Research with Diaprepes Root Weevil with an Emphasis on Classical Biological Control

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Joseph Morse[1], Kris Godfrey, Jorge Pena, Jim Bethke, Gary Bender, Lindsay Robinson[1], Alan Urena[1], & Loretta Bates

[1]Department of Entomology

University of California, Riverside

Outline of Presentation

- Background, biology of Diaprepes root weevil
- How far will Diaprepes spread in California?
- Research on classical biological control of Diaprepes eggs
  - APHIS protocol to obtain permission to release exotic biological control agents
  - Species identified by Jorge Pena (University of Florida, Homestead) for biological control of Diaprepes eggs
  - Research on chill termination of Diaprepes eggs
- Research to see if Diaprepes larvae will attack avocado roots to the degree they attack citrus roots
- Future research plans

Arthropod Pest Management on CA Citrus

- Until the 1990's, citrus pest management was relatively stable in San Diego County: California red scale, black scale, citrus rust mite, etc.
- A series of new pests emerged forcing pest management changes and adjustments
  - Periodically – various fruit fly species, esp. Medfly, Mexfly
  - 1990 – Glassy-winged sharpshooter, citrus leafminer, Mexican strain of citrus peelminer in the SJV
  - 2005 – Diaprepes root weevil
  - 2007 – Light brown apple moth (LBAM)
  - 2008 – Asian citrus psyllid (ACP) (HLB close)
  - Other new pest species should be expected

Distribution of *Diaprepes* Root Weevil

Periodic CA Introductions Since 1974

2001
Sacramento
Placer

2003
San Bernardino

2005-06
Orange, Los Angeles, and San Diego counties

1964

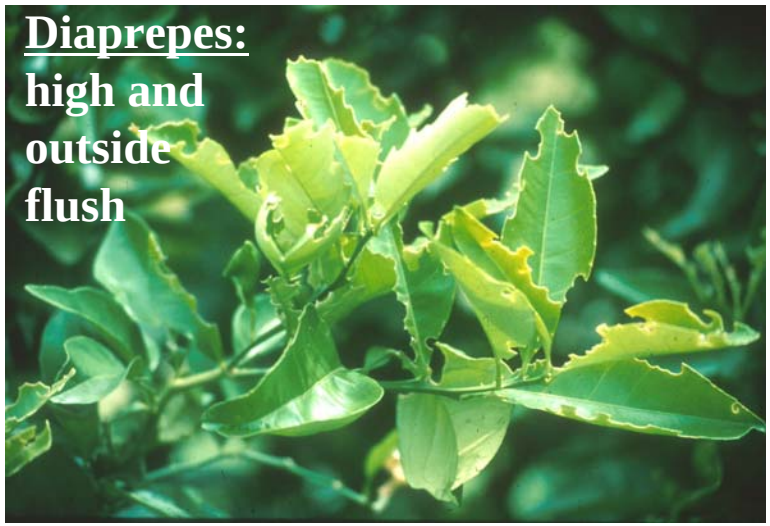
2001
in citrus

Annual losses and cost of control in citrus: $72 million; in vegetables and ornamentals: $2 million

Diaprepes Root Weevil

Bigger beetle, winged, bigger leaf notches, males and females

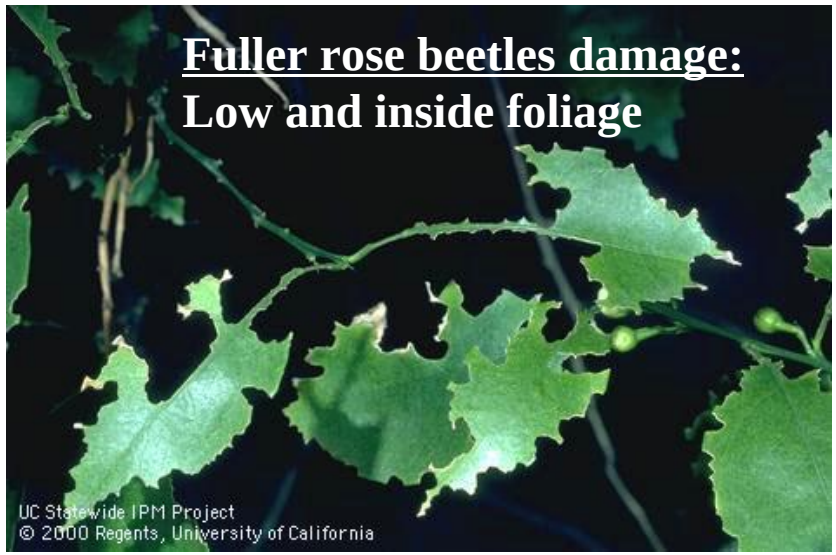
Eggs stuck between two leaves



Fuller Rose Beetle

Flightless, low on the tree, females only

Eggs hidden in the calyx of fruit or sprinkler heads



Diaprepes Root Weevil – Egg Stage

(Life cycle 5 months to 1.5 years)

Adults emerge after rainfall or irrigation events.

Deposits thousands of eggs on leaves.

