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1 **Effect of oral infection with Kashmir bee virus and Israeli acute paralysis virus**
2 **on bumblebee (*Bombus terrestris*) reproductive success**

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24 **Abstract**

25 Israeli acute paralysis virus (IAPV) together with Acute bee paralysis virus (ABPV)
26 and Kashmir bee virus (KBV) constitute a complex of closely related dicistroviruses.
27 They are infamous for their high mortality after injection in honeybees. These viruses
28 have also been reported in non-*Apis* hymenopteran pollinators such as bumblebees,
29 which got infected with IAPV when placed in the same greenhouse with IAPV
30 infected honeybee hives. Here we orally infected *Bombus terrestris* workers with
31 different doses of either IAPV or KBV viral particles. The success of the infection
32 was established by analysis of the bumblebees after the impact studies: 50 days after
33 infection. Doses of 0.5×10^7 and 1×10^7 virus particles per bee were infectious over
34 this period, for IAPV and KBV respectively, while a dose of 0.5×10^6 IAPV particles
35 per bee was not infectious. The impact of virus infection was studied in micro-
36 colonies consisting of 5 bumblebees, one of which becomes a pseudo-queen which
37 proceeds to lay unfertilized (drone) eggs. The impact parameters studied were: the
38 establishment of a laying pseudo-queen, the timing of egg-laying, the number of
39 drones produced, the weight of these drones and worker mortality. In this setup KBV
40 infection resulted in a significant slower colony startup and offspring production,
41 while only the latter can be reported for IAPV. Neither virus increased worker
42 mortality, at the oral doses used. We recommend further studies on how these viruses
43 transmit between different pollinator species. It is also vital to understand how viral
44 prevalence can affect wild bee populations because disturbance of the natural host-
45 virus association may deteriorate the already critically endangered status of many
46 bumblebee species.

47

48 Keywords: Israeli acute paralysis virus; Kashmir bee virus; *Dicistroviridae*;

49 bumblebees; multi-host pathogens

50

51 **1. Introduction**

52 The Apoidea, encompassing different families of bees, perform a valuable pollination
53 service (Garibaldi et al., 2013). With up to 80% of the plant species being dependent
54 on insect pollination, in particular by bees (Potts et al., 2010). This results in an
55 estimated value of 9.5% of the total economic value of crops that are directly used for
56 human food (Gallai et al., 2009; Potts et al., 2010). **Because of a lack of**
57 **abundance/presence of wild bees, managed bees are used to pollinate crops** (Allsopp
58 et al., 2008).

59 Because different bee species have a similar foraging behavior (gathering pollen and
60 nectar), with overlapping flower networks, sympatric distributions and direct
61 interactions between species or their stored resources, it is very likely that they are
62 exposed to each other's parasites and pathogens. Indeed, parasite networks between
63 bee species are complex and comprise a mixture of multi-host parasites (e.g. *Apicystis*
64 *bombi* (Maharramov et al., 2013), *Nosema ceranae* (Graystock et al., 2013a),
65 deformed wing virus (DWV) (Fürst et al., 2014)), as well as multi-parasite hosts
66 (Rigaud et al., 2010). However, with the exception of honeybees (*Apis* spp.), little is
67 known about the parasites and pathogens of pollinators, even less about the extent to
68 which they cross-infect different pollinators, and almost nothing about the damage of
69 such cross-infections to different hosts.

70 Here we focus on the effects of interspecific transmission of bee viruses. Most of
71 what is known about bee viruses relates to the European honeybee (*Apis mellifera*)
72 and its sister species (primarily the Asian hive bee; *A. cerana*), largely through the
73 pioneering work of Bailey and Ball (1991) during the second half of the twentieth
74 century. The evidence increasingly suggests a large degree of commonality of
75 honeybee viruses among the *Apis* species (Ai et al., 2012; Choe et al., 2012; Kojima

