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35 Pollinators as Vectors of Biocontrol Agents – the B52 Story

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Overview: Pollinating and flower-visiting insects can carry some plant diseases and can themselves be infected while foraging at flowers. This is the story of the development of the concept that pollinators could be used as carriers and disseminators of microbial biocontrol agents.

Considerations of How to Apply Symbioses to Pest Control

It is quite well known that pollinating and flower-visiting insects can carry some plant diseases and are themselves infected by diseases while foraging at flowers. Two of the best-known examples of pollinator-borne plant diseases are *Ustilago violacea*, the anther smut of various species of the pink family (Caryophyllaceae), and mummy berry (*Monilinia vaccinii-corymbosi*) on species of blueberry (*Vaccinium* spp. (Ericaceae)). Throughout Kevan's interest in insect and flower interrelationships has been an appreciation of such broader and more complex interactions. Thus, when research on pollination biology in field milkweed (*Asclepias syriaca*), in collaboration with D. Eisikowitch from Tel Aviv, ran into unexpected complications, the issue of tri-kingdom symbiosis arose. From that started our considerations of how to apply such symbioses to pest control.

The milkweed problem came about because Eisikowitch and Kevan were unable to germinate the pollinia in milkweed nectar collected from field-opened flowers, but could do so with full success in sugar solutions prepared in the laboratory or from nectar from flowers that had opened in the laboratory. The explanation seemed to reside in the yeast infections by *Metschnikowia rekaufii* in the flowers of field-opened flowers. The presence of the yeast inhibited pollinial germination (Eisikowitch *et al.*, 1990)! Review of old literature revealed that this yeast was known to be vectored by various flower-visiting insects. Other colleagues became involved in the work, notably Andre Lachance from the University

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of Western Ontario, who has since made major taxonomic and ecological discoveries about *Metschnikowia* and its relations with insects and flowers.

Because of the capacity of *M. reukauffii* to interfere with pollinial gemination in nectar, which in milkweeds is the germination medium and secreted by the stigmatic surface, Kevan and colleagues suggested that artificial application could inhibit sexual reproduction and seed-set in this important weed. Initial experiments were made, but before proper and full testing could be completed, funding was discontinued and research on potential application stopped. It is interesting that the genus (i.e. *Metschnikowia fructicola*, the 'killer yeast') has been tested as an inhibitor of grey mould on tender fruit (Kurtzman and Droby, 2001; Karabulut *et al*., 2003) but not by using insect vectors for its dissemination.

Can Pollinators Provide a Double Benefit of Combined Crop Pollination and Protection?

The idea that biological control agents could be dispersed by flower-visiting insects is hardly new if one considers that pollination itself involves the dispersal of the biological control agent of fertilization (pollen) for most flowering plants. However, that idea, combined with knowledge that some important plant diseases (e.g. grey mould, fire blight, mummy berry) were also vectored by pollinators, stimulated research into the possibility of the double benefit of combined crop pollination and protection. The B52 project, to use honeybees (*Apis mellifera*) as vectors (bombers) for *Clonystachis roseum* to the flowers of strawberries (*Fragaria* x *ananassa*) to suppress grey mould (*Botrytis cinerea*), was initiated from John Sutton's laboratory, spearheaded by Peng Gang in collaboration with Kevan's group (Peng *et al*., 1992). The dispensers used were made by adapting the design of the Nova Scotia Agricultural College Pollen Dispenser, developed for pollination in pome crops (King and Burrel, 1933). That work was followed by parallel studies with raspberries (*Rubus idaeus*), and using honeybees and bumblebees (*Bombus impatiens*) to bomb flowers with *C. roseum* (Yu and Sutton, 1997). The dispenser for bumblebees is a small box mounted to the hive entrance so that the bees have to walk a zig-zag track past baffles and through the inoculum before being able to fly out of the hive (Fig. 35.1). Again, the device directs outgoing bees through the inoculum, but incoming bees enter the hive by a different route isolated from the inoculum.

