

Discovering and diagnosing honey bee killers

What's killing the honey bees?

How might we discover which viruses are affecting bee colonies?

Background

What jobs do honeybees perform? Honeybees are known for their honey, made from the nectar they gather from flowering plants. As they gather nectar, pollen collects on their legs and is transferred to new plants. This is called pollination. Most flowering plants and many food crops rely on pollinators, the organisms that transfer pollen.

Beekeepers travel with their hives around the United States to help pollinate crops. Almond trees in California are some of the biggest customers.

Annual losses for honeybee colonies have been increasing.

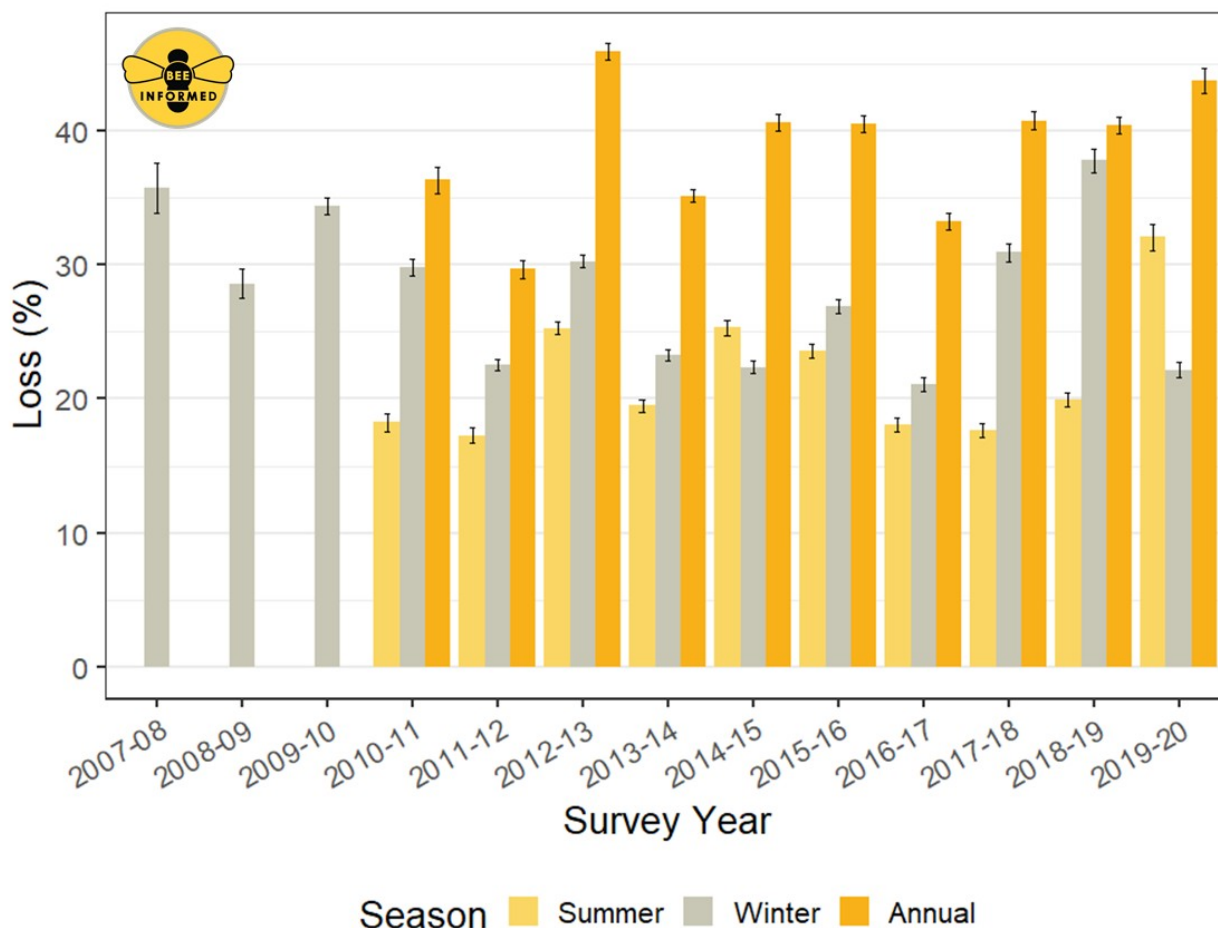


Figure 1. National Honey Bee Colony Loss Survey

<https://beeinformed.org/2020/06/22/preliminary-results-of-the-2019-2020-national-honey-bee-colony-loss-survey/>

What is causing these losses? Multiple factors contribute to colony losses: nutrition, weather, pesticide use, management of colonies, genetics, pathogens, and mites. This activity will focus on Deformed Wing Viruses (DWV), a rapidly evolving group of closely-related viruses. DWV has high infection rates associated with colony loss. These viruses are transmitted by a major honey bee pest, a mite called *Varroa destructor*.

