

Rocky Mountain *Bombus* pathogen survey:
Are invasive plants affecting pathogen prevalence and intensity?

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Abstract

Parasites have the potential to alter population dynamics by decreasing fitness and increasing mortality of their hosts. Changes in ecosystems may also create scenarios that are more conducive to higher infection or parasitism. This survey examined parasitism of *Bombus* by *Crithidia bombi*, *Nosema bombi*, *Apicystis bombi*, Phorid parasitoids, Conopid parasitoids, and tracheal mites in the region surrounding the Rocky Mountain Biological Laboratory in Gunnison County, CO, USA. This study was run over an elevational gradient, and *Bombus* workers were collected over a 5 week period. Moreover, I examined if the presence or absence of the invasive flowering plant *Linaria vulgaris* was associated with pathogen intensity. This manuscript presents the results of my preliminary data. The presence of *Linaria vulgaris* had no relationship with pathogen intensity. Moreover, pathogen intensity could not be predicted by the proportion of morphologically “closed” flowers (flowers whose corollas are not directly open to the sun), elevation, or *Bombus* species. However, trends were observed regarding time of year on pathogen load, showing a peak in *A. bombi* early in the season and a peak in *N. bombi* late in the season. In summation, *Bombus* in this region were parasitized by *N. bombi* (6% across all *Bombus* species), *A. bombi* (3.6% across all *Bombus* species), and Conopidae (3.6% across all *Bombus* species). Parasitism by *C. bombi*, and tracheal mites were less than 1% across all *Bombus* species and no Phoridae were observed.

Keywords: *Bombus*, parasites, *Linaria vulgaris*, *Crithidia bombi*, *Nosema bombi*, *Louctacarus buchneri*, mites

Introduction

Bumble bees (Order Hymenoptera, Family Apidae, Genera *Bombus*) are commonly infected, to varying intensities, with a number of pathogens or parasites, such as viruses, fungi, protozoa, nematodes, other insects, or mites (Schmid-Hempel, 1998). Much work has been done to understand the methods of transmission and the effects of these pathogens on the health of individuals and colonies (Schmid-Hempel, 1998). In many instances, parasitic infection often influences the behaviors of hosts (Gegebar et al., 2006 and Otterstatter et al., 2005). Therefore, investigations have also been conducted with bumble bees to observe how infections lead to changes in foraging behavior, reproduction, and other social behaviors (Shykoff and Schmid-Hempel 1991).

A majority of investigations on bumble bee pathogens have focused on *Crithidia bombi*. *Crithidia bombi* is a non-invasive gut trypanosome that is transmitted via the ingestion of cells in bumble bee frass (Brown et al., 2003a). Flowers are a common site of infection, where excrement is mixed with nectar, allowing for the transmission of *C. bombi* cells to uninfected bees (Imhoof and Schmid-Hempel, 1999). Contact with contaminated nest material can also transmit the pathogen

(Mallon et al., 2004). Moreover, entire colonies can be easily infected through vertical infection if the pathogen is present in a founding queen (Mallon et al., 2004), and bees can infect each other with increased rates of contact with infected individuals (Otterstatter and Thomson, 2007).

Crithidia bombi is normally relatively benign but may have lethal effects on bees that are under the stress of starvation (Brown et al., 2003a). Such situations may occur when food sources are scarce or during unfavorable environmental conditions where bees are unable to forage. Brown and colleagues (2003a) found that under food stress conditions, mortality of bees infected with *C. bombi* increased 50% and that bees allocated more resources to immune function (measured in terms of fat body development) than reproduction (measured as a function of ovary size).

Hibernation is also a stressful situation, where infection with *C. bombi* has been found to have negative effects on the fitness of new queens and their ability to found new colonies (Brown et al., 2003b). These queens lose more of their body mass and have a reduction in colony-founding success in comparison to uninfected queens (Brown et al., 2003b). In addition, infected queens may have their pattern and timing of ovarian development and oviposition altered by *C. bombi* (Shykoff and Schmid-Hempel, 1991). Furthermore, there is a decrease in colony size and production of males (Brown et al., 2003b). All of these responses to infection may lead to a smaller addition to the next generation.

