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PII: S0022-1910(16)30027-0
DOI: http://dx.doi.org/10.1016/j.jinsphys.2016.03.004
Reference: IP 3489

To appear in: *Journal of Insect Physiology*

Received Date: 22 November 2015
Revised Date: 9 March 2016
Accepted Date: 11 March 2016

Please cite this article as: Chaimanee, V., Evans, J.D., Chen, Y., Jackson, C., Pettis, J.S., Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide coumaphos, *Journal of Insect Physiology* (2016), doi: http://dx.doi.org/10.1016/j.jinsphys.2016.03.004

Journal of Insect Physiology

Sperm viability and gene expression in honey bee queens (*Apis mellifera*) following exposure to the neonicotinoid insecticide imidacloprid and the organophosphate acaricide coumaphos

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Abstract

Honey bee population declines are of global concern. Numerous factors appear to cause these declines including parasites, pathogens, malnutrition and pesticides. Residues of the organophosphate acaricide coumaphos and the neonicotinoid insecticide imidacloprid, widely used to combat *Varroa* mites and for crop protection in agriculture, respectively, have been detected in wax, pollen and comb samples. Here, we assess the effects of these compounds at different doses on the viability of sperm stored in the honey bee queens' spermatheca. Our results demonstrate that sub-lethal doses of imidacloprid (0.02 ppm) decreased sperm viability by 50%, 7 days after treatment. Sperm viability was a downward trend (about 33%) in queens treated with high doses of coumaphos (100 ppm), but there was not significant difference. The expression of genes that are involved in development, immune responses and detoxification in honey bee queens and workers exposed to chemicals was measured by qPCR analysis. The data showed that expression levels of specific genes were triggered 1 day after treatment. The expression levels of P450 subfamily genes, *CYP306A1, CYP4G11* and *CYP6AS14* were decreased in honey bee queens treated with low doses of coumaphos (5 ppm) and imidacloprid (0.02 ppm). Moreover, these two compounds suppressed the expression of genes related to antioxidation, immunity and development in queens at day 1. Up-regulation of antioxidants by these compounds in worker bees was observed at day 1. Coumaphos also caused a repression of *CYP306A1* and *CYP4G11* in workers. Antioxidants appear to prevent chemical damage to honey bees. We also found that DWV replication increased in workers treated with imidacloprid. This research clearly demonstrates that chemical exposure can affect sperm viability in queen honey bees.

Keywords: Honey bee queen; *Apis mellifera;* Sperm viability; Imidacloprid; Coumaphos; Gene expression

1. Introduction

The Western honey bee, *Apis mellifera* is the most important managed pollinator of agricultural crops worldwide (Klein et al., 2007). Currently, populations of honey bees have declined in many regions of Europe and North America (vanEngelsdorp and Meixner, 2010). In the United States, high mortality rates of honey bee colonies have been reported since 2006 (vanEngelsdorp et al., 2007, 2008, 2010, 2011, 2012). Several factors have been suggested as contributing causes including parasitic mites, pathogens, poor nutrition and exposure to pesticides (Evans and Schwarz, 2011; Cornman et al., 2012). Also, a combination of these factors has been reported to weaken honey bees (Alaux et al., 2010; Vidau et al., 2011; Pettis et al., 2012, 2013; Wu et al., 2012). Of the many factors investigated, pesticide exposure has arguably received much consideration. Pesticide residues including fungicides, insecticides, herbicides and miticides have been detected in pollen and wax combs (Mullin et al., 2010; Wu et al., 2011). Exposure to sub-lethal levels of pesticides and miticides in honey bees can affect development, behavior, learning performance, sperm viability in drones and number of sperm and body weight in queens (Haarmann et al., 2002; Decourtye et al., 2004b; Burley et al., 2008; Yang et al., 2008; Wu et al., 2011; Collins and Pettis, 2013; Williams et al., 2015). Moreover, pesticide-exposed honey bees are more susceptible to infection by the microsporidia parasite *Nosema ceranae* (Aufauvre et al., 2012; Pettis et al., 2012, 2013; Wu et al., 2012).

Among the insecticides, the organophosphate coumaphos is used to control *Varroa* mites. While effective in killing *Varroa*, coumaphos exposure also causes negative effects on honey

bees. Sub-lethal doses of coumaphos impairs olfactory learning and memory, affects locomotion as well as grooming behavior in honey bees and reduces trophallaxis of honey bees (Bevk et al., 2011; Williamson et al., 2013a, 2013b). Drone sperm viability is also reduced after coumaphos exposure (Burley et al., 2008). The effects of coumaphos on queen development, body weight and sperm volume have also been reported but only during the rearing and early life of the queen (Haarmann et al., 2002; Collins et al., 2004; Pettis et al., 2004). However, the effects of miticide exposure on sperm viability in queens have not been investigated.

To select a candidate pesticide from agriculture, the newer systemic pesticides were considered. Of theses the neonicotinoid imidacloprid is a widely used systemic insecticide that acts on the nicotinic acetylcholine receptor to interfere with the nervous system (Matsuda et al., 2001). Imidacloprid is often applied as a systemic pesticide and can be present in all parts of a plant. Thus, honey bees could be exposed to this compound when foraging and collecting nectar and pollen. Several studies have been carried out under laboratory, semi-field and field conditions to investigate negative impacts of imidacloprid on honey bees. The studies showed that exposure to imidacloprid reduces memory and learning abilities, affects hypopharyngeal glands and the respiratory function of honey bees (Decourtye et al., 2003, 2004a, 2004b; Hatjina et al., 2013).

Although honey bees are exposed to a range of xenobiotics and pesticides, they have detoxification systems for metabolizing these substances into less toxic or non-toxic compounds. Cytochrome P450 monooxygenases (P450s) are the main detoxification enzymes which allow for tolerance and resistance to all classes of insecticides (Li et al., 2007). The mediation of pyrethroid insecticides by P450s has been reported (Johnson et al., 2006). Acaricides, tau-fluvalinate and coumaphos are detoxified by three CYP9Q3 enzymes (Mao et al., 2011). The

detoxification subfamilies CYP4, CYP6 and CYP9 of honey bee larvae are induced by imidacloprid at low doses (Derecka et al., 2013). Besides the detoxification enzymes, acaricides could also reduce the immune responses of honey bees (Boncristiani et al., 2012).

To investigate the effects of pesticides on sperm viability in honey bee queens, we analyzed the viability of sperm in the spermatheca of honey bee queens exposed to the widely used organophosphate coumaphos and the neonicotinoid imidacloprid. Although the effect of coumaphos on sperm viability in young honey bee queen has been reported, here, different doses of pesticides were tested to simulate exposure later in life as queens may live in contaminated hives. Additionally, we evaluated the effects of these pesticides on gene expression related to detoxification enzymes and immunity in queens and workers. Lastly, the expressions of antioxidant-related genes, which play important roles in protecting against oxidative damage, were measured in this study.

2. Materials and methods

2.1. Insects

Mated queens (*Apis mellifera ligustica*) were obtained from a commercial queen breeder in Hawaii, USA. They were shipped in wooden Benton cages with five to six attendant bees to the Bee Research Laboratory in Beltsville, Maryland, USA within two days from Hawaii in September 2013. Frames of capped brood were obtained from a single colony at the Bee Research Laboratory apiary, USDA in Beltsville, Maryland and adult bees were allowed to emerge in an incubator held at 34 ± 1ºC. Newly emerged bees were removed and divided into groups of 20 bees in plastic cup cages (Evans et al., 2009). Worker bees were provided with 1:1

sterile sugar-water with pollen and kept in an incubator at 30 ± 1ºC for 5 days before pesticide application.

