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Original article

A DNA method for screening hive debris for the presence of small hive beetle (*Aethina tumida*)*

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Abstract – The small hive beetle (SHB) is a parasite and scavenger of honey bee colonies. It has recently become an invasive species creating the need for an efficient and reliable detection method. We present a method to screen hive debris for the presence of SHB using real-time PCR in conjunction with an automated DNA extraction protocol. The method was able to detect DNA from SHB eggs, larvae and adult specimens collected from Africa, Australia and North America. The method was used to successfully detect SHB DNA extracted from spiked and naturally infested debris. An *Apis mellifera* 18S rRNA real-time PCR assay was used as an internal positive control (IPC). The IPC showed that the method was reliable for detection as extraction efficiency was consistent between hive debris samples. If the SHB were to establish at new locations, the availability of such a method would be a valuable support tool to enable species identification and rapid screening of hive debris for delimiting surveys.

Aethina tumida **/ real-time PCR / honey bee / small hive beetle / hive debris**

1. INTRODUCTION

The small hive beetle, (*Aethina tumida* Murray: Coleoptera, Nitidulidae, = SHB), is a minor pest of African honey bee colonies (Neumann and Elzen, 2004; Neumann and Härtel, 2004), that has recently become a harmful invasive species (Hood, 2004; Neumann and Elzen, 2004; Ellis and Hepburn, 2006). The beetle has attainted pest status in around 30 states in North America (Hood, 2004) where the economic impact on the Apiculture industry has been significant (Taber and Hood, 2000; Neumann and Elzen, 2004; Hood, 2004; Ellis, 2005; Harman, 2005). The beetle has now been reported in Canada (Hood, 2004) and Australia (White, 2004). It is anticipated that a similar scale of impact is likely to occur if the beetle establishes in other areas of the world with suitable climatic conditions.

There is currently a lack of molecular techniques for rapid detection and identification of the SHB. In particular, the eggs and larvae are difficult to detect and identify by morphological techniques alone (Murihas, 2004). In addition, there has been little focus on developing methods for detection of SHB at low (cryptic) levels of reproduction within a hive, where an overt infestation (which is readily identified on hive inspection) is not evident (Spiewok and Neumanm, 2006).

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The objective of this research was to develop a fast extraction protocol in conjunction with a real-time PCR assay to enable rapid identification of the SHB life stages, as well as screening of hive debris for detection of SHB – the method may be a valuable tool to test the many thousands of hive samples that might be submitted for laboratory diagnosis in the event of an incursion. The method could also have further value for routine surveillance of hives in high-risk areas such as those close to major ports, or where climatic conditions may be more conducive for establishment of the beetle.

2. MATERIALS AND METHODS

2.1. DNA extraction from SHB beetle, larval and egg samples

DNA was extracted using a modification of the 'DNA purification system for food' kit (Promega, Southampton, UK) as follows; the magnetic particles were manipulated using a robotic magnetic particle processor and the 'total genomic DNA programme' (Kingfisher mL, Thermo Labsystems). SHB adult or larval tissue (~100 mg) or eggs (10–15), were placed in 2 mL micro-centrifuge tubes containing approximately 700 mg of 2.4 or 1.0 mm Zirconia beads (Biospec Products, Bartlesville, Oklahoma, USA) and 1 mL of Wizard® MagneSil™ extraction buffer A containing 1% antifoam B (Sigma). The tubes were shaken for 30 s at full speed on a mini bead-beater, and then centrifuged at 10000 *g* for 2 min. Clarified extract (1 mL) was transferred to a fresh 2 mL micro-centrifuge tube and 250 μL of lysis buffer B (Promega) and 750 μL of precipitation buffer (Promega) were added. After vortexing, tubes were spun at 10000 g for 10 min. Clarified extract (750 μL) was transferred to a fresh micro-centrifuge tube and 50 μL of kit MagneSil™ beads and 600 μL of isopropanol was added. Tubes were incubated at room temperature for 10 min. The Kingfisher ML 5 tube strips were loaded as follows: tube 1: 1.4 mL of the sample containing the MagneSil™ beads; tube 2: 1 mL of kit lysis buffer B; tubes 3 and 4: 1 mL of 70% ethanol; tube 5: 100 μL 1 × TE buffer. DNA collected in tube 5 was transferred to a fresh 1.5 mL micro-centrifuge tube and stored at –20 °C prior to use in real-time (TaqMan) PCR.

