HOST COLONIZATION AND FLIGHT PATTERNS OF THE WALNUT TWIG BEETLE IN NEW MEXICO

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Study Goals

Flight Pattern Study

- Determine the flight periodicity of WTB in its native range across an elevational gradient and between two local host species

Host Preference Study

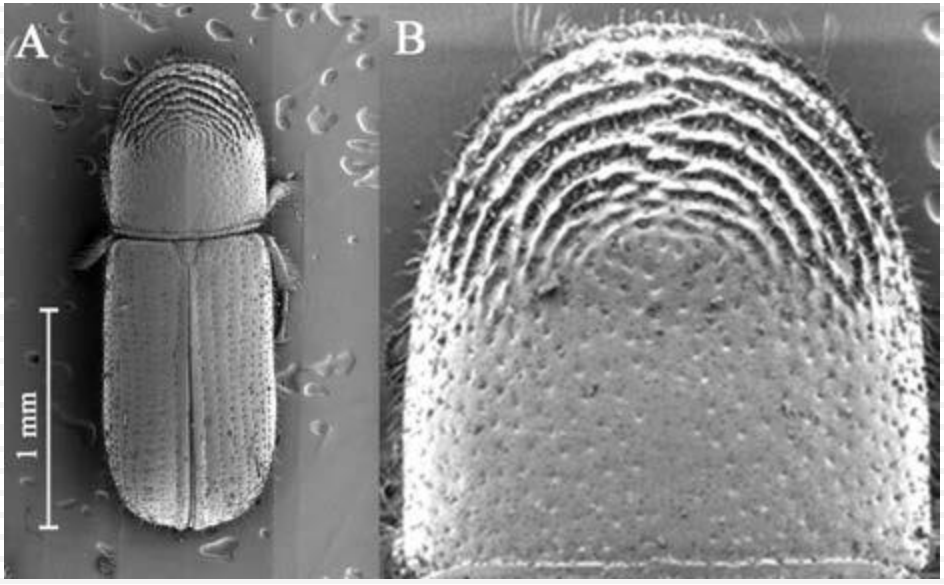
- Determine the attack density and productivity of WTB among different *Juglans* species
- Investigate Differences in WTB host preference between CA and NM populations

Nematode Study

- Investigate nematodes found inside WTB and within WTB galleries
- Are WTB-associated nematodes parasites, phytopathogens or other?

Walnut Twig Beetle

Pityophthorus juglandis

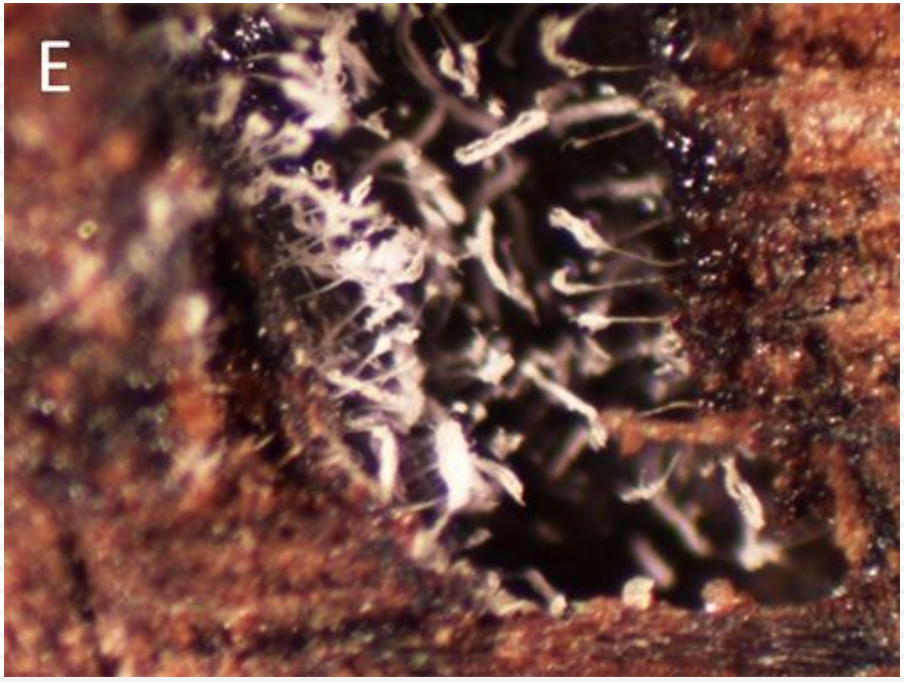


Andy Graves, USDA FS

Stacy Hishinuma, UC Davis

Thousand Cankers Disease

WTB + *Geosmithia morbida*



Ned Tisserat, CSU

Photo credit: Ned Tisserat and Whitney Cranshaw, CSU

TCD damage

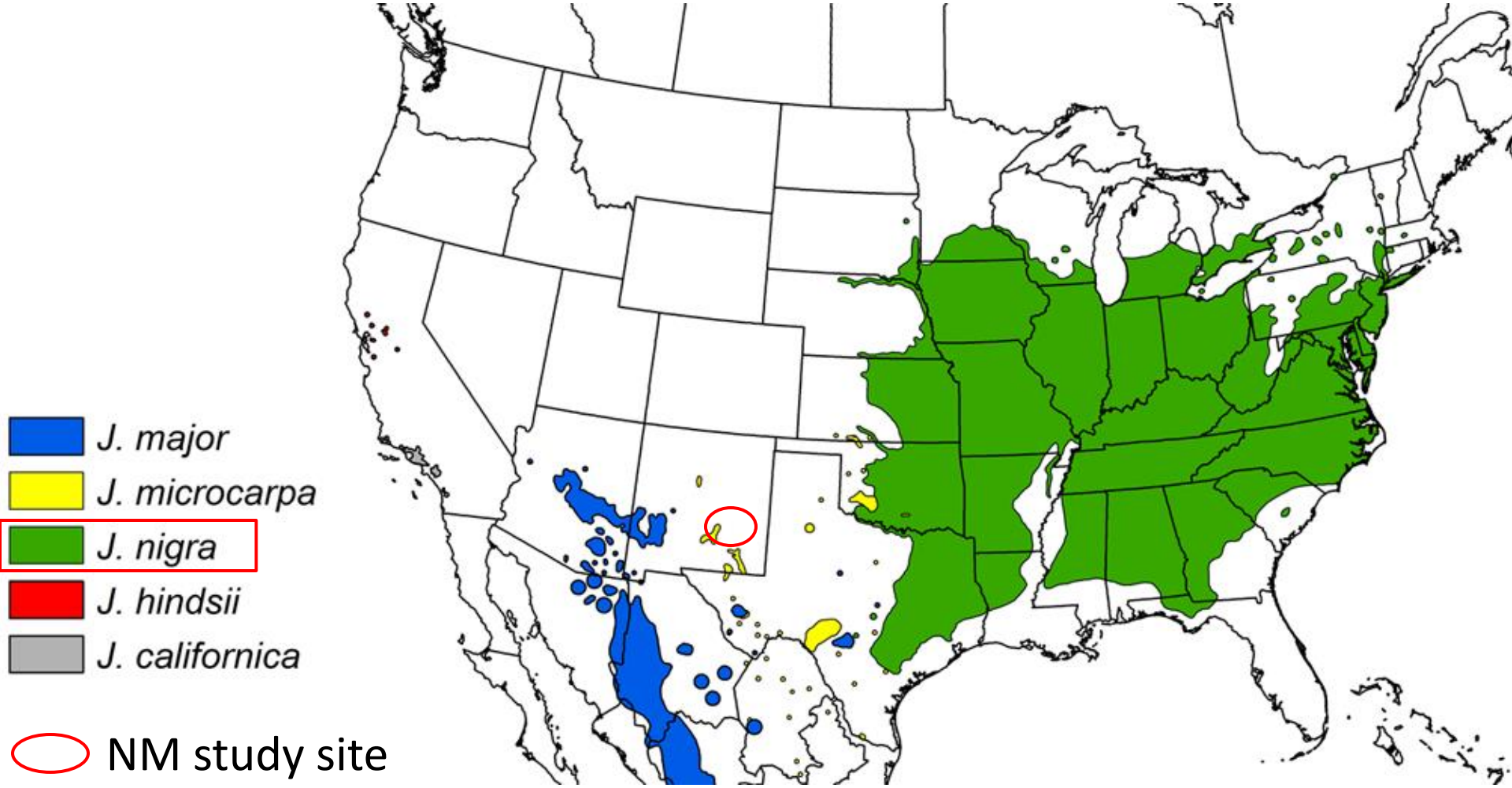
Geosmithia morbida creates oblong cankers in the phloem around beetle galleries. The cankers often coalesce and kill the branch

