(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 April 2011 (21.04.2011)

PCT

(10) International Publication Number
WO 2011/045796 A1

(51) International Patent Classification:
C12N 15/113 (2010.01) *A61P 33/14* (2006.01)
A61K 31/713 (2006.01)

(21) International Application Number:
PCT/IL2010/000844

(22) International Filing Date:
14 October 2010 (14.10.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/251,339 14 October 2009 (14.10.2009) US

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(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2011/045796 A1

(54) **Title**: COMPOSITIONS FOR CONTROLLING VARROA MITES IN BEES

(57) **Abstract**: An isolated nucleic acid agent is disclosed comprising a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite. Compositions comprising same and uses thereof are also disclosed.

COMPOSITIONS FOR CONTROLLING VARROA MITES IN BEES

FIELD AND BACKGROUND OF THE INVENTION

The invention relates to compositions for controlling *Varroa* mite infestation in bees.

Honey bees, Apis mellifera, are required for the effective pollination of crops and are therefore critical to world agriculture. Honey bees also produce economically important products, including honey and bees wax. Honey bees are susceptible to a number of parasites and pathogens, including the ectoparasitic mite, Varroa destructor.

Colony Collapse Disorder (CCD) of honeybees is threatening to annihilate U.S. and world agriculture. Indeed, in the recent outbreak of CCD in the U.S in the winter of 2006-2007, an estimated 25 % or more of the 2.4 million honeybee hives were lost because of CCD. An estimated 23 % of beekeeping operations in the United States suffered from CCD over the winter of 2006–2007, affecting an average of 45 % of the beekeepers operations. In the winter of 2007-2008, the CCD action group of the USDA-ARS estimated that a total of 36 % of all hives from commercial operations were destroyed by CCD.

CCD is characterized by the rapid loss from a colony of its adult bee population, with dead adult bees usually found at a distance from the colony. At the final stages of collapse, a queen is attended only by a few newly emerged adult bees. Collapsed colonies often have considerable capped brood and food reserves. The phenomenon of CCD was first reported in 2006; however, beekeepers noted unique colony declines consistent with CCD as early as 2004. Various factors such as mites and infectious agents, weather patterns, electromagnetic (cellular antennas) radiation, pesticides, poor nutrition and stress have been postulated as causes. To date, control of CCD has focused on Varroa mite control, sanitation and removal of affected hives, treating for opportunistic infections (such as *Nosema*) and improved nutrition. No effective preventative measures have been developed to date.

Varroa mites parasitize pupae and adult bees and reproduce in the pupal brood cells. The mites use their mouths to puncture the exoskeleton and feed on the bee's hemolymph. These wound sites in the exoskeleton harbor bacterial infections, such as Melissococcus pluton, which causes European foulbrood. In addition, to their parasitic effects, Varroa mites are suspected of acting as vectors for a number of honey bee pathogens, including deformed wing virus (DWV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV) and black queen cell virus (BQCV), and may weaken the immune systems of their hosts, leaving them vulnerable to infections. If left untreated Varroa infestations typically result in colony-level mortality.

Current methods of treating Varroa infestations are proving to be ineffective as the mites develop resistance to existing miticides. In addition, the use of such miticides may introduce injurious chemicals into honey that is intended for human consumption.

U.S. Patent Application 20090118214 teaches the use of dsRNA for prevention and treatment of viral infections in honeybees.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an isolated nucleic acid agent comprising a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite.

According to an aspect of some embodiments of the present invention there is provided a nucleic acid construct comprising a nucleic acid sequence encoding the isolated nucleic acid agent of the present invention.

According to an aspect of some embodiments of the present invention there is provided a bee-ingestible composition comprising at least one nucleic acid agent which comprises a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite.

According to an aspect of some embodiments of the present invention there is provided a method of preventing or treating a Varroa destructor mite infestation of a bee hive, the method comprising administering to the bee an effective amount of least one nucleic acid agent which comprises a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite, thereby preventing or treating a Varroa destructor mite infestation of a bee hive.

According to an aspect of some embodiments of the present invention there is provided a method of preventing or treating a Varroa destructor mite infestation of a bee hive, the method comprising administering to the bee an effective amount of the nucleic acid construct of the present invention, thereby preventing or treating a Varroa destructor mite infestation of a bee hive.

According to an aspect of some embodiments of the present invention there is provided a method of reducing the susceptibility of honeybees to Colony Collapse Disorder (CCD), the method comprising administering to the honeybee an effective amount of at least one double-stranded ribonucleic nucleic acid (dsRNA), the at least one dsRNA comprising a sequence complementary to at least 21 nucleotides of Varroa destructor mite mRNA and capable of inducing degradation of the Varroa destructor-specific mRNA.

According to some embodiments of the invention, the nucleic acid sequence is complementary to at least 21 nucleotides of Varroa destructor mite specific RNA and capable of inducing degradation of the Varroa destructor mite RNA.

According to some embodiments of the invention, the agent is selected from the group consisting of a dsRNA, an antisense RNA and a ribozyme.

According to some embodiments of the invention, the dsRNA is selected from the group consisting of siRNA, shRNA and miRNA.

According to some embodiments of the invention, the gene product is an mRNA encoding a polypeptide selected from the group consisting of ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin.

According to some embodiments of the invention, the at least one nucleic acid agent comprises at least five nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of the at least five nucleic acid agent targeting a different gene.

According to some embodiments of the invention, the at least one nucleic acid agent comprises at least six nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of the at least six nucleic acid agents for targeting a different gene.

According to some embodiments of the invention, the nucleic acid agents are as set forth in SEQ ID Nos: 1, 13, 27, 30 and 39.

According to some embodiments of the invention, the nucleic acid agents are as set forth in SEQ ID Nos: 1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39.

According to some embodiments of the invention, the nucleic acid sequence is greater than 15 base pairs in length.

According to some embodiments of the invention, the nucleic acid sequence is 19 to 25 base pairs in length.

According to some embodiments of the invention, the nucleic acid sequence is greater than 30 base pairs in length.

According to some embodiments of the invention, the composition is in solid form.

According to some embodiments of the invention, the composition is in liquid form.

According to some embodiments of the invention, the composition comprises protein.

According to some embodiments of the invention, the protein is in the form of pollen and/or soy patties.

According to some embodiments of the invention, the liquid is a sucrose solution.

According to some embodiments of the invention, the liquid is a corn syrup solution.

According to some embodiments of the invention, the liquid further comprises a carbohydrate or sugar supplement.

According to some embodiments of the invention, the bee is a honeybee.

According to some embodiments of the invention, the honeybee is a forager.

According to some embodiments of the invention, the honeybee is a hive bee.

According to some embodiments of the invention, the honeybee is a bee of a colony, and wherein the administering reduces the susceptibility of the bee colony to Colony Collapse Disorder.

According to some embodiments of the invention, the administering is effected by feeding.

According to some embodiments of the invention, the feeding comprises providing a liquid bee-ingestible composition.

According to some embodiments of the invention, the feeding comprises providing a solid bee-ingestible composition.

According to some embodiments of the invention, the Varroa destructor mite mRNA encodes a polypeptide selected from the group consisting of NADH dehydrogenase subunit 2, ATP synthetase subunit 8, ATP synthetase subunit 6, sodium channel and cytochrome oxydase subunit I.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

In the drawings:

FIG.1 is a schematic representation of the time-course of various experiment for dsRNA transfer to Varroa mites.

FIGs. 2A-E are photographs of the results of Slot blot analysis of the presence of dsRNA-GFP in ingested bees (Figure 2A), in larvae fed by adult bees (Figure 2B), in pupae (Figure 2C), and in the newly-emerge bees (Figure 2D). The presence of dsRNA-GFP and of siRNA derived from it was analyzed by Northern blots (Figure 2E). D=days after administration of dsRNA-GFP to the hive.

FIG. 3 is a photograph illustrating the results of RT-PCR analysis of Varroa-extracted RNA at the days indicated in the top row (time as indicated in Figure 1). Green numbers (top row) indicate Varroa individuals which had been placed on dsRNA-GFP-ingested bees and black numbers indicate RNA from Varroa placed on control bees. + = positive control (a GFP-carrying plasmid).

FIG. 4 is a photograph illustrating RT-PCR of Varroa RNA with primers to apoptosis inhibitor protein (IAP; sequence 27). M: size markers. Lanes 1-3: Template RNA of Varroa from hives treated with dsRNA of sequences 27. Lane 4: Template RNA of Varroa from control hives. Lane 5: Positive control (a IAP-carrying plasmid). Lane 6: Negative control (no template).

FIG. 5 is a bar graph illustrating the Varroa count per bee (adult bees plus larvae inside sealed cells) in control hives and in hives treated with dsRNA mixture I (Min) and with dsRNA mixture II (Max).

DESCRIPTION OF EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to methods and compositions for reducing the susceptibility of bees to Varroa mite infestation.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Bees are susceptible to a myriad of viral infections. Treatment of such infections by down-regulation of a particular viral gene product has shown to be successful in eliminating virally induced infections in the bee (see U.S. Patent Application 20090118214).

The present inventors now propose treatment of Varroa mite infestations in bees by down-regulating particular Varroa mite gene products.

Varroa mites parasitize pupae and adult bees and reproduce in the pupal brood cells. The mites use their mouths to puncture the exoskeleton and feed on the bee's hemolymph. The present inventors unexpectedly found that polynucleotide agents administered to the bees to treat Varroa mite infestations presented in the bee's hemolymph thereby becoming available to the mite.

