Accepted in Aquatic Toxicology on 18 September 2019

<https://doi.org/10.1016/j.aquatox.2019.105310>

Resistance to a chemical pesticide increases vulnerability to a biopesticide: effects on direct mortality and mortality by predation

Vienna Delnat^{1,a,*}, Tam T. Tran^{1,2,b}, Lizanne Janssens^{1,c} and Robby Stoks^{1,d}

¹Evolutionary Stress Ecology and Ecotoxicology, University of Leuven, Belgium

² Institute of Aquaculture, Nha Trang University, Khanh Hoa, Viet Nam

^a vienna.delnat@kuleuven.be

^b tamtt@ntu.edu.vn

^c lizanne.janssens@kuleuven.be

^d robby.stoks@kuleuven.be

* Corresponding author: Vienna Delnat, Charles Deberiotstraat 32, box 2439, 3000 Leuven, Belgium, tel. +32 16 37 65 27, vienna.delnat@kuleuven.be

Conflict of Interest: none

Funding: This work was supported by research grants from the KU Leuven (C16/17/002) and from the Fund for Scientific Research Flanders (FWO) (G.0943.15).

Abstract: Pesticide mixtures are increasingly used to fight pest species that developed resistance to pesticides. To assess the pesticide control efficiency and to reduce ecological damage to non-target species, it is important to quantify the effect of these mixtures and compare them with the effect of their single pesticides on pest species, non-target species and their predator-prey interactions. We studied the effects of the chemical pesticide chlorpyrifos (CPF), the biopesticide *Bacillus thuringiensis israelensis* (Bti) and their mixture both on the direct mortality and on the mortality by predation. We focused on larvae of a CPF-resistant and a non-resistant strain of the vector mosquito *Culex quinquefasciatus* and its predator, the pygmy backswimmer *Plea minutissima*. In the CPF-Bti mixture, both pesticides interacted antagonistically for direct mortality. Exposure to the mixture caused equal direct mortality and equal mortality by predation in both strains. As expected, exposure to CPF resulted in less direct mortality and less mortality by predation in the CPF-resistant mosquito strain compared to the non-resistant strain. Notably, Bti caused a higher mortality in the mosquito larvae of the CPF-resistant strain compared to the non-resistant strain. Furthermore, the predator killed more mosquito larvae of the resistant strain compared to the non-resistant strain when exposed before to Bti alone. These observations identify a novel cost of resistance to a chemical pesticide in terms of increased vulnerability to a biopesticide.

Keywords: antipredator behavior; integrated pest management (IPM); mixture toxicity; pesticide resistance; predator-prey interactions; vector control

1. INTRODUCTION

The intensive use of single chemical pesticides is resulting in a widespread and rapidly increasing pesticide resistance both in target and in non-target populations (Bass and Jones, 2018; Borel, 2017; Jansen et al., 2011; Liu, 2015; Raymond et al., 2001). One recommended strategy to counter this build-up of resistance in target populations is the use of pesticide mixtures (Bass and Jones, 2018; Bourguet et al., 2013). Such mixtures will be especially efficient when the mixture contains a pesticide to which the resistant strains are more sensitive than the non-resistant strains. This specific potential cost of resistance has, however, been rarely documented (Bird, 2017; Cui et al., 2018; Heim et al., 2018). Another advantage of pesticide mixtures is the possible reduction of the ecological damage to non-target species that would be expected when the dose of the pesticides could be lowered due to possible synergistic effects of the pesticides in the mixture (Pimentel and Burgess, 2014). Such ecological damage is expected to be lower when a chemical pesticide is combined with a biopesticide specifically targeting the pest species (Chattopadhyay et al., 2004; Mansour et al., 2012).

Another recommended strategy to avoid and deal with pesticide resistance in target species is the combined use of pesticides and natural control by predation. This strategy is part of

Abbreviations: AChE – acetylcholinesterase; Bti - *Bacillus thuringiensis israelensis*, CPF - chlorpyrifos, Df – degrees of freedom, glmer (lmer) – generalized (general) linear mixed model, IA – Independent Action model, IPM - Integrated pest management, L1 – first instar mosquito larvae, L2 – second instar mosquito larvae, LC_{10,48h} – lethal concentration where 10% of the population is killed after 48 hours, OECD - Organisation for Economic Co-operation and Development, SE – standard error, UPLC MS/MS - Ultra performance liquid chromatography - tandem mass spectrometer, WHOPES – World Health Organization Pesticide Evaluation Scheme

integrated pest management (IPM) which uses various tools and tactics to increase the efficiency and sustainability of pest control programs (Bukhari et al., 2013; Cloyd, 2012; Thomas et al., 2012; Wilkie et al., 2019). Biological control by predation may be especially efficient when resistant pest strains are more vulnerable to predation as has been documented for some vector species (e.g. Berticat et al., 2004; Chan and Zairi, 2013; Xu et al., 2017; but see Berticat et al., 2004). A widely acknowledged challenge is that the use of chemical pesticides with a broad-spectrum mode-of-action may interfere with biological control through negatively affecting the non-target predators that can act as natural control agents (Gutiérrez et al., 2017b; Reid et al., 2012; Relyea and Hoverman, 2008). Notably, pesticides might also impair antipredator traits (Tran et al., 2016). This will also influence how pesticides shape the outcome of the interactions between the pest species and their predators (Tran et al., 2016). Thus, pesticides that control pest populations, but that also have a low impact on their predators are required (Gutiérrez et al., 2017a). Also here, the use of taxon-specific biopesticides that target the pest species but do not affect the predator may be promising (Gutiérrez et al., 2017a; McNitt et al., 1995). In general, how the use of pesticide mixtures affects biological control by predation is poorly studied (but see Cloyd, 2011).

One group of pest species for which the widespread build-up of resistance is alarming, are vector mosquitoes (Liu, 2015; Raymond et al., 2001). By transmitting diseases to humans and wildlife they pose a strong health risk (Guarner and Hale, 2019; Huang et al., 2019; Institute of Medicine, 2008). Both the use of pesticide mixtures, and the use of biological control with predators are widely used in larval mosquito control programs (Benelli et al., 2016; Imbahale et al., 2012; Thomas, 2018) and in pesticide resistance management (Bass and Jones, 2018; Bourguet et al., 2013; Liu, 2015). Yet, the potential interference between both strategies remains

to be studied (but see Cloyd, 2011). While chemical pesticides are still mainly used to fight mosquitoes, taxon-specific biopesticides are getting more attention (Kamareddine, 2012; Raghavendra et al., 2011; Senthil-Nathan, 2015). One widely used biopesticide, especially in Europe, is the gram positive bacteria *Bacillus thuringiensis israelensis* (Bti) (e.g. Duchet et al., 2014; Land and Miljand, 2014; von Hirsch and Becker, 2009). Bti has been assumed to have a low ecological impact because of its high specificity (Lagadic et al., 2016; but see Boisvert and Boisvert, 2000). Bti forms spores that contain toxic proteins that specifically kill larvae of mosquitoes and blackflies (Becker et al., 2010). Bti has been successfully incorporated in IPM programs. For example, the number of late instar larvae of the vector mosquito *Anopheles gambiae* showed a strong decrease in man-made habitats when drainage as source reduction, Bti application and the presence of the predatory fish *Gambusia affinis* were combined (Imbahale et al., 2012).

We studied the effects of the biopesticide Bti, the chemical pesticide chlorpyrifos (CPF) and their mixture on the direct mortality and mortality by predation of larvae of a CPF-resistant and a non-resistant mosquito strain. We explicitly tested whether the direct mortality of the mosquito larvae caused by the CPF-Bti mixture was synergistic. We evaluated costs of resistance to CPF in a pesticide-free environment and when exposed to Bti, and this both in terms of direct mortality and mortality caused by predation. To explain differences in predation rates we scored effects of the pesticides on two key antipredator traits of the mosquito larvae: freezing time and escape swimming speed. Both of the chosen (bio)pesticides, Bti and CPF, are recommended for vector control of mosquito larvae by WHOPES (2016). CPF inhibits acetylcholinesterase (Eaton et al., 2008), which causes muscle spasms and eventually death (Gupta, 2011). We studied effects in the Southern house mosquito (*Culex quinquefasciatus*, Say 1823) since it is the target

of major control programs. This species is an important vector of viruses such as the Saint Louis encephalitis virus (Richards et al., 2009) and the West Nile virus (Richards et al., 2010), and can induce large economic and social costs (Barrett, 2014). We compared the non-resistant S-Lab strain and the organophosphate-resistant Ace-1R strain. A single amino-acid substitution that protects the enzyme acetylcholinesterase (AChE) from being disabled by organophosphates causes the resistance in the Ace-1R strain (Weill et al., 2003). Importantly, both strains only differ in this point mutation and share the same genetic background as a result of back crossing (e.g. Alout et al., 2016; Berticat et al., 2008, 2004, 2002). Therefore, any phenotypic differences between these strains can be directly and causally linked to the resistance allele, hence to resistance (e.g., Agnew et al., 2004; Berticat et al., 2008, 2004, 2002; Duron et al., 2006). Resistance against CPF in mosquitoes in the field is widespread and might be caused both directly due to CPF used in vector management, but also indirectly due to run-off from CPF use in the agriculture (Shin et al., 2012; Toma et al., 2011). As predator of the mosquito larvae, we used the pygmy backswimmer *Plea minutissima*. Mosquito larvae of the Ace-1R strain have been shown to be more susceptible to predation by *P. minutissima* in a pesticide-free environment than those of the S-Lab strain (Berticat et al., 2004). We here extend this study to another, largely unexplored cost of resistance in terms of direct mortality and mortality by predation in the presence of Bti and the CPF-Bti mixture.