Crithidia bombi has also been found to alter foraging behavior. Bees infected with *C. bombi* have an impaired ability to recognize and learn differences in floral rewards (Gegear et al., 2006) in comparison to uninfected bees. Moreover, infected bees also show a decreased ability to learn floral handling techniques (Gegear et al., 2005). Infected bees often collect less pollen (Imhoof and Schmid-Hempel, 1999) and visit fewer flowers than uninfected bees (Otterstatter et al., 2005). Therefore, a highly infected colony may also have a decreased ability to acquire resources or withstand food shortages, which can greatly affect survival.

A number of other parasites have been studied in less detail but can also have great impacts on the fitness of individual bees and their colonies. *Nosema bombi* is a microsporidia that lives within the

gut and fat bodies of bumble bees and can have lethal effects on entire colonies (Schmid-Hempel et al., 1998, Schmid-Hempel, 2001). This parasite reproduces primarily in the Malpighian tubules and infected workers often become sluggish, greatly impacting potential foraging abilities. In addition, *N. bombi* can be cross-infected to other bumble bee species (Schmid-Hempel, 2001). *Nosema bombi* is transmitted in a similar manner as *C. bombi*, as spores are expelled in feces.

Apicystis bombi is a protozoa that is less common but infections can have severe impacts on colonies (Otterstatter, personal communication). *Apicystis bombi* causes the disintegration of fat bodies in bees and infected colonies are unlikely to grow and reproduce (Schmid-Hempel, 1998). Large spores are present in the feces and transmission is likely to occur when these spores are ingested by uninfected bees (Schmid-Hempel, 1998).

Parasitic mites are often found in the trachea of bees and can also lead to a decrease in health of the individual. In Japan, infections of mites have resulted in decreased foraging activity and diarrhea (Goka et al., 2001). Otterstatter and colleagues (2005) found that infection may not only alter the speed of foraging activity but the foraging behavior itself. Workers infected with the tracheal mite *Locustacarus buchneri*, foraged at similar speeds as uninfected workers but varied in that they were more constant to a single flower type (Otterstatter et al., 2005). Lastly, phorid and conopid (Order Diptera) parasitoids are other insects that lay eggs on or within bees. Larvae then develop within the host. These parasitoids may change bee behavior and often lead to eventual death of the host (Otterstatter et al., 2002). Differences in levels of infection of phorids and conopids are observed for different species of bumble bees, possibility attributed to variations in behavior and foraging activity of bees (Otterstatter et al., 2002).

Although the pathogens that commonly infect bumble bees are quite familiar, few studies have comprehensively surveyed pathogen prevalence across *Bombus* species in a particular area. Taking stock of pathogens and parasites that infect bees in the Rocky Mountains is integral to understanding the current health of bee colonies in this region. Moreover, a preliminary survey can set a baseline of

pathogen prevalence and intensity in this area for future studies to utilize. With an increase in overseas shipment of commercial bees, novel parasites and introduced competitors or predators may become more common in bee populations (Goulson et al., 2008). For example, *Aethina tumida* (small hive beetle) has recently invaded North America and can cause considerable harm to bumble bee colonies (Goulson et al., 2008). “Pathogen spillover” is another phenomenon leading to an increase in pathogen prevalence and intensity in wild bee populations when commercial bees escape and infect wild populations (Colla et al., 2006). Moreover, little is known about the effects of the presence of invasive plants on pathogen prevalence or intensity. This survey may be a powerful tool to elucidate possible shifts in future pathogen prevalence and intensity in the region.

This study surveyed bumble bee pathogens among various species over an altitudinal gradient. Environmental conditions, such as temperature, humidity, available resources, and variety of plants available for foraging, may influence differences in pathogen prevalence (the number of bees infected) and intensity (the number of pathogens in a single bee). This survey will focus specifically on the prevalence of the pathogens *Apicystic bombi*, *Crithidia bombi*, *Nosema bombi*, conopid parasitoid larvae, phorid parasitoid larvae, and tracheal mites, as infections by viruses, bacteria, fungi, and nematodes are relatively unknown. I collected data on pathogen presence and intensity, bumble bee species, time of season and body variables (size and age).

