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ORIGINAL PAPER

Commercial *Bombus impatiens* as reservoirs of emerging infectious diseases in central México

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Abstract The rapid decline in range and relative abundance of some wild North American bumble bee species, combined with the commercialization of bumble bee colonies as agricultural pollinators, and recent evidence that bumble bees can be infected by honey bee viruses, suggest the possibility that invasive and emerging infectious diseases (EIDs) may play a substantial current and future role in the decline of wild bumble bee populations. Pollination in North American greenhouses is primarily mediated by industrially produced *Bombus impatiens* colonies. The use of *B. impatiens* is preferred in greenhouses because it is a native species of East North America and they are very efficient pollinators. However, *B. impatiens* is also host of various viruses that have been associated with colony collapse disorder in honey bees, as well as hosts to a number of bumble bee-specific pathogens and parasites. In this study, we used qPCR to screen adult worker bumble bees collected from 120 different greenhouses in central Mexico. Fifty-four locations were positive for one or more pathogens (45 %). The most frequently detected pathogen was *Apicystis bombi*, which was present in 32 colonies. Of these 32 *A. bombi* positive colonies, 15 were co-infected with at least one other pathogen or parasite, such as *Locustacarus buchneri*, *Nosema bombi*, or the viral pathogens ABPV, CBPV, DWV, IAPV and KBV. Routine use of this type of screening technology together with policy changes to restrict pathogen infested commercial bumble bees should help improve the selection of healthy commercial colonies of *B. impatiens* and could lead to a higher efficiency in greenhouse pollination thus providing better environmental conservation of natural *Bombus* spp. by preventing spillover of EIDs.

Keywords Colony collapse disorder · Bumble bee diagnosis · Emerging infectious diseases · Pathogen spillover

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Introduction

The importance of *B. impatiens* as a commercial pollinator

Traditionally, pollination was considered a gratuitous environmental service. A large percentage of the global food supply is naturally pollinated by wild insects, birds and mammals (Ollerton et al. 2011). The annual global economic impact of this natural service has been estimated in 200 trillion dollars (Gallai et al. 2009). The decimation of populations of natural pollinators, the increase in global food demand and the introduction of noxious species, such as the Africanized honey bee, have led to the use of commercially available pollinators, such as *Bombus impatiens* and *B. terrestris* whose demand has been growing steadily (Stubbs and Drummond 2001; Velthius and Van Doorn 2006).

Bumble bees (*Bombus* spp., Hymenoptera, Apidae) are naturally distributed throughout North America, and they are ecologically important native plant pollinators (Kearns and Thomson 2001). At the end of the last century, private companies began to massively produce and distribute *B. terrestris* and *B. impatiens* colonies for commercial pollination of *Solanum lycopersicum* (i.e., tomato) (Whittington and Winston 2004), *Capsicum annuum* (i.e., bell pepper) (Shipp et al. 1994) and *Vaccinium angustifolium* (i.e., cranberries) (Stubbs and Drummond 2001), among many other commercial species. In Europe alone, the annual market value of *B. terrestris* is estimated to be 55 million Euros (Meeus et al. 2010a). In North America 55,000 *B. impatiens* colonies were sold in 2005 representing a market of about five million US dollars (Velthius and Van Doorn 2006), and the demand seems to be growing steadily.

Global epidemiological status of *B. impatiens* pathogens

Some of the pathogens that infect honey bees and bumble bees such as *Nosema cerenae*, *Apicystis bombi* and DWV have been recently catalogued as emerging infectious diseases (EIDs) (Arbetman et al. 2012; Maharramov et al. 2013; Fürst et al. 2014). The intensive production and global dispersal of commercial *B. impatiens* can transmit hymenopteran diseases to naturally occurring honey bee and bumble bee populations (Whittington and Winston 2004; Singh et al. 2010). Previous studies have demonstrated that wild bumble bees collected near greenhouses that have commercial *B. impatiens* colonies are more likely to be infected than naturally occurring colonies by *Crithidia bombi*, a protozoan pathogen that infest the bumble bee gut, the fungus *Nosema bombi* that causes a systemic infection, and the mite *Locustacarus buchneri* (Colla et al. 2006; Morkeski and Averil 2010). *N. bombi* infects a wide range of bumble bee species (Tay et al. 2005) and has likely contributed to the recent collapse of commercial populations of *Bombus occidentalis* in North America. However it is not clear if the infection is the cause of the decline or a consequence of a reduction of genetic variation caused by the decline (Brown 2011). *C. bombi* infection lowers the reproductive yield of bumble bee colonies by up to 40 % (Brown et al. 2003) and reduces the survival of individual workers by up to 50 % when food is scarce (Brown et al. 2000). In addition, a single individual may be simultaneously infected with a variety of EIDs (Meeus et al. 2010a). Therefore, there is a possibility that these individuals could simultaneously transmit viral, protozoan and fungal pathogens (Celle et al. 2008).