The eggs are sandwiched between leaves that are glued together

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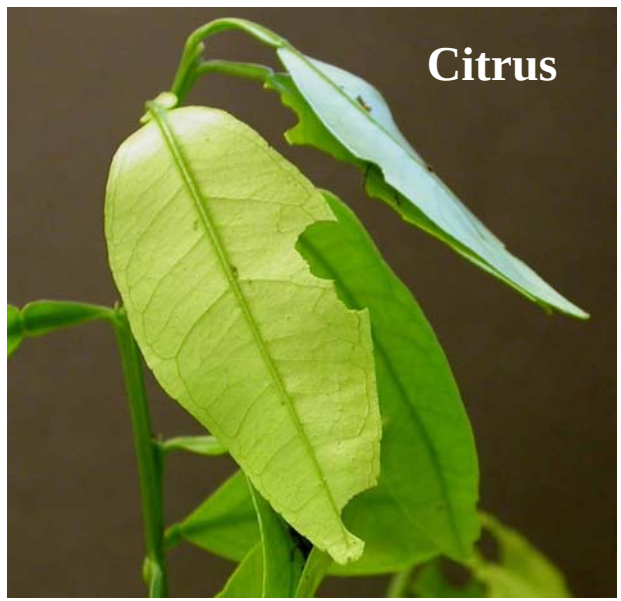
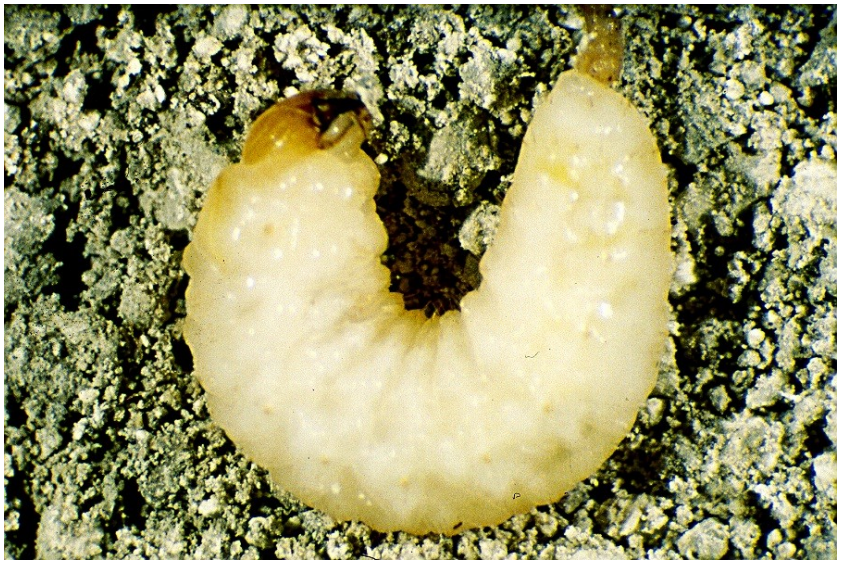


Diaprepes Root Weevil – Larval and Pupal Stages

(Life cycle 5 months to 1.5 years)



Larvae drop to the soil and go through 10-11 instars as they feed on roots.

Pupae are in chambers in the soil for 30 days.

Diaprepes Root Weevil – Adult Stage

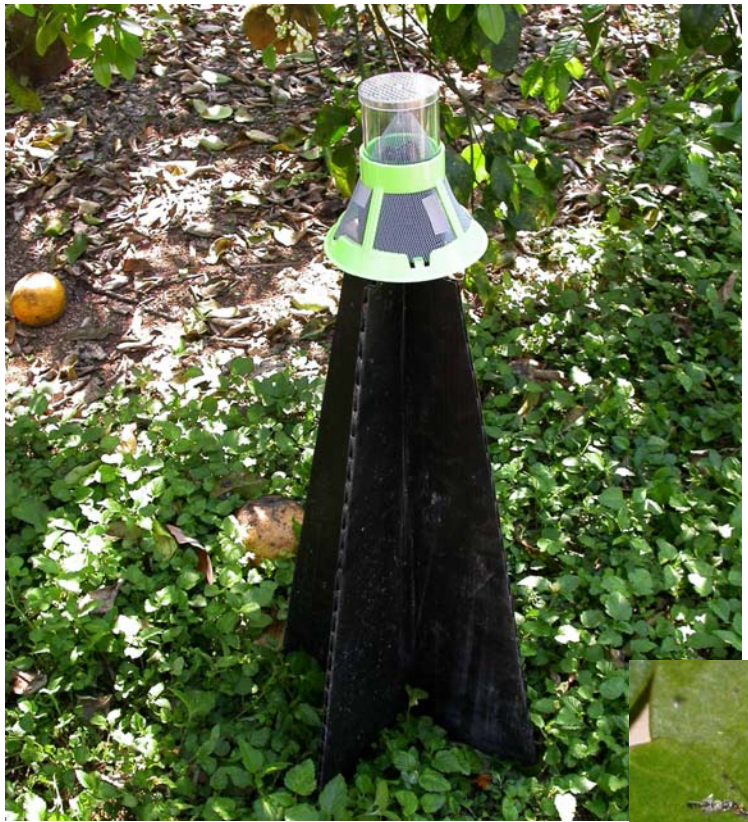
(Life cycle 5 months to 1.5 years)