2.2. DNA extraction from hive debris samples

DNA was extracted from hive debris samples by placing 10 g samples of debris into grinding mill canisters (Kleco, Visalia, California, USA). CTAB (Hexadecyltrimethylammoniumbromide, [Sigma]) lysis buffer (12% Sodium phosphate buffer pH 8.0, 2% CTAB, 1.5 M NaCl), (20 mL) containing 1% antifoam B (Sigma) was added to each canister. The canisters were sealed, loaded onto the Kleco grinding mill and ground for 2 min at top speed. The lysate from each canister was poured into a 50 mL Falcon tube and the tubes were spun at 4000 *g* for 5 min; 2 mL of the cleared lysate was removed and placed into a 2 mL micro-centrifuge tube. The tubes were spun for a further 3 min at 10000 *g*. Cleared lysate (1 mL) was transferred to a fresh 2 mL micro-centrifuge tube and 250 μL of lysis buffer B (Promega) and 750 μL of precipitation buffer (Promega) were added. After vortexing, tubes were spun at 10000 *g* for 10 min. Clarified extract (750 μL) was transferred to a fresh micro-centrifuge tube and 50 μL of kit MagneSil™ beads and 600 μL of isopropanol was added. Tubes were incubated at Room temperature for 10 min. DNA was extracted from each sample using the robotic magnetic particle processor (Kingfisher mL, Thermo Labsystems) in conjunction with the Promega DNA purification system for food kit as described previously for the beetle/larval extractions.

2.3. Validation of a method to screen hive debris for the presence of the SHB

2.3.1. SHB detection in spiked hive debris

To determine how much beetle or larval tissue was being spiked into hive debris material, 10 adult SHB and 10 larvae (Stellenbosch, South Africa) were weighed separately to obtain an average weight for each life stage. In addition, 10 adults and 10 larvae were cut in half and a half segment from each sample was weighed separately to obtain an average weight for each.

Hive debris was collected from the Central Science Laboratory apiaries during the spring and summer when the colonies were active. Samples of debris (30 g) were weighed out and spiked with ½, 1, 2, 3 or 4 *A. tumida* adults (Stellenbosch, South Africa). Prior to spiking the debris, the beetles were

cut into small (~2 mm) size pieces using a sterile scalpel. The beetle pieces were thoroughly mixed into each 30 g debris sample. Each sample of spiked hive debris was then split into three 10 g replicates. DNA was extracted from each 10 g sample as described previously.

The experiment was repeated using ½, 1, 2, 3 and 4, *Aethina tumida* larvae. (The larvae were added whole to the debris samples). Positive controls were included for both experiments as ½, 1, 2, 3 and 4 beetles or larvae ground in 20 mL of CTAB lysis buffer without hive debris. Negative controls were included as unspiked hive debris ground in 20 mL of CTAB buffer and CTAB buffer only. The DNA was extracted as previously described, and tested using the SHB CO1 and bee 18S rRNA real-time PCR assays.

2.3.2. Detection of A. tumida in debris from infested hives

Debris was collected from a hive naturally infested with SHB in Beltsville, Maryland USA. Three 10 g replicate samples of debris were weighed out and placed in Kleco grinding mill canisters and ground for 2 min as described previously. DNA was extracted from 3 replicate 1 mL samples of cleared lysate as described previously. Final DNA extracts were tested neat and after diluting 1 in 10 in molecular grade water (BDH, Lutterworth, Leicestershire, UK) using the SHB CO1 and bee 18S rRNA real-time PCR assays.