The research goals were two-fold, 1) to understand pathogen prevalence in various species of *Bombus* over a range of altitudes and over time and 2) to investigate the parasite prevalence and intensity of bumble bees foraging in areas with exclusively native plants and at sites invaded with the non-native plant *Linaria vulgaris*. This invasive species produces larger floral rewards (more sugar in its nectar and more protein in its pollen) in comparison to native species (Irwin, personal communication). This variation may influence the visitation rate by *Bombus*. Bumble bee species in the Rocky Mountains are more or less generalists and have been observed to forage at *L. vulgaris* (Irwin, personal communication). All of the pathogens and parasitoids focused on in this survey are thought to

be transmitted, in part, via flower usage or flowers being a site of infection. How does this highly rewarding invasive species affect bumble bee foraging and subsequently, disease transmission? Are plants with higher floral rewards sites of greater transmission of pathogens or higher rates of infection by parasitoids?

Furthermore, an increase in foraging bee density may have an affect on pathogen prevalence and intensity. Schmid-Hempel and colleagues (1999) found that low host densities in the field decrease the success of the pathogen *C. bombi* due to the fact that the cells of the pathogen are subject to longer intervals experiencing environmental stresses such as desiccation and UV radiation on flowers. Moreover, plants with different structures and morphologies, such as those that are closed to direct sunlight, may provide better habitats for protozoan cells as they protect cells from desiccation and UV radiation. Does the difference in morphology between *L. vulgaris* and native species influence variation in success of pathogen transmission and therefore increase pathogen prevalence and intensity?

Methods

Study sites and flower sampling

Bumble bees were collected in the summer of 2008 at a number of sites ranging a span of altitudes over a period of several weeks in areas surrounding the Rocky Mountain Biological Laboratory, Colorado. Sites contained primarily native flowering plants or were invaded with *L. vulgaris* to examine the effects of plant type on pathogen prevalence and intensity. Native sites were located at Brush Creek (approximately 2,835 m), Cooper Creek (approximately 3,048 m), and Almont Triangle (approximately 2,530 m). Sites invaded with *L. vulgaris* consisted of Lower Loop (approximately 2,713 m), RMBL Townsite--Happy Valley (approximately 2,896 m), and Slate River (approximately 2,804 m). I collected *Bombus* workers over a five week period over all sites to examine the effect of time of flowering season on levels of infection. Increased pathogen prevalence was expected later in the season as more bees become infected. However, since the timing of the emergence of workers differed at all sites, some sites were sampled over more weeks than others. The preliminary

data in this manuscript is based on the following number of collections: Almont Triangle was sampled 6 times; Brush Creek, Lower Loop, and Slate River were sampled 4 times; and Cooper Creek and RMBL Townsite were sampled 2 times (Table 1).

On each visit to every site, three 25 m transects were run haphazardly to collect data on flowering plant diversity and abundance. The total number of flowering plants and species were recorded. On each transect, I also collected data on the number of viable foraging flowers per plant to assess the number of flowers available for bees. Numbers of flowers were counted on the first 10 plants encountered of each species.

I collected bees using butterfly nets and then placed them in separate vials to reduce the possibility of pathogen transmission at collection. Bees were then put into coolers in the field and immediately freeze killed upon return to the laboratory. At time of collection, I also noted the plant on which the bee was foraging. Records were kept on site, floral abundance and diversity at each site, collection date, *Bombus* species, bee size and age of bee. Bee size was determined by measuring the length of the radial cell of right forewing. Age was determined by wing wear, bees without wing wear or with minimal wear were categorized as young and bees with extensive wing wear were categorized as older bees. *Bombus* species collected included, *B. appositus*, *B. nevadensis*, *B. californicus*, *B. bifarius*, *B. sylvicola*, *B. flavifrons*, *B. ruficinctus* and *B. mixtus*.