2. MATERIALS AND METHODS

2.1. Study animals and rearing

No permits or animal care protocols by the Animal Ethics Committee of the University of Leuven were required for this experiment since the study organisms are invertebrates.

The two strains of *C. quinquefasciatus* were acquired from a continuous culture maintained at the University Montpellier II (France). Prior to the experiment, both strains were kept in the laboratory in Leuven for >10 generations. Adults were maintained in cages (30 cm × 30 cm × 30 cm) within a climate chamber (24 °C, 70% relative humidity, L:D 14:10) and were fed with a 6% glucose solution (D-Glucose anhydrous; Sigma-Aldrich, St. Louis, Missouri, USA). Additionally, the adult females were given at least once a week defibrinated sheep blood (Thermo Fisher Scientific) that was warmed to 37 °C using a heating block. Within each cage a plastic cup filled with 100 mL aerated tap water was provided for oviposition. To start up the experiment, two egg clutches (<24h old) were transferred to each plastic cup filled with 100 mL aerated tap water. These cups were covered with netting (mesh size: 1 mm) and were kept in the same climate chamber as the culture.

The pygmy backswimmer *P. minutissima* is an important predator of crustaceans and small insects, including mosquito larvae, in shallow ponds in the Palearctic where they can be very abundant (Laird, 1988; Papacek, 2001; Vandekerkhove et al., 2012). It readily feeds on first (L1) and second (L2) instar *C. pipiens* larvae in the laboratory (Berticat et al., 2004). The similar-sized *P. leachi* is able to catch up to 20 mosquito larvae per day (Becker et al., 2010). After capturing a prey, *P. minutissima* ingests the contents by injecting a digestive saliva into the prey bodies where after the external skeleton of the empty prey remains. Adults (ca. 2-3 mm in size) were collected at a pond in Neerijse, Belgium (50°49'22.7''N 4°35'38.8''E). Predators were individually kept in plastic cups filled with aerated tap water in an incubator at a temperature of 20.48 °C (SE: ± 0.04 °C) and a light:dark regime of 14h:10h. The pygmy backswimmers were fed daily with nauplii of *Artemia* (mean ± SE: 220 ± 85 nauplii; n = 17 daily food portions counted).

2.2. General experimental procedure

In a first step, the mosquito larvae and their predators were separately exposed for 48h to one of the four pesticide treatment combinations: solvent control, CPF alone, Bti alone and the CPF-Bti mixture (Figure 1). This resulted in a full factorial design of 2 CPF treatments (absence vs presence) $\times$ 2 Bti treatments (absence vs presence). In a second step, predation trials were run in the absence of pesticides where sets of mosquito larvae were combined for 48h with single predators at the pesticide treatment combination to which both prey and predators had been exposed to during the first step. To better interpret the results of the predation trials, extra sets of mosquito larvae were exposed to the pesticide treatment combinations where we tested for (i) delayed mortality after the exposure period in the absence of pesticides and predators (see 2.6), and (ii) effects on two key antipredator behaviours of mosquito larvae (see 2.7 and 2.8).

Mosquito hatchlings (<24h old, L1) were exposed to their pesticide treatment in groups of 20 in 210 mL glass vials filled with 100 mL medium for 48 hours. The medium was refreshed every 24h. The number of replicate vials per strain ranged from 37 to 43 for each pesticide treatment combination (total of 6,440 mosquitoes). Exact replicate numbers are shown in Figure 2. Mortality was checked daily. The amount of food was adjusted daily according to the number of surviving larvae to keep the food level constant per larva (0.300 mg/day/larva). This avoided possible effects of density. Mortality was quantified until the end of the 48-hour pesticide exposure period and was expressed as the total percentage of mosquito larvae that died

After the 48-hour pesticide exposure period, the surviving larvae of each strain entered the predation trials (see 2.4). The number of replicate predation trials ranged from 13 to 15 for each pesticide treatment combination (total of 2,280 mosquitoes). Exact replicate numbers are shown in Figure 3. During the pesticide exposure period and the predation trials, animals were placed in

an incubator at 23.94 °C (SE: ± 0.01 °C) and with a 14:10 h light:dark regime. The temperature during the experiment was measured continuously every 15 min with temperature loggers (Onset HOBOWare v3.7.12).

2.3. (Bio)pesticide concentrations

The commercial Bti formulation Vectobac (WG, 37.4% Bti at 3000 ITU/mg) was purchased at Technic 3D (Paris, France). Chlorpyrifos (CPF) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA; purity grade $\geq 99\%$).

To ensure that possible synergistic interactions on mortality could be studied, we selected nominal concentrations of Bti and CPF that individually induced approximately 10% mortality after 48 hours ($LC_{10,48h}$). Based on a range finding experiment, this was 0.5 $\mu\text{g/L}$ for Bti and 0.15 $\mu\text{g/L}$ for CPF (see Appendix A). These concentrations are similar to or lower than the recommended dose to control mosquito larvae which are 25-150 $\mu\text{g/L}$ for Bti and 2.2-5 $\mu\text{g/L}$ for CPF (WHOPES, 2016). These doses were based on a water depth of 0.5 m, matching the shallow water bodies preferred by *C. quinquefasciatus* (Becker et al., 2010). A stock solution of 100 $\mu\text{g/mL}$ CPF was dissolved in ethanol absolute and was stored in the dark at 4 °C. A second stock solution of 1 $\mu\text{g/mL}$ was made from this first stock solution in Milli-Q water. Pooled water samples from three exposure vials of the CPF treatments and the CPF-Bti treatments were taken at the start and after 24h before the next renewal of the medium. The CPF samples were analysed using UPLC-MS/MS with Triple Quadrupole Mass Spectrometry at the Division of Soil and Water Management at the KU Leuven. The initial CPF concentration in the CPF treatment was 0.061 ± 0.009 $\mu\text{g/L}$ (mean $\pm$ SE, $N = 3$) and in the CPF-Bti treatment was 0.070 ± 0.000 $\mu\text{g/L}$ ($N = 3$). The CPF concentration after 24 hours was 0.017 ± 0.001 $\mu\text{g/L}$ ($N = 3$) in the CPF treatment and 0.021 ± 0.006 $\mu\text{g/L}$ ($N = 3$) in the CPF-Bti treatment. A daily stock solution of 1000 $\mu\text{g/mL}$

Vectobac dissolved in Milli-Q water was used to prepare the Bti solution. A second stock solution of 10 µg/mL was made from this first stock solution in Milli-Q water.

Only a solvent control was used since all solutions (including the Bti solution for the homogeneity of ethanol exposure) contained 1.5 µL ethanol/L. This is ca. 66 times lower compared to the maximum allowed solvent concentration recommended by the OECD (100 µL/L; OECD, 2011) and ca. 8 times lower than the lowest reported NOEC for aquatic invertebrates (12.23 µL/L; United Nations Environment Program, 2004).

2.4. Predation trials

Prior to the predation trials, the pygmy backswimmers were also individually exposed for 48 hours to the same pesticide treatment combinations and concentrations as the mosquito larvae. This increases realism since both prey and predator likely experience the same pesticide exposure in natural ponds. The pygmy backswimmers were exposed in 210 mL glass vials filled with 100 mL medium in the same incubator set at 24°C as the mosquito larvae. Note that by doing so they were acclimated to 24°C for 48 hours before the start of the predation trials. During the last 24 hours of the 48-hour pesticide exposure period, the pygmy backswimmers were starved to equalize hunger levels.

Each predation trial was conducted in a white 2 L plastic container (180 mm x 133 mm x 121 mm) filled with 1 L of aerated tap water. Per predation trial, 20 resistant Ace-1R and 20 non-resistant S-Lab mosquito larvae from the same pesticide treatment combination were placed together in a container. In each trial, larvae were used from two exposure vials per mosquito strain (10 larvae from each vial). After 10 minutes of acclimation of the 40 mosquito larvae in the containers, one pygmy backswimmer was introduced. The 2 L-containers were placed in the same incubator set at 24°C as used in the pesticide exposure period. Mortality within each

2 L-container was checked after 18, 21, 24, 42, 45 and 48 hours after the introduction of the predator. When ca. 50% of the mosquito larvae were killed by the predator (mean $\pm$ SE: 20 ± 3 larvae), the predation trial was stopped. This percentage was chosen to allow detecting a difference in the proportion of each strain that survived (see 2.5). There was no significant difference between pesticide treatments in the number of mosquito larvae killed when the predation trial was stopped and in the duration of the time to reach 50% killing (ca. 32.6 hours, SE: 1.6 hours, $N = 57$ predation trials) (see Appendix B).

2.5. Differentiation between strains

To determine the strain identity of the survivors of the predation trials, all survivors were exposed to 0.5 μ g/L CPF solution for 24 hours based on a pilot study. This CPF exposure causes 100% mortality in the non-resistant S-Lab strain and no mortality in the organophosphate-resistant Ace-1R strain. Thus, surviving larvae could be considered as being of the resistant Ace-1R strain, while larvae that died during this 24-hour CPF exposure as being of the non-resistant S-Lab strain.

2.6. Delayed mortality

We assessed whether some of the mortality during the predation trials could be due to delayed mortality caused by previous pesticide exposure. For this, we determined the delayed mortality 24 and 48 hours after the end of the 48h exposure period in the absence of pesticides and predators. Per trial, 20 mosquitoes of the same strain and pesticide treatment combination were combined in a white 2 L plastic container filled with 1 L aerated tap water. Sets of 20 larvae were obtained by grouping five larvae from four extra exposure vials of the same strain. For each strain and pesticide treatment combination, the number of replicates ranged from 3 to 10

containers for delayed mortality (total of 1,080 mosquitoes; exact replicate numbers are shown in Table C.1 in Appendix C).

