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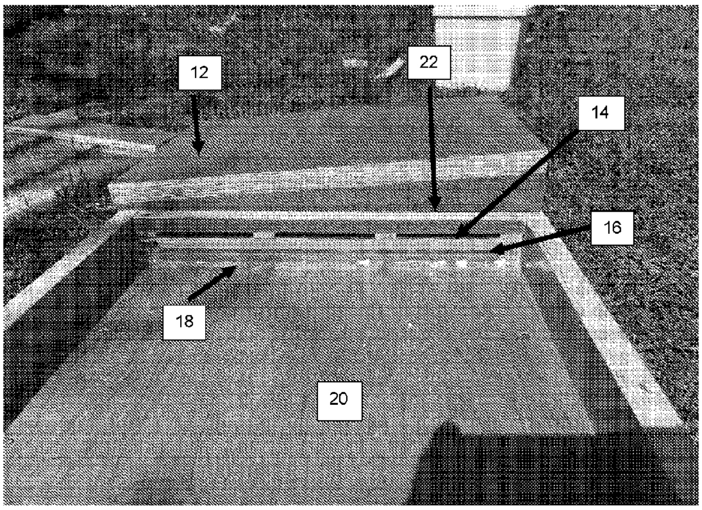

FIG. 1

(57) Abstract: The present invention relates to a biocontrol agent dispensing device for bee biovectors comprising a housing having an first passage with an entry and exit for bees to enter a hive wherein the first passage further comprises one-way flaps at the exit such that bees exiting hive can't leave via the first passage, and a second passage with an entry and exit for bees to exit the hive wherein the biocontrol agent dispensing device further comprises, above the second passage, a mesh layer onto which a biocontrol agent powder may be loaded, such that in use, as bees exit through the second passage their movement disturbs the powder and it falls onto their bodies and the second passage floor, thereby coating the bees. The invention further relates to a method for delivery of a biocontrol agent to plants with the use of the bee biovector dispenser device.

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BEE BIOVECTOR DISPENSER

FIELD OF THE INVENTION

The current invention relates to a biocontrol agent dispensing device for bee biovectors comprising a housing having an first passage with an entry and exit for bees to enter a hive wherein the first passage further comprises one-way flaps at the exit such that bees exiting hive can’t leave via the first passage, and a second passage with an entry and exit for bees to exit the hive wherein the biocontrol agent dispensing device further comprises, above the second passage, a mesh layer onto which a biocontrol agent powder may be loaded, such that in use, as bees exit through the second passage their movement disturbs the powder and it falls onto their bodies and the second passage floor, thereby coating the bees. The invention further relates to a method for delivery of a biocontrol agent to plants with the use of the bee biovector dispenser device.

BACKGROUND OF THE INVENTION

Biovectoring makes use of the ability of insects to transfer products like pollen and pathogens between places. Because of their morphology and behaviour, bees make good biovectors for transferring biopesticides and other biocontrol agents that they carry on their bodies to target plants, and have been used outside Africa for this purpose.

For example, *Apis mellifera* Linnaeus (honey bee; Hymenoptera: Apidae) was used as a biovector to disseminate *Erwinia amylovora* Burril (Enterobacteriales: Enterobacteriacea), a pathogen of pears and apples, but also vector a biocontrol agent of this bacteria, *Pseudomonas fluorescens* Flugge (Pseudomonadales: Pseudomonadaceae). In Finland, honey bees were used as biovectors to transfer *Gliocladium catenulatum* var. (Moniliaceae) to strawberries as an antagonist of *Botrytis cineria* Pers. (Sclerotiniaceae), the cause of grey mould (Smagghe *et al.* 2012). This study found that the use of biocontrol in this way gave the highest marketable yields, and a 90% increase over the untreated controls. This was not only due to a decrease in disease in the strawberries, but also to an increase in yield, due to increased pollination.

Following the success of *A. mellifera* as a biovector, other bee species including bumble bees that have large hairy bodies and make good carriers of powders and use buzz pollination thereby providing an efficient way to release the biopesticide onto target plants have been investigated. Honey bees form a good alternative for a number of reasons. They are pollinators of many plants, there are large numbers of this species and they can be easily transported (within legislation) within their habitation, and they have a large foraging range, meaning they can effectively treat large fields of crops.

Biovectoring has many advantages over traditional methods of applying pesticides. Replacing spraying with bees will make pest control using biopesticides more efficient and save farmers time, effort and resources. This may increase the number of farmers using biopesticides and thereby reduce the usage of chemical pesticides. It will also have positive effects on many ecosystems (chemical pesticides have many non-target effects) and is highly beneficial to bees, as bees are negatively impacted by many current pesticides. The extent of these impacts is large as pesticides are one of the main causes of colony collapse. It is becoming increasingly important to provide an effective alternative to chemical control methods, due to the development of resistance to pesticides. Any plant that is dependent on bee pollination is a potential candidate for bee-vectored biopesticide applications. One example is wild blueberry (*Vaccinium angustifolium* Aiton. (Ericacae)). Bees play a large role in creating a good yield for this crop, by cross-pollinating and inducing fruit set. In most areas, wild blueberries are already farmed alongside managed bees. Wild blueberry yield is also negatively impacted by a number of fungal diseases, including *Monilinia* blight, *Botrytis* blight, *Septoria* leaf spot, grey mould and leaf rust (Delbridge *et al.* 2011). *Botrytis cinerea* is the cause of two of these diseases (*Botrytis* blight and grey mould) and can be particularly damaging to blueberry yields, causing significant economic losses (Howatt 2005). Bees have been used to vector *C. rosea,* which has mycopathogenic effects against *B. cinerea* (Peng *et al.* 1992). However, application of this technology is currently hindered by technical issues with dispensers used. Getting the product onto the bee requires a dispenser. Typically such dispensers operate by placing a powder-based product containing the biopesticide in a tray at the entrance or exit of the bee hive. When the bee exits the hive, it traps the powder and product in its body hairs and subsequently transfers the product coating its body to the plants that it pollinates.

Technical issues with dispensers are the largest limitation to biovectoring. There are two main dispenser types, one-way dispensers and two-way dispensers. Two-way dispensers have proved more effective at loading honey bees with product (Bilu *et al.* 2004). However, although there has been success in this field, a number of authors have noted that the biggest limitation to the biovectoring process is the efficiency of the dispensers (Smagghe *et al.* 2012). For this process to be employed on a large scale, the process of loading the powder onto the bee needs to be highly efficient. Although the grooming behaviour of honey bees may reduce the amount of product that is carried by these bees onto the target plants, grooming behaviour can be influenced by the type of powder used in the product i.e. using flours as carriers decreased grooming in honey bees by 50%, making it more likely for the product to remain attached to the bee (Kevan *et al.* 2008).

Current dispenser designs are also not tailored to the characteristics of the African subspecies of the honey bee. The majority of the studies have been conducted outside Africa. Different races of bees have different morphological, behavioural and physiological characteristics. For example, honey bees in Africa tend to be much smaller than those in Europe and have large differences in foraging behaviour (Winston *et al.* 1983). It is therefore expected that the dispensers that work well in Europe will not be as effective in Africa. Additionally, differences in climate may affect the acquisition of powders, for example, caking of powders may become an issue in humid weather.

To commercialise biovectoring in Africa, it is therefore important for a dispenser to be designed that takes into account the characteristics of African honey bees. The dispenser also needs to be user-friendly and effective for large-scale use.

SUMMARY OF THE INVENTION

According to a first aspect of the invention there is provided a biocontrol agent dispensing device for bee biovectors comprising:

(i) optionally, a bee-hive receiving portion operably supporting a bee-hive where the bee-hive comprises an entrance/exit portal for bees to enter and exit the hive;

(ii) a substantially rectangular bioagent powder dispenser housing, adjacent to the bee-hive receiving portion when present, comprising:

A. a first, substantially rectangular, passage comprising a floor, two side-walls extending upwardly from the floor, and a roof, connecting a first bee entrance and a first bee exit,

wherein the height of the first connecting passage is between about 20 mm to about 40 mm, or any height therebetween and the length of the first connecting passage is between about 100 mm to about 200 mm or any length therebetween, and

wherein the first connecting passage comprises a plurality of elongate opaque blocks alternatingly extending upwardly from the floor of the first connecting passage and downwardly from the roof of the first connecting passage, such that a space between the top of each upwardly extending elongate block and the roof of the first connecting passage and between the bottom of each downwardly extending block and the floor of the first connecting passage is between about 5 to about 10 mm or any length therebetween, and

wherein the first exit is in communication with a bee-hive entrance/exit portal when in operation such that bees fly from the outside into the first entrance and move along the first connecting passage, then exit through the first exit into the bee-hive, and

wherein the first exit comprises a plurality of plastic one-way flaps affixed to an upper portion thereof, extending downwards towards the floor of the first connecting passage, the flaps having a thickness of between about 0.020 mm to about 0.119 mm, or about 0.040 mm to about 0.111 mm, or about 0.020 mm to about 0.060 or any thickness therebetween, whereby bees exiting the first connecting passage through the one-way flaps or exiting the hive cannot re-enter the first connecting passage;

B. situated above the first connecting passage a second, substantially rectangular, connecting passage comprising a floor, two side-walls extending upwardly from the floor and a roof, comprising a second bee entrance and a second bee exit,

wherein the height of the second connecting passage is between about 4 mm to about 10 mm or any height therebetween and the length of the second connecting passage is between about 150 mm to about 250 mm or any length therebetween,

wherein the second entrance is in communication with the bee-hive entrance/exit portal such that bees exiting the hive move through the second bee exit, along the second connecting passage and to the outside through the second bee exit;

C. situated above the second connecting passage and forming the roof of the second connecting passage, a substantially rectangular, optionally removable, mesh layer having a mesh size of between about 200,000 nm to about 2,000,000 nm or any size therebetween, and therefore capable of dispensably supporting a biocontrol agent or mixture of biocontrol agents in powder form for dispensing onto bees moving along the second connecting passage to the second bee exit; and

(iii) situated above the second connecting passage and removable mesh layer, and operably in contact with the biovector powder dispenser housing, a lid covering the second connecting passage and removable mesh layer.

