OP-226 [Beekeeping Technology and Quality]
RNAi As a Next Generation Varroa Mite Control

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Beekeepers worldwide understand the challenges the varroa mite Varroa destructor present within their hives. Colony losses due to this parasite are increasing in many countries around the globe, highlighting the need for new, safe methods of mite control. One of the most promising next generation technologies is RNA interference (RNAi), which uses double stranded RNA (dsRNA) to control varroa populations without having any negative impact on honey bee populations or in-hive dynamics.

Vadescana is a new RNA based active ingredient in NorroaTM, which is currently under review by multiple regulatory authorities. By targeting the gene that regulates the production of calmodulin protein, the foundress mite is unable to lay viable eggs, therefore maintaining low varroa populations within a colony. Years of scientific research by both GreenLight Biosciences and Victoria University of Wellington has supported both the effectiveness and safety of Norroa, showing significant reduction in mite fertility with no impact on pupal survival.

Laboratory mini-hive experiments demonstrated that Norroa induces a near-complete knockdown of mite reproduction. While female Varroa mites are not killed, their gene expression is substantially altered immediately following dsRNA exposure. By monitoring bees using Radio Frequency Identification (RFID) tags, we found that bees from hives treated with Norroa survive significantly better and forage more during their lifetime compared to bees from hives without mite control.

Non-target effects were evaluated both bioinformatically and experimentally with multiple generations of wax moths, with results suggesting that the only likely non-target species affected by Norroa would be mites related to varroa. Field trial data with commercial beekeepers in the US supports these results, showing the ability to keep varroa population under 3 mites per 100 bees for up to 18 weeks.

Given the supporting data, Norroa appears to offer a highly targeted and much needed tool in the beekeeper's toolkit for varroa control.

norroa™

RNAi as a Next Generation Varroa Mite Control

Adam Pachl- GreenLight Biosciences, USA

Dr. Phil Lester- Victoria University of Wellington, NZ

Norroa™ targets reproducing mites in the brood cells

Delivery pouch

dsRNA is formulated into a sucrose solution and packaged into a pouch with perforated holes on top. The pouch is placed on top of frames of brood or on the bottom board. Bees take the solution and distribute through the brood nest.

Mode of action

(1) The queen lays an egg in the cell. (2)Nurse bees deliver brood food containing Norroa (vadescana) to the brood cells. (3) The Varroa mites hiding in the brood cells come in contact with brood food containing Norroa, absorbing the dsRNA which targets the production of a protein in the mite. This inhibits the mite's ability to reproduce, resulting in long term control of mite populations. (4) The adult bee ecloses with no new Varroa mites attached.

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A novel biopesticide - Norroa™ for mite control

1. How does it work?
2. Is it effective in commercial bee hives?
3. What are the effects on bee longevity and foraging?
4. Are there any non-target effects?

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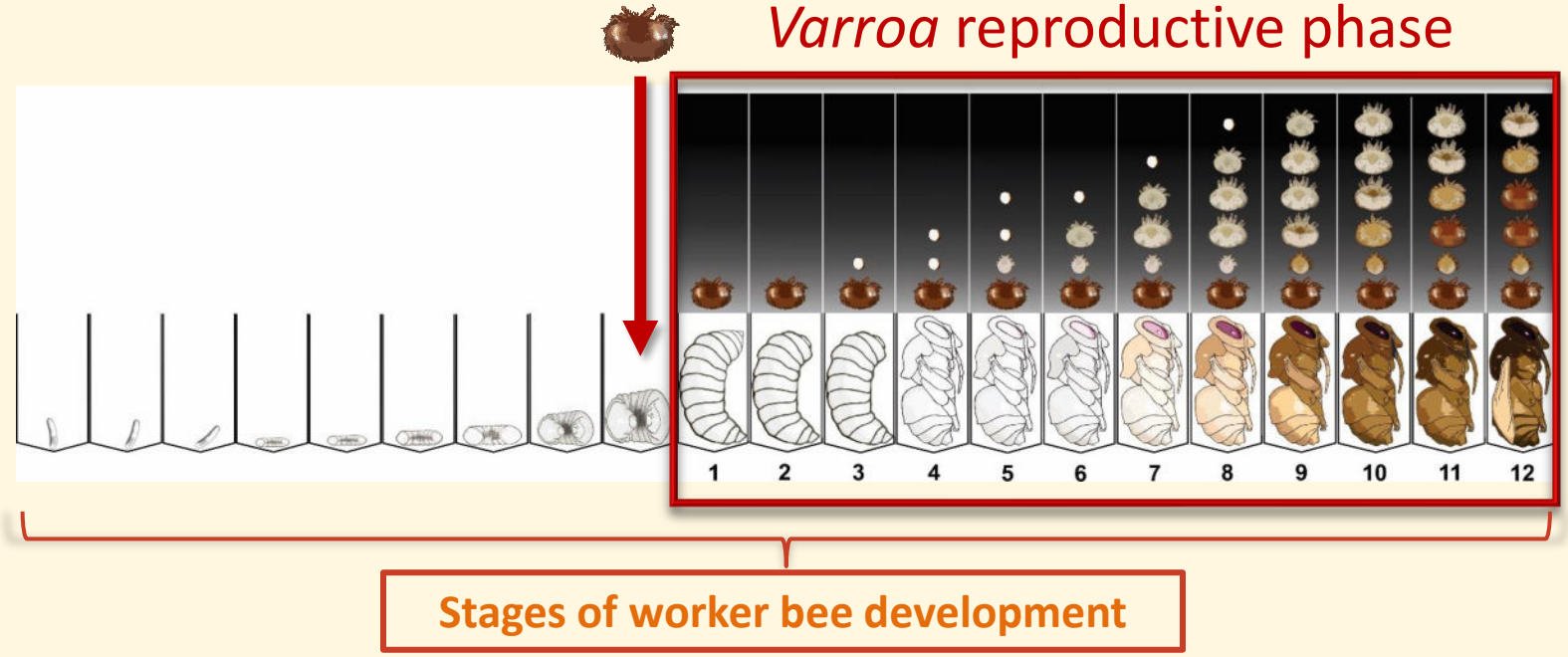
Testing the effect of Norroa™ on Varroa mite survival and reproduction