2.2. Chemical application

2.2.1. Effect of chemicals on sperm viability in honey bee queens

Analytical grade chemicals coumaphos (purity 99.4 %) and imidacloprid (purity 99.9 %) were purchased from Sigma-Aldrich, Co. (St. Louis, MO). Two microliters of acetone containing pesticide were applied topically to the abdomen using a repeating dispenser (Eppendorf Repeater® plus Manual Dispenser). Groups of 6 queens were treated with the following doses: 5, 25, 50 and 100 ppm of coumaphos; 0.02, 0.1, 0.2 and 0.4 ppm of imidacloprid. Twelve queens were treated with 2 µl of acetone as controls. After treatment each queen was placed in a wooden Benton cage with attendant bees and held in an incubator at 30 ± 2 ºC for 7 days. Mortality was recorded daily. The spermatheca was removed 7 days after treatment, by dissecting live queens, and placed in a buffer solution (Moritz, 1984) for live/dead sperm count.

2.2.2. Gene expression in pesticide-treated queens and worker bees

Mated honey bee queens from a single queen breeder were randomly divided into groups of 18 and treated with pesticides in the same manner as described above. Then, queens were transferred to new wooden Benton cages with five attendant bees and kept at 30 ± 2 ºC. For worker bees, carbon dioxide was used for anesthetization before pesticide treatment and 1 µl of acetone containing the pesticide was applied on the abdomen of each 5-d-old bee. Bees were transferred into plastic cup cages held at 30 ± 2 ºC. Mortality was checked daily and any dead bees were removed from the cages. Pesticide treatment groups were 5 ppm of coumaphos; 0.02

ppm of imidacloprid, and acetone was used for the control group. After 24 hrs and 7 days following pesticide treatment, nine of queens and worker bees from each group were collected and immediately frozen in dry ice mixed with ethanol and then held at –80 ºC for subsequent molecular analysis.

2.3. Sperm viability

The semen in the spermatheca was released by puncturing the spermatheca with a needle, then compressing and macerating it with dissection scissors. The semen was mixed in 200 µL buffer and gently agitated and the spermathecal remains were removed. The diluted semen was stained with 1 µl SYBR-14 dye and 8 µl propidium iodide (Live/Dead Sperm Viability Kit, L-7011, Molecular Probes). Then, the diluted semen was incubated at room temperature for 15 min. Three drops (2 µl each) of dilution were separately placed on a clean slide and covered with 12 mm round cover slips. Live (green) and dead (red) cells were counted using a fluorescent light source with an isothiocyanate (FITC) filter (Axioskop 2 plus, Carl Zeiss). A hundred sperm cells were counted in each of the three drops to determine the percentage of sperm viability. The first one hundred sperm encountered were scored live or dead based on color and the observer changed the field to a new area once all sperm in a given field had been scored. A two button hand counter was used that chimed after 100 numbers had been recorded.

2.4. RNA extraction and cDNA synthesis

Total RNA was isolated from individual abdomens of queen and worker bees using Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. DNA was removed using DNAse I in an 11 µl reaction containing 8 µl (1.5 µg) total RNA, 10 U DNAse I

(Invitrogen) in appropriate buffer, 20 U RNAseout (Invitrogen), poly dT(12-18), random heptamer oligonucleotides, and 2 mM dNTP. The DNAse reaction was performed at 37 ºC for 1 h followed by 75 ºC for 10 min. Then, first-stand cDNA was synthesized by using 100U Superscript II Reverse Transcriptase (Invitrogen) and incubation at 42 ºC for 50 min followed by 15 min at 70 ºC. The cDNA was diluted 1:5 in nuclease free water (~100 ng/µl).

2.5. Quantitative real-time PCR

qPCR was performed in a 20 µl reaction mixture consisting of 1X SsoAdvanced™ SYBR® Green supermix (Bio-Rad), 0.2 µM of each primer, and 1µl (~100 ng) of cDNA template. The oligonucleotide primers for qPCR are shown in Table 1 (Evans, 2006; Simone et al., 2009; vanEngelsdorp et al., 2009; Li et al., 2010; Di Prisco et al., 2013). The reaction was carried out in 96-well plates using a Bio-Rad Icycler (Bio-Rad Crop., Hercules, CA.) programmed with the following temperature profile: 95 ºC for 30sec followed by 50 cycles of 95 ºC for 5 s, 60 ºC for 30 s, melt curve from 65–95 ºC in 0.5 ºC/5s increments. The melt curve dissociation was analyzed to confirm each amplicon.

Relative expression levels were calculated by the ΔC_T method. Threshold cycle (C_T) numbers for target genes were subtracted from the reference gene for each sample. Ribosomal protein subunit 5 was chosen as the reference gene and used for normalization. According to the primer efficiencies via serial dilutions of known templates, a low transcript level (~10 copies) was detected at 42 cycles (Simone et al., 2009; Cornman et al., 2012). Thus, a C_T value of 42 cycles was assigned for samples showing C_T value above 42.

2.6. Statistical analysis

The software JMP version 11.0 for Windows (SAS Institute Inc.) was used for statistical analysis. The effect of pesticides on sperm viability was analyzed by ANOVA because the data set conformed to a normal distribution. The non-parametric Steel-Dwass test was used to compare DWV titers. To compare the mean transcript levels between the pesticide treatment group and untreated control group, the parametric unpaired *t*-test was used to analyze if the data fitted a normal distribution. However, the non-parametric Wilcoxon's test was applied when the data sets were not normally distributed. The statistically significant difference was determined at $p \leq 0.05$.

3. Results

3.1. Effect of coumaphos and imidacloprid on sperm viability in honey bee queens

Honey bee queens were treated with different doses of the organophosphate coumaphos and the neonicotinoid imidacloprid. No queens died in any of the treatments over a 7 days period. Sperm viability in queens treated with different chemicals is shown in Fig. 1. Sperm viability was not significantly affected by coumaphos at concentrations of 5, 25 and 50 ppm (Fig. 1A). The mean sperm viability value was about 64-77%. However, there was a trend toward lower viability of spermatozoa (about 33%) in queens exposed to coumaphos with the highest doses (100 ppm), but this was not significant compared to control queens. In regard to the queen groups exposed to imidacloprid, sperm viability was significantly decreased in queens treated with all four doses of imidacloprid (0.02, 0.1, 0.2 and 0.4 ppm) (ANOVA, $p = 0.0038$) (Fig.1B). There was generally a 50% increase in dead sperm after 7 days of imidacloprid treatment. There was no significant difference in sperm viability with increased imidacloprid doses (0.1, 0.2 and 0.4 ppm) (ANOVA, $p > 0.05$).

3.2. Effect of pesticides on gene expression in honey bee queens

The gene expression in queen bees exposed to 5 ppm of coumaphos and 0.02 ppm of imidacloprid was measured. Low doses of coumaphos and imidacloprid caused down-regulation of P450 subfamily genes, antioxidant, immunity and development genes 1 day after treatment (Fig. 2). *CYP306A1* and *CYP4G11* levels were significantly decreased in coumaphos-treated queens (t-test, $p = 0.0054$ and $p = 0.0014$, respectively; Fig. 2A). Also, imidacloprid reduced *CYP306A1* expression (t-test, $p = 0.0097$) (Fig. 2C). The expression of genes encoding antioxidative enzymes *superoxide dismutase (SOD)* and *thioredoxin peroxidase* was significantly down-regulated by both pesticides (Fig. 2A, C). Immunity-related genes were affected by coumaphos and imidacloprid in the same pattern. Both pesticides suppressed levels of *apidaecin* and *AmeⅣLRR* expression in honey bee queens. By contrast, *eater* expression was elevated after exposure to tested pesticides (t-test, $p < 0.0001$) (Fig. 2A, C). Further, coumaphos and imidacloprid were found to have significantly negative impacts on development genes. Transcript levels of *vitellogenin* and *hexamerin 70b* were significantly lower in pesticides-treated queens in comparison to acetone-treated queens (Fig. 2A, C).

Gene expression in queens treated with low levels of coumaphos and imidacloprid was also monitored 7 days following treatment. The expression of *CYP4G11*, *CYP6AS14*, *thioredoxin peroxidase* and *AmeⅣLRR* were significantly suppressed in imidacloprid-treated queens (Fig. 2D). Coumaphos caused a reduction of *CYP4G11* and *AmeⅣLRR* (t-test, $p = 0.0017$ for *CYP4G11* and Wilcoxon's test, $p = 0.0004$ for *AmeⅣLRR*), but *catalase* was induced by coumaphos (t-test, $p = 0.0247$) (Fig. 2B). Lastly, *vitellogenin* and *hexamerin 70b* levels were decreased by pesticide exposure, 7 days following treatment (Fig. 2B, D).