They vary in size but are around ¾ inch long

Adults vary in color from gray to yellow to red and vary in the striped patterns on their backs

Monitoring: Leaf notches, feces, adults, eggs, larvae
Visual survey for feeding and frass is the most effective method

Modified Tedders Trap



Diaprepes is Associated with a Wide Variety of Host Plants

Host plants associated with adults: > 270 species in 59 plant families

Plants associated with larval feeding: > 40 species in 20 plant families

Plants known to support development from egg to adult: 10 species in 6 families

Citrus, peanut, sorghum, corn, Surinam-cherry, Dracaena or dragon tree, sweet potato, and sugarcane

Larval damage to the root system of citrus – results in tree death

No *Diaprepes*

1 larva feeding on roots for 6 months

Larval damage to the root system of citrus – is often not discovered until it is too late – tree death

Diaprepes Root Weevil infestations escalate problems with *Phytophthora*

Florida observations:

•Root injury is cumulative over time and feeding sites can serve as infection sites for root rot diseases, thereby exacerbating economic loss.

•Non-tolerant and even normally *Phytophthora*-tolerant rootstocks are rendered highly susceptible to *Phytophthora nicotinae* and *P. palmivora* in poorly-drained soils infested with *Diaprepes* root weevil.

Encinitas Infestation Site: 30 June 2006

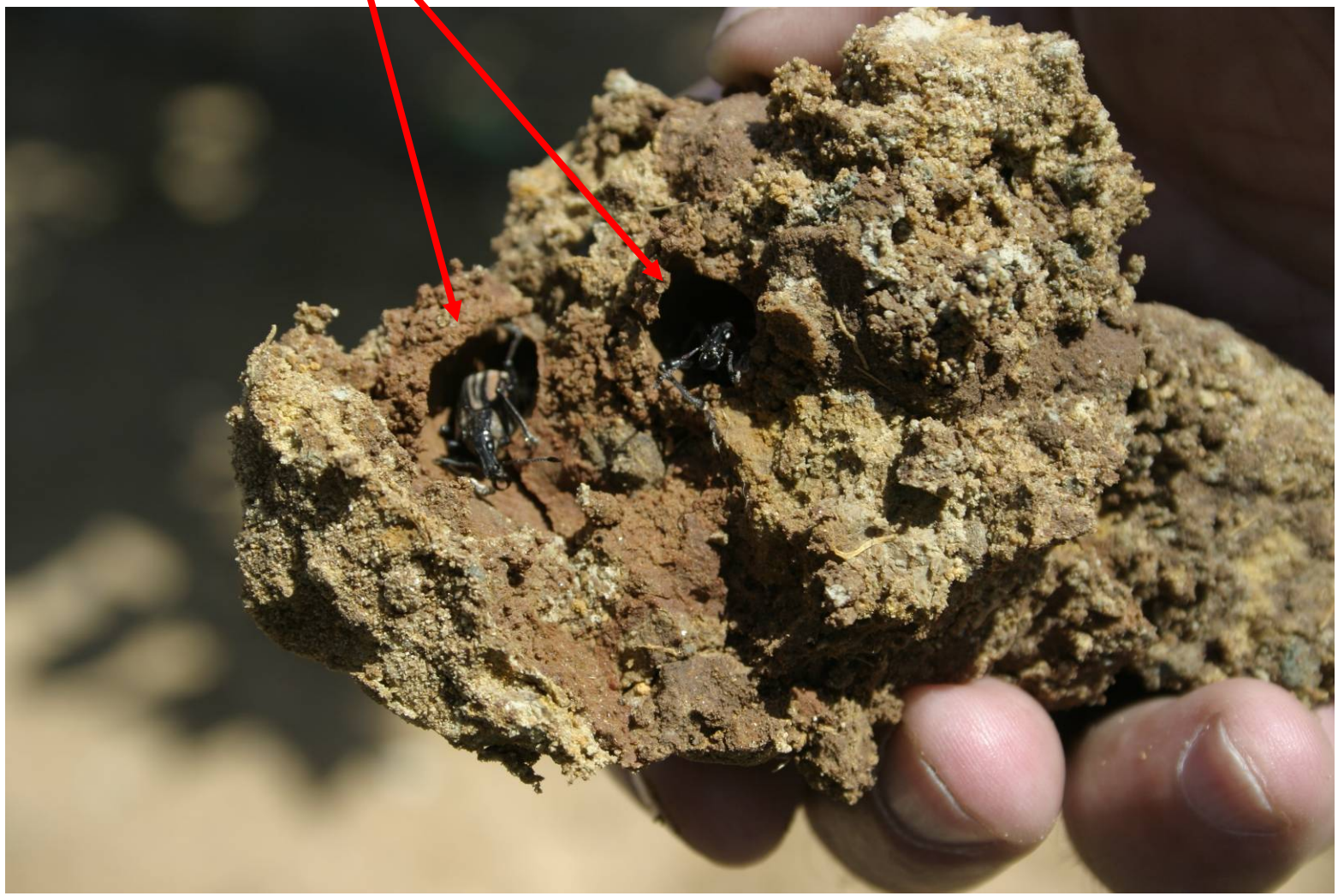
The infested orchard was 4 years old

Both healthy-looking and unhealthy trees were found to have *Diaprepes* adults and grubs among the roots.