76 et al., 2011; Yañez et al., 2012; Zhang et al., 2012), usually with similar symptoms.
77 Many honeybee viruses have also been detected in other Hymenopteran pollinators,
78 predators and scavengers, initially mostly through incidental observations (Anderson,
79 1991; Bailey and Ball, 1991) and more recently also through dedicated research
80 (Celle et al., 2008; Evison et al., 2012; Fürst et al., 2014; Genersch et al., 2006; Li et
81 al., 2011; Peng et al., 2011; Singh et al., 2010; Yañez et al., 2012). Bee viruses have
82 also been detected in non-Hymenopteran hosts associated with honeybees (Celle et
83 al., 2008; Dainat et al., 2009; Eyer et al., 2008; Gisder et al., 2009). Honeybees may
84 also be hosts or vectors of certain aphid viruses (Runckel et al., 2011), through the
85 collection of honeydew, or possibly even plant viruses (Li et al., 2014), which could
86 also be transmitted on to other pollinators, through their overlapping contact network
87 with honeybees.

88 Because of their wide foraging range, large diversity of floral resources visited, long
89 foraging seasons and extensive accumulation of stored pollen and nectar, honeybees
90 are likely to be major factors in any pathogen transmission network involving other
91 (Hymenopteran) pollinators. The worldwide trade in honeybees and bee products
92 coupled with the increasing pathogen prevalence and loads in honeybee colonies, due
93 to a variety of biological and environmental stressors (Genersch et al., 2010a;
94 vanEngelsdorp and Meixner, 2010), could therefore have potentially serious
95 consequences for local wild bee populations (Fürst et al., 2014; McCallum and
96 Dobson, 1995; Meeus et al., 2011).

97 However, the above mentioned arguments have so far been largely speculative. Other
98 than detecting honeybee pathogens in other insects, and thus establishing possible
99 transmission routes (*e.g.* (Evison et al., 2012; Li et al., 2011; Peng et al., 2011; Singh
100 et al., 2010), there has been little research as to whether these viruses are actually

101 infectious or, more importantly, cause damage to species other than honeybees. The
102 only recorded exceptions so far are the association of DWV with wing deformities
103 found naturally in both wild and commercially reared bumblebees (Genersch et al.,
104 2006), the reduced survival of bumblebees orally inoculated with DWV (Fürst et al.,
105 2014) and the rapid mortality of bumblebees injected with low doses of Israeli acute
106 paralysis virus (IAPV; Niu et al., 2014). Studies of the effects of interspecific transfer
107 of pollinator viruses are especially important for bumblebees, since bumblebee
108 diversity is diminishing rapidly in many regions of the world (Biesmeijer et al., 2006;
109 Cameron et al., 2011; Potts et al., 2010).

110 This study concerns the pathogenic effects on bumblebees (*Bombus terrestris* or the
111 buff-tailed bumblebee) of two dicistroviruses: IAPV and Kashmir bee virus (KBV),
112 which together with Acute bee paralysis virus (ABPV) form a complex of closely
113 related viruses (de Miranda et al., 2010). These three viruses share a similar
114 pathology, all being rapidly lethal after injection in honeybees. In honeybee colonies,
115 they are normally present in low titer as persistent infections. But under certain
116 environmental stress^{es}, such as for example *Varroa destructor* infestation, they can
117 undergo re-emergence toward an overt infection-type that can contribute to colony
118 failure (Ribière et al., 2008). Injection of low numbers of IAPV particles in
119 bumblebees also resulted in rapid mortality (Niu et al., 2014). However, the most
120 likely natural virus transmission route for bumblebees is oral. We therefore infected
121 newborn bumblebee workers orally with IAPV or KBV and assessed the effects of
122 this on the performance of bumblebee micro-colonies, a standardized method for
123 studying colony development and reproduction.

124

125 **2. Materials and methods**

126 *2.1. Bumblebees source*

127 All bumblebee (*Bombus terrestris*) workers were obtained from a continuous mass
128 rearing program (Biobest, Westerlo, Belgium) and were maintained on commercial
129 sugar water (BIOGLUC, Biobest) and honeybee-collected pollen (Soc. Coop.
130 Apihurdes, Pinofranqueado-C'aceres, Spain) as energy and protein source,
131 respectively. The insects were kept under standardized laboratory conditions with 29
132 – 31 °C, 60–65 % relative humidity, and continuous darkness.