3.3. Effect of pesticides on gene expression in worker honey bees

There were no significant effect on worker mortality 7 days post-treatment (percent survival = acetone 93.33%, imidacloprid 100% and coumaphos 80%). Worker bees treated with low concentrations of coumaphos (5 ppm) and imidacloprid (0.02 ppm) showed repression of the *CYP306A1and CYP4G11* transcripts at day 1, a significant difference was observed in coumaphos-exposed worker bees (t-test, $p = 0.00148$ for *CYP306A1* and $p = 0.0279$ for *CYP4G11*) (Fig. 3A). The result demonstrated that mRNA level of *CYP6AS14* was a significant up-regulation in neonicotinoid imidacloprid-treated worker bees (t-test, $p = 0.0081$) (Fig. 3C). The expression of antioxidant enzyme-coding genes, *catalase, superoxide dismutase (SOD)* and *thioredoxin peroxidase* were significantly increased in worker bees treated with 5 ppm of coumaphos and 0.02 ppm of imidacloprid when compared to acetone-treated control worker bees (Fig. 3A, C). In particular, *thioredoxin peroxidase* levels in imidacloprid-exposed worker bees were 4-fold higher than that in control, acetone-treated worker bees (t-test, $p < 0.0005$) (Fig. 3C). Also, the mRNA levels of *thioredoxin peroxidase* in workers exposed to coumaphos were 3.3 fold higher than that recorded for control worker bees (t-test, $p = 0.0009$) (Fig. 3A). The expression of all three immunity-related genes *apidaecin*, *hymenoptaecin* and *eater* in pesticide-treated workers did not significantly differ when compared to control acetone-treated bees. Negative impact of NF-κB activation was monitored by measuring the expression of *Amel/LRR*. The results showed that mRNA levels of *Amel/LRR* in workers treated with low concentrations of coumaphos and imidacloprid were significantly higher than those in the controls (t-test, $p = 0.0448$ for coumaphos and $p = 0.0002$ for imidacloprid) (Fig. 3A, C).

Low concentrations of coumaphos and imidacloprid did not affect mRNA levels of *CYP306A1, CYP4G11* and *CYP6AS14* at 7 days. Transcript levels of *eater* and *AmelΛLRR* were

significantly suppressed by both tested pesticides at seven days (Fig. 3B, D). Moreover, coumaphos reduced the expression of *thioredoxin peroxidase* and *hexamerin 70b* in worker bees at 7 days. (Fig. 3B)

3.4. Effect of pesticides on Deformed wing virus (DWV) titers in worker honey bees

The titers of DWV in worker bees increased after exposure to the neonicotinoid imidacloprid. There was a significant difference between imidacloprid-treated workers and acetone-treated control workers at 1 day. The highest levels of DWV were observed when worker bees were treated with imidacloprid at concentrations of 0.4 ppm (Steel-Dwass, $p = 0.0057$) (Fig. 4A). DWV titers were about 5-fold higher than those in control workers. Imidacloprid of 0.02 ppm also promoted DWV replication in workers (Steel-Dwass, $p = 0.017$) (Fig. 4A). Coumaphos did not affect DWV replication in bees after 7 days (Steel-Dwass, $p > 0.05$) (Fig. 4B).

4. Discussion

To determine the effect of imidacloprid and coumaphos on sperm viability, four different concentrations of each chemical were applied to honey bee queens. Our results indicate that 1/30 of the LD_{50} by contact of imidacloprid (0.02 ppm) can reduce sperm viability in queens by 50%. Although higher concentrations of imidacloprid were also used, the viability of spermatozoa was not significantly different at doses above 0.02 ppm indicating no dose-dependent response. Since, imidacloprid is a neonicotinoid insecticide which acts on the nicotinic acetylcholine receptor (nAChR) in the nervous system (Buckingham et al., 1997; Matsuda et al., 2001), we assume that the imidacloprid at the higher concentrations might affect the nervous system rather

than sperm viability of honey bee queens or simply be detoxified. Queens are likely to be exposed to imidacloprid through the food she is fed by workers (Dively and Kamel, 2012). However, it is possible that direct exposure could occur via wax. Previous studies have demonstrated that imidacloprid affects the behavior and physiology of honey bees. The effects of imidacloprid on honey bee behavior include the impairment of olfactory memory and learning performance (Decourtye et al., 2003; 2004a, 2004b; Yang et al., 2012) and abnormal foraging behavior (Colin et al., 2004; Yang et al., 2008). The negative effects on hypopharyngeal glands development and respiratory function of honey bees were also reported by exposure to imidacloprid at the sub-lethal doses (Hatjina et al., 2013). Apart from honey bees, bumble bees also are influenced by the neonicotinoid imidacloprid. Under laboratory and semi-field conditions, imidacloprid reduces bumble bee, *Bombus terrestris,* colony growth and queen production (Whitehorn et al., 2012), and Tasei et al. (2000) has been reported to affect the survival rate of *B. terrestris* workers.

Our results also showed that the viability of sperm was a downward trend (about 30 %) by coumaphos at $1/30^{th}$ of the LD_{50} by contact (100 ppm). Other research has shown that coumaphos-exposed queens had less than two million sperm as compared to normally reared queens which had more than two million sperm (Collins and Pettis, 2013). Also, queens exposed to coumaphos in the colony had significantly lower ovary and queen weights compared to control queens (Haarmann et al., 2002). In a California study, sperm number in queens was significantly decreased after coumaphos exposure (Haarmann et al., 2002). Also, coumaphos can affect developing queens (Collins et al., 2004; Pettis et al., 2004). Lastly, sub-lethal doses of coumaphos impairs olfactory learning and memory and affects the trophallaxis of honey bees (Williamson et al., 2013a; Bevk et al., 2011).

Our results about the metabolic detoxification of pesticides in queens and workers showed that the expression of *CYP306A1*, *CYP4G11* and *CYP6AS14* in both queens and workers was affected by imidacloprid and coumaphos at low concentrations. These results indicate that a small amount of pesticides can immediately trigger gene response. It is known that P450s can play an important role in insecticide resistance and the detoxification of natural products and xenobiotics (Feyereisen, 2006). A few genes in these superfamilies showed down-regulation in queens at 1 and 7 days after coumaphos and imidacloprid exposure. Although the cytochrome P450 gene tested in this study, *CYP306A1* is orthologous to genes involved in ecdysteroid biosysthesis (Claudianos et al., 2006; Niwa et al., 2004), it is possible that these two insecticide and acaricide may have indirect effect on *CYP306A1* gene expression. The mRNA levels of *CYP4G11* were also decreased by these chemicals, but the specific functions of *CYP4G11* have not been characterized in honey bees (Claudianos et al., 2006). Its functions in *A. cerana cerana*, may be involved in protecting honey bees from oxidative stresses (Shi et al., 2013). *CYP6AS14* expression was decreased in honey bee queens by imidacloprid 7 days after exposure and its role in worker bees might differ; *CYP6AS14* may play a role on detoxification (Boncristiani et al., 2012). Because workers forage outside the hive they would be expected to have a different immune response to xenobiotics as they are more likely to encounter these compounds than are queens that live within the hive and are fed processed glandular food by workers. The antioxidant-, and immunity-related genes in queens were down-regulated by coumaphos and imidacloprid treatments. These results suggest that honey bee queens do not effectively induce antioxidant and immunity in response to synthetic pesticides. It is known that immune response can have a cost at the colony level (Evans and Pettis, 2005) and the same tradeoffs are likely at the individual level in queens or workers. Possibly, pesticide components might even suppress