The results of the trials indicated that bumblebees, like honeybees, were as effective in vectoring the inoculum of the antagonistic fungus and suppressing the incidence of grey mould as was spraying fungicide at the normally recommended rates. The incidence of flowers with no inoculum was high in plots sprayed with the fungus (55–57%) compared with plots treated by bumblebee- or honeybee-vectored fungus (6–9% and 14–15%, respectively) (Yu and Sutton, 1997). Despite the levels of initial success in fruit protection matching or exceeding those achieved with conventional fungicidal sprays, funding was unexpectedly terminated. Since that time, the use of *C. roseum* has been promoted and used in various parts of the world for the protection of various tender fruits and other crops (Sutton *et al*., 1997).

Fig. 35.1. A bumblebee sitting on the edge of a dispenser coated with inoculum.

Using the same technology as described above, a commercial formulation of *Trichoderma harzianum*, another antagonist to grey mould, has been applied to strawberries by pollinating honeybees in Italy (Maccagnani *et al*., 1999) and by honeybees and bumblebees in the USA (Kovach *et al*., 2000). The conclusion from that work is that bee delivering of *T. harzianum* is also a viable option for strawberry growers interested in controlling grey mould with minimal use of fungicides.

Further advances made at the University of Guelph were the testing of various carrier/diluent agents for the biological control materials. Israel and Boland (1993) found that some carriers, such as talc and especially scented talc, were irritating to the bees, which groomed much of the formulation from their bodies. Other carriers, such as flours, were better accepted by the bees and resulted in more efficacious transport of the agent. Israel and Boland's interests were for the suppression of *Sclerotinia* on the flowers (anthoplane) of rape (*Brassica* spp.), but application of the technology to that system remains to be taken up again. The results of their diluent/carrier tests have been important in the authors' recent research programme (see below).

At about the same time as the B52 project was proceeding, research in the western USA by Sherman Thomson's team in Utah (Thomson *et al*., 1992) and Kenneth Johnson's team in Oregon (Johnson *et al*., 1993a,b) was directed at using honeybees to deliver the bacterium *Pseudomonas fluorescens* as an antagonist against fire blight *Erwinia amylovora* on pome crops. That research also met with some success, has continued at a modest pace (e.g. Nuclo *et al*., 1998) but recently has excited some renewed interest in Washington (Pusey, 2002).

An exciting development by Harry Gross and his team at the USDA laboratory in Tifton, Georgia was the first application of the B52 idea against insect pests (Gross *et al*., 1994). Honeybees were used to deliver *Heliothis* nuclear polyhedrosis virus (NPHV) to crimson clover (*Trifolium incarnatum*) to help control *Helicoverpa zea*, the corn earworm (Lepidoptera: Noctuidae). The dispenser developed for this project is elaborate and commanded its own patent. That initiative seems not to have been followed up, again despite its potential for practicality. Nevertheless, Tariq Butt and his group, working in Rothamsted, UK, revitalized the idea of using the system to control insect pests when they applied *Metarhizium anisopliae* to the flowers of rape (*Brassica napus*) to suppress populations of pestiferous pollen beetles (*Meligethes aeneus*) (Butt *et al*., 1998). They used a modified Nova Scotia Agricultural College Pollen Dispenser and demonstrated efficacy of control. Shortly after that, research in North Dakota by J.L. Jyoti and Garry Brewer (1999) demonstrated that honeybees could be used as effective vectors of *Bt* (*Bacillus thuringiensis* var. *kurstaki*) to the flowers of sunflower (*Helianthus annuus*), where control (equivalent to that obtained with manual sprays) of banded sunflower moth (*Cochylis hospes* (Lepidoptera: Tortricidae)) was achieved, along with increased pollination and seed-set.

Our research on using B52 technology against insect pests started in response to the outbreak of tarnished plant bugs (TPB) (*Lygus lineolaris*) on rape in Alberta in 1998. On rape, adult TPB and late-instar nymphs cause direct yield loss by feeding on individual seeds through the pod (silique) and indirect loss by feeding on other plant tissues. TPB are important pests of numerous crops, including those grown in greenhouses. So, when Mohammad Al-mazra'awi joined Kevan's laboratory for his doctoral studies, an expanded study that embraced the biocontrol of TPB by the B52 approach on rape as a field crop and sweet peppers as a greenhouse crop was initiated. On greenhouse sweet peppers (*Capsicum annuum*), TPB causes yield loss by feeding on the growing points of the plant as well as on the developing flower buds and reproductive structures. As the project unfolded, the potential for control of western flower thrips (WFT), *Frankliniella occidentalis,* became evident. WFT is also a major pest of greenhouse sweet pepper and causes direct yield loss by feeding on, or ovipositing in, developing fruits, which results in a bronzing and silvering of the fruit. Previous reports had already demonstrated the susceptibility of TPB and WFT to the entomopathogenic fungus *Beauveria bassiana* (Bidochka *et al*., 1993; Gindin *et al*., 1996), known to cause mortality to TPB through disintegration of the insect cuticle and muscle tissues (Bidochka *et al*., 1993), so it has been the entomopathogen central to our investigations.