Discovering and diagnosing honey bee killers

Your task

A commercial beekeeping operation has **lost 10%** of their colonies after almond pollination. Word has spread that a new variant of DWV has been discovered and is transmitted by the parasitic mite, *Varroa destructor*. The beekeeper has asked your diagnostic lab to determine if the new variant called Varroa Destructor Virus-1 (also known as DWV type B) is in their operation. Also, the local beekeeper association has asked the lab to create a new diagnostic test that will distinguish this new variant from the first known variant, called DWV type A.

To help your efforts, a researcher at the university has isolated and sequenced a portion of the new variant found in the two dying colonies (Colony 299 and Colony 300). They have sent you the raw sequences for analysis and test development.

You are expected to:

1. View, trim, and combine raw sequencing files (ABI) to create DNA sequences for further analysis.
2. Use BLAST, a basic bioinformatics tool, to search DNA databases.
3. Align sequences from the database to your unknown sequence for identification.
4. From these alignments, develop new primers that will only detect (amplify) the variant that matches your sequence.

Procedure

Download the honey bee (.ABI) sequences in the Honey Bee Colony Sequences folder to your desktop. <https://grownextgen.org/go/honeybee>

Go to: <https://dnasubway.cyverse.org/> then follow the steps below to analyze your sequences.

On the DNA subway page, enter as a guest.

Click on “Determine sequence relationships” (blue square).

You are now on the blue line of the DNA Subway. Complete the following steps:

1. Select Project Type - ‘Viral’
2. Click ‘Upload’
3. Click ‘Choose Files’
4. Select all Raw Sequence Files (.abi) from your desktop
5. Give a title under ‘Name Your Project’
6. Click ‘Continue’

STOP 1 Assemble Sequences

Once the sequences are uploaded, click ‘Sequence Viewer’.

On the window that opens, click the small icon beside the Colony identifier/name. These will display the raw sequence reads/trace files/ chromatograms of the sequences. Along the top of these graphic displays, the nucleotide sequence from the chromatogram is displayed. Below the peaks a scroll bar will appear. Scroll along the entire length of the sequence. Close out of that window by clicking on the ‘x’ in the top right corner.

Click ‘Sequence Trimmer’ to run Trimmer Tool (blinks). Click ‘Sequence Trimmer’ again to view trimmed sequences. Chromatograms and sequences have been trimmed, removing the poor resolution on the ends of each sequence. Close out of that window by clicking on the x in the top right corner.

Discovering and diagnosing honey bee killers

Click 'Pair Builder'. Click 'Auto Pair'. This tool pairs the forward (F) and reverse (R) sequences of each colony. Save your pairs. Close the window by clicking on the x in the top right corner.

Click 'Consensus Builder'. Click on the sequence name on the left. Scroll to see entire sequence – Forward (F) & Reverse (R) sequences are aligned to produce the single consensus sequence. Notice how the F and R sequences overlap in the middle. Be sure to click on the other sequence name to observe. Close the window by clicking on the 'x' in the top right corner.

STOP 2 Add Sequences

Click 'BLASTN' (Basic Local Alignment Tool – for Nucleotides) to run the algorithm.

In the window, click on 'BLAST' in the Action column for Colony-299. After a few seconds, you will see the sequence hits within the database (Accession # and Details) as well as comparison values (mismatches) between your sequence (Colony-299) and the sequences found in the database. Repeat the analysis for Colony-300.

Question: What are the names of the viruses from the BLASTN analysis?

Question: How many mismatches are there between the sequences found in Colony-299 and Colony-300 and the two viruses found in the BLASTN analysis?

Question: What virus was detected in Colony-299? in Colony-300? Explain how you know.

In the BLASTN window, select both accessions by clicking each box within the Accession # column. Then click: 'Add BLAST hits to project'.

STOP 3 Analyze Sequences

Click 'Select Data'.

Click 'Select All', then 'Save Selections'.

Click 'MUSCLE' (Multiple Sequences Comparison by Log-Expectation) to run the algorithm, then click again to view.

Zoom all the way in to see nucleotide differences. If a sequence has a different nucleotide at a position than the consensus (majority of the sequences), the unique nucleotide at that position is displayed inline.

Question: In this view, which sequence in the MUSCLE analysis shows the most differences?

Click 'Sequence Similarity' to get a matrix of similarity comparisons. The table shows the pairwise comparisons between the different sequences in the alignment. The top diagonal and bottom diagonal give the same pairwise results (mirrored).

Question: What sequence has the lowest similarity percentages with the other sequences? What is the lowest percent similarity?

*This document may be reproduced for educational purposes, but it may not be reposted or distributed without crediting GrowNextGen and The Ohio Soybean Council and soybean checkoff.