2.7. Freezing behaviour

As important mosquito antipredator behaviour we scored the freezing behaviour, this is the time a larva remains motionless after a simulated predator encounter (Awasthi et al., 2015; Ferrari et al., 2008). Prey with shorter freezing times have been shown to be more vulnerable to predation (Debecker and Stoks, 2019). After the 48-hour pesticide exposure period, the freezing behaviour was determined on these larvae based on the protocol of Reynaldi et al. (2011). A white 2 L plastic container was filled with 750 mL of aerated tap water. Mosquito larvae were allowed to acclimatize for 5 minutes in the container with lights on. During this period, they settled on the bottom of the container and were actively moving. After the acclimation period, the light was turned off for 5 seconds to simulate a predator approach from above which caused the larvae to freeze immediately to avoid detection. We determined the latency time as the freezing duration of the individual larvae following the end of the simulated predator approach (i.e. when the light was turned on again). When the larvae had still not moved after 10 minutes, they were given the maximum value of 600 seconds. Only 24 larvae (2.5 %) got this maximum value. For each strain and pesticide treatment combination, the number of replicates ranged from 9 to 31 vials for the freezing behaviour (with five larvae per vial, total of 869 mosquitoes). Exact replicate numbers are shown in Figure 4a.

2.8. Escape swimming speed

When attacked, mosquito larvae can avoid predation by escape swimming (Awasthi et al., 2015; Kikuchi and Mochizuki, 2010). After the 48-hour pesticide exposure period, the escape swimming speed was determined based on a protocol of Stoks and McPeck (2006) and Delnat et

al. (2017). A single larva was set in a plastic Petri dish filled with aerated tap water at 24 °C. After a 30 second acclimation period, an attack was simulated by gently tapping a mosquito larva with an Ent needle. The resulting swimming burst was recorded using a camera (Basler scout SCA 780-54FM) with a 12.5 mm C-mount Lens (Ricoh FL-BC1214D-VG) connected to StreamPix v7. Images of the swimming burst movies (33.5 fps) were extracted using VirtualDub v1.10.4 and analysed using ImageJ v1.48 (Schneider et al., 2012) with the plugin MTrackJ v1.5.0 (Meijering et al., 2012). The escape swimming speed was measured based on the first 10 frames. We videotaped the escape speeds of three swimming trials of a subset of larvae. The repeatability of the escape swimming speed was 0.43 ± 0.08 (repeatability $\pm$ SE, $P < 0.001$) and was calculated following Nakagawa and Schielzeth (2010) using the package rptR v0.9-22 (Stoffel et al., 2017). The estimated repeatability is in line with the average repeatability of behavioural traits across animal taxa (0.37, review of Bell et al., 2009). For each strain and pesticide treatment combination, the number of replicates ranged from 9 to 31 vials (with three larvae per vial, total of 490 mosquitoes). Exact replicate numbers are shown in Figure 4b.

2.9. Statistical analyses

All statistical analyses were performed in R v3.5.1 (R Development Core Team, 2017). The package lme4 v1.1-21 (Bates et al., 2015) was used to run the generalized/general linear mixed models (glmer and lmer), car v3.0-3 (Fox and Weisberg, 2018) was applied to run Analysis of variance (Anova), afex v0.24-1 (Singmann et al., 2017) was used to set effect coding since the model included two- and three-way interactions and the design was slightly unbalanced, emmeans v1.3-5.1 (Lenth, 2016) was applied to perform the contrasts and to extract the emmeans with standard errors for the figures, and drc v3.0.1 (Ritz et al., 2015) was used to construct the dose-response curve.

Effects of the mosquito strain, Bti and CPF exposure and their interactions on mortality (0 = alive, 1 = dead) during and after (delayed mortality) the 48 h pesticide exposure period were analysed using generalized linear mixed models with a binomial error structure and the logit link. Vial/container was added as a random factor to correct for pseudoreplication. The start date of the exposure period or the predation trial were also added as random factor. When interaction effects between Bti, CPF and/or strain were detected, contrasts with false discovery rate (fdr) correction were used to identify which treatment combinations differed.

Effects of Bti and CPF exposure on the proportion of the Ace-1R strain over the S-Lab strain that survived the predation trials were analysed using separate one-sided Wilcoxon signed rank tests for each pesticide treatment. The observed proportions were tested one-sided against a median value of 1, which indicates that there is no difference between the number of killed larvae from the Ace-1R and S-Lab strains. On the one hand, we expected that the S-Lab strain would perform better, hence had a relative higher survival than the Ace-1R strain (median value < 1), in the control and the Bti treatments due to a cost of resistance of the Ace-1R strain (Kliot and Ghanim, 2012). On the other hand, we expected that the Ace-1R strain would have a higher relative survival than the S-Lab strain (median value > 1) in the CPF and mixture treatments since the Ace-1R should not suffer from the exposure to CPF (Weill et al., 2003).

Effects of the mosquito strain, CPF and Bti exposure and their interactions on the freezing behaviour and escape swimming speed were analysed separately using general linear mixed models. Vial was added as random factor to correct for pseudoreplication. We also added the date of the behavioural trials as random factor in the models. Freezing behaviour was transformed using a Box-Cox transformation with a power of 0.122 to meet the assumption of normality. To specifically test whether the CPF-Bti mixture differed from the single pesticide

exposures and solvent control, we tested a set of a priori contrasts for both strains. When interaction effects between Bti, CPF exposure and/or strain were detected, contrasts with false discovery rate (fdr) correction were used to identify which treatment combinations differed.

To specifically identify the interaction type between both pesticides (Bti and CPF) for mortality during the 48h pesticide exposure period we used the independent action model as advocated by Schäfer and Piggott (2018). Note that linear models cannot be used for this because they may falsely identify antagonistic interaction effects between two lethal stressors since they use an additive null model. As a result, linear models do not account for the probability that an individual cannot be killed by two stressors simultaneously (Côté et al., 2016; Schäfer and Piggott, 2018). The use of the independent action (IA) model is further advised when both chemicals have a different mode of action (Bliss, 1939; Faust et al., 2000; Schäfer and Piggott, 2018). The interaction type was determined per strain since organophosphate-resistance may change the interaction type. We applied the model following the procedure detailed in Coors and De Meester (Coors & De Meester, 2008). First, the equation $E_i = \frac{(e_i - e_{control})}{(e_{max} - e_{control})}$ was used to convert the observed absolute mortality effects imposed by the single pesticides to proportional effects. Hereby, e_i is the single-pesticide effect in absolute units, $e_{control}$ is the effect of the pesticide-free control in absolute units, and e_{max} is the maximum possible effect of that single-pesticide which is 100 for mortality. Second, the predicted joint effects were calculated using the equation $E_{mix} = 1 - \prod_i (1 - E_i)$. Finally, the predicted joint effect E_{mix} was back-transformed to absolute units to allow comparison with the observed joint effects using the equation $e_{mix} = E_{mix} \times (e_{max} - e_{control}) + e_{control}$. If the predicted effect is not within the 95% confidence interval of the observed effect in absolute units, the observed effect is significantly different from the predicted joint additive effect by the model. An antagonistic interaction is indicated by a significantly

weaker observed joint effect compared to the predicted joint effect, while a synergistic interaction is indicated by a significantly stronger observed joint effect.

3. RESULTS

3.1. Mortality caused by the pesticides during and after the 48h exposure period

Both the nominal doses of 0.5 µg/L Bti and 0.15 µg/L CPF increased the mortality after 48 hours with ca. 12% in the non-resistant S-Lab strain (Strain × Bti, Strain × CPF, Table 1, Figure 2). In the organophosphate resistant Ace-1R strain, CPF did not increase the mortality compared to the solvent control (Contrast: $P = 0.23$, Strain × CPF, Table 1). Bti caused a ca. 4% higher mortality in the Ace-1R strain compared to the S-Lab strain (Contrast: $P = 0.047$, Strain × Bti, Table 1, Figure 2). In the solvent control and in the CPF-Bti mixture, both strains did not differ in mortality (Contrasts: both P -values ≥ 0.10 , Strain × Bti × CPF, Table 1, Figure 2). The mortality caused by the CPF-Bti mixture did not differ from single CPF or single Bti exposure in the S-Lab strain (Contrasts: both P -values ≥ 0.23), and was even lower compared to the single Bti exposure in the Ace-1R strain (Contrasts: $P = 0.032$). This was confirmed by the independent action models which identified the interaction type between CPF and Bti for mortality to be antagonistic for both the S-Lab strain (Predicted mean when additive effect = 20.75%; 95% CI observed effect = [8.81, 13.05]) and the Ace-1R strain (Predicted mean = 17.49%; 95% CI = [11.00, 14.59]).

Delayed mortality in the absence of a predator during the 48 hours after the pesticide exposure period was very low in both strains (on average 2.66%). It was not affected by any of the pesticide treatments (Table 1, all P -values ≥ 0.10).

3.2. Predation trials

The proportion of mosquito larvae of the Ace-1R and S-Lab strains that survived predation by *P. minutissima* did not differ from equality when mosquitoes were previously exposed to the solvent control (Wilcoxon: $V = 22$, $P = 0.31$, Figure 3) or to the CPF-Bti mixture (Wilcoxon: $V = 40$, $P = 0.66$, Figure 3). When exposed before to Bti alone, *P. minutissima* killed more mosquito larvae of the Ace-1R strain compared to the S-Lab strain (Wilcoxon: $V = 11$, $P = 0.015$, Figure 3). When exposed before to CPF alone, *P. minutissima* killed more mosquitoes of the S-Lab strain compared to the Ace-1R strain (Wilcoxon: $V = 81.5$, $P = 0.036$, Figure 3).

