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Apivectoring: Managed pollinators as vectors of biocontrol agents for diseases and pests

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Abstract

Insect pollinators are indispensable in global agriculture for their valuable pollination services. In managed pollination honey bees, bumble bees, stingless bees and megachilid bees of genus *Osmia* are most commonly introduced in fields /orchards at the blooming period of the crop in open as well as in protected cultivation for pollination services. Apivectoring/ entomovectoring, is the use of pollinating insects for the precision delivery of microbial control agents (MCA) that kill target pests. It uses an innovative method of control in which the inoculation of bees with biocontrol agents is dispersed by insects in this process and allows managing populations of pathogens and pests, mainly flowers and fruits. As pollinator leave hive, it exits through a specialized dispenser containing the MCA, coating it with a fine powder. When it alights on a flower, some of this biocontrol agent is left behind. As it flies through the field, the powder is also deposited on the leaves, such that it returns to the colony “clean” and can unload its gathered pollen and nectar. The left over MCA on flowers, leaves may go to work immediately against insects and pathogens, or it may colonize the flower and act as a prophylactic for the developing fruit and later dissemination. This technology represents a sustainable alternative for the preventive management of pests and diseases and implemented in crops such as apple (storage rot disease), blueberries (mummification, grey mould), coffee (coffee berry borer), pear (fire blight), raspberry, tomato (grey mould), sweet pepper (plant bug, western flower thrips), strawberry (grey mould), rapeseed (plant bug) and sunflower (*Sclerotinia* rot). The ultimate success will depend upon a number of interacting factors, including dispenser design for proper MCA distribution, selection of the most efficient bee species in a production system, transport and delivery of the MCA, and the safety of bio control agent for the environment and humans (Smagghe *et al.*, 2012). So, apivectoring provides dual benefits of crop pollination and crop protection by reducing pest pressure and pesticide applications, improves pollination which subsequently results in higher yields and better crop quality.

Keywords: Insect pollinators, dispenser, bio-control agents, pollination,

Introduction

The importance of pollinators, both managed and wild pollinators, cannot be overestimated. The reliance of global food production on animal pollination has increased in recent decades (Lautenbach *et al*., 2012). More than 80% of flowering plants rely on animal pollination for sexual reproduction (Kearns et al., 1998; Klein *et al*., 2007). Infestation by pests and diseases can seriously affect agriculture. For example, grey mold can damage fruit crops such as strawberries and raspberries. The fungus that causes mummification can destroy blueberry crops if left unchecked. Tomatoes and peppers in greenhouses can also be infested with clouded beetles (TPB), western flower thrips (WFT), whiteflies, and peach aphids. These pests and diseases can destroy crops and have a significant economic impact on farmers. Farmers often rely on cumbersome and costly spraying of insecticides and fungicides to control pests and diseases. Although effective in the short term, the potential adverse effects of chemical pesticides and fungicides on pollinators and natural enemies in agroecosystems resulting in the decline of their population. The ecological viability of farming is often highly questionable, regarding the health of consumers and farm workers. For bee-pollinated crops such as apples, strawberries, tomatoes, raspberries, canola, and sunflowers, etc. the use of vectors as biocontrol agents is a sustainable alternative to control the diseases and pests such as apple core rot, blight gray mold, sunflower borer, western thrips, aphids, and coffee bean borer etc. (Al-mazra's awi *et al*., 2006; Kevan *et al*.2008). This method of using pollinators to precisely deliver biological control agents to kill target pests is known as apivector, entomovector, or bee vector technology. The ultimate success lies in donor design for appropriate MCA distribution, selection of the most efficient bee species in the production system, transport and delivery of MCA,environmental and human safety of biocontrol agents, and much more interacting factors (Smagghe *et al.* a., 2012). Apivectoring therefore provides the dual benefits of crop pollination and crop protection, reducing pest pressure and pesticide use and improving pollination. This increases yield and improves crop quality.