Bee pupae were uncapped just before emergence:

- *Varroa*: Number of *Varroa* per pupal cell, number of mite offspring, survival status
- Bee pupae: Age and survival status

Photo Credit: Phil Lester

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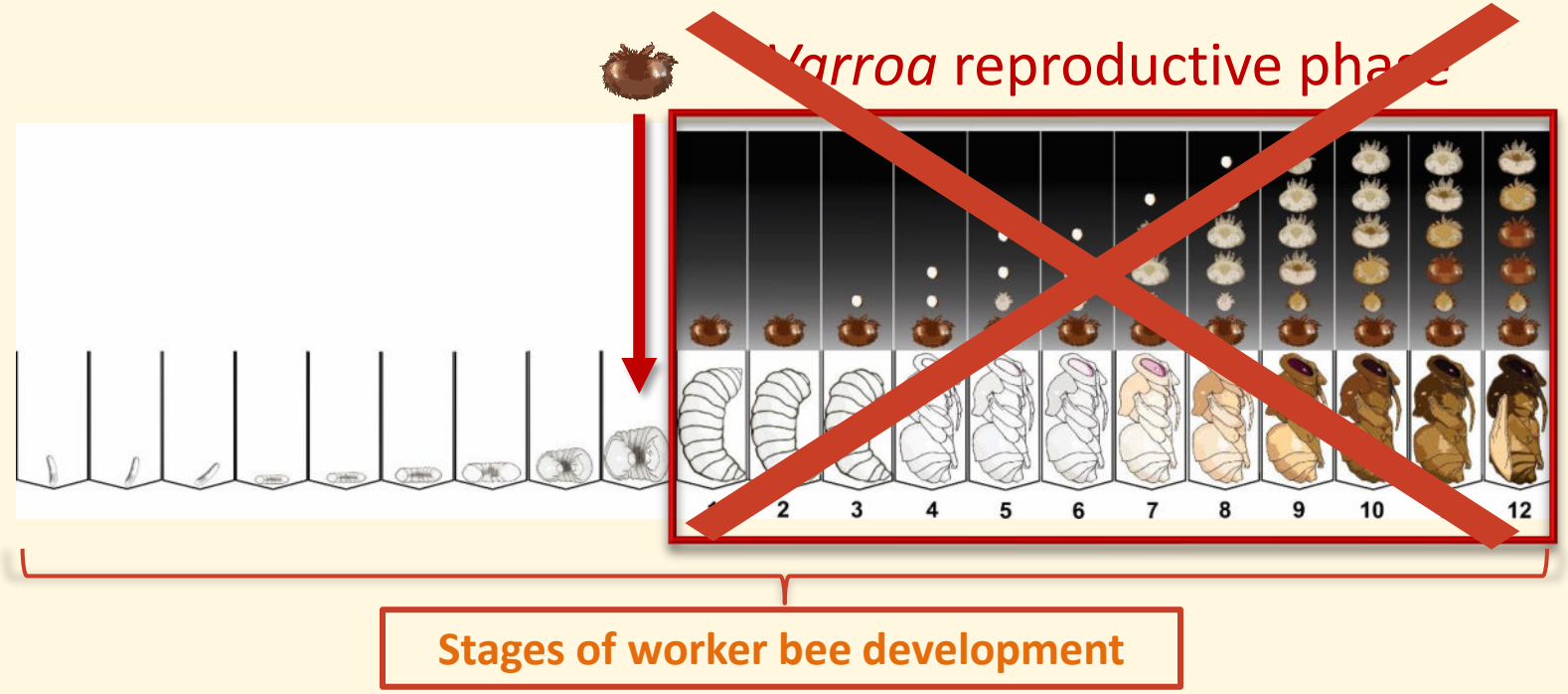
Testing the effect of Norroa™ on Varroa mite survival and reproduction