The recent development of molecular diagnostic techniques has allowed the identification of honey bee viruses that reduce production yield and have the potential to collapse honey bee hives. The most common and aggressive examples are the acute paralysis bee virus (APBV), the chronic bee paralysis virus (CBPV), the deforming wing virus (DWV), the Israel acute paralysis virus (IAPV) and the Kashmir bee virus (KBV). These viruses can infect honey bee colonies for years without producing apparent disease and then suddenly kill the entire colony for unknown reasons (Blanchard et al. 2008). CBPV was first isolated from infected honey bees in 1963 (Bailey et al. 1963), and the dispersal of this virus has been extensive in recent years (Berényi et al. 2007). Phylogenetic analysis of the IAPV genome indicated that IAPV clusters together with KBV and ABPV (Maori et al. 2007). Metagenomic studies of honey bee colonies with Colony Collapse Disorder (CCD) have demonstrated that these three viral strains were found simultaneously in collapsed colonies (Cox-Foster et al. 2007). It has been shown that these viruses can also infect *B. impatiens* colonies, this work and Meeus et al. (2010a, b).

Commercial *B. impatiens* in Mexico, use and regulatory framework

In Mexico, there are two major bumble bee producers which have a presence in over 50 countries. Tens of thousands of colonies are produced annually and used in the domestic market. The number of bumble bees colonies imported into Mexico varies each year (from 7000 in 2008 to 24,000 in 2007), and a Federal Law of Animal Health (http://www.senasica.gob.mx/) regulates the importation of agricultural products into Mexico, including requirements for three species of bumble bees: *B. impatiens*, *B. occidentalis*, and *B. ephippiatus*, even though *B occidentalis* is no longer commercially available. The restrictions require that shipments of bumble bees imported into Mexico must be accompanied with official health certificates stating that the bumble bees are free of infections, including those caused by *Nosema spp.*, *Varroa spp.*, and fungal pathogens (http://www.senasica.gob.mx/), however, there are no available data quantifying this regulatory activity. In fact, Mexican regulations about bumble bee pathogens are in general very vague. For example, there is no regulation or restriction for outdoor use of commercial bumble bees. However, to our knowledge, it is not commercially viable to use them for open crop field pollination, therefore, most commercial bumble bee colonies are only used in greenhouses. Interestingly there are no Mexican regulations regarding any hymenopteran viruses. (http://www.biodiversidad.gob.mx/pais/pdf/Estrategia_Invasoras_Mex.pdf).

Identification of pathogens in *B. impatiens*

Protozoan, acarid and fungal parasites of *B. impatiens* are traditionally identified by microscopic observation of gut and tracheal preparations (Goka et al. 2001; Colla et al. 2006; Plischuk and Lange 2009; Al-Abbadi et al. 2010). These techniques have a number of disadvantages: many parasites are difficult to detect visually when they present at a low titers, there are few qualified technicians due to the requirement for specific training, and it is difficult to discriminate between closely related parasite species, also others are impossible to culture in vitro so there are no reference strains. In addition, these techniques are time consuming, making it difficult to efficiently screen large numbers of samples. For all these reasons molecular methods are probably the best approach to monitor EIDs.

