Another Job For Busy Bees . . .

Honey bees are the perfect choice for vectoring biocontrol agents

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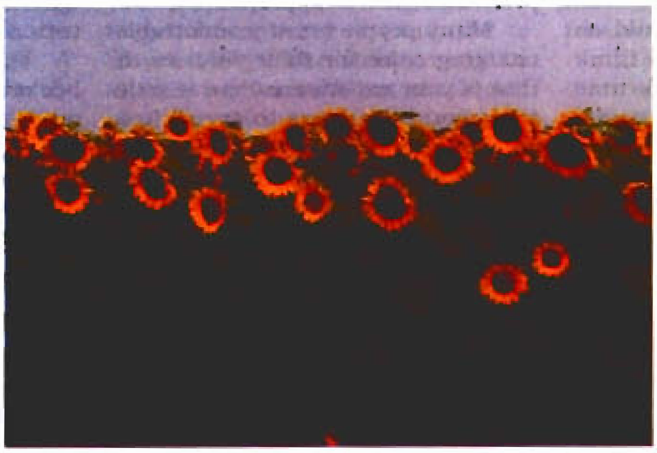
Honey bees are best known as the most important pollinator, then as the producer of honey, beeswax, and several other hive products. However, there is a new role that the honey bees are playing, this is to as a vector for dispersing biological control agents.

Answering The Call

Interest in biological control of plant pests, which may be fungal, bacterial, or in the form of an insect, has greatly increased in the past few years. To date, honey bees have been tested as possible vectors for biocontrol agents of several different pests and diseases. But why research new forms of pest control in crops? Are producers dissatisfied with the current methods of pest control? And why use honey bees as vectors?



Chemical Control: Always Reliable?

Traditionally, chemical pesticides have been the favored control for several plant pests. However, pest after pest is developing resistance to the chemical treatments used against them. For instance, streptomycin is no longer an effective control of fire blight, a bacterial disease that affects crops such as apples and pears. Chemicals used in pest control can often be harmful to other organisms in the agricultural ecosystem besides the target pest, some times beneficial parasites or pollinators. Additionally, concern for air and ground water quality is increasing and leading to the removal of many chemicals from the market. What this means is that new methods must be investigated for pest control.

Biological Control: Critters, Not Chemicals.

Biological control, which is the use of a natural, living enemy to control a plant pest, seems a promising alternative to the use of chemicals. It is often host specific, or at the least specific to a narrow range of hosts, which means it poses less of a threat to non-target organisms. Microbial plant pathogens are often controlled by other microbes that act against the pathogen. They may prevent a plant pathogen from infecting a plant by preemptive colonization and exclusion, which means they target the nutrient-rich surfaces of the plant that the pathogen would normally colonize and colonize it first, leaving no space or resources available for the pathogen. Additionally, they may produce antibiotics to the pathogen at the same time, further blocking its colonization. Biological control, or biocontrol, of insect pests acts in a different way, often by directly attacking and killing the pest, preventing it from damaging the plant in question.

It sounds as if biocontrol should work very well for plant disease or pest control. The trick is getting them to the plant where they can do their job. At first, biocontrol agents were simply being sprayed indiscriminately onto the entire plant. However, in many cases, the pest's activity on the plant can be narrowed down to a limited area, such as the flower or in some cases even as specifically as the stigmas of the flower. In such cases, spraying an entire plant with a biocontrol agent when only the flowers need to be sprayed represents a rather large waste of the biocontrol mixture, time spent on application, and money spent on the entire procedure. Additionally, the timing of biocontrol applications is crucial, as it was with chemical sprays. The flowers of plants do not all open simultaneously. In order to apply a control to the most flowers as possible, farmers had to either apply at peak bloom (and simply let any blossoms that opened later remain vulnerable), or have more than one spraying. Additional sprays would mean additional time and money spent. The million-dollar question then becomes how to inoculate only the flowers of a plant with a biocontrol (without it being very labor intensive), and would there be a way for this inoculation to proceed over an extended period of time without very high costs?