133

134 *2.2. Bumblebee fitness parameters*

135 We used micro-colonies to quantify the effects of virus infection on colony
136 development and bumblebee fitness, as well as worker mortality. The micro-colonies
137 were established by introducing 5 newborn (maximum one day old) workers in an
138 artificial 15×15×10 cm nest box. In this set-up, one worker becomes dominant, i.e. a
139 pseudo-queen, within 2 days and starts laying unfertilized eggs that develop into
140 drones. The remaining workers take care of the brood. The number and mass of the
141 (drone) offspring is a measure of colony fitness. Colony development follows a well-
142 defined pattern and timing under these controlled conditions when receiving the same
143 diet *ad libitum*. Development is measured by the time until the first oviposition, the
144 occurrence of the first developed larvae and the first pupae. Any deviation from this
145 pattern and timing is indicative of alterations in the reproductive capacity of the
146 pseudo-queen or in larval development. The micro-colonies were kept under
147 standardized rearing conditions, as reported above.

148

149 *2.3. Virus and control extracts*

150 For each extract, fifty white-eyed pupae from a healthy honeybee colony were
151 injected with previously purified IAPV or KBV and incubated at 30°C for 4 days
152 following the protocols of the virus chapter of the BeeBook (de Miranda et al., 2013).
153 The control extract was prepared from uninjected pupae incubated for the same length
154 of time. The pupae were homogenized in 10 mM phosphate buffer (pH 7.0) 0.02%
155 diethyl dithiocarbamate, clarified with chloroform and centrifuged at 8000g for 15
156 minutes (de Miranda et al., 2013). The particle concentration of each virus extract
157 was determined using transmission electron microscopy (TEM). Undiluted and 10-
158 fold diluted viral stock solutions were analyzed at the CODA-CERVA (Uccle,
159 Belgium). They were negatively stained according to the protocol described by Mast
160 and Demeestere (2009). Zones of “wet staining” could be identified on each grid
161 where the particles were evenly spread over the grid with limited competition for
162 binding sites and little overlap of particles. TEM specimens were examined using a
163 Tecnai Spirit microscope (FEI, Eindhoven, The Netherlands) operating at 120 kV, at a
164 spot size of 1. An entire grid surface 1537 nm by 1537 nm was analyzed with a
165 30.000x magnification under parallel beam conditions. The IAPV extract contained 1
166 $\times 10^6$ viral particles/ μ l and the KBV extract 2×10^6 viral particles/ μ l, while the control
167 extract was largely devoid of virus particles. The IAPV and KBV extracts had <0.1%
168 and <0.01% contamination, respectively with other common honeybee viruses, as
169 determined by RT-qPCR using specific assays for ABPV, Chronic bee paralysis virus,
170 DWV, Varroa destructor virus-1 (VDV-1), slow bee paralysis virus (SBPV), sacbrood
171 virus (SBV), black queen cell virus (BQCV), Lake Sinai virus-1 and -2 (Locke et al.,
172 2012). The control extract had similar background levels of the same viruses (mostly
173 SBV and BQCV) as the IAPV and KBV extracts.

174

175 *2.4 Experimental design and infection*

176 There were three treatment groups in this experiment; control, IAPV infection and
177 KBV infection, each with ten micro-colonies. Five newborn workers were added to
178 each micro-colony and kept under standard rearing conditions for one day. They were
179 then deprived of pollen and sugar water for 3 hours. The starved bees were then
180 placed in a feeding box (a cylinder of 1 dm diameter) containing a 30- μ l droplet
181 containing 5 μ l experimental extract plus 25 μ l of 50% sugar water solution
182 (BIOGLUC, Biobest). Therefore each bee in the IAPV treatment received 0.5×10^7
183 IAPV particles while in the KBV treatment group each bee received 1×10^7 KBV
184 particles. Additionally, 10 workers (2 micro-colonies) were fed 5 μ l of a 10-fold
185 dilution of the IAPV extract (*i.e.* 0.5×10^6 particles/bee) to assess if we could still
186 infect workers with this lower dose. After inoculation, the bees were returned to their
187 micro-colony where they immediately received *ad libitum* sugar water and after three
188 days also pollen *ad libitum*.