the metabolic pathways and immune response. Only the immune-related gene *eater* showed up-regulation after pesticide treatment. It has been shown that eater functions in the phagocytosis of bacterial pathogens in *Drosophila* (Ertürk-Hasdemir and Silverman, 2005; Kocks et al., 2005). Also, eater may play a role in the elimination of *Nosema ceranae* in honey bees (Chaimanee et al., 2014). However, there is no evidence that eater plays a role in pesticide detoxification. We assume that eater may function in cellular immunity in queens. The response of worker bees to pesticide exposure was quite different from honey bee queens in our studies. Some caution in interpretation of results between workers and queens is needed as workers were exposed to CO_2 while queens were not. Still, imidacloprid at low doses significantly induced *CYP6AS14* and antioxidant genes expression in worker bees at 1 day of treatment. The induction of antioxidant genes was also observed in workers when exposed to coumaphos. Although induction of *CYP6AS14* was observed by imidacloprid, *CYP306A1* and *CYP4G11,* which are genes in P450 subfamily, showed down-regulation in coumaphos-treated workers. The alteration of *CYP306A1* has been also shown by coumaphos exposure (Boncristiani et al., 2012). Mao et al. (2011) reported that CYP9Q enzymes detoxifies coumaphos and tau-fluvalinate. A recent study demonstrated that expression of *CYP6AS3, CYP6AS4* and *CYP9S1* in worker bees was significantly up-regulated by exposure to coumaphos and fluvalinate (Schmehl et al., 2014). CYP6 and CYP9 families are commonly involved in insecticide metabolism and resistance (Feyereisen, 2005). In this study, antioxidants seem to be the dominant components for protecting honey bees from insecticide and miticide damages.

Our results showed that the organophosphate coumaphos and the neonicotinoid imidacloprid had negative impacts on vitellogenin and hexamerin 70b in honey bee queens. Therefore, these two pesticides may cause a reduction in lifespan, reduced reproductive

capability and increased oxidative stress in honey bee queens. These developmentally- involved genes did not show a change in workers after pesticide exposure. Since, vitellogenin is a female-specific protein which is involved in the regulation of honey bee lifespan, the down-regulation of *vitellogenin* can result in a shortened lifespan in honey bees (Nelson et al., 2007). Earlier research established that vitellogenin protects honey bees from oxidative stress (Seehuus et al., 2006). As we know, hexamerins serve as storage proteins for development which function during metamorphosis (Telfer and Kunkel, 1991). They also serve as an amino acid source for gonad development and egg production (Martins et al., 2010). Thus, these issues might contribute to honey bee losses at the colony level if queens fail or have reduced life spans.

A recent study has demonstrated that the neonicotinoid clothianidin increased transcription of *AmeΛLRR* in honey bee larvae and lead to a decrease in *apidaecin* expression (Di Prisco et al., 2013). Consistently, our results demonstrate that *AmeΛLRR* levels were also up-regulated in honey bee workers after 1 day of coumaphos and imidacloprid exposure. This might cause the bee to be more susceptible to pesticides. However, down-regulation was found 7 days after exposure to both pesticides. In contrast, *AmeΛLRR* transcription was decreased in pesticide-treated queens at days 1 and 7. Leucine-rich repeat (LRR) proteins of insects have negative impacts on nuclear factor- κB (NF-κB) activation (Lich and Ting, 2007). According to our results, *AmeΛLRR* expression did not seem to correlate with the expression of the immunity-related genes *apidaecin* and *hymenoptaecin* in both honey bee queens and workers.

The study of Di Prisco and colleagues demonstrated that two neonicotinoids, clothianidin and imidacloprid promoted DWV replication in honey bees because NF- κB signaling is impaired by insecticides (Di Prisco et al., 2013). The induction of DWV proliferation did not occur with the organophosphate chlorpyriphos (Di Prisco et al., 2013). We assessed the effect of

two different pesticide classes, organophosphate and neonicotinoid on DWV replication. Although no negative effects on NF-κB activation was observed in this study, we found that DWV levels increased after imidacloprid exposure.

Acknowledgments

We thank Nathan Rice and Katie Joselow for their valuable help to conduct the experiments, I. Barton Smith for assistance with the honey bee queens, Dawn Lopez for providing technical and molecular assistance and Michele Hamilton for help with revisions of the manuscript. Funding was provided in part from the USDA-ARS Areawide Program to improve colony health.

References

Alaux, C., Brunet, J.L., Dussaubat, C., Mondet, F., Tchamitchan, S., Cousin, M., Brillard, J., Baldy, A., Belzunces, L.P., Le Conte, Y., 2010. Interactions between *Nosema* microspores and a neonicotinoid weaken honeybees (*Apis mellifera*). Environmental Microbiology 12, 774-782.

Aufauvre, J., Biron, D.G., Vidau, C., Fontbonne, R., Roudel, M., Diogon, M., Viguès, B., Belzunces, L.P., Delbac, F., Blot, N., 2012. Parasite-insecticide interactions: a case study of *Nosema ceranae* and fipronil synergy on honeybee. Scientific Reports 2, 326.

Bevk, D., Kralj, J., Čokl, A., 2011. Coumaphos affects food transfer between workers of honeybee *Apis mellifera*. Apidologie 43, 465-470.

Boncristiani, H., Underwood, R., Schwarz, R., Evans, J.D., Pettis, J., vanEngeldorp, D., 2012. Direct effect of acaricides on pathogen loads and gene expression levels in honey bees *Apis mellifera*. Journal of Insect Physiology 58, 613-620.

Buckingham, S.D., Lapied, B., Le Corronc, H., Grolleau, F., Sattelle, D.B., 1997. Imidacloprid actions on insect neuronal acetylcholine receptors. The Journal of Experimental Biology 200: 2685-2692.

Burley, L., Fell, R.D., Saacke, R.G., 2008. Survival of honey bee (Hymenoptera: Apidae) spermatozoa incubated at room temperature from drones exposed to miticides. Journal of Economic Entomology 101, 1081-1087.

Chaimanee, V., Chantawannakul, P., Chen, Y., Evans, J.D., Pettis, J.S., 2014. Effects of host age on susceptibility to infection and immune gene expression in honey bee queens (*Apis mellifera*) inoculated with *Nosema ceranae*. Apidologie 45, 451-463.

Claudianos, C., Ranson, H., Johnson, R.M., Biswas, S., Schuler, M.A., Berenbaum, M.R., Feyereisen, R., Oakeshott, J.G., 2006. A deficit of detoxification enzymes: pesticide sensitivity and environmental response in the honeybee. Insect Molecular Biology 15, 615-636.

Colin, M.E., Bonmatin, J.M., Moineau, I., Gaimon, C., Brun, S., Vermandere, J.P., 2004. A method to quantify and analyze the foraging activity of honey bees: relevance to the sublethal effects induced by systemic insecticides. Archives of Environmental Contamination and Toxicology 47, 387-395.

Collins, A.M., Pettis, J.S., 2013. Correlation of queen size and spermathecal contents and effects of miticide exposure during development. Apidologie 44, 351-356.

Collins, A.M., Pettis, J.S., Wilbanks, R., Feldlaufer, M.F., 2004. Performance of honey bee, *Apis mellifera*, queens reared in beeswax cells impregnated with coumaphos. Journal of Apicultural Research 43, 128-134.

Corman, R.S., Tarpy, D.R., Chen, Y., Jeffreys, L., Lopez, D., Pettis, J.S., vanEngelsdorp, D., Evans, J.D., 2012. Pathogen webs in collapsing honey bee colonies. PLoSOne 7, e43562.

Decourtye, A., Armengaud, C., Renou, M., Devillers, J., Cluzeau, S., Gauthier, M., Pham-Delègue, M.H., 2004a. Imidacloprid impairs memory and brain metabolism in the honeybee (*Apis mellifera* L.). Pesticide Biochemistry and Physiology 78, 83-92.

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M., Pham-Delegue, M.H., 2004b. Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. Ecotoxicoly and Environmental Safety 57, 410-419.

Decourtye, A., Lacassie, E., Pham-Delegue, M.H., 2003. Learning performances of honeybees (*Apis mellifera* L) are differentially affected by imidacloprid according to the season. Pest Management Science 59, 269-278.

