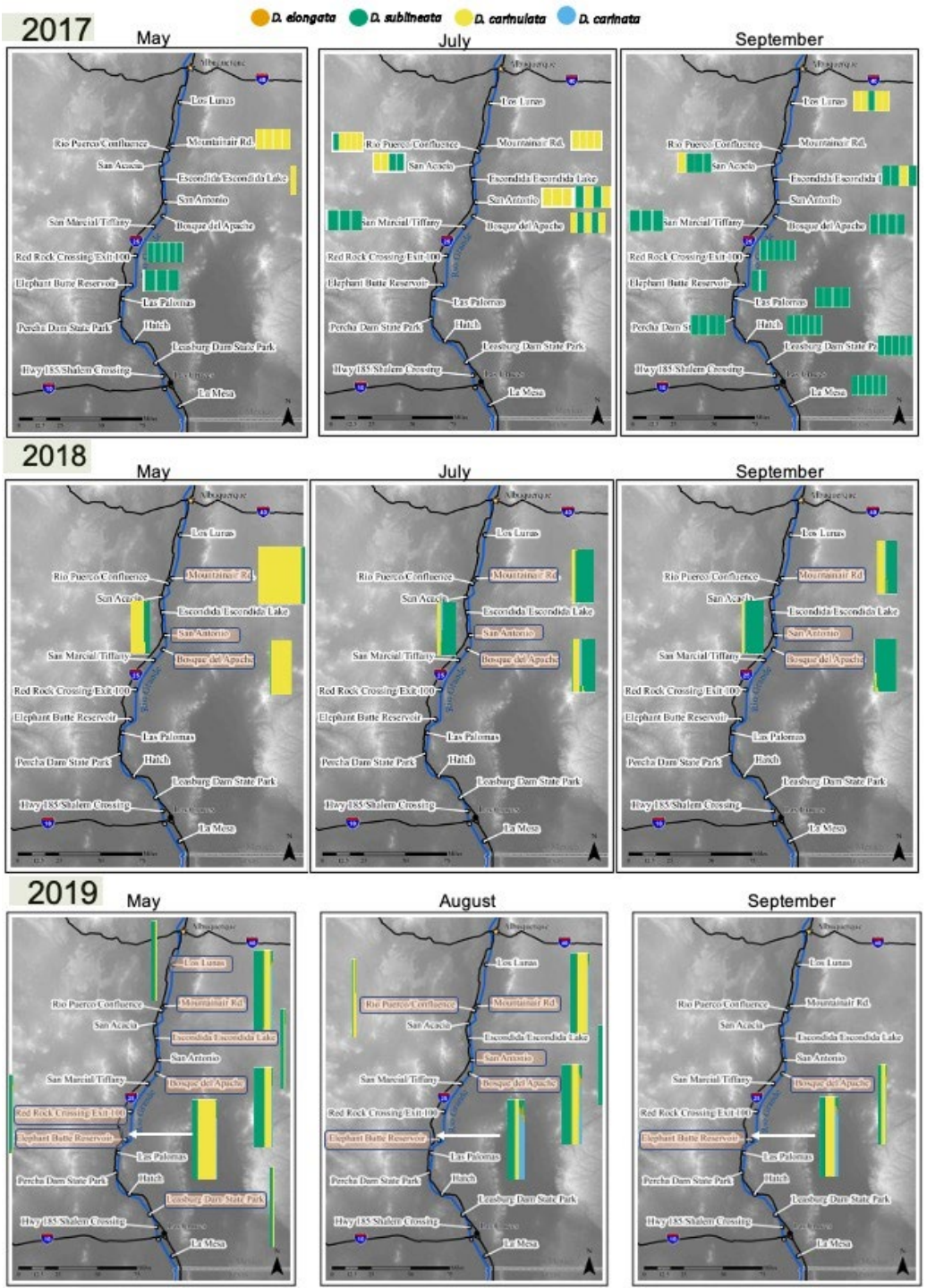
D. sublineata *D. elongata*

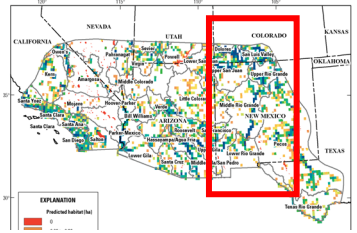
Clark EI et al., 2023. Fitness and host use remain stable in a biological control agent after many years of hybridization. *Biological Control*.



