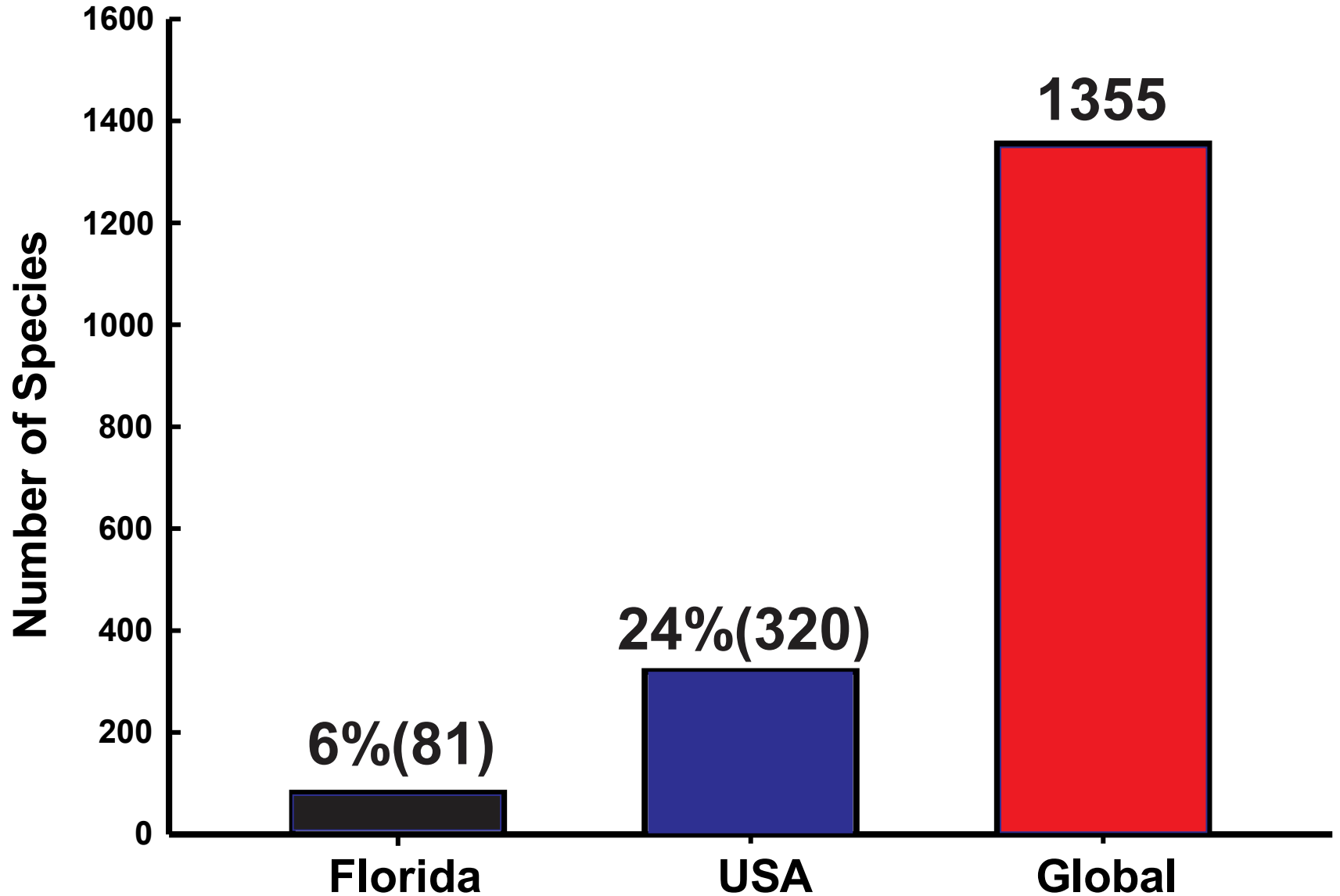


A close-up photograph of a green citrus fruit, likely a lime or lemon, showing several mealybugs. One mealybug is on the left side, and a larger cluster of mealybugs is on the right side. The mealybugs are small, white, and fuzzy insects. The background is blurred green foliage.