3.3. Freezing behaviour after a simulated predator encounter

The freezing time of the Ace-1R strain increased when exposed to Bti compared to the control treatment (Contrast: $P < 0.017$), while there was no difference in the freezing behaviour of the S-Lab strain between Bti exposure and the control (Strain $\times$ Bti, Table 2, Figure 4a, Contrast: $P = 0.75$). As a result, the Ace-1R strain had a longer freezing time compared to the S-Lab strain when exposed to Bti (Contrast: $P < 0.014$), while there was no difference in the solvent control (Contrast: $P = 0.75$). Exposure to CPF in general increased the freezing time in both strains (Table 2). For both strains, the freezing time did not differ when exposed to the CPF-Bti mixture compared to when exposed to CPF alone (a priori contrasts: both P -values ≥ 0.16), but it tended to increase compared to the solvent control (S-Lab: $P = 0.062$, Ace-1R: $P = 0.020$). The freezing time of the S-Lab strain increased when exposed to the CPF-Bti mixture compared to when exposed to Bti alone (a priori contrast: $P = 0.010$), while there was no difference in the freezing time of the Ace-1R strain ($P = 0.25$). There was no significant Bti $\times$ CPF or Strain $\times$ Bti $\times$ CPF interaction for freezing behaviour (Table 2).

3.4. Escape swimming speed after a simulated predator attack

Exposure to CPF reduced the escape swimming speed in the S-Lab strain (Contrast: $P = 0.017$), but not in the Ace-1R strain (Contrast: $P = 0.76$). This was supported by a trend for a Strain $\times$ CPF interaction ($P = 0.056$, Table 2, Figure 4b). As a result, the Ace-1R strain had a higher escape swimming speed than the S-Lab strain when exposed to CPF (Contrast: $P = 0.0087$), while both strains did not differ in the solvent control (Contrast: $P = 0.37$). There was no effect of Bti exposure on the escape speed (Table 2). The escape swimming speed for both strains did not differ when exposed to the CPF-Bti mixture compared to when exposed to CPF alone or to Bti alone (a priori contrasts: all P -values ≥ 0.38). There was also no significant Bti $\times$ CPF or Strain $\times$ Bti $\times$ CPF interaction for escape swimming speed (Table 2).

4. DISCUSSION

4.1. Mortality by pesticide exposure

In contrast to the general idea of a cost of resistance in a pesticide-free environment (Kliot and Ghanim, 2012), there was no difference in mortality between the non-resistant S-Lab strain and the organophosphate resistant Ace-1R strain in the solvent control. Yet, a cost of resistance became visible when the mosquito larvae were exposed to Bti alone. Indeed, the resistant strain did have a higher mortality (ca. 16%) compared to the non-resistant strain (ca. 12%). Note that because the genetic background of the two strains are identical except for the point mutation associated with the single amino-acid substitution which causes the resistance, this can indeed be directly interpreted as a cost of resistance. A possible explanation for the higher sensitivity to Bti in the resistant strain is its shorter gut transfer time, meaning that the rate of ingestion and digestion of food is faster which may influence the toxicity of Bti (Agnew et al., 2004; Becker et al., 2010). When exposed to 0.15 $\mu\text{g/L}$ CPF, the resistant strain was as expected not affected while the non-resistant strain had a mortality of ca. 12% (Weill et al., 2003). The strain-specific

sensitivities to Bti and to CPF apparently were balanced so that both strains did not differ in mortality when exposed to the CPF-Bti mixture.

We observed an antagonistic interaction between Bti and CPF in both strains. This antagonistic interaction is in contrast with the additive effects for mortality in *Culex pipiens* between the organophosphate malathion and Bti (Mansour et al., 2012), and between CPF and Bti at a constant temperature of 20°C (Delnat et al., 2019). The different interaction effect between the pesticides in the study by Delnat et al. (2019) compared to the current study might be explained by the following three differences: (i) a higher mean temperature was used in the current experiment (24°C instead of 20°C in the previous study) which can influence the degradation rates, (ii) a difference in the pesticide exposure regime with in the current experiment two pesticide renewals instead of only one which influences the time that both pesticides are simultaneously present, and (iii) a difference in the presence of food whereby in the current experiment *ad libitum* food was given thereby stimulating filter feeding which is the main uptake mechanism of Bti instead of no food in the previous study. Our finding also contrasts with the emerging view that the most common interaction type in pesticide mixtures is an additive effect (Cedergreen, 2014; Kamo and Yokomizo, 2015; Rizzati et al., 2016; Rodea-Palomares et al., 2015). Nevertheless, there are other examples of antagonistic effects between chlorpyrifos and for example indoxacarb (Attique et al., 2006), atrazine (Yang et al., 2015), cypermethrin or deltamethrin (Ahmad et al., 2009). General mechanisms that might explain antagonistic interaction effects are that one mixture component lowers the bioavailability (for example by complexation) or decreases the uptake rate of the other component, or that enzymatic competitive inhibition occurs between both components (Hernández et al., 2017; Reffstrup et al., 2010; Tsui et al., 2005). In our study, a possible explanation for the antagonistic interaction for

mortality could be that CPF decreased larval food intake resulting in less Bti being consumed meaning that the uptake rate decreased. Reductions in food intake under organophosphate exposure (including CPF) have been observed in other aquatic animals such as damselfly larvae (e.g. Dinh Van et al., 2014) and tadpoles (e.g. Gurushankara et al., 2007).

No mortality occurred in the pygmy backswimmers after pesticide exposure with CPF and Bti applied alone and as mixture. This was as expected since Bti is highly species specific (Becker et al., 2010) and the used concentration of CPF was 17.5 times lower compared to the $LC_{50,48h}$ of *P. minutissima* (Rubach et al., 2011).

4.2. Mortality by predation

There was no difference in the number of mosquito larvae killed by the pygmy backswimmer *P. minutissima* between both strains in the solvent control. In contrast, Berticat et al. (2004) documented in the absence of pesticides a higher predation of the resistant Ace-1R strain by the same predator as well as by the diving beetle *Guignotus pusillus* and the water measurer *Hydrometra stagnorum*. Yet, they also found no difference between both strains in the presence of the water boatman *Sigara lateralis* (Berticat et al., 2004). Prey motion is a key factor determining prey detection by backswimmers and water boatmen (Murphey and Mendenhall, 1973; Sih, 1979). Berticat et al. (2004) explained the higher predation of the resistant strain by a more frequent detection by the predator because of their higher activity associated with their shorter gut transfer time (Agnew et al., 2004). The absence of a differential mortality in the solvent control in our study is in line with no difference in the freezing behaviour and in the escape swimming speed between both strains.

Mosquito larvae from the resistant strain were more killed by the predator when exposed to Bti alone compared to mosquito larvae from the non-resistant strain. Note that differential

456 mortality in the predation trials could not be explained by differential delayed mortality between
457 strains in any of the pesticide treatments. The higher vulnerability to predation of the resistant
458 strain in the presence of Bti matches the higher vulnerability to Bti in the resistant strain
459 compared to the non-resistant strain during the pesticide exposure period. Since both strains have
460 the same genetic background except for the single point mutation which causes the resistance,
461 differences between both strains can be directly linked to resistance against the chemical
462 pesticide. This finding extends current types of the well-known cost of resistance against a
463 certain pesticide (Kliot and Ghanim, 2012) toward a higher vulnerability to predation in the
464 presence of another pesticide. Surprisingly, both quantified antipredator traits could not explain
465 this higher vulnerability to predation in the resistant strain. Indeed, freezing time of the resistant
466 strain even increased when exposed to Bti alone, while Bti did not affect the non-resistant strain.
467 This deviates from our expectation that an increase in freezing time would decrease the
468 probability of predation (Debecker and Stoks, 2019). Furthermore, there was no effect of Bti on
469 the escape swimming speed of both strains that could explain the difference in predation.
470 Although, this may not be so surprising since Bti disturbs the osmo-regulatory mechanisms of
471 the cell membrane in the gut and does not interfere with the neuromuscular junction like CPF
472 (Becker et al., 2010). Possibly, the higher baseline energetic costs of the resistant strain (Rivero
473 et al., 2011), combined with the higher energetic needs when exposed to Bti for defence and
474 repair mechanisms compared to the non-resistant strain (Canton et al., 2015), caused such an
475 increase in foraging activity that it resulted in a higher detection possibility by the predator. In
476 general, a higher foraging activity results in higher predation rates as shown in larvae of other
477 aquatic insects (e.g. Stoks et al., 2005).

As expected, mosquito larvae from the non-resistant strain were more killed by predation when exposed to CPF alone compared to mosquito larvae from the resistant strain. This can be explained by the reduced escape swimming speed of the non-resistant strain after exposure to CPF, while there was no effect on the swimming speed of the resistant strain. Similarly, larvae of the yellow fever mosquito *Aedes aegypti* decreased their swimming speed after exposure to deltamethrin (pyrethroid), imidacloprid (neonicotinoid) and spinosad (biopesticide) (Tomé et al., 2014). This reduced escape speed apparently overruled the CPF-induced increase of the freezing time. In addition, the larval activity may have increased after exposure to CPF resulting in a higher detection probability by the predator (Brodin and Johansson, 2004; Stoks and Johansson, 2000). For example, the activity of larvae of the zebrafish *Danio rerio* increased after acute exposure to chlorpyrifos (Kienle et al., 2009). Such contaminant-induced increase in activity may occur to increase food intake to compensate for the higher energetic costs associated with detoxification and repair mechanisms after pesticide exposure (Campero et al., 2007; Newman and Unger, 2003).

