Development of the Mason Bee, *Osmia cornifrons*, as a Targeted Delivery System for Biocontrol Agents in the Management of Fire Blight

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Introduction

This is a multi-disciplinary effort between entomologists and a plant pathologist to address three research priorities as set by the 2008 SHAP research committee. We propose developing a pollinator alternative to the honey bee which specializes in pollinating fruit trees and is not susceptible to the virus causing the Colony Collapse Disorder nor to the mite problems devastating the nation's honey bee industry and feral honey bee populations. As a rosaceous specialist pollinating tree fruit, and with a relatively limited foraging range, the Japanese Orchard Bee (JOB), *Osmia cornifrons*, is a good candidate to serve as a vector for delivering biological control agents to control fire blight, while limiting the spread of this disease from hosts outside the orchard and from spreading the disease between orchards. Biopesticide alternatives to Streptomycin for fire blight control have suffered from lack of efficacy most often associated with the inability to adequately deliver the biological agent directly to the flower stigma at a high enough dose with current sprayer technology. These biocontrol products are compatible with organic production and are considered of reduced risk to the environment by EPA. If their efficacy can be improved through targeted delivery to open flowers, they would form a key tool in strategies aimed at reducing the risk of resistance to streptomycin in Pennsylvania pome fruit orchards. Successful implementation would also help alleviate concerns over possible impacts of antibiotics on the environment and the possible development of cross-resistance in human pathogens.

As a more efficient pollinator of apples and pears than the honey bee and being less affected by weather, JOB may also be a more effective vector of these biological control agents than conventional sprayers. Successful pollination with JOB does not require large populations. JOB and the European Orchard Bee are 80 times more effective in pollinating apple than the honey bee, which spends most of its time collecting nectar for honey rather than pollen. Only 250-500 JOB are required per acre for pollination compared to 30,000 to 60,000 honey bees. *A single JOB can visit 15 flowers/min, setting 2,450 apples/day compared to 50 flowers set by a honey bee (Greer 1999).* This high level of pollination efficiency occurs because mason bees land directly upon the reproductive structures of the fruit tree blossom. The abdomens of foraging female bees are loaded with pollen, and the repeated and direct contact with the anthers and stamens results in higher levels of pollen transfer.

Alternative Fire Blight Management Strategies – Fire blight, caused by the bacteria *Erwinia amylovora*, is one of the most serious diseases currently limiting apple production in the eastern US. It is such a severe problem on pear in this region that the production acreage is kept quite small. Fire blight is capable of infecting blossoms, fruits, vegetative shoots, woody tissues, and rootstock crowns. There are several distinct phases on the disease including blossom blight, shoot blight, and rootstock blight. Effective management of the disease requires integrated approaches that are aimed at: a) reducing the amount of inoculum available to initiate new infections; b) imposing barriers (antibiotics & biopesticides) to the successful establishment of fire blight on its host; and c) reducing host susceptibility to infection (Norelli et al. 2003). Most

fire blight management strategies in the eastern US up to this time have focused on the reduction of inoculum in the orchard (early season sprays of copper or with the pruning of cankers and infected tissues) and with the use of antibiotic treatments to prevent infection during the blossom blight phase.

Several biocontrol products based on antagonistic bacteria that competitively inhibit *E. amylovora* have been evaluated, registered, or are at an advanced stage of registration for management of the blossom blight phase of fire blight under commercial conditions. These include Serenade® (*Bacillus subtilis*) and BlightBan® A506 (*Pseudomonas fluorescens*) which are registered for use in many states, as well as several strains of *Pantoea agglomerans* which is in the registration stage. However, except in the Pacific States, the effectiveness of the biocontrol agents has met with mixed success elsewhere in the US. For example, whereas *B. subtilis* provides effective disease control in field trials in Michigan, *P. fluroscens* which is widely used in the Pacific Northwest is not effective (http://www.ipm.msu.edu/CAT05_frt/F04-26-05.htm). These inconsistent results are possibly due to inability to target the biocontrol agents to the flowers with airblast sprayers. A targeted delivery system with *Osmia* could greatly improve efficacy, while at the same time greatly reducing costs by reducing the amount of biopesticide applied. Preliminary studies of fire blight control on pear in Italy using the European Orchard Bee, *Osmia cornuta,* support this hypothesis (Maccagnani et al. 2006)

Improving the effectiveness of the biocontrol products antagonistic to fire blight would allow them to serve as tools for streptomycin resistance management in Pennsylvania orchards.