2.4. 2.4 Design of a real-time (TaqMan®) PCR for the detection of A. tumida

Real-time PCR primers SHB207F - TCTAAATACTACTTTCTTCGACCCATC(A/G), SHB315R - TCCTGGTAGAATTAAAATATAAACTTCTGG and a probe SHB245T - ATCCAATCCTATACCAACACTTATTTTGATTCTTCGGAC were designed to the *A. tumida* cytochrome oxidase I gene (COI) from mitochondrial DNA using Primer Express™ software (PE-Biosystems). All 15 COI sequences available for *A. tumida* (Accession numbers AF227645–AF227654, AF522354–AF522358), were obtained from the EMBL sequence database and aligned using clustal V within the MegAlign package (DNASTAR INC., Madison, USA). In order to allow detection of the two haplotypes identified by these sequences, the forward primer was designed with degeneracy at nucleotide position 228 (AF227645). The fluorogenic probe was modified at the 3' end with a quencher dye TAMRA (tetramethylcarboxyrhodamine, Applied Biosystems) and at the 5' end with the fluorescent reporter dye FAM (6-carboxyfluorescein, Applied Biosystems).

A real-time PCR assay previously designed to the 18S rRNA gene of *A. mellifera* (Ward et al., 2007), was used in addition to the SHB primer in order to determine if this assay could be used as an internal positive control (IPC). An IPC is used to assess the efficiency of nucleic acid extraction and to verify false negative results. The bee 18S probe was labeled at the 3' and 5' ends as described previously for the SHB probe.

The real-time SHB assay was validated using DNA extracted from different life stages (eggs, larvae and adults) of *A. tumida.* Adults and larvae were obtained from infested hives from various locations: Australia (Queensland and Richmond, New South Wales), South Africa (Grahamstown, Heidelburg, Ixopo, Pretoria, Port Elizabeth and Stellenbosch) and North America (Maryland, Louisiana and Florida).

The real-time PCR assay was checked for general cross-reaction with DNA extracted from *Apis mellifera* L. and from a range of insect species collected inside (and around) honey bee hives (Tab. I). DNA from the bee mites *Varroa destructor* Anderson and Trueman (Acari: Varroidae) and *Tropilaelaps* spp. (Acari: Laelapidae) were included in the cross-reaction. All specimens were stored in 70% ethanol before use.

2.5. Real-time (TaqMan®) PCR reactions

All real-time (TaqMan®) reactions were set up as duplicate wells using Stratagene Brilliant core reagents (Stratagene, La Jolla, California, USA) according to the manufacturer's protocols. For each reaction, 1 μL DNA was added to 24 μL of mastermix in the appropriate well. Primers and probe concentrations were 300 nM and 200 nM respectively per 25 μL reaction. Plates were cycled at generic system conditions (50 °C for 2 min, 95 °C for 10 min and 40 cycles of 60 °C for 1 min plus 95 °C for 15 s) within the 7900 Sequence Detection System (Applied Biosystems, Branchburg, New Jersey, USA) using real-time data collection. Real-time PCR data is expressed as C_T (cycle threshold)

Table I. Average Ct values (for 3 replicate 100 mg tissue samples or 10−15 SHB eggs ± standard deviation) for the detection of DNA (CO1 gene) from *A. tumida* collected from different geographical locations. Results are also shown for other insect species (individual insects) and bee mites used in cross-reaction tests.