Bee pupae were uncapped just before emergence:

- *Varroa*: Number of *Varroa* per pupal cell, number of mite offspring, survival status
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Photo Credit: Phil Lester

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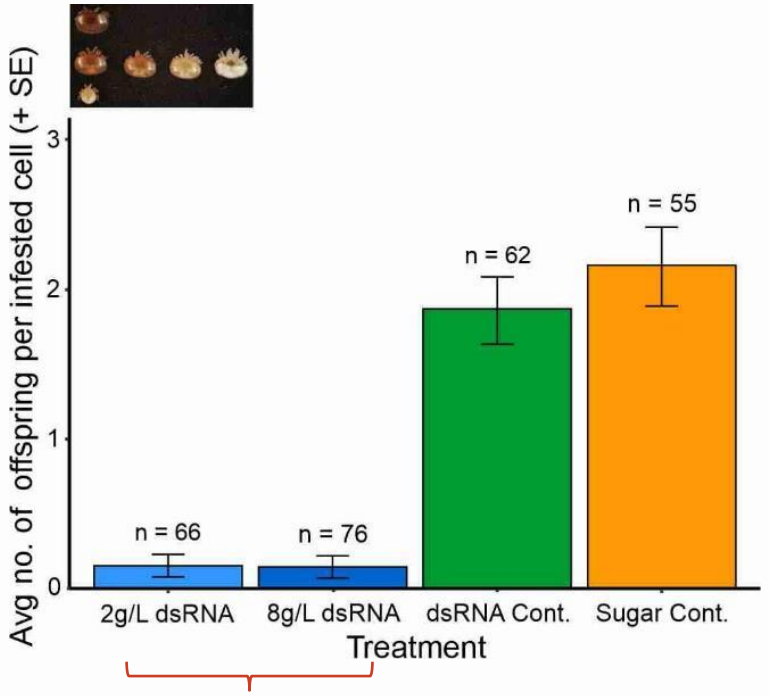
Norroa™ prevents mite reproduction in brood cells

Average number of mite offspring per infested cell

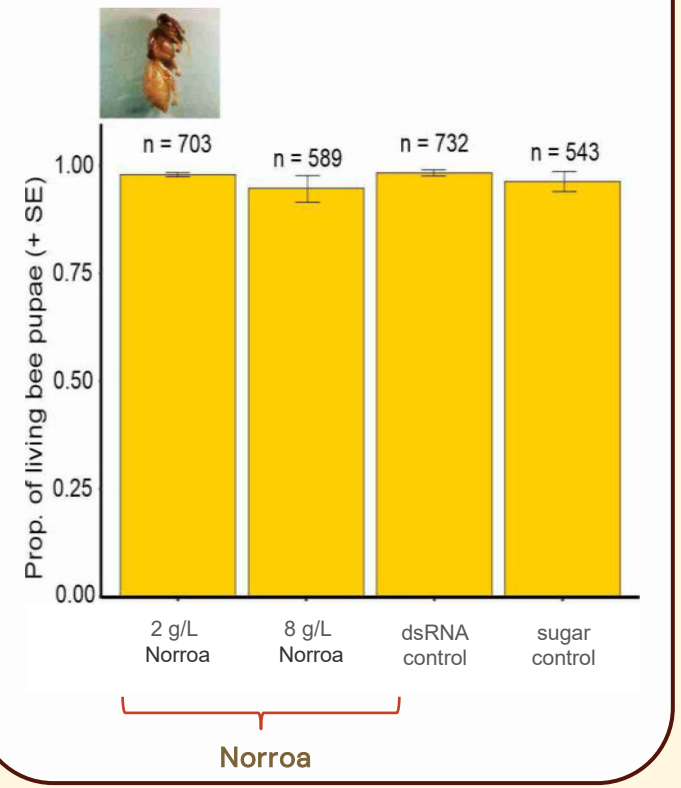


- Mini-hives with separate foraging area
- Mites added ~2-3 days before cells were capped
- Uncapped and assessed ~day 12 of pupation cycle or ~14 days after exposure to pouch

Results

- Norroa inhibited reproduction of mites
- No evidence of Norroa impacting bee pupae survival or development

