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(54) Titre : CONTROLE DES PARASITES  
(54) Title: CONTROL OF PARASITES

(57) **Abrégé/Abstract:**

The invention concerns a composition for the treatment of varroa mite infestations in honeybee colonies, comprising an effective amount of at least one compound selected from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, n-octadecyl acetate and methyl palmitate.



ABSTRACT OF THE DISCLOSURE

The invention concerns a composition for the treatment of varroa mite infestations in  
5 honeybee colonies, comprising an effective amount of at least one compound selected  
from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate,  
n-octadecyl acetate and methyl palmitate.

**CONTROL OF PARASITES**

The present invention concerns a method for the control of varroa mite infestations of honeybee colonies, and infestation controlling compositions suitable for such purpose.

5 Varroasis is an infestation of European honeybee colonies (*Apis mellifera*) with the ectoparasitic mites belonging to the order of Acari (*Varroa jacobsoni* and related species).

The varroa mite originated in Asia and from there has spread to virtually all countries where beekeeping with *A. mellifera* is practised.

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In addition, other acarine infestations such as those of *Acarapis woodii* (Tracheal mite) and *Tropilaelaps clareae* can cause significant damage to honeybee colony health.

15 The varroa mites feed off the haemolymph of the developing bees and adults alike and can result in stunted growth of bees, infection and death of bee colonies. Indeed varroa infestation is the most serious threat to beekeeping world-wide today.

20 Various treatments are available for the control of this disease. However, in certain countries the varroa mite has developed a resistance to the active ingredient of some of these various treatments, notably to products based on the pyrethroids flumethrin, fluvalinate and acrinathrin, but also to amitraz, coumaphos, malathion, chlorfenvinphos and related molecules.

25 International patent application WO90/04922 describes the use of an extract of bee larvae to attract varroa mites. Such an extract may contain, or be supplemented with, various substances including methyl palmitate. However, the extracts and substances disclosed in WO90/04922 are only stated to be useful as attractants; other substances, such as acaricides, are necessary to effect control of mite populations.

30 There is a need, therefore, for a new treatment for varroa and other infestations.

Summary of the Invention

It has now been found that the varroa mite infestation in bee-hives can be efficiently controlled by applying to the beehives an effective amount of at least one compound  
5 selected from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, n-octadecyl acetate and methyl palmitate.

In a first aspect, therefore, the invention provides a composition for controlling varroa mite infestations in a honeybee colony, comprising an effective amount of at least one  
10 compound selected from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate and n-octadecyl acetate.

Preferably, the composition consists essentially of cis-8-dodecenyl acetate. Other compounds, selected from the group consisting of 1-dodecanol, n-hexadecyl acetate, n-  
15 octadecyl acetate and excipients, may be included. Advantageously, the composition comprises about 50% cis-8-dodecenyl acetate, preferably about 60% cis-8-dodecenyl acetate, more preferably about 70% cis-8-dodecenyl acetate and ideally about 85% cis-8-dodecenyl acetate or more.

20 Preferably, the composition according to the invention is provided in a suitable dispenser, as used for dispersion of pheromones and other semiochemicals.

The invention therefore provides a dispenser for the controlled release of semiochemicals to the locus of a bee colony, said dispenser containing and releasing a composition  
25 according to the invention.

In order to achieve the treatment of varroa mite infestations, release of the composition should take place over a period of a minimum of two months. Preferably, the composition is released over a period of two to three months. This requires the dispenser  
30 to contain between 0.1mg and 15mg of the composition. Preferably, it contains 1mg to 10mg of the composition. Preferably, it contains 1mg to 5mg of the composition.

It is envisaged that a dispenser containing an amount of the composition may comprise two dispensers, each containing half said amount of the composition, for example a dispenser containing 15mg of the composition may comprise two dispensers, each containing about 7.5mg of the composition. Similarly, a dispenser containing 10mg of the composition may comprise two dispensers, each containing about 5mg of the composition.

According to a further aspect, the invention provides the use of an effective amount of at least one compound selected from the group consisting of cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, n-octadecyl acetate and methyl palmitate for the treatment of varroa mite infestations in honeybee colonies.

In accordance with the present invention, the term "treatment" means the reduction of the numbers of the mites which infest the honeybee colonies. Thus, compositions according to the invention are effective to limit population growth of the mites and, indirectly, cause the death thereof. The compositions do not merely function as attractants, requiring the presence of other agents to provide control of the mite population.

According to a still further aspect, the invention relates to a method for the control of varroa mite infestations in honeybee colonies, comprising applying to the honeybee colonies an effective amount of at least one compound selected from the group consisting of cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, n-octadecyl acetate and methyl palmitate.

Preferably, the concentration of the composition in the environment of the bee colony is held at an efficacious level for at least two months, advantageously two to three months. This may be accomplished using a single application of composition according to the invention, or may be accomplished using more than one application of a composition according to the invention, for example two applications or even more. In the case of using two or more applications, such applications may be made simultaneously, sequentially or together.