When exposed to the CPF-Bti mixture, there was no longer a difference in predation between both strains. This suggests that the opposing single pesticide exposure effects on predation between both strains cancelled out in the mixture. Indeed, while predation after Bti exposure favoured the non-resistant strain, predation after CPF exposure favoured the resistant strain.

5. CONCLUSIONS

Both to assess the pesticide control efficiency and to reduce their ecological damage to non-target species, it is important to assess the effects of pesticide mixtures compared to their single pesticides on pest species, non-target species and their predator-prey interactions. For the control

efficiency, it is also crucial to test to what extent resistant strains are more vulnerable to alternative pesticides and control strategies compared to non-resistant strains. Against this background, we exposed a vector mosquito to a pesticide mixture including a biopesticide, and to a natural predator. Hereby, we integrated two recommended strategies against the build-up of resistance in vector populations (Liu, 2015; Raymond et al., 2001). Our results suggest that the application of a taxon-specific biopesticide, which targets the vector species while not affecting the predator, is promising to target the resistant strain (Gutiérrez et al., 2017a; McNitt et al., 1995). Indeed, Bti caused a ca. 4% higher mortality in the CPF-resistant Ace-1R strain compared to the non-resistant S-Lab strain, but did not cause mortality in the predator. After exposure to Bti, the natural control agent (the pygmy backswimmer *P. minutissima*) also killed more mosquito larvae of the CPF-resistant strain. This shows a novel additional cost of resistance that was not detected in the absence of pesticides. However, the use of the pesticide mixture was not successful in having a larger impact on the resistant strain compared to the non-resistant strain. Indeed, there was an antagonistic effect between Bti and CPF in the mixture for both strains. This resulted in equal mortality of both strains in the mixture. Moreover, the preference of *P. minutissima* killing more larvae of the resistant strain also disappeared when exposed to the CPF-Bti mixture. To conclude, the integrated use of natural control by predators and Bti seems a promising strategy to counter the build-up of resistance and to keep vector mosquito populations under control.

6. ACKNOWLEDGEMENTS

We thank William Demoen, Maria Ioanna Papadaki and Ana Elisabete Pires De Lima for their practical assistance during the experiment and Margaux Casier for the image processing of the swimming speed. We are very thankful to Prof. Dr. Mylène Weill (Univ Montpellier) for

providing the mosquito strains, and two anonymous reviewers to improve our manuscript. VD is a PhD fellow of the Fund for Scientific Research Flanders (FWO). Financial support came from FWO research grant G.0943.15 and the KU Leuven grant C16/17/002.

7. REFERENCES

Agnew, P., Berticat, C., Bedhomme, S., Sidobre, C., Michalakakis, Y., 2004. Parasitism increases and decreases the costs of insecticide resistance in mosquitoes. *Evolution* (N. Y). 58, 579–586. <https://doi.org/10.1111/j.0014-3820.2004.tb01680.x>

Ahmad, M., Saleem, M.A., Sayyed, A.H., 2009. Efficacy of insecticide mixtures against pyrethroid- and organophosphate-resistant populations of *Spodoptera litura* (Lepidoptera: Noctuidae). *Pest Manag. Sci.* 65, 266–274. <https://doi.org/10.1002/ps.1681>

Alout, H., Labbé, P., Berthomieu, A., Makoundou, P., Fort, P., Pasteur, N., Weill, M., 2016. High chlorpyrifos resistance in *Culex pipiens* mosquitoes: strong synergy between resistance genes. *Heredity* (Edinb). 116, 224–231. <https://doi.org/10.1038/hdy.2015.92>

Attique, M.N.R., Khaliq, A., Sayyed, A.H., 2006. Could resistance to insecticides in *Plutella xylostella* (Lep., Plutellidae) be overcome by insecticide mixtures? *J. Appl. Entomol.* 130, 122–127. <https://doi.org/10.1111/j.1439-0418.2006.01035.x>

Awasthi, A.K., Molinero, J.C., Wu, C.H., Tsai, K.H., King, C.C., Hwang, J.S., 2015. Behavioral changes in mosquito larvae induced by copepods predation. *Hydrobiologia* 749, 113–123. <https://doi.org/10.1007/s10750-014-2154-4>

Barrett, A.D.T., 2014. Economic burden of West Nile Virus in the United States. *Am. J. Trop.*

544 Med. Hyg 90, 389–390. <https://doi.org/10.4269/ajtmh.14-0009>

545 Bass, C., Jones, C.M., 2018. Editorial overview: Pests and resistance: Resistance to pesticides in
 546 arthropod crop pests and disease vectors: mechanisms, models and tools. Curr. Opin. Insect
 547 Sci. 27, iv–vii. <https://doi.org/10.1016/j.cois.2018.04.009>

548 Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using
 549 lme4. J. Stat. Softw. 67, 1–48. <https://doi.org/10.18637/jss.v067.i01>

550 Becker, N., Petrić, D., Zgomba, M., Boase, C., Madon, M., Dahl, C., Kaiser, A., 2010.
 551 Mosquitoes and their control, Second. ed. Springer, Verlag Berlin Heidelberg.
 552 <https://doi.org/10.1007/978-3-540-92874-4>

553 Bell, A.M., Hankison, S.J., Laskowski, K.L., 2009. The repeatability of behaviour: a meta-
 554 analysis. Anim. Behav. 77, 771–783. <https://doi.org/10.1016/j.anbehav.2008.12.022>

555 Benelli, G., Jeffries, C.L., Walker, T., 2016. Biological control of mosquito vectors: Past,
 556 present, and future. Insects 7, 1–18. <https://doi.org/10.3390/insects7040052>

557 Berticat, C., Bonnet, J., Duchon, S., Agnew, P., Weill, M., Corbel, V., 2008. Costs and benefits
 558 of multiple resistance to insecticides for *Culex quinquefasciatus* mosquitoes. BMC Evol.
 559 Biol. 8, 104. <https://doi.org/10.1186/1471-2148-8-104>

560 Berticat, C., Boquien, G., Raymond, M., Chevillon, C., 2002. Insecticide resistance genes induce
 561 a mating competition cost in *Culex pipiens* mosquitoes. Genet. Res. (Camb). 79, 41–47.
 562 <https://doi.org/10.1017/S001667230100547X>

563 Berticat, C., Duron, O., Heyse, D., Raymond, M., 2004. Insecticide resistance genes confer a

564 predation cost on mosquitoes, *Culex pipiens*. Genet. Res. 83, 189–196.
565 <https://doi.org/10.1017/S0016672304006792>

566 Bird, L.J., 2017. Genetics, cross-resistance and synergism of indoxacarb resistance in
567 *Helicoverpa armigera* (Lepidoptera: Noctuidae). Pest Manag. Sci. 73, 575–581.
568 <https://doi.org/10.1002/ps.4334>

569 Bliss, 1939. The toxicity of poisons applied jointly. Ann. Appl. Biol. 26, 585–615.
570 <https://doi.org/https://doi.org/10.1111/j.1744-7348.1939.tb06990.x>

571 Boisvert, M., Boisvert, J., 2000. Effects of *Bacillus thuringiensis* var. *israelensis* on target and
572 nontarget organisms: A review of laboratory and field experiments. Biocontrol Sci.
573 Technol. 10, 517–561. <https://doi.org/10.1080/095831500750016361>

574 Borel, B., 2017. When the pesticides run out. Nature 543, 302–304.
575 <https://doi.org/10.1038/543302a>

576 Bourguet, D., Delmotte, F., Franck, P., Guillemaud, T., Reboud, X., Vacher, C., Walker, A.-S.,
577 2013. Heterogeneity of selection and the evolution of resistance. Trends Ecol. Evol. 28,
578 110–118. <https://doi.org/10.1016/j.tree.2012.09.001>

579 Brodin, T., Johansson, F., 2004. Conflicting selection pressures on the growth/predation-risk
580 trade-off in a damselfly. Ecology 85, 2927–2932. <https://doi.org/10.1890/03-3120>

581 Bukhari, T., Takken, W., Koenraadt, C.J.M., 2013. Biological tools for control of larval stages of
582 malaria vectors - a review. Biocontrol Sci. Technol. 23, 987–1023.
583 <https://doi.org/10.1080/09583157.2013.810706>

584 Campero, M., Slos, S., Ollevier, F., Stoks, R., 2007. Sublethal pesticide concentrations and
585 predation jointly shape life history: behavioral and physiological mechanisms. *Ecol. Appl.*
586 17, 2111–2122. <https://doi.org/10.1890/07-0442.1>

587 Canton, P.E., Cancino-Rodezno, A., Gill, S.S., Soberón, M., Bravo, A., 2015. Transcriptional
588 cellular responses in midgut tissue of *Aedes aegypti* larvae following intoxication with
589 Cry11Aa toxin from *Bacillus thuringiensis*. *BMC Genomics* 16, 1–17.
590 <https://doi.org/10.1186/s12864-015-2240-7>

591 Cedergreen, N., 2014. Quantifying synergy: A systematic review of mixture toxicity studies
592 within environmental toxicology. *PLoS One* 9, e96580.
593 <https://doi.org/10.1371/journal.pone.0096580>

594 Chan, H.H., Zairi, J., 2013. Permethrin resistance in *Aedes albopictus* (Diptera: Culicidae) and
595 associated fitness costs. *J. Med. Entomol.* 50, 362–370.
596 <https://doi.org/https://doi.org/10.1603/ME12182>