189

190 *2.4. Virus detection*

191 Bumblebees were dissected and the gut was grounded individually in 300 μ l of RLT
192 buffer (Qiagen, Venlo, Netherlands) supplemented with 3 μ l β -mercapto-ethanol.
193 RNA was extracted with the Qiagen RNeasy Mini Kit following manufacturer's
194 instructions, eluting the RNA in 30 μ l of RNase free water. We used reverse
195 transcriptase multiplex-ligation probe dependent amplification (RT-MLPA)
196 technology to determine the virus infection status of our samples. This technology,
197 called BeeDoctor (De Smet et al., 2012), detects 6 targets simultaneously and covers
198 10 common "honeybee" viruses: Black queen cell virus (BQCV); the acute bee
199 paralysis virus complex including ABPV, KBV and IAPV; the DWV-complex

200 including DWV, VDV-1 and Kakugo virus (KV); SBPV; SBV; and chronic bee
201 paralysis virus (CBPV). Since the BeeDoctor does not distinguish between IAPV and
202 KBV, all samples were also analyzed by RT-PCR using primers specific for either
203 IAPV (CGATGAACAACGGAAGGTTT and ATCGGCTAAGGGGTTTGTTT
204 (Cox-Foster et al., 2007) or KBV (GCCGTACAACGACGACTACA, and
205 CGTCATTTTAACCGCTGCTT). The viral identity of both amplicons was
206 confirmed by Sanger sequencing (LGC Genomics, Berlin, Germany). A two-step RT-
207 PCR protocol was used for this. The cDNA was synthesized with SuperScript-II
208 Reverse Transcriptase (Invitrogen, Merelbeke, Belgium) according to the
209 manufacturer's guidelines with 0.8 μ M virus-specific reverse primers. One microliter
210 of cDNA was added to a final 25 μ l PCR reaction mixture containing 2.5 μ l 10x PCR
211 buffer, 1.5 mM MgCl₂, 0.2 mM dNTP, 0.5 μ M primers and 1.25 U Recombinant Taq
212 DNA Polymerase (Invitrogen). The PCR reactions were run in a Sensoquest
213 Labcycler for 2 min at 94 °C followed by 30 amplification cycles of (30 s
214 denaturation at 94 °C; 30 s annealing at 56 °C; 45 s extension at 72 °C) followed by 3
215 min final extension at 72 °C.

216

217 2.5. Statistics

218 Statistical analysis of the data was conducted in SPSS v21.0 (SPSS Inc., Chicago, IL).
219 The normal distribution was confirmed by the Kolmogorov-Smirnov test ($P = 0.05$).
220 The non-normal distributed dependent variable (time until oviposition) was divided
221 into regular and delayed oviposition. A χ^2 Goodness of Fit test was used to determine
222 if virus treatment resulted in significant deviation from the control treatment. The
223 number and mass of drones produced in micro-colonies with a regular time until
224 oviposition were analyzed by one-way analysis of variance (ANOVA) and the mean \pm

225 standard error were separated with a post hoc Tukey test ($\alpha = 0.05$). The numbers of
226 drones produced by all micro-colonies, including both regular and delayed
227 oviposition, were analyzed by a non-parametric Whitney U test.

228

229 **3. Results**

230 *3.1. Infection status*

231 The pseudo-queen of a micro-colony, the one that lays the eggs, has the highest
232 impact on the performance of her micro-colony. Therefore we tested the virus
233 infection status of the pseudo-queens after following micro-colony development for
234 50 days. Six out of 10 IAPV-treated pseudo-queens and 9 out of 10 KBV-treated
235 pseudo-queens tested positive for infection with an ABPV-KBV-IAPV complex virus,
236 using the BeeDoctor RT-MLPA technology, while none of the other viruses covered
237 by BeeDoctor (De Smet et al., 2012) were detected. IAPV- KBV-specific RT-PCR
238 reactions, followed by sequencing of the RT-PCR products, confirmed that IAPV
239 treatment resulted only in IAPV infections and the KBV treatment only in KBV
240 infections. The control pseudo-queens as well as and bumblebees receiving a ten fold
241 dilution of the IAPV stock (n = 10) were entirely free of any virus covered by the
242 BeeDoctor.

243

244 *3.2. Impact of virus infection on bumblebee colony development*

245 Infection with either IAPV or KBV did not result in any major increase in mortality of
246 the bumblebee workers. The IAPV treatment resulted in 6 dead workers out of 50
247 workers by day 50; the KBV treatment only had 1 dead worker, and the control
248 treatment had 3 dead workers out of 50.