Detailed Description of the Invention

For the purpose of this invention, the locus of colonies of honeybees is usually understood to be, without implying a limitation, beehives, or similar containers where colonies of  
5 bees build their breeding places, such as honeycombs. Preferably, the locus of honeybee colonies as referred to herein is a beehive.

*Compositions*

A composition according to the present invention may be a pure compound, or a mixture  
10 of compounds. Compositions according to the invention are typically formulated from high purity starting materials, available commercially from specialist manufacturers. Typically, the compositions consist essentially of the active ingredients in substantially pure form. The preparation may also include one or more anti-oxidants such as Butylated Hydroxytoluene (BHT), 2,6-Bis(1,1-dimethyl-ethyl)-4-methylphenol.

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An "effective amount" of a compound component of the composition according to the present invention is an amount which, in the relevant composition, is sufficient for effective control of varroa mite infestations, as defined below. For example, the composition may comprise about 80% to 100% of the selected active compound(s).  
20 Advantageously, the composition comprises about 85% or more cis-8-dodecenyl acetate.

The composition may comprise or consist essentially of any one of the compounds according to the present invention, or any two or more of said compounds. In addition, the composition may include one or more excipients, which may be solvents, thickeners,  
25 and the like.

In a preferred embodiment, methods according to the present invention comprise the application of only a single semiochemical substance. Alternatively, however, mixtures of two or more semiochemical substances may be used. Optionally, the method may  
30 entail the application of a mixture of semiochemicals.

*Dispenser*

Dispensers are widely used in the art to distribute semiochemicals to lepidopteran and coleopteran populations. Generally, a dispenser may be any medium suitable for dispersing a composition according to the present invention characterised in that it provides a more regulated administration of active material over a given time-frame in comparison to raw active material. In particular, such are preferably capable of maintaining a relatively constant vapour concentration of the semiochemical(s) in the atmosphere of an enclosed volume of a bee habitat, i.e. a beehive.

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The composition of the invention is transported from the dispenser via the vapour phase to the varroa mites. In a preferred aspect of the invention, the composition evaporates or sublimates from the dispenser in a regulated concentration into the atmosphere of the bee environment (e.g. the beehive) and maintains the preselected concentration for at least a 2 month treatment period. Though the atmosphere in the bee environment is not hermetically sealed, the population increase of the varroa mites in said environment can be effectively controlled by the release of the composition from the dispenser.

The method of control of varroa infestations according to the present invention is both effective against acaricide-resistant and acaricide-susceptible varroa mite strains in beehives. In a preferred embodiment of the invention, effective control of mite populations, particularly varroa populations, may be achieved such that the infestation is reduced to a level of 50% or less of a starting infestation level. The "starting infestation level" is the infestation level before treatment is initiated. Preferably, the reduction in infestation levels to 50% is achieved within two months. Advantageously, varroa populations are reduced by between 60% and 80%, and preferably between 80% and 95%. At higher dispenser loadings in particular, the invention is capable of reducing varroa mite populations by at least 80%, preferably about 90% and optimally about 95%.

Dispensers are known in the art which may be employed with the compositions of the present invention. For example, dispensers are known as manufactured by Shin-Etsu

Chemical Co., Japan, for instance as described in US 4,325,941 and US 5,503,839; or US 5,000,987 (Nitto Denko Corporation).

US 5,750,129 describes a number of dispensers. Zeoli, L.T., et al., 1982 ("Controlled  
5 Release Technologies" in *Insect Suppression with Controlled Release Pheromone Systems*, vol. 1, ed. G. Zweig, CRC Press Inc., Boca Raton, FL. pp. 131-144) have summarised controlled release technologies used for delivering semiochemicals and have categorised the dispensers types into four groups: monolithic systems, laminated structures, reservoir systems without rate controlling membranes and reservoir systems  
10 with rate controlling membranes.

Monolithic systems are one material systems which consist of inert polymeric matrices impregnated with the semiochemical as the active ingredient. Such homogenous devices are simple and they lend themselves to a number of applications. Examples of this  
15 technology are polyvinylchloride (PVC) impregnated with semiochemical (Fitzgerald, T.D. et al., 1973: *Environmental Entomology*, vol. 2, #4, pp. 607-610) or silicone rubber impregnated with fragrances as in U.S 4,725,575 (Union Camp Co.), or silicone- urethane copolymers used as matrices for dispensing fragrances or pheromones as in U.S. 4,908,208 and 5,008,115 (Dow Corning Co.), urethane matrices for dispensing pesticides  
20 as in U.S. 4,594,380 (AT & T Bell Laboratories) or acrylate polymers impregnated with pheromones (Smith C.N. et al., 1991: *Intern. Symp. Control Rel. Bioact. Mater.*, Controlled Release Society, Inc. pp. 81-82; Kim, J. et al., 1988: *Appl. Radiat. Isot.*, vol. 39, #11, pp. 1145- 1152).