Photo credit: Corwin Parker

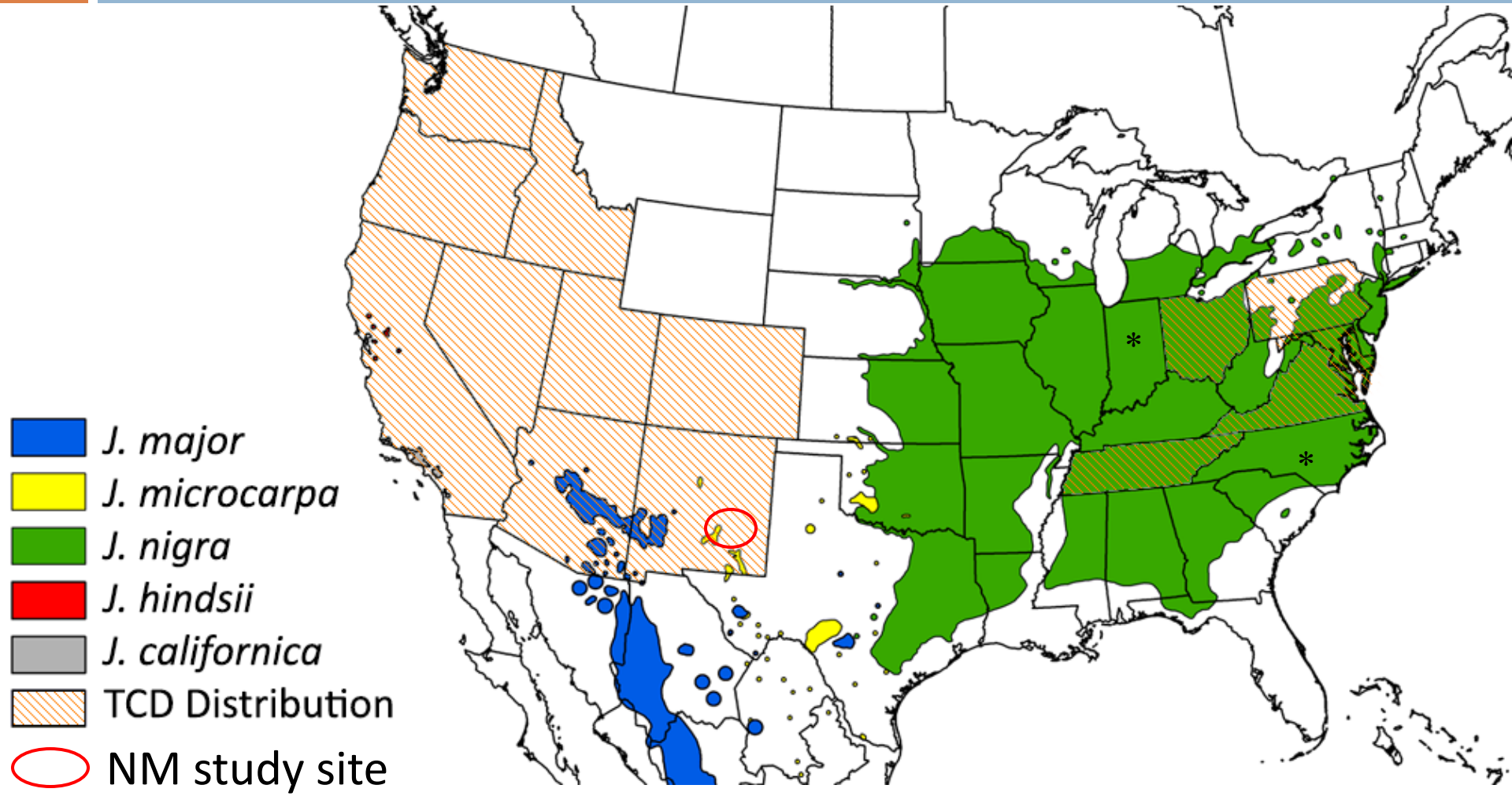
TCD damage

Crown dieback and epicormic sprouting are evident in advanced cases of TCD. Photo of *Juglans major* in Lincoln County, New Mexico.

Native walnut distributions



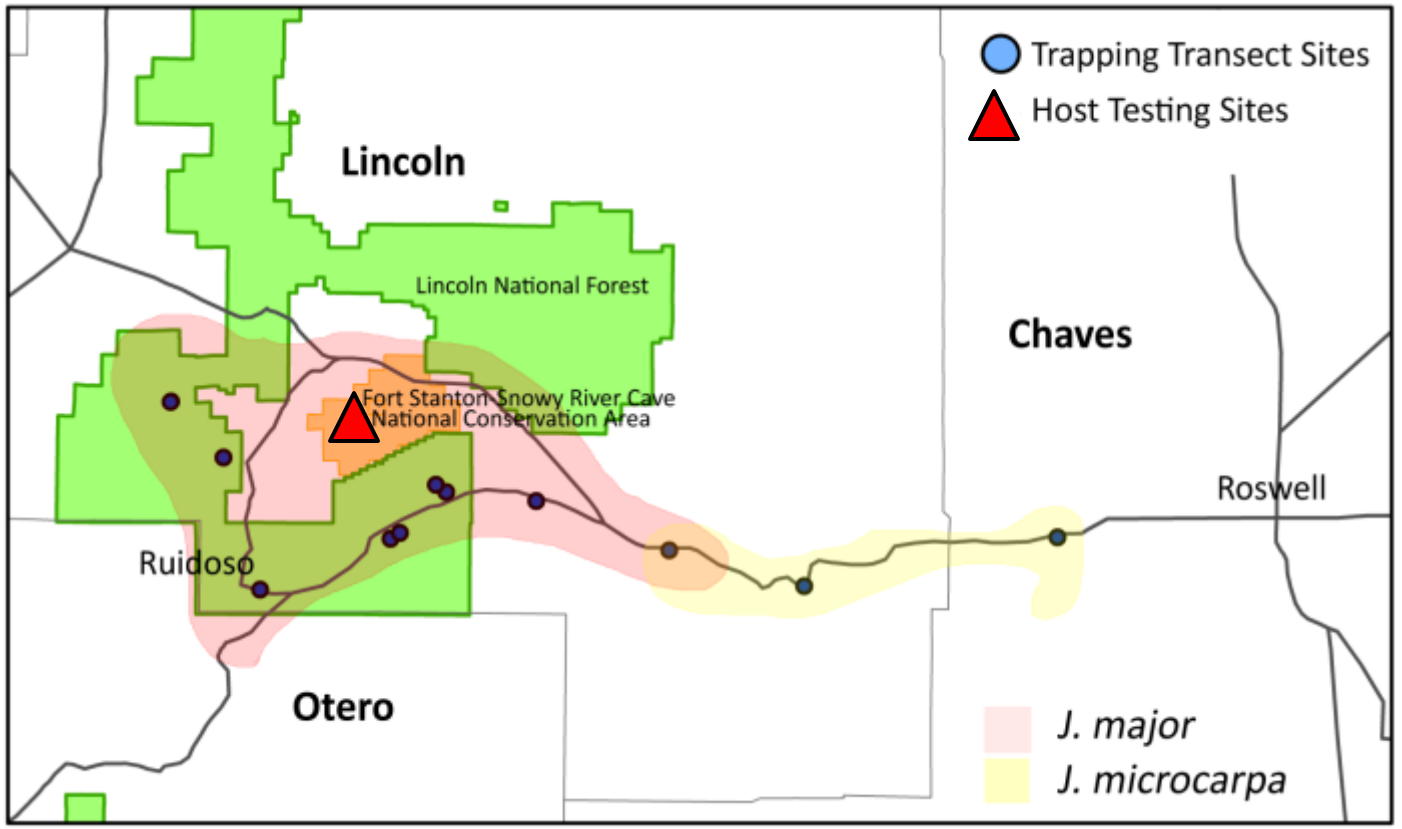
Walnut and TCD distributions



Flight pattern study

Determine the flight periodicity of WTB in its native range across an elevational gradient and between two local host species

Figure credit: Andy Graves

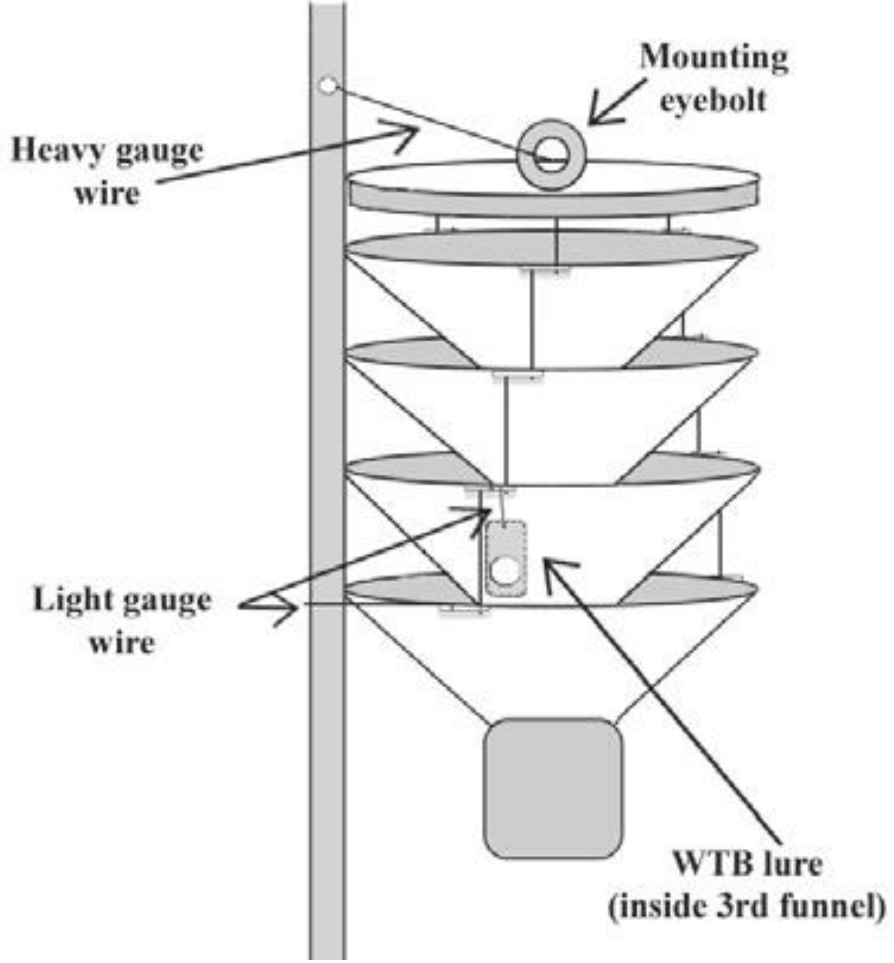


Methods

Study Site

Twelve traps were set up along a transect starting in the Sierra Blanca Mountains of New Mexico near Ruidoso (elev. 2300 m) and running eastward along U.S. route 70 to the desert near Roswell (elev. 1200 m)

Photo credit: Steven Seybold



Methods

Flight Traps

Flight traps used were 4-unit Lindgren funnel traps, baited with synthetic WTB aggregation pheromone

Collection cup filled with antifreeze

Photo credit: Steven Seybold

Methods

Flight Traps

Traps were emptied weekly from April through December, 2013, and from January to the present, 2014

Photo credit:
Joe Boggs
Corwin Parker

Methods

Sorting

WTB were sorted each week and both sexes were counted and stored in ethanol

Sometimes other insects got in the way

Photo credit: Corwin Parker

Methods

Juglans major

Highest elevation flight trap in trapping transect, illustrating trap located 3-5 m from an old growth *J. major* in a mixed oak-conifer forest.

