Introduction

Bees are well known for their ability to carry microscopic particles. After all, they are important pollinators, and most pollen is microscopic (Wodehouse, 1959). Bees also are known to carry fungal spores and bacterial cells, some of which are pathogens of the bees themselves, or of plants (Morse & Nowogrodzki, 1990; Shaw, 1999). The capacity of bees to vector spores, bacteria, and viruses can be turned to our advantage by using them to transport biological control agents, a technique known as pollinator biocontrol vector technology (Kevan et al., 2001, 2003, 2004, 2005). This chapter describes the range of biocontrol agents useful for this technique and the targets against which they can be used. These biocontrol agents all have good potential for control of weeds, plant pathogens, or insect pests. The safety of the vector is considered next, along with the fact that it must be harmonized with efficacy against the target problem. Following from and closely linked to those two parts of the pollinator biocontrol vector technology is the matter of concentration of the biocontrol agent in an effective formulation and the efficiency of its acquisition and delivery by the vector. Dispenser designs are discussed as they influence the efficiency of pickup of the formulated biocontrol agent by the pollinators and so constitute another component of the technology. Finally, we briefly consider environmental safety and issues of nontarget effects.

The elements involved in this new technology are:

1. A crop in need of protection
2. A pest (weed, disease, or herbivore) that adversely affects crop production
3. Pollinating insects that visit the flowers of that crop
4. Biocontrol agents that can be vectored by the pollinators
5. A formulation of that biocontrol agent that allows effective vectoring without unduly risking the health of the vectors but that also has demonstratable efficacy against the pest
6. Some means of dosing the vector (i.e., a dispenser)
7. Consumer and environmental safety

Even though this technology has been developed to manage plant diseases and pests, successful application requires interdisciplinary research on several fronts. The biocontrol agent should be effective against the target, yet relatively safe for the bee vectors. The agent must be dispersed by the bees in sufficient quantity to be effective against the target, yet the crop in need of protection must not be adversely affected. Plant and insect pathology must be part of research and development because microbial control agents are being used. Design of the inoculum dispensers requires insights into vector behavior, as does the technology of formulation to maximize inoculum dispersibility and dosage at the target site without negatively endangering the health of the vectors. Moreover, safety of nontarget organisms in the environment in which the technology is applied must be considered. Finally, any produce grown and protected through use of the pollinator biocontrol vector technology must be safe for consumption by people or livestock. Figure 5.1 illustrates the integrated and connected array of facets of the research and development process.

Figure 5.1 The integrated and connected array of facets of the research and development process in pollinator biocontrol vector technology.
Pollinator biocontrol vector technology has been explored by different investigators in a wide variety of ways with different biocontrol agents in various formulations, using different insect pollinators and different dispensing devices. Because few investigations report on comparisons and those that do report on only a few in any one study, this chapter cannot provide an objective and comprehensive comparison of details. Readers should be aware that pollinator biocontrol vector technology has great potential in crop protection, and those interested in using it in their own research should refer to the cited papers in deciding how they should proceed. A great deal remains to be done to discover how pollinator biocontrol vector technology should be customized to the problems at hand.

Control Agents

**Control of Seed Set in Weeds and Invasive Plants**

One of the first considerations for using this technology was to control the fruiting and seed production of field milkweed, *Asclepias syriaca* (Asclepiadaceae; Eisikowitch et al., 1990; Kevan et al., 1989a, 1989b). A yeast, *Metschnikovia reukaufii* (Ascomycetes), was found to inhabit the nectar of milkweed flowers. It is vectored by flower-visiting insects and, once in the nectar, inhibits germination of pollinia. The nectar is secreted by the stigmatic surface in *Asclepias* and is the natural germination medium for the pollinia, and so it seemed logical to propose an inundative application of the yeast, followed by dispersing it with flower-visiting insects to reduce the fecundity of the plants. Despite the potential utility of manipulating this tri-kingdom system (plant, yeast, and insect) for weed control and the evolutionary implications related to hypotheses for mate selection by plants (Morgan & Schoen, 1997), further research has yet to be done. Nevertheless, the idea did not go unnoticed. Recently, Porcella (2006) has suggested that honey bees, *Apis mellifera* (Apidae), could be used to deliver “microsite-specific gameticides,” such as the herbicide glufosinate, to interfere with seed set in weeds.