The second connecting passage may further comprise one or more internal walls or supports of the same height as the second connecting passage, thereby dividing the second connecting passage into, for example, two, three, or four passages connecting the second bee entrance and the second bee exit. For example, the one or more internal walls or supports may be parallel relative to each other and the second connecting passage walls. In one non-limiting example the internal walls or supports may define three passages connecting the second bee entrance and the second bee exit each passage having a width of about 110 mm. However, it is to be appreciated that the number and width of passages formed by the internal walls or supports may be different and selected as desired by one skilled in the art.

The mesh layer may be supported by the second connecting passage and/or the internal walls or supports thereof. The mesh layer may be inlaid within the perimeter of the second connecting passage and supported by the internal walls or supports thereof.

The biovector powder dispenser housing may comprise a substantially water-proof seal between the lid and housing such as a foam, rubber or similar water-proof seal such that when in a closed position, the lid connects with the housing preventing water from entering the biovector powder dispenser housing, thereby to water-proof the biocontrol agent dispensing device from rain and ensure that the biocontrol agent powder, when present, does not get wet.
The lid may be a hollow, triangular pyramid-shaped lid, angled to provide for run-off of rain water or dew and sized to cover the second connecting passage and mesh layer of the biovector powder dispenser housing, where one side of the base of the pyramid is hingedly connected to one side of the housing and when in a closed position, the apex of the pyramid connects with the opposite side of the housing.

The biocontrol agent dispensing device may optionally include a biocontrol agent powder capture device comprising a plurality of flat elongate slats, for example two, three, four or five slats sized to extend into the biovector powder dispenser which slots into the housing of the biocontrol agent dispensing device below the mesh layer and below the lid. Biocontrol agent is added after the biocontrol agent powder capture device is in position under the mesh layer by opening the lid of the biocontrol agent dispensing device to add the biocontrol agent powder onto the mesh. Excess powder that falls through the mesh lands on the elongate slats of the biocontrol agent powder capture device rather than on the floor of the second connecting passage and can be removed by removing the biocontrol agent powder capture device.

The first entrance may further comprise a lip for bees to land prior to entering the first entrance. The lip may be comprised of a translucent plastic, more particularly poly(methyl methacrylate) also known as Perspex®.

The second exit may further comprise a lip affixed to the top of the second connecting passage and extending downwardly at an acute angle of between about 40 to about 80 degrees, or any angle therebetween. The lip may be hingedly attached such that it may be it may be reversibly moved in the direction (**A**) by a user as desired. The lip may be comprised of a translucent plastic, more particularly poly(methyl methacrylate) also known as Perspex®. The angled lip prevents bees from entering through the second exit. The translucent plastic provides more light to enter the second passage and encourages the bees to move toward the second exit and the light outside.

The one-way flaps may be made from a transparent or translucent material including a plastic. Preferably they are made from a transparent material.

According to a further aspect of the invention, there is provided a method for delivery of a biocontrol agent to one or more plant(s) by a bee biovector with the use of the biocontrol agent dispensing device of the invention in communication with a bee-hive, comprising:

(I) one or more bee(s) entering the biocontrol agent dispensing device at the first bee entrance, moving along the first passage to the first bee exit and through the plurality of one-way flaps into the bee-hive;

(II) one or more bee(s) exiting the bee-hive and entering the second bee entrance and then moving along the second passage to the second bee exit, wherein the movement of the bee(s) along the second passage causes the biocontrol agent powder located on the mesh layer to be shaken onto the floor of the second passage and body of the bee(s), thereby coating the bee(s) in the biocontrol agent powder;

(III) biocontrol agent coated bee(s) exiting the second passage through the second bee exit to the outside;

(IV) one or more biocontrol agent coated bee(s) delivering the biocontrol agent to one or more plants when pollinating one or more flowers of the plant(s).

The method may comprise a step of inserting a biocontrol agent powder capture device into the biocontrol agent dispensing device of the invention below the mesh layer followed by opening the lid and loading one or more biocontrol agent powder(s) onto the mesh such that excess biocontrol agent powder(s) that falls through the mesh is captured by the biocontrol agent powder capture device rather than landing in the second passage, and can be removed by sliding the biocontrol agent powder capture device out of the biocontrol agent dispensing device of the invention.

The plant may be any plant that is pollinated by bees. Many such plants are known to those skilled in the art.

According to a further aspect of the invention there is provided a biocontrol agent dispensing device substantially as herein described with reference to any one of the illustrated embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be further described, by way of example, with reference to the accompanying drawings.

Figure 1 shows a photograph of the bioagent powder dispenser of the invention from the direction of the bee-hive receiving portion towards the bioagent powder dispenser housing portion, with the lid of the bioagent powder dispenser being in the closed position;

Figure 2 shows a photograph of the bioagent powder dispenser of the invention from above, with the lid in the open position and the mesh in place, along with the capture device placed alongside the bioagent powder dispenser;

Figure 3 shows a photograph of the bioagent powder dispenser of the invention from the direction of the second bee exit, with the lid of the bioagent powder dispenser being in the closed position;

Figure 4 shows a graphical illustration of a cross-sectional view of the bioagent powder dispenser of the invention; and

Figure 5 shows a graphical representation of and exploded perspective view of the bioagent powder dispenser.

DETAILED DESCRIPTION OF THE INVENTION

The current invention relates to a biocontrol agent dispensing device for bee biovectors comprising a housing having an first passage with an entry and exit for bees to enter a hive, and a second passage with an entry and exit for bees to exit the hive wherein the second passage further comprises one-way flaps at the entry such that bees entering the second passage cannot return to the hive and wherein the biocontrol agent dispensing device further comprises, above the second passage, a mesh layer onto which a biocontrol agent powder may be loaded, such that in use, as bees exit through the second passage their movement disturbs the powder and it falls onto their bodies and the second passage floor, thereby coating the bees. The invention further relates to a method for delivery of a biocontrol agent to plants with the use of the bee biovector dispenser device.

The following description of the invention is provided as an enabling teaching of the invention, is illustrative of the principles of the invention and is not intended to limit the scope of the invention. It will be understood that changes can be made to the embodiment/s depicted and described, while still attaining beneficial results of the present invention. Furthermore, it will be understood that some benefits of the present invention can be attained by selecting some of the features of the present invention without utilising other features. Accordingly, those skilled in the art will recognise that modifications and adaptations to the present invention are possible and can even be desirable in certain circumstances, and are a part of the present invention. The applicant has designed a biopesticide dispenser that meets all of the needs of an effective dispenser as set out in Table 1, while taking into account the morphology and behaviour of African honey bees that works well in the African context.

Table 1. Successful dispenser design criteria

Criterion	Rationale
MAIN ISSUE: Refill time > one day	Efficiency necessary for a commercial product, refilling many dispensers daily takes a lot of time and effort
Load bees with an adequate amount of product	Bees need to carry the correct amount of product to the plant so that it can gain protection
Does not hinder the bee	Pollination needs to still occur in order for the dispenser to be effective
Loaded in an area where it will not be cleaned off by the hygienic honey bee	The powder needs to remain on the bee until it reaches the flower
Loaded onto an area of the bee that makes contact with the target plant when the bee lands to collect nectar and pollen and is not specific to a single cropping system	The powder needs to be deposited on the flower in any cropping system.
No caking of powder	Reduces maintenance
Does not contain moving parts	Reduces maintenance

All bees coated with powder	Increases the chances of a plant receiving the biopesticide
Easy to attach to the hive	Important to save time in large scale operations

EXAMPLE 1

METHODS

General design

A number of successive prototypes of dispensers were designed. Subsequent to the first prototype dispenser, designs were based on the general model of a two-way dispenser and improvements were made from there. Mommerts *et al.* (2010) determined that the optimal path length of a dispenser is 20 cm in terms of the ability of the dispenser to load a bee with product. Shorter paths failed to saturate the bee with product, while longer paths induced grooming behavior. This was taken into account during dispenser design. The biopesticide powder generally used in dispensers was substituted with corn flour. The dispensers were fitted to hives at Rhodes University. The bees were allowed to accustom to the new dispenser for two days, as studies by Smagghe *et al.* (2012) showed that that process generally takes bees a day or two.