The Importance of Pollinators

As the population grows rapidly, so does our need for food. To cope with the future, our agricultural systems need to produce more food sustainably. Pollinators are key to these systems. Both wild and controlled pollinators provide essential pollination services, either natural or provided by humans.

Several global statistics show the magnitude of the contribution of pollinators to agriculture and food security:

- Of the world's 115 major crops for human consumption, i.e. about 35% of the crops are dependent on pollination (Beenow, 2018)

The total amount of food we eat is measured by the amount produced worldwide and is partly dependent on pollination done by animals (Brittain *et al.* , 2013)

- An estimated 5-8% of global crop production with an annual market value of $235-577 billion (Camillo, 2003) is directly attributable to animal pollination.

Animal pollination increases the quantity and quality of many crops, increasing their value to farmers. Pollinator-plant relationships range from generalists (plants with many different pollinators (such as bees) visiting or having many pollinators) to highly specialized one-to-one relationships (specialists relationship). These relationships are not fixed. Plant species may be visited by different pollinators in different regions, and relationships may change throughout the year due to changes in pollinator densities. And for the millions of people around the world who depend on pollinators for their livelihoods, it matters. Given the importance of pollinators in the agricultural sector, there is a need to increase knowledge about which crops require which pollinators and to identify the best techniques and methods to protect and enhance both wild and managed species in our ecosystem.

Background

Pollination by bees and other animals greatly improves crop yield and quality. Bees also transfer biological control agents to control crop pests and diseases, using entomovector technology Entomovector technology is an environmental friendly control strategy for economically important plant pathogens and pests, using pollinators to deliver powdered formulations of crop protection products to the flowers and foliage of crops. Bee vectoring is a technique that uses controlled pollinating bees to disperse beneficial microbial agents into flowering plants to control pests and suppress plant diseases (Peng et al. 1992; Kevan et al. 2008). Hokkanen and Menzler-Hokkanen (2007) and Hokkanen et al. (2015) used the term as 'entometeor technology' to describe the use of controlled pollinators as applicators of biological control agents against crop pests. Apivectoring technology combines knowledge developed over thousands of years and sustainable alternatives for the active management of plant pests and diseases, and multi-tropical relationships among pollinators, plants and pathogens. It uses an innovative control method: bees are inoculated with a biocontroller that is distributed by the insect when it peaks. This mechanism makes it possible to control the population of pathogens and pests, especially attacking flowers and fruits. This technology is used in crops such as strawberries, raspberries, pears, apples, sunflowers, oilseed rape and tomatoes. Increasing production challenges have led to technological advances and innovations that have enabled better crop production in many countries around the world (Plan, 2016). However, such technology needs to be validated in different regions for easy adoption. Biovector technology is one of the remarkable new technologies transforming agriculture from a labor-intensive to a capital-intensive industry (Mommaerts and Smagghe, 2011). This technology uses insects as vectors for biological control agents. The aim of this technique is to minimize the use of synthetic pesticides and pest resistance while maximizing crop quality and yield. Entomovector technology achievements are mainly realized in some developed countries. This technique is especially useful for many pollination-dependent crops. Farm bees, honeybees, and bumblebees are used to transfer fungal, bacterial, and viral inoculum from hives to flowers (Kevan *et al.*, 2003).

Can pollinators deliver a dual benefit combining pollination and protection of crops?