Honey Bees As Vectors?

Honey bees are well known vectors of not only pollen, but of bacteria, viruses, and fungi. Several studies have shown that honey bees are capable of transferring organisms that cause plant disease from plant to plant. So why not use them to transfer organisms that we want in our crops? Honey bees possess the ability to pick up and transfer a biocontrol on their bodies and to deposit it specifically onto a plant's flowers, often directly onto the stigmatal surfaces. What is more, honey bees would be able to inoculate blossoms throughout the growing season, often soon after flowers open, without additional costs to the farmer. This is due to the honey bees' foraging patterns. Additionally, biocontrol agents may spread to untreated flowers without the aid of the honey bee vector due to the movement of the biocontrol itself. Once a biocontrol agent has successfully colonized a flower, it could disperse to other flowers on the same plant by other mechanisms, such as raindrop splash.

Perfect For The Job.

Honey bees seem a natural choice for vectoring biocontrol agents to crops because of their role in crop pollination. Throughout the year, honey bees visit millions of flowers in search of nectar and pollen. The body of the honey bee is highly specialized for the collection of both. One such specialization used for the collection of pollen is the presence of setae (or branched hairs) all over the body of the honey bee. These are tiny hairs that pick up pollen particles as the bee climbs over flowers in search of rewards. They are crucial in the collection of pollen, because they represent such a large surface area to come in contact with pollen. Beekeepers have used this behavior to their advantage already, by placing pollen inserts on hives for honey bees to crawl through on their way out of a hive to pick up pollen for dispersal. These inserts are used to provide honey bees with this pollen in case insufficient pollen or lack of pollen of a compatible cultivar (which may be the case in an apple orchard) may threaten thorough pollination of a crop. The transition to biocontrol vector is now clear. By placing either powdered biocontrol compounds or pollen covered with biocontrol agents in similar pollen inserts, the honey bees exiting the hive will transport these agents to flowers, performing yet another beneficial task.

There are several other key points that make honey bees suitable as biocontrol vectors. As with any efficient organization, honey bees want to maximize the benefit from the efforts of each individual for the entire colony and want to lessen duplicated efforts. When a honey bee finds a good nectar source, she will communicate its location to the rest of the colony and then many will be exploiting this food source until it is exhausted.

It sounds as if using honey bees for biocontrol should work very well for plant disease or pest control.

This behavior is a perfect design for using honey bees as biocontrol vectors. Honey bees will remain faithful to one crop until pollination is no longer required. Thus, when that crop is in bloom and honey bees start to visit it, they will not stop until the bloom is over. If the biocontrol agent is placed in the beehive just as the target crop comes into bloom, the honey bees will deliver the biocontrol agent to that crop and only to that crop until the bloom is over.

How To Go About It.

For successful control of a plant pest, honey bees need to be properly inoculated with a biocontrol agent every time they leave the hive to go foraging. They need to pick up a sufficient amount of inoculum so that they will have enough to deposit on several flowers during that flight. Thus far, a few preparations have been used to make biocontrol agents available in a form that honey bees would be able to pick up and transport. These preparations include placing biocontrol agents on supplemental pollen, into mixtures such as talc-corn meal, or simply making dried bacterial preparations available to the honey bees. A second necessary element for inoculation is that the biocontrol agent be made available to the workers every time they exit the hive. The exit with the biocontrol agent preparation should be the only way available for the bees to leave the hive, and it is normally helpful if there is some cue to encourage them to exit that way (such as light at the end of the tray to guide them out, while light is obscured from all other directions). Additionally, there must be a separate entrance for the honey bees returning to the hive. The tray containing the biocontrol inoculum must be made long enough so that the distance through which the bees crawl will allow them to pick up sufficient inoculum. Most trays used so far have had lengths around 12 cm.