The present inventors have shown that dsRNA can successfully be transferred to Vorroa mites (Figures 2A-E), that the dsRNA can serve to down-regulate expression of a particular gene in the Varroa mite (Figure 4) and further that targeting of particular genes for down-regulation can result in a reduction in the number of Varroa mites (Figure 5)

Thus, according to one aspect of the present invention there is provided a method of preventing or treating a Varroa destructor mite infestation of a bee, the method comprising administering to the bee an effective amount of a nucleic acid agent comprising a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite, thereby preventing or treating a Varroa destructor mite infestation of a bee.

As used herein, the term "bee" refers to both an adult bee and pupal cells thereof. According to one embodiment, the bee is in a hive.

An adult bee is defined as any of several winged, hairy-bodied, usually stinging insects of the superfamily Apoidea in the order Hymenoptera, including both solitary and social species and characterized by sucking and chewing mouthparts for gathering nectar and pollen. Exemplary bee species include, but are not limited to, Apis, Bombus, Trigona, Osmia and the like. In one embodiment, bees include, but are not limited to bumblebees (Bombus terrestris), honeybees (Apis mellifera) (including foragers and hive bees) and Apis cerana.

According to one embodiment, the bee is part of a colony.

The term "colony" refers to a population of bees comprising dozens to typically several tens of thousand bees that cooperate in nest building, food collection, and brood rearing. A colony normally has a single queen, the remainder of the bees being either "workers" (females) or "drones" (males). The social structure of the colony is maintained by the queen and workers and depends on an effective system of communication. Division of labor within the worker caste primarily depends on the age of the bee but varies with the needs of the colony. Reproduction and colony strength depend on the queen, the quantity of food stores, and the size of the worker force. Honeybees can also be subdivided into the categories of "hive bees", usually for the first part of a workers lifetime, during which the "hive bee" performs tasks within the hive, and "forager bee", during the latter part of the bee's lifetime, during which the "forager" locates and collects pollen and nectar from outside the hive, and brings the nectar or pollen into the hive for consumption and storage.

According to this aspect of the present invention the agents of the present invention are used to prevent the Varroa destructor mite from living as a parasite on the bee, or larvae thereof.

The phrase "Varroa destructor mite" refers to the external parasitic mite that attacks honey bees Apis cerana and Apis mellifera. The mite may be at an adult stage, feeding off the bee, or at a larval stage, inside the honey bee brood cell.

As mentioned, the agents of the present invention are capable of downregulating expression of a gene product of a Varroa destructor mite.

As used herein, the phrase "gene product" refers to an RNA molecule or a protein.

According to one embodiment, the Varroa destructor mite gene product is one which is essential for mite viability. Down-regulation of such a gene product would typically result in killing of the Varroa mite. According to another embodiment, the Varroa destructor mite gene product is one which is essential for mite reproduction. Down-regulation of such a gene product would typically result in the prevention of reproduction of the Varroa mite and the eventual extermination of the mite population. According to yet another embodiment, the Varroa destructor mite gene product is one which is required to generate pathogenic symptoms in the bee.

Exemplary gene products that may be down-regulated according to this aspect of the present invention include, but are not limited to NADH dehydrogenase; subunit 2- Genbank accession NC_004454; ATP synthetase; subunit 8 - NC_004454; ATP synthetase; subunit 6 - NC_004454; sodium channel gene - Genbank accession No. FJ216963; Cytochrome oxydase subunit I - Genbank accession No. EF025469.

It will be appreciated that whilst the agents of the present invention are capable of downregulating expression of a gene product of a Varroa destructor mite, it is preferable that they downregulate to a lesser extent expression of the gene product in other animals, such as the bee. Accordingly, the agents of the present invention must be able to distinguish between the mite gene and the bee gene, down-regulating the former to a greater extent than the latter. According to another embodiment the agents of the present invention do not down-regulate the bee gene whatsoever. This may be effected by targeting a gene that is expressed differentially in the mite and not in the bee e.g. the mite sodium channel gene - FJ216963. Alternatively, the agents of the present invention may be targeted to mite-specific sequences of a gene that is expressed both in the mite and in the bee.

According to one embodiment the agents of the present invention target segments of Varroa genes that are at least 100 bases long and do not carry any sequence longer than 19 bases that is entirely homologous to any bee-genome sequence or human-genome sequence.

Examples of such gene segments are provided herein below:

SEQ ID NO: 1. Varroa gene homologous to ATPase subunit A (segment 1); SEQ ID NO: 2. Varroa gene homologous to ATPase subunit A (segment 2); SEQ ID NO: 3. Varroa gene homologous to ATPase subunit A (segment 3); SEQ ID NO: 4. Varroa gene homologous to ATPase subunit A (segment 4); SEQ ID NO: 5. Varroa gene homologous to ATPase subunit A (segment 5); SEQ ID NO: 6. Varroa gene homologous to ATPase subunit A (segment 6); SEQ ID NO: 7. Varroa gene homologous to ATPase subunit A (segment 7); SEQ ID NO: 8. Varroa gene homologous to ATPase subunit A (segment 8); SEQ ID NO: 9. Varroa gene homologous to ATPase subunit A (segment 9); SEQ ID NO: 10. Varroa gene homologous to RNA polymerase I (segment 1); SEQ ID NO: 11. Varroa gene homologous to RNA polymerase I (segment 2); SEQ ID NO: 12. Varroa gene homologous to RNA polymerase I (segment 3); SEQ ID NO: 13. Varroa gene homologous to RNA polymerase III (segment 1); SEQ ID NO: 14. Varroa gene homologous to RNA polymerase III (segment 2); SEQ ID NO: 15. Varroa gene homologous to RNA polymerase III (segment 3); SEQ ID NO: 16. Varroa gene homologous to RNA polymerase III (segment 4); SEQ ID NO: 17. Varroa gene homologous to RNA polymerase III (segment 5); SEQ ID NO: 18. Varroa gene homologous to RNA polymerase III (segment 6); SEQ ID NO: 19. Varroa gene homologous to RNA polymerase III (segment 7) SEQ ID NO: 20. Varroa gene homologous to RNA polymerase III (segment 8); SEQ ID NO: 21. Varroa gene homologous to RNA polymerase III (segment 9); SEQ ID NO: 22. Varroa gene homologous to toInhibitor of apoptosis (IAP; segment 1); SEQ ID NO: 23. Varroa gene homologous to to Inhibitor of apoptosis (IAP; segment 2); SEQ ID NO: 24. Varroa gene homologous to to Inhibitor of apoptosis (IAP; segment 3); SEQ ID NO: 25. Varroa gene homologous to to Inhibitor of apoptosis (IAP; segment 4); SEQ ID NO: 26. Varroa gene homologous to to Inhibitor of apoptosis (IAP; segment 5); SEQ ID NO: 27. Varroa gene homologous to Inhibitor of apoptosis (IAP; segment 6); SEQ ID NO: 28. Varroa gene homologous to Inhibitor of apoptosis (IAP; segment 7); SEQ ID NO: 29. Varroa gene homologous to Inhibitor of apoptosis (IAP; segment 8); SEQ ID NO: 30. Varroa gene homologous to FAS apoptotic inhibitor (segment 1); SEQ ID NO: 31. Varroa gene homologous to FAS apoptotic inhibitor (segment 2); SEQ ID NO: 32. Varroa gene homologous to FAS apoptotic inhibitor (segment 3); SEQ ID NO: 33. Varoa gene homologous to α-Tubulin (segment 1); SEQ ID NO: 34. Varoa gene homologous to α-Tubulin (segment 2); SEQ ID NO: 35. Varoa gene homologous to α-Tubulin (segment 3); SEQ ID NO: 36. Varoa gene homologous to α-Tubulin (segment 4); SEQ ID NO: 37. Varoa gene homologous to α-Tubulin (segment 5); SEQ ID NO: 38. Varoa gene homologous to α-Tubulin (segment 6); SEQ ID NO: 39. Varoa gene homologous to α-Tubulin (segment 7); SEQ ID NO: 40. Varoa gene homologous to α-Tubulin (segment 8); SEQ ID NO: 41. Varoa gene homologous to α-Tubulin (segment 9); SEQ ID NO: 42.NADH dehydrogenase; subunit 2 (NC_004454): bases 709 to 974; SEQ ID NO: 43. ATP synthetase; subunit 8 (NC_004454): bases 3545 to 3643; SEQ ID NO: 44. Sodium channel protein (AY259834): bases 3336-3836.

It will be appreciated that more than one gene may be targeted in order to maximize the cytotoxic effect on the Varroa mites.

Thus, according to one embodiment, the following group of genes are targeted - ATPase subunit A, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin (e.g. using nucleic acid agents having the sequence as set forth in 1, 13, 27, 30 and 39).

According to another embodiment, the following group of genes are targeted - ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin.

It will be appreciated that as well as down-regulating a number of genes, the present invention further contemplates using a number of agents to down-regulate the same gene (e.g. a number of dsRNAs each hybridizing to a different segment of the same gene). Thus, for example, the present inventors showed maximal cytotoxic activity when the following mixture of dsRNAs was used: SEQ ID Nos:1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39 and less of a cytotoxic activity when the following mixture of dsRNAs was used: SEQ ID Nos: 1, 13, 27, 30 and 39.