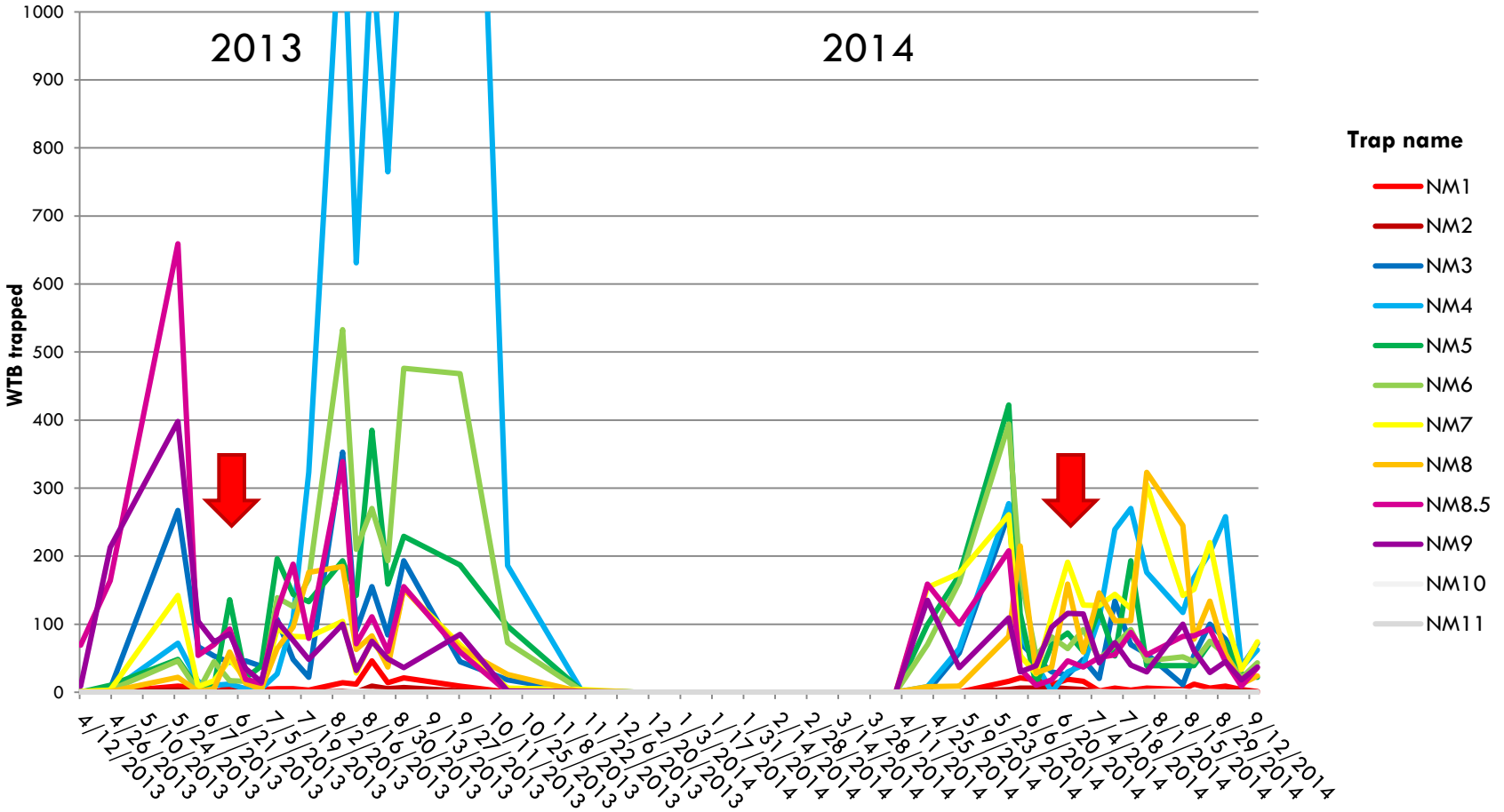
Photo credit: Corwin Parker

Methods

Juglans microcarpa

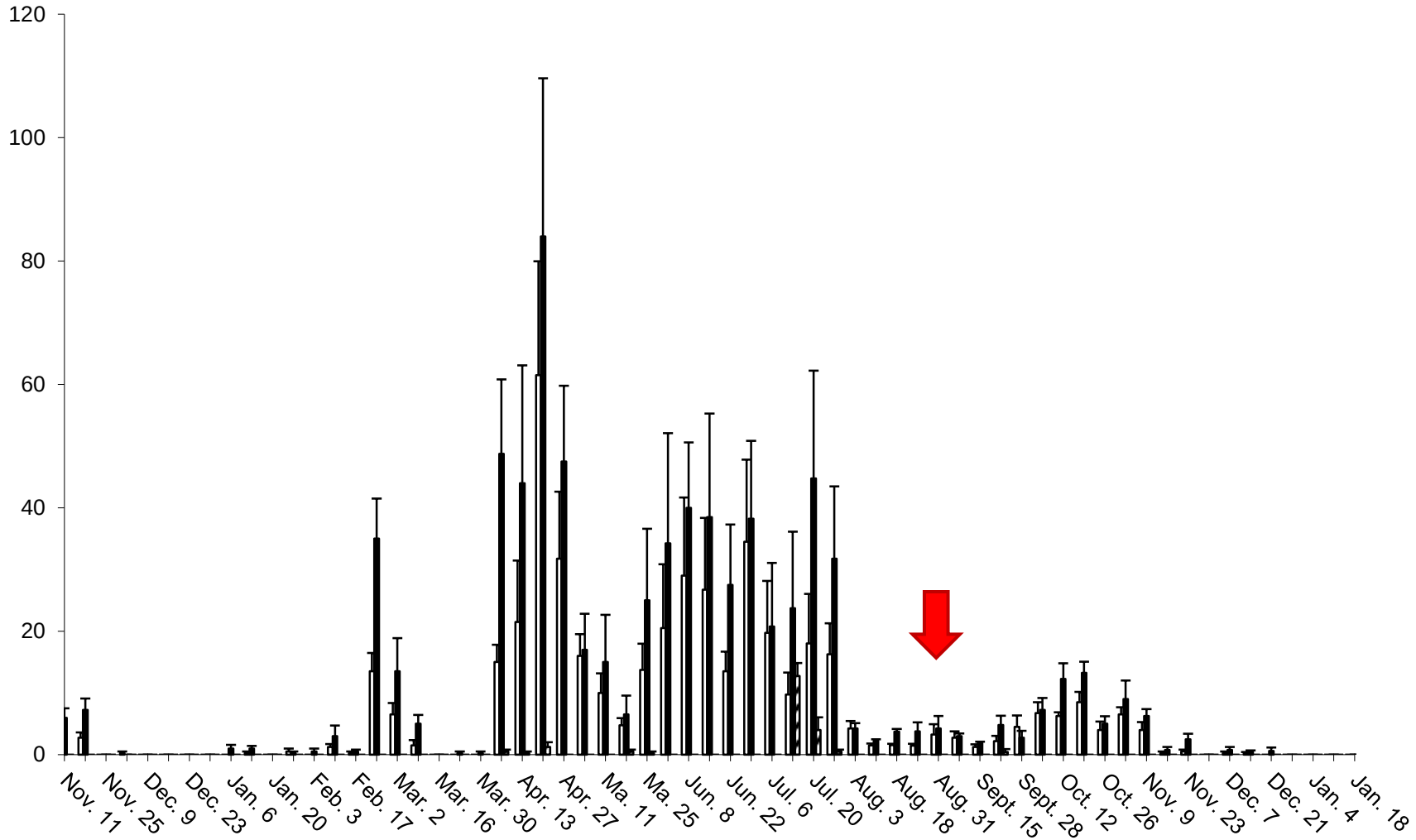
Low elevation site in the trapping transect, illustrating trap located next to a multi-stemmed *J. microcarpa* in an open desert riparian habitat.