Assessing Pathogen Prevalence and Intensity

Bees were dissected and the gut and fat bodies were examined separately for *A. bombi*, *C. bombi* and *N. bombi*. The gut tract was removed, crushed thoroughly, and placed onto a slide with a drop of water. One abdominal segment was scraped of fat bodies and put on the same slide as the gut tract but in a separate drop of water. The slide was fixed with methanol until dry and stained with Giemsa solution (9 mL phosphate buffer and 1 mL of Giemsa stock solution). The slide was examined under a compound microscope at 400x magnification. Ten haphazardly chosen fields of view for the fat bodies and ten haphazardly chosen fields of view for the gut tract were examined for *A. bombi*, *C.*

bombi and *N. bombi* cells. Only these fields were examined for pathogens. The total number of cells counted in all fields of view was defined as the measure of intensity of infection. I inspected the trachea for mite (*Louctacarus buchneri*) infection, the abdomen for conopid parasitoids, and the thorax for phorid parasitoids.

Results

The subsequent information represents preliminary data. I dissected 84 bees out of an approximately 300 collected. In an attempt to assemble a sub-sample, I chose 4 bees haphazardly from each site and date of collection.

Phorid parasitoids were never encountered in the 84 dissected bees and only one mite was found in one *B. appositus* collected on July 27, 2008 in the Townsite. Three conopid parasitoids were found, one parasitoid on each of the following bees: Almont Triangle (July 14, 2008), Lower Loop (July 23, 2008), and RMBL Townsite (July 27, 2008). Overall, protozoa were not common in many of my dissections. In qualifying protozoa infection, I used the methods of Colla et al. (2005) (light infection = 10–100 cells observed, medium infection = 101–999 cells observed, and heavy infection = 1000 or more cells observed). For my statistical analyses for this preliminary data, I did not distinguish between intensities of infection as the number of infected bees was so small. Therefore, overall 3.6% of bees in this preliminary data set were infected with *A. bombi* and 6% were infected with *N. bombi*. Moreover, I found infection by what appears to be yeast cells. However, only one of these observations were what I define as a measurable infection (one *B. bifarius* collected on July 14, 2008 from the Lower Loop site was infected with 14 cells). Subsequent confirmation on the identity of these cells will be done by Leellen Solter of the Illinois Natural History Survey.

For all data, pathogen intensity was calculated as the sum of all pathogens in a bee (i.e., the sum of the number of cells, mites, and parasitoid larvae). There seemed to be no reason to exclude mites or parasitoid larvae from this total as only one mite was found and three larvae were found. Either excluding or incorporating these parasites into the total pathogen load would not have a strong effect on

the analysis. In addition, these totals were transformed using the log + 1, to attempt to give data a more normal distribution.

A possible trend in peak *A. bombi* intensity (Figure 1) was observed early in the season ($F_{12,71}=1.17$, $p<0.32$) and a trend that *N. bombi* intensity (Figure 2) peaks late in the season ($F_{12,71}=2.24$, $p<0.02$). For these analyses, I examined the effect of date on the mean number of total protozoan cells found in each bee for all *Bombus* species. Due to the haphazard selection of my sub-samples of specimen for examination, I only investigated the relationship between three the species that were relatively abundant in the sub-sample (*Bombus appositus*, *Bombus flavifrons*, and *Bombus bifarius*) and pathogen intensity. I found no significance was in pathogen intensity among specific species of *Bombus* (Figure 3: $F_{2,67}=0.16$ $p<0.85$). Each of these species was infected at a similar intensity. Moreover, I found no significant relationships between elevation and pathogen intensity (Figure 4: $F_{1,82}=1.68$, $p<0.19$), the proportion of “closed” flowers available and pathogen intensity (Figure 5: $F_{1,19}=2.24$, $p<0.15$), and the presence or absence of *L. vulgaris* and pathogen intensity (Figure 6: $F_{1,82}=0.034$, $p<0.56$).