Tools which are capable of identifying species-specific sequences may be used for this purpose - e.g. BLASTN and other such computer programs

As used herein, the term “downregulating expression" refers to causing, directly or indirectly, reduction in the transcription of a desired gene, reduction in the amount, stability or translatability of transcription products (e.g. RNA) of the gene, and/or reduction in translation of the polypeptide(s) encoded by the desired gene.

Downregulating expression of a gene product of a Varroa destructor mite can be monitored, for example, by direct detection of gene transcripts (for example, by PCR), by detection of polypeptide(s) encoded by the gene or bee pathogen RNA (for example, by Western blot or immunoprecipitation), by detection of biological activity of polypeptides encode by the gene (for example, catalytic activity, ligand binding, and the like), or by monitoring changes in the Vaarroa destructor mite (for example, reduced proliferation of the mite, reduced virulence of the mite, reduced motility of the mite etc) and by testing bee infectivity/pathogenicity.

Downregulation of a Varroa destructor mite gene product can be effected on the genomic and/or the transcript level using a variety of agents which interfere with transcription and/or translation (e.g., RNA silencing agents, Ribozyme, DNAzyme and antisense).

According to one embodiment, the agent which down-regulates expression of a Varroa destructor mite gene product is a polynucleotide agent, such as an RNA silencing agent According to this embodiment, the polynucleotide agent is greater than 15 base pairs in length.

As used herein, the phrase "RNA silencing" refers to a group of regulatory mechanisms [e.g. RNA interference (RNAi), transcriptional gene silencing (TGS), post-transcriptional gene silencing (PTGS), quelling, co-suppression, and translational repression] mediated by RNA molecules which result in the inhibition or "silencing" of the expression of a corresponding protein-coding gene or bee pathogen RNA sequence. RNA silencing has been observed in many types of organisms, including plants, animals, and fungi.

As used herein, the term "RNA silencing agent" refers to an RNA which is capable of inhibiting or "silencing" the expression of a target gene. In certain embodiments, the RNA silencing agent is capable of preventing complete processing (e.g, the full translation and/or expression) of an mRNA molecule through a post-transcriptional silencing mechanism. RNA silencing agents include noncoding RNA molecules, for example RNA duplexes comprising paired strands, as well as precursor RNAs from which such small non-coding RNAs can be generated. Exemplary RNA silencing agents include dsRNAs such as siRNAs, miRNAs and shRNAs. In one embodiment, the RNA silencing agent is capable of inducing RNA interference. In another embodiment, the RNA silencing agent is capable of mediating translational repression.

RNA interference refers to the process of sequence-specific post-transcriptional gene silencing in animals mediated by short interfering RNAs (siRNAs). The corresponding process in plants is commonly referred to as post-transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post-transcriptional gene silencing is thought to be an evolutionarily-conserved cellular defense mechanism used to prevent the expression of foreign genes and is commonly shared by diverse flora and phyla. Such protection from foreign gene expression may have evolved in response to the production of double-stranded RNAs (dsRNAs) derived from viral infection or from the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single-stranded RNA or viral genomic RNA.

The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNAs). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 nucleotides in length and comprise about 19 base pair duplexes. The RNAi response also features an endonuclease complex, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single-stranded RNA having sequence complementary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex.

According to one embodiment, the dsRNA is greater than 30 bp. The use of long dsRNAs can provide numerous advantages in that the cell can select the optimal silencing sequence alleviating the need to test numerous siRNAs; long dsRNAs will allow for silencing libraries to have less complexity than would be necessary for siRNAs; and, perhaps most importantly, long dsRNA could prevent viral escape mutations when used as therapeutics.

Various studies demonstrate that long dsRNAs can be used to silence gene expression without inducing the stress response or causing significant off-target effects - see for example [Strat et al., Nucleic Acids Research, 2006, Vol. 34, No. 13 3803–3810; Bhargava A et al. Brain Res. Protoc. 2004;13:115–125; Diallo M., et al., Oligonucleotides. 2003;13:381–392; Paddison P.J., et al., Proc. Natl Acad. Sci. USA. 2002;99:1443–1448; Tran N., et al., FEBS Lett. 2004;573:127–134].

Another method of downregulating a Varroa mite gene product is by introduction of small inhibitory RNAs (siRNAs).

The term "siRNA" refers to small inhibitory RNA duplexes (generally between 18-30 basepairs, between 19 and 25 basepairs) that induce the RNA interference (RNAi) pathway. Typically, siRNAs are chemically synthesized as 21mers with a central 19 bp duplex region and symmetric 2-base 3'-overhangs on the termini, although it has been recently described that chemically synthesized RNA duplexes of 25-30 base length can have as much as a 100-fold increase in potency compared with 21mers at the same location. The observed increased potency obtained using longer RNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27mer) instead of a product (21mer) and that this improves the rate or efficiency of entry of the siRNA duplex into RISC.

It has been found that position of the 3'-overhang influences potency of an siRNA and asymmetric duplexes having a 3'-overhang on the antisense strand are generally more potent than those with the 3'-overhang on the sense strand (Rose et al., 2005). This can be attributed to asymmetrical strand loading into RISC, as the opposite efficacy patterns are observed when targeting the antisense transcript.

The strands of a double-stranded interfering RNA (e.g., an siRNA) may be connected to form a hairpin or stem-loop structure (e.g., an shRNA). Thus, as mentioned the RNA silencing agent of the present invention may also be a short hairpin RNA (shRNA).

The term "shRNA", as used herein, refers to an RNA agent having a stem-loop structure, comprising a first and second region of complementary sequence, the degree of complementarity and orientation of the regions being sufficient such that base pairing occurs between the regions, the first and second regions being joined by a loop region, the loop resulting from a lack of base pairing between nucleotides (or nucleotide analogs) within the loop region. The number of nucleotides in the loop is a number between and including 3 to 23, or 5 to 15, or 7 to 13, or 4 to 9, or 9 to 11. Some of the nucleotides in the loop can be involved in base-pair interactions with other nucleotides in the loop. Examples of oligonucleotide sequences that can be used to form the loop include 5'-UUCAAGAGA-3' (SEQ ID NO: 4; Brummelkamp, T. R. et al. (2002) Science 296: 550) and 5'-UUUGUGUAG-3' (SEQ ID NO: 5; Castanotto, D. et al. (2002) RNA 8:1454). It will be recognized by one of skill in the art that the resulting single chain oligonucleotide forms a stem-loop or hairpin structure comprising a double-stranded region capable of interacting with the RNAi machinery.

According to another embodiment the RNA silencing agent may be a miRNA. miRNAs are small RNAs made from genes encoding primary transcripts of various sizes. They have been identified in both animals and plants. The primary transcript (termed the "pri-miRNA") is processed through various nucleolytic steps to a shorter precursor miRNA, or "pre-miRNA." The pre-miRNA is present in a folded form so that the final (mature) miRNA is present in a duplex, the two strands being referred to as the miRNA (the strand that will eventually basepair with the target) The pre-miRNA is a substrate for a form of dicer that removes the miRNA duplex from the precursor, after which, similarly to siRNAs, the duplex can be taken into the RISC complex. It has been demonstrated that miRNAs can be transgenically expressed and be effective through expression of a precursor form, rather than the entire primary form (Parizotto et al. (2004) Genes & Development 18:2237-2242 and Guo et al. (2005) Plant Cell 17:1376-1386).

Unlike, siRNAs, miRNAs bind to transcript sequences with only partial complementarity (Zeng et al., 2002, Molec. Cell 9:1327-1333) and repress translation without affecting steady-state RNA levels (Lee et al., 1993, Cell 75:843-854; Wightman et al., 1993, Cell 75:855-862). Both miRNAs and siRNAs are processed by Dicer and associate with components of the RNA-induced silencing complex (Hutvagner et al., 2001, Science 293:834-838; Grishok et al., 2001, Cell 106: 23-34; Ketting et al., 2001, Genes Dev. 15:2654-2659; Williams et al., 2002, Proc. Natl. Acad. Sci. USA 99:6889-6894; Hammond et al., 2001, Science 293:1146-1150; Mourlatos et al., 2002, Genes Dev. 16:720-728). A recent report (Hutvagner et al., 2002, Sciencexpress 297:2056-2060) hypothesizes that gene regulation through the miRNA pathway versus the siRNA pathway is determined solely by the degree of complementarity to the target transcript. It is speculated that siRNAs with only partial identity to the mRNA target will function in translational repression, similar to an miRNA, rather than triggering RNA degradation.

In one embodiment of the present invention, synthesis of RNA silencing agents suitable for use with the present invention can be effected as follows. First, the Varroa mite target mRNA is scanned downstream of the AUG start codon for AA dinucleotide sequences. Occurrence of each AA and the 3' adjacent 19 nucleotides is recorded as potential siRNA target sites. Preferably, siRNA target sites are selected from the open reading frame, as untranslated regions (UTRs) are richer in regulatory protein binding sites. UTR-binding proteins and/or translation initiation complexes may interfere with binding of the siRNA endonuclease complex [Tuschl ChemBiochem. 2:239-245]. It will be appreciated though, that siRNAs directed at untranslated regions may also be effective, as demonstrated for GAPDH wherein siRNA directed at the 5' UTR mediated about 90 % decrease in cellular GAPDH mRNA and completely abolished protein level (wwwdotambiondotcom/techlib/tn/91/912dothtml).