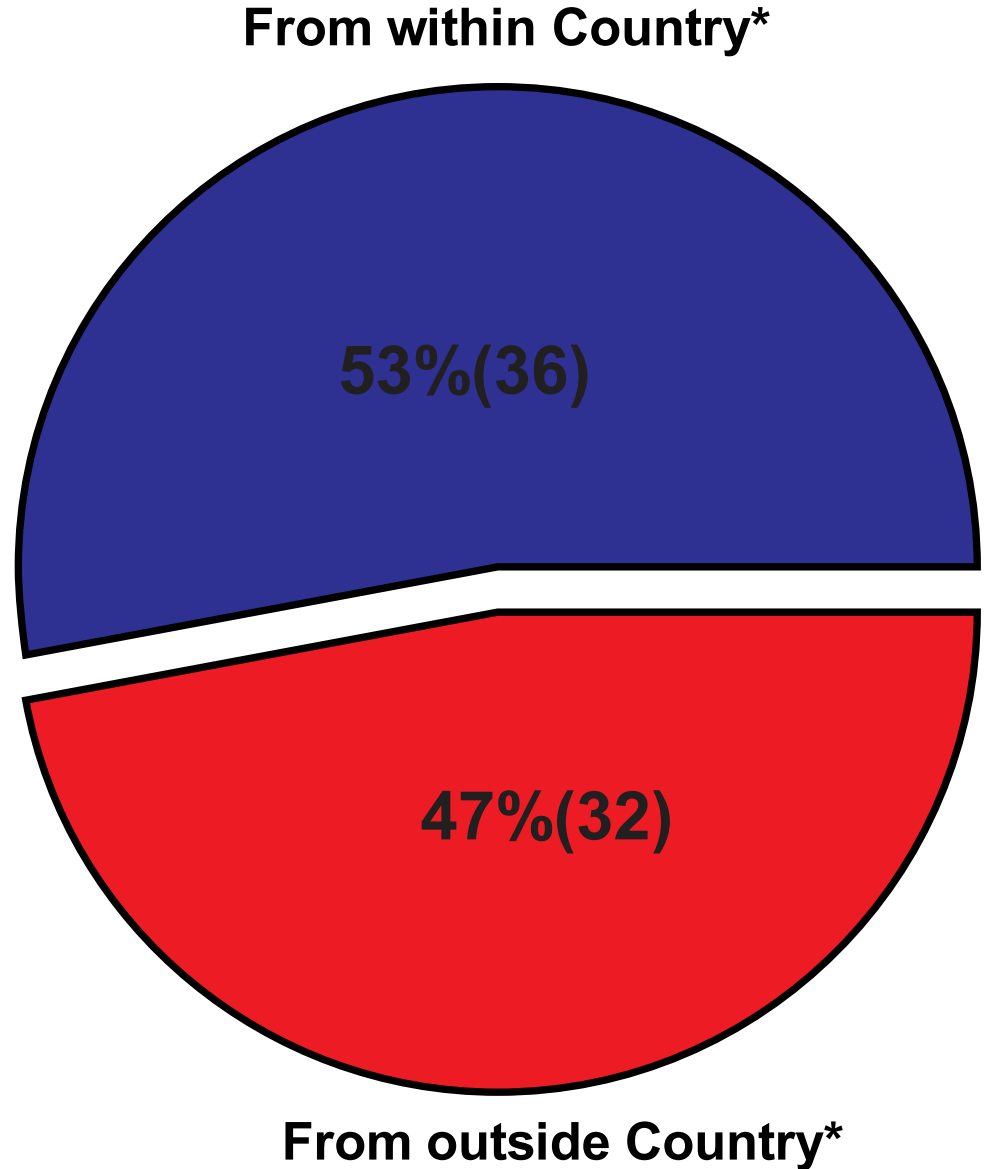
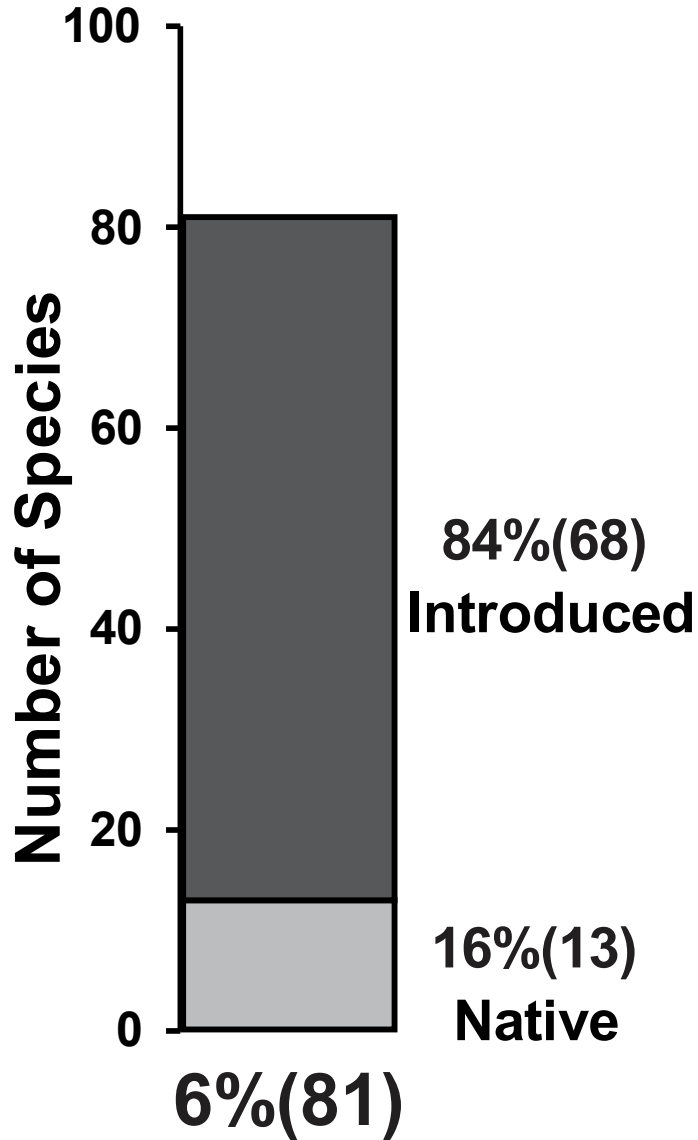
Developing Species-level Diagnostic Kit for Lebbeck Mealybug, *Nipaecoccus viridis*