The genomes of the most damaging honey bee viruses, including KBV, IAPV, APBV, DWV and CBPV have been sequenced in the last decade (Govan et al. 2000; de Miranda et al. 2004; Lanzi et al. 2006; Celle et al. 2008). The availability of these sequences has facilitated the development of molecular diagnostics for these viruses that can be used in asymptomatic organisms (Yue and Genersch 2005). There is a large amount of evidence that suggest that all these viruses and pathogens are harmful or have the potential to be harmful to bumble bees. However, the causes for the decline of wild bumble bee populations are difficult to elucidate as reviewed by Brown in 2011 (Brown 2011). These methods have yet to be extensively applied to the diagnosis of *B. impatiens* and other commercial hymenopterans. Epidemiological surveys of these species are necessary due to their ecological and economic importance. Meeus et al. (2011) suggest that the development of molecular screening protocols might immediately mitigate these threats. In this study, we developed five *de novo* molecular diagnostic tests for eukaryotic pathogens. We also used quantitative PCR to determine the prevalence, the colony co-infection rate and the infection colony load of five viruses in *B. impatiens* colonies obtained in the Mexican state of Queretaro. Finally, we discuss the potential ecological impacts of commercial *B. impatiens* populations on communities of indigenous bees and bumble bees and outline the diagnostic and certification tools that should be implemented to minimize ecological risk and maximize crop productivity.

Materials and methods

Field sampling of *B. impatiens*

Bombus impatiens samples were donated by anonymous commercial greenhouses that use commercial bumble bee colonies for pollination produced in Mexico. The authors have no affiliations with the commercial greenhouses. All samples were graciously donated by producers on the condition of anonymity. The bumble bee producers do not make any claim about the health of the hives. However they do guarantee a minimum amount of workers (80 per large hive).To avoid any site contamination, the samples were obtained from newly opened commercial

colonies. The trademark of the bumble bees was anonymized so that the technician would be blinded to the sample source. Batches of 10 *B. impatiens* individuals were collected from each colony. We assume that this population represents the overall health status of the colony. In total, 120 batches from 120 different greenhouses were analyzed. To avoid the possible contamination of the samples with native *Bombus ephippiatus,* all individuals were classified to ensure that they had a pale tergum 1 and a completely black tergum 2 (Duennes et al. 2012a). The animals were kept alive in flasks with a swab of cotton soaked in sucrose prior to processing in the laboratory.

Purification of RNA from *B. impatiens*

Bombus impatiens individuals were anesthetized with CO2 before processing. The abdomens from each location were cut in half, homogenized and pooled. The total RNA from these pools of ten individuals was extracted using the Trizol® reagent (Invitrogen, Carlsbad, CA, USA) according to manufacturer instructions. RNA was quantified using a Nanodrop 3000 spectrophotometer, and the quality of the RNA was determined by electrophoresis in a MOPS/formaldehyde 1.5 % agarose gel.

cDNA synthesis

One microgram of total RNA from each RNA pool was used for cDNA synthesis. The reaction was performed in a volume of 20 µl that contained the following: 1 µg of total RNA, 1X reverse transcriptase reaction buffer and 300 ng of random hexamers. The reaction was incubated at 65 °C for 5 min and transferred to ice. The M-MuLV reverse transcriptase (New England Biolabs) was added, and the reaction was incubated at 37 °C for 1 h. The enzyme was subsequently inactivated by incubating the reaction at 65 °C for 10 min. For qPCR, the cDNA was diluted 1:20, and 1 µl of the diluted cDNA was used as substrate.

Primer and probe design

All available sequences for each pathogen were obtained from Genbank. The Basic Local Alignment Sequence Tool (BLAST) was used to compare the pathogen sequences to sequences from close taxonomic groups. Variable regions were identified, as these regions are the most informative and the most likely to be unique for each organism. The variable regions were again subjected to BLAST to identify sequences that retrieved only the pathogen of interest and not pathogens from closely related taxa. These sequences were then analyzed using PrimerExpress 5.0 software, and the primer and TaqMan® probe set that presented the best physicochemical scores (i.e., lack of hairpin formation, duplex formation, higher stability and Tm) for each pathogen were synthesized (Applied Biosystems, USA). The sequences for all primers and TaqMan® probes are presented in Table 1.

Positive control design

The informative sequences that were identified during primer design were synthesized at GenScript (GenScript, Piscataway, NJ, USA) and cloned into pUC57 flanked by EcoRI and HindIII sites. Serial dilutions of these clones (10^{-2}–10^{-6} ng of plasmid) were performed to establish a reference curve. Target, primer and probe sequences are presented in Table 1.