Proportion of living pupae



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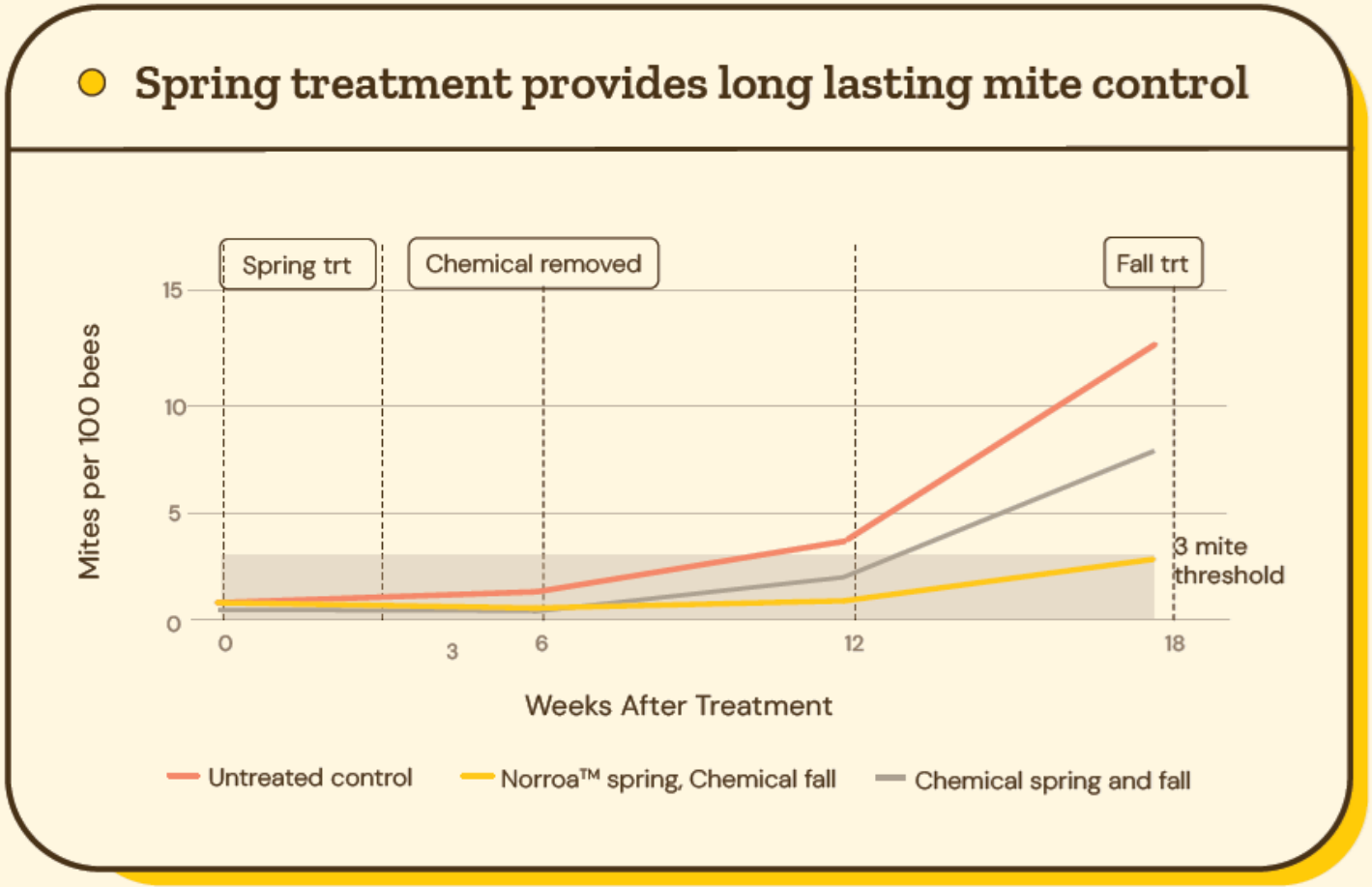
Norroa™ provides long-lasting control of mites. Up to 18 weeks of control!



- Norroa was the only treatment to maintain mite numbers below threshold (3 mites/100 bees) until the fall treatment was applied
- All treatments are better than untreated
- Norroa application in the spring provides longer and more effective mite control

Cross-trial results from 5 field trial locations in 2022 (FL, GA, ID, ME, ND) to compare Norroa to chemical treatment

Trial ID's: USICJM2022_98F (ME), USICJM2022_99F (GA), USICJM2022_100F (FL), USICJM2022_101F (ND), USICJM2022_102F (ID)