Second, potential target sites are compared to an appropriate genomic database (e.g., human, bee, mouse, rat etc.) using any sequence alignment software, such as the BLAST software available from the NCBI server (wwwdotncbidotnlmdotnihdotgov/BLAST/). Putative target sites which exhibit significant homology to other coding sequences are filtered out.

Qualifying target sequences are selected as template for siRNA synthesis. Preferred sequences are those including low G/C content as these have proven to be more effective in mediating gene silencing as compared to those with G/C content higher than 55 %. Several target sites are preferably selected along the length of the target gene or sequence for evaluation. For better evaluation of the selected siRNAs, a negative control is preferably used in conjunction. Negative control siRNA preferably include the same nucleotide composition as the siRNAs but lack significant homology to the genome. Thus, a scrambled nucleotide sequence of the siRNA is preferably used, provided it does not display any significant homology to any other gene or bee pathogen target sequence.

For example, a siRNA that may be used in this aspect of the present invention is one which targets a mite-specific gene. Exemplary siRNAs are provided in SEQ ID NOs: 45-47.

SEQ ID NO: 45: attttattcaattaaagtatt

SEQ ID NO: 46: atacctcaaatgtatccttca

SEQ ID NO: 47: ggccaatcccgattccggcga

It will be appreciated that the RNA silencing agent of the present invention need not be limited to those molecules containing only RNA, but further encompasses chemically-modified nucleotides and non-nucleotides.

In some embodiments, the RNA silencing agent provided herein can be functionally associated with a cell-penetrating peptide. As used herein, a "cell-penetrating peptide" is a peptide that comprises a short (about 12-30 residues) amino acid sequence or functional motif that confers the energy-independent (i.e., non-endocytotic) translocation properties associated with transport of the membrane-permeable complex across the plasma and/or nuclear membranes of a cell. The cell-penetrating peptide used in the membrane-permeable complex of the present invention preferably comprises at least one non-functional cystein residue, which is either free or derivatized to form a disulfide link with a double-stranded ribonucleic acid that has been modified for such linkage. Representative amino acid motifs conferring such properties are listed in U.S. Pat. No. 6,348,185, the contents of which are expressly incorporated herein by reference. The cell-penetrating peptides of the present invention preferably include, but are not limited to, penetratin, transportan, pIsl, TAT(48-60), pVEC, MTS, and MAP.

Another agent capable of downregulating a Varroa mite gene product is a DNAzyme molecule capable of specifically cleaving an mRNA transcript or DNA sequence of the bee pathogen polypeptide. DNAzymes are single-stranded polynucleotides which are capable of cleaving both single and double stranded target sequences (Breaker, R.R. and Joyce, G. Chemistry and Biology 1995;2:655; Santoro, S.W. & Joyce, G.F. Proc. Natl, Acad. Sci. USA 1997;943:4262) A general model (the "10-23" model) for the DNAzyme has been proposed. "10-23" DNAzymes have a catalytic domain of 15 deoxyribonucleotides, flanked by two substrate-recognition domains of seven to nine deoxyribonucleotides each. This type of DNAzyme can effectively cleave its substrate RNA at purine:pyrimidine junctions (Santoro, S.W. & Joyce, G.F. Proc. Natl, Acad. Sci. USA 199; for rev of DNAzymes see Khachigian, LM [Curr Opin Mol Ther 4:119-21 (2002)].

Downregulation of Varrao mite gene products can also be effected by using an antisense polynucleotide capable of specifically hybridizing with an mRNA transcript encoding the Varroa mite gene product.

Design of antisense molecules which can be used to efficiently downregulate a Varrao mite gene product must be effected while considering two aspects important to the antisense approach. The first aspect is delivery of the oligonucleotide into the cytoplasm of the appropriate cells, while the second aspect is design of an oligonucleotide which specifically binds the designated mRNA or RNA target sequence within cells in a way which inhibits translation thereof.

The prior art teaches of a number of delivery strategies which can be used to efficiently deliver oligonucleotides into a wide variety of cell types [see, for example, Luft J Mol Med 76: 75-6 (1998); Kronenwett et al. Blood 91: 852-62 (1998); Rajur et al. Bioconjug Chem 8: 935-40 (1997); Lavigne et al. Biochem Biophys Res Commun 237: 566-71 (1997) and Aoki et al. (1997) Biochem Biophys Res Commun 231: 540-5 (1997)].

In addition, algorithms for identifying those sequences with the highest predicted binding affinity for their target mRNA based on a thermodynamic cycle that accounts for the energetics of structural alterations in both the target mRNA and the oligonucleotide are also available [see, for example, Walton et al. Biotechnol Bioeng 65: 1-9 (1999)].

Such algorithms have been successfully used to implement an antisense approach in cells. For example, the algorithm developed by Walton et al. enabled scientists to successfully design antisense oligonucleotides for rabbit beta-globin (RBG) and mouse tumor necrosis factor-alpha (TNF alpha) transcripts. The same research group has more recently reported that the antisense activity of rationally selected oligonucleotides against three model target mRNAs (human lactate dehydrogenase A and B and rat gp130) in cell culture as evaluated by a kinetic PCR technique proved effective in almost all cases, including tests against three different targets in two cell types with phosphodiester and phosphorothioate oligonucleotide chemistries.

In addition, several approaches for designing and predicting efficiency of specific oligonucleotides using an in vitro system were also published (Matveeva et al., Nature Biotechnology 16: 1374 - 1375 (1998)].

Another agent capable of downregulating a Varroa mite gene product is a ribozyme molecule capable of specifically cleaving an mRNA transcript encoding the Varroa mite gene product.

Ribozymes are being increasingly used for the sequence-specific inhibition of gene expression by the cleavage of mRNAs encoding proteins of interest [Welch et al., Curr Opin Biotechnol. 9:486-96 (1998)]. The possibility of designing ribozymes to cleave any specific target RNA, including viral RNA, has rendered them valuable tools in both basic research and therapeutic applications.

An additional method of downregulating the expression of a Varroa mite gene product in cells is via triplex forming oligonuclotides (TFOs). Recent studies have shown that TFOs can be designed which can recognize and bind to polypurine/polypirimidine regions in double-stranded helical DNA in a sequence-specific manner. These recognition rules are outlined by Maher III, L. J., et al., Science,1989;245:725-730; Moser, H. E., et al., Science,1987;238:645-630; Beal, P. A., et al, Science,1992;251:1360-1363; Cooney, M., et al., Science,1988;241:456-459; and Hogan, M. E., et al., EP Publication 375408. Modification of the oligonuclotides, such as the introduction of intercalators and backbone substitutions, and optimization of binding conditions (pH and cation concentration) have aided in overcoming inherent obstacles to TFO activity such as charge repulsion and instability, and it was recently shown that synthetic oligonucleotides can be targeted to specific sequences (for a recent review see Seidman and Glazer, J Clin Invest 2003;112:487-94).

In general, the triplex-forming oligonucleotide has the sequence correspondence:

oligo	3'--A	G	G	T
duplex	5'--A	G	C	T
duplex	3'--T	C	G	A

However, it has been shown that the A-AT and G-GC triplets have the greatest triple helical stability (Reither and Jeltsch, BMC Biochem, 2002, Sept12, Epub). The same authors have demonstrated that TFOs designed according to the A-AT and G-GC rule do not form non-specific triplexes, indicating that the triplex formation is indeed sequence specific.

Triplex-forming oligonucleotides preferably are at least 15, more preferably 25, still more preferably 30 or more nucleotides in length, up to 50 or 100 bp.

Transfection of cells (for example, via cationic liposomes) with TFOs, and formation of the triple helical structure with the target DNA induces steric and functional changes, blocking transcription initiation and elongation, allowing the introduction of desired sequence changes in the endogenous DNA and resulting in the specific downregulation of gene expression.

Detailed description of the design, synthesis and administration of effective TFOs can be found in U.S. Patent Application Nos. 2003 017068 and 2003 0096980 to Froehler et al, and 2002 0128218 and 2002 0123476 to Emanuele et al, and U.S. Pat. No. 5,721,138 to Lawn.

The polynucleotide down-regulating agents of the present invention may be generated according to any polynucleotide synthesis method known in the art such as enzymatic synthesis or solid phase synthesis. Equipment and reagents for executing solid-phase synthesis are commercially available from, for example, Applied Biosystems. Any other means for such synthesis may also be employed; the actual synthesis of the polynucleotides is well within the capabilities of one skilled in the art and can be accomplished via established methodologies as detailed in, for example, “Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988) and "Oligonucleotide Synthesis" Gait, M. J., ed. (1984) utilizing solid phase chemistry, e.g. cyanoethyl phosphoramidite followed by deprotection, desalting and purification by for example, an automated trityl-on method or HPLC.

The polynucleotide agents of the present invention may comprise heterocylic nucleosides consisting of purines and the pyrimidines bases, bonded in a 3' to 5' phosphodiester linkage.

Preferably used polynucleotide agents are those modified in either backbone, internucleoside linkages or bases, as is broadly described hereinunder.

Specific examples of preferred polynucleotide agents useful according to this aspect of the present invention include polynucleotide agents containing modified backbones or non-natural internucleoside linkages. Polynucleotide agents having modified backbones include those that retain a phosphorus atom in the backbone, as disclosed in U.S. Pat. NOs: 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466, 677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050.

Preferred modified polynucleotide backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkyl phosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms can also be used.

Alternatively, modified polynucleotide backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones;

methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts, as disclosed in U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623, 070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439.