Quantitative PCR

qPCR reactions were performed using the universal TaqMan® PCR master mix (Applied Biosystems) according to manufacturer instructions. However, the reactions were scaled down to a volume of 14 µl. The TaqMan® probe was used at a final concentration of 25 nM, and 1 µl of a 1:20 dilution of the cDNA reaction was used for the detection of each pathogen. Alternatively, reactions were performed using the SYBR® Green Universal PCR Master Mix. The following PCR conditions were used: 95 °C for 5 min, 40 cycles of 95 °C for 15 s, and 59 °C for 1 min. In all cases, the specificity of the reactions was confirmed using polyacrylamide gel electrophoresis (PAGE). Positive controls (i.e., synthetic DNA) and negative controls (i.e., lack of target sequences) were included in all experiments.

Statistical analysis

Standard deviations, linear regressions and correlation indexes were calculated using Microsoft Excel.

Table 1 Pathogen sequences used in this work

Eukaryota -Quencher	Sequence	Tm °C Pri/Pro	Position bp length	Gene	Genbank Acces No.
A. bombi FAM	5'GAAACGGCTACCACATCTAAGGAAGGCAGCAGGCGCGCAAATTACCCAATCC TGACATAGGGAGGTAGTGACAAGAAA3'	59/70	341-418/77	*Rrs*	FN546182.1
C. bombi FAM	5'CTCTCCTTCTCGTCTCTCTTCTCTTCCCTTCTCTCTCCCCC TGCATGCACATACACGTGTGT3'	59/69	274-338/64	*Rrs*	GU321148.1
L.buchner VIC	5'CCATTATCAAGAACCCCATTCCAAAGAAAGTCAATAGACATAACCA TTCTCTCAATTCACCTAGCAGGAA3'	58/69	107-176/70	*Cit-Oxi*	AB052703.1
N. bombi FAM	5'TGACGGAAGAATACCACAAGGAGTGGATTGTGCGGCTTAA TTTGACTCAACGCGAGGTAACTT3'	59/69	742-804/62	*Rrs*	HM142729.1
S. bombi FAM	5'CGGTTAAAAAGCTCGTAGTTGGATCTGTGCCCGGGACTTGGTCCACCT TTTAGGTGCGCACTAGGCTTC3'	59/68	565-633/68	*Rrs*	AB250213.1
Virus Quencher	**Sequence**	**Tm°C Pri/Pro**	**Position bp length**	**Coding**	**Genbank Acces No.**
ABPV FAM	5'TGCTCGAGCTGGTAATGATGATTTCACTTTTGGATGGCTCATAG GAACACCGCAAACTCAAGGAA3'	59/68	9166-9230/64	capside	AF150629.1
CBPV VIC	5'CATCGGCAACGGATTCATCAACGCATTCATTATCTGGC TCTGTCTTCGCAAACTGCCC3'	60/68	1209-1266/57	RNApol	FJ345309.1
DWV FAM	5'CATGCATATTGGCGTGGAGATATGGAAGTTCGAGTGCAGATTAAC TCGAATAAATTCCAGGTTGGTCA3'	59/70	2094-2161/67	polypro	HM067437.1
IAPV VIC	5'GTGGAAACTCCAAATAGGATCGATACCCCCATGGCTCAGGATACT TCATCGGCTAGGAGCATGG3'	58/70	595-658 / 63	RNApol	EU436450.1
KBV FAM	5'GCATCGAGCGCATTCCAGTGCTAGGTGAAATTGCAAAGC CAGTAACCGCTGCAGTTAAATGG3'	60/69	6735-6798/1	RNApol	AY275710.1

Primer sequences are shown in gray, parasite genomic sequences are shown in black and the probe sequences are underlined. The Tm for each Primers (Pri) and Probes (Pro) Tm_s are included, together with genomic coordinates and Genbank accession numbers are also provided

Results and discussion

The objective of this work was to study the prevalence of ten common pathogens that infect bumble bees in the central Mexican state of Queretaro, where commercial bumble bees are used extensively for greenhouse production, particularly for production of the tomato *S. lycopersicum*. We screened adult worker bumble bees from 120 different greenhouses. To make the screen economically feasible, we pooled ten individuals from each location (colony) into a single sample. We can assume that each pool represents the infection status of a particular bumble bee colony at the moment the sample was taken (when the colony boxes were opened for first time in the greenhouse). The pooling strategy makes sense because it has been demonstrated that bumble bee workers can be infected by the exposure to fecal matter of bumble bees or by pollen that is contaminated by these parasites and that these infections become systemic in the colony, it has also been observed that the infection rate tends to be greater after shipment (Graystock et al. 2013). Of the 120 locations, 54 were positive for one or more pathogens (45 %).