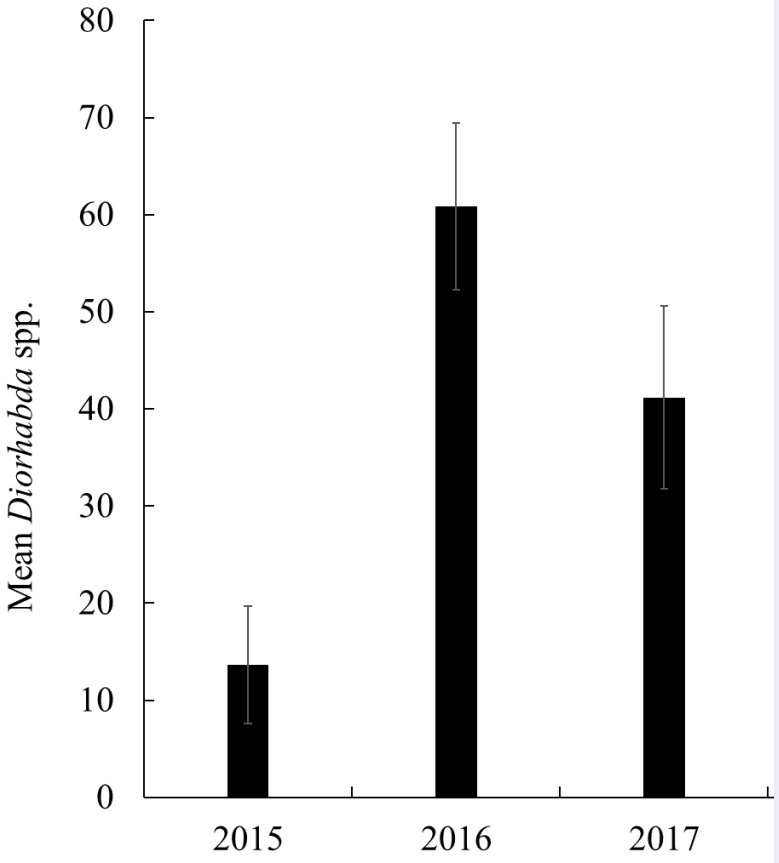
Monitoring the Rio Grande 2017-2019 with genomic data:

- Relative abundance differed between May and September until 2019
- *Dcarinu* and *Dsub* boundaries were maintained
  - Both occupied full reach





Did range overlap cause a decline in beetle populations?



Genetic Monitoring of Tamarisk Biocontrol Agents (*Diorhabda* spp.) along the Gila River using Mitochondrial CO1 Barcoding

Francisca Esquivel[1], Zeynep Ozsoy[1], Matthew J. Johnson[2], Amanda Stahlke[1]

1: Department of Biological Sciences, Colorado Mesa University, Grand Junction, CO
2: EcoPlateau Research, Bend, OR

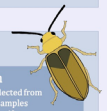
Background

Four tamarisk beetle species (*Diorhabda* spp.) were introduced to the western United States in 2001 as biological controls agent for invasive tamarisk (*Tamarix* spp.). Now widespread colonization of tamarisk beetles could impact the recovery of the federally listed southwestern willow flycatcher (SWFL, *Empidonax traillii extimus*) along the Gila River, with *D. carinulata* coming from the west and *D. sublineata* coming in from the east (Fig. 3) .

D. carinulata and *D. sublineata* were generally unable to form successful hybrids in lab. Those that successfully produced eggs had low viability (Bean et al., 2007). Despite this, hybrids have been observed along the Rio Grande: 10 in 2018 and one in 2019; though since 2020, no more have been observed (Ozsoy et al., 2020).

Based on these studies we predict that *D. carinulata* and *D. sublineata* would exhibit competitive exclusion along the Gila River, rather than hybridization, which could have major implications for the recovery of SWFL.

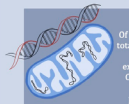
Methods



Collection

A total of 424 samples were collected from May to early September 2023. Samples were chosen based on stage (Fig. 1), location along the Gila (Figs. 2-6) , and which month they were collected in. Specific months (Table 1) were chosen as to test differences in phenology among species (Bean et al., 2013).