Other polynucleotide agents which can be used according to the present invention, are those modified in both sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for complementation with the appropriate polynucleotide target. An example for such an polynucleotide mimetic, includes peptide nucleic acid (PNA). A PNA polynucleotide refers to a polynucleotide where the sugar-backbone is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The bases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. United States patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Other backbone modifications, which can be used in the present invention are disclosed in U.S. Pat. No: 6,303,374.

Polynucleotide agents of the present invention may also include base modifications or substitutions. As used herein, "unmodified" or "natural" bases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified bases include but are not limited to other synthetic and natural bases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Further bases include those disclosed in U.S. Pat. No: 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. I., ed. John Wiley & Sons, 1990, those disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. , ed., CRC Press, 1993. Such bases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C. [Sanghvi YS et al. (1993) Antisense Research and Applications, CRC Press, Boca Raton 276-278] and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Following synthesis, the polynucleotide agents of the present invention may optionally be purified. For example, polynucleotides can be purified from a mixture by extraction with a solvent or resin, precipitation, electrophoresis, chromatography, or a combination thereof. Alternatively, polynucleotides may be used with no, or a minimum of, purification to avoid losses due to sample processing. The polynucleotides may be dried for storage or dissolved in an aqueous solution. The solution may contain buffers or salts to promote annealing, and/or stabilization of the duplex strands.

It will be appreciated that a polynucleotide agent of the present invention may be provided per se, or as a nucleic acid construct comprising a nucleic acid sequence encoding the polynucleotide agent.

Typically, the nucleic acid construct comprises a promoter sequence which is functional in the host cell, as detailed herein below.

The polynucleotide sequences of the present invention, under the control of an operably linked promoter sequence, may further be flanked by additional sequences that advantageously affect its transcription and/or the stability of a resulting transcript. Such sequences are generally located upstream of the promoter and/or downstream of the 3' end of the expression construct.

The term "operably linked", as used in reference to a regulatory sequence and a structural nucleotide sequence, means that the regulatory sequence causes regulated expression of the linked structural nucleotide sequence. "Regulatory sequences" or "control elements" refer to nucleotide sequences located upstream, within, or downstream of a structural nucleotide sequence, and which influence the timing and level or amount of transcription, RNA processing or stability, or translation of the associated structural nucleotide sequence. Regulatory sequences may include promoters, translation leader sequences, introns, enhancers, stem-loop structures, repressor binding sequences, termination sequences, pausing sequences, polyadenylation recognition sequences, and the like.

It will be appreciated that the nucleic acid agents can be delivered to the Varroa mites in a great variety of ways.

According to one embodiment, the nucleic acid agents are delivered directly to the mites (e.g. by spraying an infested hive). The nucleic acid agents, or constructs encoding same may enter the mites bodies by diffusion. In this embodiment, the promoter of the nucleic acid construct is typically operational in mite cells.

It will be appreciated that since Varroa mites use their mouths to puncture the bee exoskeleton and feed on the bee's hemolymph, the present invention contemplates delivering the polynucleotide agents of the present invention to the bees, whereby they become presented in the bee's hemolymph thereby becoming available to the mite. Thus, according to another embodiment, the nucleic acid agents are delivered indirectly to the mites (e.g. via the bee). In this embodiment, the promoter of the nucleic acid construct is typically operational in bee cells.

According to one embodiment, the nucleic acid agents are delivered to the bees by spraying. The nucleic acid agents, or constructs encoding same may enter the bees bodies by diffusion.

According to another embodiment, the nucleic acid agents are delivered to the bees via its food. The present inventors consider that following ingestion of the nucleic acid agents of the present invention, the agents will be presented in the bee's hemolymph, whereby it becomes available to the Varroa mite.

Thus the polynucleotides of the present invention may be synthesized *in vitro* and added to the food. For example double stranded RNA may be synthesized by adding two opposing promoters (e.g. T7 promoters; SEQ ID NOs: 48 and 49) to the ends of the gene segments, wherein SEQ ID NO: 48 is placed immediately 5' to the gene and SEQ ID NO: 49 is placed immediately 3' to the gene segment. The dsRNA may then be transcribed in vitro with the T7 RNA polymerase.

Exemplary sequences for synthesizing dsRNA according to embodiments of the present invention are provided in SEQ ID NOs: 50-93.

As detailed herein, bee feeding is common practice amongst bee-keepers, for providing both nutritional and other, for example, supplemental needs. Bees typically feed on honey and pollen, but have been known to ingest non-natural feeds as well. Bees can be fed various foodstuffs including, but not limited to Wheast (a dairy yeast grown on cottage cheese), soybean flour, yeast (e.g. brewer's yeast, torula yeast) and yeast products products-fed singly or in combination and soybean flour fed as a dry mix or moist cake inside the hive or as a dry mix in open feeders outside the hive. Also useful is sugar, or a sugar syrup. The addition of 10 to 12 percent pollen to a supplement fed to bees improves palatability. The addition of 25 to 30 percent pollen improves the quality and quantity of essential nutrients that are required by bees for vital activity.

Cane or beet sugar, isomerized corn syrup, and type-50 sugar syrup are satisfactory substitutes for honey in the natural diet of honey bees. The last two can be supplied only as a liquid to bees.

Liquid feed can be supplied to bees inside the hive by, for example, any of the following methods: friction-top pail, combs within the brood chamber, division board feeder, boardman feeder, etc. Dry sugar may be fed by placing a pound or two on the inverted inner cover. A supply of water must be available to bees at all times. In one embodiment, pan or trays in which floating supports-such as wood chips, cork, or plastic sponge-are present are envisaged. Detailed descriptions of supplemental feeds for bees can be found in, for example, USDA publication by Standifer, et al 1977, entitled "Supplemental Feeding of Honey Bee Colonies" (USDA, Agriculture Information Bulletin No. 413).

It will be appreciated that Varro mites cause wound sites in the exoskeleton of bees. Such wound sites harbor bacterial infections, such as Melissococcus pluton, which causes European foulbrood. In addition, to their parasitic effects, Varroa mites are suspected of acting as vectors for a number of honey bee pathogens, including deformed wing virus (DWV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV) and black queen cell virus (BQCV), and may weaken the immune systems of their hosts, leaving them vulnerable to infections.

Thus, by killing the mites (or preventing reproduction thereof), the agents of the present invention may be used to prevent and/or treat bacterial infections such as Melissococcus pluton and viral infections caused by the above named viruses.

Since Varroa mite infestation and viral infections are thought to be responsible for colony collapse disorder (CCD), the present agents may also be used to prevent or reduce the susceptibility of a bee colony to CCD.

It will be appreciated that in addition to feeding of oligonucleotides and/or polynucleotides for reduction of the bee pathogen infection and infestation, enforcement of proper sanitation (for example, refraining from reuse of infested hives) can augment the effectiveness of treatment and prevention of infections.

It is expected that during the life of a patent maturing from this application many relevant methods for downregulating expression of gene products will be developed and the scope of the term "downregulating expression of a gene product of a Varroa destructor mite" is intended to include all such new technologies *a priori*.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to". This term encompasses the terms "consisting of" and "consisting essentially of".

The phrase "consisting essentially of" means that the composition or method may include additional ingredients and/or steps, but only if the additional ingredients and/or steps do not materially alter the basic and novel characteristics of the claimed composition or method.

As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term “treating” includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Feeding Varroa-specific dsRNA prevents Varroa mite infestation

In order to determine the effectiveness of ingested dsRNA on Varroa mite infestation, honeybees are provided with Varroa mite-specific and control dsRNA in the feed for 7 days before, and 2 days following contact with the Varroa mite, as illustrated in Figure 1. Numbers of dead Varroa per experimental hive are counted, and sample live and dead Varroa are collected for molecular analysis.

MATERIALS AND METHODS

Establishment of mini-hive colonies: Young, approximately 2-month-old queens, together with approximately 200 worker bees are collected from hives in a local apiary. The bees are transferred into mini-hives fitted with one mini comb that was previously built by a regular hive. All of the mini-hives are closed and placed in a temperature-controlled room (30°C).

dsRNA preparation: Varroa mite sequences are cloned into a plasmid between two opposing T7 promoters. Following propagation of plasmid DNA, the viral fragments, including the T7 promoters, are excised and gel-purified. These serve as templates for T7-directed *in-vitro* transcription (MEGAscript™, Ambion, Austin TX). The reaction product is submitted to DNase digestion followed by phenol extraction and ethanol precipitation. The final preparation is dissolved in nuclease-free water.

dsRNA feeding in minihives: 5 gr. pollen supplement patties are placed on top of each comb and 10 ml of 50 % sucrose solution is introduced into the hive in a sterile Petri dish nightly. The feeding is continued for 9 days and subsequently only hives in which queens had begun to lay eggs are included in the trial.

Following establishment of active hives (queens laying eggs), some of the mini-hives are supplemented with Varroa mite-specific (apoptosis inhibitor (IAP) gene (SEQ ID NO: 27) or non-specific control (e.g. GFP SEQ ID NO: 91) dsRNA, which is added to the 10 ml 50 % sugar solution given to the hives, adjusted to approximately 1 microgram dsRNA per feed per bee, assuming all bees consume approximately the same amount of sucrose solution. dsRNA feeding is continued for six days.