Derecka, K., Blythe, M.J., Malla, S., Genereux, D.P., Guffanti, A., Pavan, P., Moles, A., Snart, C., Ryder, T., Ortori, C.A., Barrett, D.A., Schuster, E., Stöger, R., 2013. Transient exposure to low levels of insecticide affects metabolic networks of honeybee larvae. PlosONE 8, e68191.

Di Prisco, G., Cavaliere, V., Annoscia, D., Varricchio, P., Caprio, E., Nazzi, F., Gargiulo, G., Pennacchio, F., 2013. Neonicotinoid clothianidin adversely affects insect immunity and promotes replication of a viral pathogen in honey bees. Proceeding of the National Academy of Sciences of the United States of America 110, 18466-18471.

Dively, G.P., Kamel, A., 2012. Insecticide residues in pollen and nectar of a cucurbit crop and their potential exposure to pollinators. Journal of Agricultural and Food Chemistry 60, 4449-4456.

Ertürk-Hasdemir, D., Silverman, N., 2005. Eater: a big bite into phagocytosis. Cell 123, 190-192.

Evans, J.D., 2006. Beepath: an ordered quantitative-PCR array for exploring honey bee immunity and disease. Journal of Invertebrate Pathology 93, 135-139.

Evans, J.D., Chen, Y.P., Di Prisco, G., Pettis, J., Williams, V., 2009. Bee cups: single-use cages for honey bee experiments. Journal of Apicultural Research 48, 300-302.

Evans, J.D., Pettis, J.S., 2005. Colony-level impacts of immune responsiveness in honey bees, Apis mellifera. Evolution 59, 2270-2274.

Evans, J.D., Schwarz, R.S., 2011. Bees brought to their knees: microbes affecting honey bee health. Trends in Microbiology 19, 614-620.

Feyereisen, R., 2006. Evolution of insect P450. Biochemical Society Transactions 34, 1252-1255.

Feyereisen, R., 2005. Insect cytochrome P450. Comprehensive Molecular Insect Science 4, 1-77.

Haarmann, T., Spivak, M., Weaver, D., Weaver, B., Glenn, T., 2002. Effects of fluvalinate and coumaphos on queen honey bees (Hymenoptera: Apidae) in two commercial queen rearing operations. Journal of Economic Entomology 95, 28-35.

Hatjina, F., Papaefthimiou, C., Charistos, L., Dogaroglu, T., Bouga, M., Emmanouil, C., Arnold, G., 2013. Sublethal doses of imidacloprid decreased size of hypopharyngeal glands and respiratory rhythm of honeybees in vivo. Apidologie 44, 467-480.

Johnson, R.M., Wen, Z., Schuler, M.A., Berenbaum, M.R., 2006. Mediation of pyrethroid insecticide toxicity to honey bees (Hymenoptera: Apidae) by cytochrome P450 monooxygenases. Journal of Economic Entomology 99, 1046-1050.

Klein, A.M., Vaissiere, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., Tscharntke, T., 2007. Importance of pollinators in changing landscapes for world crops. Proceeding of the Royal Society B 274, 303-313.

Kocks, C., Cho, J.H., Nehme, N., Ulvila, J., Pearson, A.M., Meister, M., Strom, C., Conto, S.L., Hetru, C., Stuart, L.M., Stehle, T., Hoffmann, J.A., Reichhart, J.M., Ferrandon, D., Rämet, M., Ezekowitz, R.A.B., 2005. Eater, a transmembrane protein mediating phagocytosis of bacterial pathogens in *Drosophila*. Cell 123, 335-346.

Li, J., Wu, J., Begna Rundassa, D., Song, F., Zheng, A., Fang, Y., 2010. Differential protein expression in honeybee (*Apis mellifera* L.) larvae: underlying caste differentiation. PLoS One 5, e13455.

Li, X., Schuler, M.A., Berenbaum, M.R., 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. Annual Review of Entomology 52, 231-253.

Lich, J.D., Ting, J.P., 2007. CATERPILLER (NLR) family members as positive and negative regulators of inflammatory responses. Proceeding of the American Thoracic Society 4, 263-266.

Mao, W., Schuler, M.A., Berenbaum, M.R., 2011. CYP9Q-mediated detoxification of acaricides in the honey bee (*Apis mellifera*). Proceeding of the National Academy of Sciences of the United States of America 108: 12657-12662.

Martins, J.R., Nunes, F.M., Cristino, A.S., Simoes, Z.L., Bitondi, M.M., 2010. The four hexamerin genes in the honey bee: structure, molecular evolution and function deduced from expression patterns in queens, workers and drones. BMC Molecular Biology 11, 23.

Matsuda, K., Buckingham, S.D., Kleier, D., Rauh, J.J., Grauso, M., Sattelle, D.B., 2001. Neonicotinoids: insecticides acting on insect nicotinic acetylcholine receptors. Trends in Pharmacological Sciences 22, 573-580.

Moritz, R.F.A., 1984. The effect of different diluents on insemination success in the honeybee using mixed semen. Journal of Apicultural Research 23, 164-167.

Mullin, C.A., Frazier, M., Frazier, J.L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., Pettis, J.S., 2010. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. PLoS One 5, e9754.

Nelson, C.M., Ihle, K.E., Fondrk, M.K., Page, R.E.Jr., Amdam, G.V., 2007. The gene *vitellogenin* has multiple coordinating effects on social organization. PLoS Biology 5, e62.

Niwa, R., Matsuda, T., Yoshiyama, T., Namiki, T., Mita, K., Fujimoto, Y., Kataoka, H., 2004. CYP306A1, a cytochrome P450 enzyme, is essential for ecdysteroid biosynthesis in the prothoracic glands of Bombyx and Drosophila. The Journal of Biological Chemistry 279, 35942-35949.

Pettis, J.S., Collins, A.M., Wilbanks, R., Feldlaufer, M.F., 2004. Effects of coumaphos on queen rearing in the honey bee, *Apis mellifera*. Apidologie 35, 605-610.

Pettis, J.S., Lichtenberg, E.M., Andree, M., Stitzinger, J., Rose, R., 2013. Crop pollination exposes honey bees to pesticides which alters their susceptibility to the gut pathogen *N. ceranae*. PLoS One 8, e70182.

Pettis, J.S., vanEngelsdorp, D., Johnson, J., Dively, G., 2012. Pesticide exposure in honey bees results in increased levels of the gut pathogen *Nosema*. Naturwissenschaften 99, 153-158.

Schmehl, D.R., Teal, P.E.A., Frazier, J.L., Grozinger, C.M., 2014. Genomic analysis of the interaction between pesticide exposure and nutrition in honey bees (*Apis mellifera*). Journal of Insect Physiology 71, 177-190.

Seehuus, S.C., Norberg, K., Gimsa, U., Krekling, T., Amdam, G.V., 2006. Reproductive protein protects functionally sterile honey bee workers from oxidative stress. Proceeding of the National Academy of Sciences of the United States of America 103, 962-967.

Shi, W., Sun, J., Xu, B., Li, H., 2013. Molecular characterization and oxidative stress response of a cytochrome P450 gene (CYP4G11) from *Apis cerana cerana*. Zeitschrift für Naturforschung C 68, 509-521.

Simone, M., Evans, J.D., Spivak, M., 2009. Resin collection and social immunity in honey bees. Evolution 63, 3016-3022.

Tasei, J.N., Lerin, J., Ripault, G., 2000. Sub-lethal effects of imidacloprid on bumblebees, *Bombus terrestris* (Hymenoptera: Apidae), during a laboratory feeding test. Pest Management Science 56, 784-788.