The objectives of this study were the following:

1) Design, fabricate and test a dispenser to be used with nesting tubes of JOB to facilitate the delivery of the commercially available biocontrol products such as Serenade.

2) Determine if JOB exiting the dispensers effectively vector the biocontrol agents onto open crab apple flowers.

3) Determine the ability of JOB to secondarily transmit biocontrol bacteria between flowers.

Materials & Methods: Crabapple trees were used rather than apples because of the much heavier flower production on small potted trees. Several hundred potted 'Manchuria' crabapple trees were bought and used during this project. Initial trials within the PSU-FREC greenhouses failed, because the bees left the dispensers and never returned. Other closely related species of orchard bees have been used in greenhouse work before, but these were glasshouses. Bees are known to use the UV light from the sun for navigation, and we speculate that the polycarbonate material of our new greenhouses could have affected this navigation. We, therefore, moved the potted trees and the experiments outdoors to remote areas and timings that reduced bloom competition from other orchards on the station. Although JOB delivery of Streptomycin would be an interesting comparison and could greatly decrease the amount of product applied, analytical methods for the detection of residues in pollen are very expensive (Bogdanov 2003.) and beyond the scope of this project.

Our biggest problem in 2008 was in maintaining enough JOB adults at the right times to run replicated trials through time. We had planned to supplement the station's JOB colonies for this work in the spring of 2008, but found that the demand for pollinators in almonds and organic

apple in the western US was so great that all commercial dealers of JOB had sold out by the end of January. We, therefore, supplemented our populations with the use of colonies maintained by fruit grower, Barry Rice, and with populations from the PSU apiarist, Maryann Frazier. All JOB colonies, however, suffered from high mortality due to early adult emergence from unseasonably warm temperatures in early April followed by intense cold, from parasitic wasps, and from a new parasitic mite. Numbers of JOB for transmission trials were therefore low and we were only able to test the Serenade® formulation of *Bacillus subtilis*, which is well formulated for bee transmission and has been used previously with honey bees by other researchers.

Objective 1: Design and fabrication of a dispenser suitable for JOB.

We designed and tested a JOB nest dispenser suitable for JOB following a model described by Maccagnani et al. (2006). The design is of a simple wooden structure consisting of a transparent plastic exit ramp with a shallow channel at the base to hold the biocontrol product in a fine power form (Fig. 1). We scaled up this dispenser to accommodate enough JOB for an acre - 250 bees with a foraging range of about 3 acres, but at numbers considered adequate for pollination of only a single acre. We modified the upper exit ramp to allow for quick loading of the biocontrol product into the grooves with a small spoon (Fig. 2). Maccagnani et al. (2006)'s design allowed 20% of the bees to exit via the entrance holes, thus reducing their effectiveness as active carriers of the antagonistic bacteria. We modified the design to increase the efficiency of using the exit slot, by covering the screened front to allow only light entering from above and attracting the bees upward to the exit ramp. We also separated the exit slot from the entrance tubes by fitting the entrance tubes with a screen flap directing the bees upward, but restricting their movement downward, all of which greatly reduced the use of the entrance tubes by exiting bees. Our experience with JOB has shown that color coded entrance tubes are "remembered" by the bees on their initial orientation flight, so that upon returning, they fly directly to a specific tube opening.

We tested the proper use of entrances and exits in the JOB nesting dispenser by observing all bees entering and exiting the dispenser over a 15 minute period and repeated the observations three times. The observations were made with the Serenade formulation in place in the exit grooves and results (Fig. 3) indicate that, while 95% of the bees exited properly and were exposed to the Serenade, only about 50% returned to enter the lower tubes properly. The other half used the exit grooves to re-enter. Proper exit behavior and contact with the biocontrol product was by far the most important aspect of the dispenser. Entering the exit grooves is really not a problem since they would pick up more of the biocontrol product.

Objective 2: Determine if JOB using the dispenser can vector the biocontrol product.