It is nothing new, given that it involves the spread of biopesticides (pollen) in most flowering plants. This idea, combined with the knowledge that some important plant diseases (such as gray mold, rot, and mummies) are also transmitted by pollinators, has led to research into the potential dual benefits of combining crop pollination as well as crop protection. In 1992, John Sutton's lab, the work led by Peng Gang, in collaboration with Kevan group, initiated project B52 to extract honey bees (*Apis mellifera*) from *Clonostachys roseum* to strawberry flowers (*Fragaria x ananassa*) to control gray mould. They used it as a vector (bomber) for control of mold (*Botrytis cinerea*) (Peng et al., 1992). Commercial formulations of *Trichoderma harzianum*, another botrytis antagonist, are available for pollinating honeybees in Italy (Maccagnani et al., 1999) and honeybees and bumblebees in the United States (Kovach *et al.*, 2000). The conclusion from this study is that *T. harzianum* bee delivery is also a viable option for strawberry growers interested in controlling botrytis with minimal use of fungicides. Around the same time as the B52 project, research was focused in the western United States by Sherman Thomson's team in Utah (Thomson *et al*., 1992) and Kenneth Johnson's team in Oregon (Johnson *et al.*, 1993). They provide the bacterium *Pseudomonas fluorescens* as an antagonist for the fungus *Erwinia amylovora* in core cultures. This research has also met with some success and continues at a moderate pace in Washington (Pusey, 2002).

The story behind the development of the concept that pollinators can be used as carriers and propagators of microbial biological agents is as follows:

Honey bees as a vector for *Clonöstachys roseum* to the flowers of strawberries (*Clonystachis roseum*) inhibits gray mold (*Botrytis cinerea*). Yu and Sutton used bumblebees and honeybees to bombard strawberry flowers with *C. roseum* (Yu and Sutton, 1997). A commercial preparation of *Trichoderma harzianum*, another *Botrytis* antagonist, is available in Italy (Maccagnani *et al.*, 1999). The same commercial formulation of the botrytis antagonist *Trichoderma harzianum* was applied to strawberries by honeybees and bumblebees in the United States (Kovach *et al*., 2000). Honeybees were used to transmit *Heliothis* nuclear polyhedrosis virus (NPHV) to control the red clover (*Trifolium incarnatum*) from *Helicoverpa zea*, the maize earwig (Lepidoptera: Noctuidae). Bees are effective vectors of Bt (*Bacillus thuringiensis* var. *kurstaki*) can be used on sunflower blossoms (*Helianthus annuus*) for control (equivalent to hand spraying) of sunflower moths (*Cochylis hospes* (Lepidoptera: Tortricidae)). with increased pollination and seed formation. (North Dakota study by J.L. Jyoti and Garry Brewer (1999). The B52 technique was initiated in response to a cloudy plant bug (TPB) (*Lygus lineolaris*) outbreak in Canola in Alberta in 1998.

Are pollinators efficient in delivering biocontrol agent?

This approach is possible due to the interaction between crops, pests (weeds, diseases, or herbivores), pollinators (vectors), biological control agents, powder products, donors, and environmental safety and human health (Kevan *et al.* 2008). Vectors are bee species that have a high rate of flower visitation and the ability to deposit a microbial control agent (MCA) on target plants. MCA selection depends on the target crop pest or disease and should be safe for bees and the environment. In general, commercial MCA powder formulations are often used in the BAT approach (Mommaerts and Smagghe, 2011). MCA powder formulations are often mixed or diluted with a carrier to reduce concentration and maximize contact between the MCA and the body of the bee (Kevan *et al.* 2008; Al-Mazra'awi *et al.* 2006). A designed dispenser placed in front of the hive allows contact between the bees and the MCA. So, as the bees pass through the repellent in the dispenser at the entrance to the hive, they introduce an inoculum of microbial agents (fungi, bacteria and viruses) into their body and hair. The bees then visit the flowers to collect nectar and pollen, self-pollinate the leaves of the plants, and deposit the inoculum on the flowers and leaves of the target crop (Kevan *et al.*2008). Some studies have reported success with vector bee technology (Carreck *et al.* 2007; Mommaerts *et al.* 2010). According to Hokkanen *et al.* (2015) control of strawberry gray mould caused by *B. cinerea* using the biocontrol fungus *Gliocladium catenulatum*, transmitted by honeybees or bumblebees, was conducted for strawberry cultivation in five countries. The results showed that under high disease pressure, vectorization of honeybees reduced disease incidence by an average of 47%. This is similar to multiple fungicide sprays. However, at moderate disease pressure, biocontrol reduced gray mold by an average of 66% and was more effective than fungicide sprays. It was then effective against many crop pests and diseases (Kovach *et al.*, 2000; Maccagnani *et al.*, 2005; Shafir *et al.*, 2006). Management of bumblebees to deliver biological control agents has been studied for over two decades (Peng *et al.* 1992; Yu and