**Control of Plant Diseases**

Pollinator biocontrol vector technology has been applied successfully to control gray mold, *Botrytis cinerea* (Moniliaceae), on strawberries, *Fragaria x ananassa* (Rosaceae), with the fungal antagonistic, *Clonostachys rosea* (Hypocreales), using honey bees as the pollinator and biocontrol vector (Peng et al., 1992). The levels of control were similar to those obtained from application of a fungicide at the recommended dosages and frequencies. Later, the same technology was applied to raspberries, *Rubus idaeus* (Rosaceae), against the same disease pathogen using honey bees and bumble bees, *Bombus impatiens* (Apidae; Sutton et al., 1996; Yu & Sutton, 1997). The levels of initial success in fruit protection matched or exceeded those achieved with conventional fungicidal sprays. Yu and Sutton (1997) also compared the application of *C. rosea* using a compressed air sprayer versus the pollinator biocontrol vector technology. The incidence of flowers with
no inoculum (C. rosea) was higher in plots sprayed using the air sprayer (55–57 %) compared with plots treated by bumble bee- (6–9 %) or honey bee- (14–15 %) vectored C. rosea. The suppression of gray mold in the flowers was usually greater when C. rosea was applied using bees versus the sprayer. Since then, other researchers have applied the fungal mycoparasite Trichoderma harzianum (Hypocreaceae) to strawberry flowers using honey bees (Maccagnani et al., 1999) and bumble bees (Kovach et al., 2000), and the fungal antagonist Ulocladium atrum (Hypomycetes) to strawberry flowers using honey bees (van der Steen et al., 2006). All reported suppression of gray mold. Trichoderma spp. also suppressed Sclerotinia sclerotiorum (Sclerotiniaceae), an important crop pathogen. Escande, and co-workers (1994, 2002) successfully used these fungi, vectored by pollinating honey bees, to protect sunflowers, Helianthus annuus (Asteraceae), from head rot caused by S. sclerotiorum. Svedelius (2000) suppressed the pathogenic plant fungus Didymella bryoniae (Ascomycetes) on cucumber, Cucumis sativus (Cucurbitaceae), with T. harzianum vectored by the bumble bee Bombus terrestris (Apidae) in the greenhouse.

A myriad of unknown and potentially useful biocontrol agents can be tried against plant diseases using pollinators as vectors. At about the same time that our research was in progress, Thomson et al. (1992) and Johnson et al. (1993a, 1993b) were experimenting with this technology with honey bees for suppression of infection with the fire blight bacterium, Erwinia amylovora (Enterobacte riaeae), using the bacterium Pseudomonas fluorescens (Pseudomonadaceae) on apples, Malus x domestica (Rosaceae), and pears, Pyrus communis (Rosaceae). Since then, there has been some resurgence of interest in that system (e.g., Nuclo et al., 1998; Pusey, 2002). Other examples include the fungal plant pathogen mummy berry, Monilinia vaccinii-corymbosi (Pezizaceae), which itself is obligately vectored by pollinators to species of blueberry, Vaccinium spp. (Ericaceae; Batra, 1983; Woronin, 1888) and can be suppressed by a pollinator-vectored bacterium Bacillus subtilis (Bacillaceae; Dedej et al., 2004) and the so-called “killer yeast,” Metschnikovia fructicola (a newly described ascosporic yeast species), being tested as an inhibitor of gray mold on tender fruit (Kurtzman & Droby, 2001; Karabulut et al., 2003). But as far as we are aware, no one has tried using insect vectors for its dissemination.