249 Bumblebee micro-colonies develop very predictably under standard, uniform
250 nutritional conditions, with oviposition starting 7-8 days after introducing the bees
251 into their micro-colony, with usually no more than 1 day variation in oviposition
252 between colonies (Meeus et al., 2013). However, in these experiments the micro-
253 colonies were deprived from pollen for 3 days, which delayed oviposition to a mean
254 of 11 days in the control group, and also increased the variation in oviposition time
255 around this mean. Consequently, the time until oviposition in these 30 experimental
256 and control micro-colonies did not show a normal distribution (One-Sample
257 Kolmogorov-Smirnov Test, $P = 0.00014$). The control group had an interquartile
258 (IQR) of 1, everything lower than $Q1 - 1.5 \times IQR = 8.8$, and everything higher than $Q3$
259 $+ 1.5 \times IQR = 12.5$ is an outlier. Based on this we saw two groups: those with 9, 10, 11
260 or 12 days until oviposition (“regular colonies”) and those with oviposition starting at
261 day 13 or later (“delayed colonies”). There were 2 out of 10 colonies with delayed
262 oviposition in the control group; 4 out of 10 in the IAPV-treated group and 6 out of 10
263 in the KBV-treated group (Table 1a). The difference between the KBV-treated
264 colonies and control colonies is significant, as determined by a χ^2 Goodness of Fit
265 Test. KBV treatment also resulted in significantly more micro-colonies with no drone
266 production at all compared to control samples; this effect did not occur for IAPV
267 treatment (Table 1b).

268 The delay in oviposition will further influence the total number of drones produced by
269 these colonies. Therefore we only used the colonies with a “regular” oviposition time
270 (10-12 days after start-up of the experiment) to compare drone production between
271 treatments. The ANOVA indicated a significant difference in numbers of drones
272 produced between the treatments ($F_{(2,15)} = 4.127$; $P = 0.036$). Using the post hoc
273 Tukey test, to determine which treatment caused the effect, we saw that both

274 treatments (KBV and IAPV) produced fewer drones than the control colonies, with a
275 probability of 0.07 (Fig 1). These comparisons excluded the micro-colonies with
276 delayed oviposition time, which reduces the statistical power of the comparisons.
277 When we compare all IAPV-treated micro-colonies that produce drones, irrespective
278 of oviposition time, to similar micro-colonies from the control group, than we see a
279 significant drop in drone production in IAPV-treated colonies ($N = 18$; Mann Whitney
280 U test: $z = 17.5$; $P = 0.04$). Furthermore, drone production in all virus-treated colonies
281 combined (i.e. both KBV and IAPV) was significantly reduced when compared with
282 the control colonies ($F_{(1,16)} = 8.828$; $P = 0.009$) (Fig 1).

283 The same analyses applied to drone mass for all drone-producing micro-colonies,
284 revealed a lower mean mass of the drones in virus-treated colonies compared to
285 control colonies, although this difference was not significant ($F_{(2,18)} = 1.801$; $P =$
286 0.194) and $F_{(1,19)} = 1.782$; $P = 0.198$).

287

288 **4. Discussion**

289 There is extensive historical literature on the effects of ABPV and KBV on honeybees
290 (for reviews see Ribière et al. (2008) and de Miranda et al. (2010)). Both viruses have
291 been implicated in *Varroa*-associated colony losses (de Miranda et al., 2010; Ribière
292 et al., 2008). More recent European data links ABPV with honeybee winter mortality
293 (Genersch et al., 2010b; Siede et al., 2008). IAPV, which was only recently described
294 as a separate virus (Maori et al., 2007), has also been implicated as a marker for
295 Colony Collapse Disorder (CCD) in North America (Cox-Foster et al., 2007),
296 although this was re-assessed in subsequent, more comprehensive studies
297 (vanEngelsdorp et al., 2009). Instead mortalities have been linked to KBV and ABPV
298 infections (Cornman et al., 2012) and overall pathogen load as an indicator of