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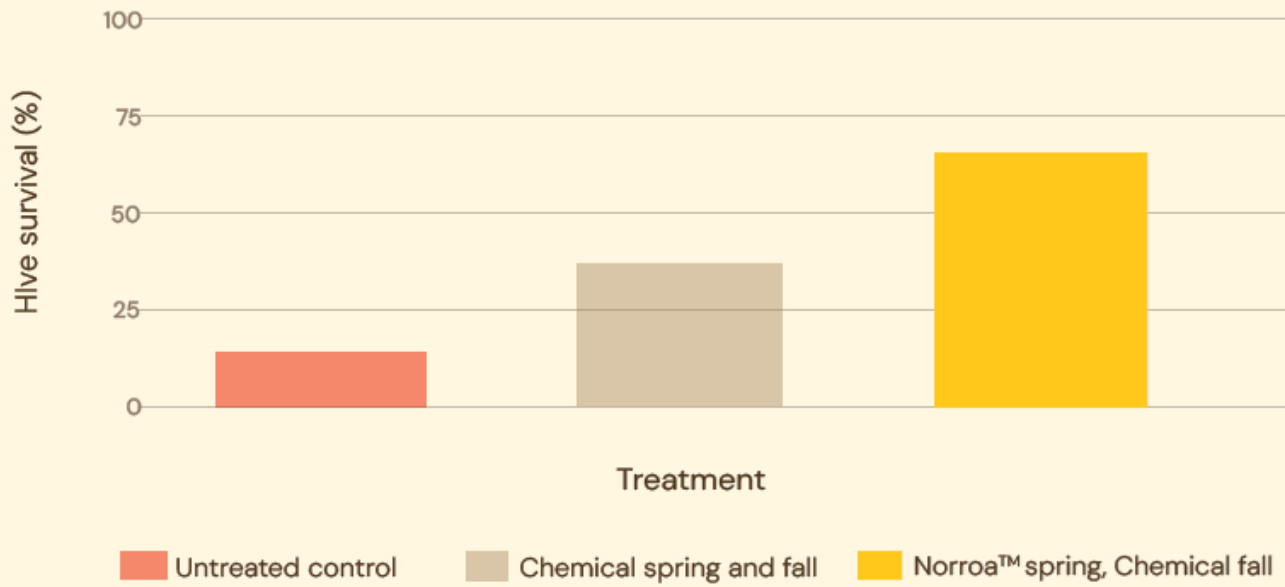
Norroa™ gave excellent mite control and resulted in much higher winter survival

Norroa™ significantly boosts overwintering survival



- Highly stressful trials with large colony losses – primarily driven by *Varroa* mites
- Norroa treatment in spring results in dramatic improvement in overwintering survival compared to chemical control and untreated control

Dead Hive: A hive is considered dead if it has less than 2 frames of bees or is hopelessly queenless and will not be requeened.

Trial ID's: USICJM2022_99F (GA), _100F (FL), _101F (ND), _102F (ID)

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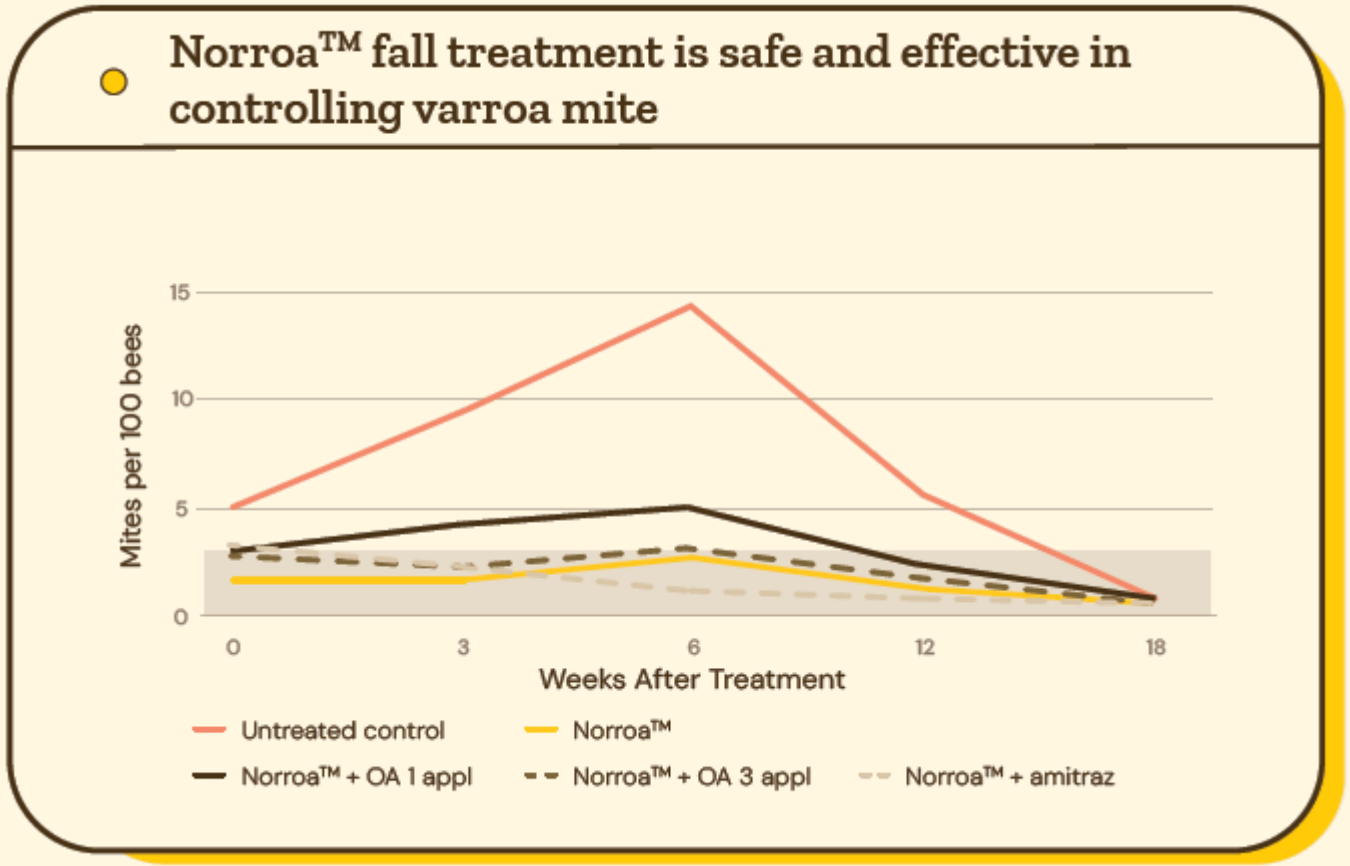
Norroa™ applied in the fall provided control of varroa mites in Florida (USA) in 2024