The roots of the unhealthy tree were completely girdled, lacking feeder roots

Diaprepes grubs found among the roots

***Diaprepes* adults were found inside earthen cells in clumps of dirt, ready to emerge**

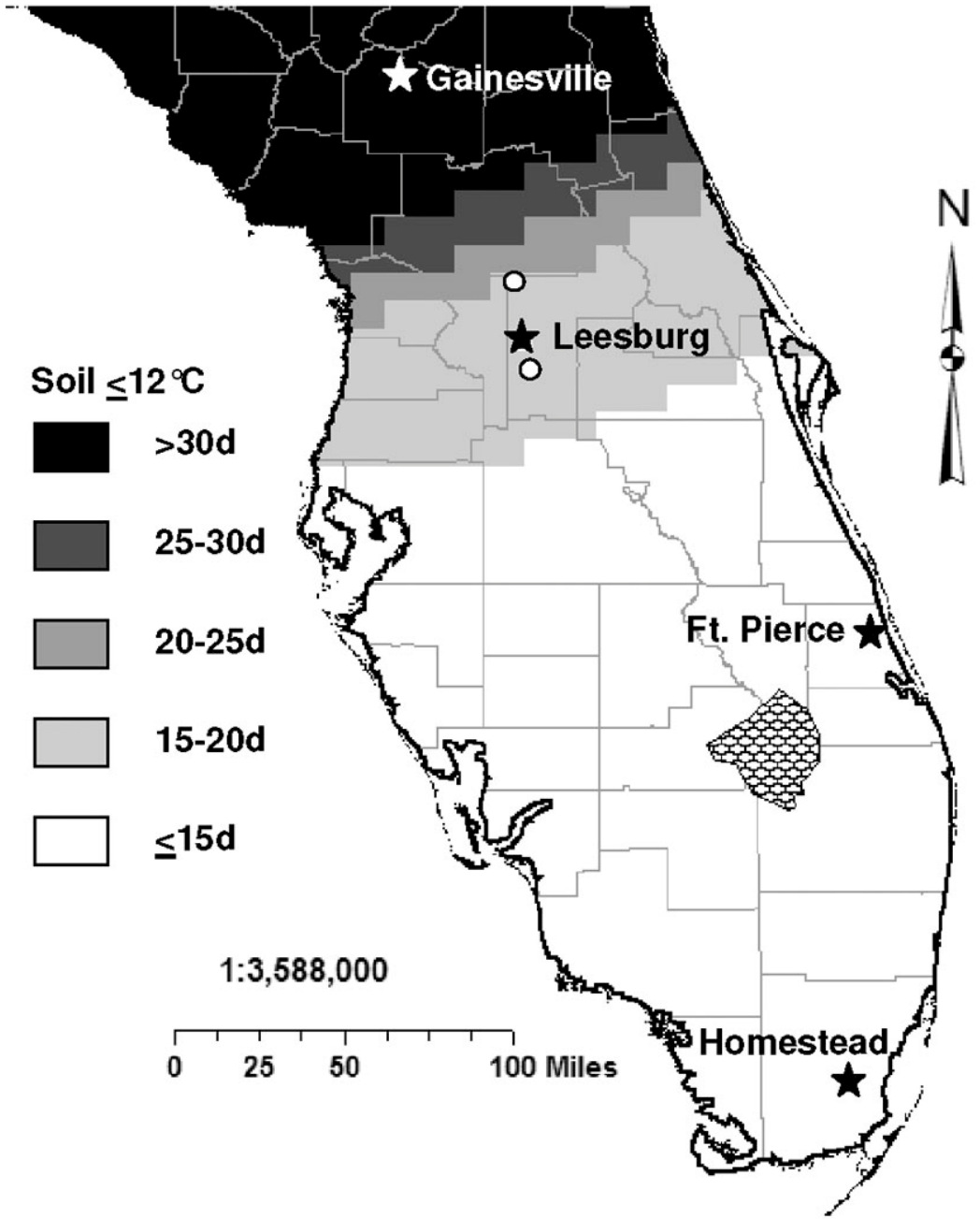


We watched this adult climb out of its earthen cell

How Far Will Diaprepes Spread in California?