Results: Flight Patterns By Time

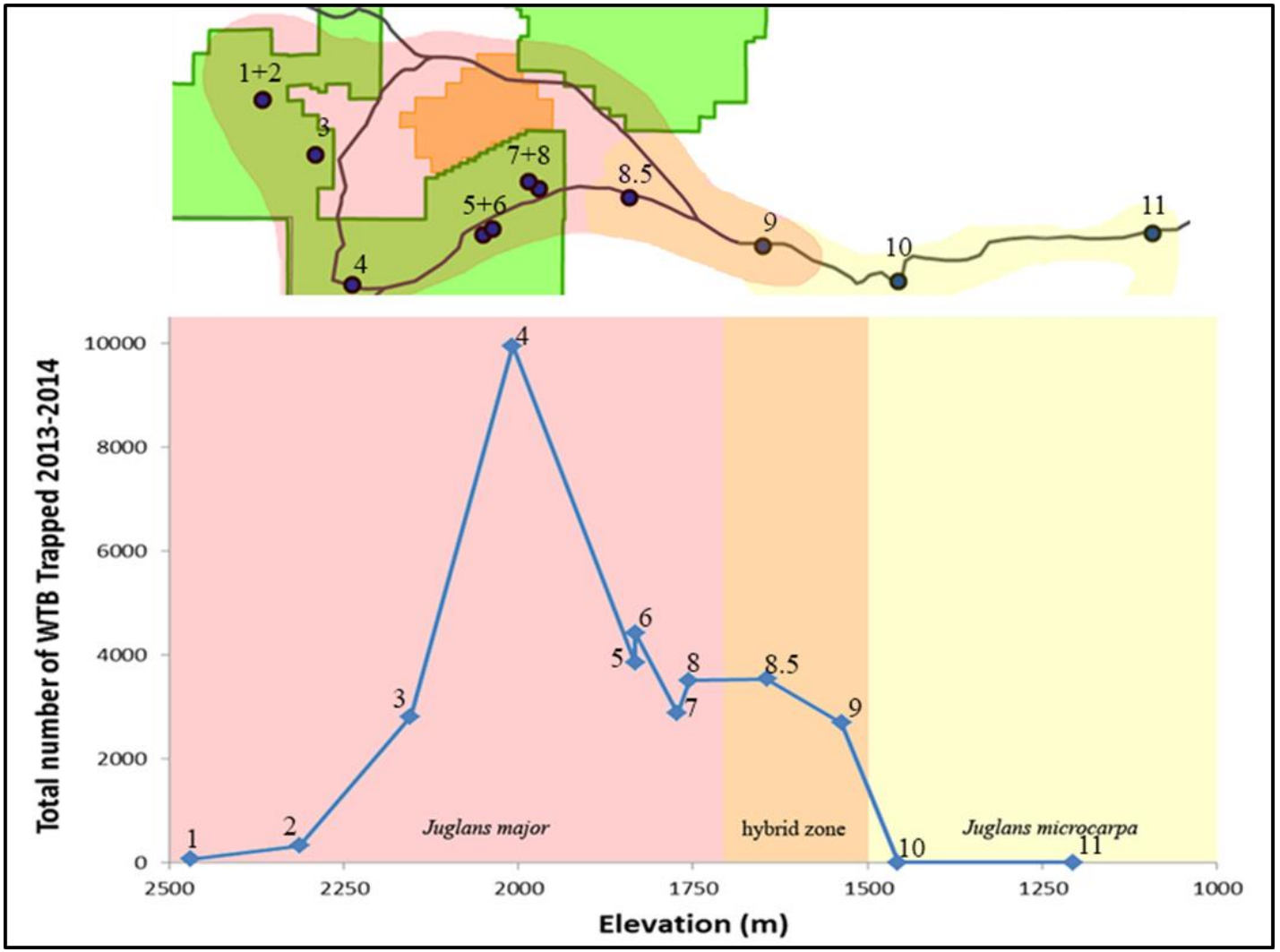


Two major flight peaks per year, separated by dry season during June (red arrows)

California Flight Patterns 2012-13



Results: Flight Patterns By Elevation



Discussion

- In New Mexico and California, WTB has two major flight peaks during the year
- The first flight peak likely represents the emergence of overwintering adults and larvae that develop in the spring
- The second flight peak likely represents adults that have developed early from the current year's brood
- Greater WTB flight activity at the higher elevation sites
- Greater flight activity associated with *J. major*
- Lower flight activity at the highest elevation sites due to low host density?

Host Preference Study

Determine the attack density and productivity of WTB among different *Juglans* species

Investigate differences in WTB host preference between CA and NM populations

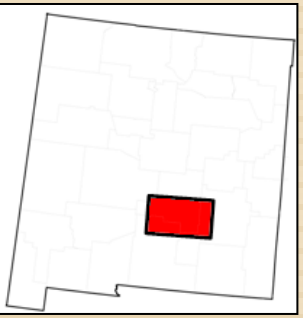
Photo credit: Stacy Hishinuma

Methods

California Study Site

California: Wolfskill Experimental Orchards, USDA ARS National Clonal Germplasm Repository, Winters, Solano County, California

Photo credit: Corwin Parker



Methods

New Mexico Study Site

Fort Stanton Snowy River Cave, National Conservation Area, Lincoln County

J. nigra
Eastern black walnut

J. microcarpa
Little black walnut

J. major
Arizona walnut

J. californica
Southern CA black walnut

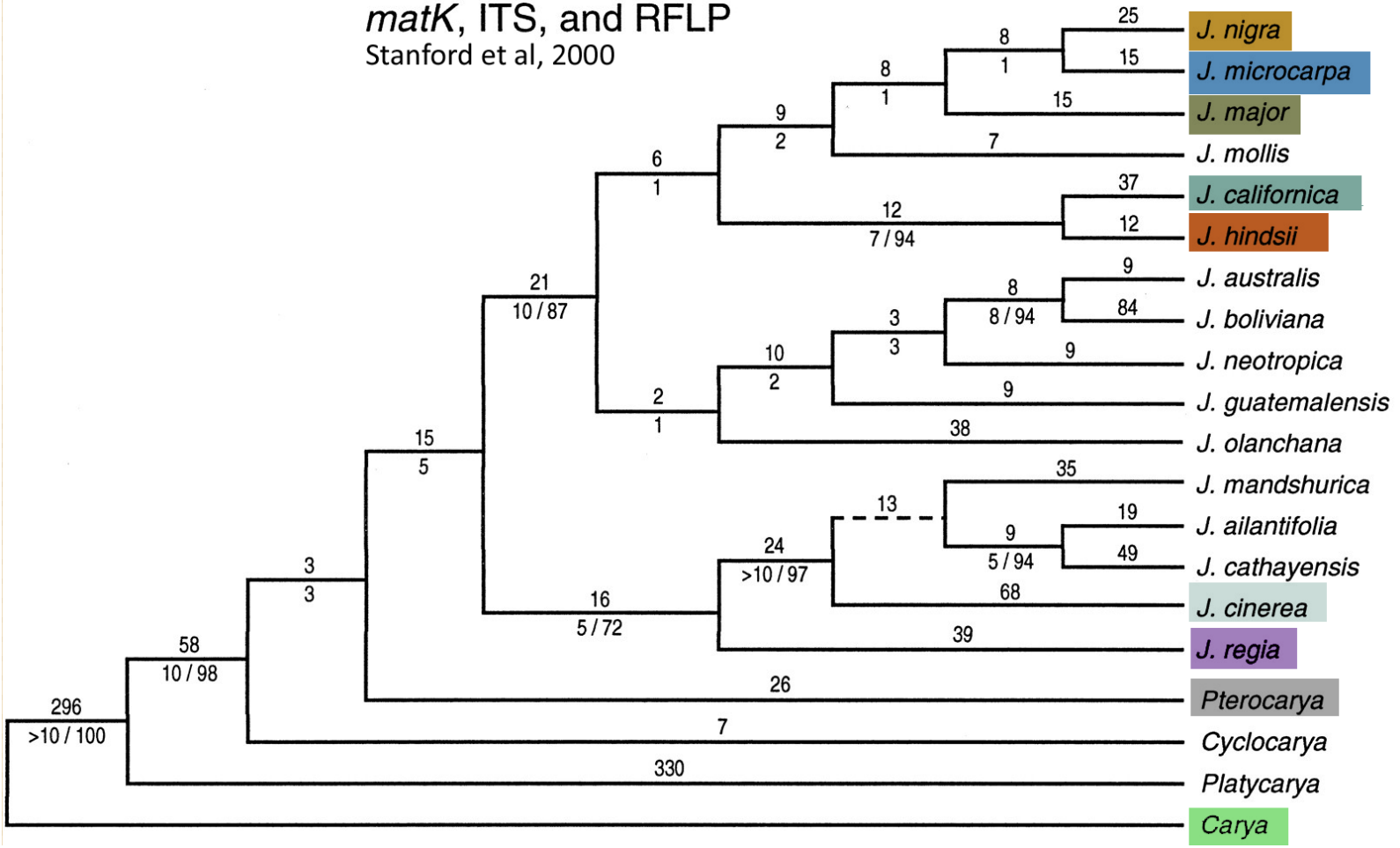
J. hindsii
Northern CA black walnut

J. regia
English walnut

J. cinerea
Butternut

P. stenoptera
Chinese wingnut

C. illinoinensis
Pecan



Methods

Tree Species

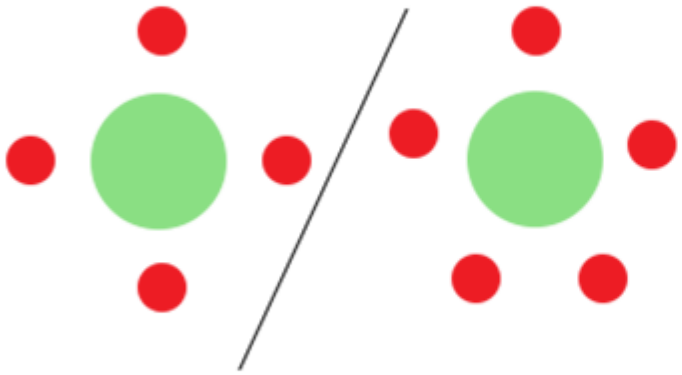
All studies included *J. major*, *microcarpa* and *hindsii*, as well as 2-3 other walnut or related species

