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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 February 2012 (09.02.2012)

PCT

(10) International Publication Number
WO 2012/018266 A1

(51) International Patent Classification:
A01K 47/06 (2006.01) *A01M 1/20* (2006.01)

(21) International Application Number:
PCT/NZ2011/000151

(22) International Filing Date:
5 August 2011 (05.08.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
587219 5 August 2010 (05.08.2010) NZ

(71) Applicant *(for all designated States except US)*: **THE NEW ZEALAND INSTITUTE FOR PLANT AND FOOD RESEARCH LIMITED** [NZ/NZ]; Mt Albert Research Centre, 120 Mt Albert Road, Mt Albert, 1025 Auckland (NZ).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **PINFOLD, Erin, James** [NZ/NZ]; 13 Chancery Close, 3216 Hamilton (NZ). **GOODWIN, Robert, Mark** [NZ/NZ]; Ruakura Road RD 6, 3286 Hamilton (NZ).

(74) Agents: **FOOTE, Humphrey, C.** et al.; A J Park, Level 22, State Insurance Tower, 1 Willis Street, P.O. Box 949, 6015 Wellington (NZ).

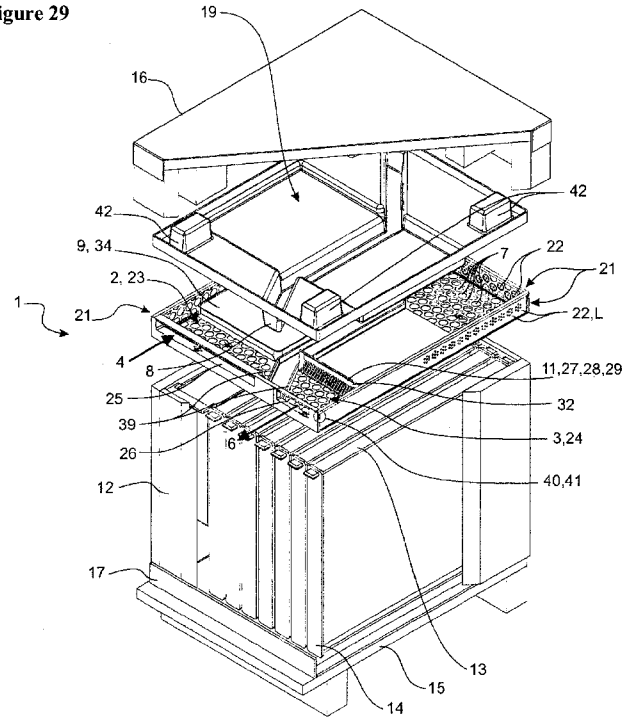
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, 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, QA, 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).

[Continued on next page]

(54) Title: METHOD AND APPARATUS FOR PEST CONTROL

Figure 29



(57) Abstract: The invention provides a method for controlling growth of a pathogen of bees, the method comprising providing a culture of a fungus inside, or proximate to a beehive, wherein the culture is positioned to be contacted by the bees to effect control of growth of the pathogen. The invention also provides for a device locatable at the entrance or exit (or both) of an insect colony. The device comprises an entranceway in communication with a passageway extending to first openings into an insect nest, and an exitway in communication with the passageway extending from the first openings or second openings out of the nest. Interposed between the entranceway and exitway, and the first openings or second openings, is a region receivable or supportable of an inoculum. The invention also provides the method for controlling growth of a pathogen of bees wherein the culture is provided in the device.

WO 2012/018266 A1

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))*

METHOD AND APPARATUS FOR PEST CONTROL

TECHNICAL FIELD

The present invention relates to methods and apparatus for controlling parasites on bees.

BACKGROUND ART

Honey bees, *Apis mellifera*, produce US$270 million worth of honey, bee's wax and other hive products in the US per year. In addition honey bees are also required for the effective pollination of crops, and are therefore critical to world agriculture. Therefore maintaining a supply of strong honey bee colonies is essential for the sustained production of crops, which are worth more than US$14 billion in the United States alone.

Honey bees are however susceptible to a number of parasites including the ectoparasitic Varroa mite, *Varroa destructor*. The Varroa mite was originally confined to the Asian honey bee, *Apis cerana*, but it has spread in recent decades to the European honey bee, *Apis mellifera*. Varroa mites are transferred to new bee colonies on adult bees. Varroa is now established in most bee keeping regions of the world and has become the world's most important pest of bees. The varroa bee mite is reducing the number of bees in feral or wild colonies in as well managed hives, heightening the pollination problems.

Varroa is reported to be present in the following countries with the date of introduction shown in brackets: Japan, USSR (early 1960's); Eastern Europe (1960s-1970s); Brazil (1971); South America (late 1970s); France (1982); Switzerland, Spain, Italy (1984); Portugal (1987); USA (1987); Canada (1989); England (1992); New Zealand (North Island) (2000); New Zealand (South Island) (2006); and Hawaiian Islands (2007).

Varroosis is an infestation caused by varroa, and can be devastating to bee populations. The mites feed on both adult bees and brood, weakening them and spreading harmful pathogens such as bee viruses. In severely attacked colonies bees may have stunted wings, missing legs or other deformities. Unless urgent action is taken, the vitality of bees in the colony declines until all are dead. Varroa mites can remain undetected for up to two years, by which time it is too late to prevent spread to other hives.

Numerous approaches have been used in attempt to control the growth and spread of Varroa mite.

Chemical measures have included use of commercially-available miticides, such as use of pyrethroid insecticide (Apistan) as strips, and organophosphate insecticide (Coumaphos (Check-mite)) as strips

However such miticides must be applied carefully to minimize the contamination of honey that might be consumed by humans. In addition development of resistance of the mites to such miticides has been reported.

Natural occurring chemicals have also been used such as: essential oils (especially lemon, mint, and thyme oil); sugar esters (Sucrocide) in spray application; oxalic acid in a trickling method or applied as vapor; formic (acid as vapor or pads); and food grade mineral oil (as vapor and in direct application on paper or cords).

Physical or mechanical methods have also been applied. Most of these controls are intended to reduce the mite population to a manageable level, rather than eliminate the mites completely.

For example, many beekeepers use a screened bottom board on their hives. When mites occasionally fall off a bee, they must climb back up to parasitize a new bee. If the beehive has a screened floor with mesh the right size, the mite will fall through and can not return to the beehive. The screened bottom board is also being credited with increased circulation of air which reduces condensation in a hive during the winter. However, studies at Cornell University done over two years found that screened bottoms have no measurable effect at all on reducing mite infestation. (Northeast Beekeeper Vol 1 #1 Jan 2004)

Use of powdered sugar (Dowda Method), talc or other "safe" powders with a grain size between 5 and 15 micrometres sprinkled on the bees has been reported. The powder does not harm the bees but allegedly interferes with the mite's ability to maintain its hold on the bee. It is also believed to increase the bees' grooming behavior. This causes a certain percentage of mites to become dislodged.

Biological control methods have also been applied. For example spores of Metarhizium fungi have been applied to bees and shown to kill varroa mites. However the effectiveness of such treatment in beehive situations has been limited. Metarhizium showed some promise in USA and England in early investigations (Kanga and James 2002, Shaw et al 2002) but later research by James et al (2006) reported variable results.

In spite of all these of attempted control measures the growth and spread of Varroa mite continues, together with associated economic damage. A significant need therefore exists for better treatments, methods and compositions for treating Varroa and other bee pathogens.

It is an object of the invention to provide improved methods and/or apparatus for the control of bee pathogens, and/or to go at least some way towards providing the public with a useful choice.

SUMMARY OF THE INVENTION

Biologican Aspects

1.0 Method

In a first aspect the invention provides a method for controlling growth of a pathogen of bees, the method comprising providing a culture of a fungus inside, or proximate to a beehive, wherein the culture is positioned to be contacted by the bees to effect control of growth of the pathogen.

In a preferred embodiment the culture is provided inside the beehive

2.0 Pathogen

Preferably the pathogen is a mite. Preferably the pathogen is a Varroa mite. Preferred Varroa species include *Varroa destructor* and *Varroa jacobsoni.* A preferred Varroa species is *Varroa destructor.*

3.0 Bees

Preferably the bees are honey bees. Preferred bee species include *Apis mellifera* and *Apis cerana.*

A preferred bee species is *Apis mellifera.*

4.0 Control of pathogen

4.1 Extent of control of growth of the pathogen

Preferably the control of growth of the pathogen, results in death of a proportion of the pathogen population. Preferably the control of growth of the pathogen, results in death of a proportion of the pathogen population in a given time period. Preferably at least 20% of the pathogen population is killed. More preferably at least 40% of the pathogen population is killed. More preferably at least 60% of the pathogen population is killed. More preferably at least 80% of the pathogen population is killed. More preferably at least 90% of the pathogen population is killed. More preferably at least 95% of the pathogen population is killed. Most preferably 100 % of the pathogen population is killed.

5.0 Fungus

5.1 Fungus – spores

Preferably the fungus is a fungus that produces spores. Preferably the spores produced by the fungus control of the growth of the pathogen.

5.2 Fungus – growth

Preferably the fungus grows when inside, or proximate, to a beehive.

5.3 Fungus – growing environment

Preferably the fungus is capable of growing in the conditions typically found in beehives.

Preferably the fungus is capable of growing in the temperature range 20 to 50°C. More preferably the fungus is capable of growing in the temperature range 25 to 45°C. More preferably the fungus is capable of growing in the temperature range 30 to 40°C. More preferably the fungus is capable of growing in the temperature range 32 to 38 °C. More preferably the fungus is capable of growing at about 35°C

Preferably the fungus is capable of growing at a relative humidity of at least 25%. More preferably the fungus is capable of growing at a relative humidity of at least 50%. More preferably the fungus is capable of growing at a relative humidity of at least 70%. More preferably the fungus is capable of growing at a relative humidity of at least 80%. More preferably the fungus is capable of growing at a relative humidity of at least 90%. More preferably the fungus is capable of growing at a relative humidity of about 95%.

5.4 Fungal genera/species

Any suitable fungus capable of controlling growth of the pathogen may be used.

Preferably the fungus is from the phylum Ascomycota. Preferably the fungus is from the family Clavicipitaceae.

Preferably the fungus is entomopathogenic.

Preferably the fungus is from the genus *Metarhizium*.

A preferred *Metarhizium* species is *Metarhizium anisopliae*.

Any suitable strain of fungus capable of controlling growth of the pathogen may be used.

Preferred *Metarhizium anisopliae* strains include A-H as herein described.

A particularly preferred *Metarhizium anisopliae* strain is *Metharhizium ansiopliae* var. *ansiopliae* FI-1045 BC603. A culture of this strain is deposited at National Measurement Institute (formerly AGAL), 1/153 Bertie Street, Port Melbourne, Victoria, Australia 3207, under International Depositry Authority Accession Number V10/0104285).

5.5 Fungal culture

The term "culture" preferably refers to a living colony of the fungus.

The culture may optionally incude a substrate.

The culture may comprise dry fungul spores. The culture may comprise dry fungul spores without a substrate.

The culture typically comprises the fungus and a substrate.

The fungus in the culture may be in the form of spores, mycelium, or a mature spore producing colony. The spores may be dry.

The fungus may be growing on the substrate.

The culture may be a mature spore producing colony.

6.0 Fungal growth substrate

Preferably the growth substrate can not be easily removed by bees. Preferably the culture can not be easily removed by bees.

A preferred substrate comprises rice. A preferred substrate is rice.

7.0 Hive conditions

Preferably the conditions in the hive are conducive to the growth of the fungus.

8.0 Providing the fungal culture

In one embodiment the method includes the step of inserting the culture into the beehive, or position proximate to the beehive.

The culture may be provided by inserting a growing colony of the fungus. The colony may be actively producing spores.

Alternatively the culture may be provide by inserting a substrate, inoculated with the fungus into the beehive, or position proximate to the beehive. The inoculated substrate may not be producing spores when inserted, but may grow to provide the mature, spore producing culture some time after insertion.

In a further embodiment the method includes the step of inserting a substrate, inoculated with the fungus into the beehive, or position proximate to the beehive.

The term "providing a culture" thus includes both

- inserting a spore-producing culture into the beehive, or position proximate to the beehive, and

- inserting an substrate inoculated with the fungus, or spores, into the beehive, or position proximate to the beehive, which will grow and produce spores some time after insertion.

In a preferred embodiment the culture is inserted dry. In this embodiment dry culture may have been produced as follows. The moist substrate is inoculated with fungal spores. The spores optionally germinate. The inoculated substrate, with spores which are optionally germinated, is then dried to prevent growth of the fungus which requires moisture.

The dry culture is then sealed in a substantially water-tight package. The term "substantially water-tight" means that entry of moisture or water into the culture is reduced, or preferably prevented. Preferably the package is completely water-tight. The substantially water-tight package is opened prior to being place into the beehive, or proximate to the beehive. Ambient moisture from the air in the beehive, or proximate to the beehive optionally wets the culture and/or substrate to allow growth of the fungus.

A preferred embodiment of the invention includes the step of inserting a dry culture, produced in this way, into the hive

9.0 Position of the culture in the hive

Preferably the culture is positioned in a separate compartment from the compartment in which the honey is produced.

Preferably the culture is positioned to take advantage of the temperature conditions, within the hive, that are suitable for growth of the fungus. Preferably the culture is positioned to take advantage of the relative humidity conditions, within the hive, that are suitable for growth of the fungus. Preferably the culture is positioned to take advantage of the temperature and relative humidity conditions, within the hive, that are suitable for growth of the fungus.

Preferably the culture is positioned above the bee colony.

Mechanical Aspects

In a second aspect, there is provided a device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony, the device comprising:

an entranceway into the device, the entranceway in communication with a first passageway extending to one or more first openings (or inlets) into a nest for insects, and

an exitway from the device, the exitway in communication with a second passageway extending from one or more second openings (or outlets) out of the nest for the insects,

wherein the first and second passageways are separated (or separable) from each other by a divider (or a barrier), and

interposed (or interposable) between the entranceway and the first opening (or inlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum, and

wherein interposed (or interposable) between the second opening (or outlet) and exitway is/are a directionally openable gate or gates, the gate(s) permitting of insects exiting from the second passageway to the exitway of the device.

In a third aspect, there is provided a device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony (preferably a beehive or beehive structure), the device supporting an inoculum or a substrate comprising an inoculum, such that, insects (preferably bees) transiting through the device contact or become inoculated or contaminated with the supported inoculum.

In a fourth aspect, there is provided a man-made structure supportive of a nest for an insect colony, the structure comprising a device attached or attachable at, or to, an or the entrance or exit (or both) of the nest of the insect colony, wherein the device comprises:

an entranceway into the device, the entranceway in communication with a first passageway extending to one or more first openings (or inlets) into a nest for insects, and

an exitway from the device, the exitway in communication with a second passageway extending from one or more second openings (or outlets) out of the nest for the insects,

wherein the first and second passageways are separated (or separable) from each other by a divider (or a barrier), and

interposed (or interposable) between the entranceway and the first opening (or inlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum, and

wherein interposed (or interposable) between the second opening (or outlet) and exitway is/are a directionally openable gates or gates, the gate(s) permitting of insects exiting from the second passageway to the exitway of the device.

Preferably, the one or more first openings is/are apertures in flooring of the first passageway, and the one or more second openings is/are apertures in flooring of the second passageway. More preferably, the apertures are sized to allow insects to pass therethrough.

Preferably, at least a part of the first passageway includes at least a constriction within which the region is located (or locatable). Even more preferably, the constriction substantially provides a crawl-space (or substantially a no-fly zone) for insects passing through the region. Most preferably, the constriction encourages insects to substantially crawl through the region (or substantially prevents flying), thereby contacting inoculum when in-situ.

In one alternative, preferably at least a part of the region (optionally being an or the inoculation region) is positioned within a (or the) constriction of the first passageway, the constriction being at least a reduced height between the passageway's flooring and ceiling relative to the height of the remainder of the first passageway. More preferably, the constriction is a first passageway height constriction.

In a second alternative, preferably the passageway surrounding the region (optionally being an or the inoculation region) is of a reduced height between flooring and ceiling of the first passageway. More preferably, the passageway's reduced height provides a crawl-space (or substantially a no-fly zone) for insects passing through the region. Most preferably, the reduced height encourages insects to substantially crawl through the region (or substantially prevents flying), thereby contacting inoculum when in-situ.

In a third alternative, preferably the constriction is provided by i) the floor of the region elevated compared with the floor of the remainder of the first passageway, or ii) the ceiling above the region lowered compared to the ceiling of the reminder of the first passageway, or iii) a combination of both i) and ii).

Preferably, the height or constriction between the flooring and ceiling about the region in the first passageway is less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm, preferably is about 9 mm, or may even be 8 mm.

Preferably, at least a portion of the second passageway is of a reduced height between flooring and ceiling. More preferably, a substantial length of the second passageway has the reduced height. Even more preferably, the reduced height is such that, in use, insects of the colony are discouraged from forming, building or nesting in the second passageway.

Preferably, the height between the flooring and ceiling in the second passageway is less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, preferably is about 9 mm, or may even be 8 mm.

Preferably, one or more external facing side walls of the device include a porous portion or portions. More preferably, the porous portion(s) are porous to light or air (or other gases) or both. Even more preferably, one or more pores in the side wall or walls form the porous portion(s). Most preferably, the pores are sized such that, in use, insects of the nest are substantially prevented from (or substantially unable to) pass therethrough. Yet more preferably, the pores are perforations in the one or more external facing side walls.

Preferably, the porous portion or portions is/are of external side walls of the second passageway. More preferably, the porous portion or portions are of side wall or walls located near, adjacent or about the one or more second openings. Even more preferably, the porous portions extend along the side walls of the second passageway

More preferably, the apertures in the side walls allow light (e.g. sunlight) to pass therethrough or are ventilators for ventilation of gases from the nest. Even more preferably, the apertures in the external facing side wall or walls are sized such that, in use, insects of the nest are substantially prevented from (or substantially unable to) pass therethrough. Even more preferably, the light transmissive portions or the apertures in the external facing side wall or walls are of external side walls of the second passageway.

Preferably, the entranceway and exitway are located adjacent one another. More preferably, the entranceway and exitway are located on the same side (or face) of the device.

Preferably, the entranceway has a larger mouth into the first passageway than the mouth provided from the second passageway at the exitway. More preferably, the entranceway mouth has a greater width than the mouth of the exitway. Even more preferably, the entranceway mouth has a height greater than the height of the exitway mouth.

Preferably, the entranceway mouth includes a lip. More preferably, the lip is a raised platform positioned above the floor of the entranceway mouth. Even more preferably, the lip is a landing zone for insects arriving at the entranceway mouth. Most preferably, the lip is located adjacent the outer-most edge of the entranceway mouth.

Preferably, at least a part of the entranceway mouth includes one or more visual attributes attractive to the insects approaching the device from outside of the nest. More preferably, at least a part of the entranceway mouth is coloured, or emits a colour, that is attractive to insects of the nest when outside of the nest. Even more preferably, the at least part of the

entranceway mouth is of a dark colour or appearance. Most preferably, the at least part of the entranceway mouth is a darker colour or appearance than the colour of the exitway mouth.

Preferably, at least a part of the entranceway mouth is a black colour.

Preferably, the visual attributes or colouring is displayed on external facing parts of the entranceway mouth.

Preferably, the visual attributes or colouring is provided by a transfer appliable to the entranceway mouth or at least a part or parts of the entranceway mouth.

Preferably, the exitway mouth includes a raised wall portion extending upwards from the floor of the exitway mouth, the wall portion providing for a display surface of one or more visual attributes.

Preferably, at least a part of the exitwayway mouth includes one or more visual attributes attractive to the insects approaching the mouth from inside of the nest (or from within the second passageway). More preferably, at least a part of the exitway mouth is coloured, or emits a colour, that is less attractive to insects of the nest when outside of the nest. Even more preferably, the at least part of the exitway mouth is of a light colour or appearance. Most preferably, the at least part of the exitway mouth is a lighter colour or appearance than the colour of the entranceway mouth.

Preferably, at least a part of the exitway mouth is a white or yellow colour.

Preferably, the visual attributes or colouring is displayed on both internal and external facing parts of the exitway mouth.

Preferably, the visual attributes or colouring is provided by a transfer appliable to the exitway mouth or at least a part or parts of the exitway mouth.

Preferably, the directionally openable gate or gates is a one-way gating system. More preferably, the directionally openable gate or gates allow nest insects to pass through the gate(s) in a substantially first direction. Even more preferably, the gate(s) allows for insects to pass through in the first direction from the second passageway to the exitway mouth. Most preferably, the gate(s) deter nest insects from passing through the gate(s) in a substantially second direction from the exitway mouth to the second opening(s).

Preferably, the one-way gating system is a one-way curtain. More preferably, the one-way curtain is locatable to substantially span the width of the second passageway and interposed between the second openings and exitway mouth. Even more preferably, the curtain comprises a series of flaps moveable from a closed gate position to an open gate position under influence of an insect, the flaps returnable from the open position to (or towards) the closed position under their own power without influence of the insect on the flaps. Most preferably, the flaps are moveable about a hinge or hinges along an edge of the flaps.

Yet more preferably, the flaps are a series of substantially vertically hanging flaps. Yet even more preferably, a block is locatable to be positioned adjacent to and at the second opening(s) side of the curtain. Yet most preferably, the block comprises one or more apertures sized to allow insects of the nest to pass therethrough.

Preferably, the flaps are spaced or dimensioned to hang at least partially obscuring the block's one or more apertures when in the closed position. More preferably, the flaps are formed of a plastics film or material, optionally being substantially light transmissive. Even more preferably, the block is formed of a substantially light transmissive material, optionally being a plastics material, such as transparent thermoplastic acrylic resins, for example polymethylmethacrylate.

Preferably, the block is dimensioned such that it substantially spans the whole of the width and height at its location within the second passageway. More preferably, the curtain is attached to the block.

Preferably, the inoculum or substrate comprising of inoculum is provided via a cartridge locatable within the region. More preferably, the cartridge is replaceable with another cartridge or is re-chargeable with fresh inoculum. Even more preferably, the cartridge is a tray receivable or loadable with the inoculum or the substrate comprising of inoculum.

Preferably, the inoculum is a fungus as described herein.

Preferably, the substrate is as described herein.

Preferably, the device is a unitary item.

Preferably, two or more of the devices are stackable atop one another.

Preferably, the device is collapsible or foldable, optionally collapsible or foldable into a flat-pack format. More preferably, parts of the device which are collapsible or foldable comprise of inter-connectable or engageable sections for connecting and engaging with one another.

Alternatively, preferably the device is constructed or constructible from a multiple parts.

Preferably, the device is an assembly constructed from or constructible from a base component, a lid component, and an internal component or components, each of the base, lid and internal components engageable with at least one other component for constructing the device,

wherein the base component is constructed or constructible to form at least each of the floors for the respective first and second passageways, the region receivable or supportable of inoculum or a substrate comprising of inoculum, the first and second openings of the passageways, the entranceway, the exitway, and external side walls for the device, and

wherein the lid component is constructed or constructible to form at least each of the ceilings for the respective first and second passageways, and the divider (or barrier) for separating the first and second passageways from each other, and

wherein the internal component(s) is constructed or constructible to form at least the directionally openable gate or gates to be locatable within the second passageway to be or so constructed.

Preferably, the base component(s) is a first sub-assembly, the lid component(s) is a second sub-assembly, and the internal component(s) is a third sub-assembly, the first and second and third sub-assemblies assemblable for forming the device. More preferably, the first and second sub-assemblies sandwich the third sub-assembly (or the internal components).

Preferably, corresponding parts of flooring and ceiling forming the second passageway include locators for locating the directionally openable gate or gates (hereinafter "gate locators"). More preferably, the flooring and ceiling locators are receivable of at least a part of directionally openable gate(s) for locating the gate(s) in a pre-determined gate position. Even more preferably, the locators and directionally openable gate(s) have respectively engageable parts. Most preferably, the respectively engageable parts are male and female (or female and male) shaped parts.

Preferably, the flooring includes a locator or locators for locating the divider (or barrier) between the first and second passageways (hereinafter "divider locator(s)"). More preferably, the flooring locator(s) is/are receivable of at least a part of the divider (or barrier) for locating the divider (or barrier) in a pre-determined divider (or barrier) position. Even more preferably, the locators and divider (or barrier) have respectively engageable parts. Most preferably, the respectively engageable parts are male and female (or female and male) shaped parts.

In an alternative, the divider (or barrier) is an integral part of the lid component. Preferably, the lid component is an integrally moulded article.

In an alternative, at least one of the external side walls of the device is a separate discrete part, such a part connectable to a respective edge of flooring of the base component and adjacent side wall(s), or the edge(s) of the entranceway and exitway. In a further alternative, one or more (or all) of the external side walls are integral parts of the base component, the side walls hingedly connected to the flooring.

In an alternative, the entranceway mouth and exitway mouth is a separate discrete part, such a part connectable to the entranceway and exitway side face or flooring edge of the base component. In a further alternative, the entranceway mouth and exitway mouth is an integral part of the base component, and hingedly connected to the flooring.

Preferably, the lid component further comprises a support or supports for supporting of a roof above the device or nest, or both. More preferably, the support or supports is/are a riser or risers for holding the roof in-situ.

Preferably, wherein internal surfaces of the flooring or external side walls or ceiling, or combinations of these, comprise of an embossed, or in-moulded, pattern. More preferably, the embossed or in-moulded, pattern is a series of regularly spaced apart hexagonal.

Preferably, the device is insertable at, or to, the entrance or the exit (or both) of a nest of an insect colony.

Preferably, the device is locatable above the nest. More preferably, at least the region (optionally an inoculation region), is locatable above the nest.

Preferably the device is located more than half-way up the nest, the device positioned where environmental conditions are more favourable or beneficial to allow growth of the inoculum.

Preferably, the insect colony is/are a colony of bees.

Preferably, the nest is a beehive or beehive structure.

Preferably, the man-made structure is a beehive structure. More preferably, the beehive structure is of the type comprising at least one super within which are located (or locatable) one or a series of top bar supported frames, the super seated on a floorboard and topped by a lid or roof, an opening into the structure provided between a edge of the super and the floorboard.

Preferably, the device or at least a part of the device is one or a combination of wood, cellulose-fibre composites, cardboard, polymers such as for example, thermoplastics or thermosetting polymers. More preferably, wherein the is selected from one of: polycarbonate (PC), polystyrene (PS), general purpose polystyrene (GPPS), polymethyl methacrylate (PMMA), thermoplastic (poly) urethane (TPU), polyethylene terephthalate (PET), polyester methacrylate (PEM), polypropylene (PP), high impact polystyrene (HIPS), acrylonitrile butadiene styrene (ABS), polyester (PES), polyamides (PA), poly(vinyl chloride) (PVC), polyurethanes (PU), polyvinylidene chloride (PVDC), polyethylene (PE), polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK) (polyetherketone), polyetherimide (PEI), polylactic acid (PLA), high impact polystyrene, aquilobutalstyrene, nylons, acrylics, amorphous polymers, polyethylene (PE), polyethylene terephthalate (PET), low density polyethylene (LDPE), low low density polyethylene (LLDPE), thermoplastic ethylene (TPE), polypropylene (PP), rubbers, phenolics and the like.