- Norroa applied at 'full dose' in the autumn gave control of varroa mites
- Starting mite numbers were at or above treatment threshold
- One trial, but addition of other products did not improve control.

Number of varroa mites per 100 bees at ~0, 3, 6, 12, and 18 WAT in Florida trial

Trial ID: USICBM2024_540F. Means with different letters are significantly different (using Tukey's at 0.05). Applications on 12 Oct, 17 Oct, 23 Oct, 1 Nov. Norroa application was a split application.

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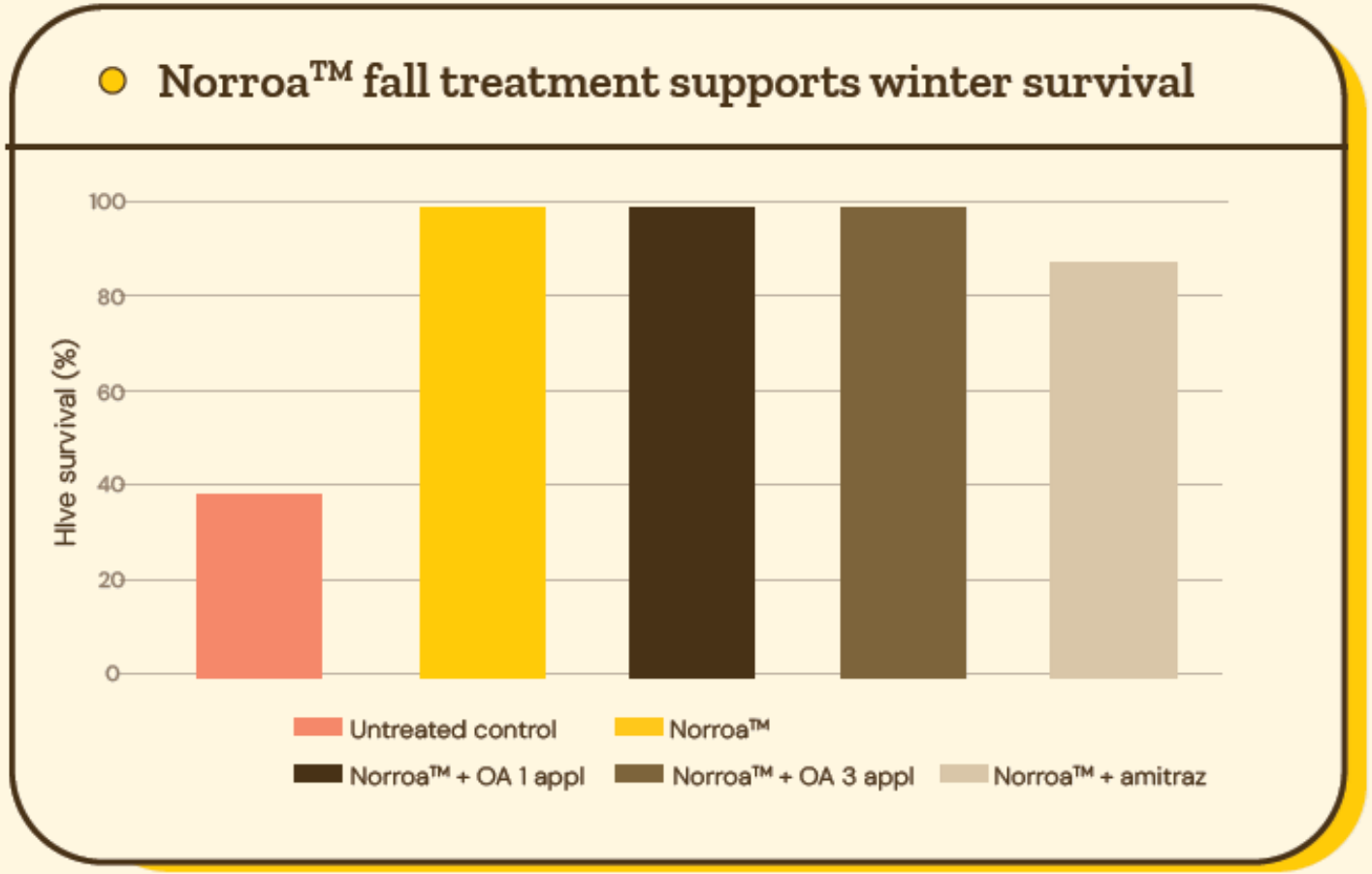
Norroa™ applied in the fall resulted in improved overwintering survival compared to untreated colonies