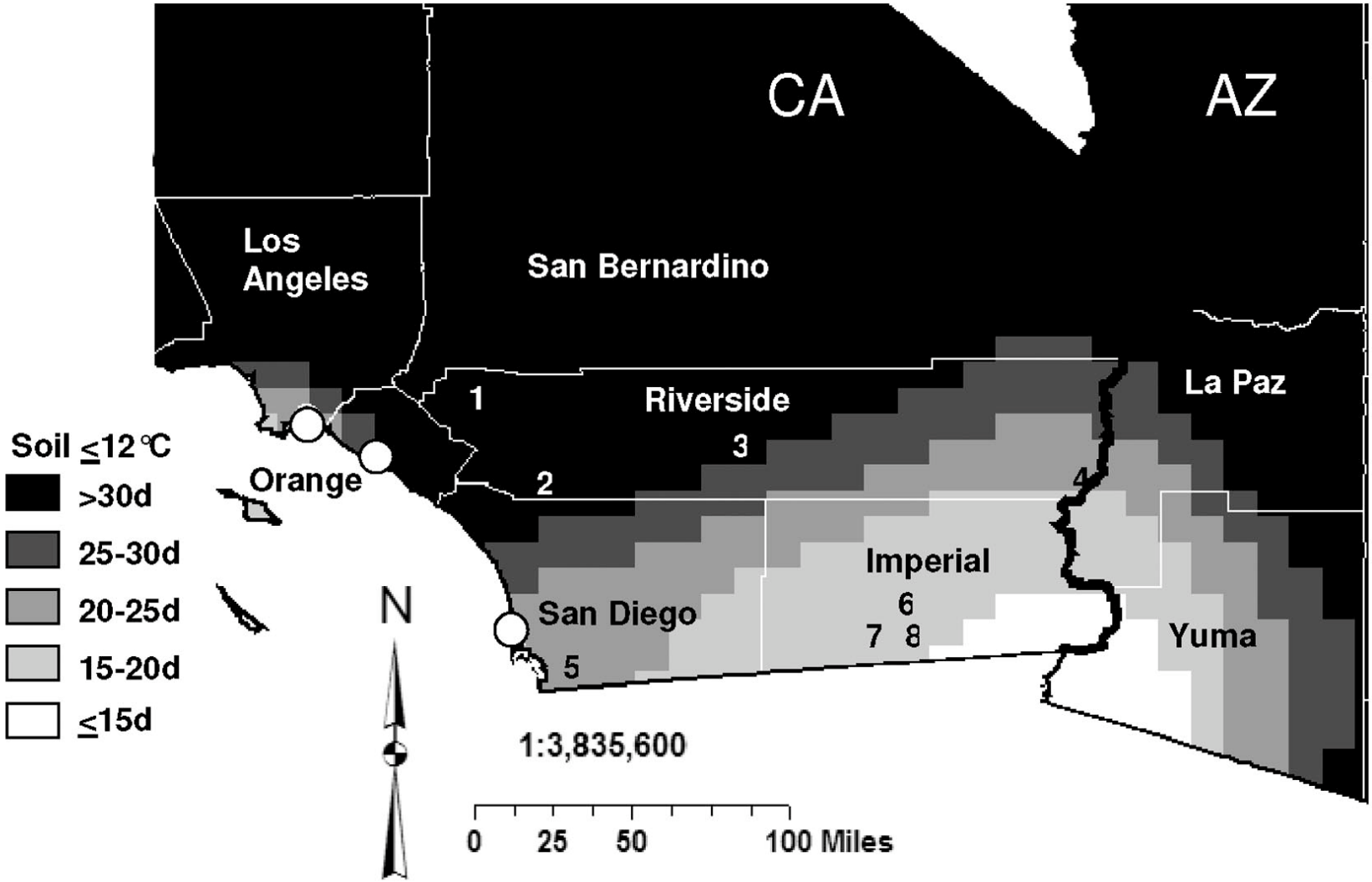
- We won't know for sure until Diaprepes has had a chance to spread into other areas of California
- Lapointe et al. (2007) tried to predict how far Diaprepes would spread in CA and TX based on the lack of movement into northern FL (has been in FL since 1964)
  - Larvae and pupae are subterranean and pupae are more sensitive to cool soil temperatures – 100% pupal mortality when held at 9°C (48.2°F) for 14 days
  - Map of FL based on 15-20 days/year ≤ 12°C (53.6°F) soil temperature for ≥ 5 of 10 years fairly closely resembles the current distribution of Diaprepes in Florida

Lapointe et al. (2007): soil temp. ≥ 5 of the last 10 years



North circle – northernmost spread to date; South circle – site of original find at Apopka, south of Leesburg

Lapointe et al. (2007): soil temp. ≥ 5 of the last 10 years



Use perhaps 15-20 days in ≥ 5 of the last 10 years to predict spread of Diaprepes in CA/AZ (circles = find sites)

Classical Biological Control - Release of Exotic Species

- Classical biological control = find effective natural enemies by searching presumed native range of an introduced pest
- USDA APHIS regulates the movement and release of exotic species into new regions of the U.S.
  - Previous policy – authorized release in the continental U.S. (FL) allowed release in the other 47 states
  - Mid 2009 – NEPO (National Environmental Policy Act) – enforcement required host specificity testing done in each state on the possible non-target impact of exotics
  - List of 22 weevils introduced into various areas of the U.S. as biological control agents of pest weeds (10 CA)

Planned host specificity testing

Species for testing in CA		
Trichosirocalus horridus	Thistle crown weevil	Various thistles (CO, KS, MO)
Phrydiuchus tau	Mediterranean sage root weevil	Mediterranean sage (Lassen, Modoc Co.)
Eustenopus villosus	Yellow starthistle hairy weevil	Yellow starthistle (widespread)
Bangasternus fausti or *B. orientalis*	Knapweed seedhead weevil or Yellow starthistle bud weevil	Knapweed (Lassen, Modoc, Shasta); Yellow starthistle (widespread)
Mecinus janthinus	Dalmation toadflax stem weevil	Dalmation toadflax (Kern Co.)
Rhinocyllus conicus	Thistle head weevil	Musk thistle (widespread)
Species for testing in FL		
Neochetina bruchi	Waterhyacinth weevil	Waterhyacinth (San Joaquin, Sacramento)
Neochetina eichorniae	Waterhyacinth weevil	Waterhyacinth (Sacr.)