299 compromised honeybee health (Ravoet et al., 2013). Despite the acute virulence of
300 these viruses in honeybees and their ability to infect other hymenopteran species,
301 including bumblebees (Bailey and Gibbs, 1964; Singh et al., 2010), few systematic
302 host-range studies have been conducted for any of these viruses. Moreover, no study
303 to date has investigated their impact on such alternative hosts. Using the buff-tailed
304 bumblebee, a generalist forager in the Palearctic region, we demonstrate that oral
305 feeding of 0.5×10^7 and 1×10^7 viral particles per bee of either IAPV or KBV,
306 respectively, results in an active infection and fitness loss. Lower doses of IAPV (0.5
307 $\times 10^6$ IAPV particles/bee) did not result in a detectable infection. Thus, our oral
308 administration dose is close to the minimum required for inducing an infection, and
309 may not have been sufficient to affect worker mortality. This may also explain the
310 slightly reduced virulence of IAPV compared to KBV in these experiments, since the
311 KBV infectious dose was twice that of IAPV. Experiments elsewhere showed that
312 oral infection of *B. terrestris* workers with 10^9 genome copies of a different honeybee
313 virus, DWV, reduced the mean survival of *B. terrestris* workers by 6 days (Fürst et
314 al., 2014).

315 With KBV-infected bumblebees, the time until oviposition was delayed and fewer
316 colonies initiated drone production than with uninfected bumblebees. We speculate
317 that the exclusion of pollen in the first 3 days of the experiment exacerbated these
318 effects, as pathogenic effects are often context dependent, with low nutritional status
319 being an important stressor for pathogen infections (Brown et al., 2003). In colonies
320 without delayed oviposition, drone production was also impaired. We can thus
321 conclude that under the experimental conditions KBV infection reduces *B. terrestris*
322 fitness.

323 For IAPV the situation is less obvious. IAPV-infected bumblebees showed deviations
324 in time until oviposition and drone production, but these were not significant.
325 However, when we only analyze micro-colonies with drone production, we see that
326 IAPV-infected colonies produce significantly fewer drones than non-infected
327 colonies. We can therefore conclude that IAPV impacts *B. terrestris* fitness as well.
328 The lower virulence of IAPV in these experiments, relative to KBV, may be partly
329 due to the lower IAPV infectious dose used (half that of KBV).

330 Here we report fitness impact of KBV and IAPV, and Fürst et al. (2014) showed
331 lower survival after DWV infection (Fürst et al., 2014) in bumblebees. The time is
332 now to clarify what this could mean for critically endangered bumblebee populations
333 (Biesmeijer et al., 2006; Cameron et al., 2011; Potts et al., 2010). Could
334 anthropogenic movement of bees disturb the natural multi-host pathogen association
335 by spilling over pathogens? And how severe is this stressor compared to other factors
336 such as pesticide use and land use change? Two potential reservoirs of pathogens
337 from which pathogens can potentially infect wild pollinators are: domesticated
338 honeybees, notorious for their viral infection loads, and commercially bred
339 bumblebees escaping greenhouses (Murray et al., 2013) can carry viruses (Graystock
340 et al., 2013b). For now the threats toward wild pollinators is unknown. A critical
341 factor in the overall risk-determination is the pathogen's infectivity (the capacity to
342 initiate an infection), virulence (the capacity to cause damage) in the wild pollinator
343 and host tolerance, genetics and condition (Casadevall and Pirofski, 1999; Casadevall
344 and Pirofski, 2001), in relation to the amount and concentration of virus produced by
345 the domesticated or bred bees. It is therefore important to know if the oral doses
346 applied here are realistic in their ecological context. This study shows that the
347 infectivity of IAPV and KBV in bumblebees is relatively low (high oral doses are

348 required to start an infection) and of the same order of magnitude as their oral
349 infectivity in honeybees (Bailey and Ball, 1991; de Miranda et al., 2013). The other
350 factors important for risk assessment are the exposure rates and probabilities, either
351 through direct contact (bumblebees feeding at honeybee hives) or through flower
352 networks. The results of Fürst et al. (2014) and Singh et al. (2010) have shown that
353 this exposure can be high for those bumblebee colonies in the immediate vicinity of
354 honeybee colonies, but that for bee viruses most of this risk is related to the primary
355 contact with honeybee colonies, with currently little evidence for independent
356 secondary proliferation within the bumblebee community itself.