Discussion

In this study, my preliminary data demonstrate that a relatively small number of bees (9.5%) had significant infection. Only 6% of these bees were infected with *N. bombi* and 3.6% with *A. bombi*. This is quite different from what Colla et al. (2006) found near commercial greenhouses in Ontario, Canada, where *N. bombi* infections were 15% and *A. bombi* infections were approximately 6%. However, Colla et al. (2006) found that *N. bombi* occurred in less than 4% of the bumble bee population in sites where greenhouses were lacking and they described *A. bombi* as relatively rare (comprising 6% or less of the population of bees surveyed) in these locations. Such differences suggest that there may be site-specific variables that result in variation in pathogen levels. Moreover, an investigation of *Bombus* pathogens in the Swiss Alps observed that *C. bombi* infected 10% of the population and tracheal mites 6% of the population, while parasitism by Conopidae and *N. bombi* were

absent (Korner and Schmid-Hempel, 2005). There is variability in pathogen loads in sites where similar surveys have been completed (Table 2). Therefore, we can speculate that different variables at each of these sites are driving the prevalence and intensities of parasites in *Bombus* communities. Further research should survey pathogens at other alpine sites to determine if there is a trend in pathogen and parasite abundances and intensities of infection in alpine habitats or if these dynamics are regionally specific. Deviations may be due, in part, to geographical variation in virulence. Based on the available literature and the preliminary data, *Bombus* near the RMBL region have less pathogens than wild bees that share an area with commercially reared bees. While this fact is not surprising, we do find slightly increased rates of pathogen intensity for *N. bombi* than in sites where commercial *Bombus* are absent.

Based on the preliminary data, we found no effect of the presence of *L. vulgaris* on pathogen intensity. Moreover, an increase in the proportion of “closed” flowers was not associated with increased pathogen intensity. Therefore, flower composition is likely not the primary indicator of pathogen levels in bees utilizing a specific foraging area, although experiments are needed to make definitive conclusions. Furthermore, the results suggest that horizontal transmission at flowers is not the primary means of transmission between bees as intensities of infection did not increase in areas with more “closed” flowers. However, before this idea is rejected, we must refine the data analysis. Since different pathogens accumulate in intensity at different rates, time lags should be incorporated into the analyses. In other words, I must examine the relationship between flowers that were abundant several weeks prior to bee collection and the pathogen intensity of the bee. However, if the results of the preliminary data remain similar upon further dissections throughout the season or with the incorporation of time lags into the analyses, it would provide support to the idea that a different mechanism of pathogen transmission is in play. What is the role and relative importance of vertical and horizontal transmission in these host-parasite dynamics?

Trends in peak pathogen intensity may help to elucidate these questions of the relative importance of vertical transmission between queen and offspring and horizontal transmission between

colonies at sites such as flowers. *Nosema bombi* takes approximately two weeks to build up in an individual bee (Schmid-Hempel 1998). Therefore, if transmission was occurring between queen and offspring during oviposition or nest construction, we might expect to see a peak in *N. bombi* also in mid-season, rather than only one peak late in the season (Figure 2). However, there were no infections with *N. bombi* in workers collected in earlier in the season, suggesting that horizontal transmission between colonies may be more important for this pathogen.

It is most likely that transmission is mediated by both horizontal and vertical transmission for the parasites in this study, excluding parasitoid Diptera. However, the primary driver of transmission may be different for each pathogen. The peak we observe in *A. bombi* (Figure 1) early in the season suggests that vertical transmission may be the primary mechanism of transmission. If horizontal transmission between colonies were the driving force, an early peak would mean infections should spread to other colonies. However, there is no reemergence of the pathogen later in the season.

Transmission may also be more clearly understood if we knew the rates at which horizontal transmission might be occurring within the hive itself, not merely between workers of different colonies as sites such as flowers. Otterstatter and Thomson (2007) investigated the role of contact networks for within-hive transmission of *C. bombi*. They found that an individual bee's rate of contact with infected nestmates predicted its risk of infection (Otterstatter and Thomson, 2007). However, these rates may be different for colonies of different densities. Nor is it known the relative importance of this mode of transmission in relationship to others. Lastly, the phenomenon of drifting workers is another factor that should be taken into account. Birmingham et al. (2004) found that workers from commercial greenhouses drifted into the hives of wild colonies but they did not investigate whether or not hives with drifting bees had higher pathogen loads.