Control of Insect Pests

The pollinator biocontrol vector technology has been evaluated successfully against several insect pests on crops. Gross and colleagues (1994) used honey bees to deliver Heliothis nuclear polyhedrosis virus (NPHV) to crimson clover, Trifolium incarnatum (Fabaceae), to help control Helicoverpa zea (Noctuidae), the corn earworm. Although this initiative seems not to have been followed up in the United States, Butt et al. (1998) and, more recently, Carreck et al. (2007) revitalized the idea by applying Metarhizium anisopliae (Clavicipitaceae) to the flowers of canola, Brassica napus (Brassicaceae), to suppress populations of pestiferous pollen beetles, Meligethes aeneus (Nitidulidae), and later, cabbage seed weevils, Ceutorhynchus assimilis (Curculionidae). Research by Jyoti and Brewer (1999) demonstrated that honey bees could also be used as effective vectors of the bacterium Bacillus thuringiensis var. kurstaki (Bt) (Bacillaceae) for control of the
banded sunflower moth, *Cochylis hospes* (Tortricidae), in sunflowers, *H. annuus*. The level of control achieved, along with greater pollination efficiency and seed-set, was better than or equivalent to spray applications of Bt.

Our research on using pollinator biocontrol vector technology for insect control started in response to an outbreak of tarnished plant bugs, *Lygus lineolaris* (Miridae), on canola in Alberta, Canada, in 1998 (Cárcamo et al., 2003). We used the entomopathogenic fungus *Beauveria bassiana* (Clavicipitaceae; Bidochka et al., 1993; Gindin et al., 1996) known to cause mortality through disintegration of the insect cuticle and muscle tissues (Bidochka et al., 1993). Realizing that tarnished plant bugs are important pests of numerous crops, including those grown in greenhouses, we expanded our study to include biocontrol of plant bugs on canola as a field crop (figure 5.2) and on sweet pepper, *Capsicum annuum* (Solanaceae), as a greenhouse crop (figure 5.3). Al-mazra‘awi et al. (2006a) reported mortalities of tarnished plant bug on canola caged with honey bees using the pollinator biocontrol vector technology at 22–56% versus only 9–22% in the controls. *Beauveria bassiana* conidia were recovered from 100% of the bees sampled in our trials, 67–77% of the flowers, and 70% of the leaves. The mean concentration of inoculum recovered from plant bug samples ranged from 1,411 to 3,803 colony-forming units (CFU) per tarnished plant bug over the 2-year study. For greenhouse peppers, we used bumble bees to vector the *B. bassiana* inoculum, and tarnished plant bug mortality ranged from 34 to 45% versus 9 to 15% in the controls (figure 5.4; Al-mazra‘awi.

![Figure 5.2](image-url)  
Figure 5.2 Experimental field set up to evaluate the pollinator biocontrol vector technology using honey bees to vector *Beauveria bassiana* to canola for control of tarnished plant bug. Inset shows honey bee nucleus hive with inoculum dispenser placed inside the cage.
Figure 5.3 Bumble bee hive with inoculum dispenser attached to the front of the hive used in a greenhouse trial set up with sweet peppers.

Figure 5.4 Percent infection for western flower thrips (WFT) and percent mortality for tarnished plant bugs (TPB) exposed to bumble bee-vectored Beauveria bassiana in greenhouse sweet pepper trials; dark gray bars = treatments in which B. bassiana was vectored by bumble bees; light gray bars = treatments with bumble bees only; and medium gray bars = controls.
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2004; Al-mazra'awi et al., 2006b). Again, 97–99% of the bees, 90–96% of the flowers, and 87–91% of the leaf samples contained detectable concentrations of B. bassiana. In this study, the mean concentration of B. bassiana recovered from the tarnished plant bug samples ranged from 587–708 CFUs per tarnished plant bug.