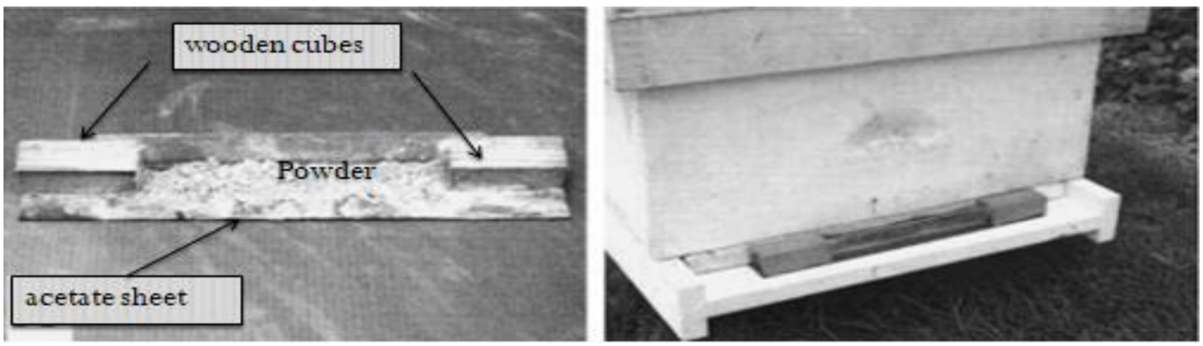
Sutton 1997). However, most studies have been conducted primarily in laboratory or greenhouse conditions (Kevan *et al.* 2003; Mommaerts *et al.* 2011). The reason of using honeybees as vectors for biocontrol agents (BCAs) is due to their morphological and behavioral characteristics. Bumblebees have a relatively large body surface area covered with split ends that aid in the capture and transport of pollen grains (Free and Williams 1972; Batra *et al.* 1973). The commercial availability of bumblebee colonies has led to increased use of *Bombus terrestris* L. in Europe and the common eastern bumble bee *B. impatiens* Cresson (Hymenoptera: Apidae) in North America, not only in greenhouses (Mommaerts *et al*. 2011) (Kovach *et al.* 2000; Dedej *et al.* 2004; Carise *et al.* 2016). Results from the BICOPOLL project shows that bumblebees successfully infuse the biofungicide Prestop-Mix formulation of *Gliocladium catenulatum* strain J1446 as active organism, Verdera OY, Finland) under field conditions. Prestop Mix is a safe biological product for both humans and beneficial organisms visiting the field (Verdera, 2015). The distribution of bumblebees on the field was uniform over a distance of 100 m.