Varroa mite infestation in minihives: 7 days after feeding in active hives, some of the colonies are placed in contact with a population of Varroa mites. Thereafter, dsRNA treatment is continued for a further 2 days. Samples of live and dead bees (larvae and adults) are collected daily from each mini-hive post introduction of the Varroa mite population for 32 consecutive days. Every bee collected is frozen in liquid nitrogen and preserved at -70 °C pending molecular analysis. Vitality of the colonies are monitored by opening the hives (without smoke), withdrawing the mini-comb and photographing the mini-comb from both sides. The hive-combs are photographed daily, and the numbers of remaining live bees are monitored. The photographs are downloaded onto a computer and the total number of bees is counted for every mini-hive.

To test dsRNA toxicity, another group of hives are provided with Varroa mite-specific dsRNA, but is not placed in contact with the Varroa mite population. Two sets of hives serve as additional controls: hives that are not treated with dsRNA and are not inoculated with Varroa mites, and hives that were not treated with dsRNA, but were inoculated with Varroa mites.

RT-PCR analysis:

Extraction of Nucleic Acids: Total RNA is extracted from the preserved bees using the TRIREAGENT method (Sigma, St. Louis MO, USA). Briefly, RNA is extracted by precipitation and separation by centrifugation, then resuspended in RNAsecure solution.

Real-Time RT-PCR: Measured amounts of RNA (100 ng for viral expression analyses and 100 pg for 18S rRNA internal controls) are subjected to one-step RT-PCR using the SYBR Green PCR master mix with Taqman reverse transcriptase (Applied Biosystems, Foster City, CA). Real-time RT-PCR is conducted in GeneAmp PCR System 5700 (Applied Biosystems). Reactions performed without reverse transcriptase or without template should not result in any product.

Northern-Blot Analysis: Total RNA is extracted from treated and control bees. Formaldehyde is added to the RNA to 1.8 % and warmed to 65 °C. The RNA, 15 μg per lane is electrophoresed on a 1.2 % agarose gel at 70 V, 4 °C with stirring. The previously described amplified Varroa mite-RNA product is digoxigenin labeled and serves as a probe for hybridization. Detection is performed with the DIG luminescent detection kit (Roche Diagnostics GmbH, Mannheim, Germany). RNA sizes are estimated by comparison to electrophoresed RNA Molecular Weight Markers I (Roche). Hybridization is carried out at high stringency (0.1x SSC; 65°C).

The fate of ingested Varroa mite-specific dsRNA in honeybees: In order to better understand the mechanism(s) of action by which dsRNA-Varroa mite protects the bees against Varroa mite infestation and its consequences, total RNA is extracted from dsRNA-Varroa mite treated, and non-treated control bees, submitted to digestion by a panel of nucleases, and separated on PAGE

RESULTS

The presence of dsRNA in the adult bee body in the bee larvae (fed by adult bees), in the bee pupa was determined by slot-blot hybridization with a probe for GFP. The processing of the dsRNA to siRNA was determined by Northern blots detecting small RNAs (Figures 2A-E).

Varroa individuals were placed on adult bees that had been fed for 7 days with dsRNA-GFP and on control (unfed) bees. RNA was extracted from Varroa at the indicated times (Figure 1) and subjected to RT-PCR with GFP primers. The results are illustrated in Figure 3.

Bees were fed with a segment of dsRNA for apoptosis inhibitor (IAP) gene (SEQ ID NO: 27). Varroa collected from that hive were analyzed by RT-PCR for the expression of the IAP gene (Figure 4).

EXAMPLE 2

MATERIALS AND METHODS

Hives were fed by two different mixtures of dsRNAs corresponding to Varroa gene segments. All dsRNA were corresponding to gene segments that are not homologous to bee or human sequences (not carrying stretches of homologous sequences longer than 19 bases). Mixture I (Minimum treatment) contained SEQ ID NOs: 1, 13, 27, 30 and 39. Mixture II (Maximum treatment) contained SEQ ID NOs: 1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39. Thirty Varroa individuals were placed in each hive and two months later Varroa and bees were counted in each hive. Each treatment was repeated 3 times.

RESULTS

No visible damage to the strength of the hive was noticed among the various hives. Figure 5 demonstrates the reduction of Varroa population following treatment with dsRNAs of Varroa's gene sequences.

EXAMPLE 3

Large-scale field trials of Varroa-specific dsRNA for prevention of Varroa mite-associated disease of honeybees

In order to determine the effectiveness of ingested Varroa mite dsRNA on Varroa mite infestation under actual field conditions, and to assess effects on important parameters of colony health, bees in sample full size hives are provided with Varroa mite-specific dsRNA in the feed for 5 days before, and 4 days following infestation with Varroa mite.

MATERIALS AND METHODS

Insect material:

Pools of five bees from the following treatments; Remote control, Varroa mite-dsRNA only, Varroa mite only and Varroa mite-specific dsRNA+ Varroa mite at each time point day 0-(day of virus application), day 7 and end point (day 42). The test was repeated several times.

RNA extraction:

RNA is extracted using Tri-Reagent (Sigma,USA) according to protocol provided by the manufacturer. All samples are treated with DNaseI and resuspended with loading buffer (90 % Formamide, 0.05 Bromophenol Blue, 0.05 % Xylene cyanol) prior to loading on gel.

Gel electrophoresis and Blot:

10 ug of freshly prepared RNA is measured using the nanodrop spectrophotometer and loaded on 12 % Acrylamide gel (1:19 acrylamide:Bis acrylamide ratio) in danturation environment (gel contains 7M Urea). After electrophoresis samples are transferred to positively charged nylon membrane (Roch,USA) using electrobloting method.

Hybridization and signal detection:

Membrane is hybridized with freshly prepared DNA probe of Varroa mite segment, taken from a region that does not correspond to the dsRNA of the Varroa mite-specific dsRNA itself. This is made using DIG PCR probe preparation Kit (Roch,USA) o/n 42°C in DIG easyhyb solution (Roch, USA) according to manufacturer protocol. The membrane is washed twice with 2 X SSC/0.1 % SDS, than washed for stringency with 0.1 X SSC/0.1 % SDS in 65 °C. Membranes are further washed using DIG Wash and Block Kit (Roch, USA) according to manufacturer protocol. Detection is preformed using CSPD-star substrate (Roch, USA). Positive control is 21nt DNA primers corresponding to the hybridized sequence.

Signal is detected using membrane exposure for 2-12 hours in chemiluminator manufactured by Kodak.

Basic parameters of bee colony health (numbers of capped brood, numbers of bees in the hive, returning foragers and honey production) are assessed in hives fed Varroa mite-dsRNA and control hives, in the absence of Varroa mite infestation.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. An isolated nucleic acid agent comprising a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite.

2. The isolated nucleic acid agent of claim 1, wherein said nucleic acid sequence is complementary to at least 21 nucleotides of Varroa destructor mite specific RNA and capable of inducing degradation of said Varroa destructor mite RNA.

3. The isolated nucleic acid agent of claim 1, wherein the agent is selected from the group consisting of a dsRNA, an antisense RNA and a ribozyme.

4. The isolated nucleic acid agent of claim 3, wherein said dsRNA is selected from the group consisting of siRNA, shRNA and miRNA.

5. The isolated nucleic acid agent of claim 1, wherein said gene product is an mRNA encoding a polypeptide selected from the group consisting of ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin.

6. A nucleic acid construct comprising a nucleic acid sequence encoding the isolated nucleic acid agent of claim 1.

7. The isolated nucleic acid agent of claim 1, wherein said nucleic acid sequence is greater than 15 base pairs in length.

8. The isolated nucleic acid agent of claim 7, wherein said nucleic acid sequence is 19 to 25 base pairs in length.

9. The isolated nucleic acid agent of claim 7, wherein said nucleic acid sequence is greater than 30 base pairs in length.

10. A bee-ingestible composition comprising at least one nucleic acid agent which comprises a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite.

11. The bee-ingestible composition of claim 10, wherein said at least one nucleic acid agent comprises at least five nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least five nucleic acid agent targeting a different gene.

12. The bee-ingestible composition of claim 10, wherein said at least one nucleic acid agent comprises at least six nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least six nucleic acid agents for targeting a different gene.

13. The bee-ingestible composition of claim 11, wherein said nucleic acid agents are as set forth in SEQ ID Nos: 1, 13, 27, 30 and 39.

14. The bee-ingestible composition of claim 12, wherein said nucleic acid agents are as set forth in SEQ ID Nos: 1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39.

15. The bee-ingestible composition of claim 10, wherein said composition is in solid form.

16. The bee-ingestible composition of claim 10, wherein said composition is in liquid form.

17. The bee-ingestible composition of claim 10, wherein said composition comprises protein.

18. The bee-ingestible composition of claim 17, wherein said protein is in the form of pollen and/or soy patties.

19. The bee-ingestible composition of claim 16, wherein said liquid is a sucrose solution.

20. The bee-ingestible composition of claim 16, wherein said liquid is a corn syrup solution.

21. The bee-ingestible composition of claim 16, wherein said liquid further comprises a carbohydrate or sugar supplement.

22. A method of preventing or treating a Varroa destructor mite infestation of a bee hive, the method comprising administering to the bee an effective amount of at least one nucleic acid agent which comprises a nucleic acid sequence which downregulates expression of a gene product of a Varroa destructor mite, thereby preventing or treating a Varroa destructor mite infestation of a bee hive.