To determine the amount of the biocontrol product collected by an individual insect, bees exiting the dispensers were captured and placed in glass vials containing 2ml of sterile 0.1M potassium phosphate buffer (KPB) amended with 0.1ml of Tween-20 surfactant per liter. The vials were shaken vigorously for 30 seconds followed by incubation in a sonicating bath for another 30 seconds to dislodge the bacteria adhered to the body of the vectors. The wash buffer was subjected to serial dilution and plated either on nutrient yeast extract dextrose agar (NYDA) medium for *B. subtilis* (Scherm et al., 2004). The plates were incubated at 28˚C and number of colony forming units (CFUs) determined within 3 days of plating. Bees exiting dispensers in which no biocontrol product was placed were used as controls. Eight bees were assayed for the Serenade treatment in each of 3 replicates. Also collected for preliminary data on primary

transmission, 8 flowers from each trial were collected from 6 potted trees immediately after being visited by JOB carrying Serenade and placed singly into vials (replicates) for assaying the quantity of *B. subtillus* by dilution plating as described above. JOB colonies were very strong during this preliminary trial and the data are represented in Figs. 4 & 5.

A much larger trial to examine the amount of Serenade deposited from primary transmission was carried out about 2 weeks later. Two nest dispensers with at least 25 tubes of JOB were placed about 500 yards apart near potted 2-year-old apple or crab apple plants from the greenhouses which were at 25-50% bloom. Following JOB visitation, flowers were harvested and placed in vials containing 5 ml of KPB. Eight flowers per tree so visited were placed singly into vials (replicates) for assaying the quantity of *B. subtillus* by dilution plating as described above. The experiment was repeated twice with a total of 10 potted trees in the first site and 4 trees at the second site due to shortages of trees at the proper stage of bloom. A total of 120 blossoms were thus, collected, serially diluted 4 times and 480 agar plates were incubated for counting CFUs. The JOB colonies for this second trial were very weak due to parasitic wasps, mites and being kept cooled for several weeks. This data is represented in table 1.

Results and Discussion

The amount of Serenade carried by JOB exiting the dispenser in the preliminary trial with strong colonies of JOB can be seen in Fig. 5. Compared to the amount of Serenade carried by honey bees for protection of blueberries for mummy berry disease (Scherm et al. 2004), JOB carried approximately 20 times more product upon leaving the dispenser. The amount of Serenade that JOB actually delivered to the flowers was high (Fig. 5) - about 18 times higher than was found on blueberry flowers after delivery by honey bees (Scherm et al. 2004). The data for primary transmission in the larger trial using the weak JOB colonies is presented in Table 1, and was much lower probably because we had weak JOB colonies for this trial. It serves primarily to illustrate the levels present in the blossoms prior to the secondary transmission trial in Objective 3. Maccagnani et al. (2006) found the European Orchard Bee, *Osmia cornuta*, to carry up to 1,000 times more *B. subtilis* than the honey bee and delivered much higher dose to the flowers. We would expect that with strong JOB colonies we would see much higher levels of transmission in future work.

Objective 3: Determine the ability of JOB to secondarily transmit biocontrol bacteria between flowers.

In this experiment, trees that had been directly exposed to JOB carrying Serenade were moved about 5 miles to an isolated location in a wooded area where JOB colonies from fruit grower, Barry Rice were kept. The level of transmission of Serenade to these exposed trees can be seen in Table 1. All unopened blossoms on these trees were removed prior to transport so that only flowers that had been exposed to JOB with Serenade were left for the secondary transmission trial. Potted crabapple trees at 25-50% bloom which had never been exposed to bees or Serenade were moved from the greenhouse and placed within 2 meters of the primary exposure trees. The experiment was repeated twice with a total of 10 potted trees of each treatment in the first replicate and 4 trees in the second. After 24 hours of exposure to the new JOB colonies, 8 random blossoms were collected and the dilution and agar plating protocol of Objective 2 was repeated. A total of 120 blossoms were collected, serially diluted 4 times and 480 plates were incubated for counting CFUs. Log transformations of the data are also present in Table 1 to better show standard errors of the trials due to the large values of the data collected.

Flowers collected from 4 unexposed trees from the greenhouse prior to movement to the Barry Rice site were used as controls to make sure there was no possibility of prior contamination of the trees with Serenade.