Once the dispenser was working, quantitative data was collected to quantify differences between small changes to the dispenser.

Dispenser entrance flaps

In order to ensure that bees enter the hive through the hive entrance and exit through the hive exit, flaps that the bees pass through were attached to the entrance of a prototype dispenser in such a way that the bees could push them open to pass into the hive but could no longer exit through the entrance. In order to determine the ability of bees to push open plastic flaps a device was developed that allowed for the bees to be confined in a small area and presented with the flaps and their behaviour was noted (passing through the flaps or remaining in the confined area). Ten bees were sampled and the proportion of bees that moved through the flaps were recorded. Flaps of varying thickness (0.040 mm, 0.111 mm, 0.119 mm, 0.140 mm) and transparency (translucent and transparent) were tested. The first flaps tested were translucent. To eliminate the chance that the bees did not pass through the flaps because they were translucent, transparent flaps were used in the rest of the tests. The first transparent flaps were 0.140 mm, increasingly thinner flaps were then used until the bees were able to pass through. Flaps were measured using a micrometre screw gauge (Moore & Wright, model 200-01, 0.01 mm precision).

Bee morphology

The morphology of the African honey bee was also incorporated into the design of the prototype dispensers by considering the bee space and cell size of *Apis mellifera scutellata* (Faji *et al*. 2018). Bees commonly found in South Africa are from the subspecies *capensis* or *scutellata,* or are hybrids of these two subspecies (Steele et al 1998). However, there is very little available information of the bee space and cell size of these bees. The average size of *A. mellifera capensis* bees is very similar to that of A*. mellifera scutellata* bees (Faji et al 2018; Hepburn & Crewe 1991), so information from this subspecies was used to determine the exit height for the prototype dispensers.

Bee space should allow for two bees to easily pass each other, while a single bee should just be able to fit into the cell space. The applicant has found that the ideal height of the exit path of a dispenser should lie somewhere between the cell diameter and half the distance of the bee space to ensure that the bee makes contact with both sides of the exit channel, and therefore gets powder on both sides of its body, but does not struggle to fit through the pathway. Bee space for the South African subspecies van vary, but generally lies between 6-10mm. Therefore, initially the design of the dispensers was made with the exit path representing bee space at 10 mm and the hive exit height was lowered to 5 mm to ensure that the dispenser was tailored for South African bees, so that they acquired powder on both the dorsal and ventral sides of their bodies.

Assessment

The prototype dispensers were observed after two days of being attached to identify any issues. The number of bees leaving the exit, entering the exit, entering the entrance and leaving the entrance were recorded in one minute intervals. This was repeated 30 times over 3 days (10 random times per day) and the results were recorded. These tests were only carried out on warm days so that an adequate number of bees were leaving and entering to make the test feasible. The proportion of bees exiting the exit and entering the entrance were then calculated to compensate for the effect of the weather on the bees. A general linear model was run in Statistica (TIBCO Software Inc. 2018) to determine if the powder had a significant effect on the movement of the bees, and whether any dispensers were working better than others in terms of the bees entering and exiting via the correct areas.

RESULTS

Each new dispenser was an improvement on the previous one, targeting specific issues that were realized once each dispenser had been attached (Table 2).

Table 2: Applicant dispenser designs and their corresponding issues

Dispenser	Design	Issue
1	Spring loaded powder holder Powder applied from the bottom- pushed up through mesh Single gap for entrance and exit- one way dispenser.	Bees walked on the roof and avoided the powder Powder wasted as it was being brought back into the hive Support bar compacted the power preventing it from moving through the mesh
2	Dispenser inverted Springs removed Powder applied through mesh from the top Separate entrance added	Bees entered and exited through the entrance and struggled to get back into the hive Powder leaked out of the sides Issues with aligning the dispenser with the hive
3	Landing Platform added at the entrance Plexiglass added at the exit at an angle to prevent bees entering through this opening	Bees exiting through entrance in large numbers

	Canopy clips added on sides to prevent powder leaking out Pins added to the front of the dispenser to aid in aligning the dispenser with the hive	
4	One-way flaps added on the entrance of the dispenser that can move to let bees into the hive from the outside of the hive but not from the inside of the hive, preventing the use of the entrance as an exit. Ramp added onto the entrance to limit the light entering the hive from the entrance to decrease the likelihood of bees leaving via the entrance	Bees entering the entrance failed to push open the flaps and turned around and left. This led to bees searching for an alternative entrance into the hive. This dispenser was promptly removed to avoid putting the hive at risk.
5	Two of the flaps were taped up to provide an area from which bees could enter into the hive, while still reducing the likelihood of bees exiting from this path, as the area from which they could do so was vastly reduced in comparison to the exit channel Powder removed from the dispenser	Bees exiting through entrance and entering through exit
6	Powder added	Bees exiting through entrance and entering through exit, but to a lesser extent as proved by the time tests
7	Previous flaps removed and very thin transparent flaps that the bees were able to push through (as shown by the flap tests) added to the entrance. Height of exit path decreased so that the average bee will touch both sides of the exit path when leaving the hive.	Bees using the dispenser as intended. No bees entering into the exit, extremely low number of bees infrequently exiting out of the entrance

Large issues were identified with the first four dispenser designs that made them impractical. Dispensers 5, 6 and 7 all worked adequately, dusting the bees with powder on both side of their bodies when they left the dispenser from the exit. However, bees were still entering and exiting from the wrong areas of Dispenser 6. For the dispenser to be effective, this needed to be minimised. Significantly more bees entered through the entrance of Dispenser 6 than Dispenser 5 ($F_{(2,177)}$ =

177.72; p-value = 0.000022) and significantly more bees entered through the entrance of Dispenser 7 than both Dispensers 5 and 6 ($F_{(2, 177)}$ = 177.72; p = 0.000 (Table 3). Because these values are proportions of the total number of bees entering, significantly fewer bees were entering the exit of Dispenser 7 than Dispensers 5 and 6 and significantly fewer bees entered the exit of Dispenser 6 than Dispenser 5. In Dispenser 6 a number of bees were observed flying into the exit and turning around and flying out when they made contact with the powder.

Table 3: Results of Tukey's post hoc tests done on the data of bees entering through the entrance, showing the p-values for each dispenser compared to others.

Dispenser	P-value Dispenser 5	P-value Dispenser 6
Dispenser 6	0.000022	
Dispenser 7	0.000022	0.000023

Significantly more bees exited through the exit of Dispenser 6 than for Dispenser 5 ($F_{(2, 87)}$ = 61.400; p = 0.000) and significantly more bees exited through the exit of Dispenser 7 than for both Dispensers 5 and 6 ($F_{(2, 87)}$ = 61.400; p = 0.006) (Table 4). Significantly fewer bees were exiting the entrance of Dispenser 7 than Dispensers 5 and 6, and significantly fewer bees exited the entrance of Dispenser 6 than Dispenser 5. Although Dispenser 6 worked fairly effectively, bees were still entering and exiting incorrectly. Dispenser 7 decreased these numbers.

Table 4: Results of Tukey's post hoc tests done on the data of bees leaving through the exit showing the p-values for each dispenser compared to others.

Dispenser	P- value Dispenser 5	P- value Dispenser 6
Dispenser 6	0.000107	
Dispenser 7	0.000107	0.006800

Bees failed to make contact with both sides of the exit channel in Dispensers 1-6. After the reduction in exit path height in Dispenser 7, bees made contact with both sides, increasing powder load (statistical results not shown). The dispensers created in this study have a much longer refill period than dispensers that have been previously reported on. Dispenser 7 can hold roughly 11 times as much powder as many of the previous dispensers. Clumping was minimal and only became a problem after days where there was rain. In the flap tests, all 10 bees were able to push through the thinnest flaps (0.040 mm). The bees failed to push through the 0.140 mm, 0.119 mm and 0.111 mm flaps in all of the repetitions.