Mediterranean Sage Weevils released onto Mediterranean Sage

Italian star thistle received as bare root plants and potted at UCR

Sample of plants shipped from CDFA for weevil host specificity testing. Plants have not survived shipment from Sacramento to UCR very well. Currently receiving bare rooted weeds and re-pot these at UCR.

Egg Predator for Diaprepes Weevil Control

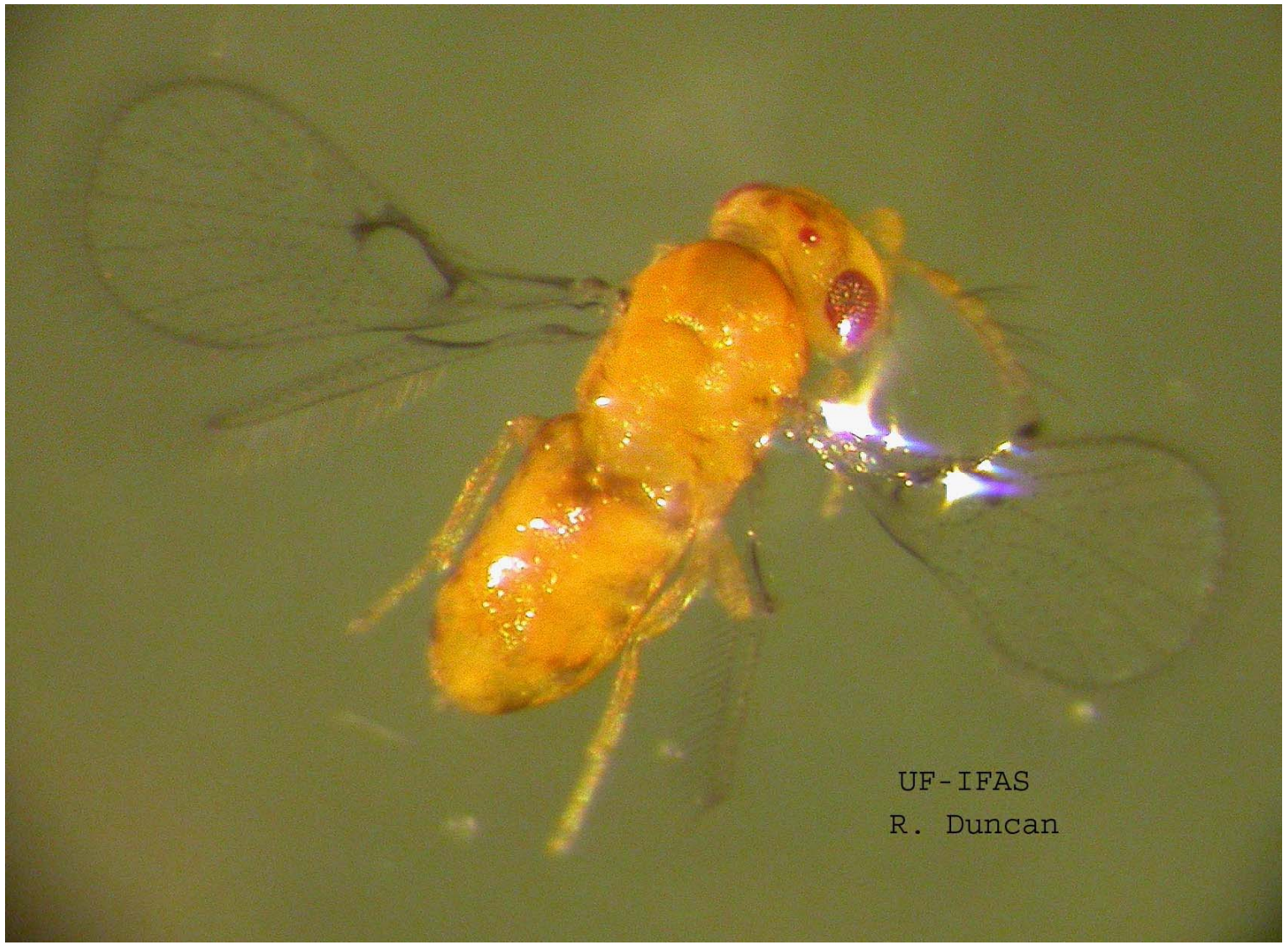
- *Aprostocetus vacquitarum* (Eulophidae) – one of the more important natural enemies of Diaprepes in the Caribbean
- Collected from the island of Dominica in 2000; released in FL 2000-2003 and considered well established – does best in the warmer areas of southern FL
- Obtained permit and released in CA before NEPO rules went into effect
- Really more an egg predator than a parasitoid – female lays her eggs inside a Diaprepes egg mass; prefers 0-3 d old eggs; larva must feed on 2-3 eggs to complete their development
- Adult females live about 15 days, lay a mean of 53 eggs (86% female)