As the project unfolded, the potential for control of western flower thrips, Frankliniella occidentalis (Thripidae), a major pest of greenhouse crops, became evident (Al-mazra'awi et al., 2006b). Using bumble bee pollinator biocontrol vector technology, infection rates of western flower thrips ranged from 34 to 40% compared with only 3% in the controls (figure 5.4). Since then, we have further expanded the potential of pollinator biocontrol vector technology to include pests such as greenhouse whitefly, Trialeurodes vaporariorum (Aleyrodidae), green peach aphid, Myzus persicae (Aphididae), and gray mold in other greenhouse crops, especially tomatoes, Lycopersicon esculentum (Solanaceae) (Kapongo et al., 2005; Shipp et al., 2005). Recently, we have demonstrated that pollinator biocontrol vector technology can be used to simultaneously deliver multiple biocontrol agents for insect pest control and disease management (Shipp et al., 2006). Bumble bees have been used to vector an inoculum containing B. bassiana and C. rosea for tarnished plant bug and whitefly control and for suppression of gray mold on greenhouse sweet pepper and tomato.

Safety for the Vectors and Efficacy Against the Target Pest

When choosing a biocontrol agent and formulation, one must be careful not to unduly endanger the health of the vectors. The potentially useful control agents that suppress seed set in weeds appear to have no adverse effects on the vectors. No matter what sort of agent is considered for use, safety to the vector must be assessed before these biocontrol agents are registered as part of the natural assemblage of microorganisms encountered by pollinators.

The fungal biocontrol agent T. harzianum that suppresses the incidence of gray mold has been tested for safety and seems safe to both honey bees and bumble bees (van der Steen et al., 2004). The other plant fungal agents so far used, and potentially useful, are thought to be similarly safe, but they must be tested for vector safety.

However, when considering the use of entomopathogenic agents, one must assume that the risks to the vectors can be measured. Bt has been tested for its effects against adult honey bees (Vandenberg & Shimanuki, 1986) and is regarded as safe; tests on bumble bees have not been made. We have evaluated the risks to pollinators associated with B. bassiana. We found that Botanigard 22 WP (Laverlam International Corp., Butte, MT), a commercial formulation of B. bassiana, had to be diluted from $2 \times 10^{11}$ conidia/g of product in the commercial formulation to $6 \times 10^{10}$ conidia/g to achieve minimum mortality of bumble bees (B. impatiens) and maximum mortality of the pests (Kapongo et al., 2005; Shipp et al., 2006). At that lower concentration, B. bassiana is known to have little effect on honey bees (Vandenberg, 1990; Goettel & Jaronski, 1997). Moreover, it would not be expected to infect at the temperature of the brood chamber (~35°C) of the hive. In general, it seems that bumble bees are a little more susceptible than honey
bees to developing mycosis from \textit{B. bassiana}, but the risks are small (Al-mazra‘awi 2004; Kapongo, unpublished data). \textit{Metarhizium anisopliae} also carries risks to the vectoring bees (Macfarlane, 1976). It is being considered for biological control of \textit{Varroa} mites in honey bee colonies (Kanga et al., 2003; James et al., 2006), so the risks are probably low and similar to those posed by \textit{B. bassiana}. The NPHV used by Gross et al. (1994) is host-specific to Lepidoptera, and as such is probably safe.

**Diluents and Formulation**

The biocontrol agents used with pollinator biocontrol vector technology are concentrated commercial formulations. They must be diluted to be cost effective and to maximize dispersability and dosage at the target site. Various diluents have been used in preparing inoculum formulations that are placed into the dispensers for use. These diluents can vary greatly in their properties, which can thus affect their utility.