Preferably, the device or at least a part of the device is extrusion formed (extruded) or injection moulded.

Preferably the inoculum is a fungus as described herein.

Preferably the substrate is as described herein.

Package of separate apparatus components

In a fifth aspect, there is provided a package comprising:

one or more base components as previously defined,

one or more lid components as previously defined, and

one or more internal components as previously defined,

the base, lid and internal components constructible as the device defined in any one of the second, third or fouth aspects, and

one or more cartridges, the cartridges receivable of inoculum or substrate comprising inouclum, cartridges inoculatable with inoculum or the substrate on-site or prior to use within the device.

Preferably the inoculum is a fungus as described herein.

Preferably the substrate is as described herein.

Method of installation of apparatus

In a sixth aspect, there is provided a method for installing or attaching at, or to, an or the entrance or exit (or both) of a man-made structure supporting a nest of an insect colony a device, the device comprising an entranceway into the device, the entranceway in communication with a first passageway extending to one or more first openings (or inlets) into a nest for insects, and an exitway from the device, the exitway in communication with a second passageway extending from one or more second openings (or outlets) out of the nest for the insects, wherein the first and second passageways are separated (or separable) from each other by a divider (or a barrier), and interposed (or interposable) between the entranceway and the first opening (or inlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum, and wherein interposed (or interposable) between the second opening (or outlet) and exitway is/are a directionally openable gate or gates, the gate(s) permitting of insects exiting from the second passageway to the exitway of the device, the method comprising the steps of:

positioning the device relative the man-made structure and nest such that the respective first and second openings of the first and second passageways are communicable with the inside of the nest, and

blocking entrances to and exits from the nest, such that access to and from the nest by the nest's insects is/are via the entranceway and exitway of the device.

Preferably, the method further comprising locating the device more than half way up the man-made structure or nest within the structure.

Preferably, the method further comprising locating the device at the top of the man-made structure or above the top of the nest within the structure.

Preferably, the method further comprising locating the device at where conditions are more favourable or beneficial for allowing or facilitating growth of the inoculum. More preferably, locating the device where the environmental conditions adjacent the device, or at least the region, comprises of temperatures in the range 20 to 50 °C, more preferably in the range 25 to 45 °C, more preferably in the range 30 to 40 °C, more preferably in the range 32 to 38 °C. More preferably the temperature is about 35°C.

More preferably, the device is located where the environmental conditions adjacent the device, or at least the region, comprises relative humidity of at least 25 %, more preferably at least 50%, preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and more preferably about 95 %.

Additional embodiment – tray design

In a sixth aspect, there is provided a device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony, the device comprising:

an entranceway into the device, the entranceway in communication with a passageway extending to one or more first openings (or inlets) into a nest for insects, and

an exitway from the device, the exitway in communication with the passageway extending from the one or more openings or one or more second opening (or outlet) out of the nest for the insects,

wherein interposed (or interposable) between the entranceway and exitway, and the first opening (or inlet) or second opening (or outlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum.

Preferably, the one or more first (or second, or both first and second) openings is/are apertures in flooring of the passageway. More preferably, the apertures are sized to allow insects to pass therethrough.

Preferably, at least a part of the passageway includes at least a constriction within which the region is located (or locatable). Even more preferably, the constriction substantially provides

a crawl-space (or substantially a no-fly zone) for insects passing through the region. Most preferably, the constriction encourages insects to substantially crawl through the region (or substantially prevents flying), thereby contacting inoculum when in-situ.

Preferably, the constriction may comprise of a side wall or walls of the passageway shaped or positioned to channel or restrict the width of the passageway. More preferably, such side wall or walls assist in encouraging insectis to substantially i) provides a crawl-space (or substantially a no-fly zone) for insects passing through the region, or ii) encourage insects to substantially crawl through the region (or substantially prevents flying), or both i) and ii), thereby contacting inoculum when in-situ.

In one alternative, preferably at least a part of the region (optionally being an or the inoculation region) is positioned within a (or the) constriction of the passageway, the constriction being at least a reduced height between the passageway's flooring and ceiling relative to the height of the remainder of the passageway. More preferably, the constriction is a passageway height constriction.

In a second alternative, preferably the passageway surrounding the region (optionally being an or the inoculation region) is of a reduced height between flooring and ceiling of the passageway. More preferably, the passageway's reduced height provides a crawl-space (or substantially a no-fly zone) for insects passing through the region. Most preferably, the reduced height encourages insects to substantially crawl through the region (or substantially prevents flying), thereby contacting inoculum when in-situ.

In a third alternative, preferably the constriction is provided by i) the floor of the region elevated compared with the floor of the remainder of the first passageway, or ii) the ceiling above the region lowered compared to the ceiling of the reminder of the passageway, or iii) a combination of both i) and ii).

Preferably, the height or constriction between the flooring and ceiling about the region in the passageway is less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm, preferably is about 9 mm, or may even be 8 mm.

Preferably, the reduced height is such that, in use, insects of the colony are discouraged from forming, building or nesting in the passageway.

Preferably, the entranceway and exitway are located adjacent one another, or are a communal space. More preferably, the entranceway and exitway are located on the same side (or face) of the device.

Preferably, the entranceway mouth or exitway mouth (or both) includes a lip. More preferably, the lip is a raised platform positioned above the floor of the entranceway mouth or exitway mouth. Even more preferably, the lip is a landing zone for insects arriving at the entranceway mouth or departing from the exitway mouth.

Preferably, at least a part of the entranceway mouth includes one or more visual attributes attractive to the insects approaching the device from outside of the nest. More preferably, at least a part of the entranceway mouth is coloured, or emits a colour, that is attractive to insects of the nest when outside of the nest. Even more preferably, the at least part of the entranceway mouth is of a dark colour or appearance. Most preferably, the at least part of the entranceway mouth is a darker colour or appearance than the colour of the exitway mouth.

Preferably, at least a part of the entranceway mouth is a black colour.

Preferably, the visual attributes or colouring is displayed on external facing parts of the entranceway mouth.

Preferably, the visual attributes or colouring is provided by a transfer appliable to the entranceway mouth or at least a part or parts of the entranceway mouth.

Preferably, the exitway mouth includes a raised wall portion extending upwards from the floor of the exitway mouth, the wall portion providing for a display surface of one or more visual attributes.

Preferably, at least a part of the exitwayway mouth includes one or more visual attributes attractive to the insects approaching the mouth from inside of the nest (or from within the second passageway). More preferably, at least a part of the exitway mouth is coloured, or emits a colour, that is less attractive to insects of the nest when outside of the nest. Even more preferably, the at least part of the exitway mouth is of a light colour or appearance. Most preferably, the at least part of the exitway mouth is a lighter colour or appearance than the colour of the entranceway mouth.

Preferably, at least a part of the exitway mouth is a white or yellow colour.

Preferably, the visual attributes or colouring is displayed on both internal and external facing parts of the exitway mouth.

Preferably, the visual attributes or colouring is provided by a transfer appliable to the exitway mouth or at least a part or parts of the exitway mouth.

Preferably, the inoculum or substrate comprising of inoculum is provided via a cartridge locatable within the region. More preferably, the cartridge is replaceable with another cartridge or is re-chargeable with fresh inoculum. Even more preferably, the cartridge is a tray receivable or loadable with the inoculum or the substrate comprising of inoculum.

Preferably, the inoculum is a fungus as described herein.

Preferably, the substrate is as described herein.

Preferably, the device is a unitary item.

Preferably, two or more of the devices are stackable atop one another.

Preferably, the device is collapsible or foldable, optionally collapsible or foldable into a flat-pack format. More preferably, parts of the device which are collapsible or foldable comprise of inter-connectable or engageable sections for connecting and engaging with one another.

Alternatively, preferably the device is constructed or constructible from a multiple parts.

Preferably, the device is an assembly constructed from or constructible from a base component, a lid component, and an internal component or components, each of the base, lid and internal components engageable with at least one other component for constructing the device,

wherein the base component is constructed or constructible to form at least the floor for the passageway, the region receivable or supportable of inoculum or a substrate comprising of inoculum, the first or second, or both first and second openings of the passageway, the entranceway, the exitway, and external side walls for the device, and

wherein the lid component is constructed or constructible to form at least each of the ceilings for the passageway, and

wherein the internal component(s) is constructed or constructible to form at least the constriction within the passageway to be or so constructed.

Preferably, the base component(s) is a first sub-assembly, the lid component(s) is a second sub-assembly, and the internal component(s) is a third sub-assembly, the first and second and third sub-assemblies assemblable for forming the device. More preferably, the first and second sub-assemblies sandwich the third sub-assembly (or the internal components).

Preferably, the lid component is an integrally moulded article.

In an alternative, at least one of the external side walls of the device is a separate discrete part, such a part connectable to a respective edge of flooring of the base component and adjacent side wall(s), or the edge(s) of the entranceway and exitway. In a further alternative, one or more (or all) of the external side walls are integral parts of the base component, the side walls hingedly connected to the flooring.

In an alternative, the entranceway mouth and exitway mouth is a separate discrete part, such a part connectable to the entranceway and exitway side face or flooring edge of the base component. In a further alternative, the entranceway mouth and exitway mouth is an integral part of the base component, and hingedly connected to the flooring.

Preferably, the lid component further comprises a support or supports for supporting of a roof above the device or nest, or both. More preferably, the support or supports is/are a riser or risers for holding the roof in-situ.

Preferably, wherein internal surfaces of the flooring or external side walls or ceiling, or combinations of these, comprise of an embossed, or in-moulded, pattern. More preferably, the embossed or in-moulded, pattern is a series of regularly spaced apart hexagonal.

Preferably, the device is insertable at, or to, the entrance or the exit (or both) of a nest of an insect colony.

Preferably, the device is locatable above the nest. More preferably, at least the region (optionally an inoculation region), is locatable above the nest.

Preferably the device is located more than half-way up the nest, the device positioned where environmental conditions are more favourable or beneficial to allow growth of the inoculum.

Preferably, the insect colony is/are a colony of bees.

Preferably, the nest is a beehive or beehive structure.

Preferably, the man-made structure is a beehive structure. More preferably, the beehive structure is of the type comprising at least one super within which are located (or locatable) one or a series of top bar supported frames, the super seated on a floorboard and topped by a lid or roof, an opening into the structure provided between a edge of the super and the floorboard.

Preferably, the device or at least a part of the device is one or a combination of wood, cellulose-fibre composites, cardboard, polymers such as for example, thermoplastics or thermosetting polymers. More preferably, wherein the is selected from one of: polycarbonate (PC), polystyrene (PS), general purpose polystyrene (GPPS), polymethyl methacrylate (PMMA), thermoplastic (poly) urethane (TPU), polyethylene terephthalate (PET), polyester methacrylate (PEM), polypropylene (PP), high impact polystyrene (HIPS), acrylonitrile butadiene styrene (ABS), polyester (PES), polyamides (PA), poly(vinyl chloride) (PVC), polyurethanes (PU), polyvinylidene chloride (PVDC), polyethylene (PE), polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK) (polyetherketone), polyetherimide (PEI), polylactic acid (PLA), high impact polystyrene, aquilobutalstyrene, nylons, acrylics, amorphous polymers, polyethylene (PE), polyethylene terephthalate (PET), low density polyethylene (LDPE), low low density polyethylene (LLDPE), thermoplastic ethylene (TPE), polypropylene (PP), rubbers, phenolics and the like.

Preferably, the device or at least a part of the device is extrusion formed (extruded) or injection moulded.

Preferably the inoculum is a fungus as described herein.

Preferably the substrate is as described herein.

Additional embdoimemt - Package of separate apparatus components – simplified design

In a seventh aspect, there is provided a package comprising:

one or more base components as previously defined in the sixth aspect,

one or more lid components as previously defined in the sixth aspect, and

one or more internal components as previously defined in the sixth aspect,

the base, lid and internal components constructible as the device defined in the sixth aspect, and

one or more cartridges, the cartridges receivable of inoculum or substrate comprising inouclum, cartridges inoculatable with inoculum or the substrate on-site or prior to use within the device.

Preferably the inoculum is a fungus as described herein.

Preferably the substrate is as described herein.

Additional embdoimemt - Method of installation of apparatus– simplified design

In an eighth aspect, there is provided a method for installing or attaching at, or to, an or the entrance or exit (or both) of a man-made structure supporting a nest of an insect colony a device, the device comprising an entranceway into the device, the entranceway in communication with a passageway extending to one or more first openings (or inlets) into a nest for insects, and an exitway from the device, the exitway in communication with the passageway extending from the one or more openings or one or more second opening (or outlet) out of the nest for the insects, wherein interposed (or interposable) between the entranceway and exitway, and the first opening (or inlet) or second opening (or outlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum, the method comprising the steps of:

positioning the device relative the man-made structure and nest such that the respective first or second (or both) openings of the passageway is/are communicable with the inside of the nest, and

blocking entrances to and exits from the nest, such that access to and from the nest by the nest's insects is/are via the entranceway and exitway of the device.

Preferably, the method further comprising locating the device more than half way up the man-made structure or nest within the structure.

Preferably, the method further comprising locating the device at the top of the man-made structure or above the top of the nest within the structure.

Preferably, the method further comprising locating the device at where conditions are more favourable or beneficial for allowing or facilitating growth of the inoculum. More preferably,

locating the device where the environmental conditions adjacent the device, or at least the region, comprises of temperatures in the range 20 to 50 °C, more preferably in the range 25 to 45 °C, more preferably in the range 30 to 40 °C, more preferably in the range 32 to 38 °C. More preferably the temperature is about 35°C.

More preferably, the device is located where the environmental conditions adjacent the device, or at least the region, comprises relative humidity of at least 25 %, more preferably at least 50%, preferably at least 70%, more preferably at least 80%, more preferably at least 90%, and more preferably about 95 %.

Combined Biological and Mechanical Aspects

In a preferred embodiment of the method of the invention, the culture is provided in the device of the invention, positioned inside the beehive. Preferably the culture is provided in the region of the device.

DETAILED DESCRIPTION

In this specification where reference has been made to patent specifications, other external documents, or other sources of information, this is generally for the purpose of providing a context for discussing the features of the invention. Unless specifically stated otherwise, reference to such external documents is not to be construed as an admission that such documents, or such sources of information, in any jurisdiction, are prior art, or form part of the common general knowledge in the art.

Definitions

The term "comprising" as used in this specification means "consisting at least in part of". When interpreting each statement in this specification that includes the term "comprising", features other than that or those prefaced by the term may also be present. Related terms such as "comprise" and "comprises" are to be interpreted in the same manner.

Detailed description of biological aspects

The present invention provides a method for controlling a parasite of bees, particularly controlling growth of Varroa mite. The present invention involves growing a fungus, which controls growth of the pathogen, into or close to a beehive. In a preferred embodiment, the fungus is grown inside the beehive and produces spores to control growth of the parasite.

Although fungal spores have previously been used to control bee parasites, the success of this approach has been limited, in part due to the bees active removal of such spores from the hive. The present invention takes advantage of the temperature and humidity conditions in the hive to grow the fungus, on a suitable substrate which cannot be easily removed by the bees, and produce a continous supply of spores to control growth of the parasite.

1.0 Method

In a first aspect the invention provides a method for of controlling growth of pathogen of bees, the method comprising providing a culture of a fungus inside or proximate to a beehive, wherein the culture is positioned to be contacted by the bees to effect control of growth of the pathogen.

In a preferred embodiment the culture is provided inside the beehive

2.0 Pathogen

Preferably the pathogen is a mite. Preferably the pathogen is a Varroa mite. Preferred Varroa species include *Varroa destructor* and *Varroa jacobsoni*. A preferred Varroa species is *Varroa destructor*.

3.0 Bees

Preferably the bees are honey bees. Preferred bee species include *Apis mellifera* and *Apis cerana*.

A preferred bee species is *Apis mellifera*.

4.0 Control of pathogen

4.1 Extent of control of growth of the pathogen

Preferably the control of growth of the pathogen, results in death of a proportion of the pathogen population in a given period. Preferably at least 20% of the pathogen population is killed. More preferably at least 40% of the pathogen population is killed. More preferably at least 60% of the pathogen population is killed. More preferably at least 80% of the pathogen population is killed. More preferably at least 90% of the pathogen population is killed. More preferably at least 95% of the pathogen population is killed. Most preferably 100 % of the pathogen population is killed.

5.0 Fungus

5.1 Fungus – spores

Preferably the fungus is a fungus that produces spores. Preferably the spores produced by the fungus control of the growth of the pathogen.

The term "spores" as used in this specification includes conidia, conidiospores and mitospores. The term typically refers to the asexual non-motile spores of a fungus.

5.2 Fungus – growth

Preferably the fungus grows when inside, or proximate, to a beehive.

The term "grows" refers to either growth of the fungal myclellium or production of spores, or both.

5.3 Fungus – growing environment

Preferably the fungus is capable of growing in the conditions typically found in beehives.

Preferably the fungus is capable of growing in the temperature range 20 to 50°C. More preferably the fungus is capable of growing in the temperature range 25 to 45°C. More preferably the fungus is capable of growing in the temperature range 30 to 40°C. More preferably the fungus is capable of growing in the temperature range 32 to 38 °C. More preferably the fungus is capable of growing at about 35°C

Preferably the fungus is capable of growing at a relative humidity of at least 25%. More preferably the fungus is capable of growing at a relative humidity of at least 50%. More preferably the fungus is capable of growing at a relative humidity of at least 70%. More preferably the fungus is capable of growing at a relative humidity of at least 80%. More preferably the fungus is capable of growing at a relative humidity of at least 90%. More preferably the fungus is capable of growing at a relative humidity of about 95%.

5.3.1 Fungus – growth rate

Preferably the strain of fungus used in the method is suitably fast growing strain, under the conditions described above. Preferably the fungus when provided innoculated onto the substrate, will spread quickly to form an established spore producing culture. Preferably the

fungus is capable of substantially out-competing other organisms that might colonise the substrate in the non-sterile environment of the hive.

5.4 Fungal genera/species

Any suitable fungus capable of controlling growth of the pathogen may be used.

Preferably the fungus is from the phylum Ascomycota. Preferably the fungus is from the family Clavicipitaceae. Preferably the fungus is entomopathogenic.

Preferably the fungus is from the genus *Metarhizium*. Preferred *Metarhizium* species include *M. anisopliae* (that includes many isolates previously described as *M. anisopliae var. anisopliae*), *M. guizhouense* (syn. *M. taii*), *M. pingshaense*, *M. acridum* stat. nov. (= *M. anisopliae var. acridum*), *M. lepidiotae* stat. nov. (= *M. anisopliae var. lepidiotae*), *M. majus* stat. nov. (= *M. anisopliae var. major*), *M. globosum* sp. nov., *M. robertsii* sp. nov. , *M. brunneum*, *M. album*, *M. flavoviride*, and *M. frigidum*.

A preferred *Metarhizium* species is *Metarhizium anisopliae*.

Any suitable strain capable of controlling growth of the pathogen may be used.

Preferred *Metarhizium anisopliae* strains include A-H as herein defined.

A particularly preferred *Metarhizium anisopliae* strain is Metharhizium ansiopliae var. ansiopliae FI-1045 BC603. A culture of this strain is deposited at National Management Institute (formerly AGAL), 1/153 Bertie Street, Port Melbourne, Victoria, Australia 3207, under International Depositry Authority Accession Number V10/0104285).

5.5 Fungal culture

The term "culture" preferably refers to a living colony of the fungus. The fungus is typically growing on a substrate. Thus the culture typically comprises the fungus and a substrate.

The fungus in the culture may be in the form of spores, mycelium, or a mature spore producing colony. Preferably the fungus is a mature spore-producing colony.

Methods for handling and culturing fungi are known in the art and are described for example in Dorta *et al.*, 1998, Enzyme and Microbial Technology, 23: 501-505.

6.0 Fungal growth substrate

Preferably the fungus is provided on a growth substrate. Preferably the growth substrate can not be easily removed by bees.

In one embodiment the substrate is artificial. Suitable artificial growth substrates include but are not limited to nutrient impregnated membranes, Cray granules, and Vermiculite.

In an alternative embodiment the substrate is plant-based. Suitable plant-based substrates include but are not limited to barley, millet, sorghum and rice.

Preferably the substrate is plant-based. Preferably the substrate is rice. Preferably the substrate is long grain white rice.

6.1 Supplements to fungal growth substrate

The substrate may be supplemented with at least one antimicrobial to prevent, or slow, the growth of competing organisms. Suitable antimicrobials include antibiotics.

A preferred antimicrobial is an antibiotic, such as streptomycin or chloramphenicol. A preferred antibiotic is chloramphenicol. A suitable concentration is 0.2g/l.

6.2 Condition of fungal growth substrate

Preferably the substrate is sterilised prior to being inoculated with the fungus. Preferably the substrate is autoclaved prior to being inoculated with the fungus. Preferably the substrate is moist prior to being inoculated with the fungus. In one embodiment the substrate is moist as a result of being autoclaved.

7.0 Hive conditions

Preferably the conditions in the hive are conducive to the growth of the fungus.

Preferably the temperature in the hive is in the range 20 to 50 °C. More preferably the temperature in the hive is in the range 25 to 45 °C. More preferably the temperature in the hive is in the range 30 to 40 °C. More preferably the temperature in the hive is in the range 32 to 38 °C. More preferably the temperature in the hive is about 35°C.

Preferably the relative humidity in the hive is at least 25 %. More preferably the relative humidity in the hive is at least 50%. More preferably the relative humidity in the hive is at least 70%. More preferably the relative humidity in the hive is at least 80%. More preferably the relative humidity in the hive is at least 90%. More preferably the relative humidity in the hive is about 95 %.

8.0 Providing the fungal culture

In one embodiment the method includes the step of inserting the culture into the beehive, or position proximate to the beehive.

The culture may be provided by inserting a growing colony of the fungus. The colony may be actively producing spores.

Alternatively the culture may be provide by inserting a substrate, inoculated with the fungus into the beehive, or position proximate to the beehive. The inoculated substrate may not be producing spores when inserted, but may grow to provide the mature, spore producing culture some time after insertion.

In a further embodiment the method includes the step of inserting a substrate, inoculated with the fungus into the beehive, or position proximate to the beehive.

The term "providing a culture" thus includes both

- inserting a spore-producing culture into the beehive, or position proximate to the beehive, and
- inserting an substrate inoculated with the fungus, or spores, into the beehive, or position proximate to the beehive, which will grow and produce spores some time after insertion.

In a preferred embodiment the culture is provided dry. In this embodiment dry culture may have been produced as follows. The moist substrate is inoculated with fungal spores. The spores optionally germinate. The inoculated substrate, with spores, is then dried to prevent growth of the fungus which requires moisture.

The dry culture is then sealed in a substantially water-tight package. The substantially water-tight package is opened prior to being place into the beehive, or proximate to the beehive.

Ambient moisture from the air in the beehive, or proximate to the beehive wets the substrate to allow growth of the fungus

9.0 Position of the culture in the hive

Preferably the culture is positioned in a separate compartment from the compartment in which the honey is produced.

Preferably the culture is positioned to take advantage of the temperature conditions, within the hive, that are suitable for growth of the fungus. Preferably the culture is positioned to take advantage of the relative humidity conditions, within the hive, that are suitable for growth of the fungus. Preferably the culture is positioned to take advantage of the temperature and relative humidity conditions, within the hive, that are suitable for growth of the fungus.

Preferably the culture is positioned above the bee colony.

Detailed description of mechanical aspects

Preferred embodiments of the invention will now be described with reference to figures 29 to 47.

This invention recognises significant advantages in providing a device 1 suitable for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony. In particular, a device 1 capable of directing or allowing entry for the insects of the colony into the nest via a first passageway 2, and capable of directing or allowing exit for the insects of the colony from the nest via a second passageway 3. The device provides for a system of contact between an agent, for example an inoculum, and insects passing through the device. Further, this invention contemplates the inoculation of insects passing through the first passageway 2 and transporting of the inoculants (inoculum) into the nest of the insect colony. Most advantageously, the colony of insects is/are bees and the nest is a beehive or a man-made structure within which the bees have constructed or are constructing a hive.

In one embodiment there is provided a device 1 for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony. The device 1 comprises of an entranceway 4 into the device 1. The entranceway 4 communicating or being in communication with a first passageway 2 that extends to one or more first openings (or inlets) 5 into a nest (not shown) for insects (for example bees, B). Also provided is an exitway 6 from the device 1, the exitway 6 communicating or being in communication with a second passageway3 extending

from one or more second openings (or outlets) 7 out of the nest for the insects. The first and second passageways 2, 3 are separated (or separable) from each other by a divider (or a barrier) 8, and interposed (or interposable) between the entranceway 4 and the first opening(s) (or inlet(s)) 5 is a region (optionally an inoculation region) 9. The region 9 is receivable or supportable of inoculum or a substrate comprising of inoculum 10. Interposed (or interposable) between the second opening(s) (or outlet(s)) 7 and exitway 6 is/are a directionally openable gate or gates 11. The gate(s) 11 permitting or controlling of the direction of movement, or exiting direction, of insects from or through the second passageway 3 to the exitway 6 of the device 1.

In a second embodiment there is provided a device 1 for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony (preferably a beehive or beehive structure), the device 1 supporting an inoculum or a substrate comprising an inoculum 10, such that, insects (preferably bees) transiting through the device 1 contact or become inoculated or contaminated with the supported inoculum.

In a third embodiment there is provided a man-made structure (such as that a beehive within which bees make a nest) supportive of a nest for an insect colony, the structure comprising a device 1 attached or attachable at, or to, an or the entrance or exit (or both) of the nest of the insect colony. Such a device 1 comprises of an entranceway 4 into the device 1, the entranceway 5 communicating, or in communication, with a first passageway 2 extending to one or more first openings (or inlets) 5 into a nest for insects. The device 1 also comprises of an exitway 6 from the device 1, the exitway 6 communicating, or in communication, with a second passageway 3 extending from one or more second openings (or outlets) 7 out of the nest for the insects. The first and second passageways 2, 3 are separated (or separable) from each other by a divider (or a barrier) 8. And, interposed (or interposable) between the entranceway 4 and the first opening(s) (or inlet(s)) 5 is a region (optionally an inoculation region) 9. The region 9 receivable or supportable of inoculum or a substrate comprising of inoculum. Interposed (or interposable) between the second opening(s) (or outlet(s)) 7 and exitway 6 is/are a directionally openable gate or gates 11. The gate(s) 11 permitting or controlling of the direction of movement, or exiting direction, of insects from or through the second passageway 3 to the exitway 6 of the device 1.

