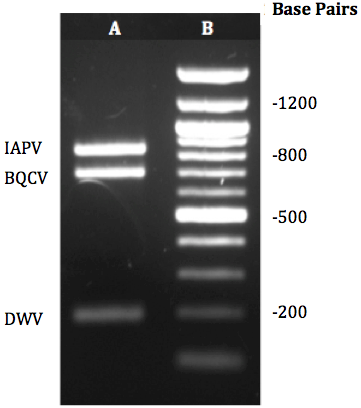
RNA viruses: prevalence, transmission, and effect on native bumble bees in Vermont

Keywords: bumble bee, virus, pollination

**Intellectual Merit.** The decline of honeybees is of national and international concern among scientists and the public alike. Indeed, the loss of pollinators resulted in the President issuing a memorandum last summer to create a “Federal Strategy to Promote the Health of Honey Bees and Other Pollinators” (1). The public is strongly aware that honeybees are in decline. Less appreciated, perhaps, is that many of the threats to honeybees are also affecting native bees (2,3,4,5,6). In particular, the spread of pathogens including viruses is emerging as a serious threat. Once considered to be specific to European honeybees, RNA viruses have been detected in bumble bee species (2,6). With their high mutation rates and short generation time, RNA viruses are likely to cross species-barriers and adapt rapidly to new environments(2,7). Indeed, RNA viruses have been detected in 11 non-*Apis* hymenoptera (2). **My goal is to examine the propensity, means of transmission, and effects of RNA viruses on bumble bees in Vermont**. Eighteen viruses are known to affect bees (2). I will focus on two of the most common—deformed wing virus, and black queen cell virus—as well as Israeli acute paralysis virus, a recently described virus of managed honeybees affected by colony collapse disorder. I will examine:

**The prevalence of RNA viruses across native bumble bee species in Vermont 2. The synergistic effects of virus infection and pesticide exposure on bumble bee behavior and colony health,** and **3. The role of flowering plants in virus transmission.** No previous study to date has defined native bee viral host range or closely examined the role of flowers in transmission. Filling these knowledge gaps is important from an ecological and conservation perspective.

Figure 1. Agarose gel electrophoresis. A. honey bee positive for Israeli acute paralysis virus (IAPV), black queen cell virus (BQCV), and deformed wing virus (DWV). B. 100 base pair ladder.

**1. Prevalence.** To determine the prevalence of RNA viruses across native bumble bees in Vermont, I conducted a pilot survey in 2014. **Approach.** I collected over 300 bumble bees from 15 field sites across northern VT. Because shared flowers are suspected bridges for “spill over” of viruses from honeybees to wild bumble bees (2), I conducted surveys of flowering plants and honeybees at each field site. I am currently testing bees and their pollen loads for viral infection using RT-qPCR (8). I have already identified primers and developed protocols that are highly specific to the viruses of interest (**Fig. 1**). I will determine if virus prevalence can be explained by honeybee abundance or flower density/diversity using honeybee counts and flower measurements as covariates in analyses with bumble bee viral load as my response variable. Results from 2014 will inform my 2015 surveys; I plan to collect bees and census plants in at least 30 field sites across VT.

**2. Effects.** Already, RNA viruses are known to infect bumble bees (2,6) but their effects on individual behavior and colony health are largely unknown. Viruses can persist as subclinical infections and replicate quickly when the host is stressed, resulting in observable symptoms such as deformed wings, paralysis and mortality. Stressors include neonicotinoids, a class of neurotoxic pesticide found to increase honeybees’ susceptibility to pathogens and parasites (9). I hypothesize that pesticide exposure reduces bees’ ability to tolerate viruses and the synergistic effects of pesticides and viruses will severely affect bee behavior and colony health. Understanding these effects is critical to pollinator conservation. **Approach.** Using field and lab experiments, I will test the effects of RNA viruses and pesticide exposure on bumble bees. In 2014, I successfully reared seven bumble bee colonies using wild-caught queens and relocated them to the field where viral load was measured throughout the summer. In addition, I measured colony size and weight, number of sexuals, and timed bee foraging trips. In future summers, I will rear 10-20 colonies/year and use these to measure how viral load affects foraging behavior and colony fitness. To examine how pesticide exposure affects viral load, I will expose 5-10 colonies to field-relevant levels of neonicotinoids. Differences in viral load and fitness (colony size, weight, sexuals, foraging behavior) will be determined using an analysis of variance.

**3. Transmission.** Viruses may be transmitted via pollen from honeybees to wild bees (2,6).Transmission of these viruses through the use of shared flowers has been assumed but remains untested. I hypothesize that viruses may be transferred by floral visitors that carry infected pollen from flower to flower and by systemic expression in nectar and pollen. RNA viruses cross species, and even kingdom boundaries, as with the tobacco ring spot virus, an RNA plant virus recently found replicating within honeybee hosts (7). I will investigate if the RNA viruses I study replicate within both insect and plant hosts, and if their infectivity varies across plant species. **Approach.** To test if viruses can be detected on flowers, I allowed infected bumble bee colonies to forage on ‘clean’ flowers within a screen enclosure. Flowers were collected and will be tested using RT-qPCR. In future greenhouse experiments, I will use multiple flowering plant species to determine how plants act as viral reservoirs, and if certain plant or floral traits affect viral transmission. After exposure to infected bees, I will test flowers, leaves and stems for viruses to determine if viruses are persisting systemically within the different plant tissue.

**Broader impacts.** In discovering how non-crop flowers might act as reservoirs for viruses, and how multiple insults affect bee behavior and colony health, my work will lead directly to management recommendations for farmers dependent on bees for the pollination of their crops. I have already reached out to a variety of constituencies: I am working with the VT apiculturist to update the state’s database on managed honeybee hives and am offering testing of hives for viruses through the VT beekeepers association. I will hold workshops to spread awareness among beekeepers about pathogens and suggest practices that may lesson pathogen spillover from honeybees to native bees. Results will be published in scientific journals. Lastly, I will mentor at least two undergraduate assistants each summer and academic year, giving them opportunities to develop independent research projects in the field or in the laboratory.

**1)** The White House, Office of the Press Secretary (2014). Presidential Memorandum—Creating a federal strategy to promote the health of honey bees and other pollinators. **2)** Singh, R *et al.* 2010. RNA viruses in hymenopteran pollinators: evidence of inter-taxa virus transmission via pollen and potential impact on non-apis hymenopteran species. *PlosOne.* 5 (12). **3)** González-Varo, JP et al. 2013. Combined effects of global change pressures on animal-mediated pollination. *TREE*. 28, 524-530. **4)** Potts, SG *et al.* 2010. Global pollinator declines: trends, impacts and drivers. *TREE.* 25, 345-353. **5)** Williams, NM *et al.* 2010. Ecological and life-history traits predict bee species responses to environmental disturbances. *Biol. Con*. 143, 2280-2291. **6)** Fürst, M *et al.* 2014. Disease associations between honeybees and bumblebees as a threat to wild pollinators *Nature* 506, 364-373. **7)** Li J. *et al.* 2013. Systemic spread and propagation of a plant-pathogenic virus in European honeybees, *Apis mellifera*, *mBio, 5* e00898-13. **8)** Chen Y. *et al.* 2005. Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.), Appl. Environ. Microbiol. 71, 436–441. **9)** Hopwood *et al.* 2012. Are Neonicotinoids killing bees? A review of research into the effects of neonicotinoid insecticides on bees, with recommendations for action. The Xerces Society for Conservation.