Extraction



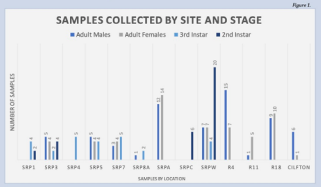
Of the 424 samples received, a total of 178 were used for DNA extraction. After DNA extraction, the mitochondrial CO1 sequence was amplified using PCR. The resulting products were then sent for Sanger sequencing.

Identification

The samples were then compared to the GenBank database available through the NIH NCBI BLAST for identification.

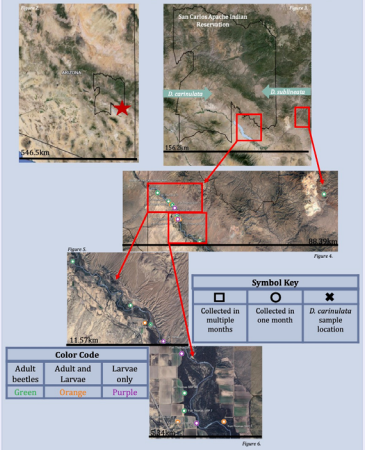
Results

Of the 178 samples extracted for DNA:
163 were identified as *D. sublineata*,
and one was identified as *D. carinulata*.
15 samples failed to amplify via PCR. Maps (Figs. 2 -6) and Table 1 below indicate sampling locations, dates, and life stage collected at each site.



Collection Sites by Month & Stage

	May		June		July		August
							31 adult males (1 *D. carinulata*),
SRP_7	5 adult Male, 5 2nd instar	Clifton	6 adult male, 5 adult female	SRP 5	2 adult males, 5 adult female	FT SRPA	14 adult females
SRP 4	5 3rd instar	SRP5	3 Adult males, 3 adult females	R4	15 Adult males, 7 adult females	R18	5 adult males, 5 adult females,
SRP C	6 2nd instar	R11	5 adult male, 5 adult females			SRP W	7 adult males, 7 adult females, 3 3rd instar, 15 2nd instar
	5 third instar,		4 adult males,				
SRP W	5 2nd instar	R18	5 adult females				
			5 adult males,				
SRPS	3 3rd instar	SRP 3	4 adult females				
	4 3rd instar,		2 adult males,				
SRP 1	2 2nd instar	SRP 7	4 adult females				
	2 3rd instar,						
SRP 3	4 2nd instar						
SRP8	1 adult male, 2 3rd instar						



Discussion

Historically, *D. carinulata* were not predicted to establish at lower latitudes due to premature diapause during the early summer (Bean et al., 2007). However, evolution in *D. carinulata* diapause has allowed the species to expand its range (Bean et al., 2013). The continued expansion of *D. carinulata* from the west was predicted to eventually collide with the *D. sublineata* from the east as shown in figure 2 (Ozsoy et al., 2020).

We were surprised to detect only a single *D. carinulata* in our sampling and almost no overlap with *D. sublineata*.

We may be observing a similar outcome in southern Arizona along the Gila River as was observed along the Rio Grande. Indeed, 2023 field observations suggested that *Diorhabda* populations declined between Phoenix and the San Carlos Reservation. This could be due to competitive exclusion between the two species.

D. sublineata has the potential to become the dominant species along the Gila River, as nearly all samples identified as *D. sublineata*, with only one exception. *D. sublineata* could have a greater impact on tamarisk and SWFL due to its larger size, greater fecundity, and longer seasons compared to *D. carinulata*.

Future Directions

We will select a subset of samples to be processed for whole genome sequencing analysis. Whole genomes will provide insight to hybridization status.
Mitochondrial CO1 sequencing will continue to be used in future projects as it an effective method to gain accurate results quickly.

Acknowledgements

We thank Adam Stein (Arizona State U) for his help in collecting the 424 samples we received. We also thank RiversEdge West and USDA-APHIS for the funding provided to perform this work.

References

Bean, D. W., Dalin, P., & Dudley, T. L. (2012). Evolution of Critical Day length for Diapause Induction Enables Range Expansion of Diorhabda carinulata, a Biological Control Agent against Tamarisk (Tamarix spp.). *Evolutionary Applications*, 13.