In a fourth embodiment there is provided a package comprising one or more base components, one or more lid components, and one or more internal components, the base, lid and internal components are constructible together as the device 1 as defined in any one of the first, second

or third embodiments described above. The package further comprising of one or more cartridges receivable of inoculum or substrate comprising inoculum, the cartridges inoculatable with inoculum on-site or prior to use within the device 1.

In a fifth embodiment there is provided a method for installing or attaching at, or to, an or the entrance or exit (or both) of a man-made structure (such as a beehive) supporting a nest of an insect colony (such as bees) a device 1. The device 1 comprises of an entranceway 4 into the device 1. The entranceway 4 communicating, or being in communication, with a first passageway 2 extending to one or more first openings (or inlets) 5 into a nest for insects. The device further comprises of an exitway 6 from the device 1. The exitway 6 communicating, or being in communication, with a second passageway 3 extending from one or more second openings (or outlets) 7 out of the nest for the insects. The first and second passageways 2, 3 are separated (or separable) from each other by a divider (or a barrier) 8. And, interposed (or interposable) between the entranceway 4 and the first opening(s) (or inlet(s)) is a region (optionally an inoculation region) 9. The region 9 is receivable or supportable of inoculum or a substrate comprising of inoculums. Further, interposed (or interposable) between the second opening(s) (or outlet(s)) 7 and exitway 6 is/are a directionally openable gate or gates 11. The gate(s) 11 permitting or controlling of the direction of movement, or exiting direction, of insects from or through the second passageway 3 to the exitway 6 of the device 1. Such that the method comprises the steps of positioning the device 1 relative the man-made structure and nest such that the respective first and second opening or openings 5, 7 of the first and second passageways 2, 3 are communicating, or communicable, with the inside of the nest (preferably the internal of the man-made structure). The method further comprising the steps of blocking entrances to and exits from the nest, such that access to and from the nest by the nest's insects is/are via the entranceway 4 and exitway 6 of the device 1.

For example, as shown in figures 29-31, the device 1 is installable as a part of the man-made structure of a beehive. Man-made structures for beehives may vary somewhat across the industry. One form of a beehive structure is that illustrated in figure 29, being of the type comprising supers 12 within which are located (or locatable) a series of top bars 13 supporting or supportable of frames 14, the super seated on a floorboard 15 and topped by a lid or roof 16, an opening 17 into the structure provided between a edge of the or a super 12 and the or a floorboard 15.

Figure 29 illustrates the layout and configuration an embodiment of the device 1 relative to a man-made structure, such as the beehive shown. The device is seated or positioned at the top

of the beehive, taking advantage of the environmental conditions generated by the insect nest. The view is partially exploded and partially cut-away to illustrate various features of the invention.

The device can be installed at a variety of positions relative to the man-made structure and insect's nest; however it is contemplated that positioning of the device 1 more than half-way up the man-made structure or nest provides most favourable conditions. Of course, the device can be located at the top of the man-made structure or above the top of the or each nest within the structure. Advantageously, positioning of the device 1 is determined to be those where conditions are more favourable or beneficial for allowing or facilitating growth of the inoculums when provided in region 1.

Turning back to the device 1, as is more clearly shown by figures 32, 33 and 38-40, there one or more first openings (or inlets) 5 is/are apertures in the flooring 18 of the first passageway 2. Likewise, the one or more second openings (or outlets) 7 is/are apertures in flooring 18 of the second passageway 3. Figures 32 and 33 illustrate one embodiment in which single larger sized apertures are provided in the flooring 18 for allowing insects (e.g. bees) to enter or exit their nest (e.g. hive for bees) via respective inlets and outlets.

Figures 38-40 illustrate an alternative embodiment in which a plurality of smaller sized apertures are provided in the flooring 18. In each embodiment, the aperture(s) provided are sized, at minimum, to allow an insect of the nest (to which the device 1 is attachable) to pass therethrough. The apertures may be sized according to the dimensions of the insects which the device 1 is to be employed. In this manner, larger sized insects can be prevented from entering the nest via the openings of the device 1.

Where multiple apertures are provided, they can be spaced adjacent or apart from each other. Advantageously, there is provided a larger second opening (or outlet) 7 or greater number of individual second openings (or outlets) 7 compared to the size or number of first openings (or inlets) 5.

At least a part of the first passageway 2 includes a constriction within which the region 9 is located (or locatable). The constriction substantially provides for a crawl-space (or substantially a no-fly zone) for the nest's insects passing through the region 9. In this manner, the constriction encourages insects to crawl through the region 9 (or the constriction substantially prevents flying by the nest's insects), the insects thereby coming in to contact

with inoculum when in-situ in the region 9. At least a part of the region 9 which is receivable of inoculum or a substrate comprising inoculum is positioned within a (or the) constriction of the first passageway 1 such that the nest's insects passing through the constricted part of the first passageway come into physical contact with the inoculum.

In one example, the constriction is a reduction in the height between the passageway's flooring 18 and its ceiling 20 relative to the height of the remainder of the first passageway 2.

In another example, the passageway 2 surrounding the region 9 is of a reduced height between flooring and ceiling of the first passageway. In yet a further example, constriction can provided by i) the floor 18 of the region 9 being of an elevated position compared with the floor 18 of the remainder of the first passageway 2, or ii) the ceiling 20 above the region 9 is lower (or lowered) compared to the ceiling 20 of the reminder of the first passageway 2, or iii) a combination of both i) and ii).

The height or constricted gap (i.e. internal gap within the passageway) between the flooring 18 and ceiling 20 about the region 9 in the first passageway 2 is, in various embodiments, less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm. Advantageously however the height is about a 9 mm gap, but may also be an 8 mm gap. It is considered such a distance encourages a majority of bees to be prevented (or discouraged) from flying through the region 9, and instead to crawl through the region 9. It is considered a "majority" means more than about 50% of the insect population passing through the region 9. A "majority" for these purposes means more than about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% of the insects passing through the region 9.

Most advantageously however, at least a part of the first passageway 2 surrounding the region 9 includes a constriction for encouraging the insects to come into contact with inoculum when in-situ. In this manner, the insects contacting the inoculum become inoculated.

In a similar manner, at least a portion of the second passageway 3 is of a reduced height (i.e. internal gap within passageway) between flooring 18 and ceiling 20. However, a greater length of the second passageway 3 compared to the constriction of the first passageway may have such a reduced height or constriction. In various embodiments, the height between the flooring 18 and ceiling 20 in the second passageway 3 may be less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm. Advantageously the height is about a 9 mm gap, or may be an 8 mm gap. The reduced height or constriction is however utilized for the

purposes of discouraging (or preventing) a majority of insects passing through the second passageway 3 of the colony from forming, building or nesting in the second passageway 3. It is considered a "majority" means more than about 50% of the insect population passing through the second passageway 3. A "majority" for these purposes means more than about 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% of the insects passing through the region 9.

In one particularly preferred embodiment, the physical height of the device 1 (Z) is about 48 mm, while the length from the entranceway/exitway to the rearmost facing side wall is about 505 mm long, and the width (i.e. from side wall to side wall) is about 405 mm.

In addition, one or more external facing side walls 21 of the device 1 include a porous portion or portions 22. Such porous portion(s) 22 allow light (e.g. sunlight) or gas(es) (e.g. air) or both to transmit from the surrounds of the device into the passageways 2, 3. As the passageways 2, 3 are in communication with the nest via the openings 5, 7; the porous portions allow transmission of gases (for example as ventilation) between the nest and surrounding environment. In one embodiment, the porous portions 22 are one or more pores in the side wall or walls 21. Such pores are sized such that, in use, insects of the nest are substantially prevented from (or substantially unable to) pass therethrough, however light or air (or other gases) or both are able to do so. As shown in the drawings, it will be appreciated the pores can be a series of perforations or apertures in one or more of the external facing side walls 21.

In one particularly preferred embodiment, for example as shown by figure 40, the porous portion or portions 22 are along a length L of an external side wall of the second passageway 3. Locating such a portion or portions along the length L near, adjacent or about the one or more second openings 7 allows for transmission of light from outside of the device into a area near of the second passageway 3. In this manner, it is understood the light allowed into the second passageway 3 helps to encourage insects (e.g. bees) exiting the nest to exit via the second openings 7 and into the second passageway 3. Further, resulting from this encouragement the insects may then sight or be drawn to the exitway 6.

The first passageway 2 on the other hand may not be provided with porous portions in its external facing side walls 21. In this manner, the insects (e.g. bees) are encouraged or drawn further into the first passageway 2 and toward the first openings 5 and the nest.

As shown in the accompany drawings, the entranceway 4 and exitway 6 are advantageously located adjacent one another, and located on the same side (or face) of the device 1.

In one embodiment the entranceway 4 has a larger mouth 23 into the first passageway 2 than the mouth 24 provided from the second passageway 3 at the exitway 6. In other configurations, the entranceway mouth 23 has a greater width than the exitway mouth 24, or the entranceway mouth 23 can have a height greater than the height of the exitway mouth 24.

As illustrated by figure 39, the entranceway mouth preferentially includes a lip 5. The lip 5 is for example a form of a raised platform positioned generally above the floor 18 of the entranceway mouth 23. The lip 25 can be used as a landing zone for flying insects arriving at the entranceway mouth 23, in this manner therefore the lip is located adjacent an outer-most edge of the entranceway mouth 23.

Encouraging of discouraging of the insects into the preferred passageway 2, 3 of the device 1 may be influence by a variety of factors. At least a part of the entranceway mouth 23 can therefore include one or more visual attributes attractive to the insects approaching the device 1 from outside of the nest. For example, at least a part of the entranceway mouth can be coloured, or can emit a colour, that is attractive to insects of the nest when outside of the nest (of the colour is indicative to the insects of the entrance into their nest). For this reason at least part of the entranceway mouth 23 is of a dark colour or appearance, or darker colour or appearance than the colour of the exitway mouth 24.

In one particular example, the entranceway mouth 23 is coloured black. Such a visual attribute or colouring is displayed on external facing parts of the entranceway mouth 23 (i.e. on display to insects approaching the device). A transfer, such as an adhesive sticker or other covering can be placed over parts of the entranceway mouth 23 or about the mouth 23. Alternatively, the entranceway mouth 23 or surrounding surfaces may be formed of a suitably coloured material.

Similarly, surfaces about the exitway mouth 24 can be provided with one or more other visual attributes attractive to the insects approaching the mouth from inside of the nest (or from within the second passageway 3). For example, at least a part of the exitway mouth 24 can be coloured, or emits a colour, that is less attractive to insects outside of the nest or is indicative of a pathway to outside of the nest. It is understood for bees that a light colour or appearance is indicative of a way out of a nest. Therefore, at least part of the exitway mouth 24 can be of

a lighter colour or appearance than the colour of the entranceway mouth to provide such an indication. Colouring the exitway mouth or parts of the second passageway 3 in, for example, white or yellow, may produce the desired encouragement for bees. Such visual attributes or colouring can be displayed on both internal and external facing parts of the exitway mouth. As with the entranceway 4, the colours can be via transfers such as adhesive stickers or the relevant or desired parts can be formed of materials having such colouring or properties.

Yet further, the exitway mouth 24 can include a raised wall portion 26 extending upwards from the floor 18 of the exitway mouth 24, the wall portion providing for a display surface of the one or more visual attributes.

The directionally openable gate or gates 11 can also be formed of suitable materials that allow the insects to observe the visual attributes (for example, the gates 11 may be formed of suitable coloured materials or are coloured as such, or may be substantially transparent or light transmissive allowing the insects to observe the colours or light at or near at the exitway mouth 24.

The directionally openable gate or gates are preferably a one-way gating system. Such gates or system allow nest insects to pass through the gate(s) in a substantially first direction (towards the exitway mouth 24). The gate(s) or system is used to deter nest insects (or other insects) from entering or passing through the gate(s) in a direction from the exitway mouth 25 toward the second openings 7.

One such example of the one-way gating system is a one-way curtain 27 (such as that illustrated by figures 36, 37). Such a one-way may be curtain 27 is locatable to substantially span the width of the second passageway 3 and be interposed between the second openings 7 and exitway mouth 25. The curtain 27 comprises a series of flaps 28 moveable from a closed gate position (position 29) to an open gate position (position 30) under influence of or from an insect. The flaps 28 are returnable from the open position 30 to (or towards) the closed position 29 under their own power without influence of the insect on the flaps 28 further. The flaps 28 are optionally moveable about a hinge or hinges 31 along an edge of the flaps 28.

As shown in figures 36, 37 the flaps 28 can be a series of substantially vertically hanging flaps. The flap 28 are attached via the hinges 31 to a block 32 that is itself locatable to be positioned adjacent to and at the second opening(s) side of the curtain 27. Such a block 32

acts as a frame for the flaps 28 and optionally comprises one or more block apertures 33 sized for allowing insects of the nest to pass therethrough.

It will be appreciated the flaps can be spaced apart from each other or dimensioned to hang at least partially obscuring the block's one or more apertures 33 when in their closed position 29.

The flaps 28 can be formed of a variety of materials, for example including plastic film or similar light-weight materials. The flaps 28 are additionally optionally of a light transparent or transmissive material. The block itself can be optionally formed of light transparent or transmissive materials. The block may be formed of suitably transparent thermoplastic acrylic resins, for example polymethylmethacrylate.

It will be appreciated the block 32 (or gate(s) 11) is/are dimensioned such that substantially the whole of the width and height of the second passageway 3 is spanned.

The inoculum or substrate comprising of inoculum can be provided for insertion or placement at the region 9 in a variety of forms. One such embodiment is a cartridge 34, such as a portable tray, that is locatable or seatable within the region 9. Such a cartridge 34 is a suitable carrier or support for a substrate comprising the inoculum or the inoculum in whatever form it may be provided. Portability is a particularly useful characteristic as the cartridge 34 can then be readily replaceable with another cartridge or is able to be removed and re-charged with fresh inoculums or fresh substrate comprising of the inoculum.

In one example, the cartridge 34 is a tray receivable or loadable with the inoculum or the substrate comprising of inoculum. The cartridge 34 can be loaded or seated into position within the region 9. It will be appreciated the region 9 may comprise of locating walls or other arrangements for locating of the cartridge in position within the first passageway 2.

In one form, the device of this invention can be constructed and provided as a substantially unitary item, for example with all features integral (apart from the replaceable cartridge which is portable, being removable and receivable in the region 9). Such a device can be formed allowing one device to be stacked atop of another device for transport or storage purposes (the devices may be considered to be nestable with each other). In such an embodiment, the device 1 can be of a collapsible or foldable or flat-pack format. The parts of the device which are collapsible or foldable or flat-packable comprise of inter-connectable or engageable

sections for connecting and engaging with one another when constructed into the operational form of the device.

In another form, the device of this invention is constructible from a multiple parts. For example, the device 1 can be provided in multiple parts for assembly or constructed from or constructible from a base component 35, a lid component 36, and an internal component or components 37. Each of the base 35, lid 36 and internal components 36 are engageable with at least one other of these components in construction of the device 1.

Figures 38-40 illustrate details of a base component 35. As shown, the base component 35 is constructed or constructible to form at least each of the floors 18 for the respective first and second passageways 2, 3, the region 9 that is receivable or supportable of inoculum or a substrate comprising of inoculums (for example a cartridge carrying the inoculum or substrate with inoculums), the first and second openings 5, 7 in the passageways, the entranceway 4, the exitway 6, and external side walls 21 for the device. The base component 35 forms a first sub-assembly.

Figures 42 and 43 illustrate details of a lid component 36. As shown, the lid component 36 is constructed or constructible to form at least each of the ceilings 20 for the first and second passageways 2, 3, having a divider (or barrier) (optionally integrally formed with the lid component) for separating the first and second passageways 2, 3 from each other. In an alternative however, the divider (or barrier) may be a separate component forming one of the internal components, such a divider (or barrier) being locatable between the flooring 18 and ceiling 20 and positionable between the passageways 2, 3. Such a separate barrier (or divider) can be inserted into a recess of the flooring 18 and ceiling 20 for purposes of positioning.

Figure 36 and 37 illustrate details of an internal component 37. As shown, at least one internal component 37 is constructed or constructible to form the directionally openable gate or gates 11 to be locatable within the second passageway 3. In an alternative however, the internal component 37 can include the separate divider (or barrier) as described above.

It will be appreciated that the base component(s) 35 provides for a first sub-assembly, the lid component(s) 37 provides for a second sub-assembly, and the internal component(s) 37 provides for a third sub-assembly, each of the sub-assemblies engageable with another sub-assembly or a part thereof. The sub-assemblies can be assembled for forming the device. As

shown, for example in figures 29 and 44, the first and second sub-assemblies sandwich the third sub-assembly (or the internal components).

As the device 1 can be an assembly of different components (e.g. the lid component, the base component and the internal components), there can be provided a package 43 comprising each of these components in a ready-to-ship format. Such a package can provide replacement parts, spares or all of the the necessary parts for constructing the device 1. In addition, such a package can include one or more cartridges 34 into which the inoculum or a substrate comprising the inoculums can be placed or charged. Alternatively, the cartridge 34 may comprise of a suitable substrate to which the inoculum can charged or is inoculatable with inoculum on-site or prior to use within region 9 of the device 1.

Figure 47 illustrates one form of a package 43 in which five base components 35, five lid components 36, five sets of internal components 36 and ten cartridges 34 (eight of which are shown, two of which are not shown) are provided together. Such a package 43 can be prepared for shipping or transport. It will however be appreciated that figure 47 illustrates only one example of a packaged arrangement. Different numbers of parts can be included in a package depending on the needs or demands of a user or customer. Usefully however, figures 45-47 illustrate the nestability and stabability of each of the components for ease of storage and transport. Significant cost benefits result from such flat-pack formats and nestabilities.

Where the device 1 is provided in separate components (i.e. as a series of separate sub-assemblies) there can be corresponding parts of flooring 18 and ceiling 20 forming the second passageway 3 that include locators for locating the directionally openable gate or gates (hereinafter "gate locators" 38), and divider locators (hereinafter "divider locators" 39).

The gate locators 38 may be in the form of a slot receivable and supportable of the block 32 or another part of the gates 11 in the second passageway 3. A similar gate locator 38 can be provided in the ceiling of the second passageway 3 to receive and support the upper edge of the block 32 or another upper part of the gates 11. The gate locators 38 are receivable of at least a part of directionally openable gate(s) for locating the gate(s) in a pre-determined gate position within the second passageway 3. The gate locators 38 and directionally openable gate(s) can be provided with respectively engageable parts, for example respectively engageable male and female shaped parts engageable with the block 32 or gates 11.

The divider locators 39 may be in the form of a channel or recess receivable and supportable of the divider (provided either as an integral part of the lid component or as a separate component as part of the internal components 37 or third sub-assembly). See for example figure 44 showing the arrangement of the gates 11 in the second passageway 3 and divider 8 positioned and located by the divider locator 39 in the form of a channel.

The flooring 18 can include the divider locators 39 for locating the divider (or barrier) 8 in a suitable position between the first and second passageways. The divider locators 39 can be provided either (or both) on the flooring 18 and ceiling 20 and be receivable or engageable with at a part of the divider (or barrier) 8. In this manner, the divider locators 39 help to position and locate the divider (or barrier) 8 in a pre-determined divider (or barrier) position between the passageways 2, 3. Such divider locators 39 may be provided with respectively engageable parts, for example respectively engageable parts are male and female shaped parts engageable with parts of the divider 8.

For ease of manufacturing or construction purposes, a preferable arrangement can be that where the divider (or barrier) 8 is formed an integral part of (e.g. integrally moulded with) the lid component 36. See for example figure 43.

In a alternative form, at least one of the external side walls 21 of the device 1 can be provided as separate discrete parts, such parts connectable to respective edges of flooring 18 of the base component 35 and respective adjacent side wall(s), or the edge(s) of the entranceway 4 and exitway 6. In this manner, the base component 35 is formed by assembling together a number of separate parts. However, in a preferred embodiment, one or more (or all) of the external side walls 21 can be integral parts of the base component 35, the side walls 21 being hingedly connected to the flooring 18. Figure 41 provides an enlarged view of a hinged type arrangement between side walls 21 and flooring 18. Such parts can be formed by plastic moulding processes. The side walls 21 can include engageable parts or portions that engage or clip together with adjacent side walls 21, see for example the protrusions 40 extending from an edge of the side walls 21 in figures 38 and 46. Such protrusions 40 are slottable or engagingly receivable within correspondingly alignable recesses or slots 41of adjacent side walls 21, see for example figures 38 and 46. In this manner, the side walls 21 with hinged sections can be provided in a flat-pack or folded format, then raised and engaged with their adjacent side walls 21 into an operational construction of the device 1. Figure 38 illustrates the base component 35 in a flat-pack format with side walls 21 disengaged from one another.

Figures 39 and 40 illustrate the base component 35 in a constructed mode (excluding the directionally openable gates 11) where side walls 21 are engaged with adjacent side walls.

As with the side walls 21, the entranceway and exitway can be provided as a separate discrete part which is connectable to the flooring 18 or adjacent side walls 21. Alternatively however, such a part is an integral part of the base component, connected to the flooring 18 via a hinged section, such as that illustrated in figure 41. A similar mode of connection to adjacent side walls 21 is provided, as described above, for the entranceway and exitway from a flat-pack format into a constructed operational mode. Again, compare flat-pack mode of figures 38 and 46 with constructed mode of figure 40.

In yet a further option, the lid component 36 can include a support or supports 42 (see figures 42-45) for supporting of a roof 16 above the device 1 or nest, or both. Such supports 42 are in the form of one or more risers (optionally risers at each corner of the top surface of the lid component 36) for holding or supporting the roof 16 of a man-made structure in-situ above the device 1.

The internal surfaces of the flooring 18 or external side walls 21 or ceiling 20, or combinations of these, can be smooth surfaces or may be of an embossed or in-moulded pattern, type. For example, one such embossed or in-moulded pattern may be a series of regularly spaced apart circles or hexagonal as generally indicated in the figures.

As shown by figure 29, the device 1 is insertable at, or to, the entrance or the exit (or both) of a nest of an insect colony, for example a beehive. Generally, it is preferred that the device 1 be locatable above the nest. In particular, it is particularly advantageous if at least the region 9 (optionally an inoculation region) is locatable above the nest.

In other configurations, the device can be located more than half-way up the nest, most preferably positioned such that environmental conditions are more favourable or beneficial to allow growth of the inoculums which can be placed within the region 9.

The device can be formed of a number of different materials. However those that may be most preferable at those that are capable of enduring the environmental conditions surrounding the nest and insect colony. In addition, where the insect colony is a food producing colony, the materials chosen may need to comply with suitable food grades. Without these specific limitations however, examples of certain materials include a device 1 comprising of various parts formed of one or more of a combination of wood, cellulose-fibre

composites, cardboard, polymers such as for example, thermoplastics or thermosetting polymers. More preferably, wherein the is selected from one of: polycarbonate (PC), polystyrene (PS), general purpose polystyrene (GPPS), polymethyl methacrylate (PMMA), thermoplastic (poly) urethane (TPU), polyethylene terephthalate (PET), polyester methacrylate (PEM), polypropylene (PP), high impact polystyrene (HIPS), acrylonitrile butadiene styrene (ABS), polyester (PES), polyamides (PA), poly(vinyl chloride) (PVC), polyurethanes (PU), polyvinylidene chloride (PVDC), polyethylene (PE), polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK) (polyetherketone), polyetherimide (PEI), polylactic acid (PLA), high impact polystyrene, aquilobutalstyrene, nylons, acrylics, amorphous polymers, polyethylene (PE), polyethylene terephthalate (PET), low density polyethylene (LDPE), low low density polyethylene (LLDPE), thermoplastic ethylene (TPE), polypropylene (PP), rubbers, phenolics and the like.

Preferably, the substrate is as herein described.

Preferably, the inoculum is a fungus as herein described.

In a further alternative embodiment of the invention, there is provided a device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony. Such a device comprising of an entranceway into the device, the entranceway in communication with a passageway extending to one or more first openings (or inlets) into a nest for insects, and an exitway from the device, the exitway in communication with the passageway extending from the one or more openings or one or more second opening (or outlet) out of the nest for the insects. And, interposed (or interposable) between the entranceway and exitway, and the first opening (or inlet) or second opening (or outlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum.

Such a further alternative embodiment is illustrated by figures 48 to 52. As illustrated, this alternative embodiment has a single, communal passageway 100. Apertures 101 are shown, these apertures 101 can be used by insects accessing their nest/hive, or when exiting from their nest/hive.

Ramps 102 provide crawl-in access for the insects to and from the region 103. The region 103 is shown in the figures to include a tray 104 of inoculum.

The internal side walls 105 channel or guide the insects passing through the passageway 100 to enter the region 103, thereby increasing the chance of insects coming into contact with the inoculum when in-situ.

A lid 106 provides for a depressed portion 107 that, when the lid 106 is in place over the passageway 100, further encourages insects passing through the region 100 to crawl, rather than fly. Again, further enhancing the likelihood of the insects coming into conact with the inoculum, when in-situ. The lid optionally includes a series of legs 108 which can be used to structurally support a more substantive or other lid plaed atop of the lid 106. A more substantive or other lid may for example be in the form of lid 109, such as a typical lid of a beeive as illustrated by the figures.

In respect of materials and other features of this alterantive embodiment, see the above description.

The foregoing description of the invention includes preferred forms thereof. Modifications may be made thereto without departing from the scope of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

The present invention will be better understood with reference to the accompanying drawings in which are described as follows:

Figure 1 shows *Metarhizium anisopliae* growing on Difcotm Sabouraud Dextrose Agar

Figure 2 shows a metal cage containing 50 adult honey bees

Figure 3 shows Varroa being transferred into a glass vial.

Figure 4 shows pink-eyed pupae that have been removed from a frame.

Figure 5 shows the average (+ SE) number of dead varroa after treatment with Metarhizium isolates A, B, C.

Figure 6 shows the percentage (+ S.E.) of adult bees surviving after being treated with Metarhizium isolates A, B, and C. The vertical lines are standard error bars.

Figure 7 shows the effect of Metarhizium concentration on varroa mortality after 3, 24, 48 and 72 hours.

Figure 8 shows the proportion of dead varroa after treating with Metarhizium isolates over time.

Figure 9 shows the effect of using icing sugar on mite survival. ■ = varroa removed from cells and placed into icing sugar, ♦ = varroa removed from cells and ▲ = varroa removed from bees using icing sugar. The vertical lines are standard error bars.

Figure 10 shows the effect on varroa survival over 72 hours with and without the presence of pupae and with and without damp filter paper in a bioassay. ■ = Petri dish containing 10 mites with damp filter paper and no pupae ■ = Petri dish containing damp paper, three pupae and ten mites ■ = Petri dish containing 10 mites without damp filter paper or pupae ■ = Petri dish containing three pupae and ten mites without damp filter paper. The vertical lines are standard error bars.

Figure 11 shows the effect of using icing sugar, dead Metarhizium, live Metarhizium or no treatment on mite survival in a bioassay. ■ = Dead Metarhizium ■ = live Metarhizium ■ = icing sugar ■ = no treatment. The vertical lines are standard error bars.