10 branches per species per trial

18 branches per species in the 2014 NM trial

New Mexico study site set-up

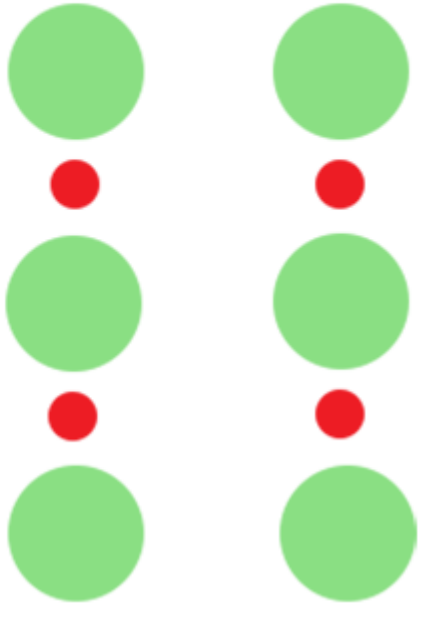
2013 2014



Juglans tree
(*J. major* in NM, *J. hindsii* in CA)

Baited branch

California study site set-up



Methods

Branch Set-up

Completely randomized design

Photo credit: Corwin Parker

Methods

Branch Set-up

Photo illustrating a branch trap with *J. hindsii* with pheromone bait pinned to the bottom

Branches were left in field for 2-3 weeks, after which all entrance holes were counted

Photo credit: Corwin Parker

Methods

Rearing Cages

After branches were removed from field, they were cut in half and placed in ventilated rearing cages

Photo credit: Corwin Parker

Methods

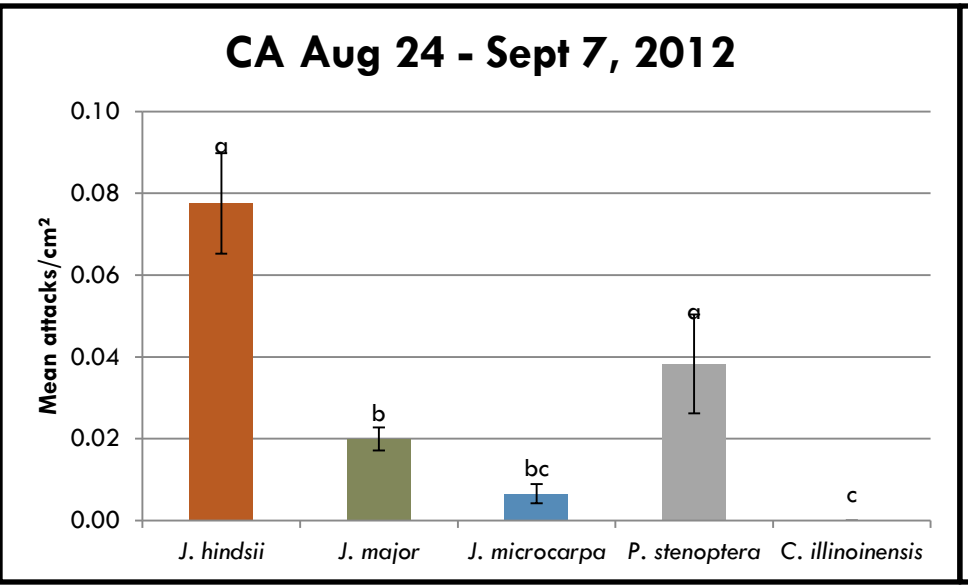
Emerging Adults

Every week emerged adults were removed from cages and the sexes were counted

Parental vs brood adults

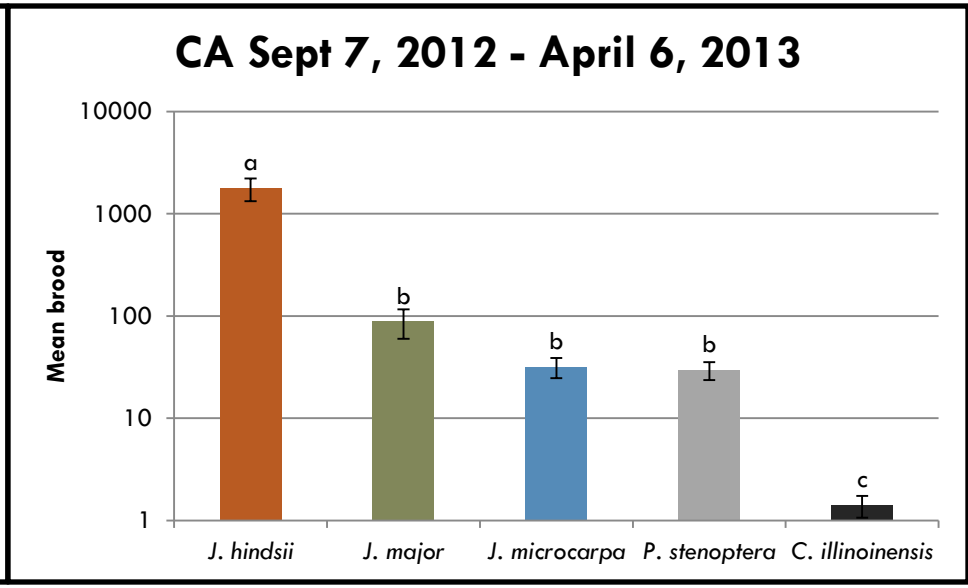
Response of WTB to Five Hosts in the Field, Wolfskill Experimental Orchards, California, August, 2012

Attack Density



Kruskal-Wallis Test;
$\chi^2 = 41.571$; $DF = 4$; $P<0.001$

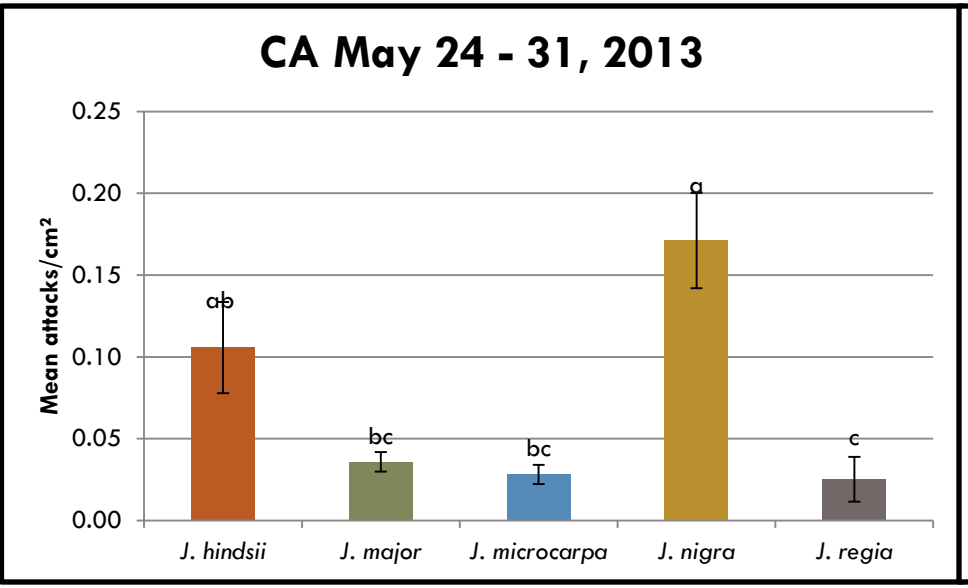
Productivity



ANOVA on transformed (data = log(brood+0.1));
$F=57.18$; $DF=4$; $P<0.001$

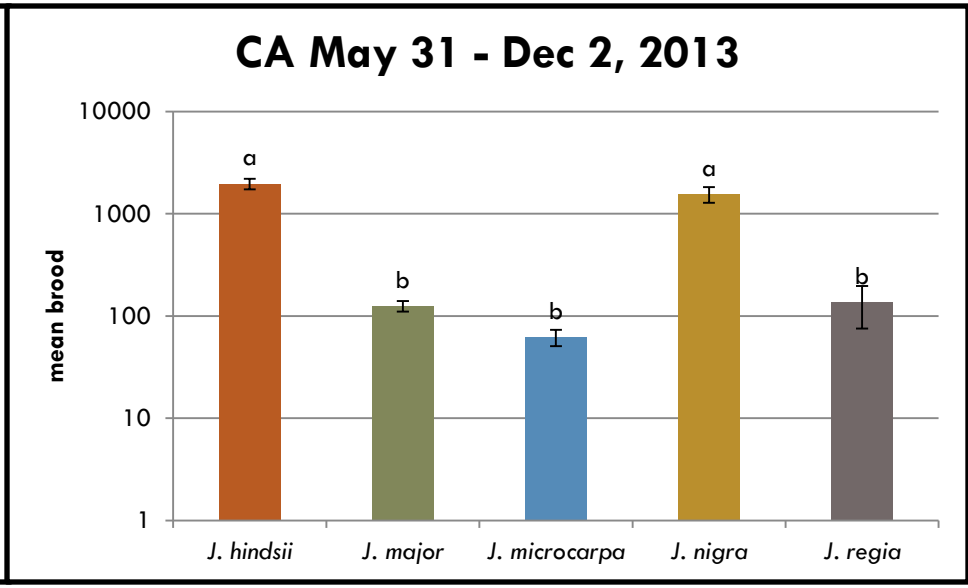
Response of WTB to Five Hosts in the Field, Wolfskill Experimental Orchards, California, May, 2013

