

Surveillance for avocado sunblotch viroid utilizing the European honey bee

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Avocado sunblotch viroid (ASBVd)

- ❑ Circular, single-stranded RNA molecule, 247 nt.
- ❑ Adopts a rod-shaped secondary structure *in planta*
- ❑ Natural host range is limited to avocado.
- ❑ Seed-transmitted, no vectors, mechanically transmissible.
- ❑ Reduces quantity and size of fruit and affects marketability through unsightly scarring on skin.
- ❑ Major trade impediment because of quarantine restrictions on fresh fruit.
- ❑ Very rare in Australia due to clean planting material scheme.

Costa Rica bans avocado imports from 9 countries



By The Tico Times May 12, 2015



Surveillance for ASBVd

Evidence of pest-freedom required to facilitate trade.

Question: How can an orchard containing 30,000 trees, each 10 x 5 m in size, be rapidly and inexpensively surveyed for ASBVd?

Potential solution: Bee-assisted surveillance!

- ASBVd is pollen-transmitted.
- Bee hives brought in to boost pollination.
- ASBVd is a very resilient molecule in the environment.
- Worker bees forage on thousands of flowers and store pollen in hive.



Leaf stored at room temp for 4 weeks and still strongly positive (Ct = 17.6)

Methods

- ❑ Study done in Australia and South Africa, but only Australian results presented here.
- ❑ Block of avocados containing 343 trees, including a tight clump of four infected trees.
- ❑ Bee hives at c. 100, 200, 300 and 400 m distance from infected trees.
- ❑ Sampling done at end of avocado flowering period – 28 Sep 2020.
- ❑ Worker bees collected from flowers on infected tree.
- ❑ Worker bees and pollen samples collected from hives.
- ❑ Diagnosis done using three methods:
 - RT-qPCR using Taqman™ probe.
 - Illumina sequencing of ribo-depleted total RNA and small RNA (21-22 nt) – *de novo* assemblies and mapping of reads to reference genomes (CLC Genomics Workbench v20).



Arrangement of hives (QLD1-4) relative to clump of four infected trees (red dot)