DISCUSSION

The applicant was able to design a bee biovector dispenser that solved all of the major issues faced by current biopesticide dispensers as discussed in Mommerts & Smagghe (2011). One-way dispensers are extremely inefficient, failing to load the bees with an adequate amount of powder and needed to be refilled daily. The lack of a separate entrance in these dispensers leads to the bees searching for an alternative way to enter into the hive to avoid the powder. Two-way dispensers designed to deal with the issues faced by one-way dispensers have proved effective at loading bees with adequate amounts of powder (Bilu *et al* 2004), but there are other issues that contribute to the efficiency of a good dispenser that still need to be addressed (Table 1). In particular, in order for all of the existing two-way dispensers to load bees with biovector product powder effectively, they need to be loaded frequently; requiring at least daily reloading.

Regular refills are a major issue with current biopesticide dispensers, as a commercial product needs to run for long periods of time without needing to be refilled. Dispenser 7 was able to hold roughly 11 times as much powder as many of the current dispensers, meaning that it will last 11 times as long and need to be refilled far less frequently. Dispenser 7 loaded the bees with a large amount of product (statistical results not shown), without the ability of the bees to fly being hindered. Regardless, the amount of powder on the bee can be easily adjusted by changing the size of the mesh in the applicant's dispenser. To determine the exact amount of powder desired to be on each bee in a particular case, the number of conidia of a particular flower per gram must be taken into account, as well as the type and purpose of the biopesticide, the amount needed per plant, and the type of crop. The ability to adjust the amount of biopesticide powder deposited on each bee by changing the mesh size is therefore an advantage of the applicant's dispenser.

The applicant determined that coating the bees with the powder from both the top and bottom reduced the likelihood of the powder being cleaned off by the bees. This will therefore increase the efficiency of the biovectoring process. Cornflour was used in all the dispensers as it has a very small particle size, which makes it more likely to lodge between the hairs of a bee. Acquisition of conidia is strongly linked to the particle size and shape of the product, with smaller, irregularly-shaped particles leading to more conidia being picked up per bee (Al-Mazra'Awi *et al.* 2007). This is due to the higher surface area per unit mass of small particles, leading to greater adhesion to both the bee and the conidia (Al-Mazra'Awi *et al.* 2007) Using a flour instead of a talc powder has been shown to reduce grooming of the honey bees by up to 50% (Kevan *et al*. 2008). For these reasons, a number of biovectoring systems mix corn flour with the biopesticide to act as a carrier (Al-Mazra'Awi *et al.* 2007). The transition from Dispenser 7 applying only corn flour to applying a biopesticide will be easy to achieve.

Due to differences in flower morphology, it is not practical for a commercial dispenser to target one specific area of the bee, as different flowers will make contact with different parts of the bee. Many of the existing dispensers have been created for a single cropping system and therefore target specific areas of the bees. However, the applicant's dispenser aimed to get the powder on both the dorsal and ventral side of the bees so that the dispenser can be used for a number of cropping systems. This was achieved by changing the way the powder was deposited onto the bees. The applicant observed that most of the bees walked on the ceiling, meaning that where powder is placed on the floor of the dispenser, although the first few bees got the powder on their backs as they walked through, once the powder had all been pushed out, the bees were able to largely avoid the powder and no powder was dusted onto the underside of the bees at any point.

The applicant was able to determine that inversion of the dispenser, so that the powder came through the mesh from the top, meant that as the bees passed through and brushed the mesh with either their backs or walked on the mesh, some powder would fall through onto the solid bottom of the exit so that the next bee that walks through will be dusted with powder on both its dorsal and ventral side. Additionally, by decreasing the height of the exit path for Dispenser 7 the applicant was able to ensure that the bees were touching both sides of the exit when leaving such that powder was knocked down from the mesh each time a bee walked through, coating them on both sides.
The applicant's Dispenser 7 was also designed such that clumping of the biovector powder was minimal, as the powder is kept in a box above the exit, and only a small amount is knocked down each time a bee passes through. The powder on the exit path is constantly replaced as the bees exit and never builds up to be too thick, meaning that is does not accumulate moisture and clump. The powder inside the box does not come into contact with the bees until it is knocked down, so does not accumulate moisture from the bees which has previously been an issue (Kovach 1999).

The applicant's dispensers also have no movable parts and do not require input from a user until the powder is finished, while being easy to attach to the hive. This means maintenance costs are low and efficiency is high. The results of the tests that looked at the number of bees entering the entrance and exiting the exit gave good insight into the effect of the powder on the bees' behaviour.

Dispenser 7 was designed to decrease the number of bees exiting and entering incorrectly. The height of the exit path was decreased to match the average height of an African honey bee. This increased the amount of powder knocked down and therefore deposited on each bee, while decreasing the number of bees using the exit channel as an entrance. This was effective as it was more difficult for the bees to fly into the small gap and more powder was present around the exit, making it less attractive to the bees from the outside. Thin 0.040 mm one-way flaps were also added to the entrance after the flap tests proved that the bees could push these open from the correct side. This greatly decreased the number of bees leaving from the entrance as bees approaching the entrance from inside the hive were unable to pass through into the entrance channel due to the length of the flaps preventing them moving in reverse. Bees leaving from the wrong channels decrease the efficiency of the dispenser in a number of ways. A bee leaving from the entrance will not be dusted with powder, meaning that the plant that it lands on will not receive the biopesticide (Bilu *et al.* 2004). If a large proportion of the bees are doing this, a large proportion of pollinated flowers will not be treated. If the bees enter from the exit, they will be moving product into the hive where it will either be incorporated into the honey, decreasing its quality, or cleaned off (Bilu *et al.* 2004). Both of these scenarios lead to the expensive biopesticide being wasted, impairing efficiency of the dispenser. In Dispenser 7 the vast majority of bees exited and entered from the correct channels, increasing efficiency and decreasing wastage. By targeting all of the issues faced by current biopesticide dispensers and being created to fit the dimensions of the African honey bee, the applicant, with Dispenser 7 has developed a prototype dispenser that will allow for effective biovectoring of biopesticides in Africa.

EXAMPLE 2

The applicant continued with development of Dispenser 7 to design a further iteration of the dispenser for commercial use. The design of the dispenser for commercial use includes the following changes relative to Dispenser 7.

- The exit and entrance pathways have been swapped around so that the bees exit from the top and enter from the bottom.
- The mesh sheet used has been changed and can be a polymer plastic or metal mesh
- The mesh sheet is now removable

- The biocontrol agent powder filling method has been modified from having to remove the dispenser and invert it to add the powder, to inserting a removable slat into the dispenser below the mesh sheet layer, adding the biocontrol agent powder onto the mesh such that excess powder that falls through the mesh is captured by the removable slat which can then be removed with the excess powder, all while the dispenser remains on the hive
- The height of the exit pathway has been to between about 4 mm - 10 mm
- The height, length and design of the entrance pathway has been modified
- The way in which the dispenser is attached to the hive has been modified
- Changing the shape of the lid is now angled to allow for adequate runoff of rain or dew
- Sealing the powder container with foam thereby to ensure that it is water-tight
- Adapting the poly(methyl methacrylate) exit flap such that it is hingedly attached and modifying the acute angle of the poly(methyl methacrylate) exit flap to between about 40 degrees - 80 degrees
- Adding internal supports to the exit pathway of the dispenser
- Using translucent plastic at the exit of the dispenser to let in more light

The design of the improved dispenser of the invention for commercial use is shown in Figure 4. In this embodiment of the invention, the biocontrol agent dispensing device (**10**) for bee biovectors comprises a bee-hive receiving portion (**20**) for supporting a bee-hive (**38**) where the bee-hive (**38**) comprises an entrance/exit portal for bees to enter and exit the hive (**38**).

In addition, the biocontrol agent dispensing device (**10**) comprises a substantially rectangular bioagent powder dispenser housing, adjacent to the bee-hive receiving portion (**20**) when present, comprising a first, substantially rectangular, passage (**34**) comprising a floor, two side-walls extending upwardly from the floor, and a roof and comprising a first bee entrance (**32**) and a first bee exit (**16**) wherein the height of the first connecting passage (**34**) is between about 20 and about 40 mm and the length of the first connecting passage (**34**) between the first bee entrance (**32**) and first bee exit (**16**) is between about 100 to about 200 mm, more typically between about 120 to about 160 mm.

The first exit (**16**) is in communication with a bee-hive entrance/exit portal when in operation such that bees fly from the outside into the first entrance (**32**) and move along the first connecting passage (**34**), then exit through the first exit (**16**) into the bee-hive. The first connecting passage (**34**) comprises a number of elongate blocks (**40**) made from an opaque material extending alternatingly upwardly from the floor or extending downwardly from the roof of the first connecting passage (**34**) such that the bees that enter through the first entrance (**32**) walk up and over the elongate blocks (**40**) and along the first connecting passage (**34**) towards the hive (**38**). The space between the top of each upwardly extending elongate block (**40**) and the roof of the first connecting passage (**34**) and between the bottom of each downwardly extending block (**40**) and the floor of the first connecting passage (**34**) is between about 7 to about 10 mm. The elongate blocks are typically between about 10 mm to about 33 mm in height x 8.5 mm width x 8.5 mm depth. The opaque elongate blocks (**40**) function to block the light from the outside from entering the hive (**38**) through the first exit (**16**) so that bees inside the hive (**38**) do not try to leave the hive through the first connecting passage (**34**) due to being attracted to the light.