Attack Density



Kruskal-Wallis Test; $\chi^2 = 25.9645$; *DF*=4; *P*<0.001

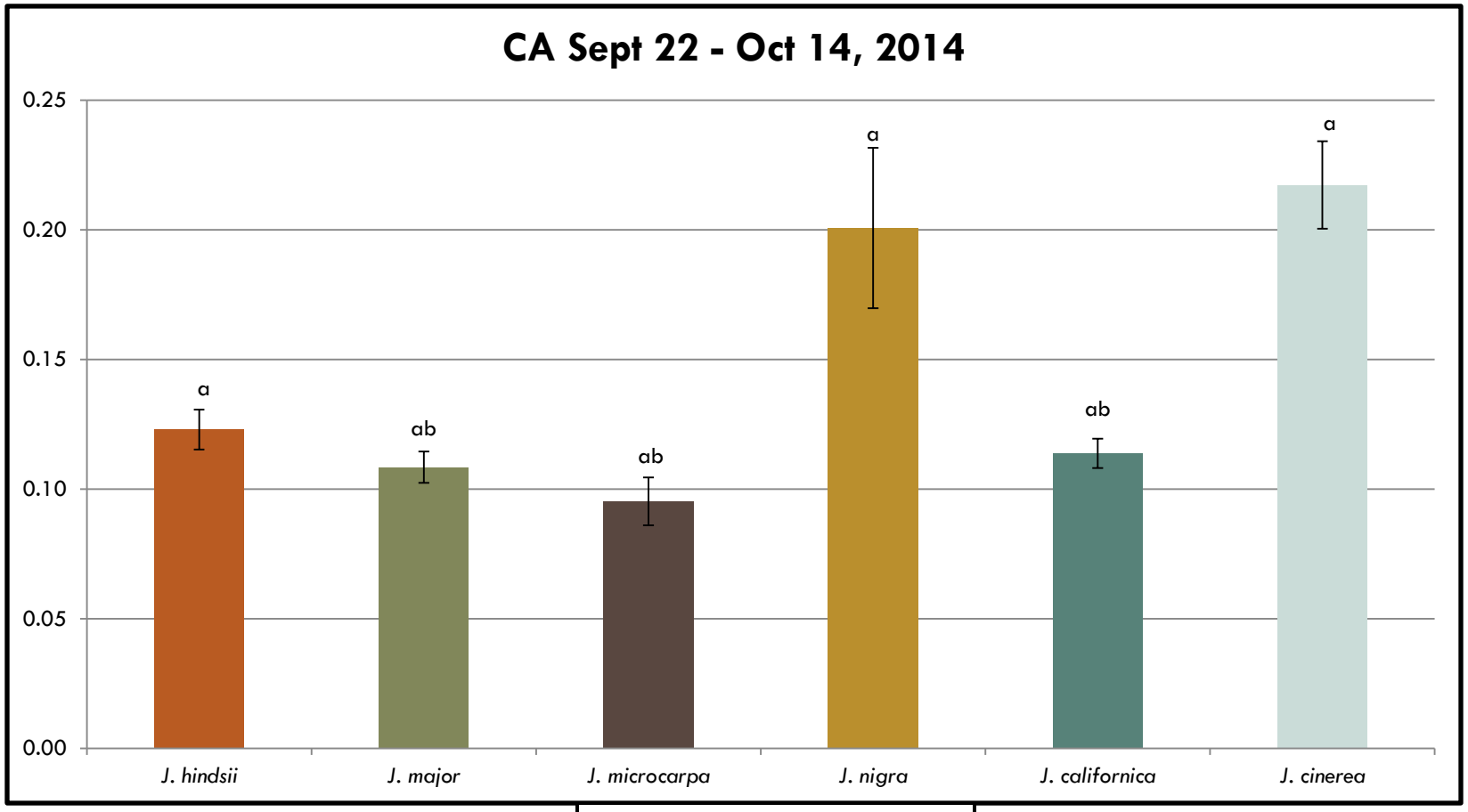
Productivity



ANOVA; *F*=31.83; *DF*=4; *P*<0.001

Response of WTB to Six Hosts in the Field, Wolfskill Experimental Orchards, California, September, 2014

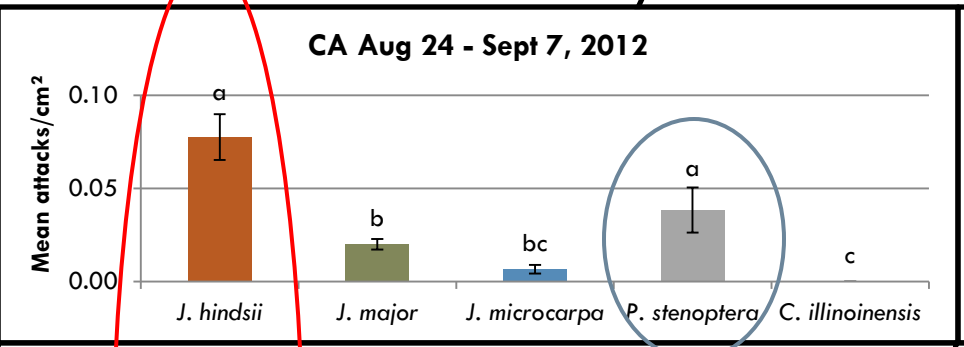
Attack Density

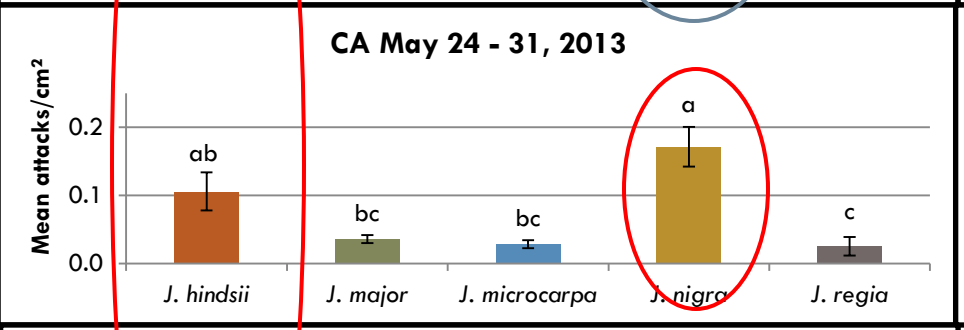


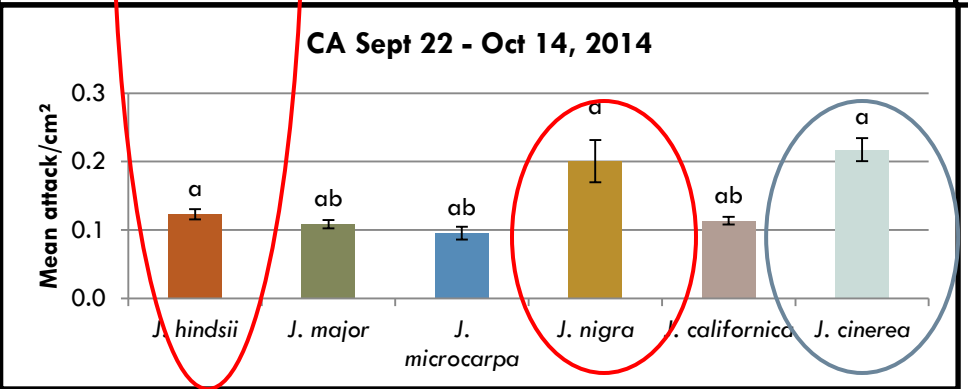
Kruskal-Wallis Test;
$\chi^2=21.9121$; $DF=5$; $P<.001$

Response of WTB to Five or Six Hosts in the Field, Wolfskill Experimental Orchards, California, 2012-2014