Are the Entomopathogens Safe for the Pollinators?

One of our concerns has been the safety or the possibility of killing the messengers (bombers) with the biocontrol fungus. The latter also involves the nature of the diluent/carrier and the dispenser design (see Bilu *et al*., 2004 for recent discussion of dispensers).

Safety tests can be made in various ways, in the laboratory with caged pollinators and in experimental hives. The biological control agents so far tested seem safe for honeybees and bumblebees if used at appropriate concentrations. Even entomopathogenic agents have been shown to be safe, except in the extremely high concentrations as in the commercially sold formulations of powders or liquids (Vandenberg, 1990; Alves *et al*., 1996; Al-mazra'awi, 2004). For example, we have found that we need to dilute Botanigard 22WP7, a formulation of *B. bassiana*, from 2×10^{11} conidia/g of product to 6×10^{10} to achieve minimum mortality of the bees and maximum mortality of the pests. At that concentration, *B. bassiana* has little effect on honeybees and it would not be expected to survive in the heat of the brood chamber (ca. 35°C) of the hive. It seems that bumblebees are a little more susceptible to developing mycosis from *B. bassiana* but that the risks are small.

In developing our formulation, we first evaluated factors affecting the acquisition of conidia of *B. bassiana* by honeybees in the laboratory using inoculum dispensers that allowed the bees to walk through different formulations of the agent. The number of conidia carried by bees emerging from the dispensers differed according to the type of formulation used. Honeybees that passed through maize flour acquired more conidia (e.g. 1.5×10^6 CFU (colony forming units)/bee) than did bees that passed through wheat flour, durum semolina, maize meal, potato starch, potato flakes, oat flour and barley flour. We find that 2 g of 6.24×10^{10} conidia of *B. bassiana*/g of formulation in dispensers on hives of 50 workers of *B. impatiens* minimizes risk to the bees while optimizing pest control. As a general rule, we found that the density of conidia carried by the bees increased with decreasing particle size and moisture content of the carrier and with increasing density of *B. bassiana* conidia in the formulation. Time required for honeybees to pass through the dispenser did not significantly affect the acquisition of conidia. After those trials, we chose maize flour as our favoured diluent/carrier. All in all, and although we continue to refine our formulation, it is evident that bees walking through such diluted inocula become sufficiently dusted with spores to control pestiferous populations of TPB on rape (Fig. 35.2) and WFT on greenhouse-grown sweet peppers, as well as green peach aphid (*Myzus persicae*), also on the latter.

Are Pollinators Efficient in Delivering the Biocontrol Agent?

Our second concern has been the efficacy of delivery by pollinators to field (i.e. honeybees, *A. mellifera*) and greenhouse crops (i.e. bumblebees, *B. impatiens*).

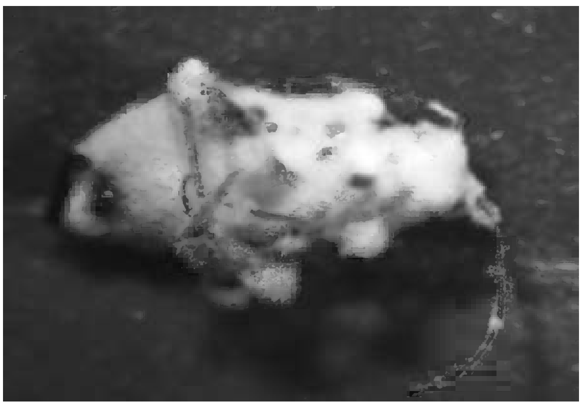

Fig. 35.2. A dead lygus bug killed by *Beauveria bassiana*. The fungal inoculum was brought to the host by a bee.