23. The method of claim 22, wherein said bee is a honeybee.

24. The method of claim 23, wherein said honeybee is a forager.

25. The method of claim 23, wherein said honeybee is a hive bee.

26. The method of claim 23, wherein said honeybee is a bee of a colony, and wherein said administering reduces the susceptibility of said bee colony to Colony Collapse Disorder.

27. The method of claim 22, wherein said administering is effected by feeding.

28. The method of claim 27, wherein said feeding comprises providing a liquid bee-ingestible composition.

29. The method of claim 27, wherein said feeding comprises providing a solid bee-ingestible composition.

30. The method of claim 23, wherein said at least one nucleic acid agent comprises at least five nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least five nucleic acid agent targeting a different gene.

31. The method of claim 23, wherein said at least one nucleic acid agent comprises at least six nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least six nucleic acid agents for targeting a different gene.

32. The method of claim 30, wherein said nucleic acid agents are as set forth in SEQ ID Nos: 1, 13, 27, 30 and 39.

33. The method of claim 31, wherein said nucleic acid agents are as set forth in SEQ ID Nos:1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39.

34. A method of preventing or treating a Varroa destructor mite infestation of a bee hive, the method comprising administering to the bee an effective amount of the nucleic acid construct of claim 6, thereby preventing or treating a Varroa destructor mite infestation of a bee hive.

35. A method of reducing the susceptibility of honeybees to Colony Collapse Disorder (CCD), the method comprising administering to the honeybee an effective amount of at least one double-stranded ribonucleic nucleic acid (dsRNA), said at least one dsRNA comprising a sequence complementary to at least 21 nucleotides of Varroa

destructor mite mRNA and capable of inducing degradation of said Varroa destructor-specific mRNA.

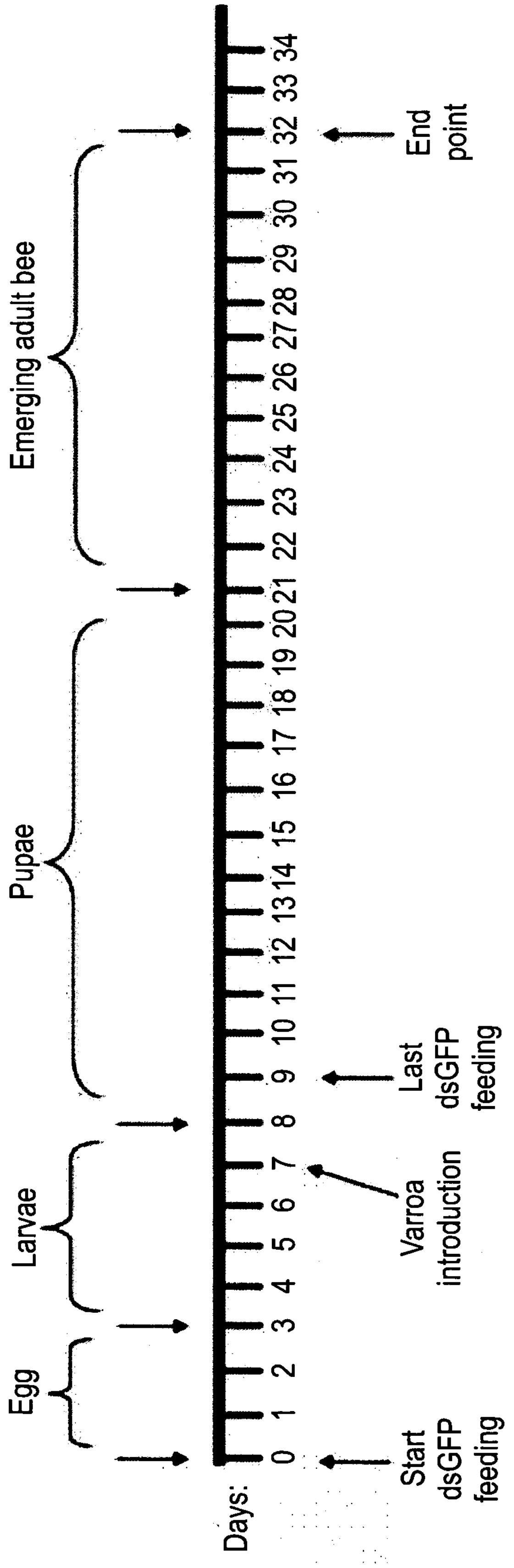
36. The method of claim 35, wherein said Varroa destructor mite mRNA encodes a polypeptide selected from the group consisting of ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin.

37. The method of claim 35, wherein said at least one nucleic acid agent comprises at least five nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least five nucleic acid agent targeting a different gene.

38. The method of claim 35, wherein said at least one nucleic acid agent comprises at least six nucleic acid agents, for down-regulating ATPase subunit A, RNA polymerase I, RNA polymerase III, Inhibitor of apoptosis (IAP), FAS apoptotic inhibitor and α-Tubulin, each of said at least six nucleic acid agents for targeting a different gene.

39. The method of claim 37, wherein said nucleic acid agents are as set forth in SEQ ID Nos: 1, 13, 27, 30 and 39.

40. The method of claim 38, wherein said nucleic acid agents are as set forth in SEQ ID Nos:1, 4, 7, 10, 13, 16, 19, 22, 25, 27, 30, 33, 36 and 39.


Days:
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1
2
3
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5
6
7
8
9
10
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12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
Egg
Larvae
Pupae
Emerging adult bee
Start dsGFP feeding
Varroa introduction
Last dsGFP feeding
End point