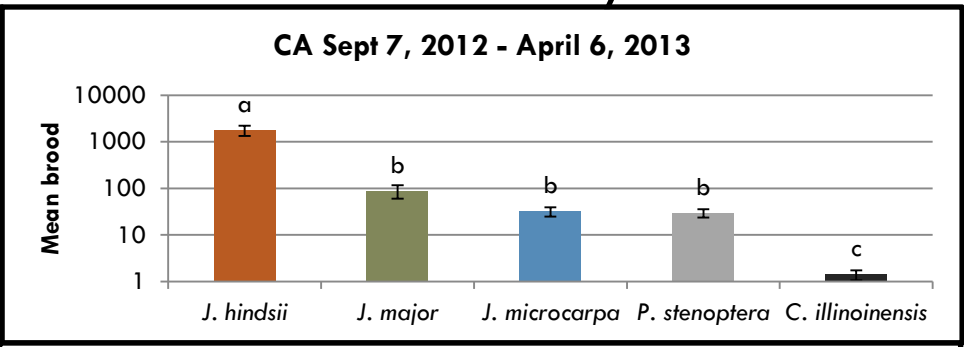
Attack Density

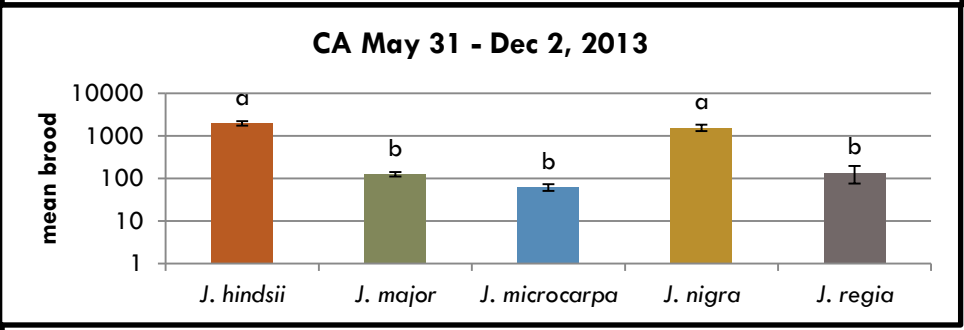






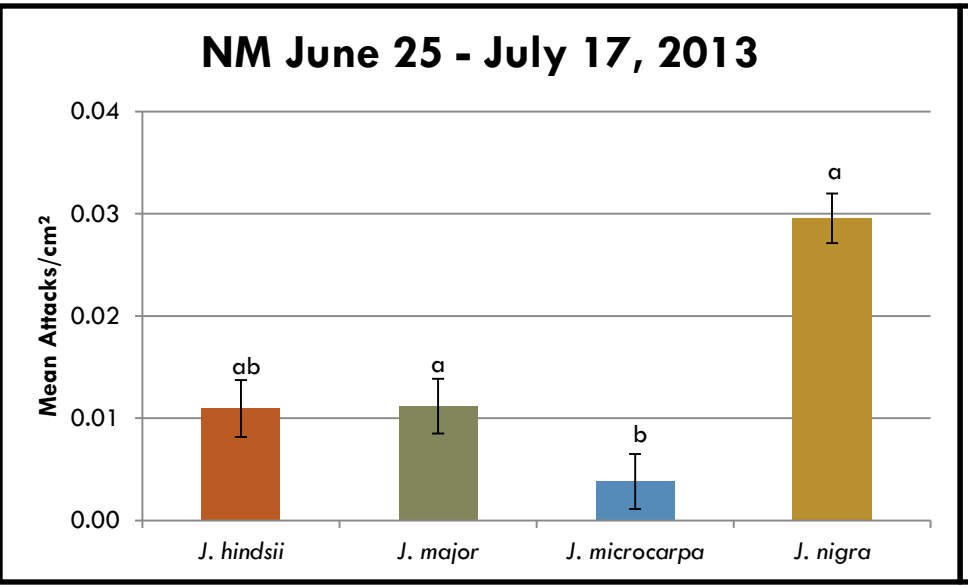
Productivity





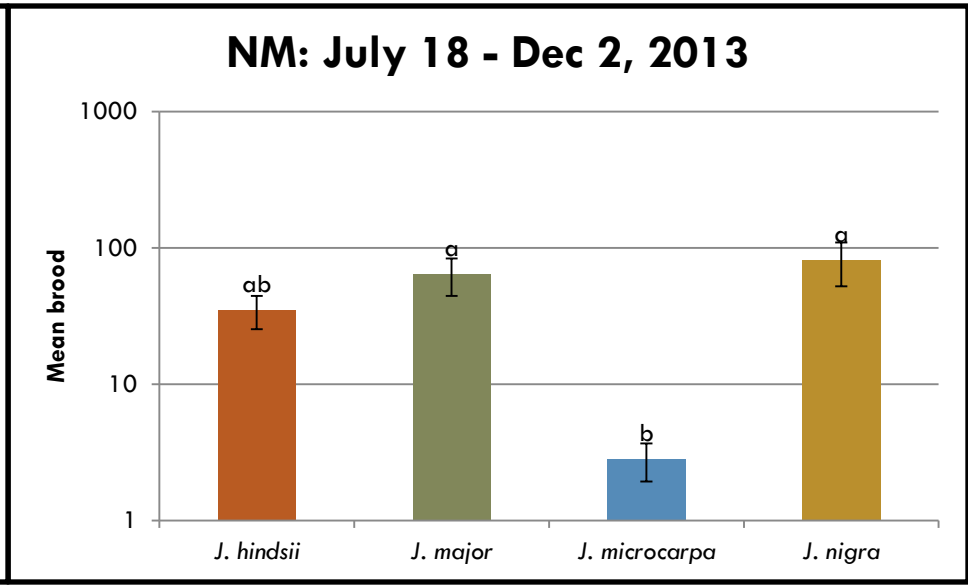
Response of WTB to Four Hosts in the Field, Fort Stanton, New Mexico, June, 2013

Attack Density



PROC GLMMIX; $F=4.72$; $DF=3$; $P<0.01$

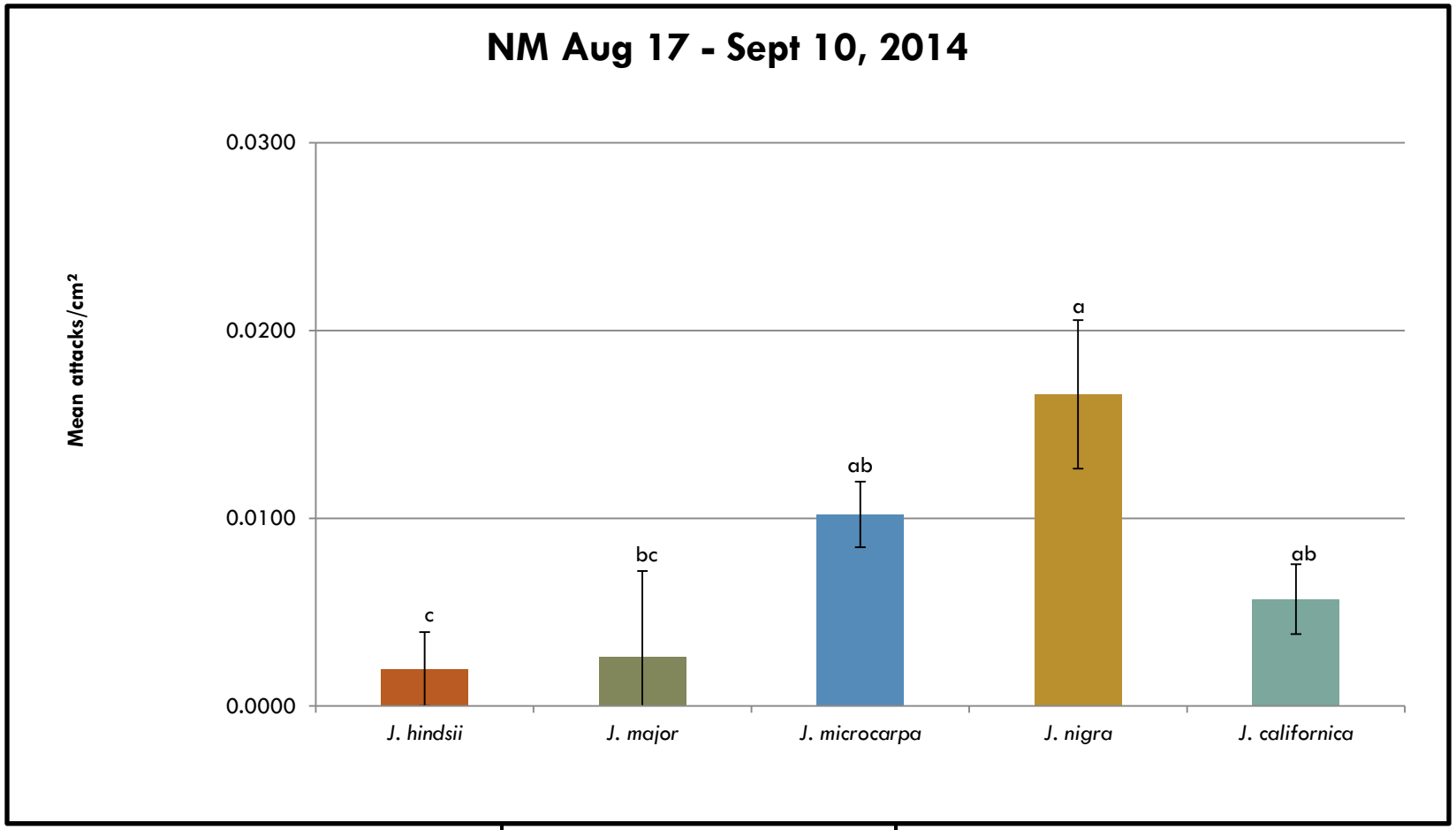
Productivity



Kruskal-Wallis Test; $\chi^2=25.7180$; $DF=4$; $P<.001$

Response of WTB to Five Hosts in the Field, Fort Stanton, New Mexico, August, 2014