Species	Family	Sampling localities	Life stage	Average Ct value
A tumida	Nitidulidae	Heidelburg, South Africa,	Adult	20.77 ± 0.13
A. tumida	"	Ixopo, South Africa	Adult	24.48 ± 0.18
A. tumida	"	Port Elizabeth, South Africa	Adult	22.31 ± 0.01
A. tumida	"	Grahamstown, South Africa	Adult	22.47 ± 0.04
A. tumida	"	Pretoria, South Africa	Adult	22.87 ± 0.09
A. tumida	"	Stellenbosch, South Africa	Adult	25.21 ± 0.13
A. tumida	"	Beltsville, USA	Adult	23.72 ± 0.03
A. tumida	"	Louisiana, USA	Adult	21.66 ± 0.06
A. tumida	"	Florida, USA	Adult	22.66 ± 0.23
A. tumida	"	Queensland, Australia	Adult	26.19 ± 0.06
A. tumida	"	Richmond, NSW, Australia	Adult	22.17 ± 0.08
A. tumida	"	Stellenbosch, South Africa	Larvae	28.56 ± 0.31
A. tumida	"	Florida, USA	Larvae	26.45 ± 0.19
A. tumida	"	Beltsville, USA	Larvae	22.56 ± 0.06
A. tumida	"	Beltsville, USA	Eggs	19.68 ± 1.41
Dorcus parallelopipdus	Lucanidae	UK	Adult	Negative
Agabus bipustulatus	Dytiscidae	UK	Adult	Negative
Antherophagus nigricornis	Cryptophagidae	UK	Adult	Negative
Melgethes rotundicollis	Nitidulidae	UK	Adult	Negative
Bombus (species uknown)	Apidae	UK	Adult	Negative
Pterostichus spp.	Carabidae	UK	Adult	Negative
Otiorhynchus claviceps	Curculionoidea	UK	Adult	Negative
Glischrochilus hortensis	Nitidulidae	UK	Adult	Negative
Apis mellifera	Apidae	UK	Adult	Negative
Varroa destructor	Varroidae	UK	Adult	Negative
Tropilaelaps spp.	Laelapidae	Chiang mai, Thailand	Adult	Negative

values. This is the PCR cycle at which PCR products are first detected above a baseline threshold.

2.6. Cloning and sequencing of real-time PCR products

Real-time PCR products (2 μL) were directly ligated into plasmid pGEM®-T Easy Vector (Promega, Madison, USA), and transformed into *E. coli* JM109 High Efficiency competent cells (Promega, Madison, USA) following the manufacturer's protocols. White bacterial colonies containing plasmids with inserts were selected and plasmid DNA was purified using the Wizard® Plus SV miniprep DNA purification system (Promega, Madison, USA). Purified plasmid concentrations were adjusted to 20 ng μL^{-1} for sequencing. Sequencing was carried out by the DNAseq sequencing service, University of Dundee, Scotland.

Table II. Average C_T values for the detection of *A. tumida* CO1 and bee 18s (IPC) in spiked spring/summer debris (the results show the average values ± standard deviation for 3 replicate samples for each treatment). Positive controls are beetles and larvae ground in an equivalent amount of lysis buffer without hive debris.

Replicate/Sample	Average C_T value *A. tumida*	Average C_T value Bee IPC	Average C_T value Positive controls
Unspiked debris	40.00 ± 0.00	15.45 ± 0.01	–
CTAB only buffer control	40.00 ± 0.00	40.00 ± 0.00	–
½ beetle	40.00 ± 0.00	15.63 ± 0.04	24.70 ± 0.01
1 beetle	38.36 ± 0.14	15.67 ± 0.01	23.32 ± 0.05
2 beetle	26.22 ± 0.04	15.36 ± 0.01	23.58 ± 0.24
3 beetle	25.43 ± 0.26	14.96 ± 0.08	23.50 ± 0.08
4 beetle	23.81 ± 0.07	14.96 ± 0.08	23.40 ± 0.09
½ larvae	35.32 ± 0.00 (1/3)*	14.98 ± 0.03	28.09 ± 1.08
1 larvae	36.24 ± 0.29 (1/3)*	15.73 ± 0.01	26.46 ± 0.15
2 larvae	31.66 ± 0.13 (2/3)*	15.47 ± 0.03	24.71 ± 0.21
3 larvae	29.74 ± 0.28 (2/3)*	15.52 ± 0.04	23.74 ± 0.23
4 larvae	30.27 ± 0.12 (2/3)*	15.08 ± 0.01	23.54 ± 0.13

(–) Indicates data not collected. * Indicates number of positive replicates.