We developed a spectrum of pathogen probes for the detection of the acaroid *Locustacarus buchneri*, the nematode *Sphaerularia bombi*, the protozoans *Apicistis bombi* and *Crithidia bombi*, the fungus *Nosema bombi*, together with the viral pathogens ABPV, CBPV, DWV, IAPV and KBV. The most frequently found pathogen was *A. bombi,* which was present in 32 locations. Of the 32 samples infected with *A. bombi*, 15 were co-infected with another pathogen: four samples were co-infected with the parasite *L. buchneri,* one sample was co-infected with *N. bombi*, three samples were co-infected with ABPV, three samples were co-infected with CBPV, one sample was co-infected with DWV, one sample was co-infected with IAPV, one sample was co-infected with KBV, and a final colony sample had a triple infection with *A. bombi,* DWV and IAPV. Interestingly the triple-infected sample was also the sample that carried the highest infection load for the three detected pathogens. Twelve locations were infected with *C. bombi,* and two of these colony samples were co-infected with ABPV. Ten other locations had single infections: 3 ABPV infections, 5 CBPV infections, 1 DWV infection and 1 KBV infection (Table 2; Fig. 1). All reactions were performed in triplicate using TaqMan® and SYBR® Green. The specificity of the TaqMan® and SYBR® Green results was always confirmed using PAGE, which only detected a single band of the expected molecular weight (Fig. 2). In this sample population, no false positives were detected.

Table 2 Positive samples positive for one or more pathogens

Pathogen	*A. bombi*	*C. bombi*	*L. buchneri*	*N. bombi*	*S. bombi*	ABPV	CBPV	DWV	IAPV	KBV	DWV/IAPV
A. bombi	17	–	4	1	–	3	3	1	1	1	1
C. bombi		10	–	–	–	2	–	–	–	–	–
L. buchneri			–	–	–	–	–	–	–	–	–
N. bombi				–	–	–	–	–	–	–	–
S. bombi					–	–	–	–	–	–	–
ABPV						3	–	–	–	–	–
CBPV							5	–	–	–	–
DWV								1	–	–	–
IAPV									1	–	–
KBV										1	–

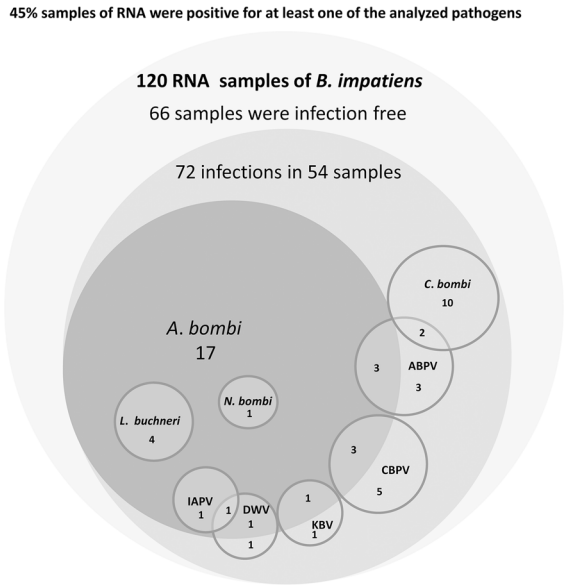

Fig. 1 Proportional diagram of representing the infections and co-infections detected of in 120 RNA samples. *Circles* represent the population size and the relationships of between samples presenting with one or more infections