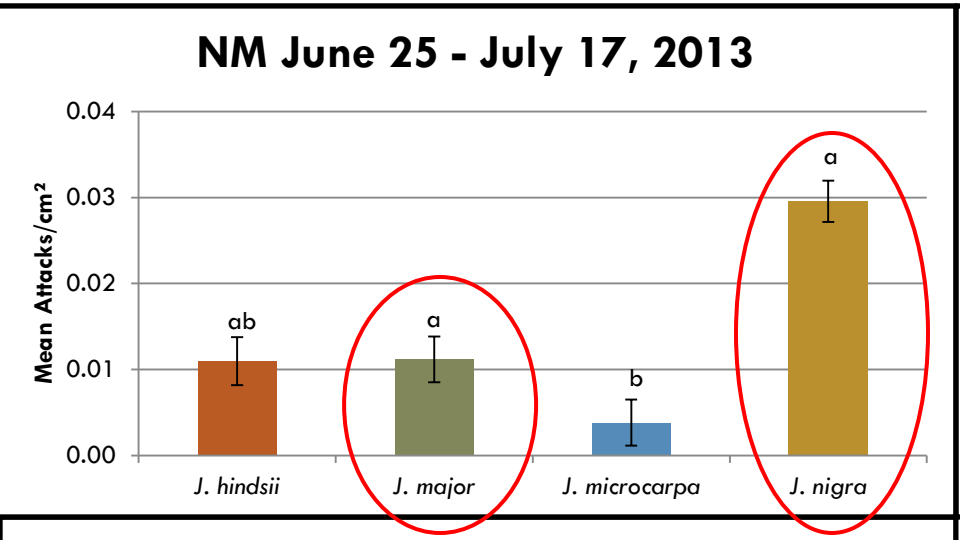
Attack Density

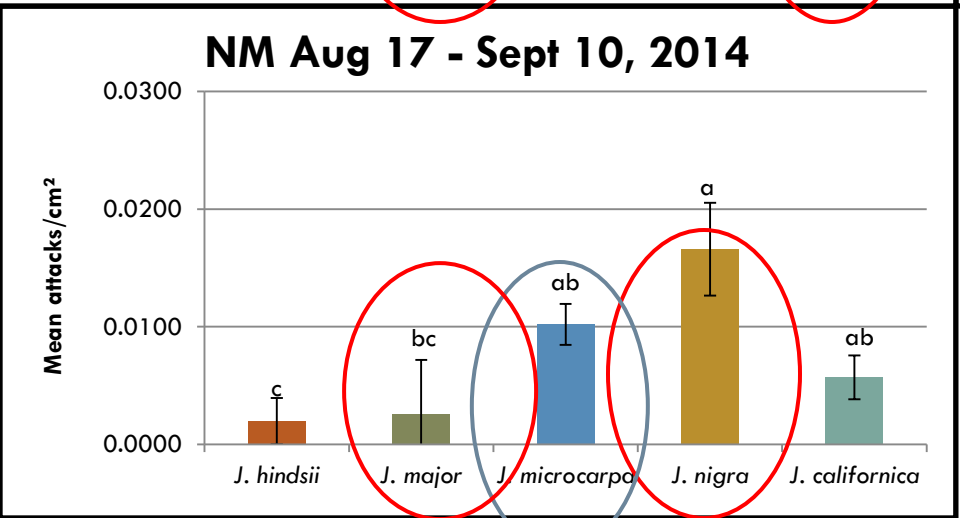


Kruskal-Wallis Test;
χ^2=25.7180; *DF*=4; *P*<.001

Response of WTB to Four or Five Hosts in the Field, Fort Stanton, New Mexico 2013-2014

Attack Density





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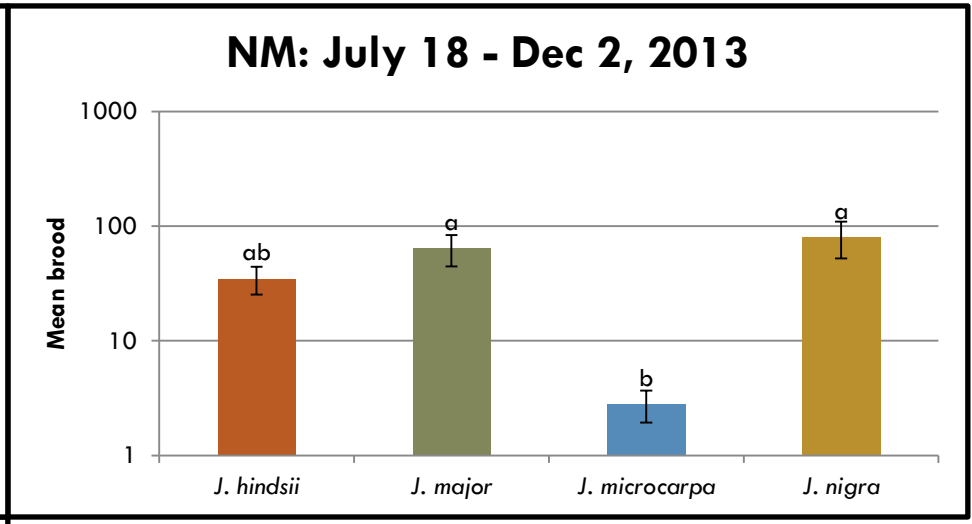


Discussion

- Attack density on *J. nigra* highest in both states
- *J. hindsii* had higher attack rate in CA
- Higher WTB population density in California site may have led to higher attack densities
- Variation in NM 2014 may be due to branch quality

Synthesis

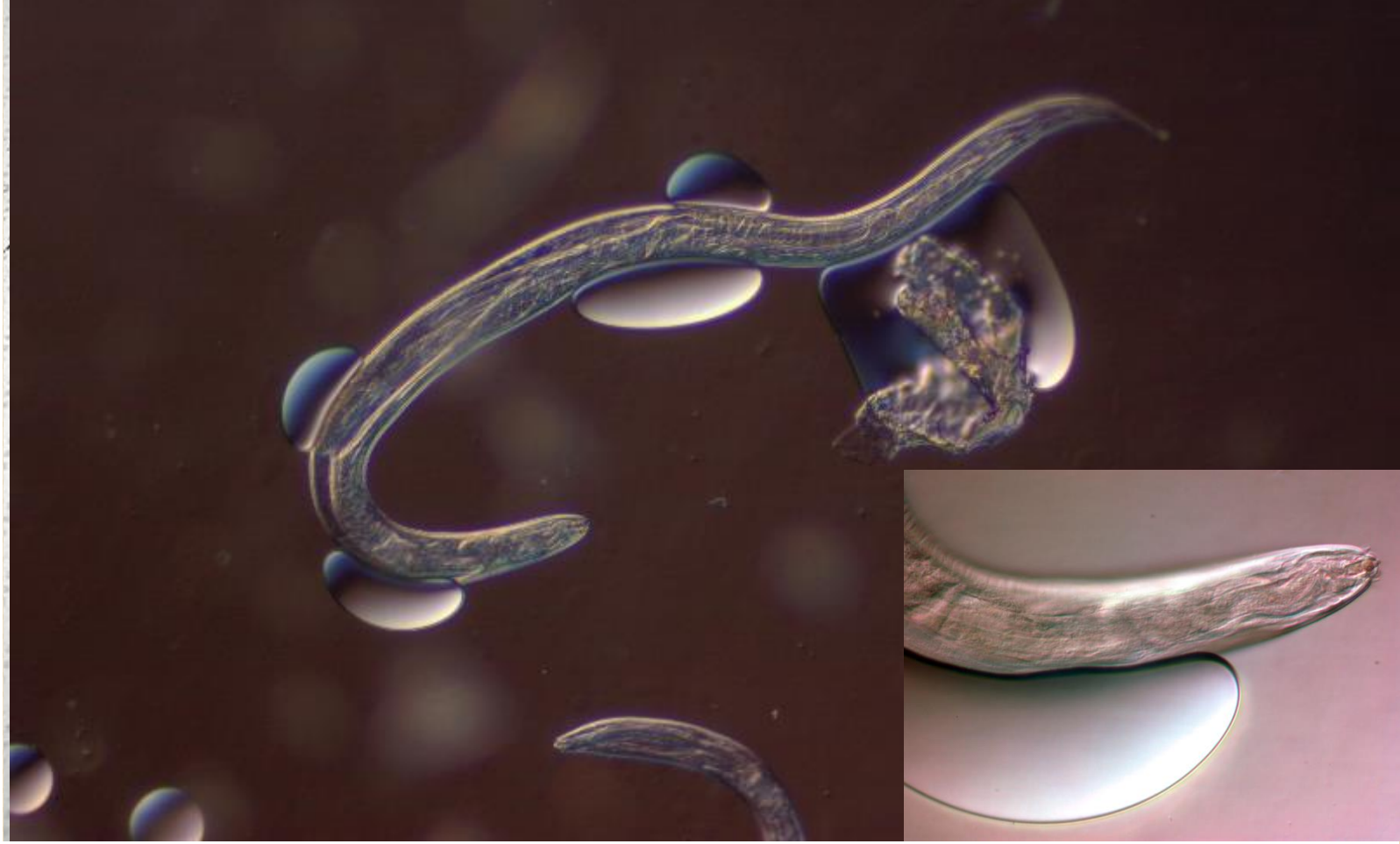
- WTB flight patterns similar outside of native range
- Higher attack rate on local trees in both CA and NM
- Eastern black walnut and butternut highly preferred by WTB
- Explains rapid spread of WTB through Western USA
- Suggests extremely high expansion potential for Eastern USA

Nematode Study

Investigate nematodes found inside WTB and within their galleries

Are WTB-associated nematodes parasites, phytopathogens or other?

Photo credit: Corwin Parker



Methods

Nematode extraction and PCR

1. Branches from CA and NM walnut trees were dissected
2. Gallery tissue was placed in a Baermann funnel for 12-18 hours
3. Aliquots of liquid containing nematodes were transferred to clean water
4. Representative nematodes were heat-killed, photographed, analyzed by PCR (18S) and sequenced

Results

- Several nematode morphospecies isolated from WTB and galleries
- Morphological analysis: Tylenchs and Cephalobs in CA, Rhabdites in NM
- Nematodes found in the beetles appear to be 1st instars, with mostly adults in the galleries
- Poor sequencing results
- More work needs to be done describing the nematodes morphologically and molecularly

Acknowledgments

Thank you:

- Steven Nadler
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- Andy Graves
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- Ivana Li
- Irene Lona
- Scott McCluen
- Bruce Moltzan

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- USDA Forest Service Lincoln National Forest, Smokey Bear Ranger District
- USDA ARS National Clonal Germplasm Repository
- UC Davis Department of Entomology and Nematology