Telfer, W.H., Kunkel, J.G., 1991. The function and evolution of insect storage hexamers. Annual Review Entomology 36, 205-208.

vanEngelsdorp, D., Caron, D., Hayes, J., Underwood, R., Henson, M., Rennich, K., Spleen, A., Andree, M., Synder, R., Lee, K., Roccasecca, K., Wilson, M., Wilkes, J., Lengerich, E., Pettis, J., 2012. A national survey of managed honey bee 2010-11 winter colony losses in the USA: results from the bee informed partnership. Journal of Apicultural Research 51, 115-124.

vanEngelsdorp, D., Evans, J.D., Saegerman, C., Mullin, C., Haubruge, E., Nguyen, B.K., Frazier, M., Frazier, J., Cox-Foster, D., Chen, Y., Underwood, R., Tarpy, D.R., Pettis, J.S., 2009. Colony collapse disorder: A descriptive study. Plos One 4, e6481.

vanEngelsdorp, D., Hayes, J.Jr., Underwood, R.M., Caron, D., Pettis, J., 2011. A survey of managed honey bee colony losses in the USA, fall 2009 to winter 2010. Journal of Apicultural Research 50, 1-10.

vanEngelsdorp, D., Hayes, J.Jr., Underwood, R.M., Pettis, J., 2008. A survey of honey bee colony losses in the U.S., fall 2007 to spring 2008. PLoS One 3, e4071.

vanEngelsdorp, D., Hayes, J.Jr., Underwood, R.M., Pettis, J.S., 2010. A survey of honey bee colony losses in the United States, fall 2008 to spring 2009. Journal of Apicultural Research, 7-14.

vanEngelsdorp, D., Meixner, M.D., 2010. A historical review of managed honey bee populations in Europe and the United States and the factors that may affect them. Journal of Invertebrate Pathology 103, S80-95.

vanEngelsdorp, D., Underwood, R., Caron, D., Hayes, J.Jr., 2007. An estimate of managed colony losses in the winter of 2006-2007. American Bee Journal 147, 599-603.

Vidau, C., Diogon, M., Aufauvre, J., Fontbonne, R., Vigues, B., Brunet, J.L., Texier, C., Biron, D.G., Blot, N., Alaoui, H.E., Belzunces, L.P., Delbac, F., 2011. Exposure to sublethal doses of fipronil and thiacloprid highly increases mortality of honeybees previously infected by *Nosema ceranae*. PLoS One 6, e21550.

Whitehorn, P.R., O'Connor, S., Wackers, F.L., Goulson, D., 2012. Neonicotinoid pesticide reduces bumble bee colony growth and queen production. Science 336, 351-352.

Williams, G.R., Troxler, A., Retschnig, G., Roth, K., Yañez, O., Shutler, D., Neumann, P., Gauthier, L., 2015. Neonicotinoid pesticides severely affect honey bee queens. Scientific Reports 5, 14621.

Williamson, S.M., Baker, D.D., Wright, G.A., 2013a. Acute exposure to a sublethal dose of imidacloprid and coumaphos enhances olfactory learning and memory in the honeybee *Apis mellifera*. Invertebrate Neuroscience 13, 63-70.

Williamson, S.M., Moffat, C., Gomersall, M.A., Saranzewa, N., Connolly, C.N., Wright, G.A., 2013b. Exposure to acetylcholinesterase inhibitors alters the physiology and motor function of honeybees. Frontiers in Physiology 4, 13.

Wu, J.Y., Anelli, C.M., Sheppard, W.S., 2011. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (*Apis mellifera*) development and longevity. PLoS One 6, e14720.

Wu, J.Y., Smart, M.D., Anelli, C.M., Sheppard, W.S., 2012. Honey bees (*Apis mellifera*) reared in brood combs containing high levels of pesticide residues exhibit increased susceptibility to *Nosema* (Microsporidia) infection. Jounal of Invertebrate Pathology 109, 326-329.

Yang, E.C., Chuang, Y.C., Chen, Y.L., Chang, L.H., 2008. Abnormal foraging behavior induced by sublethal dosage of imidacloprid in honey bee (Hymenoptera: Apidae). Journal of Economic Entomology 101, 1743-1748.

Yang, E.C., Chang, H.C., Wu, W.Y., Chen, Y.W., 2012. Impaired olfactory associative behavior of honeybee workers due to contamination of imidacloprid in the larval stage. PLoS One 7, e49472.

Figure Legends

Fig. 1. The viability of sperm from honey bee queens after exposure to the pesticides coumaphos and imidacloprid. Letters indicate statistically significant differences (ANOVA, $p > 0.05$).

Fig. 2. Differential gene expression in honey bee queens exposed to sub-lethal doses of pesticides. Gene expression in queens treated with 5 ppm of coumaphos at A) 1 day and B) 7 days after treatment. Gene expression in queens treated with 0.02 ppm of imidacloprid at C) 1 day and D) 7 days after treatment. Asterisks indicate significant differences, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ (t-test and Wilcoxon's test).

Fig. 3. Differential gene expression in honey bee workers exposed to sub-lethal doses of pesticides. Gene expression in workers treated with 5 ppm of coumaphos at A) 1 day and B) 7 days after treatment. Gene expression in workers treated with 0.02 ppm of imidacloprid at C) 1 day and D) 7 days after treatment. Asterisks indicate significant differences, *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$(t-test and Wilcoxon's test).

Fig. 4. Effect of sub-lethal doses of pesticides imidacloprid (A) and coumaphos (B) on DWV titers in infected honey bee workers. Vertical bars with different letters show statistically significant differences (Steel-Dwass test).

Table 1 Oligonucleotide sequences of qPCR used in this study.

Gene	Forward Sequence (5'-3')	Reverse Sequence (5'-3')	Reference
Ribosomal protein S5	AATTATTTGGTCGCTGGAATTG	TAACGTCCAGCAGAATGTGGTA	Evan (2006)
Cytochrome P450 306A1	CGTCGATGGGAAGGATAAAA	TCGGTGAAATATCCCGATTC	
Cytochrome P450 4G11	CAAAATGGTGTTCTCCTTACCG	ATGGCAACCCATCACTGC	
Cytochrome P450 6AS14	TGAAACTCATGACCGAGACG	AAAATTTGGGCCGCTAATAAA	
Catalase	GTCTTGGCCCAAACAATCTG	CATTCTCTAGGCCCACCAAA	
Superoxide dismutase	TACCAATTCCGTGAAGGTCA	TTGAAATGTGCACCAGCACT	
Hymenoptaecin	CTCTTCTGTGCCGTTGCATA	GCGTCTCCTGTCATTCCATT	
Hexamerin 70b	AACTCGCTCAACTTTCCACAA	GGCTCACATAACTAACCTCACC	
Apidaecin type 22	TTTTGCCTTAGCAATTCTTGTTG	GTAGGTCGAGTAGGCGGATCT	
NimC1, Eater-like	CATTTGCCAACCTGTTTGT	ATCCATTGGTGCAATTTGG	
Thioredoxin peroxidase 1	CAGCATCTACTGATTCTCACTTC	AGACCACGAAATGGAACTCC	Li et al. (2010)
Leucine-rich repeat-containing protein 16A-like	CTTGGTGAAGGCCTTGATG	ATGCAAAGAGCTATCATCA	Di Prisco et al. (2013)
Vitellogenin	AGTTCCGACCGACGACGA	TTCCCTCCCACGGAGTCC	Simone et al. (2009)
Deformed wing virus	GAGATTGAAGCGCATGAACA	TGAATTCAGTGTCGCCCATA	VanEngelsdorp et al. (2009)

Fig. 1. The viability of sperm from honey bee queens after exposure to the pesticides (A) coumaphos and (B) imidacloprid. Letters indicate statistically significant differences (ANOVA, $p > 0.05$).