Dispensers for entomovectoring

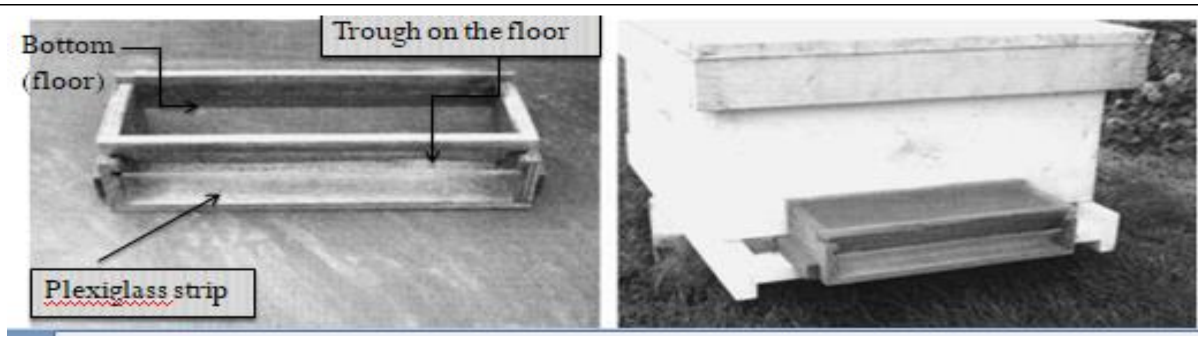
An important step in transferring additional pollen and/or biological control organisms (BCO) to pollinators is efficient loading of the vector with microbial agent to ensure adequate loading so that it can be delivered to target site in appropriate amount. A suitable dispenser must be designed to achieve this goal. The main purpose of the dispenser is to fill the carrier (bees) with powdered product (pollen and/or formulated BCO product) on its way out of the hive and distribute it to the target crop. Efficient dispensers should not only optimize vector loading, but should also have short dispenser, refill intervals, be easily attached to hives, and should not affect vector foraging behavior (Mommaerts and Smaghe, 2011). Dispensers previously used in entomovector studies can be divided into two groups: single-use and double-use dispensers (Smagghe *et al.* 2012). In single-use dispensers, the chamber in which the vector exits the dispenser is the same or not completely separate from the chamber in which the vector enters the dispenser. The vector therefore passes through the powder both when exiting and entering the hive. In a two-way dispenser, the outlet and inlet chambers are completely separated, and only the vector leaving the nest is in contact with the powder. For bees, disposable dispensers such as Harwood dispensers and Tub dispensers were originally used to fill bees with pollen to achieve cross-pollination.

Harwood Container: A Harwood container consists of a wooden box with an inner roof that curves down to the ground. The bees have to crawl through the gap that exists between the bottom of the box and the roof, crawl over the powder placed in the floor trough, and climb over the perspex strip to exit the dispenser.

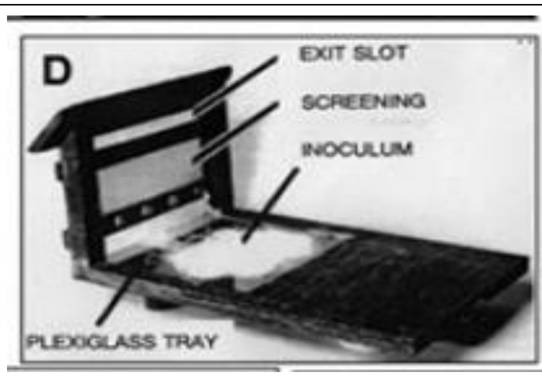
Tub Tray: This tub tray consists of two wooden cubes that hold flexible acetate sheets to form tubs that can be filled with powder. Such donors used for biological control resulted in poor management of the honeybees used in the study. Bill *et al*. (2004) confirmed that this is mainly due to the bees opening up the powder ducts, concentrating bee activity there, resulting in less contact with the powder and less exposure. This phenomenon has also been previously observed in pollen eaters (Legge 1976). The performance of disposable dispensers is relatively low, so we need better dispensers. Various types of dispensers suitable for honeybee experiments include the Peng dispenser (Peng et al. 1992), the Gross dispenser (Gross et al. 1994), the Triwaks dispenser (Bilu et al. 2004), and the Houledispenser (Albano et al. 2009).