25 Laminated structures are devices which have relatively good release characteristics, approaching zero order (constant) release. The manufacture of these devices requires multiple processing to produce the laminate. An example of this technology are laminated dispensers described in US 4,639,393 (Herculite Protective Fabrics), and DE 3524180 (Montedison S.p.A.).

30

Reservoir systems without rate controlling membranes can be exemplified by capillary release devices as described in US 4,017,030 (Albany International Co.).

Reservoir systems with a rate controlling membrane generally provide near constant release characteristics which are governed by a permeable membrane. The membrane composition and surface area can be varied to effect changes in the release rates. Examples are described in US 4,323,556 (Montedison S.p.A.), US 4,445,641 (Bend Research Inc.), US 4,979,673 (I. J. Wilk), US 4,923,119 (Shin-Etsu Chemical Co.), U.S. 4,793,555 (Dow Corning Corp.) and DE 2945655 (Celamerck G.m.b.H.).

Dispensers such as rubber septa or polyisoprene type septa of a variety of size/dimension as are traditionally used to liberate lepidopteran and coleopteran pheromones may be employed in this invention. In addition traditional "Long Life" lepidopteran or coleopteran pheromone dispensers, such as small polyethylene or other polymer tubes with or without internal wicks and closures may be used as dispensers for this invention.

Dispensers according to the invention are preferably loaded with 0.1mg to 10mg or more of the composition of the invention. Although amounts as low as 0.1mg (or lower) may be effective, higher loadings are more effective over a longer time period. The minimum treatment period is of the order of two months, with between two and three months being preferred.

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#### *Control of varroa mites*

The present invention is effective in the control of varroa mites in the presence of bee brood. In the normal varroa life cycle, fecund female varroa mites enter the cells of the brood before they are capped over by the worker bees. After a period of rest the female mite begins to feed off the developing bee pupa and only then can lay eggs.

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In the presence of compositions according to the invention, the mites are deterred from going into the bee brood cells. They do not feed off the brood and therefore cannot lay eggs. This probably leads to starvation of the mites, as they are unable to feed effectively off adult bees, with possible disturbance to other metabolic processes of the mites. After about 8 days of treatment with the invention, mites begin to die and accumulate at the bottom of the hive.

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Thus, the invention successfully alters varroa mite behaviour such that they are unable to reproduce or feed, and leads to control of varroa infestations without the use of traps, acaricides or other toxic chemicals.

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The invention is further described below, for the purpose of illustration only, in the following examples.

**Example 1: *Preparation of a Composition comprising cis-8-dodecenyl acetate***

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The starting materials (individual pheromone components) are obtained commercially in a highly pure form. A blend of the components to the prescribed ratio and formula is made up as a dosing solution. This is analysed by chromatographic methods as part of the Quality Assurance procedure prior to dosing the septa.

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**Example 2: *Dispersion Formulations in a dispenser***

A dosing solution will have been determined and verified previously under the Quality Assurance procedure. The individual dispensers are then dosed to the correct loading using accurate micropipette or automated dosing devices. Samples of such dosed dispensers are examined and analysed afterwards against the known specification. Provided the specification is met the dispensers are released for use.

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All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in the art are intended to be within the scope of the following claims.

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WHAT IS CLAIMED IS:

1. A composition for the treatment of varroa mite infestations in honeybee colonies, comprising an effective amount of at least one compound selected from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate and n-octadecyl acetate.  
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2. A composition according to Claim 1, consisting essentially of cis-8-dodecenyl acetate.  
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3. A composition according to claim 2, comprising about 85% by volume of cis-8-dodecenyl acetate.
4. A dispenser for the controlled release of semiochemicals to the locus of a bee colony, said dispenser containing and releasing a composition according to any preceding claim.  
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5. A dispenser according to claim 4, which is capable of delivering an effective amount of the composition over a period of at least two months.  
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6. A dispenser according to claim 4 or claim 5, containing 0.1-15mg of the composition.
7. A dispenser according to claim 6, containing 1-10 mg of the composition.  
25
8. A dispenser according to claim 7, containing 1-5 mg of the composition.
9. A method for the treatment of varroa mite infestations in colonies of honeybees, comprising the application of an effective amount at a composition comprising least one compound selected from the group consisting of: cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, methyl palmitate and n-octadecyl acetate of to the locus of the honeybee colonies.  
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10. A method according to claim 9, wherein the concentration of the composition in the environment of the bee colony is kept at an efficacious level over a 2-3month treatment period.

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11. Use of an effective amount of at least one compound selected from the group consisting of cis-8-dodecenyl acetate, 1-dodecanol, n-hexadecyl acetate, n-octadecyl acetate and methyl palmitate for the treatment of varroa mite infestations in honeybee colonies.

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