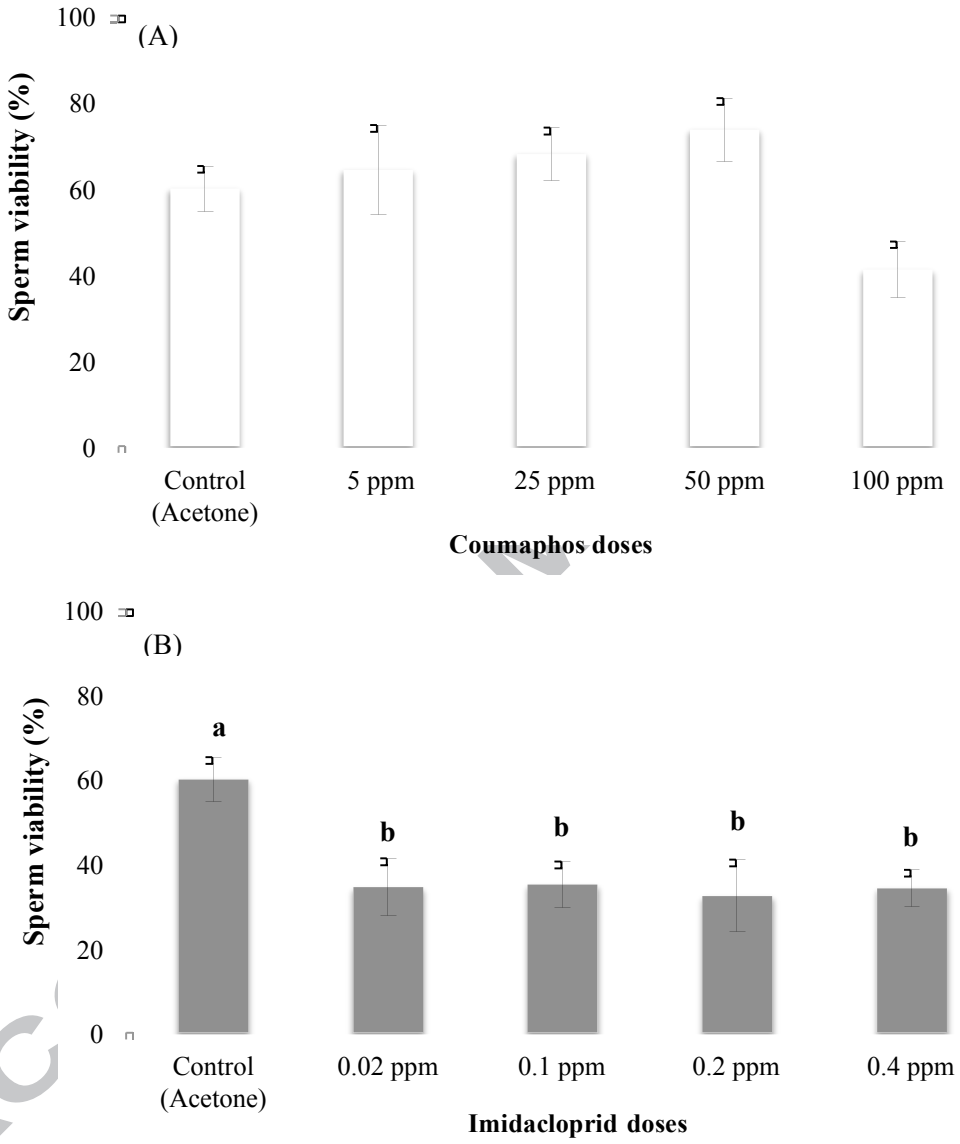


Fig. 2. Differential gene expression in honey bee queens exposed to sub-lethal doses of pesticides. Gene expression in queens treated with 5 ppm of coumaphos at A) 1 day and B) 7 days after treatment. Gene expression in queens treated with 0.02 ppm of imidacloprid at C) 1 day and D) 7 days after treatment. Asterisks indicate significant differences, *p < 0.05, **p < 0.01 and ***p < 0.001 (t-test and Wilcoxon's test).

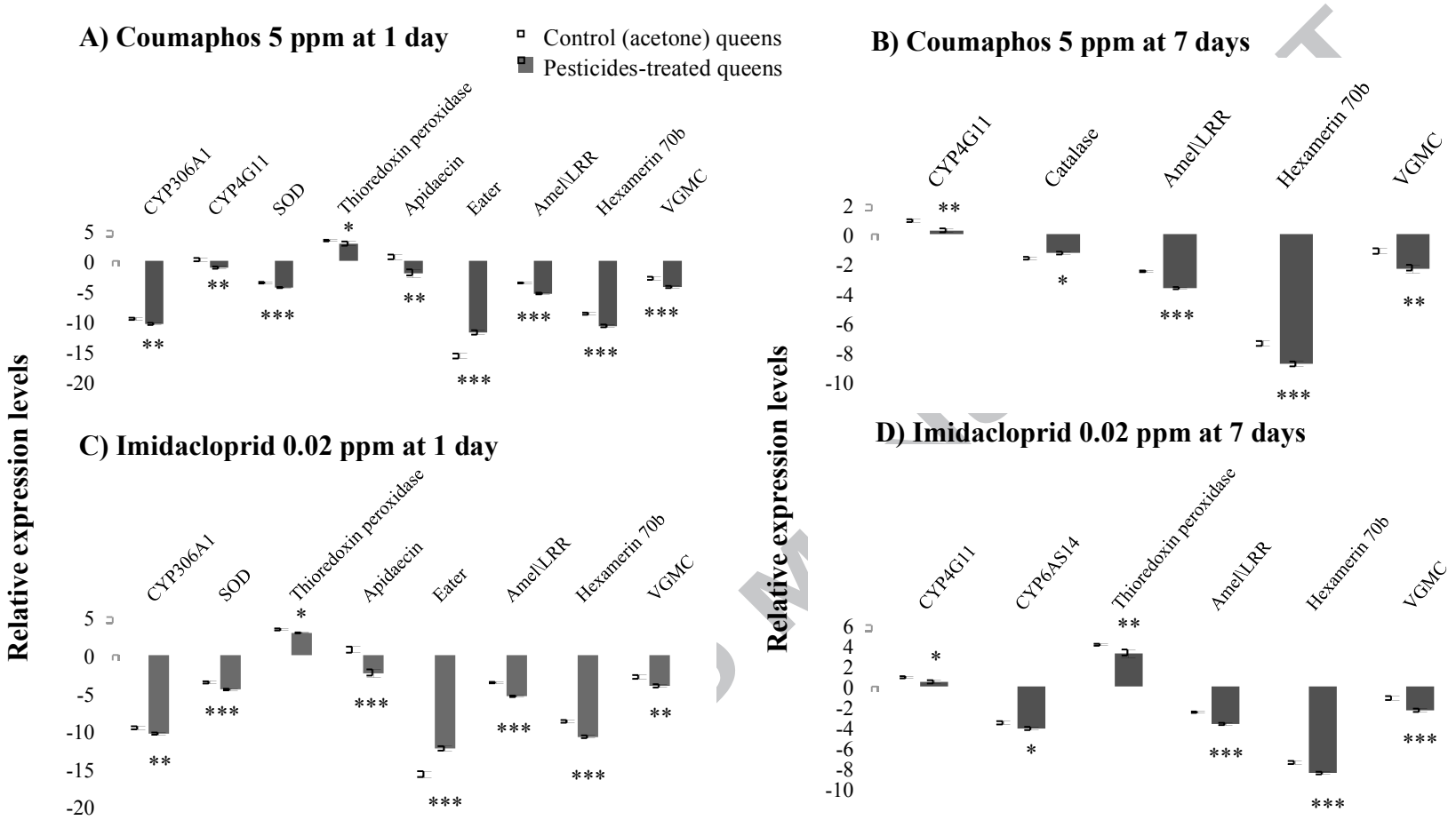


Fig. 3. Differential gene expression in honey bee workers exposed to sub-lethal doses of pesticides. Gene expression in workers treated with 5 ppm of coumaphos at A) 1 day and B) 7 days after treatment. Gene expression in workers treated with 0.02 ppm of imidacloprid at C) 1 day and D) 7 days after treatment. Asterisks indicate significant differences, *p < 0.05, **p < 0.01 and ***p < 0.001(t-test and Wilcoxon's test).