Israel and Boland (1993) found that some carriers, such as talc and especially scented talc, were irritating to honey bees, which can spend as much as 1 minute grooming much of the formulation from their bodies. Other carriers, such as flours, were better accepted, stimulating less than half the amount of time in grooming, and resulted in more efficacious transport of the agent. Spores of \textit{B. bassiana} can be diluted in substrates such as corn flour, corn starch, talc, fungi, or other materials to maximize their dispersability and life span during delivery. Al-mazra‘awi (2004) found that honey bees that passed through corn flour acquired more conidia than did bees that passed through wheat flour, durum semolina, corn meal, potato starch, potato flakes, oat flour, and barley flour. As a general rule, the number of conidia carried by the bees increased with decreasing particle size and moisture content of the carrier and with increasing density of \textit{B. bassiana} conidia in the formulation. The time required for a honey bee to pass through the dispenser did not significantly affect the acquisition of conidia. Van der Steen et al. (2006) compared the relative utility of other carrier materials (cellulose, quartz, talc, diatomaceous earth, and clay materials, such as bentonite and kaolin) as to how they affected the adherence of inoculum to honey bees and hence to the petals of strawberry flowers. They suggest that bentonite adheres well to the bodies of bees and is the best in adhering to petals. Polystyrene beads have also been used experimentally (Butt et al., 1998) but may be too costly for use in commercial formulations.

Although full sets of comparisons of various vegetable and mineral diluents remain to be made, we suggest that mineral diluents are likely to be less efficacious because of the irritation that they cause to the bees.

**Dispenser Designs**

Researchers have used a range of different dispenser designs. Dispensers that direct the pollinators/vectors through the inoculum as they leave the hive and isolate them
from the inoculum on entering the hive minimize wastage of the inoculum. This design also reduces the amount of inoculum that enters the hive. At the University of Guelph, we developed a dispenser for dusting honey bees with fungal spores (Peng et al., 1992) that was based on the design of the Nova Scotia Agricultural College pollen dispenser (Townsend et al., 1958; Hatjina, 1988; King & Burrel, 1933). This type of dispenser was used by Butt et al. (1998) and Carreck et al. (2007), among others. Our over-and-under dispenser design forces the bees to pass through the inoculum tray before directing them to depart above the inoculum tray, and bees reenter the hive by passing through the inoculum-free section of the dispenser. The side-by-side dispenser design used by van der Steen et al. (2006) directs the bees to pass through one side to pick up inoculum as they exit but to enter on the other (inoculum-free) side of the dispenser apparatus. Thomson et al. (1992) and Johnson et al. (1993a, 1993b) used the Antles (1953) pollen dispenser, again developed for pollination in pome crops, but it requires bees to both exit and enter the hive through the inoculum. The dispenser developed by Gross et al. (1994) for honey bee delivery of NPHV against H. zea on crimson clover is elaborate and commanded its own patent. It was used by Dedej et al. (2004) for dispensing B. subtilis. Recently, Bilu et al. (2004) evaluated several types of dispensers for use with honey bees, and they indicate that the University of Guelph dispenser performs well, but that their “Triwaks” has the best overall performance in dusting honey bees with inoculum of T. harzianum.

An over-and-under dispenser for bumble bee hives was developed at the University of Guelph (figure 5.5; Yu & Sutton, 1997). In addition, Maccagnani (2005) reported on the comparison of two dispensers, one a side-by-side and the other over-and-under, for the dissemination of fungal antagonists using bumble bees. She reported that the latter is the more effective for delivering fungal biocontrol agents.

Figure 5.5 Dispenser placed in front of a bumble bee hive with the inoculum tray extended.
More studies of dispenser designs for use with both honey bees and bumble bees are needed to address the practical issues of inoculum loading, duration of availability of the inoculum to the bees, rates of inoculum acquisition by the bees, and interference of the dispenser with bee and colony activity, especially through restriction of the hive entrance. The potential also exists for using other insects, including other pollinators, for dispensing biocontrol agents, but few trials have been conducted.