The first exit (**16**) comprises a plurality of plastic one-way flaps (**18**) affixed to an upper portion thereof, extending downwards towards the floor of the first connecting passage (**34**), the flaps (**18**) having a thickness of between about 0.020 mm to about 0.119 mm, or about 0.040 mm to about 0.111 mm, or about 0.020 mm to about 0.060, whereby bees exiting the first connecting passage (**34**) through the one-way flaps (**18**) or exiting the hive (**38**) cannot re-enter the first connecting passage (**34**). Situated above the first connecting passage (**34**) is a second, substantially rectangular, connecting passage (**36**) comprising a floor and two side-walls extending upwardly from the floor, comprising a second bee entrance (**14**) from the hive (**38**) and a second bee exit (**28**), wherein the height of the second connecting passage (**36**) is between about 4 mm to about 10 mm and the length of the second connecting passage (**36**) is between about 150 and about 250 mm between the second bee entrance (**14**), and the second bee exit (**28**), wherein the second bee entrance (**14**) is in communication with the bee-hive entrance/exit portal such that bees exiting the hive (**38**) move through the second bee entrance (**14**), along the second connecting passage (**36**) and to the outside through the second bee exit (**28**); and

Situated above the second connecting passage (**36**) and forming the roof of the second connecting passage (**36**) is a substantially rectangular, optionally removable, mesh layer (**24**) having a mesh size of between about 200,000 nm to about 2,000,000 nm and therefore capable of dispensably supporting a biocontrol agent or mixture of biocontrol agents in powder form for dispensing onto bees moving along the second connecting passage (**36**) to the second bee exit (**28**).

In addition, situated above the second connecting passage (**36**) and removable mesh layer (**24**), and operably in contact with the biovector powder dispenser housing, is a hinged lid (**12**) covering the second connecting passage (**36**) and removable mesh layer (**24**). The mesh layer (**24**) may be supported by the second connecting passage and/or the internal walls or supports thereof. The mesh layer may be inlaid within the perimeter of the second connecting passage (**36**) and supported by the internal walls or supports thereof.

The biovector powder dispenser housing may comprise a substantially water-proof seal (**22**) between the lid (**12**) and housing such as a foam, rubber or similar water-proof seal such that when in a closed position, the lid (**12**) connects with the housing preventing water from entering the biovector powder dispenser housing, thereby to water-proof the biocontrol agent dispensing device from rain and ensure that the biocontrol agent powder, when present, does not get wet. The lid (**12**) may be a hollow, triangular pyramid-shaped lid such that the angle of the lid allows for rain or dew water run-off. The lid (**12**) is sized to cover the second connecting passage (**36**) and mesh layer (**24**) of the biovector powder dispenser housing, where one side of the base of the pyramid is hingedly connected to one side of the housing and when in a closed position, the apex of the pyramid connects with the opposite side of the housing.

The biocontrol agent dispensing device (**10**) may optionally include a biocontrol agent powder capture device (**26**) comprising a plurality of flat, elongate slats which, in operation, slots into the housing of the biocontrol agent dispensing device below the mesh layer (**24**) and onto which excess biocontrol agent powder may be captured.

Biocontol agent powder can be added to the biocontrol agent dispensing device (**10**) by slotting the capture device (**26**) into the biovector powder dispenser underneath the mesh layer (**24**), then opening the lid (**12**) and adding the biocontrol agent powder onto the mesh such that most of the powder that is not retained on top of the mesh layer (**24**) lands onto the capture device (**26**) rather than into the second connecting passage. Once loaded, the capture device (**26**) holding the excess powder is slid out of the biovector powder dispenser. It is also possible, when the biocontrol agent dispensing device (**10**) is not in operation, for the capture device (**26**) to be stored in the slotted position under the mesh layer in the biovector powder dispenser. The second exit (**28**) may further comprise a lip (**30**) affixed to the roof of the second connecting passage (**36**) and extending inwardly at an acute angle. The lip (**30**) may be hingedly attached such that it may be reversably moved in the direction (**A**) by a user as desired. For example, the lip (**30**) may be moved inwardly in the direction (**A**) such that lies flat against the roof of the second connecting passage (**36**) for insertion of the biocontrol agent powder capture device (**26**) and then once the capture device (**26**) has been withdrawn, the lip (**30**) can be moved back to its original position. The lip (**30**) may be comprised of a translucent plastic, more particularly poly(methyl methacrylate) also known as Perspex®. The acutely angled lip (**30**) prevents bees from entering through the second exit (**28**). The translucent plastic provides more light to enter the second connecting passage (**36**) and encourages the bees to move toward the second exit (**28**) and the light outside. The one-way flaps (**18**) may be made from a transparent or translucent material including a plastic. Preferably they are made from a transparent material.

EXAMPLE 3

Effect of biovectoring of a biopesticide, against botrytis in blueberries

Aim

To determine if a test product (a biofungicide having active ingredients *Ampelomyces quisqualis* and *Gliocladium catenulatum*) shown to be effective *in vitro* in inhibiting *Botrytis* growth can be delivered to blueberry plants by bees that have been coated with the biofungicide using the dispenser of the invention, and if delivery of the biofungicide by coated bees is effective against *Botrytis* infection in blueberry plants.

Introduction

Biological fungicidal agents or biofungicides are beneficial microorganisms formulated into a product that, when sprayed onto a plant, impair fungal pathogen development (Kilian & Steiner 2003). *Ampelomyces quisqualis* is a parasitic fungus that has antagonistic effects against a variety of fungal pathogens. In our study we made use of one of the modes of antagonism that creates competition for nutrients or space on leaves in order to induce resistance to other pathogens. *A. quisqualis*is has been shown to produce a biosurfactant that affects the surface tension of water droplets on plants. This causes a reduction in water availability on the surface of the plants and thereby limits germination of other pathogenic fungi such as *Botrytis* (Kilian & Steiner 2003). *Gliocladium* spp. are antagonistic against many pathogens, including *Botrytis* spp, and have been shown to prevent sporulation of *Botrytis* (Köhl 2004). This active ingredient has been used extensively in strawberries for the control of grey mold. Greenhouse trials have demonstrated that *G. catenulatum* vectored by bumblebees can reduce *Botrytis* infection and improve shelf-life of strawberries, resulting more sustainable control with healthier strawberries with fewer residues (Köhl 2004).

Test 1:

To test if bees effectively transferred a test product from inside the dispenser onto blueberry flowers, five healthy blueberry plants were grown inside a tunnel including a hive and a dispenser containing a fluorescent coloured dye powder for delivery to the plants. The dye was placed in the dispenser after one day of the bees getting used to the dispenser. That night the bushes were observed with a UV torch to visually detect traces of the fluorescent coloured dye powder on the flowers.

Test 2:

To test if the biofungicide was effective, three test groups and a control of healthy blueberry plants were used. The first test group of blueberry plants had biofungicide powder applied in a spray formulation in accordance with the current conventional application. The second test group of blueberry plants had the biofungicide powder applied to each flower with a paintbrush in a dry formulation. This was to rule out lack of moisture as a cause for the biofungicide product not activating.

The third test group of blueberry plants was placed inside a tunnel including a hive with a dispenser according to the invention containing the dry powdered biofungicide product. Lack of *Botrytis* in this test group would show that the bees effectively transferred the biofungicide product to the flowers. The final group of blueberry plants was a negative control group. These blueberry plants served to show that lack of *Botrytis* infection was not due to any other variables and was in fact due to the biofungicide product applied in the tests above.

Method

All blueberry plants used were the same age and variety (18 months old, *Corina* variety).

Test 1

Five blueberry plants placed inside a tunnel including a hive and a dispenser containing the fluorescent coloured dye.

Test 2

Five blueberry plants sprayed with a liquid formulation of the biofungicide product
Five blueberry plants dusted with the dry biofungicide product
Five blueberry plants placed inside a tunnel with a hive and a dispenser according to the invention containing the dry biofungicide product
Five blueberry plants used as a control
1 week after the biofungicide product was applied, the plants were all equally infected with a dose of *Botrytis.*

Results

Test 1
The fluorescent powder was found in high concentrations on all of the blueberry plants inside the tunnel.

Test two
- 1/5 of the blueberry plants that were sprayed with the biofungicide product showed signs of *Botrytis* infection,
- 0/5 of the plants that had been dusted with the dry biofungicide product showed signs of *Botrytis*.
- 0/5 of the plants inside the tunnel that had the dry biofungicide product applied via bees showed signs of *Botrytis* infection.
- 5/5 of the control plants showed signs of *Botrytis* infection.