Our results demonstrate that we can routinely, quantitatively and reliably detect as little as 10^{-5} ng of synthetic DNA, which represents approximately 35000 copies of each target, using conventional qPCR technologies (Table 3). At lower concentrations, the target can be reliably detected but not reliably quantified (Yukl et al. 2013). Synthetic DNA constructs served dual purposes, acting as both positive controls and quantified standards that facilitated the creation of standard curves to define the lower limit of detection. Our probes have high sensitivity and specificity, as evidenced by the absence of false positives. We also included negative controls in which we used cDNA from the unrelated hymenoptera *Scaptotrigona mexicana* and the dipteran *Drosophila melanogaster*. Both of these controls were negative for all of the tested pathogens (data not shown). It is important to note that viruses cannot be directly visualized by conventional microscopy, therefore electron microscopy, molecular or immunological techniques are the only option to detect these pathogens, for this reason, we think that the best available technique to detect a wide range of pathogens and parasites are probably methodologies involving molecular biology. The high sensitivity and specificity of the test was reproduced for all pathogens tested. Because the viral pathogens tested in this study also infect the honey bee *Apis mellifera*, we generated a powerful tool that will be helpful for screening the health and the incidence of EIDs of honey bee and bumble bee colonies. Our results indicate that 45 % of commercial *B. impatiens* populations are infected with at least one pathogen. Importantly, 40 % of the detected infections were caused by viruses that are associated with colony collapse disorder (Table 4).

In most cases, *A. bombi* was found at very low titer (Table 3). This result is inconsistent with previous studies in which *A. bombi* was not found in commercial bumble bees (Colla et al. 2006). However, the authors of that study suggested that the absence of this parasite in their samples may be due to the greenhouse