Bombus can be infected with a variety of pathogens and such infections can increase mortality and decrease the fitness of individuals and colonies (Gegear et al., 2006 and Otterstatter et al., 2005). Increases in pathogen loads have been observed in areas where commercially reared bumblebees have

been imported to aid in pollination for agriculture (Goka et al. 2001, Colla et al. 2006, and Birmingham et al. 2004). These bees tend to be more heavily infected with a variety of pathogens than wild bees (Colla et al., 2006). This kind of “pathogen spillover,” (Colla et al., 2006) from commercial bees to wild bees, is absent from the region surrounding the Rocky Mountain Biological Laboratory in Gothic, Colorado. Currently, there are no known agricultural sites in close proximity to any of my study sites that utilize commercially reared *Bombus* for pollination services. However, small scale “spillover” may be occurring at flowers between wild colonies. Therefore, this investigation may be useful in understanding pathogen dynamics in wild *Bombus* populations in an alpine habitat removed from the possible transmission from commercial bees.

Host-parasite dynamics are complex and this complexity is confounded when examining these dynamics in social insects. Social bees such as *Bombus* have various means of transmitting parasites to one another via horizontal and vertical transmission (Birmingham et al. 2004, Otterstatter and Thomson 2007). It is difficult to tease apart these various modes of transmission. However, by attempting to observe the effect of one variable, such as flower diversity composition in this investigation, we hope to gain an understanding of the relative importance of each component. Furthermore, it is difficult to assume that all other variables are constant, as we had no information about the sizes of the colonies or the health of the queens. This study shows that although we can not rule out the importance of horizontal transmission at flowers, the presence of an invasive plant has no significant effect on altering host-parasite dynamics.

Acknowledgments

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Figures

Table 1: Sites surveyed and species collected

Site	GPS Coordinates	Weeks surveyed	<i>Bombus</i> species collected
Almont Triangle	n/a	6	<i>B. Appositus</i> , <i>B. Bifarius</i> , <i>B. Californicus</i> , <i>B. Flavifrons</i> , <i>B. Sylvicola</i>
Brush Creek	n/a	4	<i>B. Appositus</i> , <i>B. Bifarius</i> , <i>B. Californicus</i> , <i>B. Flavifrons</i> , <i>B. Nevadensis</i>
Copper Creek	38°57'52.133"N 106°58'17.185"W	2	<i>B. Appositus</i> , <i>B. Flavifrons</i>
Lower Loop	n/a	4	<i>B. Appositus</i> , <i>B. Bifarius</i> , <i>B. Californicus</i> , <i>B. Flavifrons</i> , <i>B. Mixtus</i>
Slate River	n/a	4	<i>B. Appositus</i> , <i>B. Bifarius</i> , <i>B. Californicus</i> , <i>B. Nevadensis</i> , <i>B. Rufocintus</i> , <i>B. Sylvicola</i>
Townsite	38°57'33.867"N 106°59'22.628"W	2	<i>B. Appositus</i> , <i>B. Bifarius</i> , <i>B. Flavifrons</i> , <i>B. Sylvicola</i>