Bean, D. W., Dudley, T. L., & Keller, J. C. (2007). Seasonal Timing of Diapause Induction Limits the Effective Range of Diorhabda elongata deserticola (Coleoptera: Chrysomelidae) as a Biological Control Agent for Tamarisk (Tamarix spp.). *Entomological Society of America*, 10.

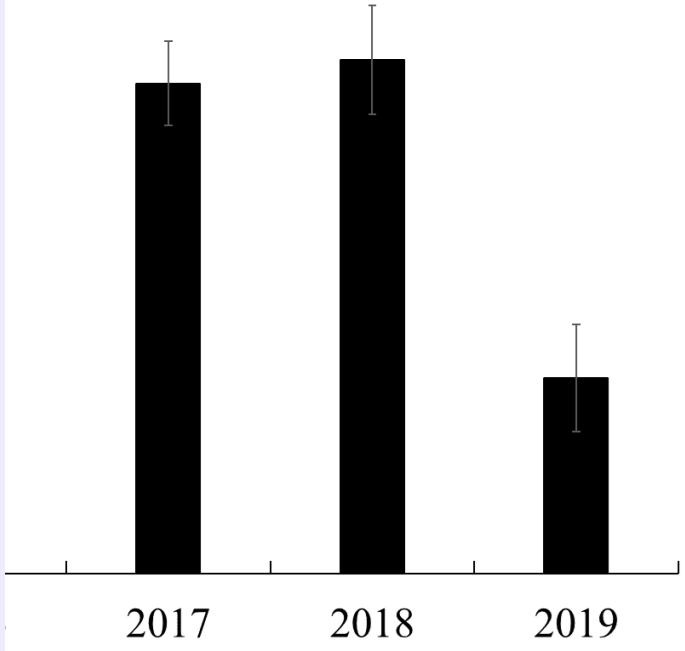
Ozsoy, Z., Stahlke, A., & Johnson, M. J. (2020). *Species Genetic Identification and Hybridization Analysis of Tamarisk Beetle (Diorhabda spp.) in the Lower Colorado Basin and along the Rio Grande, NM Watershed*. 2020. Grand Junction.

Ozsoy AZ, Stahlke AR, Johnson M. (2021). Genetic Identification and Hybrid Analysis of Tamarisk Leaf Beetles (Diorhabda spp.) along the Rio Grande River NM watershed. Contract No. W912PP-15-D-0023, Task Order No. W912PP17T0013. Delivered to Tetra Tech, Albuquerque, NM.

Stahlke, A., Bitume, E. V., Ozsoy, Z. A., Bean, D. W., Vellet, A., Clark, M. L., . . . Heinlein, P. A. (2021). Hybridization and Range Expansion in Tamarisk Beetles (Diorhabda spp.) Introduced to North America for Classical Biological Control. *Wiley*, 15.

COLORADO MESA UNIVERSITY

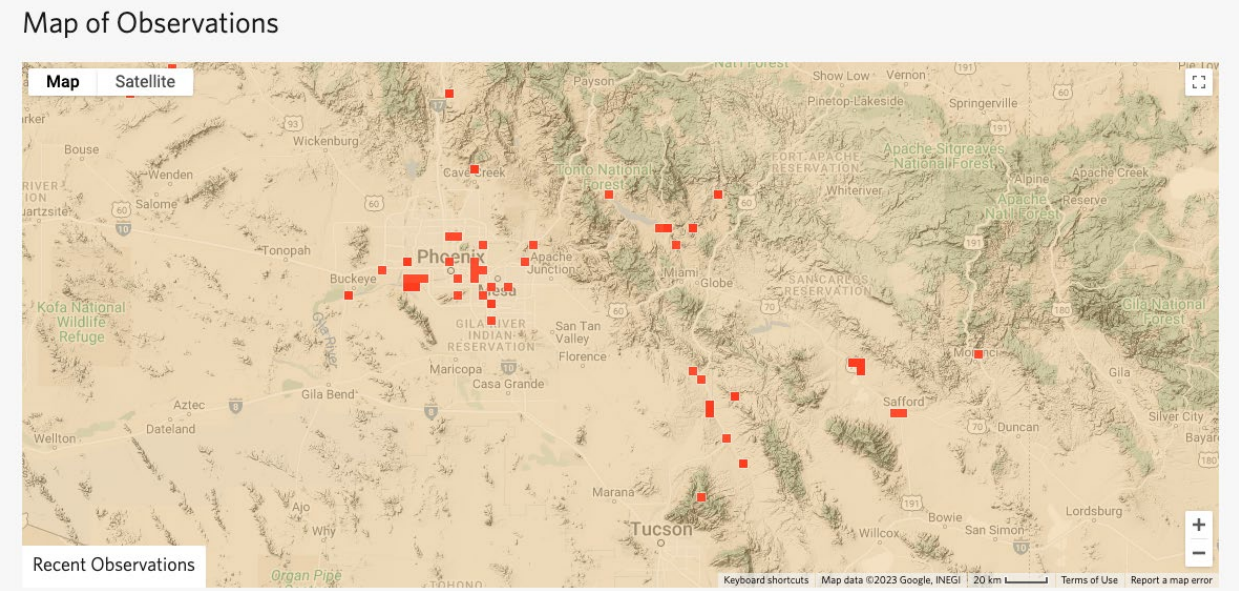
RiversEdge West
RESTORE + CONNECT + INNOVATE