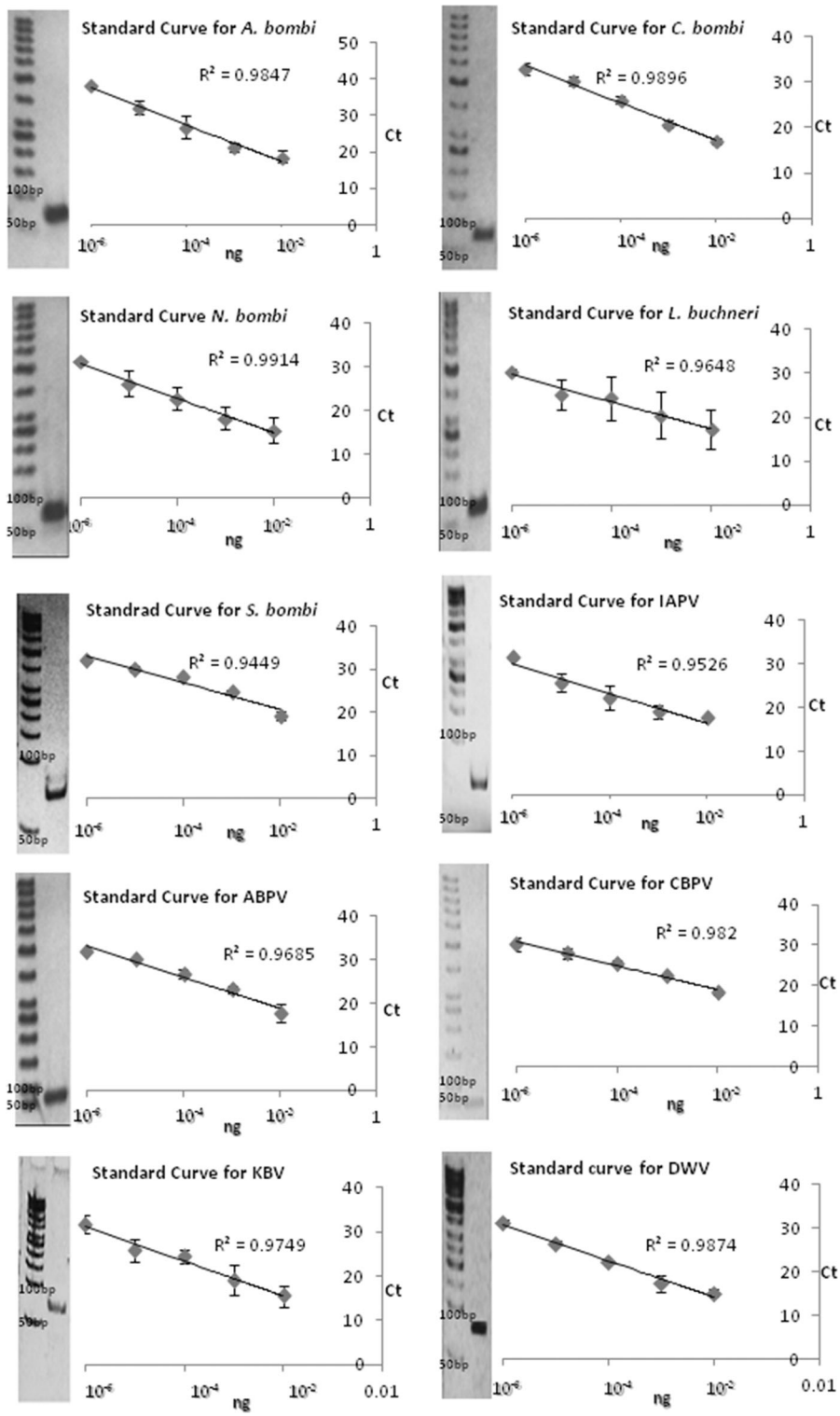

Fig. 2 Standard curves for each pathogen target. Standard deviations and linear regressions are shown (n ≥> = 3). SYBR® Green amplification specificity for the pathogen targets is shown presented on in the panels on to the left of each amplification curve

Table 3 Ranges for the number of molecules detected of for each pathogen

Number of parasite molecules detected per sample (Taqman assay)								
A. bombi	*C. bombi*	*L. buchneri*	*N. bombi*	ABPV	CBPV	DWV	IABPV	KBV
6.3×10^5 to 2.4×10^{12}	9.7×10^7 to 2.7×10^{10}	$\geq 2.5 \times 10^3$* to 4.3×10^3	1.2×10^6	2.0×10^5 to 8.0×10^5	2.0×10^3 to 7.7×10^4	1.4×10^4 to 4.6×10^5	3.6×10^4 to 4.7×10^5	1.3×10^4 to 9.1×10^4

* The low titer in of these samples does not allow the assay to be quantitative because it is below the detection limit of the qPCR. However, the parasite is clearly present in these samples, because no signal was detected in the negative controls, the signal was very robust in the positive controls, a single product was observed in via PAGE and results were reproducible in field samples

bumble bees restrictive diet, which leads to a diminished amount of fat tissue. In addition, the sensitivity of molecular techniques is several orders of magnitude higher than that of microscopic techniques, which are not sensitive enough to detect very low titers of parasites. Future studies may develop antibodies and use immunostaining techniques to identify the organs infected by *A. bombi* in commercial bumble bees, although these experiments are beyond the scope of the present work.

On the other hand, it has recently been described that *A. bombi* has travelled in commercially produced *B. terrestris* colonies and invaded new ecosystems such as Patagonia. The introduced European species *B. ruderatus* and the patagonian native *B. dahlbomii* were infection free in Patagonia previous to the introduction of *B. terrestris* and became positive for this parasite thereafter. Thus suggesting that this parasite can infect native *Bombus* species that are different from their original host (Arbetman et al. 2012; Maharramov et al. 2013). This evidence also indicates that *A. bombi* has recently been transferred at least once from Europe to South America (Maharramov et al. 2013). Other important examples of pathogen spillover in different parts of the world include: the dissemination of European *L. buchneri* by *B. terrestris* to wild Japanese bumble bee populations (Yoneda et al. 2008); the role commercial *B terrestris* as reservoir of the parasites *C. bombi*, *A. bombi* and *N. bombi* in Scotland (Whitehorn et al. 2013) and the high infection rate of wild bumble bees whose colonies are close to commercial greenhouses in Ontario, Canada (Otterstatter and Thomson 2008). Colonies with multiple infections have also been reported in other studies (Rutrecht and Brown 2008).