Examples

The following are examples of plant pests and the biocontrol agents that have been vectored by honey bees in an attempt to control them. Of the four examples, two involve plant pathogens, where preemptive colonization is key to proper control, and the other two deal with the control of plant insect pests with biocontrols.

Fire Blight. Fire blight, which chiefly appears in the commercial production of apple and pear crops, is caused by the bacterial plant pathogen *Erwinia amylovora*. This disease not only will destroy the harvestable crop for a single year, but if the case is severe enough it may kill the entire fruit tree. Death of the entire tree occurs when the pathogen spreads from the flower, site of initial infection, to the twig and subsequently to the branch and trunk of the tree.

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Clearly, the blossom blight phase of this disease cycle is the most readily targeted for control of the pathogen, at which time it colonizes the stigmatic surfaces of the flower. The biocontrol agents used for control of this pest have been *Pseudomonas fluorescens* and *Erwinia herbicola*, both of which are bacteria. They control the pathogen by preemptive colonization of the flower stigma, though *Erwinia herbicola* also produces inhibitory compounds that further help to prevent the establishment of the fire blight disease. Though this is the biological control/honey bee vector model that has received the most attention so far, more research is still required before farmers can successfully initiate this system for fire blight control.

Grey Mold. Grey mold is a pest of several crop plants, ranging from flowers such as begonias, peonies, and geraniums to fruits and vegetables such as apples, pears, peaches, raspberries, blueberries, grapes, tomatoes, and beans. It is also a pest in strawberries, the crop in which use of a biocontrol has been attempted. Grey mold is caused by the fungus *Botrytis cinerea*, which will first invade the targeted plant's flower and then proceed on to the fruit. The fungus *Gliocladium roseum* was the biocontrol agent used for attempted control of this pathogenic fungus. Results were encouraging, but more research is required before this biocontrol/vector system can be successfully used.

Banded Sunflower Moth. The banded sunflower moth, *Cochylis hospes* is an important pest of sunflower crops. It is present in many of the temperate areas of North America, but causes its most significant economic damage to crops in North Dakota. The crop is damaged when larval stages of the insect feed on disk flowers and seeds. Control of this pest was attempted with the bacteria *Bacillus thuringiensis*. The bacteria was vectored by honey bees to several flowers within the composite sunflower head, where larval death followed. Outcomes were very favorable, but more work is required to ensure sufficient control with the system.

Corn Earworm and Tobacco Budworm. Both corn earworm *Helicoverpa zea* and tobacco budworm *Heliothis virescens* are pests of several crops. The corn earworm is a pest in crops such as sweet and field corn, tomatoes, soybeans, tobacco, and cotton, to name a few. In these crops, damage is done by the larvae, which will feed on blossoms, buds, and fruits. Corn is its most common and economically impacted crop. Here it begins damage by feeding on the silks and interfering with pollination, and then by proceeding to the ear where it consumes kernels and leaves excrement. The tobacco budworm, like the corn earworm, will attack tobacco, cotton, and soybeans, as well as flax and alfalfa; additionally, it has the potential to attack several vegetable and flower crops. Crop damage is often seen on the buds and fruit of these crops. Biocontrol of these pests was attempted simultaneously on crimson clover, another crop that both pests attack and which is also readily foraged by the honey bee. Control was attempted with the *Heliothis nuclear polyhedronsis* virus (HNPV). The study found that control with HNPV that is vectored by honey bees is a definite possibility, however more research is required first before this occurs.

Winning Combinations.

In all of the aforementioned attempts at biocontrol, the controls used and vectored by honey bees provided as good or better control of the crop pest than the biocontrols applied as sprays and/or chemical control methods.

◇ In the case of fire blight, the success of the biocontrol was noteworthy because many orchards are developing pathogens resistant to streptomycin, the most popular chemical control. A somewhat limited control of frost injury and russeting on pears also occurred as secondary benefits.