3. RESULTS

3.1. Real-time PCR primers and probes

The sequence for the SHB COI real-time PCR product (109 bp) was compared to sequences on the NCBI sequence database using a BLAST search (http://www.ncbi.nlm.gov/BLAST) to identify homologous sequences. The amplicon sequence matched all 15 COI sequences available on the database for *A. tumida.* (Accession numbers AF227645–AF227654, AF522354–AF522358) confirming the real-time PCR product to be *A. tumida*.

3.2. Validation of the SHB real-time (TaqMan®) PCR assay

The SHB real-time primers and probe were validated using DNA extracted from SHB adults, larvae and eggs collected from various geographical locations. The real-time PCR assay was able to detect all life stages and all beetles from the different geographical locations. In addition, no cross-reaction was observed when the primers were tested with DNA extracted from a range of insects including *A. mellifera* and from the bee mites, *Varroa destructor* and *Tropilaelaps clareae*. (Tab. I).

3.3. Detection of the SHB in spiked hive debris

SHB DNA could be detected in all hive debris samples spiked with adult beetles, with the exception of half a beetle. This would suggest that the sensitivity of the current assay is limited to detecting approximately 17.28 ± 2.84 mg of SHB tissue (one beetle) in 30 g of hive debris. For both beetle and larval spikes, the C_T values decreased (i.e. DNA levels increasing) with increasing amounts of SHB tissue spiked in the debris. The exception to this was the four larvae spike. In this instance, the C_T value rose slightly (C_T 30.27) in comparison to the three larvae spike (C_T 29.74) (Tab. II). The number of positives out of 3 varied for the larval samples, however, as the larvae were added whole to the debris, this would reflect the grouping of more than one larva in certain replicates and none in others. When the TaqMan C_T values for *A. tumida* detection were plotted against the number of

Table III. Average C_T values for the detection of *A. tumida* CO1 and bee 18s (IPC) in debris collected from SHB infested hives from the USA. The results show the average values ± standard deviation for 3 replicates.

Sample number (10 g hive debris)	Extraction replicate number	Average C_T value *A. tumida*	Average C_T value Bee IPC
1	1	28.66 ± 0.52	22.29 ± 0.11
	2	30.66 ± 0.10	23.91 ± 0.35
	3	28.96 ± 0.18	22.37 ± 0.06
2	1	29.51 ± 0.13	22.05 ± 0.39
	2	33.11 ± 0.38	24.21 ± 0.11
	3	30.00 ± 0.62	21.99 ± 0.28
3	1	28.51 ± 0.43	22.88 ± 0.16
	2	32.02 ± 1.01	28.75 ± 0.28
	3	24.77 ± 0.26	22.49 ± 0.10

beetles or larvae spiked into the hive debris, a good correlation was observed, indicated by a straight line where the R value was 0.84 for both beetle and larvae spikes.

TaqMan C_T values for *A. tumida* detection in spiked debris were higher than the control samples with no hive debris. C_T values for hive debris spiked with larvae were 7 to 10 cycles lower in comparison to the control samples (100 to 1000 fold decrease in sensitivity). For the hive debris samples spiked with beetles, the decrease in sensitivity was more variable. A difference of 1 to 15 cycles was observed (3.3 to a 10^4 fold decrease in detection sensitivity).

DNA extracts from the debris samples were tested with the bee IPC assay. The C_T values for the extracts averaged 15.39 ± 0.28 for the beetle spikes and 15.36 ± 0.31 for the larval spikes (Tab. II).

3.4. Detection of *A. tumida* in naturally infested hive debris

A. tumida was successfully detected in all three replicate samples of naturally infested hive debris. The bee IPC was also successfully detected in all three replicates. The average TaqMan C_T values for three replicate samples of hive debris was 29.69 ± 2.55 and 23.71 ± 2.08 for *A. tumida* and the bee IPC respectively (Tab. III).