Results and Discussion

Plates from the controls showed there was no prior contamination of the crabapple trees used for the secondary transmission trials. Secondary transmission by JOB from blossoms previously inoculated with Serenade to new unexposed blossoms was successful. The 24 hour growth of Serenade colonies on these new flowers is demonstrated by the 6.7 to 61-fold increase in CFUs from that found on the primary transmission trees (Table 1). Successful secondary transmission and subsequent increases in Serenade colonies on flowers is very encouraging since it means the system is self-perpetuating. Refilling the nest dispensers with Serenade on a daily basis may not be necessary and all pollinators visiting inoculated flowers, not just JOB, should be able to move the Serenade colonies to new flowers as they open. Further work, however, is needed in managing strong colonies of JOB. The dispenser and concept of using JOB as a vector works and hold promise for dispersing biocontrol agents for fire blight control. Strong JOB colonies are needed carry and deliver products at high enough concentrations to give effective field control. We feel we can overcome this problem in the future with better sanitation procedures and storage of bees in coolers. The next step would be to test different products in addition to Serenade to overcome the biggest hurdle of using biocontrol agents over that of using Streptomycin – efficacy. Potted plants are fine for preliminary trials, but the next step would be to scale up JOB colonies and develop efficacy data under field conditions.

Selected References

Dedej, S., Delaplane, K.S., and Scherm, H. 2004. Effectiveness of honey bees in delivering the biocontrol agent *Bacillus subtilis* to blueberry flowers to suppress mummy berry disease. Biological Control 31:422-427.

Greer, L. 1999. Alternative pollinators: native bees. ATTRA Horticulture Technical Note. http://www.attra.org/attra-pub/PDF/nativebee.pdf

Johnson, K. B., and Stockwell, V. O. 1998. Management of fire blight: a case study in microbial ecology. Annual Review of Phytopathology 36:227–248.

Jones, A.L., and Schnabel, E.L. 1999. Streptomycin and oxytetracycline resistance determinants detected among bacteria from Michigan apple orchards and their potential importance. Acta Hort. 489:673.

Maccagnani, B., Bazzi, C., Biondi, E., Tesoriero, D., and Maini, S. 2006. Potential of *Osmia cornuta* as a carrier of antagonistic bacteria in biological control of fire blight: a comparison with *Apis mellifera*. Acta Horticulaturae 704:379-386.

Maccagnani,B., M. Mocioni, E. Ladurner, M. L. Gullino, and S. Maini. 2005. Investigation of hive-mounted devices for the dissemination of microbiological preparations by *Bombus terrestris*. Bull. Insectology 58: 3-8.

Ngugi, H. K., S. Dedej, K. S. Delaplane, A. T. Savelle, and H. Scherm. 2005. Effect of flower applied Serenade biofungicide (*Bacillus subtillus*) on pollination-related variables in rabbiteye blueberry. Biological Control 33: 32-38.

Norelli, J. J., A. L. Jone, H. S. Aldwinckle. 2003. Fire blight management in the 21st century. Plant Disease. http://www.ars.usda.gov/SP2UserFiles/Place/19310000/0424-02F.pdf

Scherm, H., Ngugi, H.K., Savelle, A.T. and Edward, R.J. 2004. Biological control of infection of blueberry flowers caused by *Monilinia vaccinii-corymbosi*. Biological Control 29:199-206.

Vanneste, J. L. 1996. Honey bees and epiphytic bacteria to control fire blight, a bacterial disease of apple and pear. Biocontrol News and Information 17(4): 67N-78N.

