DETECTION OF SMALL HIVE BEETLE (*AETHINA TUMIDA* MURRAY) IN NATURALLY INFESTED HIVES USING DNA ANALYSIS OF HIVE DEBRIS AND SCRAPS

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INTRODUCTION

Aethina tumida or the small hive beetle (SHB) is an emerging threat in the beekeeping world. Reliable and quick methods have to be developed to detect low levels of infestation. Detecting DNA from tissues of SHB by screening hive debris is a promising new method, as adults and mature larvae can be found on the bottom board.

Objective

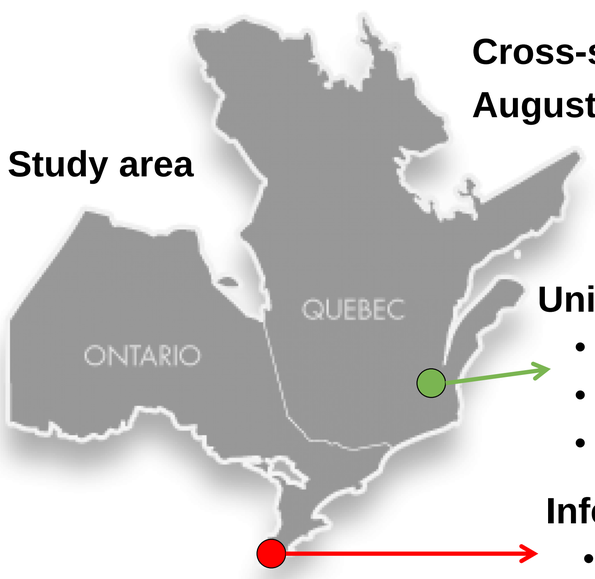
Estimate the sensitivity and specificity of PCR to detect SHB in debris and scraps from naturally infested colonies

METHODS

Cross-sectional study

August 23rd to September 2nd, 2017

Study area



Uninfested area, Deschambault

- Negative control
- 1 apiary (C)
- 21 colonies

Infested area, Essex County

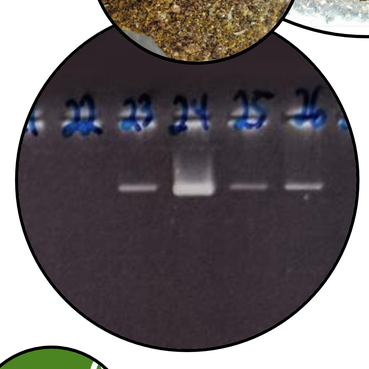
- 3 apiary (G, S, T)
- 36, 38 and 25 colonies

Visual hive inspection

- Assess SHB status
- Modified top bar inspection (MAPAQ)
  - Under the top lid
  - Top of each brood chamber
  - Bottom board

Collection of bottom board debris and scraps

- Frozen at -80ºC



DNA extraction and conventional PCR amplification

- On 60 randomly selected samples
- Tested for honey bee actin to confirm DNA extraction

Long set of primers (1080-bp)

- Mitochondrial cytochrome c oxidase subunit I (COI) *A. tumida* gene
- AT1904S - 59- GGTGGATC TTCAGTTGATTTAGC-39
- AT2953A - 59-TCAGCTGG GGGATAAAATTG-39
- Evans *et al* 2000

Short set of primers (109-bp)

- Mitochondrial cytochrome oxidase I (COI) *A. tumida* gene
- SHB207F - TCTAAATACTA CTTTCTTCGACCCATC (A/G)
- SHB315R - TCCTGGTAGAA TTAAAATATAAACTTCTGG
- Ward *et al* 2007

Statistics

Specificity of PCR testing for the two sets of primers was evaluated in colonies from the negative apiary. In infested apiaries, the probability of a positive PCR according to the level of infestation based on visual inspection was described and compared using exact chi-square tests.

RESULTS AND DISCUSSION

Visual inspection

Québec: no SHB were found in the 21 colonies
Ontario: 47/97 (48.5%) colonies had SHB visually detected

Short primers (109-bp)

- False positive in all uninfested colonies from Québec
- Honey bee actin detected in all samples
- Poor specificity of detection
- Short set of primers are not appropriate for conventional PCR

Long primers (1080-bp)

- Specificity estimated to 100% in the 11 uninfested colonies from Québec
- Honey bee actin detected in all samples

Distribution of colonies according the visual inspection and PCR test (1080-bp) results, subset of colonies selected for PCR-testing

		Visual inspection			PCR (1080-bp)	
Apiary	Number of colonies	Number of SHB-positive colonies	% of SHB-positive colonies	Median (min-max) of SHB in positive colonies	Nb positive colonies	% positive colonies
C[1]	11	0	0%	N/A	0	0%
G[2]	21	16	76%	2.5 (1-19)	8	38%
S[2]	16	5	31%	2 (1-3)	6	38%
T[2]	12	5	42%	1 (1-1)	4	33%

[1] Uninfested apiary from Québec. [2] Infested apiaries from Ontario.

Percentage of PCR-positive (1080-bp) colonies according to the level of SHB infestation in three SHB-positive apiaries

Nb SHB in colonies	Nb colonies	Nb positive colonies	% PCR-positive (1080-bp) colonies	
			Estimate	95% CI
0	23	5	21.7[a]	4.8-38.6
1	11	4	36.4[ab]	7.9-64.8
≥2	15	9	60.0[b]	32.2-83.7

[ab] Percentages with different superscripts are statistically different ($p<0.05$, exact χ^2 test)

- The quality and quantity of material sampled may affect PCR sensitivity, as debris and scraps are usually removed by honey bees. DNA from debris might also deteriorate overtime, before being collected for analysis.

CONCLUSION

Collection of debris for PCR testing was quick and can be done by less trained inspectors compared to visual inspection. Our results suggest that PCR testing for SHB with the long set of primers (1080-bp) was specific, with a sensitivity increasing with the level of SHB infestation at the colony level. Hive debris screening of multiple colonies in an apiary is a promising method to assess the SHB status of the apiary, and warrant future development and validation for early detection of SHB infestations.

REFERENCES

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Pictures: S Gingras, M. Bernier, P-L. Mercier and J. Moisan DeSerres

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