597 Chattopadhyay, A., Bhatnagar, N.B., Bhatnagar, R., 2004. Bacterial insecticidal toxins. *Crit.*
598 *Rev. Microbiol.* 30, 33–54. <https://doi.org/10.1080/10408410490270712>

599 Cloyd, R., 2012. Indirect effects of pesticides on natural enemies, in: *Pesticides - Advances in*
600 *Chemical and Botanical Pesticides*. p. 13. <https://doi.org/10.5772/48649>

601 Cloyd, R.A., 2011. Pesticide mixtures, in: *Pesticide-Formulations*. IntechOpen, p. 22.

602 Coors, A. & De Meester, L., 2008. Synergistic, antagonistic and additive effects of multiple
603 stressors: predation threat, parasitism and pesticide exposure in *Daphnia magna*. *J. Appl.*

604 Ecol. 45, 1820–1828. <https://doi.org/10.1111/j.1365-2664.2007.0>

605 Côté, I.M., Darling, E.S., Brown, C.J., M., C.I., S., D.E., J., B.C., 2016. Interactions among
606 ecosystem stressors and their importance in conservation. *Proc. R. Soc. B Biol. Sci.* 283,
607 20152592. <https://doi.org/10.1098/rspb.2015.2592>

608 Cui, L., Wang, Q., Qi, H., Wang, Q., Yuan, H., Rui, C., 2018. Resistance selection of indoxacarb
609 in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae): Cross-resistance, biochemical
610 mechanisms and associated fitness costs. *Soc. Chem. Ind.* 1–9.
611 <https://doi.org/10.1002/ps.5056>

612 Debecker, S., Stoks, R., 2019. Pace of life syndrome under warming and pollution: integrating
613 life history, behavior and physiology across latitudes. *Ecol. Monogr.* 89, e01332.
614 <https://doi.org/10.1002/ecm.1332>

615 Delnat, V., Debecker, S., Stoks, R., 2017. Integrating trait multidimensionality, predation and
616 autotomy to explain the maintenance of boldness. *Anim. Behav.* 130, 97–105.
617 <https://doi.org/10.1016/j.anbehav.2017.06.014>

618 Delnat, V., Tran, T.T., Janssens, L., Stoks, R., 2019. Daily temperature variation magnifies the
619 toxicity of a mixture consisting of a chemical pesticide and a biopesticide in a vector
620 mosquito. *Sci. Total Environ.* 659, 33–40.
621 <https://doi.org/10.1016/J.SCITOTENV.2018.12.332>

622 Dinh Van, K., Janssens, L., Debecker, S., Stoks, R., 2014. Temperature- and latitude-specific
623 individual growth rates shape the vulnerability of damselfly larvae to a widespread
624 pesticide. *J. Appl. Ecol.* 51, 919–928. <https://doi.org/10.1111/1365-2664.12269>

625 Duchet, C., Tetreau, G., Marie, A., Rey, D., Besnard, G., Perrin, Y., Paris, M., David, J.P.,
 626 Lagneau, C., Després, L., 2014. Persistence and recycling of bioinsecticidal *Bacillus*
 627 *thuringiensis* subsp. *israelensis* spores in contrasting environments: evidence from field
 628 monitoring and laboratory experiments. *Microb. Ecol.* 67, 576–586.
 629 <https://doi.org/10.1007/s00248-013-0360-7>

630 Duron, O., Labbé, P., Berticat, C., Rousset, F.O., Guillot, S., Raymond, M., Weill, N., 2006.
 631 High Wolbachia density correlates with cost of infection for insecticide resistant *Culex*
 632 *pipiens* mosquitoes. *Evolution* (N. Y). 60, 303–314. [https://doi.org/10.1111/j.0014-](https://doi.org/10.1111/j.0014-3820.2006.tb01108.x)
 633 [3820.2006.tb01108.x](https://doi.org/10.1111/j.0014-3820.2006.tb01108.x)

634 Eaton, D.L.L., Daroff, R.B.B., Autrup, H., Bridges, J., Buffler, P., Costa, L.G.G., Coyle, J.,
 635 McKhann, G., Mobley, W.C.C., Nadel, L., Neubert, D., Schulte-Hermann, R., Spencer,
 636 P.S.S., 2008. Review of the toxicology of chlorpyrifos with an emphasis on human
 637 exposure and neurodevelopment. *Crit. Rev. Toxicol.* 38, 1–125.
 638 <https://doi.org/10.1080/10408440802272158>

639 Faust, M., Altenburger, R., Backhaus, T., Bödeker, W., Scholze, M., Grimme, L.H., 2000.
 640 Predictive assessment of the aquatic toxicity of multiple chemical mixtures. *J. Environ.*
 641 *Qual.* 29, 1063. <https://doi.org/10.2134/jeq2000.00472425002900040005x>

642 Ferrari, M.C.O., Messier, F., Chivers, D.P., 2008. Variable predation risk and the dynamic nature
 643 of mosquito antipredator responses to chemical alarm cues. *Chemoecology* 17, 223–229.
 644 <https://doi.org/10.1007/s00049-007-0380-1>

645 Fox, J., Weisberg, S., 2018. An {R} companion to applied regression, Three. ed, Sage

646 Publications. SAGE Publications, Inc, Thousand Oaks CA.
647 <https://doi.org/10.1177/0049124105277200>

648 Guarner, J., Hale, G.L., 2019. Four human diseases with significant public health impact caused
649 by mosquito-borne flaviviruses: West Nile, Zika, dengue and yellow fever. *Semin. Diagn.*
650 *Pathol.* 36, 170–176. <https://doi.org/10.1053/j.semdp.2019.04.009>

651 Gupta, R.C., 2011. Toxicology of organophosphate and carbamate compounds. Academic Press.

652 Gurushankara, H.P., Krishnamurthy, S. V., Vasudev, V., 2007. Effect of malathion on survival,
653 growth, and food consumption of indian cricket frog (*Limnonectes limnocharis*) tadpoles.
654 *Arch. Environ. Contam. Toxicol.* 52, 251–256. <https://doi.org/10.1007/s00244-006-0015-5>

655 Gutiérrez, Y., Ramos, G.S., Tomé, H.V.V., Oliveira, E.E., Salero, A.L., 2017a. Bti-based
656 insecticide enhances the predatory abilities of the backswimmer *Buenoa tarsalis*
657 (Hemiptera: Notonectidae). *Ecotoxicology* 26, 1147–1155. [https://doi.org/10.1007/s10646-](https://doi.org/10.1007/s10646-017-1840-1)
658 [017-1840-1](https://doi.org/10.1007/s10646-017-1840-1)

659 Gutiérrez, Y., Tomé, H.V.V., Guedes, R.N.C., Oliveira, E.E., 2017b. Deltamethrin toxicity and
660 impaired swimming behavior of two backswimmer species. *Environ. Toxicol. Chem.* 36,
661 1235–1242. <https://doi.org/10.1002/etc.3645>

662 Heim, J.R., Weston, D.P., Major, K., Poynton, H., Huff Hartz, K.E., Lydy, M.J., 2018. Are there
663 fitness costs of adaptive pyrethroid resistance in the amphipod, *Hyaella azteca*? *Environ.*
664 *Pollut.* 235, 39–46. <https://doi.org/10.1016/j.envpol.2017.12.043>

665 Hernández, A.F., Gil, F., Lacasaña, M., 2017. Toxicological interactions of pesticide mixtures:

666 an update. Arch. Toxicol. 91, 3211–3223. <https://doi.org/10.1007/s00204-017-2043-5>

667 Huang, Y.J.S., Higgs, S., Vanlandingham, D.L., 2019. Emergence and re-emergence of
668 mosquito-borne arboviruses. Curr. Opin. Virol. 34, 104–109.
669 <https://doi.org/10.1016/j.coviro.2019.01.001>

670 Imbahale, S.S., Githeko, A., Mukabana, W.R., Takken, W., 2012. Integrated mosquito larval
671 source management reduces larval numbers in two highland villages in western Kenya.
672 BMC Public Health 12, 362. <https://doi.org/10.1186/1471-2458-12-362>

673 Institute of Medicine, 2008. Vector-borne diseases: Understanding the environmental, human
674 health, and ecological connections, workshop summary, Forum on Microbial Threats.
675 National Academies Press, Washington, D.C.

676 Jansen, M., Coors, A., Stoks, R., De Meester, L., 2011. Evolutionary ecotoxicology of pesticide
677 resistance: A case study in *Daphnia*. Ecotoxicology 20, 543–551.
678 <https://doi.org/10.1007/s10646-011-0627-z>

679 Kamareddine, L., 2012. The biological control of the malaria vector. Toxins (Basel). 4, 748–767.
680 <https://doi.org/10.3390/toxins4090748>

681 Kamo, M., Yokomizo, H., 2015. Explanation of non-additive effects in mixtures of similar mode
682 of action chemicals. Toxicology 335, 20–26. <https://doi.org/10.1016/j.tox.2015.06.008>

683 Kienle, C., Köhler, H.R., Gerhardt, A., 2009. Behavioural and developmental toxicity of
684 chlorpyrifos and nickel chloride to zebrafish (*Danio rerio*) embryos and larvae. Ecotoxicol.
685 Environ. Saf. 72, 1740–1747. <https://doi.org/10.1016/j.ecoenv.2009.04.014>

686 Kikuchi, K., Mochizuki, O., 2010. Consideration of thrust in escaping motion of a mosquito
687 larva. *J. Aero Aqua Bio-mechanisms* 1, 111–116. <https://doi.org/10.5226/jabmech.1.111>

688 Kliot, A., Ghanim, M., 2012. Fitness costs associated with insecticide resistance. *Pest Manag.*
689 *Sci.* 68, 1431–1437. <https://doi.org/10.1002/ps.3395>

690 Lagadic, L., Schäfer, R.B., Roucaute, M., Szöcs, E., Chouin, S., de Maupeou, J., Duchet, C.,
691 Franquet, E., Le Hunsec, B., Bertrand, C., Fayolle, S., Francés, B., Rozier, Y., Foussadier,
692 R., Santoni, J.B., Lagneau, C., 2016. No association between the use of Bti for mosquito
693 control and the dynamics of non-target aquatic invertebrates in French coastal and
694 continental wetlands. *Sci. Total Environ.* 553, 486–494.
695 <https://doi.org/10.1016/j.scitotenv.2016.02.096>

696 Laird, M., 1988. The natural history of larval mosquito habitats. Academic Press, London.

697 Land, M., Miljand, M., 2014. Biological control of mosquitoes using *Bacillus thuringiensis*
698 *israelensis*: A pilot study of effects on target organisms, non-target organisms and humans.
699 *Mistra EviEM Pilot Stud.* PS4.