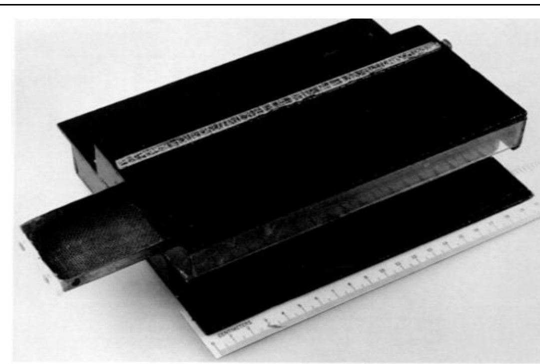
Harwood Dispenser



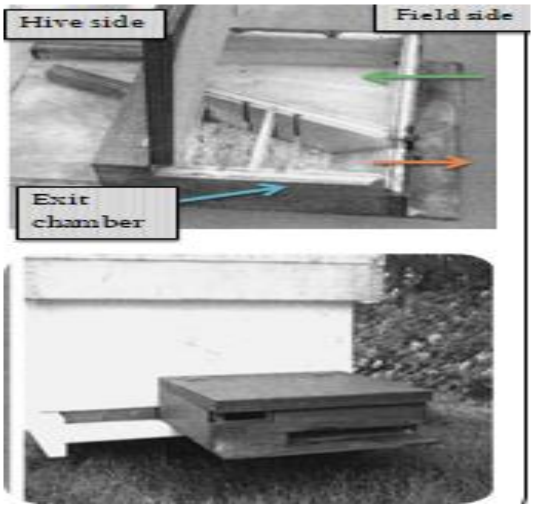
Tub Dispenser



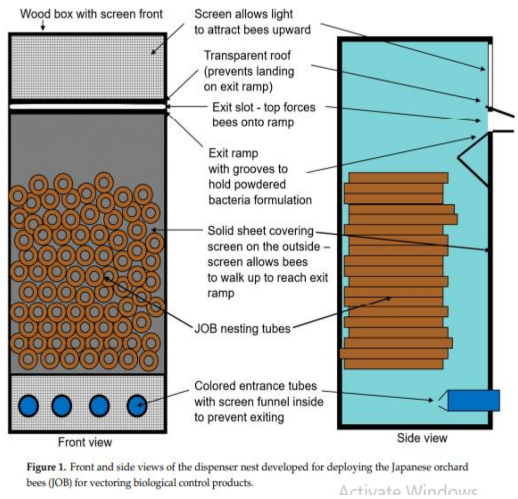
Peng Dispenser



Gross Dispenser



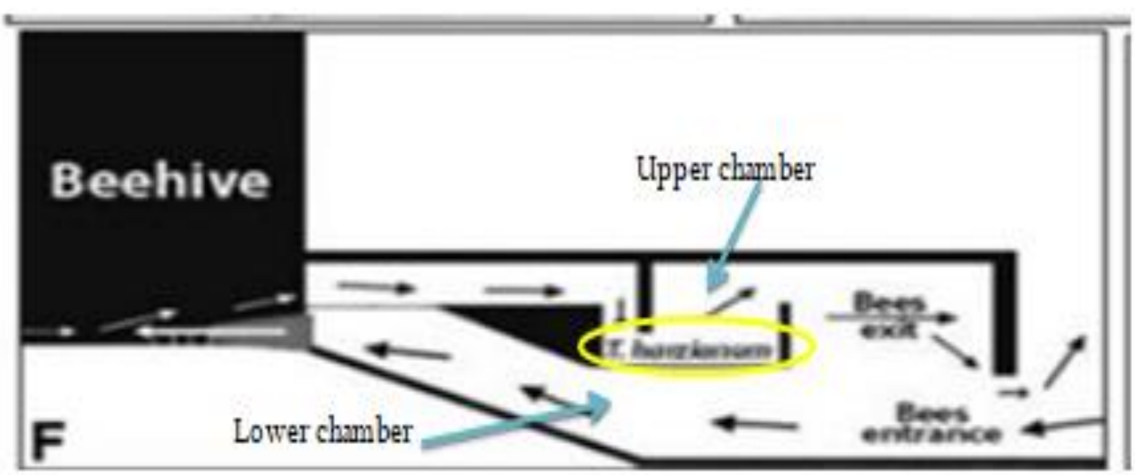
Triwaks Dispenser



Osmia dispenser

Flying doctors

Bee Treat



Houle dispenser

Figure 1 Different types of dispensers

Peng box: The Peng box consists of a wooden platform with a plexiglass tub containing powder that can be placed on the floor of the hive. Plexiglass panels are mounted vertically on the platform, and light coming in from outside the hive lures the bees through the powder and up the panel toward an exit slot a few inches above the wooden platform. Returning bees enter the hive through a slot under the wooden platform to avoid walking through the dust as they enter the hive.