4. DISCUSSION

To date there have been few molecular studies on the SHB and no published PCR detection assays currently exist. In this study, real-time (TaqMan®) PCR was used successfully in conjunction with an automated DNA extraction protocol to enable species identification and detection of SHB in hive debris. The methods developed are particularly suited for high-throughput diagnostic work. Using the automated extraction protocol, DNA can be extracted from up to 96 samples within 1 hour. Real-time PCR has several advantages over conventional PCR, no post-PCR manipulations are required and many samples can be analysed simultaneously, for example, 96 samples can be tested on most available instruments within a 2 hour period, giving a total throughput of 96 samples every three hours. More elaborate instruments are also available allowing 384 samples to be tested by real-time PCR within the 2 hour period. Although the equipment is potentially expensive, the cost has dropped 10 fold in the last 5 years. The savings in time, reagents and equipment as a result of removing gel running and interpretation of results from gel photographs, has resulted in a lower per-assay cost when compared to conventional PCR. This has caused a proliferation in the availability of real-time PCR equipment in routine diagnostic laboratories and a subsequent replacement of conventional PCR techniques.

In our study, the real-time primers and probe were designed to sequence within the COI gene, and with degeneracy in the forward primer to allow detection of the two haplotypes for which sequence is available on the EMBL sequence database (Evans et al., 2000, 2002, 2003). The primers were shown to be specific for SHB as no cross-reaction was observed with DNA extracted from nine genera of insects (some insects being from the same family as the SHB), or with DNA from *Varroa* and *Tropilaelaps* mites that may form part of the hive debris in mite infested bee colonies. The results suggest that the real-time assay would be reliable for screening hive debris for the presence of the SHB.

The assay was successful in amplifying DNA extracted from egg, larval and adult life stages of the SHB and from beetle specimens collected from different geographical regions. These results suggest that the SHB assay should be able to detect new incursions or low-level infestations regardless of where the invading beetle/larvae/eggs originate from. However, the endemic distribution of SHB in sub-Saharan Africa is vast (Hepburn and Radloff, 1998; Neumann and Elzen, 2004), and new sequence data may emerge in light of molecular studies on SHB populations. In this instance the SHB primer and probe may require further testing to determine their detection capabilities.

A. tumida can be present at low (cryptic) levels (Spiewok and Neumann, 2006) within bee hives rather than as overt infestations (Lundie, 1940). Our experiments showed that it was possible to detect small fragments of a single beetle in a starting volume of 30 g of hive debris. Of great importance also, was the success of the method in detecting SHB in debris collected from naturally infested hives from the USA.

Real-time PCR C_T values for SHB DNA extractions in naturally infested hives were relatively consistent across samples showing the method to be efficient at extracting *A. tumida* DNA. Also, in spiked samples, when the amount of beetle tissue in hive debris was plotted against real-time C_T values obtained for detection, the resulting correlation curve indicated that most of the beetle tissue spiked into the hive debris was retrieved during the extraction procedure. The consistency in extraction efficiencies was further demonstrated using a real-time PCR assay to detect the honey bee 18S rRNA gene. There was enough background bee DNA in the hive debris, to allow good PCR amplification. In each experiment, the C_T values for bee 18S detection were all within 1 cycle showing that extraction efficiency was similar for all samples. It was concluded that the bee 18s rRNA assay is a suitable and reliable internal positive control to use for hive debris extractions.

Some decrease in real-time PCR detection sensitivity was observed in the spiked hive debris DNA extracts compared to the positive controls with no hive debris, this is most likely to be attributed to the presence of PCR inhibitors in the DNA extracts. In this study, clean-up procedures were not tested.

In summary, the method developed was shown to be successful in detecting SHB in spiked and naturally infested debris. The technology is directly transferable to other diagnostic laboratories with real-time PCR capabilities, and could therefore benefit overseas researchers and inspection/surveillance programmes, as well as legislative and government bodies, by providing the means to monitor, or test suspect hive samples for the presence of the SHB. There is scope to improve and refine the method further including the reduction of PCR inhibitors and increasing the volume of debris tested at any one time. In addition, the method needs further validation with more hive samples naturally infested with SHB to verify its reliability before large-scale use.