Figure 1: *Apicystis bombi* intensity over time

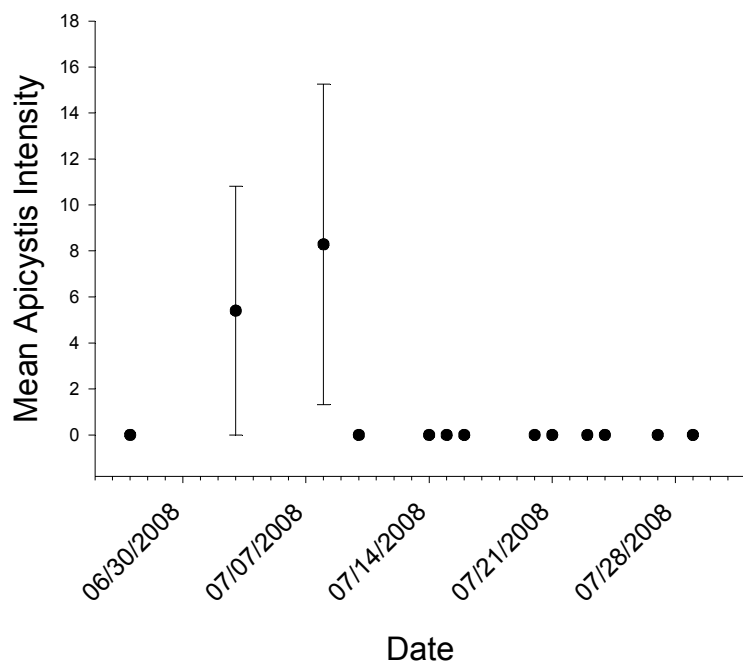


Figure 2: *Nosema bombi* intensity over time

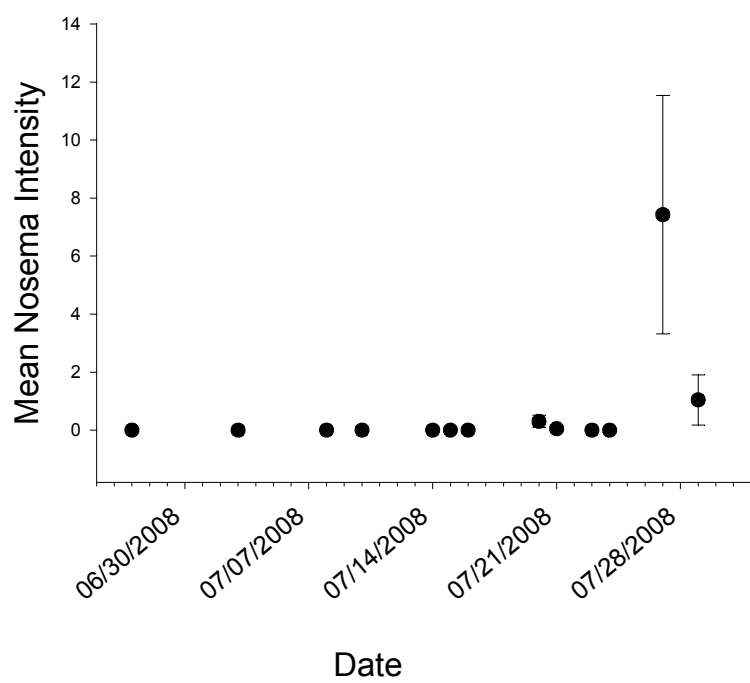


Figure 3: Pathogen load in *Bombus* species

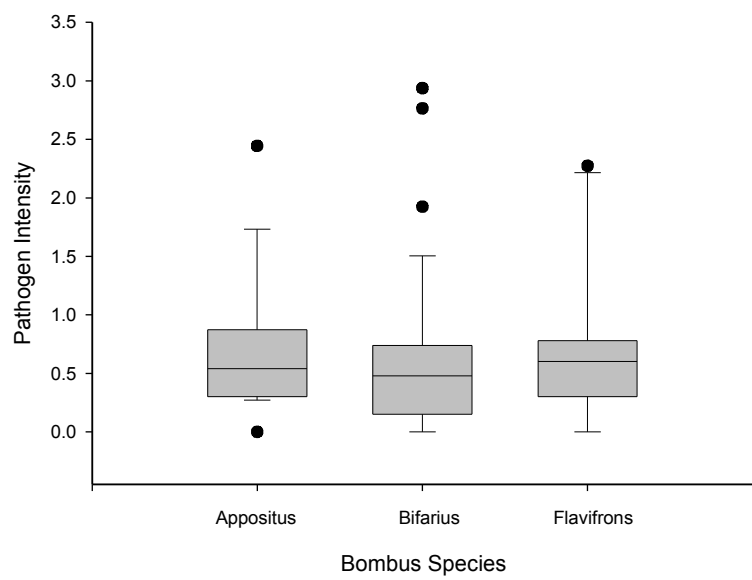


Figure 4: Elevation and pathogen intensity

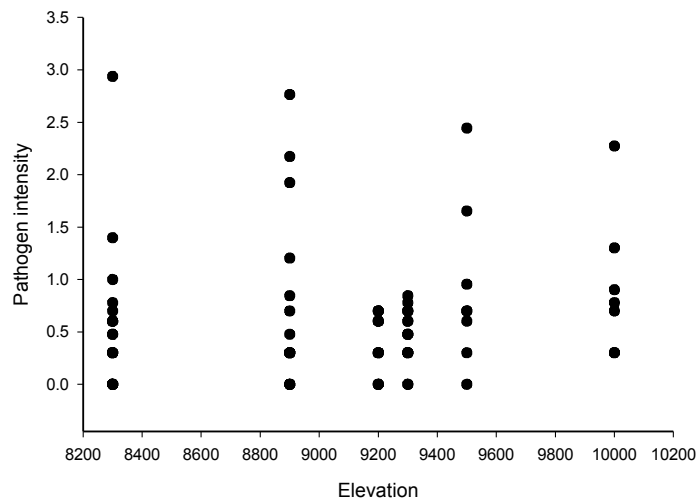


Figure 5: Proportion “closed” flowers and pathogen intensity

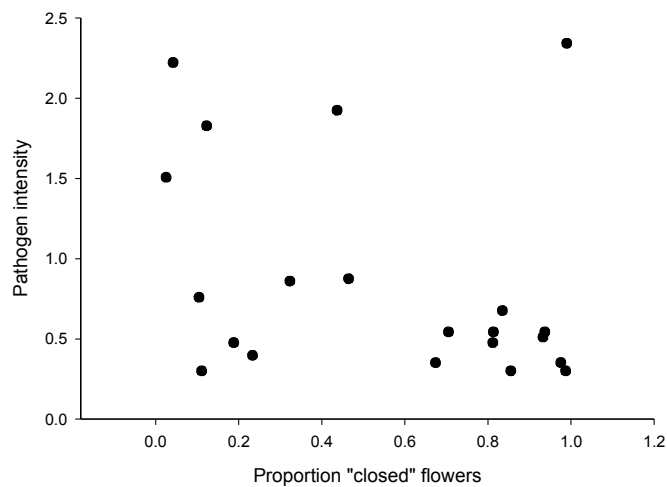


Figure 6: *Linaria vulgaris* invaded sites and pathogen intensity for all pathogens

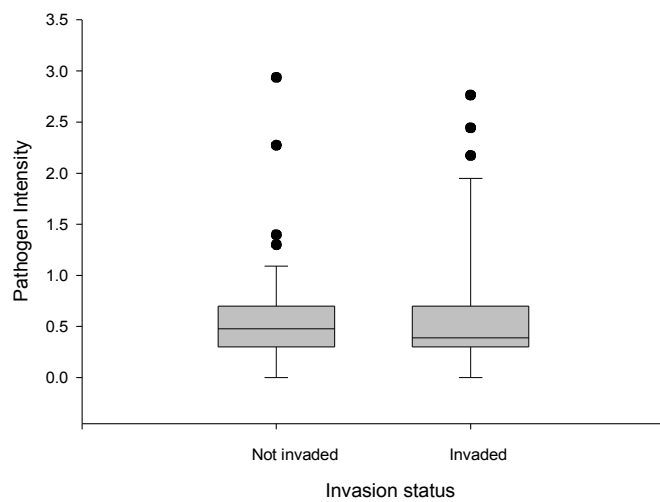


Table 2: Comparison of proportions of *Bombus* populations infected with pathogens

Investigator(s)	Site	Commercial <i>Bombus</i> Absent						Commercial <i>Bombus</i> Present	
		Population infected with <i>Crithidia bombi</i>	Population infected with <i>Nosema bombi</i>	Population infected with <i>Apicystis bombi</i>	Population infected with tracheal mites	Population infected with Conopidae	Population infected with Phoridae	Population infected with <i>Nosema bombi</i>	Population infected with <i>Apicystis bombi</i>
Diaz (2008)	Gunnison County, CO, USA	0.00%	6.00%	3.60%	1.20%	3.60%	0.00%	--	--
Colla et al. (2006)	Ontario, Canada	--	4.00%	6.00%	--	--	--	15.00%	6.00%
Korner & Schmid-Hempel (2005)	Swiss Alps	10.00%	0.00%	--	6.00%	0.00%	--	--	--
Otterstatter (2004)	Alberta, Canada	--	--	--	--	12.30%	--	--	--

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