Figure 12 shows Metarhizium being sprinkled across the top bars of a hive.

Figure 13 shows the average number of dead mites on the sticky boards from each treatment before the Apistan® was applied. Control = untreated, dead = hives treated with dead Metarhizium and live = hives treated with live Metarhizium. The vertical lines are standard error bars.

Figure 14 shows the average percentage of varroa in the hives that died during the treatment period. Control = untreated, dead = hives treated with dead Metarhizium and live = hives treated with live Metarhizium. The vertical lines are standard error bars.

Figure 15 shows a Mason jar with a wire mesh lid that prevented bees from escaping containing approximately 300 bees with 10 g of icing sugar being added before being shaken.

Figure 16 shows the effect of total mites killed after Bayvarol® treatment on sugar shake mite levels pre Metarhizium treatments: control (◊), isolate D (◊), and isolate H (◊). Hives 9 (H), 56 (H) and 58 (c) were too weak to sample completely at the post treatment sugar shakes are included.

Figure 17 shows the effect of total mites killed after one week treatment of Bayvarol® treatment on sugar shake mite levels post Metarhizium treatments. Black diamonds ◊ = control. Red diamonds ◊ = Metarhizium isolate D. Green diamonds ◊ = Metarhizium isolate H.

Figure 18 shows the total mites killed after Bayvarol® treatment on sugar shake mite levels post Metarhizium treatments. Black diamonds ◊ represent the control hives. The red diamonds ◊ represent the hives treated with Metarhizium isolate D. The green diamonds ◊ represent Metarhizium isolate H treated hives.

Figure 19 shows a top dry feeder containing Metarhizium growing on white rice raw sugar (Right) and a plastic bag (Left) containing 60% sugar syrup.

Figure 20 shows the effect of the differing amounts of Metarhizium on the percent of varroa killed in honey bee colonies.

Figure 21 shows a dry feeder on top of number five hive containing a Hortplus™ temperature probe and two Petri dishes, one dish containing inoculated white rice (Right) and one inoculated dish of Potato Dextrose Agar fortified with 2 mg chloramplenicol/L (PDA+) (Left).

Figure 22 shows the daily mean (■) temperature for hive five over the 19 days (14/02/2007 – 4/03/2007) of the trial. The vertical bars represent the range of the minimum and maximum temperature.

Figure 23 shows a top dry feeder containing white rice inoculated with Metarhizium (Left) with a bag of 60% sugar syrup and raw sugar (Right) after one week on a honey bee hive.

Figure 24 shows (a) the relationship between the number of frames of bees before (3 April 2007) and after (17 May 2007) treatment and (b) between the number of frames of brood before and after treatment. ♦ represent frames of bees and brood from control colonies and ■ represent frames of bees and brood from Metarhizium colonies.

Figure 25 shows the effect of Metarhizium on the percent of varroa killed in honey bee colonies. ■ Represents colonies that were treated with Metarhizium and ■ represent the control colonies.

Figure 26 shows a purple eyed bee pupae.

Figure 27 shows a Safeseal Microcentrifuge tube (2 ml) Sorenson™.

Figure 28 shows the effect of varroa survival over 72 hours using *Metarhizium* treatment in a bioassay. The vertical lines are standard error lines.

Figure 29 is an exploded view of one embodiment of a device according to the invention in a top installed position above a beehive structure,

Figures 30 and 31 are side views of a beehive structure with the device of the invention in-situ,

Figure 32 illustrates a perspective view of the device according to the invention, exclusive of its ceiling,

Figure 33 illustrates a plan view of the device according to the invention, exclusive of its ceiling,

Figures 34 and 35 are, respectively, front and side views of figure 5,

Figures 36 and 37 are, respectively, perspective and side views of a directionally openable gate according to the invention,

Figures 38, 39 and 40 are, respectively, according to the invention, an unassembled or unconstructed base component in a flat-pack or stackable format, a plan view of an assembled or constructed base component, a perspective view of the assembled or constructed base component,

Figure 41 is an enlarged view of one embodiment of a floor to side wall (or entranceway and exitway face) hinge enabling the flat-pack or stackable format,

Figures 42 and 43 are, respectively, a plan view of the top of a lid component that is engageable with a base component, and a perspective view of that lid component,

Figure 44 illustrates a partially cut—away perspective view of a device according to the invention constructible from assembly of a base component, a lid component and internal components,

Figure 45 illustrates the stackability or nestible capabilities of lid components,

Figure 46 illustrates the stackability or nestible capabilities of base components, and

Figure 47 illustrates a package comprising multiple sets of lid, base and internal components for construction or assembly of a device according to this invention, ready for transport or sale.

Figure 48 is an exploded and partial cross sectional view through a beehive including an embodiment of the present invention.

Figure 49 is a plan view of one embodiment according to the invention.

Figure 50 is a perspective view of the embodiment of the invention as shown in figure 49.

Figure 51 is a partial cross sectional view through an embodiment of the invention as shown in figures 49-50 including a lid/roof riser with corner posts.

Figure 52 is a side view of the lid/roof riser with corner posts as shown in figure 51.

EXAMPLES

The invention will now be illustrated with reference to the following non-limiting example.

EXAMPLE 1: Identification of fungal strains strains that effectively control the growth of Varroa mite.

Culture of strains

Three Metarhizium anisopliae isolates (A, B and C) were sourced from the Landcare Research collection (Landcare Research, 231 Morrin Road, St Johns, Auckland, New Zealand) and five isolates (D–H) were sourced from the AgResearch collection (AgResearch, 5th Floor, Tower Block, Ruakura Research Centre, East Street, Hamilton 3240, New Zealand). Information on the strains is summarized in the table below:

Strain	Source	Strain reference
A	Landcare Research collection	149
B	Landcare Research collection	3095
C	Landcare Research collection	3259
D	AgResearch collection	F 16
E	AgResearch collection	F 36
F	AgResearch collection	F 87

G	AgResearch collection	F 137
H	AgResearch collection	F 151

Cultures A, B and C were cultured on Difcotm Sabouraud Dextrose Agar (Figure 1) and isolates D–H onto Potato Dextrose Agar. The conidia were harvested from isolates by scraping mycelia mats with a sterile spatula.

Effect of Metarhizium on the survival of caged bees

Adult honey bees were collected from a hive and lightly anoxiated with CO_2. Fifty bees were counted into each of 20 metal cages (105 mm x 100 mm x 40 mm) that consisted of mesh on one side and glass on the other. The cages were placed in an incubator at 18-20°C. Honey was provided on the floor of the cage in an upturned lid (Figure 2). Five cages were not treated with Metarhizium and fifteen cages were treated Metarhizium spores. Three isolates (A, B and C) were tested and approximately 10 g of each were placed on the floor of ten cages. The number of dead bees were counted after one hour and then every 12 hours a total of 60 hours.

There was no significant ($P > 0.05$) difference between the number of dead bees in any of the cages from the three isolates or the control cages after 60 hours. The percentage of surviving adults bees were the control at 96% (S.E. 2.3), isolate A 99% (S.E. 0.5), isolate B 99% (S.E. 0.5), and isolate C 98% (S.E. 1.2).

Effect of Metarhizium on varroa

The aim of this trial was to determine if the Metarhizium isolates (A, B and C) would kill varroa.

Varroa were collected from adult bees using the sugar shake method. This consisted of placing adult bees in a container with a mesh lid that would allow varroa to pass through and not the bees. Icing sugar was added and the jar inverted and shaken so the icing sugar and the dislodged varroa fell out of the container. Ten varroa were then transferred using a paint brush into each of 40 glass vials (Figure 3).

Approximately 10 mg of three Metarhizium isolates (A, B and C) were then randomly placed into each of ten of the vials. This totalled 30 vials. An additional ten vials were not inoculated with Metarhizium to serve as controls.

Pink and purple-eyed pupae were extracted from a frame that had been removed from a hive within the preceding hour by removing the cell caps with tweezers, inverting the frame and then banging the edge on a table. The pupae that fell onto the table were then sorted and undamaged ones were selected (Figure 4). Any varroa that were present on the pupae were removed. Three healthy pupae were then placed in each of 40 Petri dishes (35 mm diameter).

The vials with the varroa were then shaken so the Metarhizium and the varroa came in contact. The varroa were then tipped from each jar onto a piece of paper and transferred with a paintbrush to a Petri dish containing pupae. A different paintbrush and a new piece of paper were used for the three isolates and the untreated control varroa. The varroa were not tipped directly into the Petri dish so as to limit the transfer of Metarhizium as much as possible. The Petri dishes were then placed into a 30oC incubator and varroa survival was checked after 15 and 24 h.

The Metarhizium treatments killed significantly more varroa than the controls, as almost all the varroa were killed (Figure 5). This indicates that all three Metarhizium isolates can kill varroa.

Effect of Metarhizium on varroa living on adult honey bees

The purpose of this trial was to determine the effectiveness of Metarhizium against varroa living on adult honey bees.

Adult honey bees were collected from a hive, lightly anoxiated with CO_2 and an average of 299 (S.E. = 6.83) bees were placed in 40 metal cages as described above. These bees were carrying an average of 21.43 (S.E. = 0.49) varroa. The cages were divided among four treatments. The treatments were an untreated control or approximately 5 mg of isolate A, B or C placed on a 90 mm x 20 mm plastic strip covered with double sided sticky tape. The strips hung in the centre of the cages and a bottle of 2 M sugar syrup was inverted in the top. A sugar cube provided additional food. The cages were placed in open paper bags to reduce cross contamination between treatments and to collect the varroa that left the cages.

The cages were placed in an incubator at 25°C for 140 h. They were then removed and the number of dead and live bees and varroa were counted.

There was no significant difference in honey bee survival between treatments (Figure 6). There was also no significant difference in the number of varroa surviving between treatments ($P > 0.05$). Only 28.1% (S.E. = 5.4) of varroa on the untreated bees survived.

These results support the previous cage trial results where Metarhizium had no effect on honey bee survival at the amounts and method of application tested.

The poor survival of varroa in the untreated cages suggested that the bioassay method used was not accurate enough to test varroa survival.

Lack of a significant effect of the treatments on varroa survival may be a function of the bioassay design or the relatively small amounts of Metarhizium used.

Development of a bioassay for the effect of Metarhizium

The purpose of this project was to develop a bioassay to determine the most effective concentration of Metarhizium against varroa.

Phoretic varroa were collected from adult honey bees using a sugar shake method.

Metarhizium from a single isolate (A) was placed into a series of vials then diluted with icing sugar to give a range of 10 concentrations (0–100%). Twenty varroa were place in each vial, which was shaken and allowed to stand for 10 minutes. The varroa were then removed with a paintbrush.

Pink and purple-eyed pupae were extracted from a frame that had been removed from a hive within the preceding hour by removing the cell cappings with tweezers, inverting the frame and then banging the edge on a table to extract the pupae. The pupae that fell onto the table were then sorted and undamaged ones were selected. Any varroa that were present on the pupae were removed (Figure 4). Three healthy pupae were then placed in each of twenty Petri dishes (35 mm diameter).

The varroa from each vial were added to a Petri dish. The Petri dishes were then placed into a 30°C incubator and varroa survival was checked after 3, 24, 48, and 72 hours.

The number of dead varroa increased with time and also with Metarhizium concentration (Figure 7).

Effect of seven Metarhizium isolates on varroa survival

The purpose of this trial was to determine which of the Metarhizium isolates would kill varroa.

Varroa were collected from adult bees using the sugar shake method. The varroa were picked up using a paint brush and 200 were placed in each of ten 70 ml yellow screw-cap specimen jars.

Metarhizium from each isolate (A, B, C, D, E, F, G and H) was placed into a specimen jar and diluted with icing sugar to give a 1% concentration. Two grams of this concentration was then randomly placed into each of the eight specimen jars. The other two specimen jars were controls, of which one had 2 g of icing sugar added and the other one had nothing added.

Pink and purple eyed pupae were removed from a frame that had been taken from a hive within the preceding hour. The pupae were removed by first removing the cell cappings with tweezers, inverting the frame and banging the edge on a table. The pupae then fell onto the table were then sorted and undamaged ones selected. Any varroa that were present on the pupae were removed. Three healthy pupae were then placed in each of 40 Petri dishes (35 mm diameter).

The specimen jars with the varroa were shaken so the Metarhizium or icing sugar and the varroa came in contact. The varroa were then tipped from each jar onto a piece of paper and 20 were transferred with a paintbrush into each of 10 Petri dishes per treatment. A different paint brush and a new piece of paper were used for each isolate, icing sugar and the 'None' control varroa. The varroa were tipped onto paper first rather than directly into the Petri dish to transfer as little of the Metarhizium into the Petri dish as possible. The Petri dishes were then placed into an incubator set at 30oC and varroa survival checked after 24, 48 and 72 hours.

All Metarhizium isolates (except E) and icing sugar treatments in Petri dishes had higher varroa mortality after 72 h than the 'None' control dishes ($P < 0.05$) (Figure 8). None of the Metarhizium isolates significantly ($P > 0.05$) differed from the icing sugar control treatment. As both the untreated varroa and the varroa treated with icing sugar had relatively high mortality this indicated that the bioassay method was not effective. The reason for this was not established.

Effect of icing sugar on varroa survival

The purpose of this trial was to determine if, as indicated from earlier studies, there is an effect of icing sugar on varroa survival in a bioassay.

Varroa were extracted from a frame that had been removed from a hive within the preceding hour by removing the cell caps with tweezers, inverting the frame and then banging the edge on a table. Any varroa that were present on the pupae were removed with a paint brush and placed into twenty glass vials (10 varroa per vial). Ten of the vials had icing sugar added and then shaken so the varroa came in contact with the icing sugar. Phoretic varroa were collected from adult bees using the sugar shake method as described above, ten varroa were added to 10 glass vials. The varroa were then tipped from each jar onto a piece of paper and the varroa transferred with a clean paintbrush to a Petri dish containing three pupae. A different paint brush and a new piece of paper were used for each treatment.

There were significantly less ($P < 0.05$) varroa surviving at each time point, from the treatment where varroa was removed from cells (with icing sugar) than in the treatment where varroa were removed from bees. Up to and including 48h the varroa that were removed from bees using icing sugar had significantly lower mortality than varroa removed directly from cells (without icing sugar).

Each treatment was not significantly ($P > 0.05$) different (Figure 16). Both the treatments where varroa were removed from cells had higher mite mortality than the normal practice of removing varroa from adult bees using the sugar shake method. There was no significant difference (except for 3h) between the mortality of varroa collected from brood with and without icing sugar (Figure 9).

This suggests that using icing sugar to collect varroa does not significantly increase varroa mortality and that collecting varroa off bees provides better survival for use in a bioassay.

Development of a bioassay to increase survival of untreated varroa

The purpose of this investigation was to develop a method to increase the survival of untreated varroa in a bioassay.

Varroa were collected from adult bees using the sugar shake method. Ten varroa were then transferred using a paint brush into each of 40 glass vials.

Pink and purple-eyed pupae were extracted from a frame that had been removed from a hive within the preceding hour by removing the cell caps with tweezers, inverting the frame and then banging the edge on a table. The pupae that fell onto the table were then sorted and undamaged ones were selected. Any varroa that were present on the pupae were removed. Three healthy pupae were then placed in each of 20 Petri dishes (35 mm diameter). Ten of these Petri dishes had damp filter paper (Whatman® size 1 (4.25 cm)) added near the pupae to increase the humidity of the dish. A further twenty Petri dishes had no pupae with ten of these dishes containing damp filter paper folded into eights.

The vials with the varroa were tipped from each jar and transferred with a paintbrush into Petri dish either containing pupae with or without damp filter paper or not containing pupae with or without damp filter paper. The Petri dishes were then placed into a 30oC incubator and varroa survival was checked after 24, 48 and 72 hours.

There were significantly more ($P > 0.05$) varroa deaths at each time during the treatments where there were no pupae present than in the treatment where pupae were present. Both the treatments where there were pupae present there were no significant difference in mite mortality (Figure 10) at each time point. Also the non pupae treatment with damp filter paper had significantly ($P < 0.007$) more varroa deaths at the 24 and 48 hour times than the other non pupae treatment. But there was no significant difference ($P = 0.17$) after 72 hours between the two non pupae treatments.

Effect of Metarhizium on varroa in hives

The purpose of this trial was to determine whether Metarhizium (A) would kill varroa in beehives.

Eighteen colonies housed in single super Langstroth hives were selected and fitted with mite collectors and sticky boards on 23 March 2005. The hives were then randomly divided between three treatments. The treatments were:

1) Two treatments of 5 g of Metarhizium sprinkled across the top bars, eight days apart (Figure 7)

2) Two treatments of 5 g of dead Metarhizium sprinkled across the top bars, eight days apart

3) Control (untreated).

The sticky boards were replaced weekly for six weeks. Apistan® was inserted at the recommended rate in the hives after three weeks.

There was significantly ($P < 0.03$) more dead mites deposited on the sticky boards from the hives treated with live Metarhizium than the untreated control hives (Figure 13).

Although more mites were present on the sticky boards treated with dead Metarhizium, this was not significantly more than the untreated controls ($P = 0.11$).

The live Metarhizium treatment also significantly ($P = 0.017$) increased the percentage of the varroa in the hives that died during the treatment period compared to the untreated colonies (Figure 14). The survival rates of varroa in untreated and dead Metarhizium treatments were not significantly different ($P = 0.25$).

Effect of two Metarhizium isolates on varroa in hives

The purpose of this trial was to determine whether Metarhizium isolates D and H would kill varroa in beehives. Isolates D and H were used because they showed the best results in the previous investigation.

Twenty-four colonies housed in two-depth Langstroth hives were selected. The hives were then randomly divided between three treatments. The treatments were:

1) Two treatments of 5 g of Metarhizium isolate D sprinkled across the top bars, eight days apart

2) Two treatments of 5 g of Metarhizium isolate H sprinkled across the top bars, eight days apart

3) No treatment (control).

The number of phoretic mites in the colonies at the start, before applying the treatments (15 May 2006), before the second application of the isolates (24 May 2006), and on day 16 (1 June 2006) of the trial was estimated using the sugar shake method (Figure 15). This consisted of collecting approximately 300 bees from the brood frames of each colony in a Mason jar with a wire mesh lid that prevented the bees from escaping. Approximately 10 g of icing sugar was then added to each jar and left to stand for approximately 5 minutes. The jars were then inverted and shaken vigorously over a white tray until no further mites were dislodged. All dislodged mites were counted and were return with the bees to their hives.

Each colony had three sugar shakes at each point in time except for three hives which were too weak to sample completely at the post treatment sugar shakes.

On day 16, the hives were fitted with a mesh screen to prevent bees removing mites, a sticky board and Bayvarol® strips were inserted into the hives at the recommended rate. The sticky boards were replaced weekly for three weeks.

During the trial, one colony from the isolate D treatment died and three colonies were too weak to sample completely at the post treatment sugar shakes (day 16), two colonies from treatment H and one from the control could not be sampled.

The number of mites collected by sugar shake is a bee measurement of phoretic mites whereas the total mites on sticky boards (over seven days) from the post Bayvarol® treatment is a hive measurement that includes mites emerging from the brood during this time (Figure 16). There was a correlation between the mean log number of mites by sugar shake (1 June 2006) and the mean log number of mites on the boards after one week of Bayvarol® r = 0.53 (Figure 17). The mean log total number of mites on the boards after three weeks of Bayvarol® had a correlation of 0.56 (Figure 18).

Effect of differing amounts of Metarhizium on varroa survival using top feeders in hives

The purpose of this investigation was to test the effect of differing amounts of Metarhizium on varroa mortality when the Metarhizium was supplied to the hives in a top feeder.

Eleven colonies housed in two-depth Langstroth hives with dry sugar feeders on the top super were selected on 25 October 2006 and fitted with mesh screened mite collectors and sticky boards placed underneath. The sticky boards were replaced weekly for eight weeks. The dry feeders were removed after four weeks and Bayvarol® strips were inserted in the hives at the recommended rate for a further four weeks.

Metarhizium from a single isolate was grown onto Potato Dextrose Agar (PDA) fortified with 0.2 g/L chloramplenicol to reduce contamination from other fungi and bacteria. To produce an inoculum for solid substrate production the conidia from three week old plates were harvested by scraping mycelia mats with a sterile spatula into 0.03% sterile Tween 80. The inoculum was then filtered using a 100 μm BD Falcon™ cell strainer to remove hyphal fragments.

Mushroom spawn bags (205mm x 570mm) containing 300g Sun Rice® Sunwhite Calose Medium Grain Rice and 80 ml deionised water were sterilised by autoclaving at 121°C for 20 minutes. After cooling, the bags of rice were inoculated with 60 ml of inoculum, mixed thoroughly and put into a growth room at 22°C for 10 days. The Metarhizium grown on the solid substrate was diluted with autoclaved white rice and raw sugar (1:1 W:W) to achieve a rate (50, 45, 40, 35, 30, 25, 20, 15, 10, 5 and 0% Metarhizium infected rice). The sugar was added to encourage bee actively in the rice to increase the likelihood of the bees being coated in the Metarhizium and therefore transferring onto the mites. This was then put into the dry feeders (Figure 19) with a Snell-Lock™ re-sealable plastic bag (155 mm x 250 mm) of 60% sugar syrup to further stimulate bees into the feeders. The brand of rice used was different to what had been used previously.

During the trial, the 5% colony died. All the treatments had lower number of varroa on the sticky boards compared to the 0% (natural mite fall) treatment ($P > 0.05$) (Figure 20). However the percentage of varroa that died during the 0% (natural mite fall) colony was higher than would normally be expected and the reason is unknown. These results do however demonstrate that applying differing amounts of Metarhizium in top feeders as carried out in this trial does not result in effective control of varroa.

Effect of growth and survival of a Metarhizium isolate in dry feeders

The purpose of this trial was to investigate whether temperature and white rice brand (Budget®) effected the survival and growth of Metarhizium in honey bee colonies.

Ten colonies housed in two-depth Langstroth hives were selected on 14 February 2007. Dry sugar feeders were placed over mesh on the top super to prevent access by bees. The feeders were covered with black plastic to increase the humidity and to promote the growth of the Metarhizium. A Hortplus™ temperature probe was placed into each dry feeder to record the temperature every hour over the time of the investigation. Also included in each feeder were two Petri dishes, one which contained inoculated Budget® long grain white rice and one containing Potato Dextrose Agar fortified with 2 mg chloramplenicol/L (PDA+) (Figure 21).

Hortplus™ temperature probes recorded the temperature in each hive every hour for the 19 days of the trial. The hives had an average temperature of 30.5°C which ranged from 16–45.5°C. The daily mean temperature for one of the hives (number five) over the 19 days of the trial is shown in Figure 22.

Metarhizium (A) was recovered from the rice by washing the rice in 0.03% sterile Tween 80. The inoculum was then filtered using a 100 μm BD Falcon™ cell strainer and plated onto PDA+ plate and incubated for 14 days at 22°C. The inoculum from each hive grew, therefore the temperature in the feeders didn't seem to effect the growth and viability of the fungi.

The effect of Metarhizium growing on rice in hives on varroa mortality

The purpose of this trial was to determine whether Metarhizium isolate A would kill varroa in honey bee colonies using dry sugar feeders.

Metarhizium from a single isolate (A) was grown onto Potato Dextrose Agar (PDA) fortified with 0.2 g/L chloramplenicol to reduce contamination from other fungi and bacteria. To produce an inoculum for solid substrate production the conidia from three week old plates were harvested by scraping mycelia mats with a sterile spatula into 0.03% sterile Tween 80. The inoculum was then filtered using a 100 μm BD Falcon™ cell strainer to remove any hyphal fragments.

Mushroom spawn bags containing 300 g Budget® long grain white rice and 160 ml deionised water were sterilised by autoclaving at 121°C for 20 minutes.

Thirteen colonies housed Langstroth hives were selected on 3 April 2007 and fitted with mite collectors and sticky boards. The sticky boards were replaced weekly for ten weeks.

Eight colonies had dry sugar feeders on the top super which were covered with black plastic to increase the humidity which promotes the growth of the Metarhizium. After cooling the bags of rice they were inoculated with 60 ml of inoculum and mixed thoroughly and put into the top feeders. Raw sugar (500 g) was added to the top feeder to encourage bee activity. Also a bag of 60% sugar syrup to further simulate bees was placed into the top feeder which was replaced weekly (Figure 23). After two weeks a further 300 g of autoclaved Budget® long grain white rice and 500 g of raw sugar were added to the rice already present in the feeders and mixed thoroughly. The extra rice was added for the Metarhizium to continue having a medium to grow on. As the bee activity was low in the infected rice the raw sugar

was added to simulate activity in the infected rice. After six weeks the dry feeders were removed and Bayvarol® was inserted in the hives at the recommended rate for a further four weeks.

The other five colonies were used as controls to monitor the levels of varroa coming into the colonies during the trial period. These colonies had Bayvarol® strips inserted at the recommended rate for six weeks after which they were replaced with Apistan® for a further four weeks.

All colonies had their strength evaluated by visual assessment of the amount of brood and bees by inspecting each frame prior to the Metarhizium being applied to the treated hives on 3 April 2007. The hives were re-assessed at the end of the fungal treatment on 16 May 2007. At the time of the post treatment two Metarhizium treated colonies had died and were removed from the analysis. One of the treated colonies was queenless when reassessed.

Bee numbers in hives that were treated with Metarhizium dropped from an average of 9.4 to 3.7 (range 2 to 4.5 frames). Bee numbers in the control hives remained stable. There were significantly fewer frames of bees ($P < 0.0002$) and brood ($P < 0.002$) in the Metarhizium treated colonies after treatment compared to pre-treatment levels (Figure 24). Even though there was a reduction (39%) in the average number of frames of bees in the Metarhizium hives compared to the control colonies they were not significantly different ($P = 0.06$). It is normal for there to be a reduction in the amount of bees and brood at this time of the year as the temperature and food sources are reduced.

The average percentage (63% S.E. = 10.5) of varroa mortality in the Metarhizium treatment colonies was significantly ($P = 0.03$) lower than the control colonies (94% S.E. = 2.9) (Figure 25). However, applying inoculating Metarhizium directly into top feeders as carried out in this trial does demonstrates that it can result in effective control of varroa (hive 2 and 5).

A sample of rice with Metarhizium was taken from each dry feeder when they were removed from the hives. Metarhizium was recovered from the rice by washing with 0.03% sterile Tween 80. The inoculum was then filtered using a 100 μm BD Falcon™ cell strainer and plated onto PDA+ plate and incubated for 14 days at 22°C. The inoculum sample from each hive grew and therefore the conidium was still active at the time the rice was removed from the colonies.

CONCLUSIONS

When testing the isolates the results prove the concept that Metarhizium can successfully kill varroa in bioassay as well as having no effect on adult honey bee survival. The bioassay tested seems to be useful to determine the treatments even though the death rates of varroa in untreated samples can be high.