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Méthode basée sur l'ADN pour rechercher dans les débris de la colonie la présence du Petit coléoptère des ruches (*Aethina tumida*).

***Aethina tumida* / parasite / *Apis mellifera* / dépistage / PCR en temps réel / méthodologie**

Zusammenfassung – Eine DNA-Methode zur Durchrasterung von Stockmüll auf die Präsenz des Kleinen Beutenkäfers (*Aethina tumida*). Ziel der gegenwärtigen Studie war die Entwicklung und Validierung eines schnellen Extraktionsprotokolls, das in Verbindung mit einem quantitativen PCR-Ansatz die artspezifische Erkennung des Kleinen Beutenkäfers (KBK) und seine Detektion im Stockmüll erlauben sollte. Wir gingen davon aus, dass angesichts der raschen Verbreitung des KBK ein schnelles und zuverlässiges Detektionsverfahren ein wertvolles Werkzeug in der KBK-Kontrolle darstellen könnte.
Das quantitative PCR-Verfahren wurde auf die Detektion des Cytochrom c-Oxidase I Gens *von A. tumida* angelegt. Zur Validierung wurden KBK-Eier, Larven und Imagines aus Afrika, Australien und Nordamerika eingesetzt. Das Verfahren wurde auch auf Kreuzreaktionen gegen die Milben *Varroa destructor* and *Tropilaelaps clareae,* sowie gegen eine Reihe an gängig in Stockmüll vorkommenden Insektenarten getestet. Es erwies sich als spezifisch für KBK und erlaubte die Detektion aller Stadien des Lebenszyklus und auch von KBK-Proben verschiedener geographischer Herkunft.
Anschliessend wurde das Verfahren auf die Erkennung von KBK in Stockmüllproben optimiert. Dazu wurde Stockmüll mit unterschiedlichen Mengen an Käfern und Larven versetzt und in Lysepuffer zermahlen. DNA wurde aus diesen Proben mittels eines automatisierten Verfahrens extrahiert, bevor die KBK-DNA-Menge dieser Proben im zuvor etablierten quantitativen PCR-Protokoll bestimmt wurde. Weitere Methodentests wurden mit natürlichen Stockmüllproben durchgeführt und mit Proben, denen KBK zugesetzt worden war. Die Ergebnisse zeigten, dass es möglich war, eine KBK-Menge von 17 mg in einer Gesamtmenge von 30 g Stockmüll zu detektieren. Die Zuverlässigkeit der Extraktionsmethode wurde ebenfalls mittels quantitativer PCR getestet, und zwar für das 18S rRNA Gen der Honigbiene, das ebenfalls aus Stockmüllproben amplifiziert werden konnte. Die C_T-Werte jedes Experiments lagen im Schwankungsbereich von je einem Zyklus, was darauf hinweist, dass die Effizienz des Extraktionsverfahrens für alle Proben ähnlich war. Die Amplifizierung des 18S rRNA-Gens stellt somit eine geeignete interne positive Kontrolle für die Stockmüllextraktion dar. Eine Verringerung der Sensitivität der PCR-Methode wurde in DNA-Proben registiert, denen Stockmüll zugesetzt worden war, was auf die Anwesenheit von PCR-Inhibitoren in den DNA-Extrakten hinweist.
Wir stellen in dieser Arbeit ein Hochdurchsatzverfahren zur Detektion von KBK vor, das besonders dann von Nutzen sein kann, wenn ein KBK-Ausbruch an neuen Standorten zu verzeichnen ist. Das Verfahren kann noch weiter verfeinert werden, so dass noch grössere Probenvolumina gleichzeitig getestet werden können. Dies setzt allerdings eine weiter Validierung voraus. Die gegenwärtige Methode ist jedoch direkt einsetzbar und transferierbar für Forschungs- und Inspektions- und Überwachungsprogramme und stellt ein Mittel dar, KBK-verdächtige Stockproben zu testen.

***Aethina tumida* / quantitative PCR / Honigbiene / Kleiner Beutenkäfer / Stockmüll**

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