- All Norroa fall treatments resulted in improved overwintering survival compared to untreated colonies
- Combination treatments with single or double OA treatment or amitraz treatments did not improve overwintering survival compared to Norroa alone.

Percentage of dead colonies at ~18 WAT (17 Feb '25)

Dead Hive: A hive is considered dead and removed from the study if it reaches 15 mites/100 bees, has less than 2 frames of bees or is hopelessly queenless and will not be requeened.

Trial ID: CAICBM2024_228F. Means with different letters are significantly different (using Tukey's at 0.05).

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A novel biopesticide - Norroa™ for mite control

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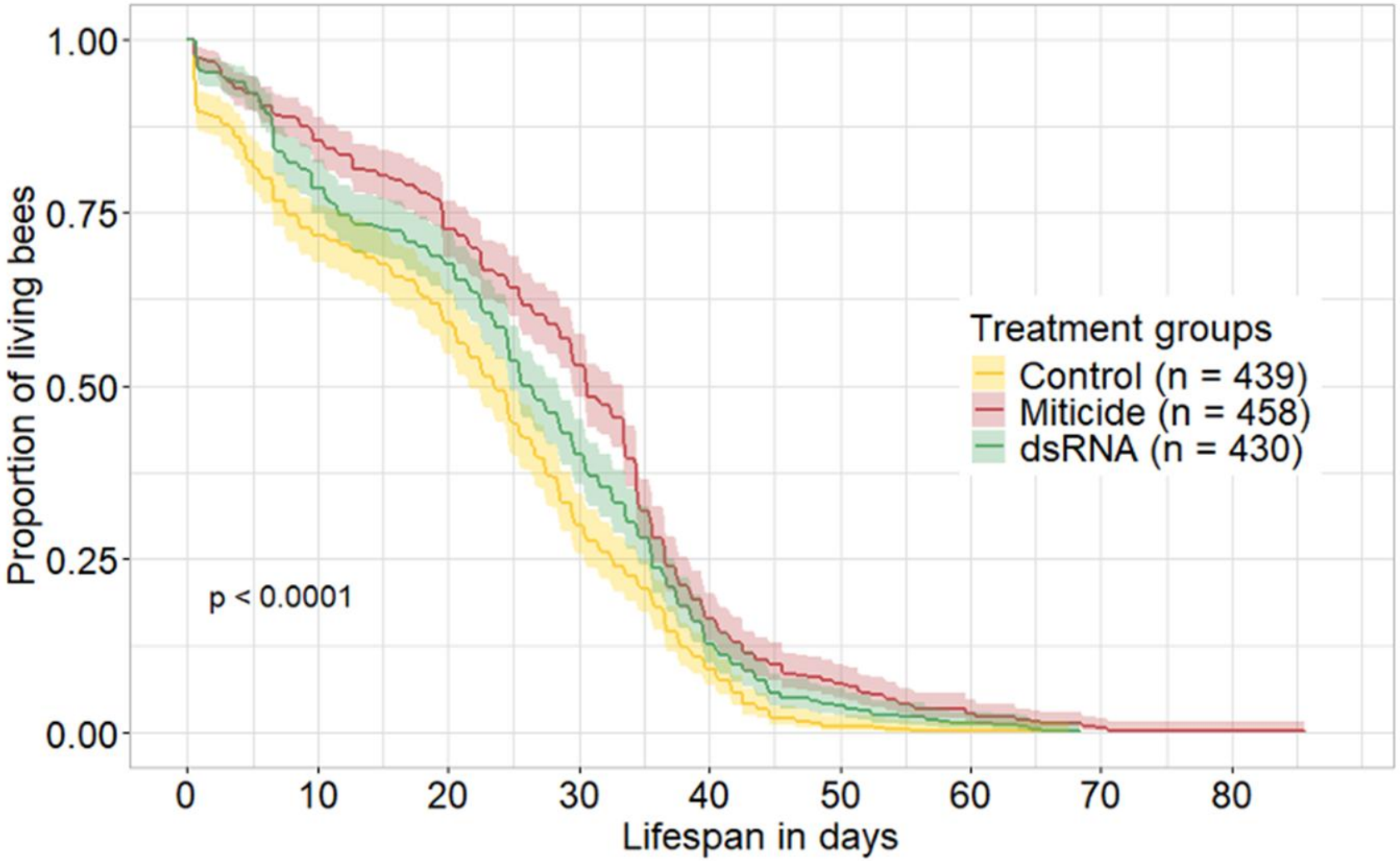
Honey bee longevity using RFID tags