Fig. 1 JOB Nest Dispenser

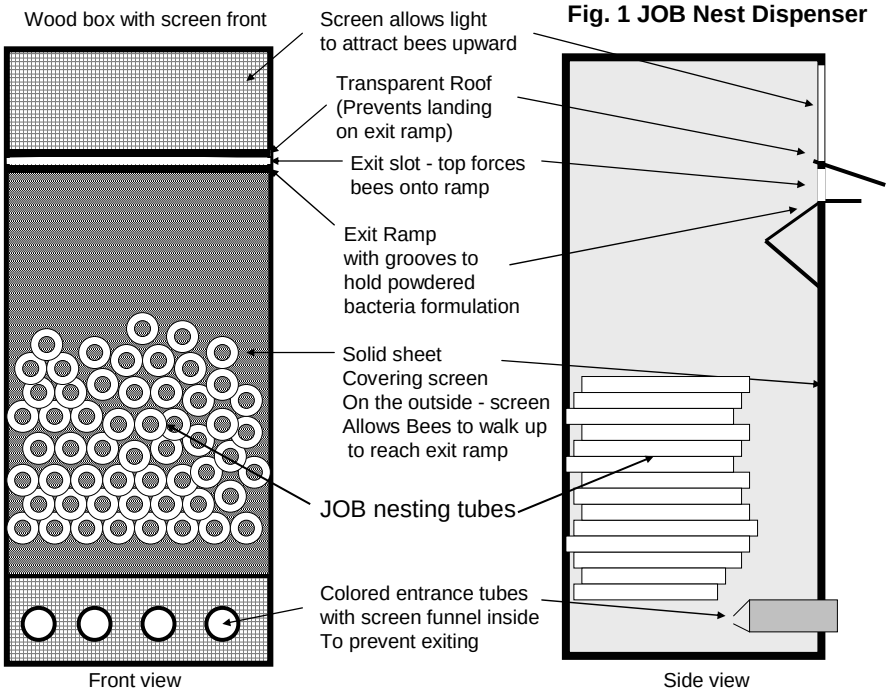
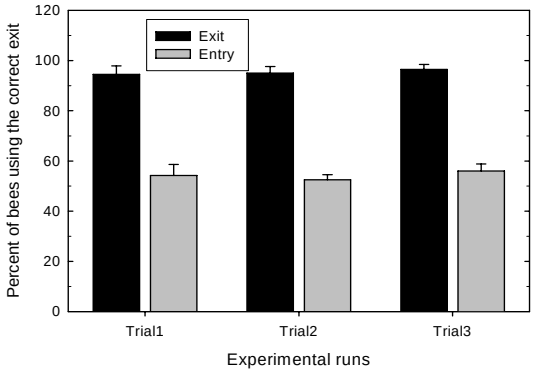


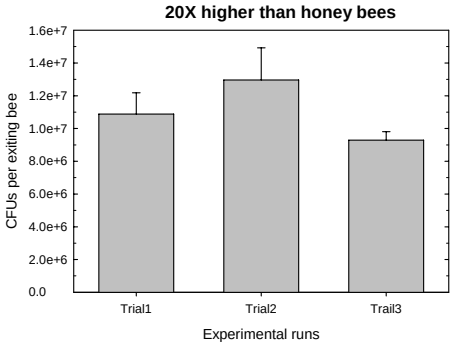
Fig. 2 Japanese Orchard Bee Nest Dispenser and Bees Moving through Serenade

Fig. 3. Utilization of the dispenser by Osmia cornifrons



***Most bees used the right exit but only the about a half returned into the dispenser using the correct entrance**

Fig. 4. Amount of *Bacillus subtillus* carried by the Japanese Orchard Bees Exiting the Nest Dispenser



***For comparison, honey bees exiting hives in GA carried 6.4 x 10^5 CFUs of the same product (Scherm et al., 2004).**

Fig. 5 Amount of *Bacillus subtillus* the Japanese Orchard Bees Deposited On Crabapple Blossoms.

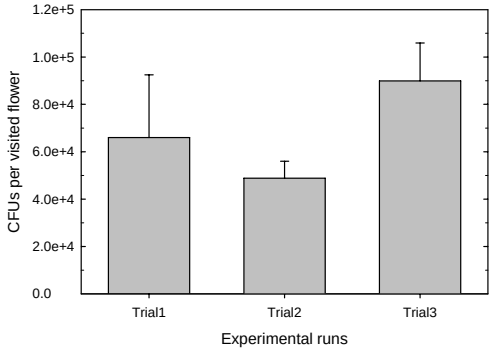


Table 1. Secondary transmission of *B. subtilis* by the Japanese Orchard Bee

Treatment	Av. # Colony Forming Units (SE)	Av. # CFU's Log Transformed (SE)
Primary Transmission Rep 1	6,818 (4,113)	0.58 (0.16)
Primary Transmission Rep 2	27,813 (11,428)	2.42 (0.39)
Secondary Transmission Rep 1	415,511 (218,531)	1.27 (0.24)
Secondary Transmission Rep 2	185,156 (129,613)	2.78 (0.43)