◇ In the control of grey mold, the ability of the fungal biocontrol to affect the pest was helped by the fact that the conidia, or fruiting structures, of *Gliocladium roseum* has a rather sticky surface, enhancing its ability to stick to both the honey bees and subsequently the flowers of the strawberry plants.

◇ In the case of the banded sunflower moth, control was improved by the fact that honey bees are primary pollinators of sunflowers and readily visited the plants. Additionally, the plants treated with the biocontrol experienced increased seed set and the production of seeds with slightly higher oil content. However, *Bacillus thuringiensis* is susceptible to inactivation by sunlight and this must be taken into account when future use is considered.

◇ In the case of control of insect pests in crimson clover, results were quite favorable. Again this is in part due to the fact that clover is readily foraged by honey bees.

The success of all of these studies highlighted some noteworthy, though expected, ideas. Control of the pests was better in every case when the weather conditions at the time of the monitoring favored foraging by the honey bees (warm, sunny weather as opposed to cool, cloudy weather when honey bees are normally not active). Secondly, success of the biocontrol is increased when vigorous honey bee colonies are used to deliver the inoculum.

Future Plans.

The future of honey bees as successful biocontrol vectors is somewhat contingent on the successful use of biocontrols in the future. Biocontrols, in general, have a history of inconsistent performance, due to the fact that it is often hard to control colonization of the plants by the biocontrols and to maintain uniformity. One way to try to ensure more uniform performance by a biocontrol product is use a mix of biocontrol agents that thrive under different climatic conditions, produce different antibiotics, or use different control mechanisms to increase the likelihood that you will have an effective biocontrol out in the field that season. Additionally, the use of different biocontrol agents will lessen the likelihood of the target pests developing resistance to the control. It is also clear that the various biocontrol agents used in a treatment must not interfere with the growth of each other in order to control the pests effectively.

There are some additional challenges specifically facing the future of biocontrol vectoring with honey bees. The application of biocontrols with honey bees must be critically timed to be effective. Furthermore, weather conditions must be conducive to honey bee foraging for the biocontrol to be dispersed. This means clear skies, little wind, and relatively high temperatures for good control. However, remember that all of these requirements for proper dispersal are not as restrictive as they seem because the same conditions are needed for the implementation of most pest control programs, especially chemical controls. Some general challenges are that certain concentrations of biocontrols are required to successfully inoculate plants against their pests. Merely increasing the concentration of the biocontrols in the exit tray of the hive means there must be some way for the organisms to survive until they are dispersed by the honey bees. Placing the organisms on pollen grains, which will provide them with temporary sustenance, is one way to achieve this. Another issue is that when vigorous honey bee colonies are used for dispesal, the inoculum in the exit tray may be quickly depleted and in need of refilling often. In one study, 10 grams of inoculum was used up in 15 minutes. At the other end of the spectrum, inoculum not used quickly enough may absorb moisture and develop clumps or a crust providing the bees with a path through the exit tray on which they will not pick up inoculum. The bees will take this path if they can, because they do not like being covered with the inoculum, and thus they will leave the hive without picking it up. Another limitation to keep in mind is that honey bees will preferentially forage crops that they like, such as peach and cherry. Unfortunately, pears and strawberries are crops that they will not visit if preferred crops are blooming. However, the use of chemical attractants may be used to help overcome this limitation.

There is great potential for the use of biocontrols to control both plant pathogens and insect pests of plants through the use of honey bee vectors. Many plant pathogens colonize the flower of the plant it affects, right where honey bees will visit the flower and carry out their activities. Control of a great number of plant insect pests is also possible, for honey bees forage on several plants that are subject to damage from insect pests. However, for successful control, a better understanding of the relationships among the flower, pest, and biocontrol(s) concerned in each particular situation is required. With a little more research and time, however, honey bees will soon be ready to fly onto the scene of plant pathology with an entire arsenal of microbes to send those pesky pests packing! BC

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