Muhammad Z. Ahmed, PhD
Research Entomologist
USDA, Fort Pierce

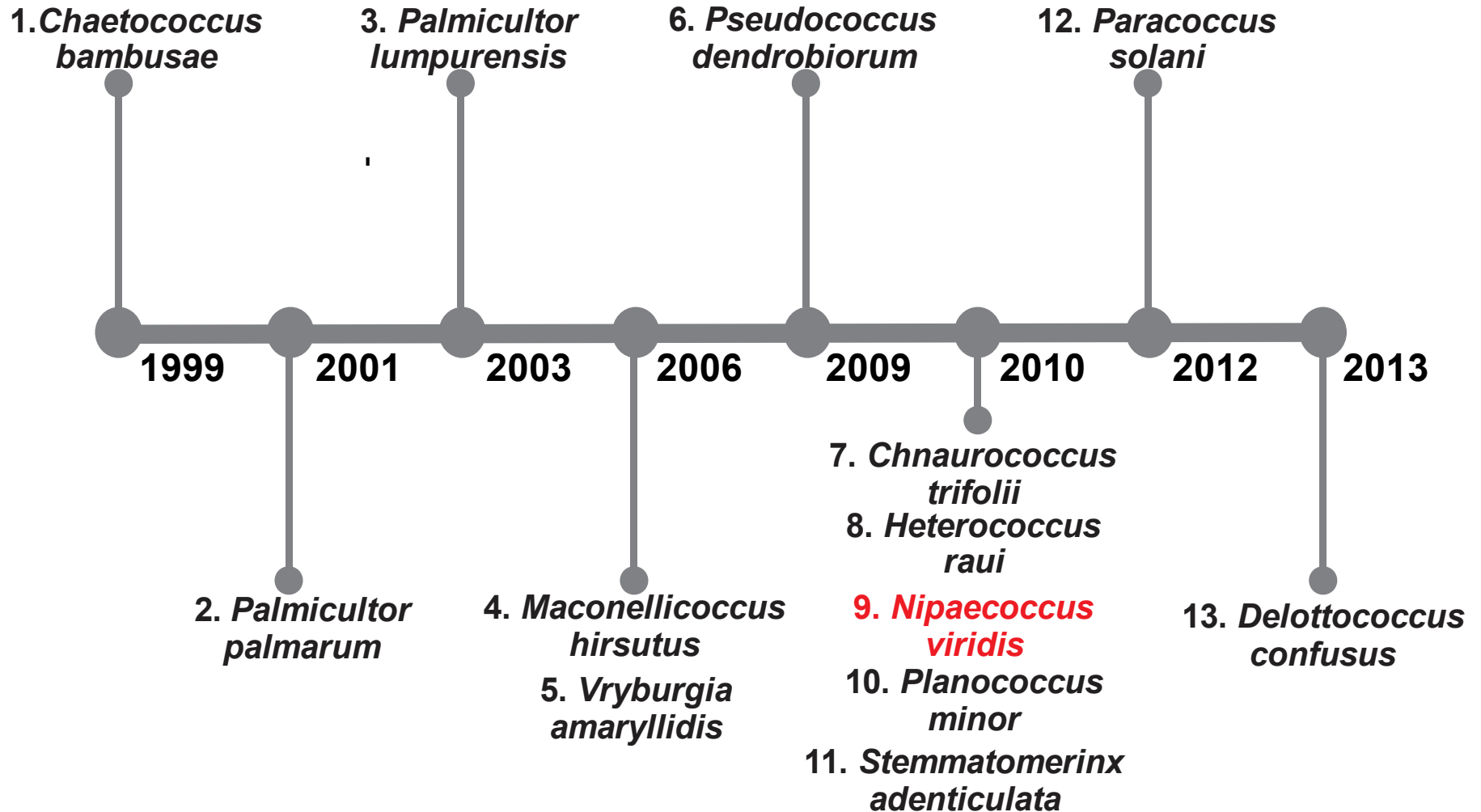
Mealybug Species



Mealybug Species



Mealybug Species





Lebbeck Mealybug

PEST ALERT

FDACS-P-02116
Pest Alert created June 2019

Florida Department of Agriculture and Consumer Services
Division of Plant Industry

Nipaecoccus viridis (Newstead), lebbeck mealybug, (Coccoidea: Pseudococcidae),
updated pest alert