FIG. 1

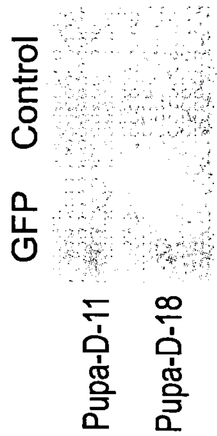


FIG. 2C

FIG. 2D

FIG. 2E

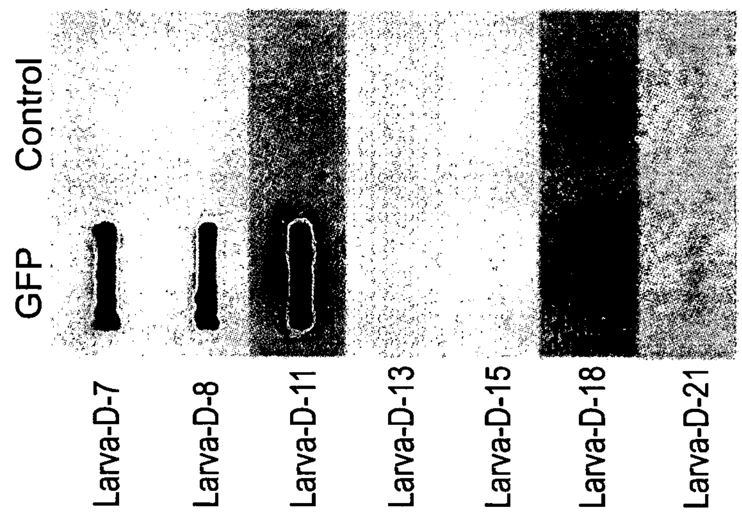


FIG. 2B

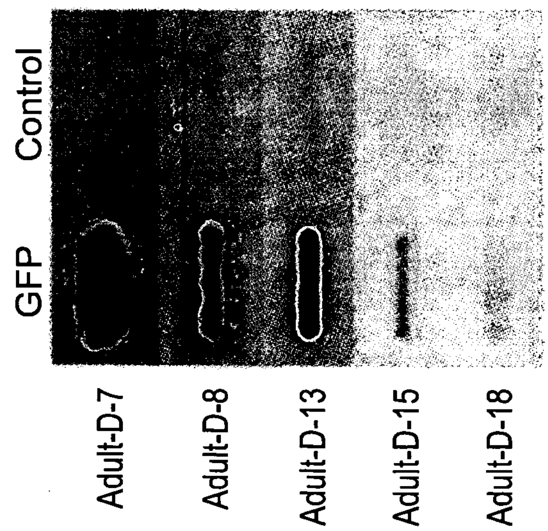


FIG. 2A

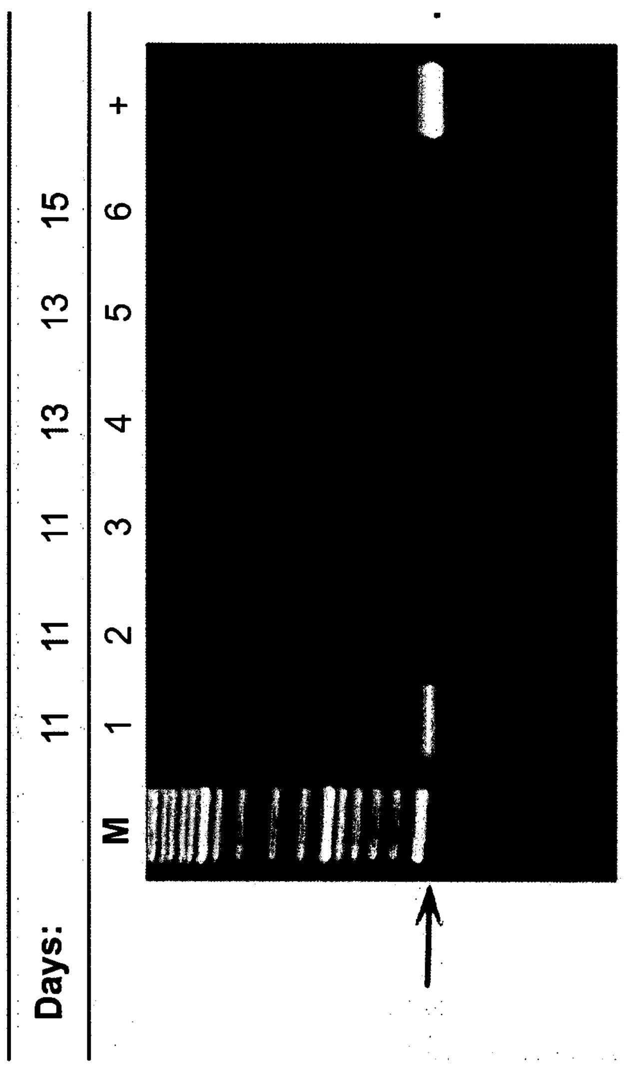
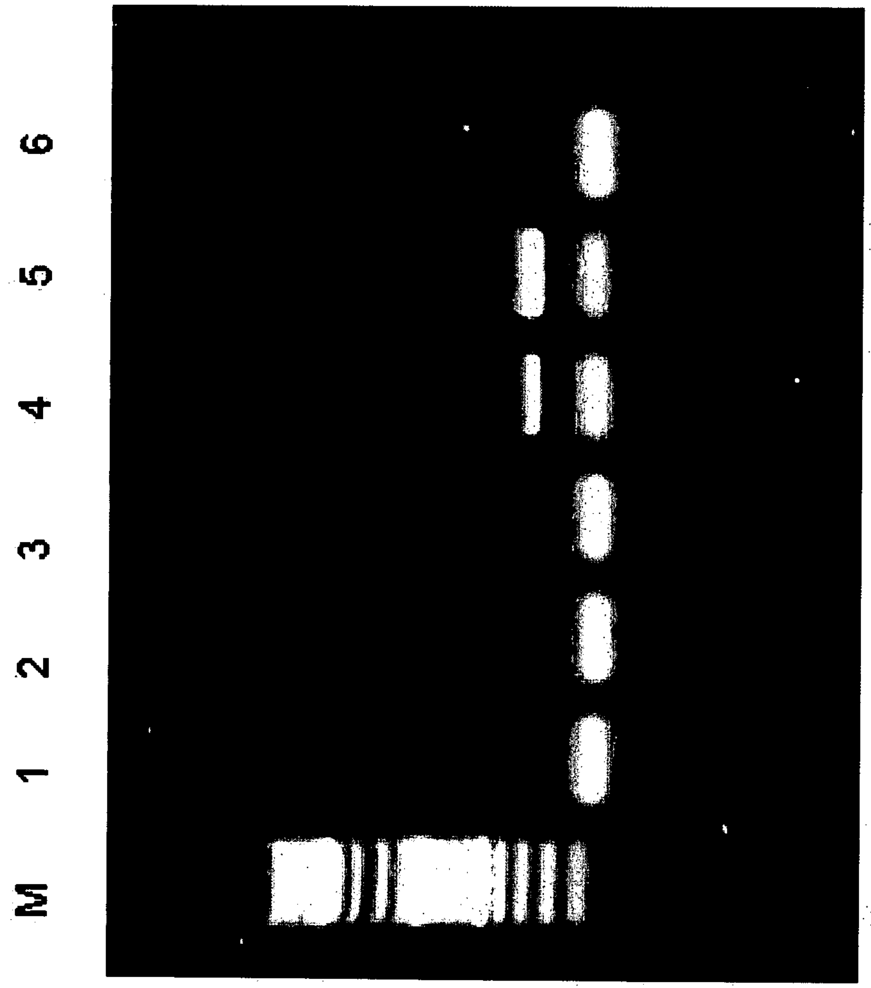


FIG. 3

FIG. 4



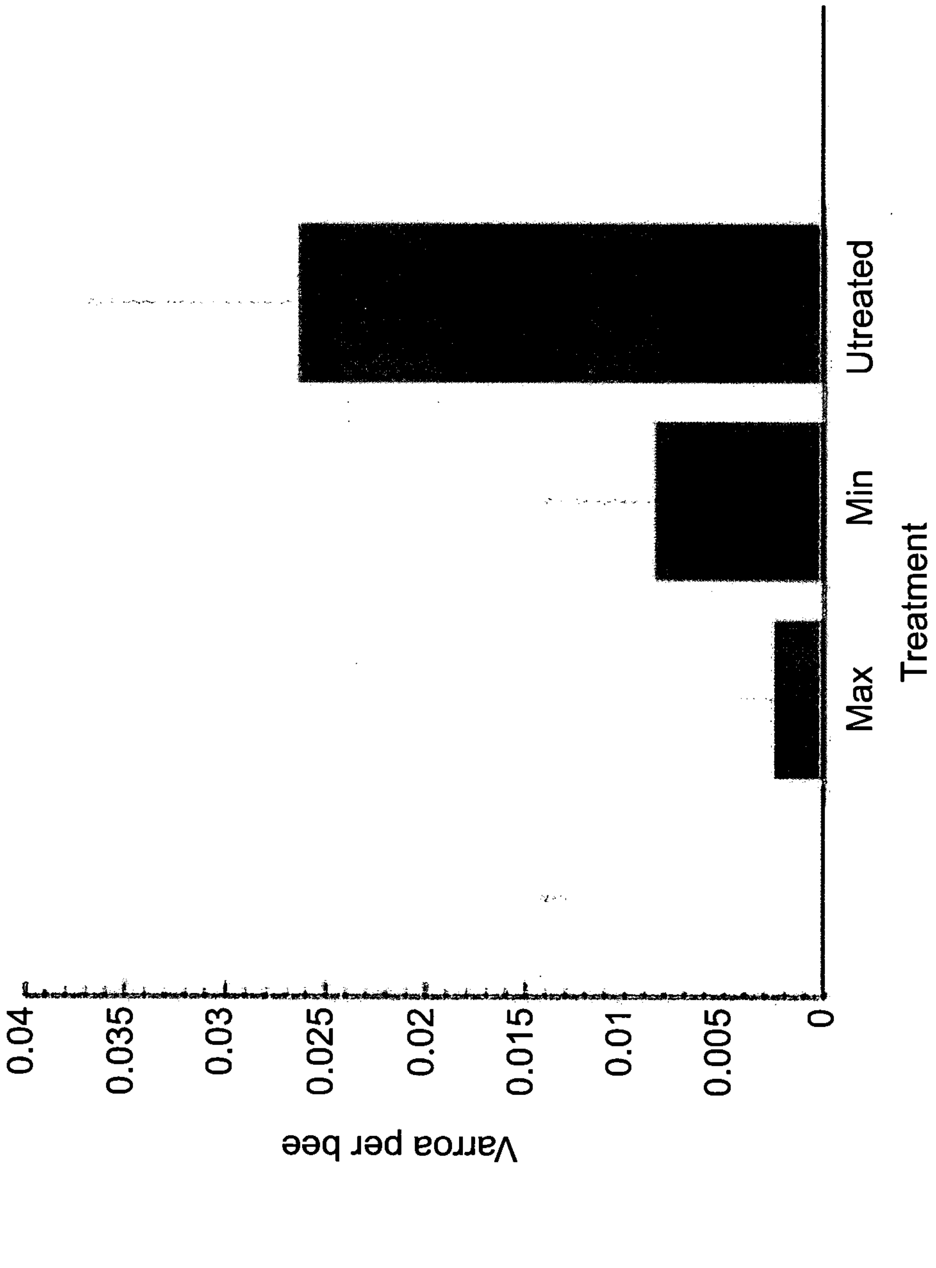

Varroa per bee
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
Max
Min
Utreated
Treatment

FIG. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/IL2010/000844

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/713 A61P33/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WANG RUIWU ET AL: "Molecular characterization of an arachnid sodium channel gene from the varroa mite (Varroa destructor)." INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 33, no. 7, July 2003 (2003-07), pages 733-739, XP002621492 ISSN: 0965-1748 abstract -----	1-4, 6-10, 15-21

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[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 February 2011	24/02/2011

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Vreugde, Sarah

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LIU Z ET AL: "Effect of a fluvalinate-resistance-associated sodium channel mutation from varroa mites on cockroach sodium channel sensitivity to fluvalinate, a pyrethroid insecticide" INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, vol. 36, no. 11, 1 November 2006 (2006-11-01), pages 885-889, XP025014535 ELSEVIER SCIENCE LTD, GB ISSN: 0965-1748 DOI: 10.1016/J.IBMB.2006.08.006 [retrieved on 2006-11-01] abstract -----	1-40
A	MAORI E ET AL: "IAPV, a bee-affecting virus associated with Colony Collapse Disorder can be silenced by dsRNA ingestion" INSECT MOLECULAR BIOLOGY, vol. 18, no. 1, 1 February 2009 (2009-02-01), pages 55-60, XP002523701 BLACKWELL SCIENTIFIC, OXFORD, GB ISSN: 0962-1075 DOI: 10.1111/J.1365-2583.2009.00847.X abstract -----	1-40
X,P	CAMPBELL EWAN M ET AL: "Gene-knockdown in the honey bee mite Varroa destructor by a non-invasive approach: studies on a glutathione S-transferase." PARASITES & VECTORS, vol. 3, 73, 16 August 2010 (2010-08-16), pages 1-10, XP002621493 ISSN: 1756-3305 abstract -----	1-4, 6-10, 22-26, 34,35

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