When a bioassay on the concentration of Metarhizium was examined the number of dead varroa increased with the amount of Metarhizium applied. Some of the varroa died within 3 hours of being treated. This suggests their deaths might not be due to the effect of a Metarhizium infection. When all the isolates were tested the varroa died and the best two isolates were selected for easy of use, amount of conidia produced and level of mite mortality for further study.

Icing sugar appears not to effect the survival of varroa when used as a method of harvesting mites for bioassays. However icing sugar increases varroa mortality when they are treated after being removed for bees. This may be due to the amount of fine particles present. Collecting mites from adult honey bees provided better survival of varroa for laboratory evaluation than collecting varroa from larvae.

Although trying to improve bioassay by increasing the humidity in the dishes it didn't result in better varroa survival. It did show that the presences of pupae were necessary as a food source for the mites to survive, even for 24 hours.

Using dry sugar feeders as a method for transferring differing amounts of Metarhizium spores to varroa appeared to be a problem due to insufficient contact to infect and kill the mites. Even though raw sugar was added to encourage bee activity to spread the spores throughout the colony it was possible that the varroa had little or no exposure to the spores. Unfortunately the viability of the Metarhizium grown on the Sun Rice® Sunwhite Calose Medium Grain rice wasn't verified post treatment in the colonies. Therefore whether this brand of rice had been coated with an additive such as a free-flowing agent which inhibited the growth and spore activity of the Metarhizium is unknown.

Further investigations to develop a method of application using dry sugar feeders showed that the temperature do not appear to be a limiting factor for growing Metarhizium. Metarhizium could grow in top feeders whether on PDA+ plates or on autoclaved white rice.

The final field investigation using dry feeders showed some promise with high mite mortality in a number of colonies. Adding sugar syrup to encourage bee activity achieved a positive result. This could indicate that to overcome the previous problems when treating colonies with Metarhizium could be more effective in the spring when colonies are expanding.

The lack of consistent results in the majority of these investigations could be that the spores of the Metarhizium were not coming into direct contact with the cuticle of varroa. But the variation in the efficacy of Metarhizium that occurred seems similar to most organic products in use in New Zealand. The method of applying this fungus so that it would contact the majority of mites needs further research.

Compared to earlier attempts used to apply Metarhizium to hives the result of this trial indicates the Metarhizium can be used to effectively control varroa. Also that the top feeder application method shows merit. It should be relativity simple to reduce the variation in the effectiveness of the treatment.

Example 2: Improved bioassay for fungal effects on survival of varroa

Summary

Because of uncertainties around the identification of the *Metarhizium* tested that surfaced after the trials were completed and a problem with keeping untreated varroa alive during the bioassay, the bioassays were repeated with an improved method to increase the survival of untreated varroa and to ensure use of a verified strain of *Metarhizium*.

The results show that the changes to the bioassay were effective in increasing the survival of untreated varroa. They also demonstrate that *Metarhizium* kills varroa.

It appears that there may be two different mechanisms allowing *Metarhizium* to kill varroa. The first kill occurred in less than 24 h and because of the short time frame, may possibly have been a physical response. The second occurred after 48 hours and may reflect mycelial growth.

Materials and Methods

Cultured Metarhizium

The *Metarhizium anisopliae* (strain 149) that was used for this assay was plated on PDA+ (15 May 2009) from a sample stored in sterile deionised water (from 24 March 2009).

The *Metarhizium* spores were harvested from two plates by scraping mycelia mats into a vial with a sterile spatula.

Pupae collection

Previously the standard container used for bioassays was a Corning® cell culture dish (35 mm x 10 mm), which contained three pupae and ten phoretic varroa mites that had been removed from adult bees using the sugar shake method. To have a bioassay simulating the natural situation within a honey bee colony like a cell on a frame, 2-ml tubes were tested.

Twenty Safeseal Microcentrifuge tube (2 ml) Sorenson™ each had a piece of tissue paper (45 mm x 48 mm) rolled up and placed into the base. Each of the tubes had a pin hole made in the cap to help with the humidity within the tube's environment.

Pink/purple eye pupae (Figure 26) were extracted from a frame that had been removed from a honey bee colony within the previous hour. The pupae were removed from the cell after the capping had been removed with tweezers. One healthy pupa was then placed into each of the tubes (Figure 27).

Varroa collection

In the previous bioassays, varroa were removed from adult bees using icing sugar. For the new bioassay, the varroa were extracted directly from pupae.

The brood frames from several colonies heavily infested with varroa were uncapped with tweezers. The frames were then inverted and banged on the edge of the laboratory bench, causing pupae and varroa to fall out of the frames. Female varroa were collected using a clean paintbrush (Humbrol No. 00) and temporarily held on healthy pupae to ensure that the mites could feed before they were used in the bioassay.

Inoculation of varroa

Ten varroa were removed from their temporary host into a clean vial. Approximately 2 mg of *Metarhizium* was placed into the vial using a sterile tooth pick. The vial with the varroa was then shaken so the *Metarhizium* and the varroa came in contact. The varroa were then tipped from the vial, transferred onto a piece of clean tissue paper and then transferred with a clean paintbrush on a pupa in a tube (Figure 27). The varroa were not tipped directly into the tube, to limit the transfer of *Metarhizium* as much as possible. Ten replicates were carried out. An additional ten vials were not inoculated with *Metarhizium* but were shaken to serve as controls. The tubes were then placed into a 35°C incubator and varroa survival was checked after 24, 48 and 72 h.

Results

Approximately 30% of the varroa were dead after 24 h exposure to *Metarhizium*. The death rate had not significantly increased at 48 h. Almost all the varroa that were not treated with *Metarhizium* were still alive after 72 hours (Figure 28). The *Metarhizium* treatment killed significantly more ($P < 0.001$) varroa than died in the controls. Only 13% of the *Metarhizium*-treated varroa were still alive after 72 h.

Discussion

The results show that the changes to the bioassay methodology were effective in increasing the survival of untreated varroa. They also demonstrate that *Metarhizium* kills varroa. It appears that there may be two different mechanisms allowing *Metarhizium* to kill varroa. The first kill occurred in less than 24 h and because of the short time frame, may possibly have been a physical response. The second occurred after 48 hours and may reflect mycelial growth. Further assays are scheduled and potential mechanisms for the observed death of varroa are being investigated.

REFERENCES

James RJ, Hayes G, Leland J 2006. Field trials on the microbial control of varroa with the fungus Metarhizium anisopliae. American Bee Journal. Vol. 146 No. 11: 968-972

Kanga LHB, James RR 2002. Hirsutella thompsonii and Metarhizium anisopliae as potential microbial control agants of Varroa destructor, a honey bee parasite. Journal of Invertebrate Pathology 81: 175-184.

Roberts DW and St.Leger RJ 2004. Metarhizium spp., cosmopolitan insect-pathogenic fungi: mycological aspects. Advances in Applied Microbiology 54: 1-70.

Shaw KE, Davidson G, Clark SJ, Ball BV, Pell JK, Chandler D, Sunderland KD 2002. Laboratory bioassays to assess the pathogenicity of mitosporic fungi to Varroa destructor (Acari: Mesostigmata), an ectoparasitic mite of the honey bee, *Apis mellifera*. Biological Control 24: 266 – 276.

CLAIMS:

1. A method for controlling growth of a pathogen of bees, the method comprising providing a culture of a fungus inside, or proximate to a beehive, wherein the culture is positioned to be contacted by the bees to effect control of growth of the pathogen.

2. The method of claim 1 wherein the culture is provided inside the beehive

3. The method of claim 1 or 2 wherein the pathogen is a mite.

4. The method of any preceding claim where the pathogen is a Varroa mite

5. The method of any preceding claim wherein the control of growth of the pathogen, results in death of at least 20% of the pathogen population.

6. The method of any preceding claim wherein the fungus is a fungus that produces spores, and the spores produced by the fungus control of the growth of the pathogen.

7. The method of any preceding claim wherein the fungus grows when inside, or proximate, to a beehive.

8. The method of any preceding claim wherein the fungus is capable of growing in the conditions typically found in beehives.

9. The method of any preceding claim wherein the fungus is capable of growing in the temperature range 30 to 40°C.

10. The method of any preceding claim wherein the fungus is capable of growing at a relative humidity of at least 90%.

11. The method of any preceding claim wherein the fungus is from the phylum Ascomycota.

12. The method of any preceding claim wherein the fungus is from the genus *Metarhizium*.

13. The method of any preceding claim wherein the fungus is from species is *Metarhizium anisopliae*.

14. The method of any preceding claim wherein the culture is to a living colony of the fungus.

15. The method of any preceding claim wherein the the culture comprises the fungus and a substrate.

16. The method of any preceding claim wherein the fungus is in the form of spores.

17. The method of any preceding claim wherein the fungus is in the form of a mycellium.

18. The method of any preceding claim wherein the fungus is in the form of a mature spore producing colony.

19. The method of any one of claims 15 to 18 wherein the substrate can not be easily removed by bees.

20. The method of claim 19 wherein the substrate comprises rice.

21. The method of any preceding claim that includes the step of inserting the culture into the beehive, or the position proximate to the beehive.

22. The method of claim 21 wherein the culture is inserted as a growing colony of the fungus.

23. The method of claim 22 wherein the colony is actively producing spores.

24. The method of claim 21 wherein the culture is inserted as a substrate, inoculated with the fungus.

25. The method of claim 24 wherein the culture is not producing spores when inserted, but grows to provide a mature, spore producing culture some time after insertion.

26. The method of claim 24 or 25 wherein the culture is inserted dry.

27. The method of claim 26 wherein the dry culture is produced as follows:

a) a moist substrate is inoculated with fungal spores,

b) the spores optionally germinate,

c) the inoculated substrate, with spores which are optionally germinated, is then dried to prevent growth of the fungus which requires moisture, to produce the dry culture.

28. The method of claim 26 or 27 including the step of sealing the dry culture in a substantially water-tight package.

29. The method of claim 28 wherein the substantially water-tight package is opened prior to being place into the beehive, or proximate to the beehive.

30. The method of any one of claims 26 to 29 wherein ambient moisture from the air in the beehive, or proximate to the beehive wets the substrate to allow growth of the fungus.

31. The method of any preceding claim wherein the culture is positioned in a separate compartment from the compartment in which the honey is produced.

32. The method of any preceding claim wherein the culture is positioned to take advantage at least one of:

i) the temperature conditions, within the hive, that are suitable for growth of the fungus, and

ii) the relative humidity conditions, within the hive, that are suitable for growth of the fungus.

33. The method of claim 32, wherein the culture is positioned to take advantage of the temperature and relative humidity conditions, within the hive, that are suitable for growth of the fungus.

34. The method of any preceding claim wherein the culture is positioned above the bee colony.

35. A device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony, the device comprising:

an entranceway into the device, the entranceway in communication with a passageway extending to one or more first openings (or inlets) into a nest for insects, and

an exitway from the device, the exitway in communication with the passageway extending from the one or more openings or one or more second opening s(or outlet) out of the nest for the insects,

wherein interposed (or interposable) between the entranceway and exitway, and the first opening (or inlet) or second opening (or outlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum.

36. The device as claimed in claim 35, wherein the first and second passageways are separated (or separable) from each other by a divider (or a barrier).

37. The device as claimed in claim 35 or claim 36, wherein interposed (or interposable) between the entranceway and the first opening (or inlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculums.

38. The device as claimed in any one of claims 35 to 37, wherein interposed (or interposable) between the second opening (or outlet) and exitway is/are a directionally openable gate or gates, the gate(s) permitting of insects exiting from the second passageway to the exitway of the device.

39. The device as claimed in any one of claims 35 to 38, wherein the device is a man-made structure supportive of a nest for an insect colony.

40. The device as claimed in any one of claims claim 35 to 39, wherein the one or more first openings is/are apertures in flooring of the first passageway, and the one or more second openings is/are apertures in flooring of the second passageway.

41. The device as claimed in claim 40, wherein the apertures are sized to allow insects to pass therethrough.

42. The device as claimed in any one of claims 35 to 41, wherein at least a part of the first passageway includes at least a constriction within which the region is located (or locatable).

43. The device as claimed in claim 42, wherein the constriction substantially provides a crawl-space (or substantially prevents flying) for insects passing through the region, thereby contacting inoculum when in-situ.

44. The device as claimed in any one of claims 35 to 43, wherein at least a part of the region (optionally being an or the inoculation region) is positioned within a (or the) constriction of the first passageway, the constriction being at least a reduced height between the passageway's flooring and ceiling relative to the height of the remainder of the first passageway.

45. The device as claimed in any one of claims 35 to 43, wherein the constriction is provided by i) the floor of the region elevated compared with the floor of the remainder of the first passageway, or ii) the ceiling above the region lowered compared to the ceiling of the reminder of the first passageway, or iii) a combination of both i) and ii).

46. The device as claimed in any one of claims 35 to 45, wherein the height or constriction between the flooring and ceiling about the region in the first passageway is less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm or is about 8 mm.

47. The device as claimed in any one of claims 35 to 46, wherein at least a portion of the second passageway is of a reduced height between flooring and ceiling.

48. The device as claimed in claim 47, wherein at least a substantial length of the second passageway has the reduced height, the reduced height is such that, in use, insects of the colony may be discouraged from forming, building or nesting in the second passageway.

49. The device as claimed in claim 48, wherein the height between the flooring and ceiling in the second passageway is less than about 15 mm, 14 mm, 13 mm, 12 mm, 11 mm, 10 mm, 9 mm, 8 mm, or is about 8 mm.

50. The device as claimed in any one of claims 35 to 49, wherein one or more external facing side walls of the device include a porous portion or portions.

51. The device as claimed in claim 50, wherein the porous portion(s) are porous to light or air (or other gases) or both, and may be one or more pores in the side wall or walls for forming the porous portion(s).

52. The device as claimed in claim 47 or claim 51, wherein the pores are sized such that, in use, insects of the nest are substantially prevented from (or substantially unable to) pass therethrough.

53. The device as claimed in any one of claims 50 to 52, wherein the porous portion or portions is/are of one or more of: external side walls of the second passageway, side wall or walls located near, adjacent or about the one or more second openings, extending along the side walls of the second passageway

54. The device as claimed in any one of claims 35 to 53, wherein the entranceway and exitway are located adjacent one another, and/or the entranceway and exitway are located on the same side (or face) of the device.

55. The device as claimed in any one of claims 35 to 54, wherein the entranceway has one or more of: a larger mouth into the first passageway than the mouth provided from the second passageway at the exitway, or the entranceway mouth has a greater width than the mouth of the exitway, or the entranceway mouth has a height greater than the height of the exitway mouth.

56. The device as claimed in any one of claims 35 to 55, wherein the entranceway mouth includes a lip, the lip being a raised platform positioned above the floor of the entranceway mouth.

57. The device as claimed in any one of claims 35 to 56, wherein the lip is a landing zone for insects arriving at the entranceway mouth.

58. The device as claimed in any one of claims 35 to 57, wherein at least a part of the entranceway mouth includes one or more visual attributes attractive to the insects approaching the device from outside of the nest.

59. The device as claimed in any one of claims 35 to 58, wherein at least a part of the entranceway mouth is coloured, or emits a colour, that is attractive to insects of the nest when outside of the nest.

60. The device as claimed in any one of claims 35 to 59, wherein the at least part of the entranceway mouth is of a dark colour or appearance, or at least part of the entranceway mouth is of a darker colour or appearance than the colour of the exitway mouth, the darker colour or appearance may be the colour black.

61. The device as claimed in any one of claims 58 to 60, wherein the visual attributes or colouring is displayed on external facing parts of the entranceway mouth.

62. The device as claimed in any one of claims 58 to 61, wherein the visual attributes or colouring is provided by a transfer appliable to the entranceway mouth or at least a part or parts of the entranceway mouth.

63. The device as claimed in any one of claims 35 to 62, wherein the exitway mouth includes a raised wall portion extending upwards from the floor of the exitway mouth, the wall portion providing for a display surface of one or more visual attributes attractive to insects approaching the mouth from inside of the nest (or from within the second passageway).

64. The device as claimed in any one of claims 35 to 63, wherein at least a part of the exitway mouth is coloured, or emits a colour, that is one or more of: being less attractive to insects of the nest when outside of the nest, a light colour or appearance, or at least part of the exitway mouth is of a lighter colour or appearance than the colour of the entranceway mouth., the colour or emitted colour may be a white and/or or yellow colour.

65. The device as claimed in any one of claims 63 or claim 64, wherein the visual attributes or colouring is displayed on both internal and external facing parts of the exitway mouth.

66. The device as claimed in any one of claims 63 to 64, wherein the visual attributes or colouring is provided by a transfer appliable to the exitway mouth or at least a part or parts of the exitway mouth.

67. The device as claimed in any one of claims 35 to 66, wherein the directionally openable gate or gates is a one-way gating system allowing nest insects to pass through the gate(s) in a substantially first direction.

68. The device as claimed in claim 67, wherein the gate(s) allows for insects to pass through in the first direction from the second passageway to the exitway mouth.

69. The device as claimed in any one of claims 64 or claim 68, wherein the one-way gating system is a one-way curtain.

70. The device as claimed in claim 69, wherein the one-way curtain is locatable to substantially span the width of the second passageway and interposed between the second openings and exitway mouth.

71. The device as claimed in claim 69 or claim 70, wherein the curtain comprises a series of flaps moveable from a closed gate position to an open gate position under influence of an insect, the flaps returnable from the open position to (or towards) the closed position under their own power without influence of the insect on the flaps.

72. The device as claimed in claim 71, wherein the flaps are moveable about a hinge or hinges along an edge of the flaps.

73. The device as claimed in claim 71 or claim 72, wherein the flaps are a series of substantially vertically hanging flaps.

74. The device as claimed in any one of claims 71 to 73, wherein the flaps are formed of a plastics film or material, and/or are a substantially light transmissive material.

75. The device as claimed in any one of claims 71 to 74, wherein a block is locatable to be positioned adjacent to and at the second opening(s) side of the curtain, the block comprises one or more apertures sized to allow insects of the nest to pass therethrough.

76. The device as claimed claim 75, wherein the flaps are spaced or dimensioned to hang at least partially obscuring the block's one or more apertures when in the closed position.

77. The device as claimed in claim 75 or claim 76, wherein the block is formed of a plastics material, such as transparent thermoplastic acrylic resins, for example polymethylmethacrylate, and/or is a substantially light transmissive material.

78. The device as claimed in any one of claims 75 to 77, wherein the block is dimensioned such that it substantially spans the whole of the width and height at its location within the second passageway.

79. The device as claimed in any one of claims 75 to 78, wherein the curtain is attached to the block.

80. The device as claimed in any one of claims 35 to 79, wherein the inoculum or substrate comprising of inoculum is provided via a cartridge locatable within the region.

81. The device as claimed claim 80, wherein the cartridge is replaceable with another cartridge or is re-chargeable with fresh inoculum.

82. The device as claimed claim 80 or claim 81, wherein the cartridge is a tray receivable or loadable with the inoculum or the substrate comprising of inoculum.

83. The device as claimed in any one of claims 35 to 82, wherein the device is a unitary item.

84. The device as claimed in any one of claims 35 to 83, wherein two or more of the devices are stackable atop one another.

85. The device as claimed in any one of claims 35 to 84, wherein the device is collapsible or foldable, such as collapsible or foldable into a flat-pack format.

86. The device as claimed in any one of claims 35 to 82, wherein the device is constructed or constructible from multiple parts.

87. The device as claimed in any one of claims 1a to 82, wherein the device is an assembly constructed from, or constructible from, a base component, a lid component, and an internal component or components, each of the base, lid and internal components engageable with at least one other component for constructing the device.

88. The device as claimed in claim 87, wherein the base component is constructed or constructible to form at least each of the floors for the respective first and second passageways, the region receivable or supportable of inoculum or a substrate comprising of inoculum, the first and second openings of the passageways, the entranceway, the exitway, and external side walls for the device.

89. The device as claimed in claim 87 or claim 88, wherein the lid component is constructed or constructible to form at least each of the ceilings for the respective first and second passageways, and the divider (or barrier) for separating the first and second passageways from each other.

90. The device as claimed in any one of claims 87 to 89, wherein the internal component(s) is constructed or constructible to form at least the directionally openable gate or gates to be locatable within the second passageway to be or so constructed.

91. The device as claimed in any one of claims 87 to 90, wherein the base component(s) is a first sub-assembly, the lid component(s) is a second sub-assembly, and the internal component(s) is a third sub-assembly, the first and second and third sub-assemblies assemblable for forming the device.

92. The device as claimed in any one of claims 87 to 91, wherein the first and second sub-assemblies sandwich the third sub-assembly (or the internal components).

93. The device as claimed in any one of claims 84 to 89, wherein corresponding parts of flooring and ceiling forming the second passageway include locators for locating the directionally openable gate or gates (hereinafter "gate locators").

94. The device as claimed claim 93, wherein the flooring and ceiling locators are receivable of at least a part of directionally openable gate(s) for locating the gate(s) in a pre-determined gate position.

95. The device as claimed claim 93 or claim 94, wherein the locators and directionally openable gate(s) have respectively engageable parts, such respectively engageable parts may be male and female (or female and male) shaped parts.

96. The device as claimed any one of claims 93 to 95, wherein the flooring includes a locator or locators for locating the divider (or barrier) between the first and second passageways (hereinafter "divider locator(s)").

97. The device as claimed in claim 96, wherein the flooring locator(s) is/are receivable of at least a part of the divider (or barrier) for locating the divider (or barrier) in a pre-determined divider (or barrier) position.

98. The device as claimed in claim 96 or claim 97, wherein the locators and divider (or barrier) have respectively engageable parts, such respectively engageable parts may be male and female (or female and male) shaped parts.

99. The device as claimed in any one of claims 96 to 98, wherein the divider (or barrier) is an integral part of the lid component.

100. The device as claimed in any one of claims 87 to 99, wherein the lid component is an integrally moulded article.

101. The device as claimed in any one of claims 87 to 100, wherein at least one of the external side walls of the device is a separate discrete part, such a part connectable to a respective edge of flooring of the base component and adjacent side wall(s), or the edge(s) of the entranceway and exitway.

102. The device as claimed in any one of claims 87 to 100, wherein one or more (or all) of the external side walls are integral parts of the base component, the side walls hingedly connected to the flooring.

103. The device as claimed in any one of claims 87 to 100, wherein the entranceway mouth and exitway mouth is a separate discrete part, such a part connectable to the entranceway and exitway side face or flooring edge of the base component.

104. The device as claimed in any one of claims 87 to 100, wherein the entranceway mouth and exitway mouth is an integral part of the base component, and hingedly connected to the flooring.

105. The device as claimed in any one of claims 87 to 104, wherein the lid component further comprises a support or supports for supporting of a roof above the device or nest, or both.

106. The device as claimed in any one of claims 35 to 105, wherein one or more of: internal surfaces of flooring, external side walls, or ceiling, or combinations of these, comprise of an embossed, or in-moulded, pattern.

107. The device as claimed in any one of claims 35 to 106, wherein the device is insertable at, or to, the entrance or the exit (or both) of a nest of an insect colony.

108. The device as claimed in any one of claims 35 to 107, wherein the device is locatable above the nest. More preferably, at least the region (optionally an inoculation region), is locatable above the nest.

109. The device as claimed in any one of claims 35 to 108, wherein the device is to be located more than half-way up the nest, the device positioned where environmental conditions are more favourable or beneficial to allow growth of the inoculum.

110. The device as claimed in any one of claims 35 to 109, wherein the insect colony is/are a colony of bees.

111. The device as claimed in any one of claims 35 to 110, wherein the nest is a beehive or beehive structure.

112. The device as claimed in any one of claims 35 to 111, wherein the man-made structure is a beehive structure. More preferably, the beehive structure is of the type comprising at least one super within which are located (or locatable) one or a series of top bar supported frames, the super seated on a floorboard and topped by a lid or roof, an opening into the structure provided between a edge of the super and the floorboard.

113. The device as claimed in any one of claims 35 to 112, wherein the device or at least a part of the device is one or a combination of wood, cellulose-fibre composites, cardboard, polymers such as for example, thermoplastics or thermosetting polymers. More preferably, wherein the is selected from one of: polycarbonate (PC), polystyrene (PS), general purpose polystyrene (GPPS), polymethyl methacrylate (PMMA), thermoplastic (poly) urethane (TPU), polyethylene terephthalate (PET), polyester methacrylate (PEM), polypropylene (PP), high impact polystyrene (HIPS), acrylonitrile butadiene styrene (ABS), polyester (PES), polyamides (PA), poly(vinyl chloride) (PVC), polyurethanes (PU), polyvinylidene chloride (PVDC), polyethylene (PE), polytetrafluoroethylene (PTFE), polyetheretherketone (PEEK) (polyetherketone), polyetherimide (PEI), polylactic acid (PLA), high impact polystyrene, aquilobutalstyrene, nylons, acrylics, amorphous polymers, polyethylene (PE), polyethylene terephthalate (PET), low density polyethylene (LDPE), low low density polyethylene (LLDPE), thermoplastic ethylene (TPE), polypropylene (PP), rubbers, phenolics and the like.

114. The device as claimed in any one of claims 35 to 113, wherein the device or at least a part of the device is extrusion formed (extruded) or injection moulded.

115. A device for attachment at, or to, an or the entrance or exit (or both) of a nest of an insect colony (preferably a beehive or beehive structure), the device supporting an inoculum or a substrate comprising an inoculum, such that, insects (preferably bees) transiting through the device contact or become inoculated or contaminated with the supported inoculum.

116. The device as claimed in any one of claims 35 to 115, wherein the inoculum is a fungus as defined in any one of claims 1 to 34.

117. The device as claimed in any one of claims 35 to 115, wherein the substrate is as as defined in any one of claims 15 to 20.

118. A package comprising:

one or more base components,

one or more lid components, and

one or more internal components,

the base, lid and internal components constructible as the device as defined in any one claims 35 to 117, and

one or more cartridges, the cartridges receivable of inoculum or substrate comprising inouclum, cartridges inoculatable with inoculum or the substrate on-site or prior to use within the device.

119. The package as claimed in claim 118, wherein the base component is constructed or constructible to form at least each of the floors for the respective first and second passageways, the region receivable or supportable of inoculum or a substrate comprising of inoculum, the first and second openings of the passageways, the entranceway, the exitway, and external side walls for the device.

120. The package as claimed in claim 118 or claim 119, wherein wherein the lid component is constructed or constructible to form at least each of the ceilings for the respective first and second passageways, and the divider (or barrier) for separating the first and second passageways from each other.

121. The package as claimed in any one of claims 118 to 120, wherein the internal component(s) is constructed or constructible to form at least the directionally openable gate or gates to be locatable within the second passageway to be or so constructed.

122. The package as claimed in any one of claims 118 to 121, wherein the inoculum is a fungus as defined in any one of claims 1 to 34.