Aprostocetus vaquitarum “egg predator” (Pena lab, UF)

Egg Parasitoids for Diaprepes Weevil Control

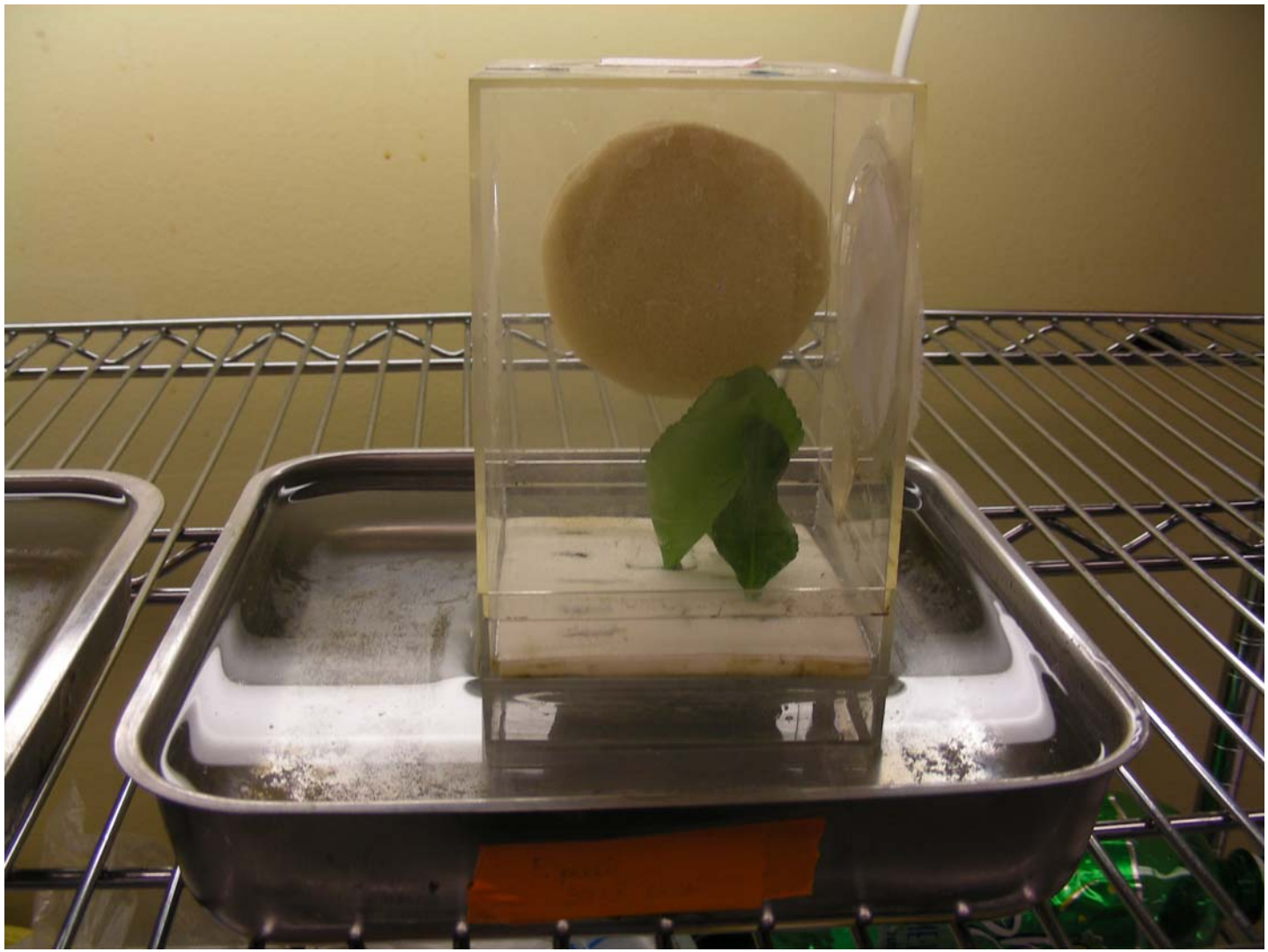
- *Haeckeliania sperata* (Trichogrammatidae) – very small, internal, gregarious (3-5 or more per egg) egg parasitoid
- Collected from the island of Dominica in 2003; released in FL but recovered only in sleeve cages
- Adults short-lived – live 2-5 days after emergence if provided honey (80% female); eggs develop in several hours, larvae in 14 days, pupae in 2 days
- *H. sperata* does not like to parasitize eggs laid between pubescent (hairy) leaves (inserts eggs through the upper surface of the leaf)
- Very small and quite susceptible to pesticides; given how short-lived adults are, its potential in CA is unclear