357 As a final point, healthy domesticated honeybee hives and bred bumblebee colonies
358 are desirable. It has been proposed that relatively clean commercial bumblebees may
359 actually dilute the natural occurrence of *Crithidia bombi* (Whitehorn et al., 2013). It is
360 clear that studies on viral dynamics within and between different pollinators
361 communities are needed to better understand the risks associated with allopatric and
362 sympatric transport of bees to determine if these transports could deteriorate the
363 endangered status of wild bees.

364

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369

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527

528

529 **Legend of figure**

530 Fig. 1.

531 The mean number of drones produced (\pm SE) and their mean mass (\pm SE) for Israeli
532 acute paralysis virus- and Kashmir bee virus-infected bumblebee micro-colonies
533 versus their control. Dicistroviruses represents the pooled data of both IAPV and
534 KBV infection.

535

Table 1: The number of micro-colonies with a regular and delayed time until oviposition (a), and with a without drone production (b).

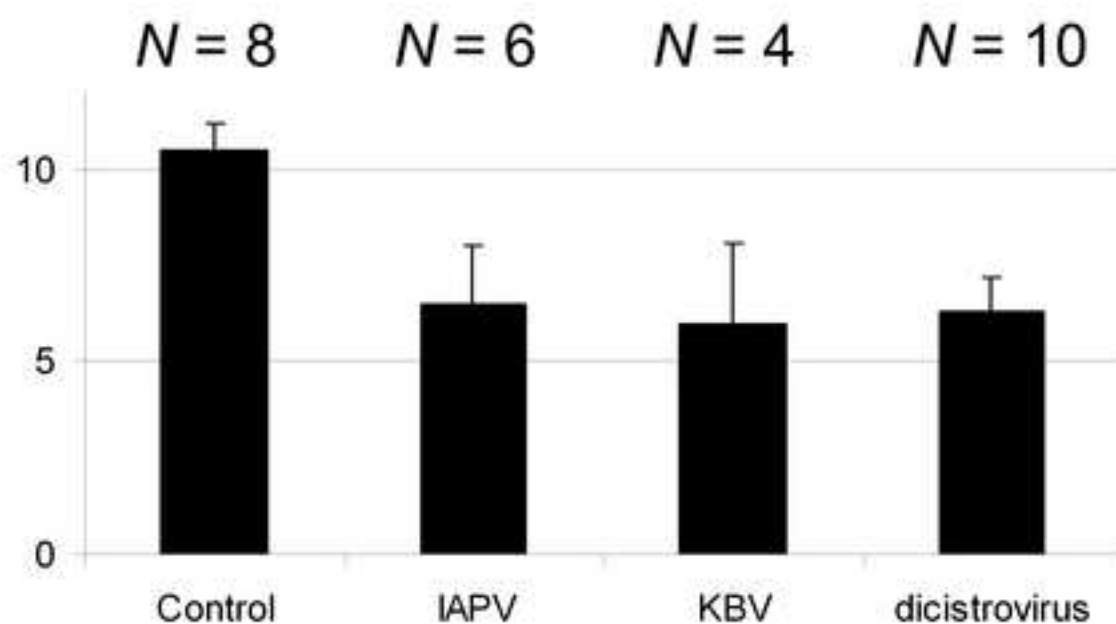
a)	The number of micro-colonies (mean oviposition day)			χ^2
	regular oviposition	delayed oviposition		
Control	8 (10.5)	2 (16.5)	Expected	
IAPV	6 (10.5)	4 (14)	Observed	$\chi^2 = 2.5, df = 1, P = 0.11$
KBV	4 (10.5)	6 (16.3)	Observed	$\chi^2 = 10, df = 1, P = 0.002$

b)	The number of micro-colonies			χ^2
	with drone production	without drone production		
Control	9	1	Expected	
IAPV	9	1	Observed	$\chi^2 = 0, df = 1, P = 1$
KBV	5	5	Observed	$\chi^2 = 17.778, df = 1, P < 0.001$

Figure1

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Mean number of drones per micro-colony (N)



Mean drone mass (mg) per micro-colony (N)