700 Lenth, R. V., 2016. Least-squares means: The R package lsmeans. *J. Stat. Softw.* 69, 1–33.
701 <https://doi.org/10.18637/jss.v069.i01>

702 Liu, N., 2015. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions.
703 *Annu. Rev. Entomol.* 60, 537–559. <https://doi.org/10.1146/annurev-ento-010814-020828>

704 Mansour, S.A., Foda, M.S., Aly, A.R., 2012. Mosquitocidal activity of two *Bacillus* bacterial
705 endotoxins combined with plant oils and conventional insecticides. *Ind. Crops Prod.* 35, 44–

706 52. <https://doi.org/10.1016/j.indcrop.2011.06.001>

707 McNitt, L., Espelle, K.E., Miller, L.K., 1995. Assessing the safety of toxin-producing
708 baculovirus biopesticides to a nontarget predator, the social wasp *Polister metrixus* Say.
709 Biol. Control 5, 267–278. <https://doi.org/https://doi.org/10.1006/bcon.1995.1033>

710 Meijering, E., Dzyubachyk, O., Smal, I., 2012. Methods for cell and particle tracking, in:
711 Methods in Enzymology. pp. 183–200. [https://doi.org/10.1016/B978-0-12-391857-4.00009-](https://doi.org/10.1016/B978-0-12-391857-4.00009-4)
712 4

713 Murphey, R.K., Mendenhall, B., 1973. Localization of receptors controlling orientation to prey
714 by the back swimmer *Notonecta undulata*. J. Comp. Physiol. 84, 19–30.
715 <https://doi.org/https://doi.org/10.1007/BF00694144>

716 Nakagawa, S., Schielzeth, H., 2010. Repeatability for Gaussian and non-Gaussian data: A
717 practical guide for biologists. Biol. Rev. 85, 935–956. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-185X.2010.00141.x)
718 185X.2010.00141.x

719 Newman, M.C., Unger, M.A., 2003. Fundamentals of ecotoxicology. Lewis Publishers, Boca
720 Raton, Florida.

721 OECD, 2011. OECD guideline for the testing of chemicals - *Chironomus* sp., acute
722 immobilisation test. OECD 1–11. <https://doi.org/10.1787/9789264069947-en>

723 Papacek, M., 2001. Small aquatic and ripicolous bugs (Heteroptera: Nepomorpha) as predators
724 and prey: The question of economic importance. Eur. J. Entomol. 98, 1–12.
725 <https://doi.org/10.14411/eje.2001.001>

726 Pimentel, D., Burgess, M., 2014. Chapter 5: Environmental and economic benefits of reducing
727 pesticide use, in: Integrated Pest Management. Springer, Dordrecht, pp. 127–139.
728 <https://doi.org/https://doi.org/10.1007/978-94-007-7796-5>

729 R Development Core Team, 2017. R: A language and environment for statistical computing. R
730 Found. Stat. Comput. [https://doi.org/ISBN 3-900051-07-0](https://doi.org/ISBN%203-900051-07-0)

731 Raghavendra, K., Barik, T.K., Reddy, B.P.N., Sharma, P., Dash, A.P., 2011. Malaria vector
732 control: From past to future. Parasitol. Res. 108, 757–779. [https://doi.org/10.1007/s00436-](https://doi.org/10.1007/s00436-010-2232-0)
733 [010-2232-0](https://doi.org/10.1007/s00436-010-2232-0)

734 Raymond, M., Berticat, C., Weill, M., Pasteur, N., Chevillon, C., 2001. Insecticide resistance in
735 the mosquito *Culex pipiens*: What have we learned about adaptation? Genetica 8, 287–296.
736 <https://doi.org/10.1007/978-94-010-0585-2>

737 Reffstrup, T.K., Larsen, J.C., Meyer, O., 2010. Risk assessment of mixtures of pesticides.
738 Current approaches and future strategies. Regul. Toxicol. Pharmacol. 56, 174–192.
739 <https://doi.org/10.1016/j.yrtph.2009.09.013>

740 Reid, W.R., Zhang, L., Liu, F., Liu, N., 2012. The transcriptome profile of the mosquito *Culex*
741 *quinquefasciatus* following permethrin selection. PLoS One 7, 1–12.
742 <https://doi.org/10.1371/journal.pone.0047163>

743 Relyea, R.A., Hoverman, J.T., 2008. Interactive effects of predators and a pesticide on aquatic
744 communities. <https://doi.org/10.1111/j.1600-0706.2008.16933.x>

745 Reynaldi, S., Meiser, M., Liess, M., 2011. Effects of the pyrethroid fenvalerate on the alarm

746 response and on the vulnerability of the mosquito larva *Culex pipiens molestus* to the
 747 predator *Notonecta glauca*. *Aquat. Toxicol.* 104, 56–60.
 748 <https://doi.org/10.1016/j.aquatox.2011.03.017>

749 Richards, S.L., Lord, C.C., Pesko, K., Tabachnick, W.J., 2009. Environmental and biological
 750 factors influencing *Culex pipiens quinquefasciatus* Say (Diptera: Culicidae) vector
 751 competence for Saint Louis encephalitis virus. *Am. J. Trop. Med. Hyg.* 81, 264–72.
 752 <https://doi.org/10.1016/j.bb.2008.05.010>

753 Richards, S.L., Lord, C.C., Pesko, K.N., Tabachnick, W.J., 2010. Environmental and biological
 754 factors influencing *Culex pipiens quinquefasciatus* (Diptera: Culicidae) vector competence
 755 for West Nile Virus. *Am. J. Trop. Med. Hyg.* 83, 126–134.
 756 <https://doi.org/10.4269/ajtmh.2010.09-0776>

757 Ritz, C., Baty, F., Streibig, J.C., Gerhard, D., 2015. Dose-response analysis using R. *PLoS One*
 758 10, e0146021. <https://doi.org/10.1371/journal.pone.0146021>

759 Rivero, A., Magaud, A., Nicot, A., Vézilier, J., 2011. Energetic cost of insecticide resistance in
 760 *Culex pipiens* mosquitoes. *J. Med. Entomol.* 48, 694–700. <https://doi.org/10.1603/ME10121>

761 Rizzati, V., Briand, O., Guillou, H., Gamet-Payrastre, L., 2016. Effects of pesticide mixtures in
 762 human and animal models: An update of the recent literature. *Chem. Biol. Interact.* 254,
 763 231–246. <https://doi.org/10.1016/j.cbi.2016.06.003>

764 Rodea-Palomares, I., González-Pleiter, M., Martín-Betancor, K., Rosal, R., Fernández-Piñas, F.,
 765 2015. Additivity and interactions in ecotoxicity of pollutant mixtures: some patterns,
 766 conclusions, and open questions. *Toxics* 3, 342–369. <https://doi.org/10.3390/toxics3040342>

767 Rubach, M.N., Crum, S.J.H., Van Den Brink, P.J., 2011. Variability in the dynamics of mortality
768 and immobility responses of freshwater arthropods exposed to chlorpyrifos. Arch. Environ.
769 Contam. Toxicol. 60, 708–721. <https://doi.org/10.1007/s00244-010-9582-6>

770 Schäfer, R.B., Piggott, J.J., 2018. Advancing understanding and prediction in multiple stressor
771 research through a mechanistic basis for null models. Glob. Chang. Biol. 1–10.
772 <https://doi.org/https://doi.org/10.1111/gcb.14073>

773 Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image
774 analysis. Nat. Methods 9, 671–675. <https://doi.org/10.1038/nmeth.2089>

775 Senthil-Nathan, S., 2015. A review of biopesticides and their mode of action against insect pests,
776 in: Thangavel, P., Sridevi, G. (Eds.), Environmental Sustainability. Springer, New Delhi, p.
777 324. <https://doi.org/10.1007/978-81-322-2056-5>

778 Shin, E.H., Kim, N.J., Kim, H.K., Park, C., Lee, D.K., Ahn, Y.J., Chang, K.S., 2012. Resistance
779 of field-collected populations of *Culex pipiens pallens* (Diptera: Culicidae) to insecticides in
780 the Republic of Korea. J. Asia. Pac. Entomol. 15, 1–4.
781 <https://doi.org/10.1016/j.aspen.2011.07.009>

782 Sih, A., 1979. Stability and prey behavioural responses to predator density. J. Anim. Ecol. 48,
783 79–89.

784 Singmann, H., Bolker, B., Westfall, J., Aust, F., 2017. afex: Analysis of factorial experiments.

785 Stoffel, M.A., Nakagawa, S., Schielzeth, H., 2017. rptR: Repeatability estimation and variance
786 decomposition by generalized linear mixed-effects models. Methods Ecol. Evol. 8, 1639–