123. The package as claimed in any one of claims 118 to 122, wherein the substrate is as as defined in any one of claims 15 to 20.

124. A method for installing or attaching at, or to, an or the entrance or exit (or both) of a man-made structure supporting a nest of an insect colony a device, the device comprising an entranceway into the device, the entranceway in communication with a passageway extending to one or more first openings (or inlets) into a nest for insects, and an exitway from the device, the exitway in communication with the passageway extending from the one or more openings or one or more second opening (or outlet) out of the nest for the insects, wherein interposed (or interposable) between the entranceway and exitway, and the first opening (or inlet) or second opening (or outlet) is a region (optionally an inoculation region), the region receivable or supportable of inoculum or a substrate comprising of inoculum,

the method comprising the steps of:

positioning the device relative the man-made structure and nest such that the respective first or second (or both) openings of the passageway is/are communicable with the inside of the nest, and

blocking entrances to and exits from the nest, such that access to and from the nest by the nest's insects is/are via the entranceway and exitway of the device.

125. The method as claimed in claim 124, wherein the device is located more than half way up the man-made structure or nest within the structure.

126. The method as claimed in claim 124 or claim 125, further comprising locating the device at the top of the man-made structure, or above the top of the nest within the structure.

127. The method as claimed in any one of claims 124 to 126, further comprising locating the device at a position where conditions are more favourable or beneficial for allowing or facilitating growth of the inoculum.

128. The method as claimed in any one of claims 124 to 127, wherein the device is located where the environmental conditions adjacent the device, or at least the region, comprises of temperatures of about 20°C to about 50 °C.

129. The method as claimed in any of one claims 124 to 128, wherein the device is located where the environmental conditions adjacent the device, or at least the region, comprises relative humidity of at least 25 %.

130. The method of the any one of claims 1 to 34 wherein the culture is provided in the device of any one of claims 35 to 115, positioned inside the beehive.

131. The method of claim 130 wherein the culture is provided in the region of the device.

Figure 1

Figure 2

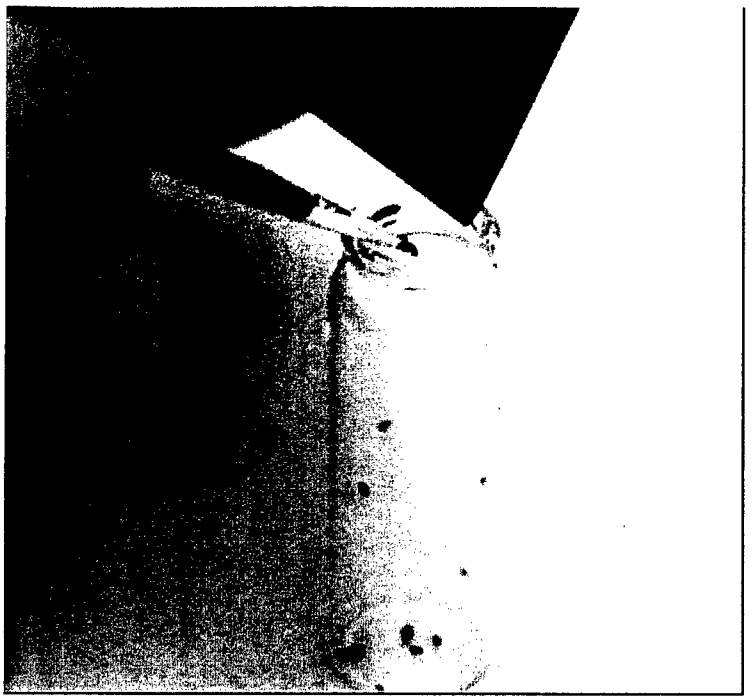


Figure 4

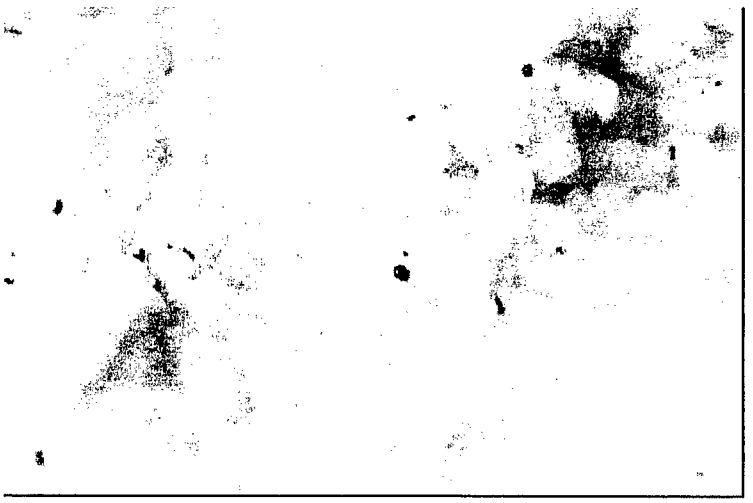


Figure 5

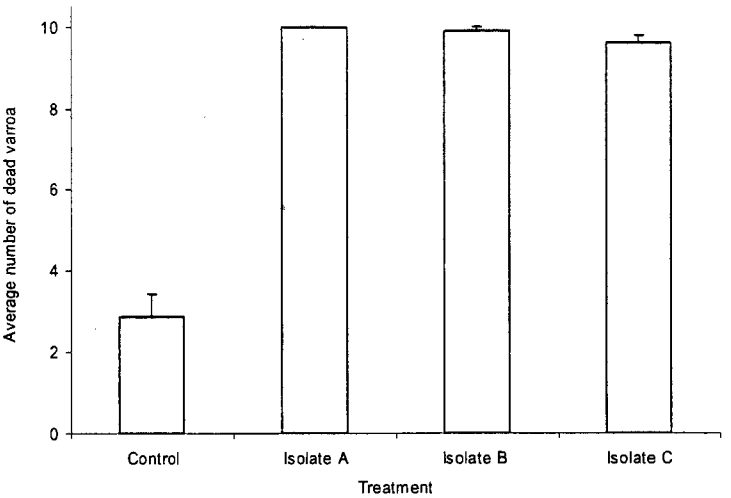


Figure 6

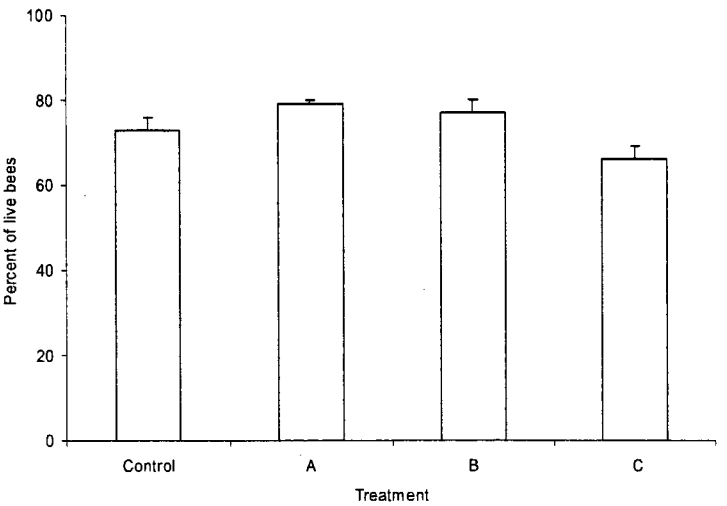


Figure 7

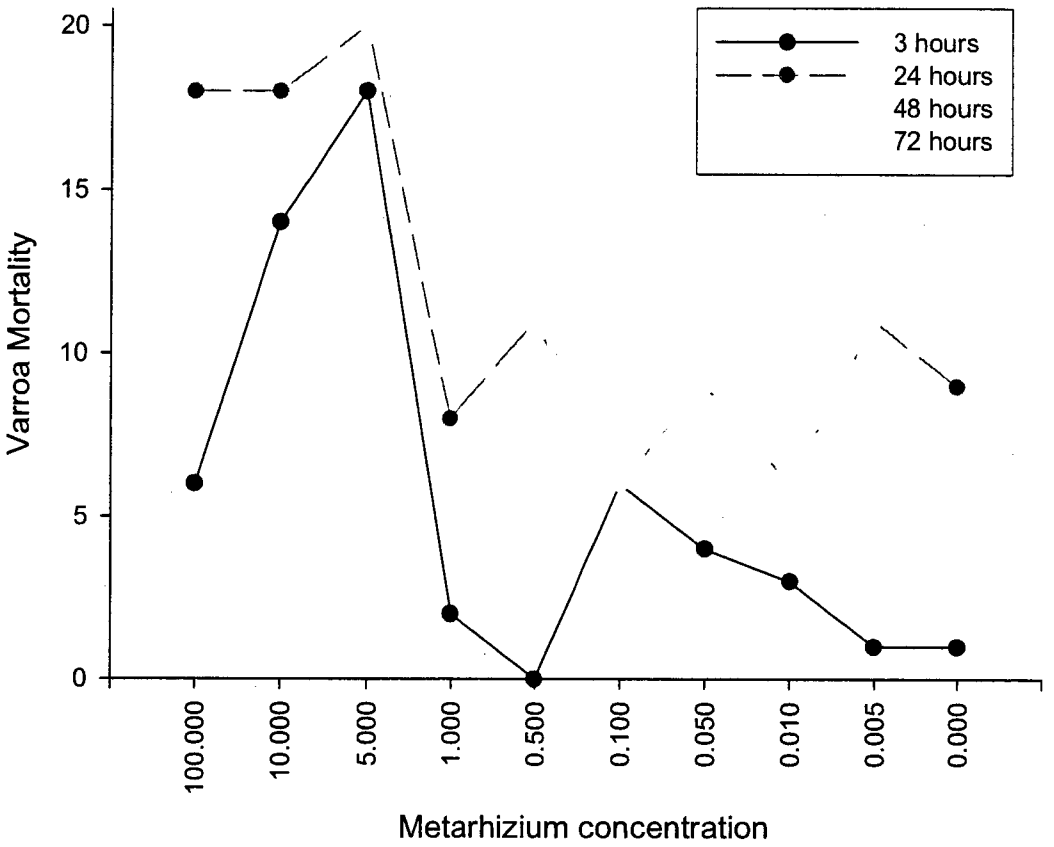

20
15
10
5
0
Varroa Mortality
3 hours
24 hours
48 hours
72 hours
100.000
10.000
5.000
1.000
0.500
0.100
0.050
0.010
0.005
0.000
Metarhizium concentration