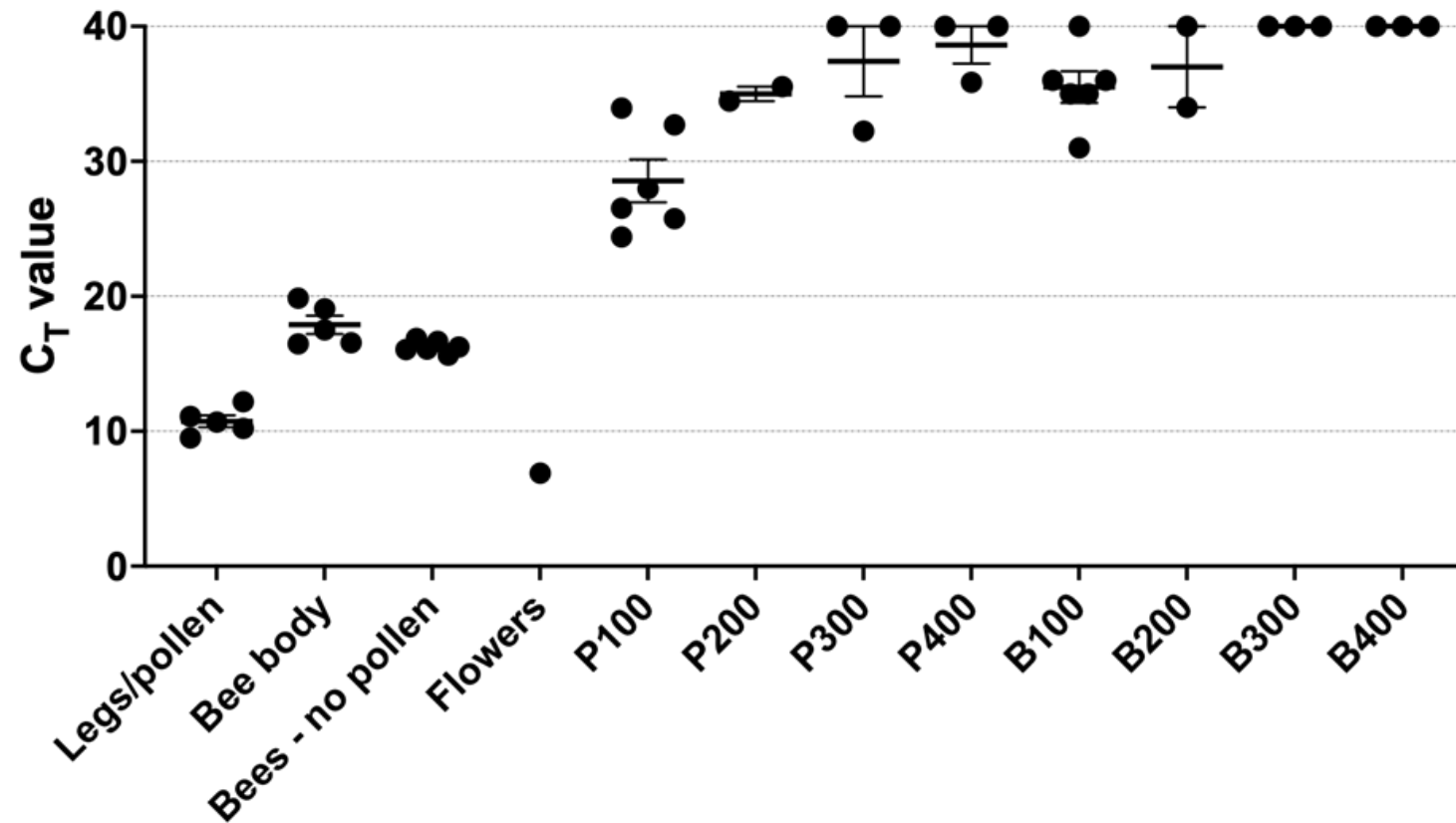
Methods



Identification of most common plant species represented in hive pollen (# sequence contigs)

Plant species (closest match)	QLD1 hive	QLD2 hive	QLD3 hive	QLD4 hive
<i>Eucalyptus grandis</i>	4,143	3,638	3,672	2,927
<i>Syzygium oleosum</i>	701	220	557	562
<i>Citrus</i> spp.	440	227	638	8
<i>Raphanus sativus</i>	738	138	127	99
<i>Rhodamnea argentea</i>	396	82	276	284
<i>Brassica</i> spp.	31	136	13	48
<i>Medicago truncatula</i>	64	2	56	6
<i>Camellia sinensis</i>	47	0	6	0
<i>Gossypium</i> spp.	0	32	13	2
<i>Cicer arietinum</i>	19	2	21	4
<i>Pyrus x bretschneideri</i>	11	0	8	24

RT-qPCR detection of ASBVd



High throughput sequencing (HTS) detection of ASBVd in hive samples

Sample name	Sample type	HTS type	Normalized sequence copy no.
QLDforagers	Forager bees on infected tree	RNA-seq	0.6
QLDforagers	Forager bees on infected tree	Small RNA-seq	289
QLD1	Hive bees	Small RNA-seq	0.3
QLD1	Hive pollen	Small RNA-seq	8.7
QLD2	Hive pollen	Small RNA-seq	0
QLD3	Hive pollen	Small RNA-seq	0
QLD4	Hive pollen	Small RNA-seq	0

Other viruses and viroids that were detected in hives

Virus/viroid	Family	% max nt identity	% genome coverage	RNAseq		sRNA	
				Bees	Pollen	Bees	Pollen
Persea americana alphaendornavirus 1**	<i>Endornaviridae</i>	99	99	✓	✓	✓	✓
Persea americana chrysovirus**	<i>Chrysoviridae</i>	99	98	✓	✓	✓	✓
Pelargonium zonate spot virus	<i>Bromoviridae</i>	99	98	✓	✓	✓	✓
Tomato ringspot virus**	<i>Secoviridae</i>	97	63	✓	✓		
Solanum nigrum ilarvirus**	<i>Bromoviridae</i>	99	16	✓	✓		
Peanut stunt virus**	<i>Bromoviridae</i>	99	78	✓			
Turnip mosaic virus	<i>Potyviridae</i>	91	37	✓			
Pear blister canker viroid*	<i>Pospiviroidae</i>	99	100	✓			




Status
**Exotic
*New for QLD

Conclusions

- ❑ Bee-assisted surveillance is a valuable tool for determining ASBVd pest status of an avocado orchard.
- ❑ Stored pollen is the best sample type.
- ❑ Optimal when trees are no further than 100 m from any tree (normal stocking rate is 2-3 hives/ha).
- ❑ Multi-purpose surveillance method – plant and bee viruses, viroids, maybe fungi, and varroa mites.
- ❑ Biased surveillance method.
 - Plants must be flowering at the time of sample collection.
 - Bees prefer some flowers over others and you can't dictate where they go!
 - Favours pollen-transmitted viruses/viroids and high titre mechanically transmissible viruses (e.g. ilarviruses, sobemoviruses and tobamoviruses).
- ❑ Best done by specialists!



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