787 1644. <https://doi.org/10.1111/2041-210X.12797>

788 Stoks, R., De Block, M., Van De Meutter, F., Johansson, F., 2005. Predation cost of rapid
789 growth: Behavioural coupling and physiological decoupling. *J. Anim. Ecol.* 74, 708–715.
790 <https://doi.org/10.1111/j.1365-2656.2005.00969.x>

791 Stoks, R., Johansson, F., 2000. Trading off mortality risk against foraging effort in damselflies
792 that differ in life cycle length. *Oikos* 91, 559–567. [https://doi.org/10.1034/j.1600-](https://doi.org/10.1034/j.1600-0706.2000.910318.x)
793 [0706.2000.910318.x](https://doi.org/10.1034/j.1600-0706.2000.910318.x)

794 Stoks, R., McPeck, M., 2006. A tale of two diversifications: reciprocal habitat shifts to fill
795 ecological space along the pond permanence gradient. *Am. Nat.* 168, S50–S72.
796 <https://doi.org/10.1086/509045>

797 Thomas, M.B., 2018. Biological control of human disease vectors: a perspective on challenges
798 and opportunities. *BioControl* 63, 61–69. <https://doi.org/10.1007/s10526-017-9815-y>

799 Thomas, M.B., Godfray, H.C.J., Read, A.F., Berg, H. Van Den, Tabashnik, B.E., Lenteren, J.C.
800 Van, Waage, J.K., Takken, W., van den Berg, H., Tabashnik, B.E., van Lenteren, J.C.,
801 Waage, J.K., Takken, W., 2012. Lessons from agriculture for the sustainable management
802 of Malaria vectors. *PLoS Med.* 9, 7–10. <https://doi.org/10.1371/journal.pmed.1001262>

803 Toma, L., Menegon, M., Romi, R., De Matthaeis, E., Montanari, M., Severini, C., 2011. Status
804 of insecticide resistance in *Culex pipiens* field populations from north-eastern areas of Italy
805 before the withdrawal of OP compounds. *Pest Manag. Sci.* 67, 100–106.
806 <https://doi.org/10.1002/ps.2039>

807 Tomé, H.V., Pascini, T. V, Dângelo, R.A., Guedes, R.N., Martins, G.F., 2014. Survival and
808 swimming behavior of insecticide-exposed larvae and pupae of the yellow fever mosquito
809 *Aedes aegypti*. Parasit. Vectors 7, 195. <https://doi.org/10.1186/1756-3305-7-195>

810 Tran, T.T., Janssens, L., Dinh, K. V., Op de Beeck, L., Stoks, R., 2016. Evolution determines
811 how global warming and pesticide exposure will shape predator-prey interactions with
812 vector mosquitoes. Evol. Appl. 9, 818–830. <https://doi.org/10.1111/eva.12390>

813 Tsui, M.T.K., Wang, W.X., Chu, L.M., 2005. Influence of glyphosate and its formulation
814 (Roundup®) on the toxicity and bioavailability of metals to *Ceriodaphnia dubia*. Environ.
815 Pollut. 138, 59–68. <https://doi.org/10.1016/j.envpol.2005.02.018>

816 United Nations Environment Program, 2004. OECD - Screening Information DataSet: initial
817 assessment report ethanol cas No: 64-17-5. UNEP Publ. 1–341.

818 Vandekerkhove, J., Namiotko, T., Hallmann, E., Martens, K., 2012. Predation by
819 macroinvertebrates on *Heterocypris incongruens* (Ostracoda) in temporary ponds: impacts
820 and responses. Fundam. Appl. Limnol. 181, 39–47. [https://doi.org/10.1127/1863-](https://doi.org/10.1127/1863-9135/2012/0310)
821 [9135/2012/0310](https://doi.org/10.1127/1863-9135/2012/0310)

822 von Hirsch, H., Becker, N., 2009. Cost-benefit analysis of mosquito control operations based on
823 microbial control agents in the upper Rhine valley (Germany). Eur. Mosq. Bull. 27, 47–55.

824 Weill, M., Lutfalla, G., Mogensen, K., Chandre, F., Berthomieu, A., Berticat, C., Pasteur, N.,
825 Philips, A., Fort, P., Raymond, M., 2003. Insecticide resistance in mosquito vectors. Nature
826 423, 136–137. <https://doi.org/10.1038/423136a>

827 WHOPEs, 2016. Recommended compounds and formulations for control of mosquito larvae
828 [WWW Document]. World Heal. Organ. <https://doi.org/10.1371/journal.pone.0170079>

829 Wilkie, M.P., Hubert, T.D., Boogaard, M.A., Birceanu, O., 2019. Control of invasive sea
830 lampreys using the piscicides TFM and niclosamide: Toxicology, successes & future
831 prospects. *Aquat. Toxicol.* 211, 235–252. <https://doi.org/10.1016/j.aquatox.2018.12.012>

832 Xu, Z., Liu, Y., Wei, P., Feng, K., Niu, J., Shen, G., Lu, W., Xiao, W., Wang, J., Smagghe, G.J.,
833 Xu, Q., He, L., 2017. High gamma-aminobutyric acid contents involved in abamectin
834 resistance and predation, an interesting phenomenon in spider mites. *Front. Physiol.* 8, 1–
835 11. <https://doi.org/10.3389/fphys.2017.00216>

836 Yang, G., Chen, C., Wang, Y., Cai, L., Kong, X., Qian, Y., Wang, Q., 2015. Joint toxicity of
837 chlorpyrifos, atrazine, and cadmium at lethal concentrations to the earthworm *Eisenia*
838 *fetida*. *Environ. Sci. Pollut. Res.* 22, 9307–9315. <https://doi.org/10.1007/s11356-015-4097-3>

839

840

8. TABLES

Table 1. Results of the generalized linear mixed model (mortality and delayed mortality) testing for the effects of exposure to chlorpyrifos (CPF) and to *Bacillus thuringiensis var. israelensis* (Bti) in *Culex quinquefasciatus*. *P*-values indicated in bold are significant ($P < 0.05$).

	Mortality			Delayed mortality		
	χ^2	Df	<i>P</i> -value	χ^2	Df	<i>P</i> -value
Strain	0.10	1	0.76	2.18	1	0.14
Bti	113.95	1	< 0.001	1.59	1	0.21
CPF	13.85	1	< 0.001	2.66	1	0.10
Strain $\times$ Bti	3.95	1	0.047	0.55	1	0.46
Strain $\times$ CPF	14.58	1	< 0.001	0.0020	1	0.96
Bti $\times$ CPF	37.86	1	< 0.001	1.98	1	0.16
Strain $\times$ Bti $\times$ CPF	10.11	1	0.0015	0.0022	1	0.96

Table 2. Results of the general linear mixed models (freezing behaviour and initial swimming speed) testing for the effects of exposure to chlorpyrifos (CPF) and to *Bacillus thuringiensis var. israelensis* (Bti) in *Culex quinquefasciatus*. *P*-values indicated in bold are significant ($P < 0.05$).

	Freezing behaviour			Swimming speed		
	χ^2	Df	<i>P</i> -value	χ^2	Df	<i>P</i> -value
Strain	4.20	1	0.040	7.20	1	0.0073
Bti	3.64	1	0.056	0.0083	1	0.93
CPF	10.86	1	< 0.001	2.05	1	0.15
Strain $\times$ Bti	6.78	1	0.0092	1.42	1	0.23
Strain $\times$ CPF	0.30	1	0.59	3.35	1	0.067
Bti $\times$ CPF	1.16	1	0.28	1.25	1	0.26
Strain $\times$ Bti $\times$ CPF	0.33	1	0.57	0.12	1	0.73

850 9. FIGURE LEGENDS

851 Figure 1. A visualisation of the experimental design including treatments and endpoints used.

852 Figure 2. Effects of chlorpyrifos (CPF), *Bacillus thuringiensis* var. *israelensis* (Bti) and the CPF-
853 Bti mixture on mortality in the non-resistant S-Lab strain and the organophosphate-resistant Ace-
854 1R strain of *Culex quinquefasciatus*: (A) mortality during the 48 hour pesticide exposure period
855 and (B) the delayed mortality afterwards. Means are given with their standard error. Numbers
856 inside the bars indicate the number of vials (20 larvae/vial).

857 Figure 3. The proportion of the surviving larvae of the non-resistant S-Lab strain and the
858 organophosphate-resistant Ace-1R strain of *Culex quinquefasciatus* after predation by *Plea*
859 *minutissima*. The black horizontal line at 1 indicates where the same amount of larvae of both
860 strains were predated. More S-Lab larvae were predated above this line, while more Ace-1R
861 larvae were predated below this line. Means are given with their 95% confidence interval.
862 Numbers indicate the number of predation trials.

863 Figure 4. Effects of chlorpyrifos (CPF), *Bacillus thuringiensis* var. *israelensis* (Bti) and the CPF-
864 Bti mixture on two antipredator traits of in the non-resistant S-Lab strain and the
865 organophosphate-resistant Ace-1R strain of *Culex quinquefasciatus*: (A) freezing behaviour and
866 (B) escape swimming speed. Means are given with their standard error. Numbers inside the bars
867 indicate the number of vials (freezing behaviour: 4-5 larvae per vial; swimming speed: 1-3 larvae
868 per vial).

870

871 Figure 1.

872

873

874 Figure 2.

875

876 Figure 3.

877

Figure 4.