Figure 8

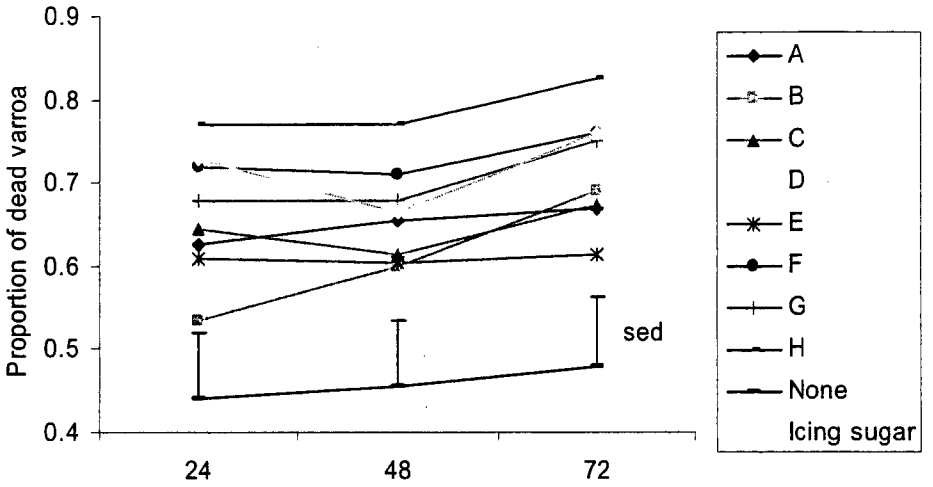


Figure 9

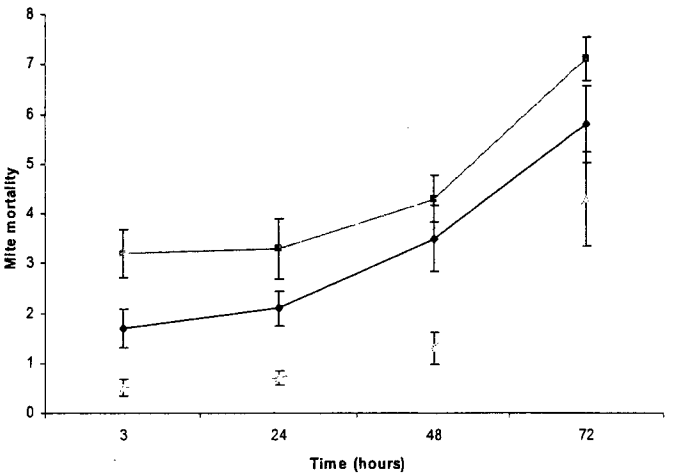


Figure 10

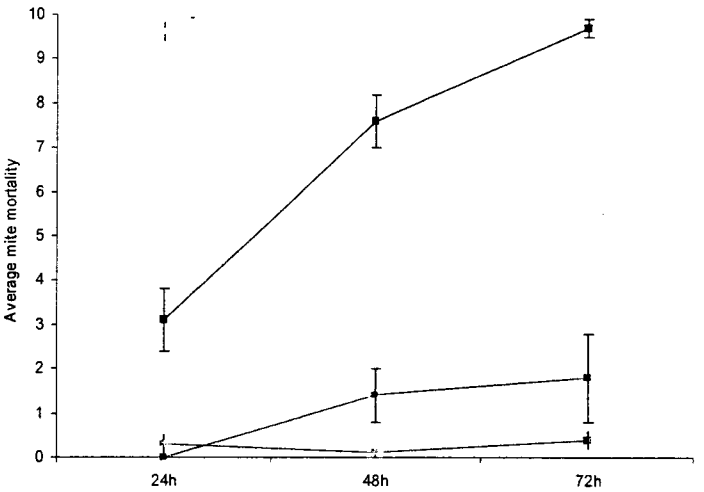


Figure 11

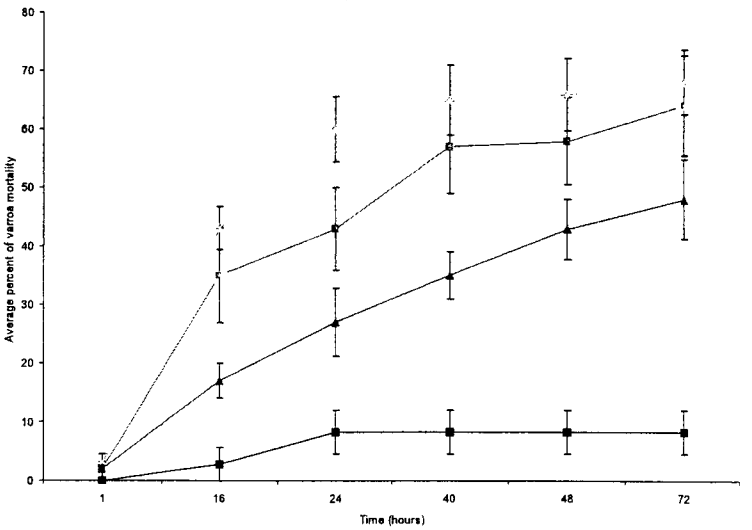


Figure 12

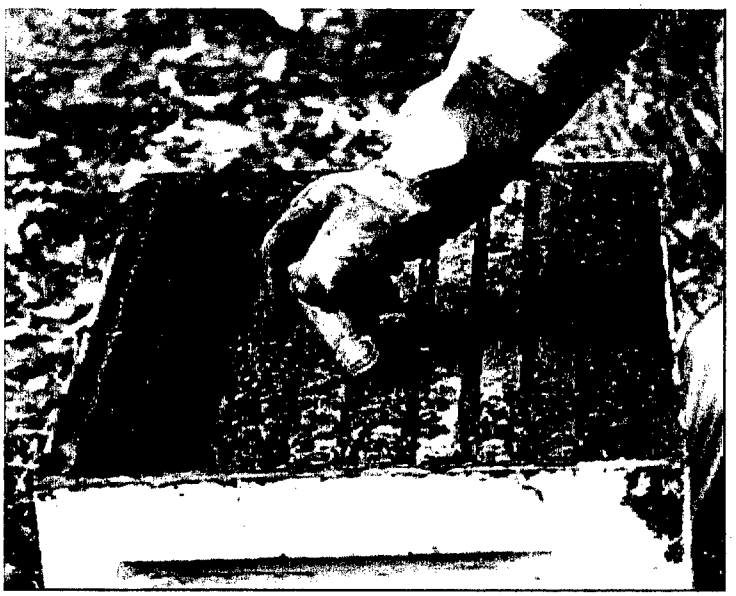


Figure 13

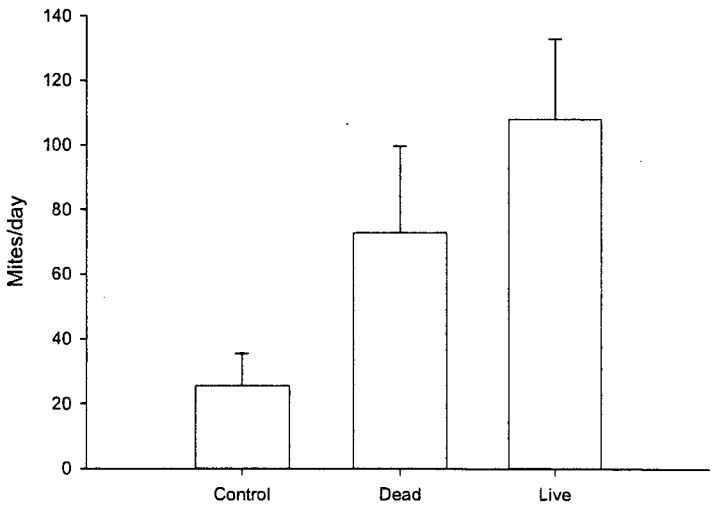


Figure 14

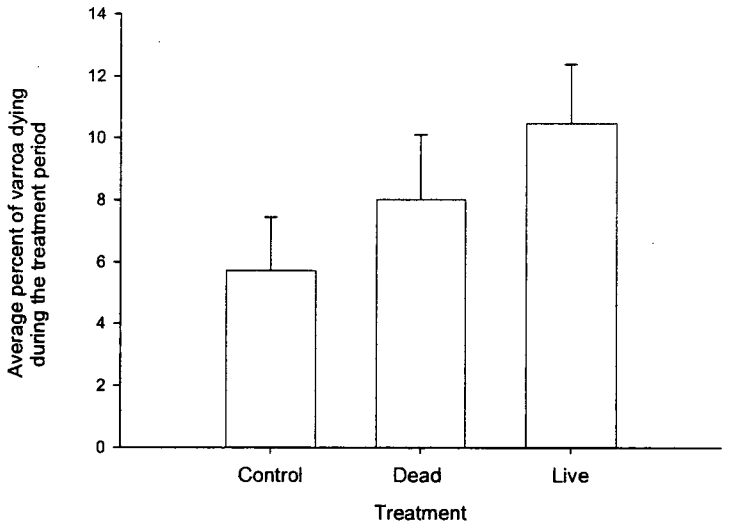


Figure 15

Figure 16

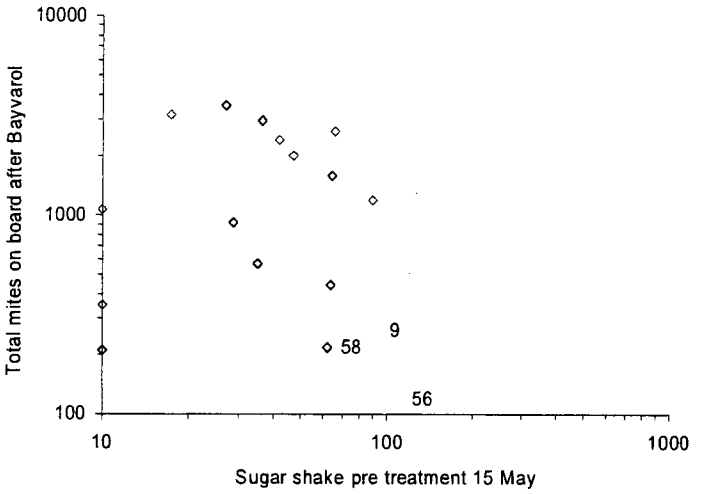


Figure 17

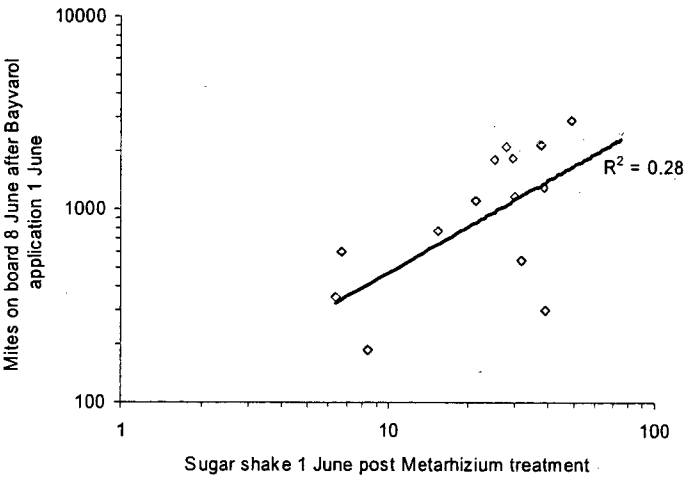


Figure 18

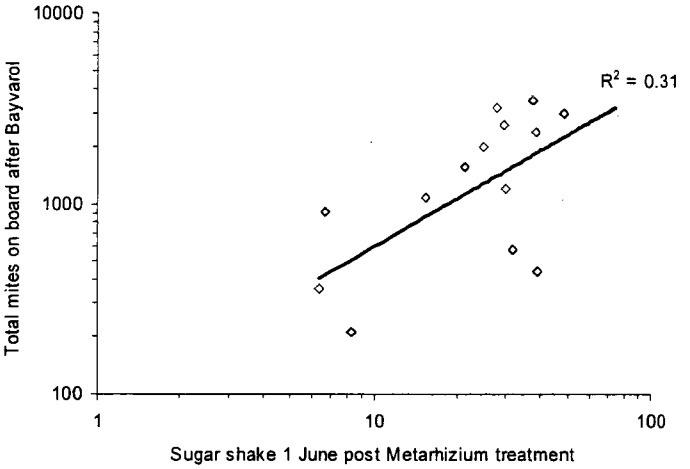


Figure 19

Figure 20

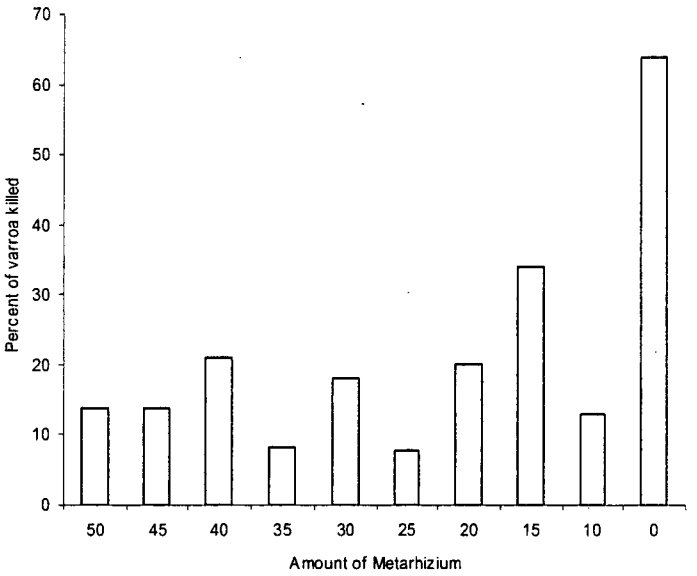


Figure 21

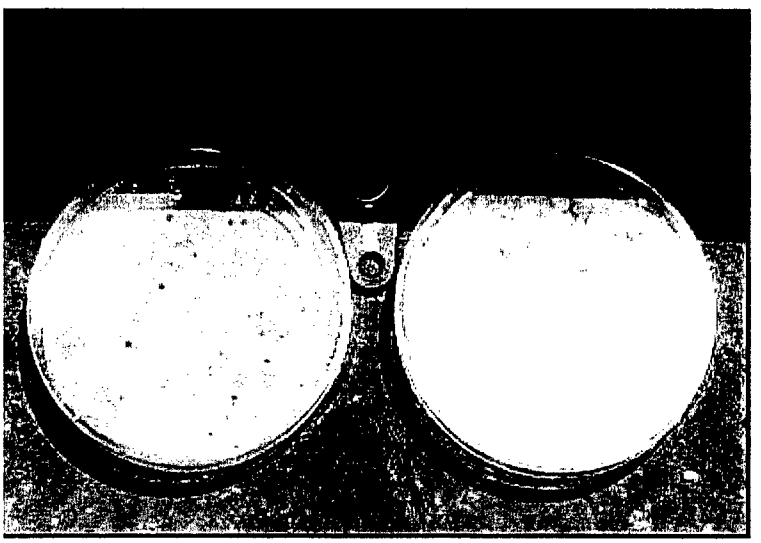


Figure 22

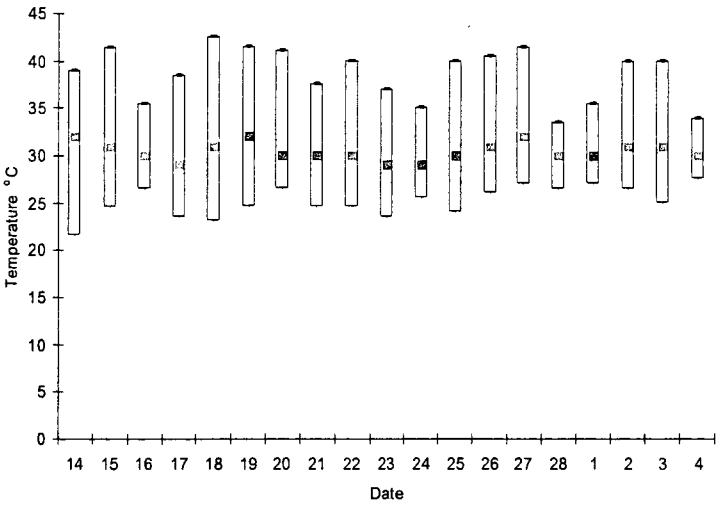


Figure 23

Figure 24

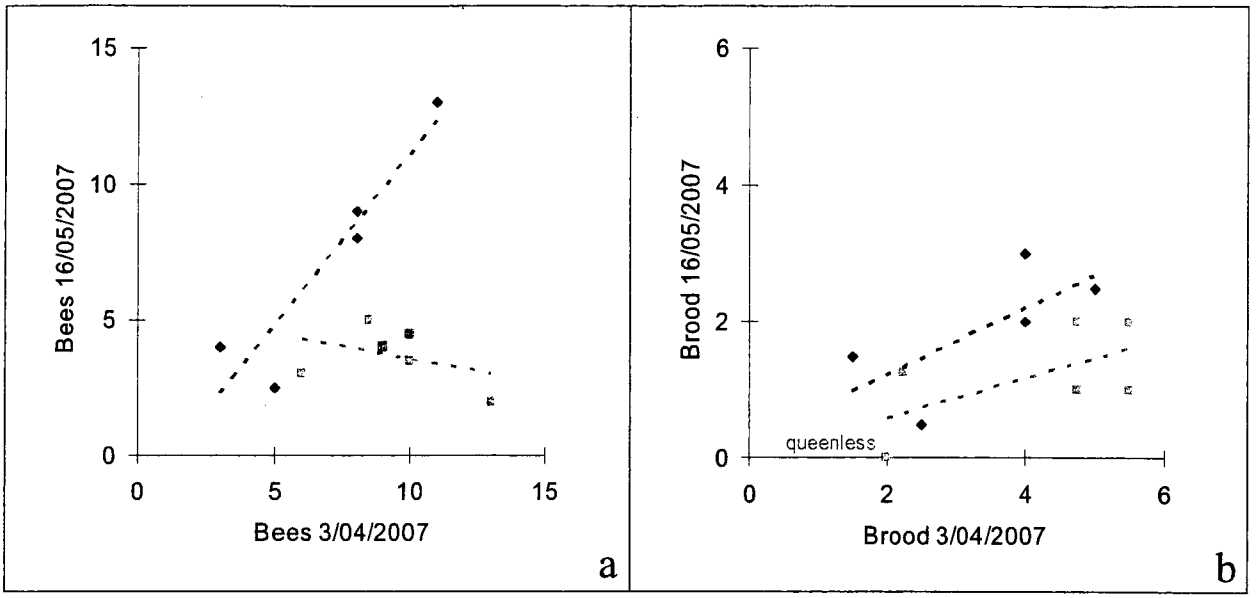


Figure 25

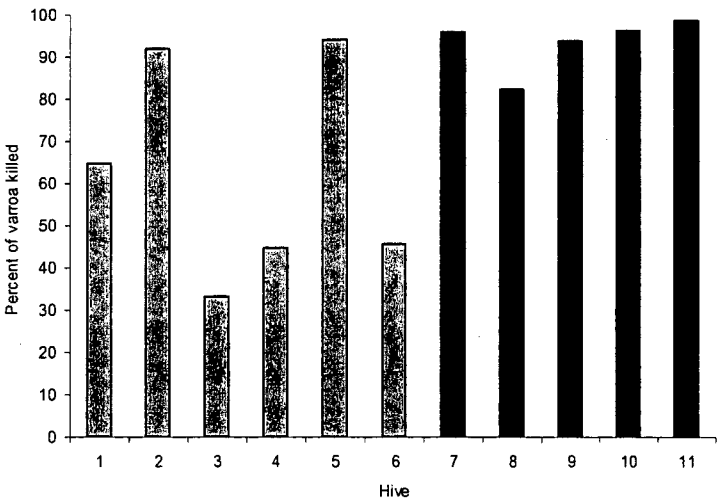


Figure 26

Figure 27

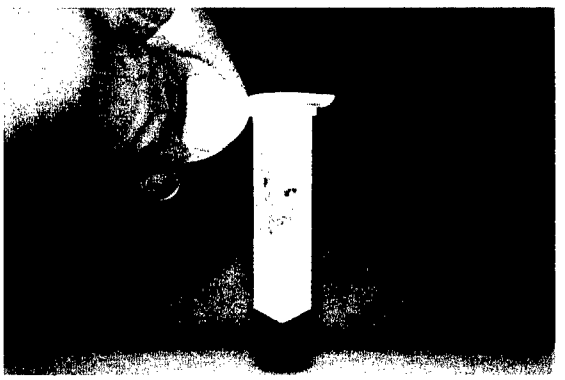


Figure 28

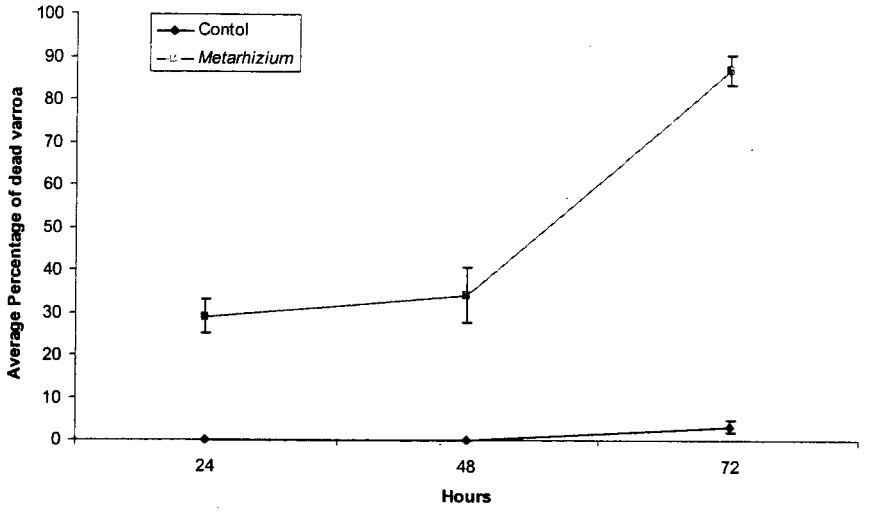

Contol
Metarhizium
Average Percentage of dead varroa
100
90
80
70
60
50
40
30
20
10
0
24
48
72
Hours