Restoration practitioners can still use beetle monitoring to:

- Prepare for the onslaught
  - Better opportunities to plant
  - Divert precious resources elsewhere
  - Monitor and work to protect vulnerable wildlife to assess impact
- Diagnose need for intervention
  - Ramp up manual removal
  - Pheromones to attract, keep beetles in area longer, or deter

Presence/absence is expensive and insufficient. We need to and can do better (with less?)



- iNaturalist
  - You know the Christmas bird count… 4th of July Beetle Count?!
  - An opportunity to increase public awareness & easy/fun outreach event

https://www.inaturalist.org/projects/tamarisk-beetles-biocontrol-agents

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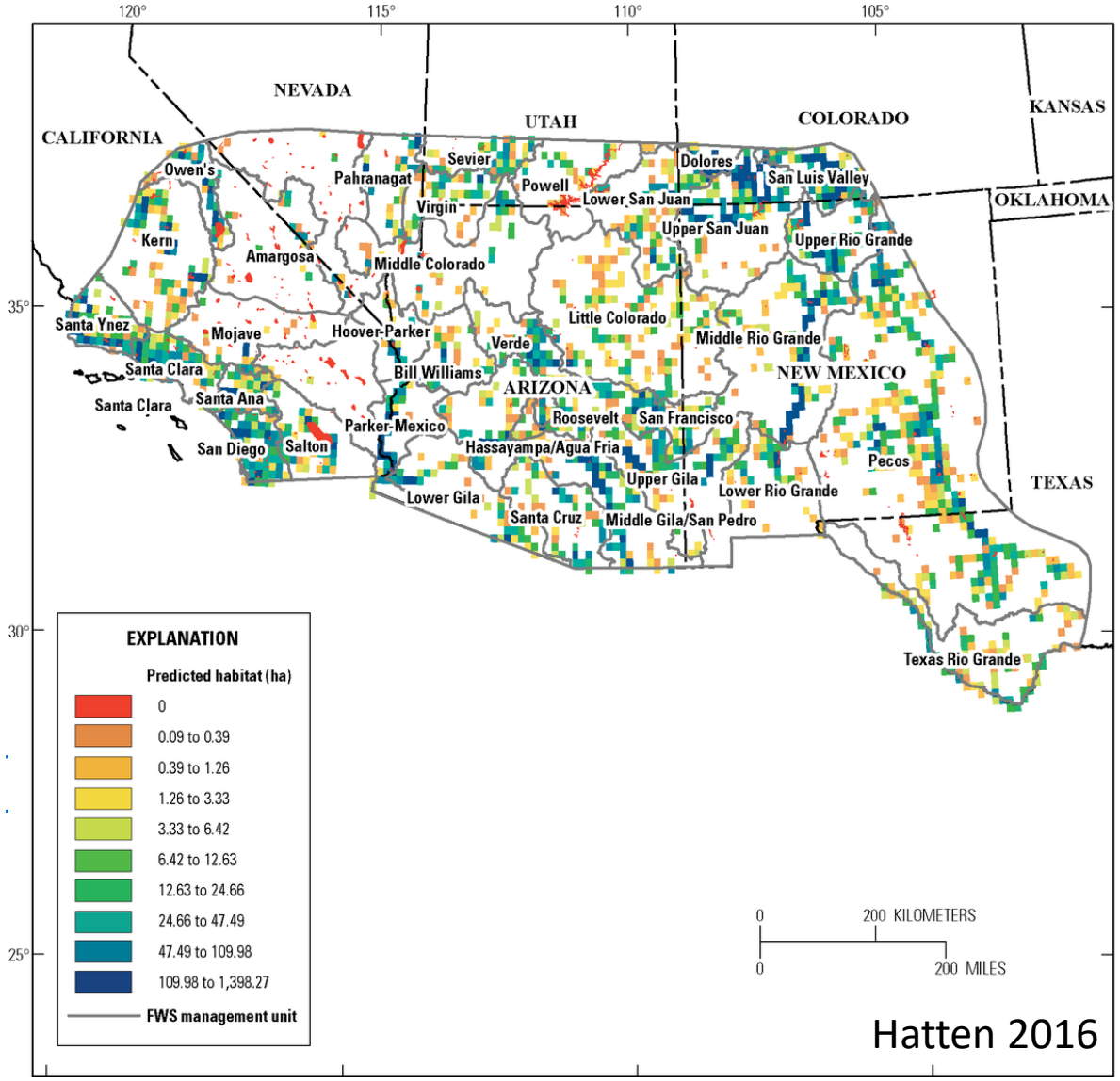