Haeckeliana sperata parasitoid (Pena lab, UF)



Two types of vial set-ups containing Japanese boxwood with one day old Diaprepes egg masses and vials of emerging *H. sperata*

One day old Diaprepes eggs on Indian Hawthorn- *H. sperata* released into cage



Egg Parasitoids for Diaprepes Weevil Control

- *Fidiobia* sp. from Columbia (Platygasteridae) – Pena trying to obtain specimens from a commercial insectary in Columbia so as to start a colony in Quarantine in Florida
- Would need to run host specificity trials prior to requesting release in California
- *Fidiobia citri* – egg parasitoid recovered 15 years ago during research with Fuller rose beetle
- We need to test this species against Diaprepes root weevil eggs – a species that would readily move between Fuller rose beetle and Diaprepes eggs might be ideal (and no permits needed to release if found in CA)

Fidiobia dominica (male on left, female right)

Fidiobia citri **(Platygasteridae) egg parasitoid (please report finding)**

Long ovipositor of *Fidiobia citri* – must reach eggs in crevices

Chill Termination Research with Diaprepes Eggs

- The problem – Diaprepes eggs are sometimes in short supply; it would be nice to have a way of "storing" eggs for future use
- Method of "chill termination" developed by Roger Leopold and colleagues at USDA-ARS in Fargo, ND
- Protocol for GWSS eggs – hold at 2°C (35.6°F) for 5 days and then eggs were good as host eggs for *Gonatocerus ashmeadi* when held for up to 70 days at 10°C (50.0°F)
  - Can hold eggs up to 30 days without side effects on parasitoid population growth parameters
  - 30-50 days – start to see negative impacts but not severe

Sheets of wax paper put in Diaprepes cage to collect eggs

Diaprepes rearing cage with wax paper strip containing egg masses

Will Diaprepes larvae attack avocado roots?

- A major problem with Diaprepes in Florida is larval root damage providing easy entry to *Phytophthora* spp.
- *Phytophthora* spp. are the #1 pest problem on avocados – thus, Diaprepes larvae readily attacking avocado roots could make root rot much worse
- Research designed to determine if Diaprepes larvae could feed on and develop on avocado rootstocks to the same degree they do on citrus rootstocks
- Citrus seedlings used as controls – neonates (first instars) do well on citrus seedlings
  - Would they do as well on the roots of avocados?
  - Are there differences between different avocado rootstocks?

Trial 1 – Larvae shipped on diet from FL to CA

Plant type	Instar	% larval survival
Lemon on *Citrus macrophylla*	first	35%
	fifth	47%
Navel on C-35	first	40%
	fifth	43%
Hass on Dusa	first	28%
	fifth	33%
Hass on Duke 7	first	13%
	fifth	40%

Trial set up 6-20-07; 50 1st or 10 5th instars left for 90 days; 8 reps each

Trial 2 – Larvae, egg masses shipped from FL to CA

Plant type	Instar	% larval survival	Larval weight (g)
Lemon on *Citrus macrophylla*	1st from diet	54%	0.27
	1st from eggs	37%	0.19
Hass on clonal Toro Canyon	1st from diet	35%	0.11
	1st from eggs	3%	0.02
Hass on clonal Dusa	1st from diet	37%	0.11
	1st from eggs	0%	--

Trial set up 5-7-08; 50 neonates left for 93 d; 6 reps each

Lindsay Robinson isolating 10 Diaprepes into each vial

Neonate (first instar) Diaprepes larvae from FL rearing program

Neonate (first instar) Diaprepes larva used to infest citrus, avocado

Tapping 10 neonate Diaprepes larvae into a hole in soil around roots

Greenhouse room inside Quarantine used to expose Diaprepes to citrus and avocado seedlings (randomized arrangement of treatments)

Citrus root ball being sifted to look for Diaprepes larvae, damage

Diaprepes larva collected from the soil around citrus seedlings

Diaprepes pupae isolated from soil around a citrus seedling

Sifting through roots in Quarantine looking for Diaprepes larvae, pupae

Diaprepes feeding damage on citrus roots

Control (no Diaprepes) avocado seedling

Trial 3 – Egg masses shipped from FL to CA

Plant type	% larval survival	Larval weight (g)
Lemon on *C. macrophylla*	19.4 %	0.172
Hass on Latas	6.3 %	0.051
Hass on clonal Dusa	1.6 %	0.027
Hass on Duke 7	0.8 %	0.019
Hass on Zutano seedling rootstock	0.0 %	--

Trial set up 5-15-09; 200 neonates left for 88-93 d; 7 reps ea.
Increased to 200 neonates per plant to “force” feeding

Future Diaprepes weevil research

- Look for funding once the CDFA Specialty Crop funding expires 6-30-12
- How far will Diaprepes have spread by then, how much damage is it causing, to what degree should research shift to ACP and HLB?
- Finding and releasing effective classical biological control agents would remain a priority
  - How effective is *Fidiobia* sp. (Columbia), *F. citri*?
- More research on entomopathogenic nematodes is probably warranted
- Substantial research on Diaprepes in Florida has not found an easy/effective method of control to date

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