Figure 29

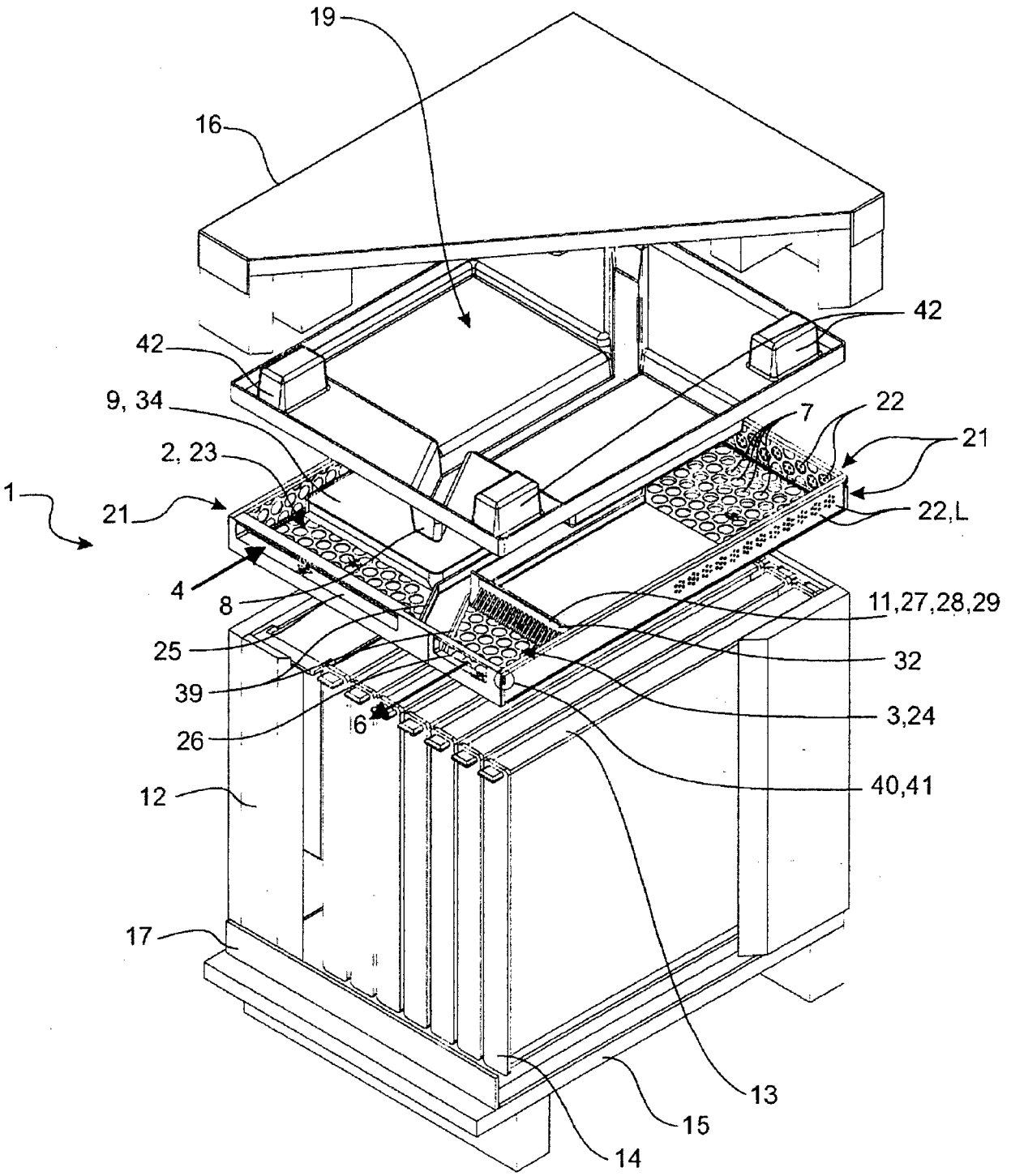

19
16
42
42
9, 34
2, 23
1
21
4
8
25
39
26
12
17
6
7
22
21
22,L
11,27,28,29
32
3,24
40,41
13
15
14

Figure 30

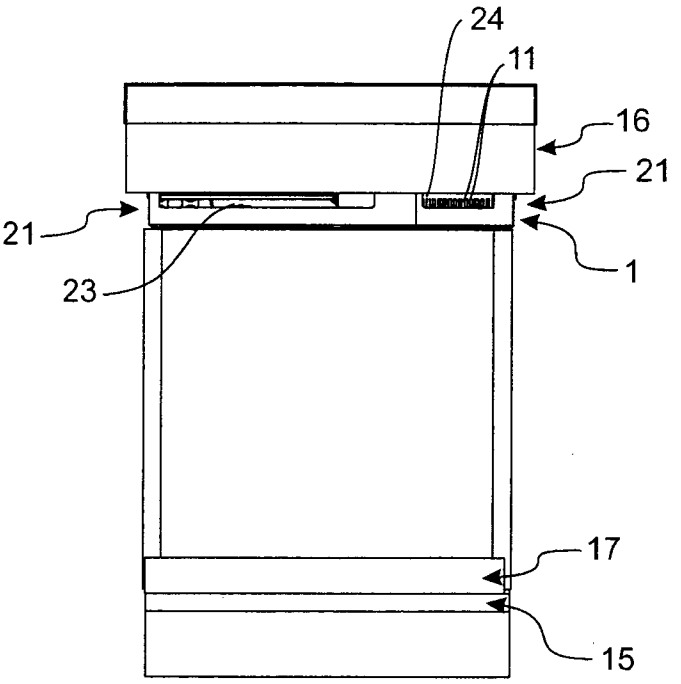


Figure 31

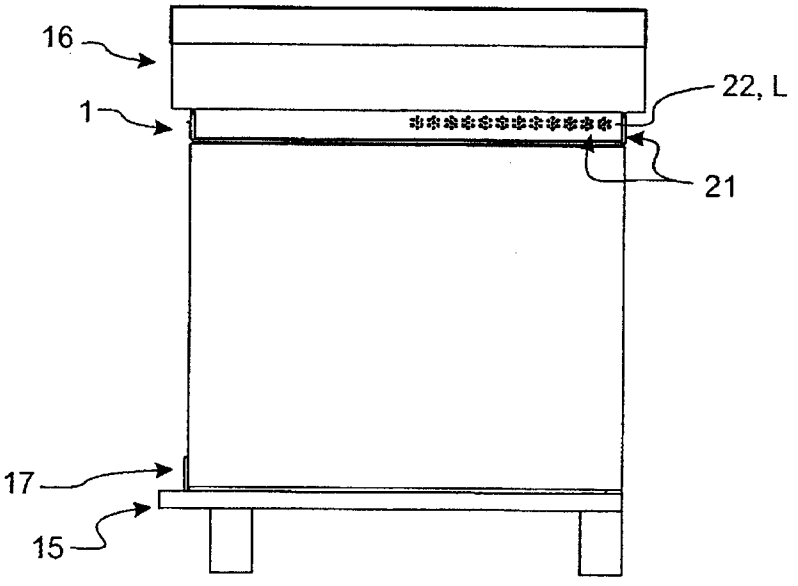


Figure 32

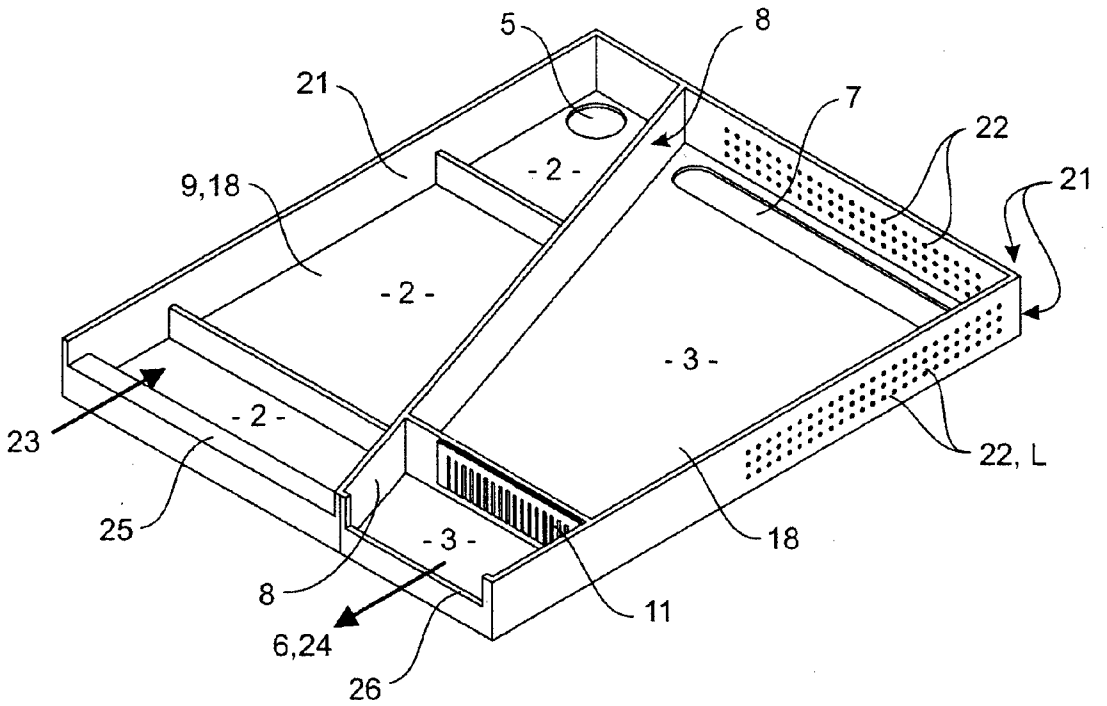


Figure 33

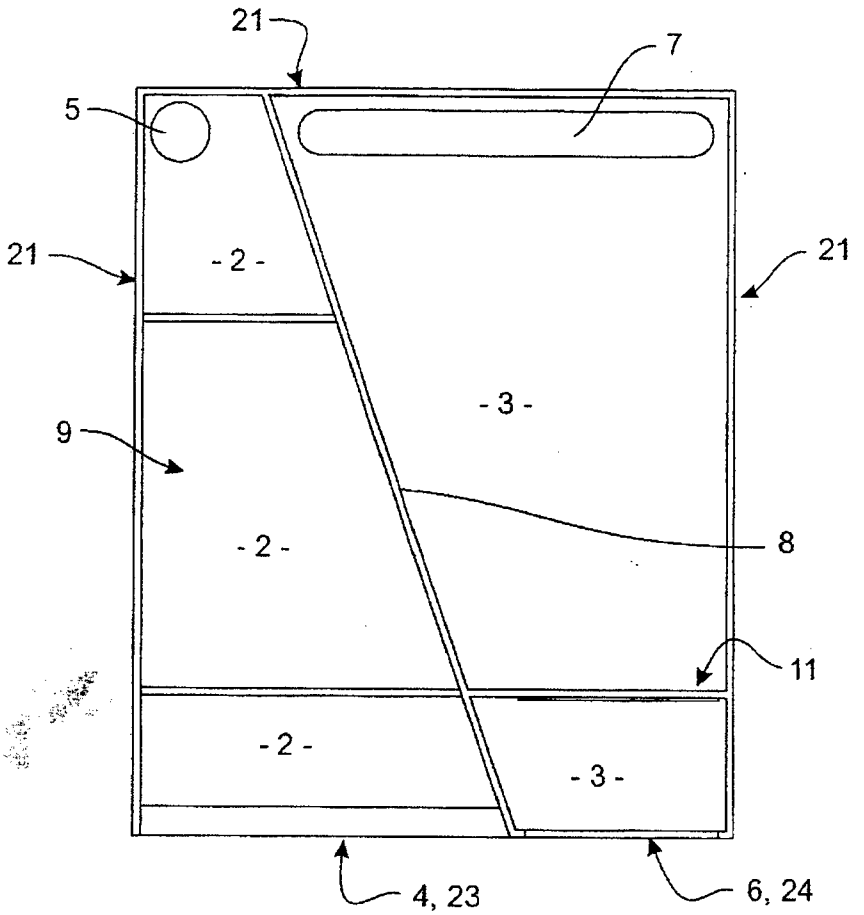


Figure 34

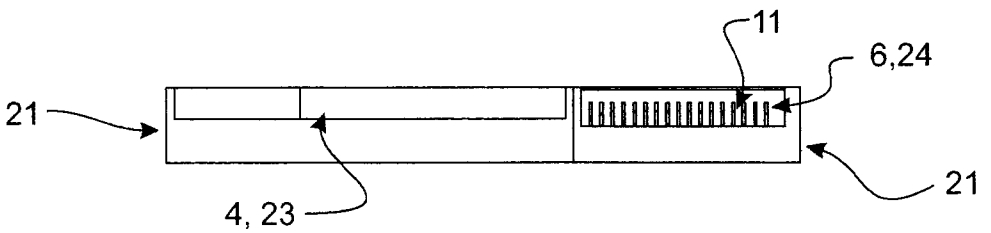


Figure 35

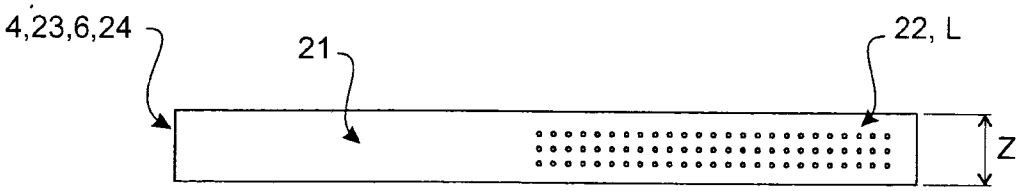


Figure 36

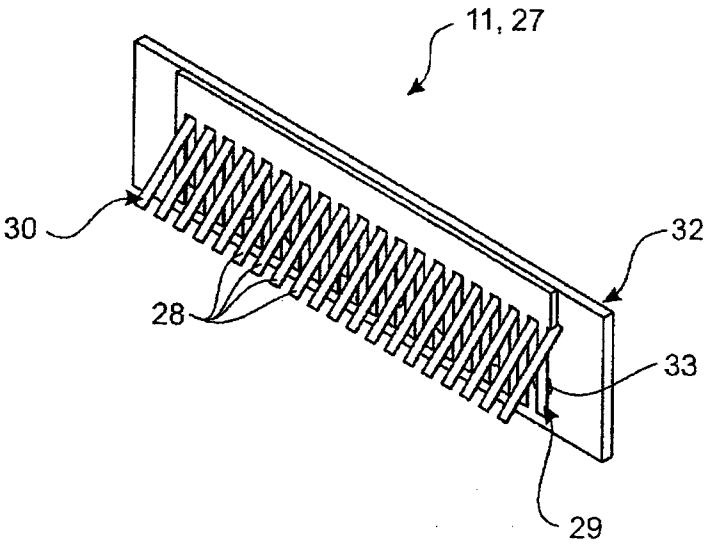


Figure 37

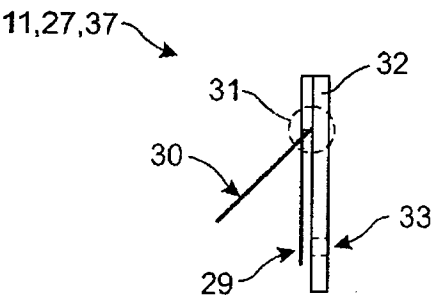


Figure 38

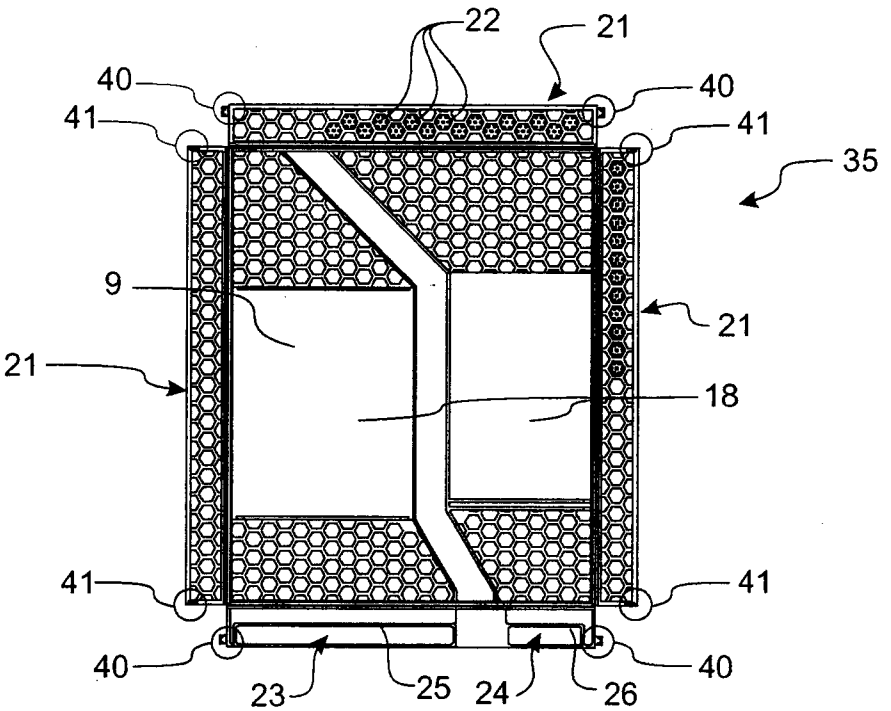


Figure 39

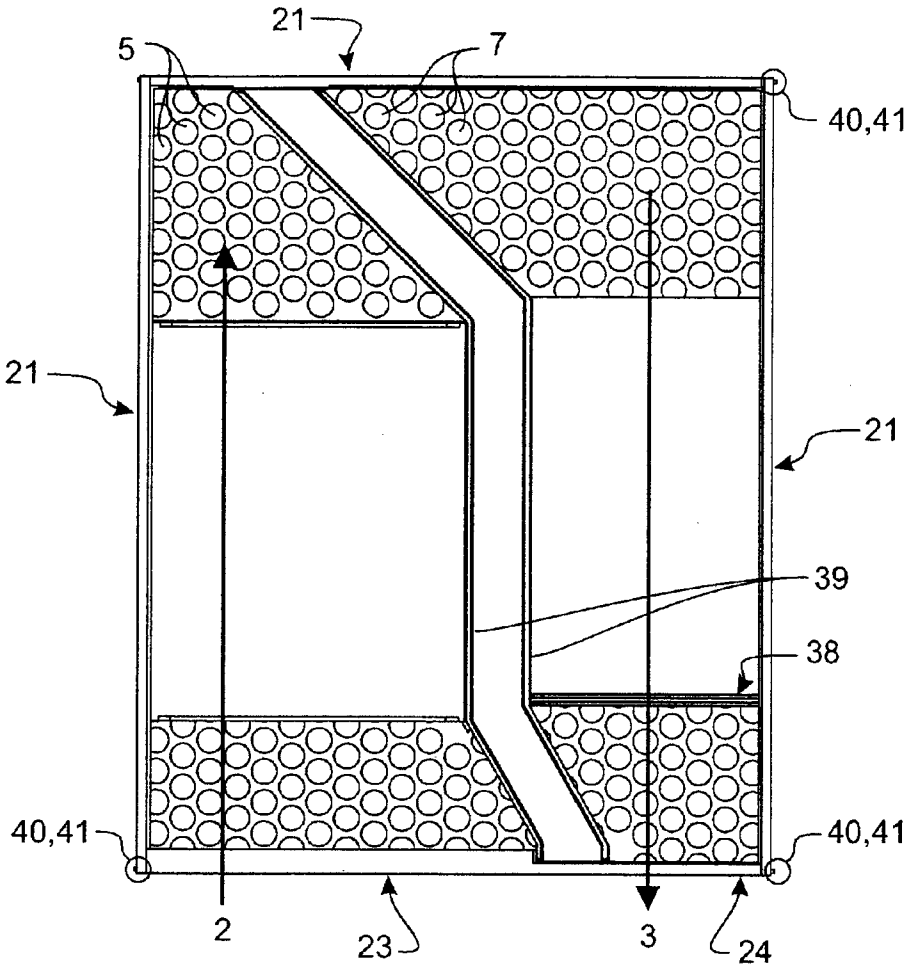


Figure 40

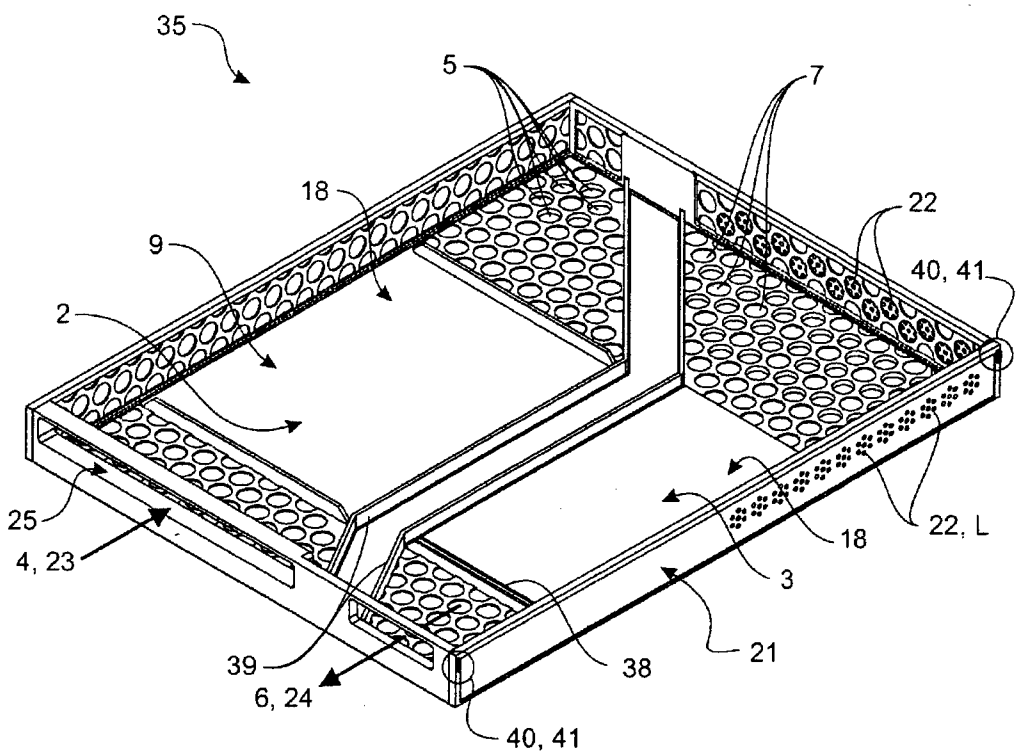


Figure 41

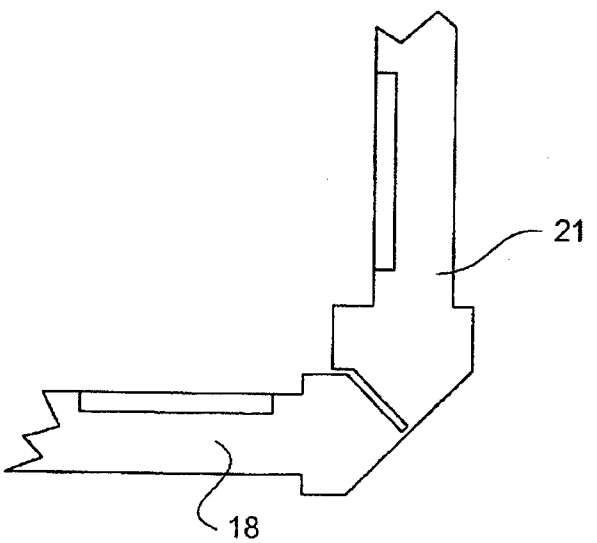


Figure 42

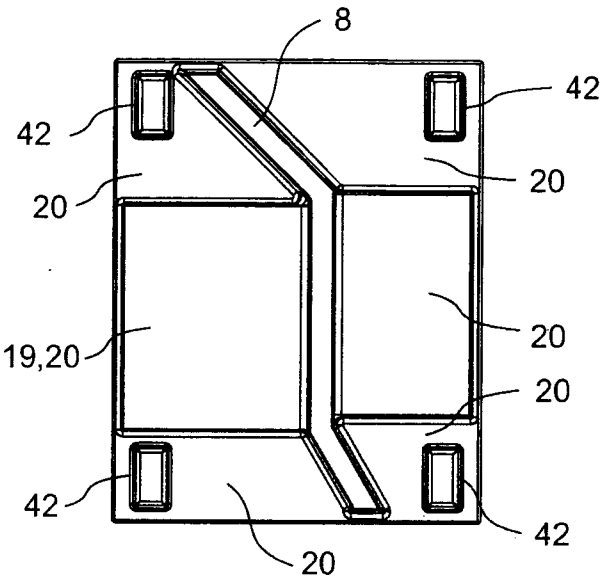


Figure 43

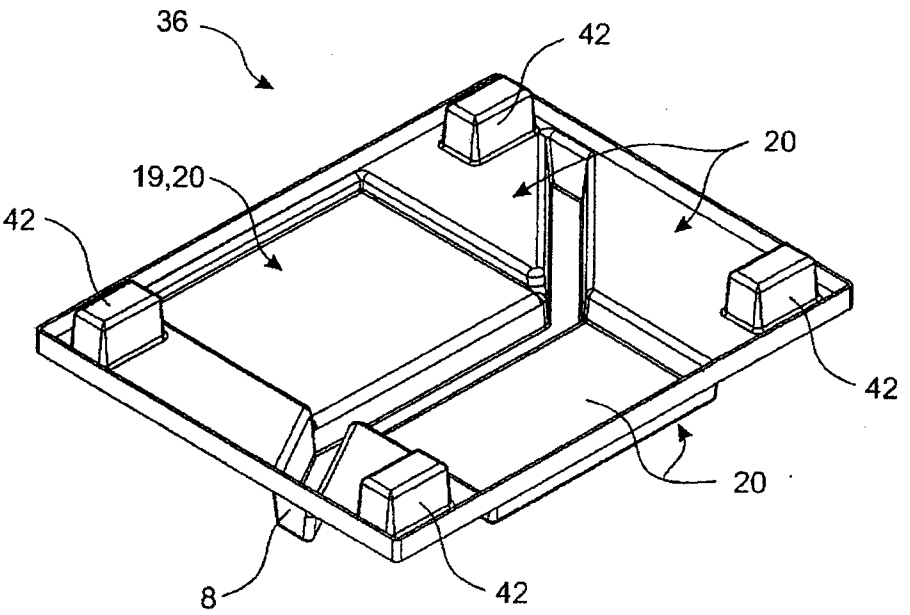


Figure 44

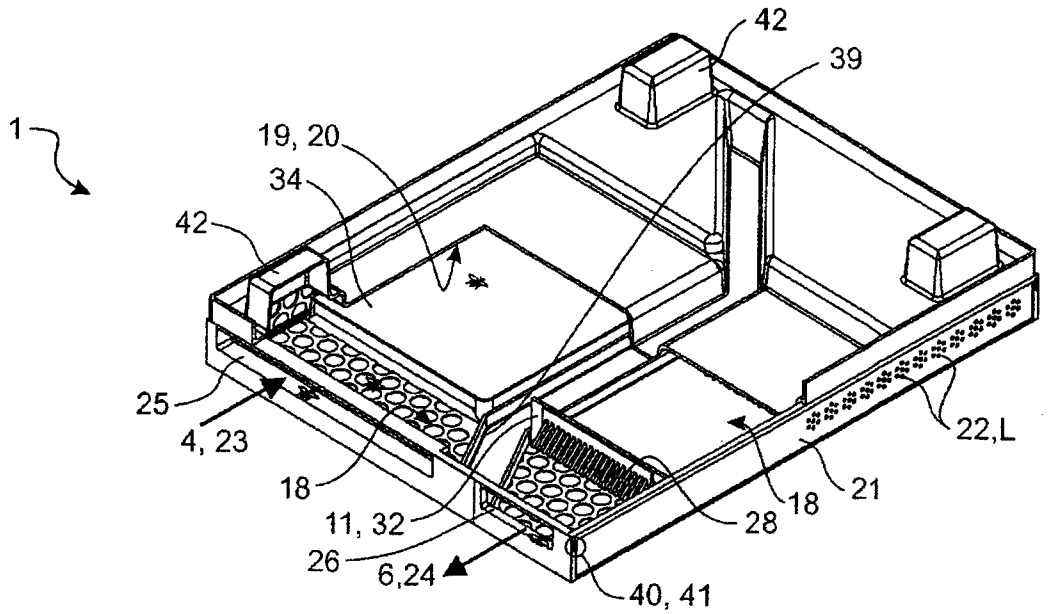


Figure 45

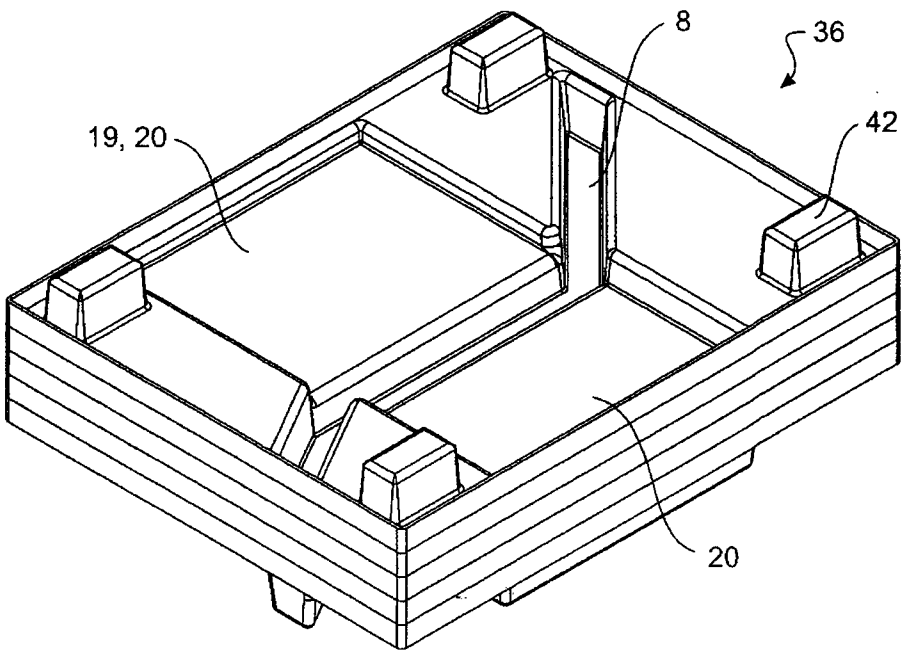


Figure 46

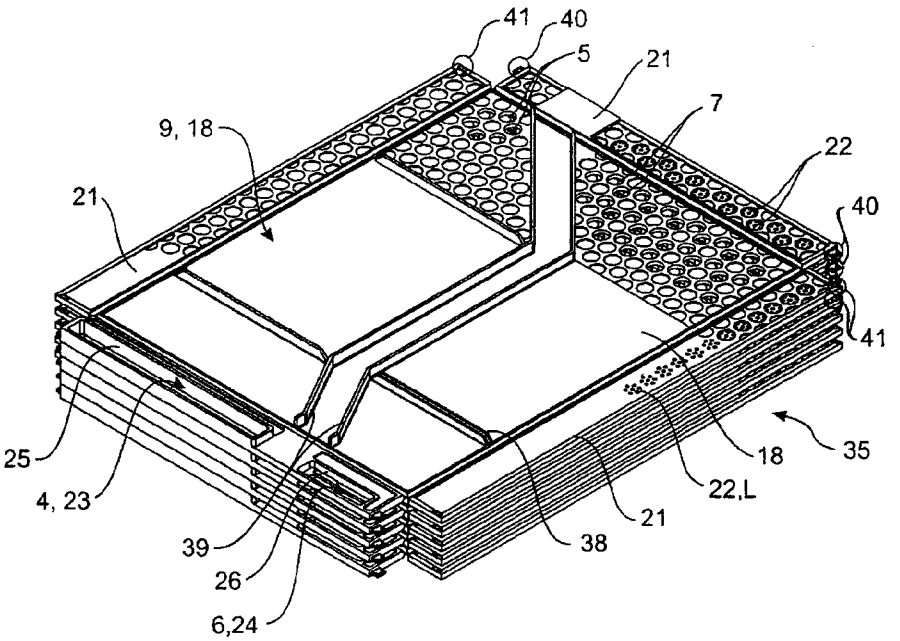


Figure 47

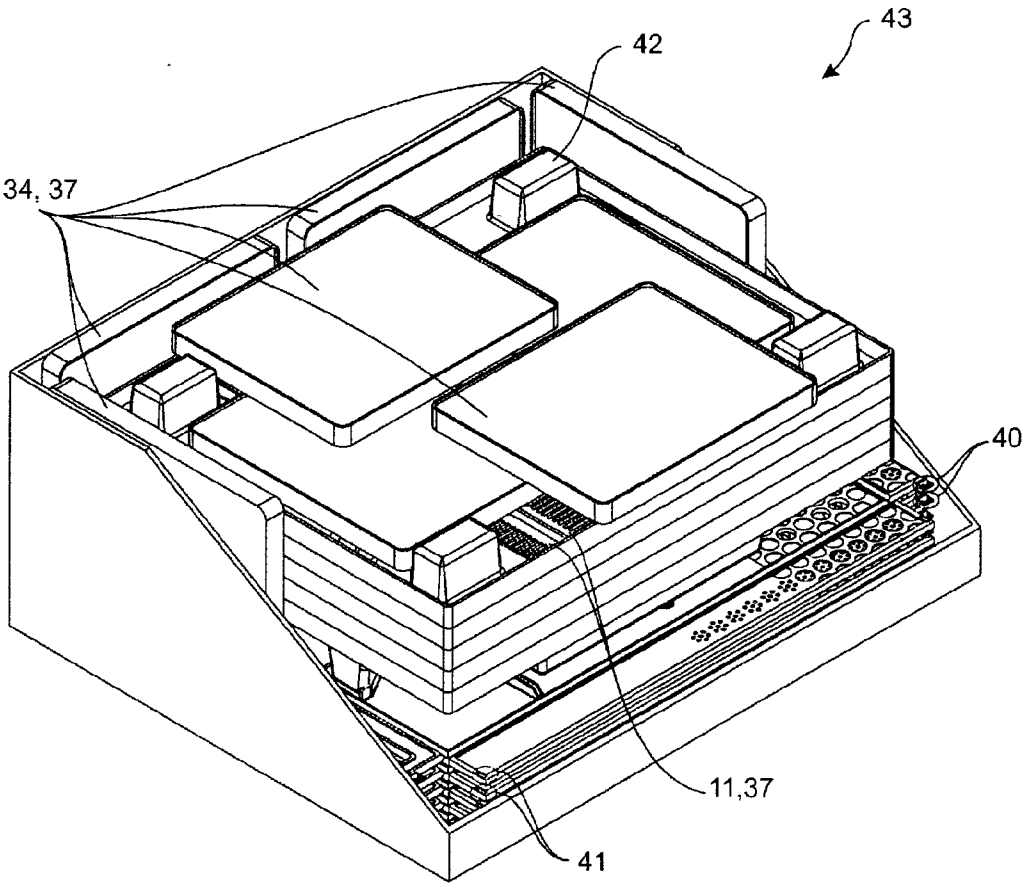


Figure 48

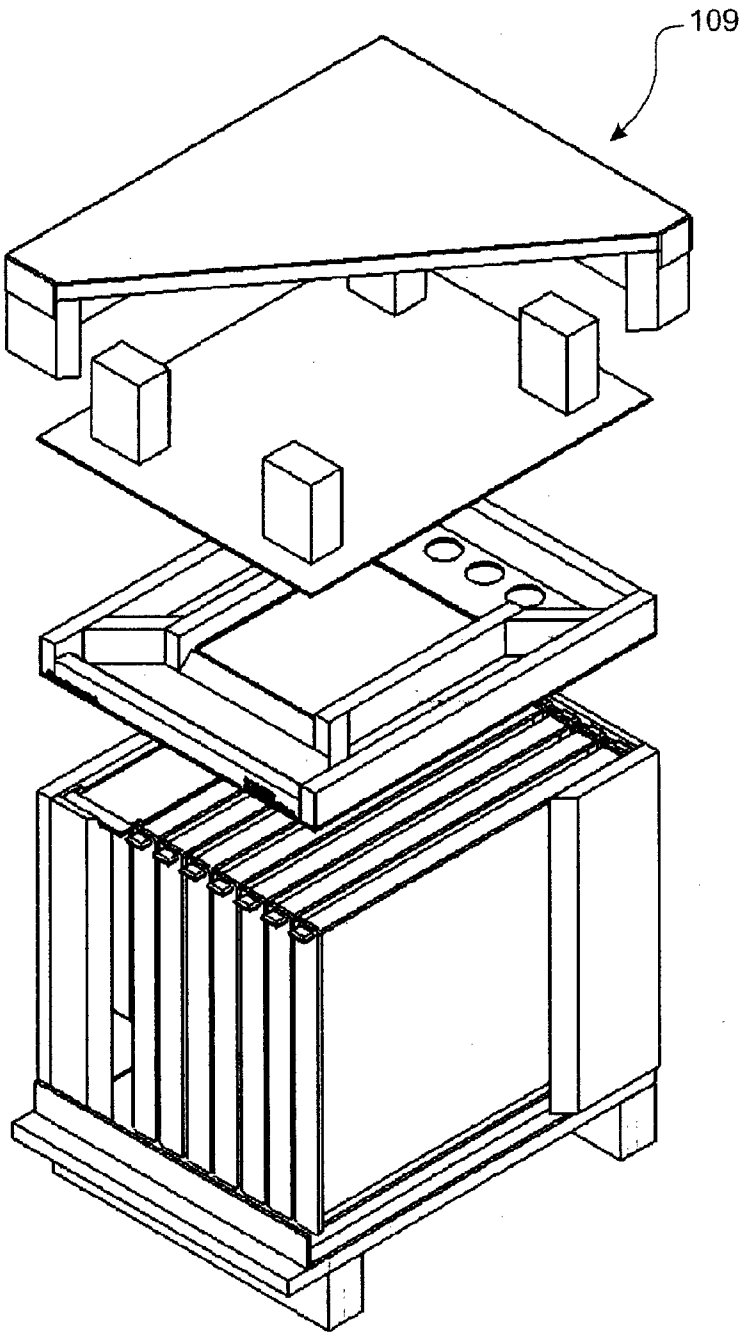


Figure 49

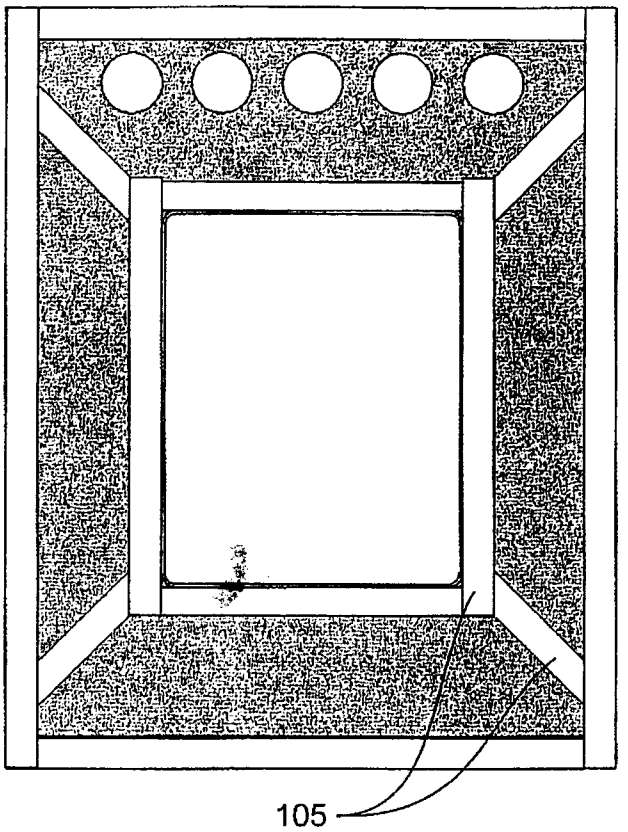


Figure 50

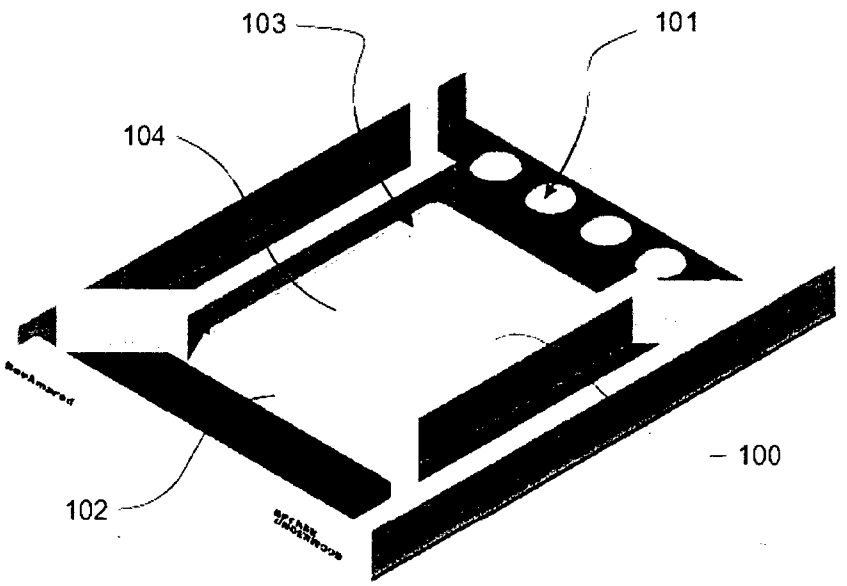


Figure 51

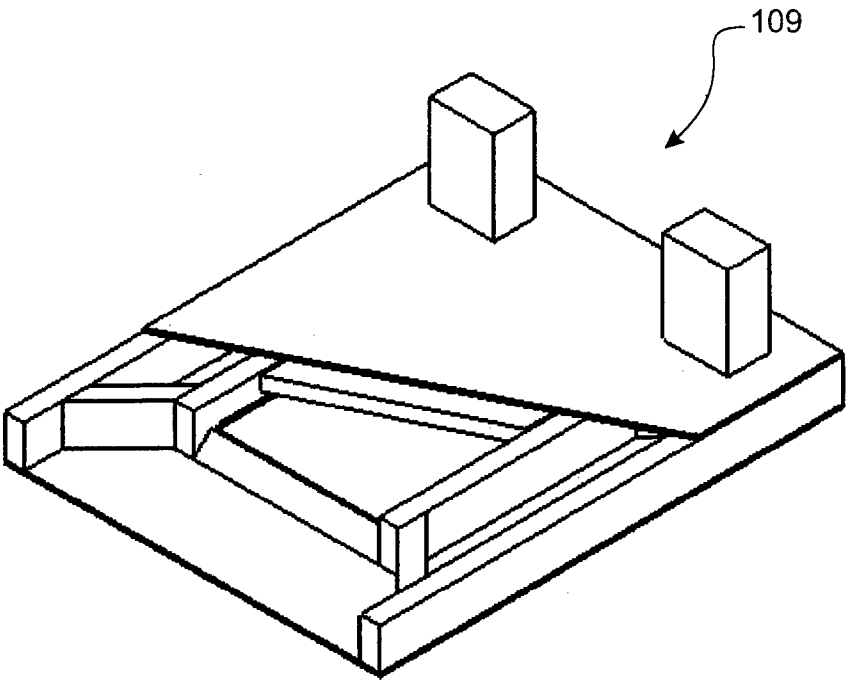
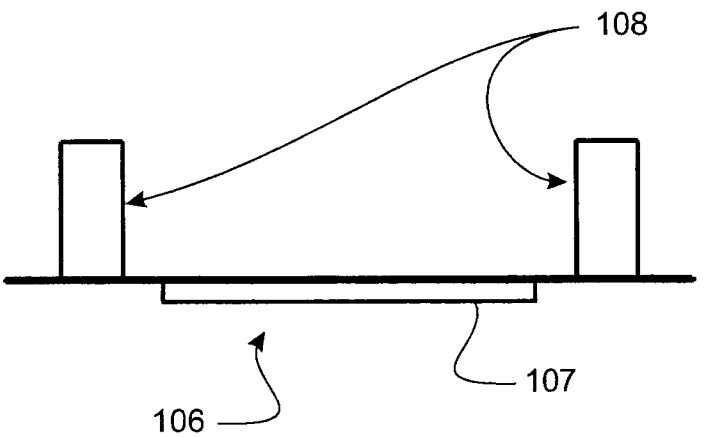


Figure 52



INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2011/000151

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.

A01K 47/06 (2006.01) ***A01M 1/20*** (2006.01)

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)

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 practicable, search terms used)
WPI, EPODOC: A01K 47/06 with Keywords (metarhizium, anisopliae, varroa, anisoplae, bees, bee hive, inoculate) and like terms
STN:FSTA, CAPLUS, BIOSIS, AGRICOLA, Google and Google Scholar with Keywords (ascomycota, metarhizium, anisopliae, entomophthora, varroa, anisoplae, bees, bee hive)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	M. Rodriguez et al. 2009. EVALUATION OF *Metarhizium anisopliae* var. *anisopliae* Qu-M845 ISOLATE TO CONTROL *Varroa destructor* (Acari: Varroidae) IN LABORATORY AND FIELD TRIALS. 69(4): p541-547. Abstract, pages 541-547	1-34
X	I. Stolz. 1999. The effect of *Metarhizium anisopliae* (Metsch.) Sorokin (=*flavoviride*) Gams and Rozsypal var. *acridum* (Deuteromycotina: Hyphomycetes) on non-target Hymenoptera. Pages 10-13	1, 2, 5-16, 18-25
X	Walker. A. Jones. 2004. Fungus Found To Attack Varroa Mites. Page 18. USDA-ARS Beneficial Insects Research Unit. Reported by A. Flores in the Agricultural Research October 2004. Page 18	1-19, 21-34

[X] Further documents are listed in the continuation of Box C [X] 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 application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"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
5 December 2011	08/12/2011
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. +61 2 6283 7999	Authorized officer **RONISH CHAUDHARY** AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No : +61 2 6283 2722

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2011/000151

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	US 5348511 A (GROSS et al.) 20 September 1994	
X	Abstract, col 3 lines 9-30, col 4 lines 24-56, figs. 1-5b	1, 9, 10, 35-37, 39-66, 80-83, 85-89, 91-92, 100-103, 106-120, 122-131
Y	Abstract, col 3 lines 9-30, col 4 lines 24-56, figs. 1-5b	121
	JP 2007-143428 A (KANEKO YUKIYOSHI) 14 June 2007. English abstract retrieved from EPODOC database	
X	Abstract, figs. 1-8b	1, 9, 10, 35-39, 50-53, 67-79, 83, 85, 87, 89, 90, 93-100, 107-115
Y	Abstract, figs. 1-8b	121
	US 5989100 A (KOVACH) 23 November 1999	
X	Abstract, col 3 lines 14-56, col 4 lines 17-30, figs. 1-5	1, 9, 10, 35-37, 39-66, 80-89, 100-102, 104-120, 122-131
Y	Abstract, col 3 lines 14-56, col 4 lines 17-30, figs. 1-5	121

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2011/000151

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[See Supplemental Box 1]

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Supplemental Box 1
(To be used when the space in any of Boxes I to IV is not sufficient)

Continuation of Box No: III

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

This Authority has found that there are different inventions based on the following features that separate the claims into distinct groups:

- Claims 1-34. The feature of *a method for controlling growth of a pathogen of bees* is specific to this group of claims.

- Claims 35-131. The feature of *a device for attachment at, or to, an or the entrance or exit of a nest of an insect colony* is specific to this group of claims.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

When there is no special technical feature common to all the claimed inventions there is no unity of invention.

In the above groups of claims, the identified features may have the potential to make a contribution over the prior art but are not common to all the claimed inventions and therefore cannot provide the required technical relationship. Therefore there is no special technical feature common to all the claimed inventions and the requirements for unity of invention are consequently not satisfied *a priori*.

It is considered that search and examination for the second invention will require more than negligible additional search and examination effort over that for the first invention, and therefore an additional search fee is warranted.