Discussion

The trial (Test 2) was successful in showing that the biofungicide product dry formulation is effective at preventing infection with *Botrytis* in the blueberries. Furthermore, the trials (Tests 1 and 2) showed that the bees can effectively transfer a dry product to the blueberry flowers via the dispenser.

Effect of the product on the bee colony
The biofungicide product had a negligible effect on the health of the bees. The hives used in the trial continued to grow in strength during and after the trial.

No bee mortalities were observed within the hives. The only cause of bee mortality in the trial was the effect of the netting in our trial tunnel on the navigation system of the bees causing a large number of forager bees to die in the corner of the net structure.

EXAMPLE 4

Products for use with the bee biovector dispenser of the invention

Non-limiting examples of bioproducts that may be dispensed with the use of the biovector dispenser of the invention include:

- Plant hormones;
- *Beauveria*;
- *Trichoderma*;
- *Bacillus*;
- *Metarhizium*; and
- Gliocladium.

EXAMPLE 5

With reference to Figure 5, there is provided a further embodiment of the bee biovector dispenser comprising a 50 housing or beehive for receiving a 52 lid with a piercing section for a 54 dispensing tray, where the dispensing tray 54 is for reloading the powder. Further included is a 56 mesh; a 58 walkway for the bees or the exit frame and an 60 entrance frame for the hive. This design has the advantages summarised below. A bracket 62 is also provided. Firstly, application of powder to the bees is done from above via a mesh 56 when the bees walk under the mesh 56 the powder falls through coating them on the top of their bodies but also falling to the floor and therefore coating them on the underside of their bodies as they walk through. One way flaps (the frames) are provided on return as the hive the bees have to pass through a one way flap. This prevents the bees from exiting from the entrance pathway and avoiding the powder. Light blocking steps are provided as the return path include blocks on alternating sides (top and bottom) that serve to block out the light entering into the hive from this pathway. This helps to further regulate the flow of the bees into the entrance and out of the exit. As a further measure, there could be included a loading system which is capable of involving dispensing trays that are pierced by the lid when closing to release the product onto the mesh 56.

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CLAIMS

1. A biocontrol agent dispensing device for bee biovectors comprising:
   (i) a bee-hive receiving portion operably supporting a bee-hive where the bee-hive comprises a portal for allowing the bees to enter and exit the hive;
   (ii) a substantially rectangular bioagent powder dispenser housing, adjacent to the bee-hive receiving portion when present, comprising:
      A. a first, substantially rectangular, passage comprising a floor, two side-walls extending upwardly from the floor, and a roof, connecting a first bee entrance and a first bee exit,
      wherein the height of the first connecting passage is between 20 and 40 mm, and the length of the first connecting passage is between 100 and 200 mm, and
      wherein the first connecting passage comprises a plurality of elongate opaque blocks extending upwardly from the floor of the first connecting passage and downwardly from the roof of the first connecting passage, such that a space between the top of each upwardly extending elongate block and the roof of the first connecting passage and between the bottom of each downwardly extending block and the floor of the first connecting passage is between 5 and 10 mm or any length therebetween, and
      wherein the first exit is in communication with a bee-hive portal when in operation such that bees fly from the outside into the first entrance and move along the first connecting passage, then exit through the first exit into the bee-hive, and
      wherein the first exit comprises a plurality of plastic one-way flaps affixed to an upper portion thereof, extending downwards towards the floor of the first connecting passage, the flaps having a thickness of between 0.020 and 0.119 mm, whereby bees exiting the first connecting passage through the one-way flaps or exiting the hive cannot re-enter the first connecting passage;

B. situated above the first connecting passage a second, substantially rectangular, connecting passage comprising a floor, two side-walls extending upwardly from the floor and a roof, comprising a second bee entrance and a second bee exit,

wherein the height of the second connecting passage is between 4 and 10 mm and the length of the second connecting passage is between 150 and 250 mm,

wherein the second entrance is in communication with the bee-hive portal such that bees exiting the hive move through the second bee exit, along the second connecting passage and to the outside through the second bee exit;

C. situated above the second connecting passage and forming the roof of the second connecting passage, a removable substantially rectangular mesh layer having a mesh size of between 200,000 and 2,000,000 nm, and therefore capable of dispensably supporting a biocontrol agent or mixture of biocontrol agents in powder form for dispensing onto bees moving along the second connecting passage to the second bee exit; and

(iii) situated above the second connecting passage and removable mesh layer, and operably in contact with the biovector powder dispenser housing, a lid covering the second connecting passage and removable mesh layer.

2. The biocontrol agent dispensing device according to claim 1 wherein the portal is capable of being used as both an entrance portal and an exit portal.

3. The biocontrol agent dispensing device according to claim 1 wherein the second connecting passage further comprises one or more internal walls or supports of the same height as the second connecting passage, thereby dividing the second connecting passage into two or more passages connecting the second bee entrance and the second bee exit.

4. The biocontrol agent dispensing device according to claim 3 wherein the one or more internal walls or supports are parallel relative to each other and the second connecting passage walls.

5. The biocontrol agent dispensing device according to claim 3 wherein the internal walls or supports define three or more passages connecting the second bee entrance and the second bee exit each passage having a width of between 80 and 120 mm.

6. The biocontrol agent dispensing device according to claim 1 wherein the mesh layer is supported by the second connecting passage.

7. The biocontrol agent dispensing device according to claim 1 wherein the mesh layer is supported by the internal walls or the supports thereof.

8. The biocontrol agent dispensing device according to claim 1 wherein the mesh layer is inlaid within the perimeter of the second connecting passage and supported by the internal walls or supports thereof.

9. The biocontrol agent dispensing device according to claim 1 wherein the biovector powder dispenser housing comprises a substantially water-proof seal between the lid and housing.

10. The biocontrol agent dispensing device according to claim 9 wherein the substantially water-proof seal is foam or rubber, or foam and rubber in combination, such that when in a closed position, the lid connects with the housing preventing water from entering the biovector powder dispenser housing to water-proof the biocontrol agent dispensing device from rain and ensure that the biocontrol agent powder is not exposed to moisture.

11. The biocontrol agent dispensing device according to claim 1 wherein the lid is a hollow, triangular pyramid-shaped lid and angularly shaped to provide for run-off of rain water or dew.

12. The biocontrol agent dispensing device according to claim 11 wherein the angled lid is sized to cover the second connecting passage and mesh layer of the biovector powder dispenser housing, where one side of the base of the pyramid is hingedly connected to one side of the housing and when in a closed position, the apex of the pyramid connects with the opposite side of the housing.

13. The biocontrol agent dispensing device according to claim 1 further comprising a biocontrol agent powder capture device comprising a plurality of flat elongate slats, for example two, three, four or five slats sized to extend into the biovector powder dispenser which slots into the housing of the biocontrol agent dispensing device below the mesh layer and below the lid.

14. The biocontrol agent dispensing device according to claim 12 wherein the biocontrol agent is added after the biocontrol agent powder capture device is in position under the mesh layer by opening the lid of the biocontrol agent dispensing device to add the biocontrol agent powder onto the mesh.

15. The biocontrol agent dispensing device according to claim 13 and 14 wherein excess powder that falls through the mesh lands on the elongate slats of the biocontrol agent powder capture device rather than on the floor of the second connecting passage and can be removed by removing the biocontrol agent powder capture device.

16. The biocontrol agent dispensing device according to claim 1 wherein the first entrance comprises a lip for bees to land prior to entering the first entrance.

17. The biocontrol agent dispensing device according to claim 16 wherein the lip is comprised of a translucent plastic.

18. The biocontrol agent dispensing device according to claim 1 wherein the translucent plastic is poly(methyl methacrylate) also known as Perspex®.

19. The biocontrol agent dispensing device according to claim 1 wherein the second exit further comprises a lip affixed to the top of the second connecting passage and extending downwardly at an acute angle of between 40 and 80 degrees.

20. The biocontrol agent dispensing device according to claim 19 wherein the lip is hingedly attached such that it is reversibly moved in the direction (**A**) by a user as desired.

21. The biocontrol agent dispensing device according to claim 19 wherein the lip is comprised of a translucent plastic comprising poly(methyl methacrylate) also known as Perspex®.

22. The biocontrol agent dispensing device according to claim 21 wherein the translucent plastic provides more light to enter the second passage and encourages the bees to move toward the second exit and the light outside.

23. The biocontrol agent dispensing device according to claim 19 wherein the angled lip prevents bees from entering through the second exit.

24. The biocontrol agent dispensing device according to claim 1 wherein the one-way flaps is made from a transparent or translucent material comprising a plastic material.