Environmental and Human Safety

Some of the biocontrol agents that have been shown to be useful with this technology are already considered safe from the viewpoint of human health and have been registered with the appropriate agencies in various countries for application to crops. Pollinator biocontrol vector technology is just a different way of applying the biocontrol agent. Nevertheless, registration of microbial control agents is usually specific to the target crop and the method of application. Other biocontrol agents that are under development will require more evaluation for nontarget organism safety (including vector safety) and with respect to residues on food destined to be consumed by human beings or livestock before they can be registered. Thus some environmental and human risk assessment is required before biocontrol agents are to be widely used with this technology. The only registered application of the pollinator biocontrol vector technology that we are aware is Binab (BINABBio-Innovation AB, Helsingborg, Sweden). Binab is registered for the application of *Trichoderma* spp. using bumble bees for control of gray mold on strawberries and on greenhouse vegetable crops in several European countries (Biobest B.V., 2006).

Because a number of the biocontrol agents tested for pollinator biocontrol vector technology have rather broad spectra of potential hosts, nontarget risks must be considered. For some agents, such as Bt and NPHV, the risks to nontarget insects are probably small. For others, too few data are available to guide further development. It must be remembered that when considering field crops, the pollinating vectors are unlikely to forage only on the target crop. Thus the same issues that confront conventional spray applications apply: nontarget organisms will be exposed. One may argue that the use of this technology against plant pathogens may be neutral or beneficial to nontarget plants. However, when using pollinator biocontrol vector technology against insect pests, clearly the risks to nontarget, beneficial insects or insects of aesthetic value (Lepidoptera particularly) are real and need investigating. Also, the delivery of microsite-specific gameticides, such as the herbicide glufosinate, to interfere with seed set in weeds (Forcella, 2006) could prove to be problematic to the reproductive output of non-weedy, short-lived plants that flower coincidentally with the target weed.

Discussion and Conclusions

Developing a pollinator vector technology for the management of insect and fungal pests on field crops, such as canola, and on greenhouse crops, such as sweet pepper,
provides the benefits of reducing pest populations and pesticide use while improving pollination of the crop. For example, insect pollination of canola improves seed quality and germination rate (Kevan & Eisikowitch, 1990), results in higher seed set and yields (Langridge & Goodman, 1975) and is required for hybrid seed production. Similarly, using bumble bees for the pollination of greenhouse sweet pepper results in increased fruit weight, volume, seed weight, and percentage of extra-large and large fruits and reduced the number of days to harvest (Shipp et al., 1994). Both honey bees and bumble bees can effectively vector biocontrol agents such as C. rosea, T. harzianum, B. subtilis, and P. fluorescens against plant pathogens for disease suppression and B. bassiana, M. anisopliae, Bt, and NPHV to field and greenhouse crops for insect pest control.

Pollinator biocontrol vector technology is a win-win situation because the technology not only reduces pest pressure and pesticide applications but can also improve pollination. Mostly, this technology seems to be safe for the bees, but more laboratory tests, followed by the monitoring of colonies during and after exposure to the biocontrol agents, must be conducted to show no adverse effect on the bees. The development of appropriate formulations and dispensers are key considerations for the success of this technology. The mixture of the dry infective propagules of microbial control agents with diluents and carriers must be made with care to maximize safety and dissemination. Well-formulated agents remain viable for extended time periods in the field and are cost effective. Trials are needed to test each combination of the biocontrol agent and its formulation, the type of pollinator used, the crop to be protected and the pest targeted by the technology, and the sort of dispenser considered to be the most appropriate. Of course, concerns for human and livestock food safety have to be included in the development and registration of any pollinator-biocontrol vector use and need to be coupled with environmental risk assessments with respect to nontarget organisms.

Pollinator biocontrol vector technology is a multidisciplinary pest management approach that incorporates different ecosystem components such as pollinators, microbial control agents and insect pests in the crop production system. It brings the benefits of a new, reduced-risk pest management tool, reduced chemical use, and better pollination of the crop, all of which subsequently result in higher yields and better crop quality.

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