Average individual bee lifespan:

- Control: 22.0 days
- dsRNA: 25.5 days
- Miticide: 28.8 days

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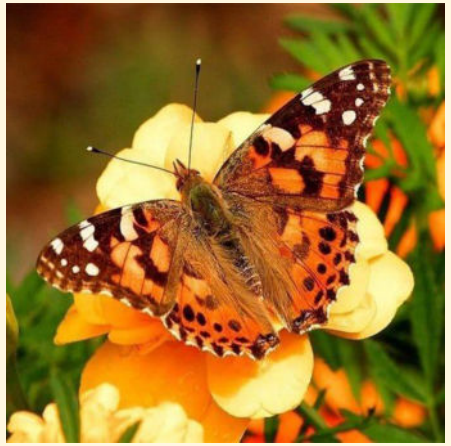
Off-target effects

1. Experimentally determined vadescana presented no off-target effects in the greater wax moth (*Galleria mellonella*) in feeding assays

2. Using bioinformatics, we examined the potential for off-target effects of the dsRNA on 39 arthropods known to be associated with beehives and insects of conservation concern



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Off-target effects using bioinformatics

Only 6 species – siRNA hits at 21 nt length:

- Monarch butterfly
- Fruit fly
- European earwig
- *Stratiolaelaps scimitus*
- *Tetranychus urticae*
- *Tropilaelaps mercedesae*

In monarchs, one hit was predicted to silence a calmodulin gene. But no off-target effects in feeding experiments

PLOS ONE

RESEARCH ARTICLE

Evaluating toxicity of Varroa mite (*Varroa destructor*)-active dsRNA to monarch butterfly (*Danaus plexippus*) larvae

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Data Availability Statement: The raw data are available at https://github.com/Niranjana296/Evaluating-Toxicity-of-Varroa-dsRNA-to-Monarch-Larvae.

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Abstract

Varroa mites (*Varroa destructor*) are parasitic mites that, combined with other factors, are contributing to high levels of honey bee (*Apis mellifera*) colony losses. A Varroa-active dsRNA was recently developed to control Varroa mites within honey bee brood cells. This dsRNA has 372 base pairs that are homologous to a sequence region within the Varroa mite calmodulin gene (*cam*). The Varroa-active dsRNA also shares a 21-base pair match with monarch butterfly (*Danaus plexippus*) calmodulin mRNA, raising the possibility of non-target effects if there is environmental exposure. We chronically exposed the entire monarch larval stage to common (*Asclepias syriaca*) and tropical (*Asclepias curassavica*) milkweed leaves treated with concentrations of Varroa-active dsRNA that are one- and ten-fold higher than those used to treat honey bee hives. This corresponded to concentrations of 0.025–0.041 and 0.211–0.282 mg/g leaf, respectively. Potassium arsenate and a previously designed monarch-active dsRNA with a 100% base pair match to the monarch *v-ATPase A* mRNA (leaf concentration was 0.020–0.034 mg/g) were used as positive controls. The Varroa mite and monarch-active dsRNA's did not cause significant differences in larval mortality, larval or pupal development, pupal weights, or adult eclosion rates when compared to negative controls. Irrespective of control or dsRNA treatment, larvae that consumed approximately 7500 to 10,500-mg milkweed leaf within 10 to 12 days had the highest pupal weights. The lack of mortality and sublethal effects following dietary exposure to dsRNA with 21-base pair and 100% base pair match to mRNAs that correspond to regulatory genes suggest monarch mRNA may be refractory to silencing by dsRNA or monarch dsRNase may degrade dsRNA to a concentration that is insufficient to silence mRNA signaling.

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Thank you

- Victoria University of Wellington
- GreenLight Biosciences
- Countless Beekeepers, monitors, students, and cooperators

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Questions?

Thank you!

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