25. A method for delivery of a biocontrol agent to one or more plant(s) by a bee biovector with the use of the biocontrol agent dispensing device according to claim 1 in communication with a bee-hive, comprising:

(I) one or more bee(s) entering the biocontrol agent dispensing device at the first bee entrance, moving along the first passage to the first bee exit and through the plurality of one-way flaps into the bee-hive;

(II) one or more bee(s) exiting the bee-hive and entering the second bee entrance and then moving along the second passage to the second bee exit, wherein the movement of the bee(s) along the second passage causes the biocontrol agent powder located on the mesh layer to be shaken onto the floor of the second passage and body of the bee(s), thereby coating the bee(s) in the biocontrol agent powder;

(III) biocontrol agent coated bee(s) exiting the second passage through the second bee exit to the outside; and

(IV) one or more biocontrol agent coated bee(s) delivering the biocontrol agent to one or more plants when pollinating one or more flowers of the plant(s).

26. The method according to claim 25 comprising a further step of inserting a biocontrol agent powder capture device into the biocontrol agent dispensing device of the invention below the mesh layer followed by opening the lid and loading one or more biocontrol agent powder(s) onto the mesh such that excess biocontrol agent powder(s) that falls through the mesh is captured by the biocontrol agent powder capture device rather than landing in the second passage, and is capable of being removed by sliding the biocontrol agent powder capture device out of the biocontrol agent dispensing device.