Gross magazine: Designed to fit in the front center of a modified beehive floorboard, the Gross Magazine features a removable magazine that can be inserted from the side to load powder into the magazine.The hatched bees pass through the tub on their way back to the hive on their own.

Triwaks dispenser: The Triwaks consist of a wooden box with an extended bottom that fits in the opening of a standard Langstroth hive. The dispenser is divided diagonally into two triangular compartments, one entering the hive and one exiting the hive. The exit chamber is divided into three sub-chambers containing powder formulations. It has the longest side in the hive and ends with the shortest side forming the outlet part of the dispenser that attracts the hatched bees thanks to the light coming from the outside. Returning foragers find a large landing platform that forms the longest side of the receiving box and terminates on the shortest side of the hive, ensuring that the bees enter the hive through the powder-free portion of the dispenser.

Houle Dispenser: The Houle Dispenser can be mounted on a hive and is divided horizontally into an upper compartment with a powder container and a lower compartment without powder. Abandoned bees leave the hive through the upper compartment, but returning bees avoid the dust and enter the hive through the lower compartment. There are many new types of dispensers on the market, including: B. Cartridge Applicator and Beet Treats for bees, Flying Doctor for bumblebees.

Pollinator safety

The biological pest control agents used in Apivectors are considered safe and are registered. Microbial control agent registrations are usually specific to the target culture and method of application. Even entomopathogenic pathogens have been shown to be safe unless in very high concentrations such as commercial powder or liquid formulations (Al-mazra'awi, 2004). *B*eauveria *bassiana* was formulated from 2×10^{11} conidia/g product to 6×10^{10} to minimize bee mortality and maximize pest mortality. Bees that passed through cornmeal acquired more conidia (e.g., 1.5×10^6 CFU (colony forming units) per bee) than if bees passed wheat flour, durum wheat semolina, corn flour, potato starch, potato flakes, oat flour, barley flour.

Conclusions

Apivectoring techniques is an interdisciplinary approach to pest management that integrates diverse ecosystem components such as pollinators, microbial agents and pests into one crop production system. It offers the dual benefits of crop pollination and crop protection as it reduces pest pressure and pesticide use and improves pollination. The development of pollination vector technologies to control insect and fungal pests reduces pest populations and pesticide use while improving crop pollination. Benefits from new pesticides that reduce risk, use less chemicals, and improve crop pollination, leading to higher yields and better crop quality.

Future Prospects

Apivectoring offers the advantage of increasing yields by producing higher quality and quantity of plants through a combined pollination and biocontrol agent service. This technology has been tested in field and greenhouse production. Other benefits include reduced need for biocontrol agents compared to spraying, lower fuel consumption and less heavy equipment use, lowering costs for farmers, and a continuous supply of crop protection products during flowering. Benefits include reduced water and synthetic pesticide usage. Making populations resistant to insecticides makes apivectoring technology economically viable. Apivector registrations have begun in several countries thanks to intensive studies by researchers who have shown the technology to be safe for vectors, the environment and human consumption. Government contributions and support are required to implement apivectoring technology and its many benefits. There are many ways to expand research in apivectoring. Testing this technology on new crops such as gourds, almonds, apples, peaches and cherries is essential. Apivector research has also expanded to include solitary stingless bees and the use of donors to treat colonies internally against disease and parasites. More information, can be obtained from the International Organization for Biological Control (IOBC) and the International Commission on Plant Pollinator Relations (ICPPR).

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