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Hemp

Blueberries



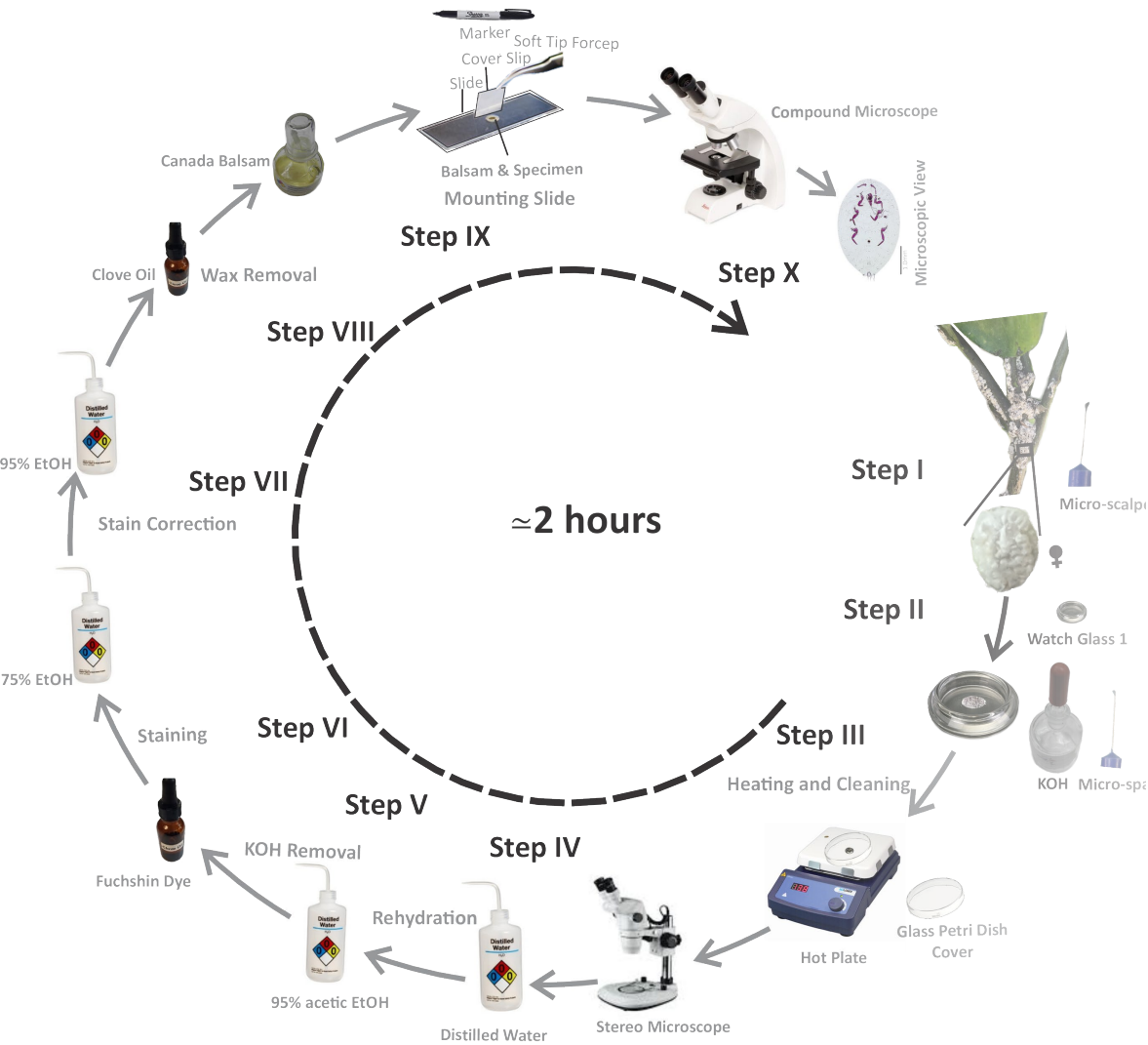


Jetropha



Oleander

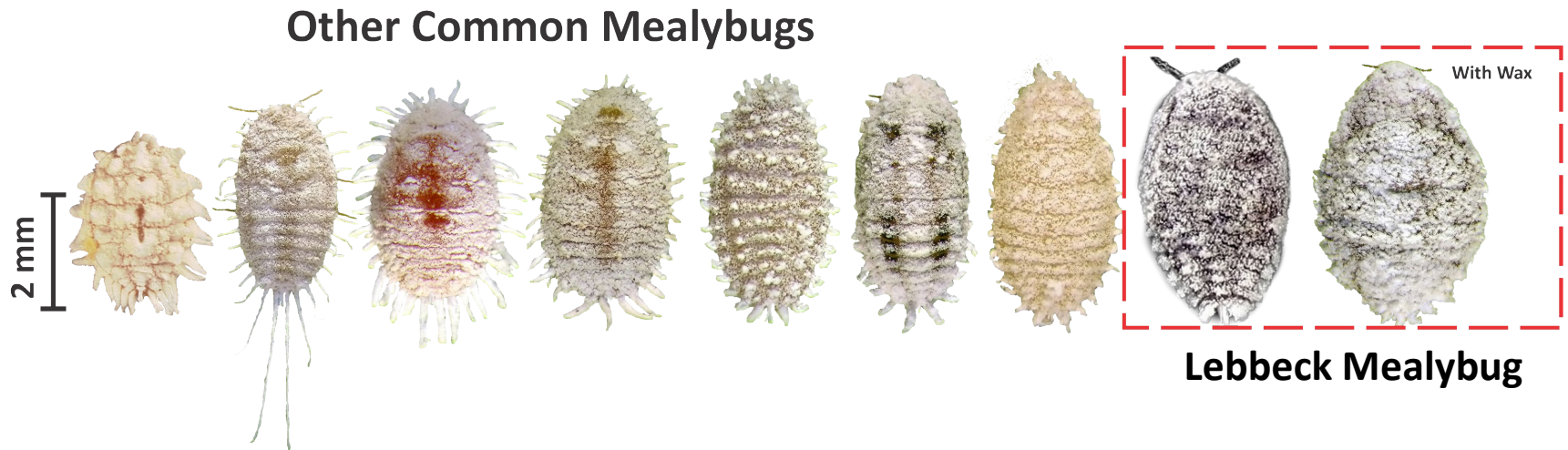
Microscopic Slide Mounting



- Mandatory for Species-level Identification of Mealybugs
- Only in Laboratory Setting
- Require Several Technical & Tedious Steps
- Take up to 2 hrs.
- Most Mealybugs Taxonomic Literature Based on Adult Females

Alternative 1

Compare Size & Appearance (Adult Females)



Challenges

- Require Technical Training
- Require Binocular Microscope
- Require Adult Female Adult Stage Ideally Without Wax
- Challenging if in Mixed Species Infestations
- May Not Valid be in the Future in Florida

Alternative 2

Adult Female Color Test in 75% EtOH

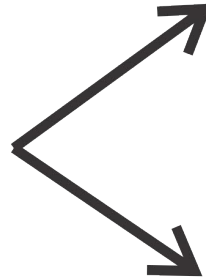


Stains Ethanol Red



Adult Female

75% EtOH



Soaked in 75% EtOH

Stocks & Hodges, 2010
Ahmed et al., 2019



Black Body



Crushed in 75% EtOH

Diepenbrock &
Ahmed, 2020



Dark Purple
Hemolymph

Challenge

Several other scale insects and mealybug species

- Stain the ethanol red
- Turn black in ethanol
- Released dark purplish body liquid



Margarodes Guilding sp. (Margarodidae), *Icerya purchasi* Maskell (Monophlebidae), *Amonostherium lichtensioides* (Cockerell), *Ferrisia dasylii* (Cockerell), *Ferrisia virgata* (Cockerell) (Pseudococcidae), *Stigmacoccus asper* Hempel (Stigmacoccidae)

Alternative 3

Dactylopius viridis, n. sp.

Pl. III, fig. 1.

♀ Adult prior to gestation (fig. 1*a*.) covered with white mealy wax, margin all round thickly set with narrow white waxy appendages, except at anal extremity where there is a slight division; in the more advanced stage the insect covers its body with a thick layer of white waxy felting which forms a complete oval sac in which the eggs are laid. When treated with potash it is of a beautiful emerald green, which is retained to a certain extent after mounting in Canada Balsam. Antenna (fig. 1*b*.) short of 7 joints, last joint the longest, and in length equal to 2 and 3 together; the rest are of nearly equal length: all with very fine short hairs; Legs (fig. 1*c*.) short, very little longer than antenna; tarsi much shorter than tibiæ; digitules of claw and tarsus slender: all are often wanting. Rostrum biarticulate, basal joint with two stiff hairs at apex, and two more on the middle of the apical joint. Anal ring with six hairs. Anal lobes obsolete with one long hair: often broken away, and two or three short spines. Dermis with a few short delicate spines, more numerous beneath in front near the antennæ.