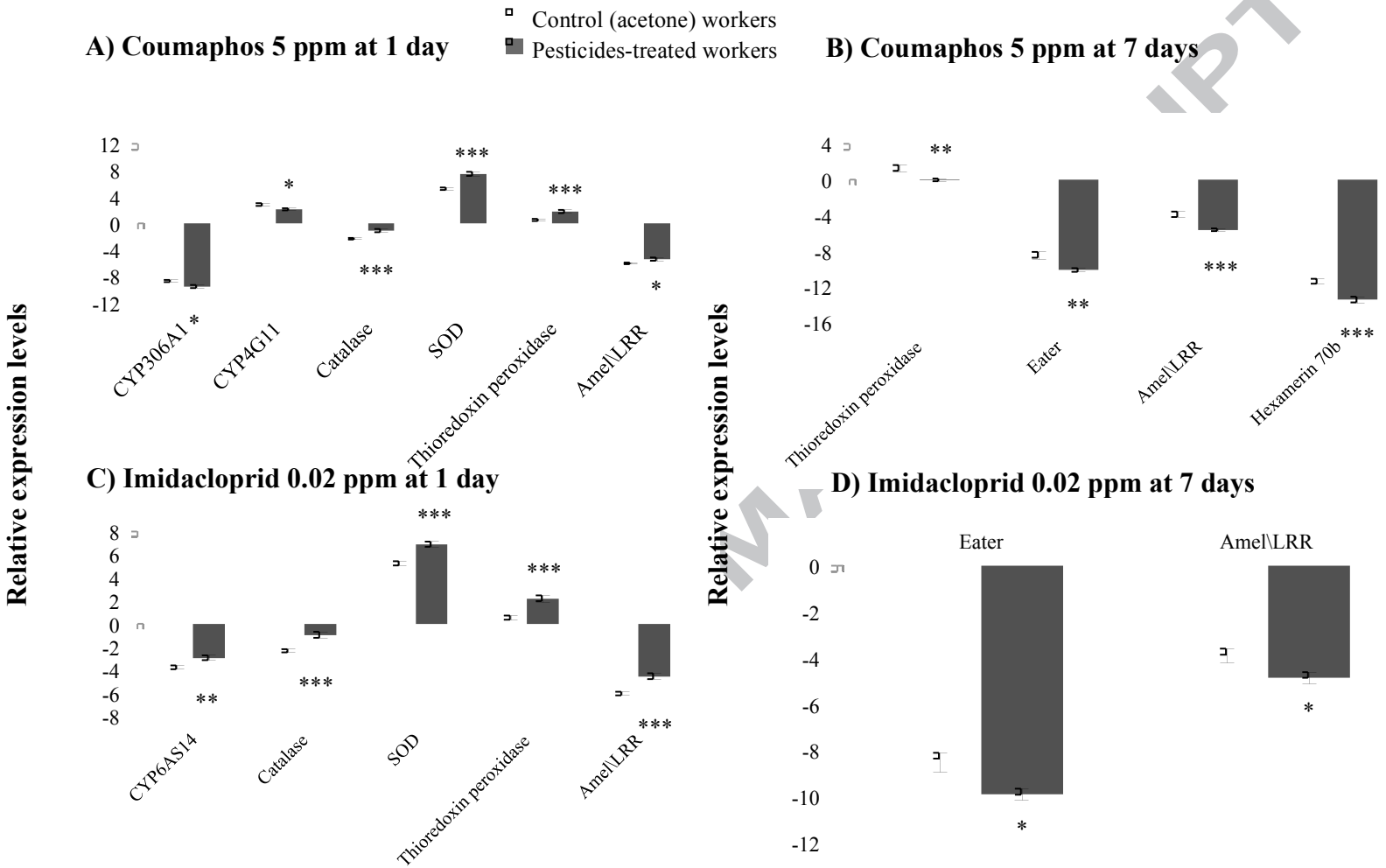
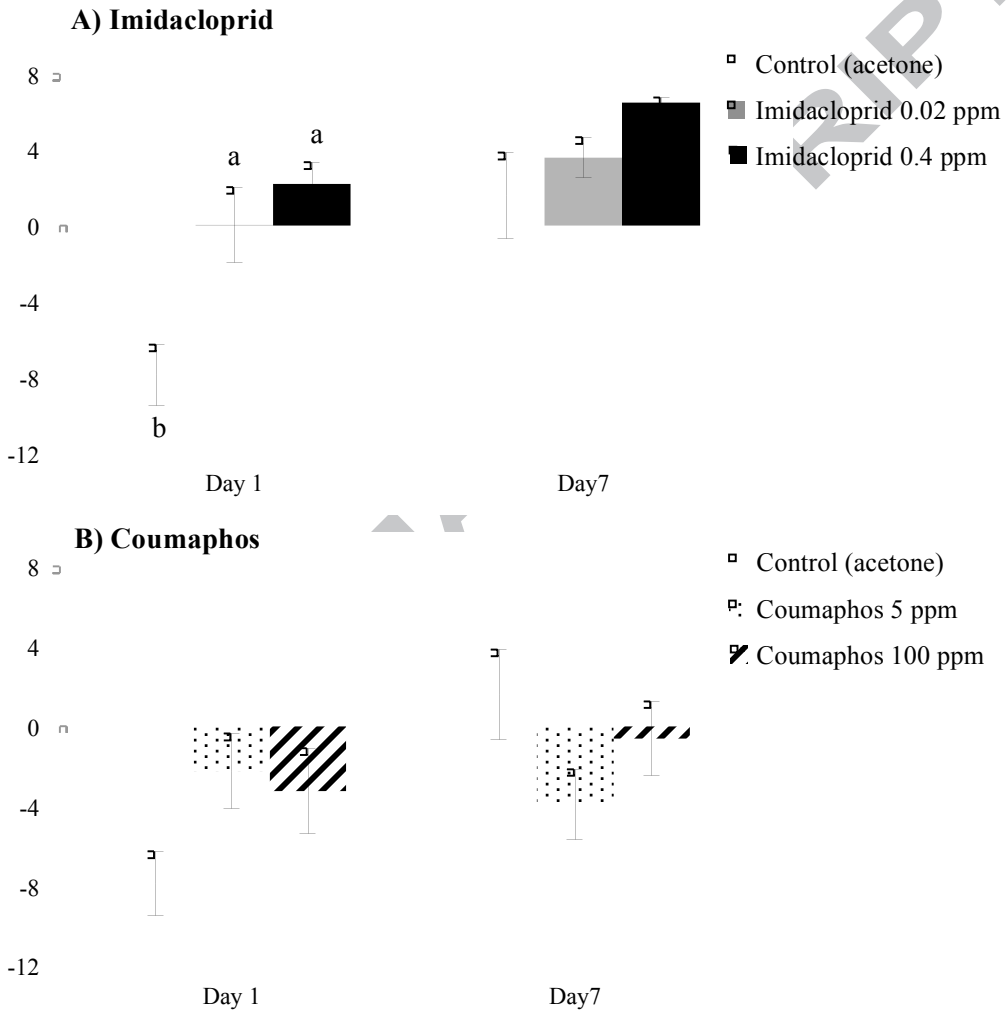


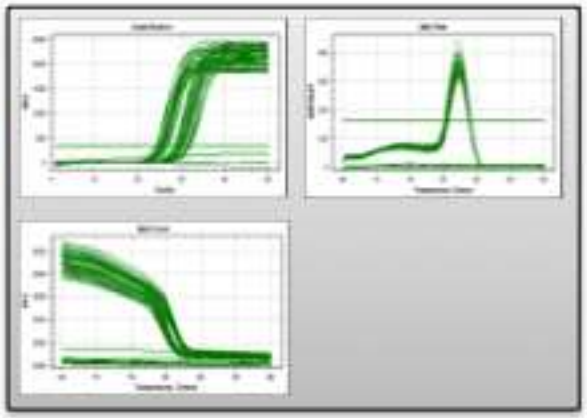
Fig. 4. Effect of sub-lethal doses of pesticides imidacloprid (A) and coumaphos (B) on DWV titers in infected honey bee workers. Vertical bars with different letters show statistically significant differences (Steel-Dwass test).



Honey bee queen

Treated with imidacloprid and coumaphos

Live/Dead Sperm Viability



Gene Expression

Highlights

- All doses of imidacloprid decreased sperm viability in queens by 50%, after 7 days.
- Sperm viability was reduced ca. 33% in queens treated with coumaphos (100 ppm).
- P450 subfamily genes decreased in pesticide-treated queens.
- Both compounds suppressed the expression of antioxidant genes.
- Immunity and development gene activity was decreased by both compounds.