Hatten 2016

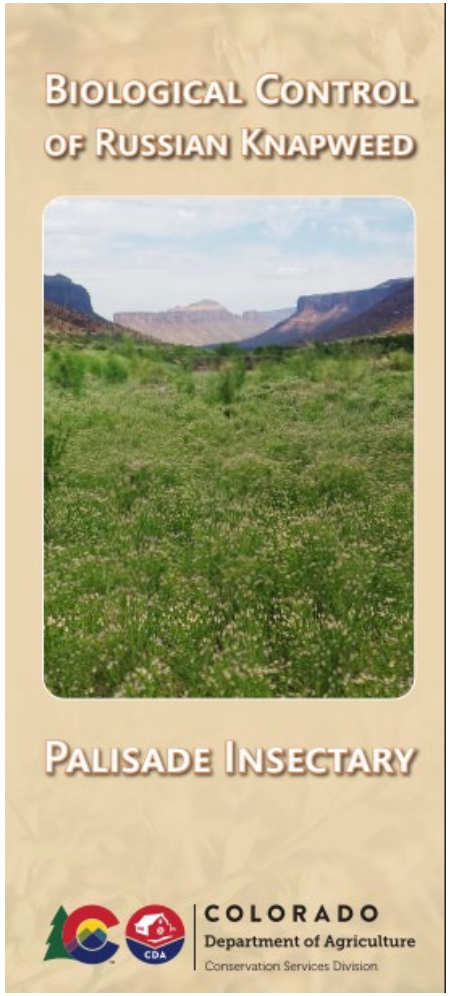
- iNaturalist
  - You know the Christmas bird count… 4th of July Beetle Count?!
  - An opportunity to increase public awareness & easy/fun outreach event
- Remote sensing (Changes in NDVI through Landsat)
- Collaboration, collaboration, collaboration

Alright, the beetles are basically everywhere now. This is difficult to fund and maintain.

But we still need to monitor and maintain these data to understand the variation and evolution within the system and biocontrol more broadly.

We can be more strategic.

The data we gather about *Diorhabda* inform biocontrol broadly, and they are not the only players in riaparian systems



https://www.cabi.org/projects/stemming-the-spread-of-russian-olive/

Thanks!
Questions, feedback, and collaboration welcome at:

astahlke@coloradomesa.edu

Check out REW's interactive 2023 AGOL map

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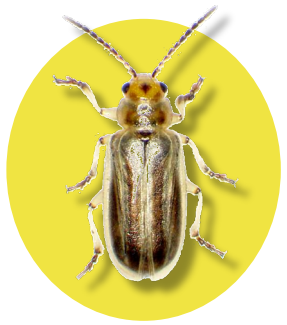

Long days

Dcarinu has adapted to shorter days and longer hot summers in the south