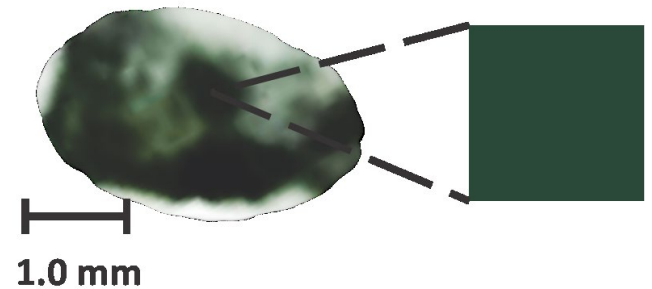
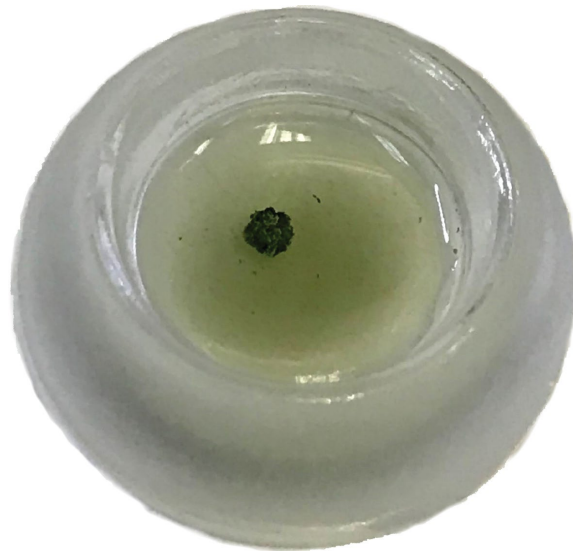
♀ Long 2-3 mm., wide 1-1½ mm., Sac long 3½ mm.

Larva, anal lobes nearly obsolete, each with one long and two or more short hairs. Antenna of 6 joints, 1 to 5 short and sub-equal, 6 about one-third the length of the whole. Anal ring with 6 hairs. Rostrum biarticulate, filaments very long.

Adult Female Color Test in 10% KOH



10% KOH



We tested 2,635 specimens of 57 mealybug species from 26 genera of mealybugs collected from all over Florida for two consecutive years and concluded green coloration of lebbeck mealybug in 10% KOH as a reliable alternative to slide-mounting.

Question 1

Can alternative 3 be applied to eggs and immature stages?





Question 2
Can alternative 3 be applied to adult male?

Immature/Adult Male Color Tests

Eggs
Nipaecoccus viridis



10% KOH



Green
Coloration

1st Instar
Nipaecoccus viridis



10% KOH



Green
Coloration

Adult Male
Nipaecoccus viridis



10% KOH



Green
Coloration

Microscopic Slide Mounting



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METHODOLOGICAL ARTICLE |  Full Access

Rapid species-level hemolymph color test for all life stages of *Nipaecoccus viridis* (Newstead) (Hemiptera: Pseudococcidae), an invasive and regulatory pest in the United States

Muhammad Z. Ahmed  Lily Deeter

First published: 26 February 2022 | <https://doi.org/10.1111/jen.12985>

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Question 3

Is it safe to use KOH in the field?



10% KOH

Causes damage to the skin, eyes, and mucous membranes (National Research Council 1995). Main limitations in developing a species-level field diagnostic kit.

An unanswered question in the study was whether potassium in the 10% KOH solution or the pH turns hemolymph green.

If hemolymph turning green is not associated with potassium but with pH, then finding a safe alternative to KOH that can change the pH could resolve the situation.

Question 4

Can this kit distinguish between alive and dead infestations?



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