To test the dissemination of *B. bassiana* to rape against TPB, we used honeybees from nuclear hives equipped with inoculum dispensers (as developed for use with *C. roseum*) filled with our developed dry formulation of *B. bassiana*. The bees were allowed to forage on blooming rape plants inside large screened cages (1.8 × 6 × 1.8 m high) in a greenhouse and in open field plots. Samples of honeybees, flowers, leaves and TPB were collected from the cages on two sampling dates separated by 6-day intervals. The samples were subject to serial dilution plating to determine densities of *B. bassiana* conidia. TPB adults were also collected from the cages to assess mortality over time. Results showed that the bees effectively vectored the inoculum from the hives, as conidia of the fungus were recovered from 100%, 67% and 77% of bees, flowers and TPB, respectively, collected on the first sampling date, and from 100%, 77% and 83%, respectively, on the second sampling date in 2002. In 2003, 100%, 64%, 70% and 47% of sampled bees, flowers, leaves and TPB had *B. bassiana* conidia on the first sampling date, and 100%, 72%, 82% and 57% on the second sampling date. Mean mortalities of TPB collected from treated rape plants in the greenhouse trial were significantly higher (37%) than of TPB collected from the control plants (18%). In the open field trials, mean mortalities of TPB collected from the treated rape were 56% and 48% compared with 9% and 10% in the controls on the first and second sampling dates in 2002, and 22% and 45% in the treated rape compared with 15% and 22% in the controls on the first and the second sampling dates, respectively, in 2003.

Our group has tested, and is testing, the ability of bumblebees to disseminate conidia of *B. bassiana* from hive-mounted dispensers (noted above) to the flowers of greenhouse sweet pepper for the control of TPB and WFT. Evaluations were made inside large screened cages (1.8 × 4 × 1.8 m high) placed inside a greenhouse. Our most recent results, from samples collected from the cages on two dates, showed that 97% of the collected bees, 90% of the flowers, 91% of the leaves and 42% of the collected TPB showed detectable densities of *B. bassiana* on the first sampling date. On the second sampling date, 99%, 96%, 87% and 30% of collected bees, flowers, leaves and TPB showed detectable densities of the fungus. Mean mortalities of TPB collected from plants treated with *B. bassiana* were significantly higher (34% and 45%) than in the controls (9% and 15%) on the first and the second sampling dates. Mean infection rates of WFT collected from treated plants were 40% and 35% compared with 3% and 2% in the controls on the first and the second sampling dates. Our research is continuing with trials against green peach aphid and greenhouse whitefly (*Trialeurodes vaporariorum*) on greenhouse crops, including tomato.

Discussion and Conclusions

Developing a pollinator–vector technology for the management of insect and fungal pests on field crops, such as rape, and on greenhouse crops, such as sweet pepper, brings the benefits of reducing pest populations and pesticide use while improving pollination of the crop.

Insect pollination of rape is necessary for high seed germination rate, higher seed-set and yields. Similarly, using bumblebees for the pollination of greenhouse sweet pepper resulted in increased fruit weight, volume, seed weight and percentage of extra-large and large fruits and reduced the number of days to harvest. Both honeybees and bumblebees effectively vector biocontrol agents such as *C. roseum*, *T. harzianum*, *B. subtilis* and *P. fluorescens* against plant pathogens and *B. bassiana*, *M. anisopliae*, *Bt* and NPHV to field and greenhouse crops and to orchards, where the populations of the various pests have shown to be reduced (review in Kevan *et al*., 2003). This is a win–win situation because the pollinator vector technology not only reduces pest pressure and pesticide applications but also improves pollination. However, for this technology to be practical, the biocontrol agent must be shown to be safe for the bees. Laboratory tests followed by monitoring of the colonies during and after the trials must both show no adverse effect on the bees. The development of appropriate formulations and dispensers are key considerations for the success of the pollinator vector technology (Kevan *et al*., 2003). The mix of the dry infective propagules of microbial control agents with diluent/carriers must be made with care to maximize safety and bee dissemination. Well-formulated agents have extended use in the field, and adjustments provide flexibility in preparing inocula with different concentrations to maximize the cost:benefit ratio. We caution that trials are needed for each combination of 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. Thus, pollinator–vector technology is a multi-disciplinary pest management approach that incorporates different ecosystem components such as pollinators, microbial biological control agents and insect pests in crop production. It brings the benefits of pest management, reduced chemical use and better pollination for the crop, which subsequently results in higher yields and better crop quality.

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