It should be noted that greenhouse bumble bees live in stressful conditions that likely increase their susceptibility to disease. It has been proposed that a combination of colony co-infection with multiple parasites and environmental stresses are the causes of the diseases that are decimating bumble bee colonies in North America (Kissinger et al. 2011; Graystock et al. 2013, 2014). It has also been suggested that the presence or introduction of *N. bombi* correlates with the decline of native North American bumble bee populations (Cameron et al. 2010). Therefore, it is most worrisome that several of these pathogens can be transferred between commercially produced bumble bees and wild bumble bees (Colla et al. 2006). Bumble bees are traded globally for commercial purposes. In Mexico, the biggest producers are Koppert (http://www.koppert.com.mx/) and Bio-Best (http://www.biobest.be/vestigingen/0/4/). Both of these companies are present in at least 50 countries. Honey beehives are frequently rented and transported over large geographic distances. Bumble bee colonies are also transported across continents and sold annually by the hundreds of thousands (Velthius and Van Doorn 2006; Watanabe 2006; Morkeski and Averil 2010). The life cycles of these colonies involves annual colony foundation by fertilized queens. Most of these queens are unlikely to survive greenhouse agricultural cycles, but there is the potential for queens to escape. Instances of escape have already been reported in central Mexico (Pitts-Singer and James 2008) and other countries such as Japan (Goka 2010a). The transportation of thousands of individuals across continents presents an opportunity for the spread of emergent pathogens into new environments and danger to biodiversity (Colla et al. 2006; Fürst et al. 2014). For example, genetic dilution of native bumble bees has been demonstrated in Japan following the introduction of *B. terrestris* (Kondo et al. 2009). Additionally there are several other factors of ecological concern that may result from the introduction of alien pathogens and insect species, among these are: (1) Native pathogens may evolve a

Table 4 Comparison of reported pathogen infection percentages in for honey bees and bumble bees reported in literature

Reference/pathogen (%)	Samples (n)	*A. bombi*	*C. bombi*	*L. buchneri*	*N. bombi*	*S. bombi*	ABPV	CBPV	DWV	IAPV	KBV	Techinque	Genera
This work	120	26.6	14.4	3.3	0.83	0	6.6	6.6	2.5	1.6	1.6	RT-qPCR	*Bombus*
Al-Abbadi et al. (2010)	100	–	–	–	–	–	–	–	–	13	12	RT-PCR	*Apys*
Kukielka and Sánchez-Vizcaíno (2010)	484	–	–	–	–	–	–	–	–	0.2	–	RT-PCR	*Apys*
Plischuk and Lange 2009	552	3.6	21.6	–	–	–	–	–	–	–	–	Microscopy	*Bombus*
Rutrecht and Brown 2008	160	3.8	23	33.3	0	15.6	–	–	–	–	–	Microscopy	*Bombus*
Colla et al. (2006)	628	1.8	7	–	5.1	–	–	–	–	–	–	Microscopy	*Bombus*
Goka et al. (2001)	367	–	–	16	–	–	–	–	–	–	–	Microscopy	*Bombus*
Gillespie (2010)	867	–	37.5	–	12	–	–	–	–	–	–	Microscopy	*Bombus*
Morkeski and Averil (2010)	801/86	–	16.8	–	26.7	–	–	–	9.3	–	–	Microscopy/ qPCR	*Bombus*
Kissinger et al. (2011)	1380	–	8.4	8.3	11.3	–	–	–	–	–	–	Microscopy/ qPCR	*Bombus*

higher virulence in captivity. (2) Managed bumble bees may cause native pathogens to increase in prevalence, which could cause an epizootic in wild bumble bees. (3) There are concerns about genetic pollution. For example, recent research has found that *B. ephippiatus* is potentially comprised of three distinct species. Managed *B. ephippiatus* could breed with closely related wild species (Duennes et al. 2012b). (4). Non-native commercial bumble bee species may establish in the wild and compete with native bumble bee species for food sources or nesting areas.

The centralized production of bumble bee colonies has clear advantages and disadvantages. The economic benefit of using these organisms in agricultural practices cannot be denied. On the other hand there is the latent risk that the transportation of infected bumble bees may transmit pathogens to other commercially important species, such as honey bees or other wild hymenopterans. As it has been demonstrated that greenhouse bumble bees transmit these viruses and eukaryotic pathogens to other hymenopteran species, such as *Apis mellifera* and vice versa (Graystock et al. 2013; Fürst et al. 2014).

Rigorous policy and routine pathogen monitoring should be implemented in a global scale (Meeus et al. 2011; Fürst et al. 2014). As it has been done in parts of Europe, where bumble bees producers must rigorously screen their production in order to maintain their import license (Murray et al. 2013; Graystock et al. 2013). Japan also requires that farms that use commercially reared bumble bees must also screen them routinely to break any possible transmission route (Goka 2010b). In conclusion, the routine pathogen screening using molecular technologies should become governmental policy and must be adopted by commercial bumble bee producers as soon as possible to avoid environmental dispersal of hymenopteran emerging infectious diseases, to maintain the sustainable production of bumble bees and the natural pollination services that different bumble bees species provide worldwide.

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