27. The method according to claim 25 wherein the plant is a plant that is capable of being pollinated by bees.

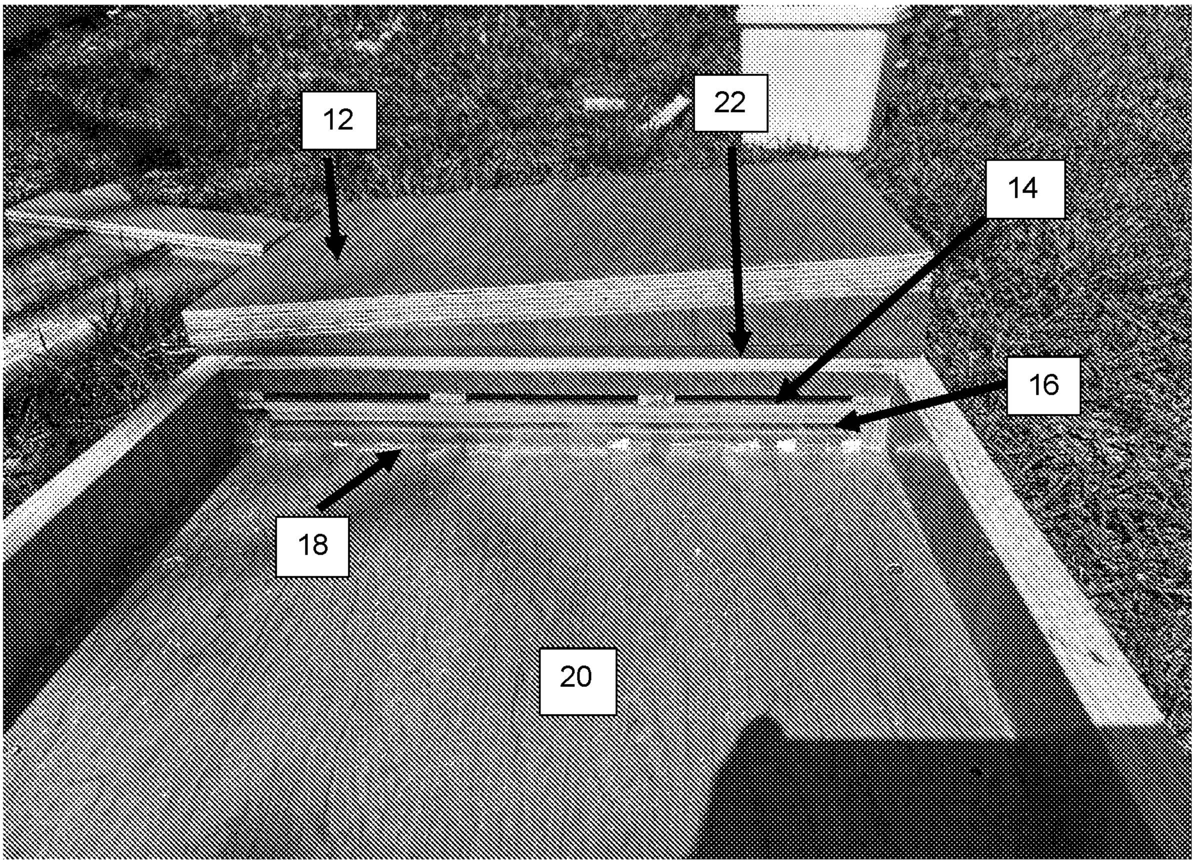

FIG. 1

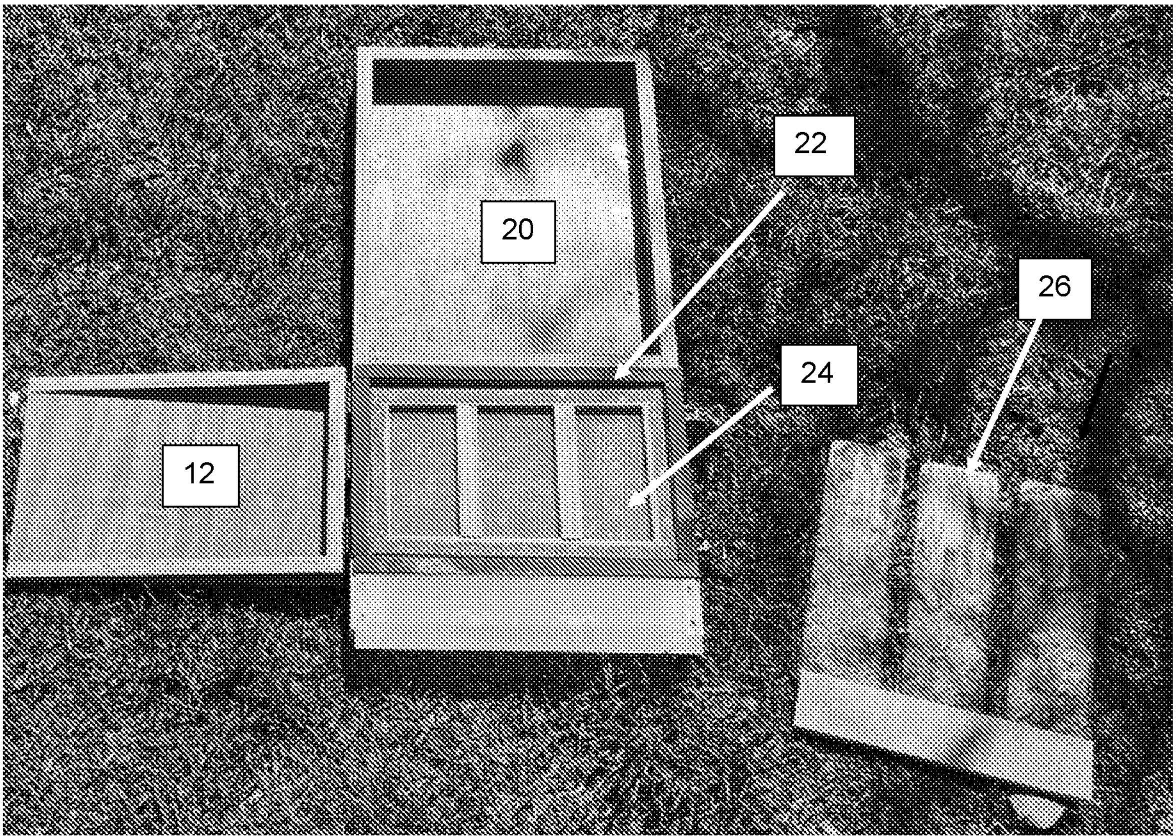

FIG. 2

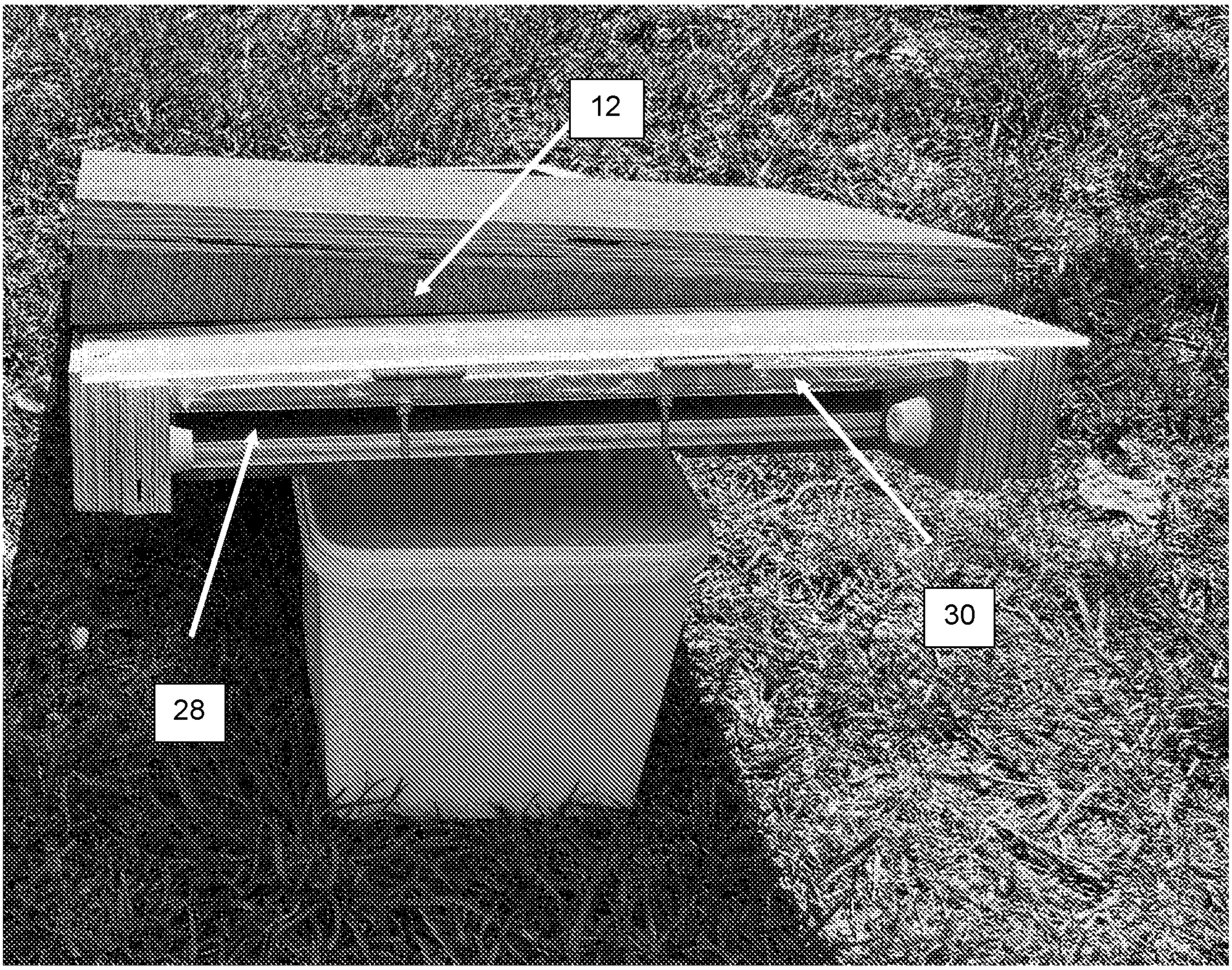

FIG. 3

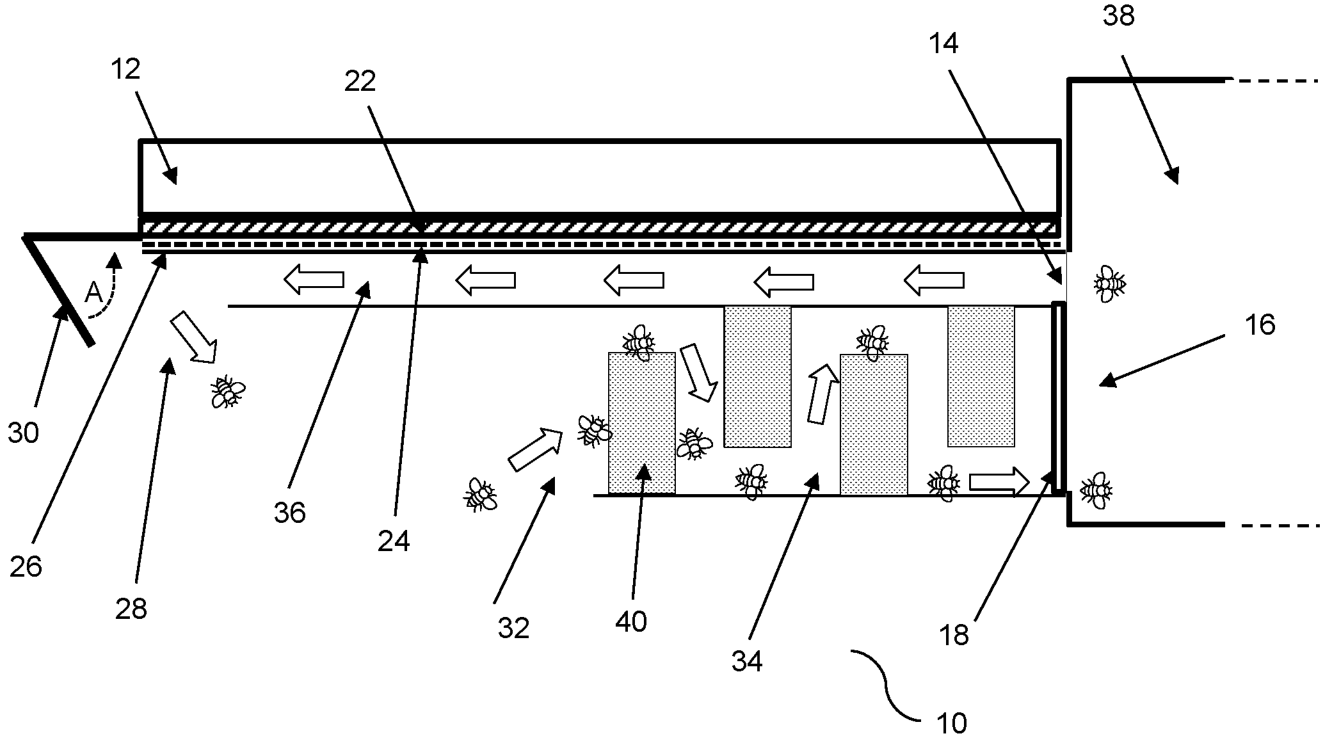

FIG. 4

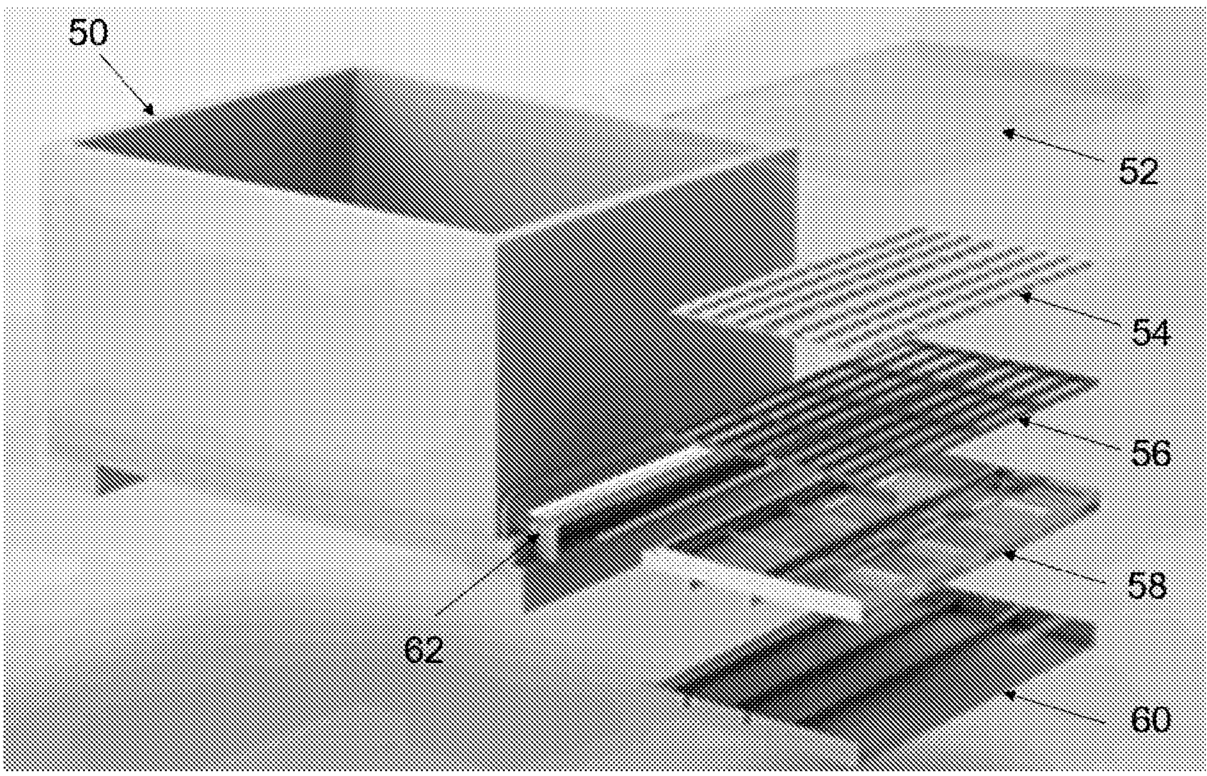

FIG. 5