

JYU DISSERTATIONS 11

Aigi Margus

Adaptation to Stressful Environments: Invasion Success of the Colorado Potato Beetle (*Leptinotarsa decemlineata*)



UNIVERSITY OF JYVÄSKYLÄ
FACULTY OF MATHEMATICS
AND SCIENCE

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**Adaptation to Stressful Environments:
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ABSTRACT

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Yhteenvedo: Stressinsietokyky ja vieraslajin invaasio

Diss.

Biological invasions, specifically human-induced dispersals, are one of the major threats to our biodiversity and are predicted to increase. Invasive pests provide an opportunity to study whether adaptation to human-induced environments could promote invasions to other human-induced environments. One major anthropogenic selection pressure is created by pesticides, and pests can be exposed to various pesticides in their native, as well as introduced, ranges. I investigated whether exposure to anthropogenic selection (i.e. insecticides and herbicides) and exposure to multiple anthropogenic stressors selects for higher stress tolerance. I also tested whether parental prolonged diapause or insecticide exposure can increase larval stress tolerance, using an invasive pest (*Leptinotarsa decemlineata*) as a study species. First, I found that populations differ in their insecticide resistance, which could be due to their past invasion history, as well as past intensity of selection by insecticides. Differences in the resistance were due to both qualitative and quantitative changes in the insecticide target sites, but also due to higher antioxidant defence. These differences also lead to differences in stress tolerance when the individuals were exposed to other pesticides. Second, I found that exposure to both herbicide and insecticide can have interactive effects (both antagonistic and synergistic). Third, I found that prolonged diapause could be a strategy to skip environmental conditions the species is poorly adapted to, however, it carries a sex-specific fertility cost, which could be mitigated by higher offspring body mass. Last, I found that exposure to sublethal doses of insecticide had positive within- and transgenerational effects, by means of increased female body mass and survival. To conclude, exposure to anthropogenic stress could select in addition to pesticide resistance for higher stress tolerance and can induce changes in fitness-related traits and thus contribute to the invasion success of the *Leptinotarsa decemlineata*.

Keywords: Adaptation, herbicide, insecticide stress, pest species, prolonged diapause, species invasions, stress tolerance.

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LIST OF ORIGINAL PUBLICATIONS

The thesis is based on the following original studies, which will be referred to in the text by their Roman numerals I-V.

- I Margus A., Piironen S., Lehmann P., Grapputo A., Chen Y., Ovčarenko I., Gilbert L., & Lindström L. Roles of acetylcholinesterase genes in organophosphate and carbamate resistance in *Leptinotarsa decemlineata*. Manuscript.
- II Rainio M., Margus A., Lehmann P., Helander M., & Lindström L. Effects of a glyphosate-based herbicide on survival and oxidative status of a non-target herbivore, the Colorado potato beetle (*Leptinotarsa decemlineata*). Submitted manuscript.
- III Margus A., Rainio M. & Lindström L. Multiple stressors: can indirect herbicide exposure modify the response to organophosphate insecticide in an invasive pest? Submitted manuscript.
- IV Margus A. & Lindström. Age does matter: prolonged diapause has sex-specific fertility and fitness costs in the Colorado potato beetle. Submitted manuscript.
- V Margus A., Piironen S., Lehmann P., Tikka S., Karvanen J. & Lindström L. Sublethal pyrethroid insecticide exposure carries positive fitness effects over generations in an invasive pest. Submitted manuscript.

This table shows the contributions to the original papers.

Study	I	II	III	IV	V
Original idea	LL, SP	AM, LL, MH	AM, LL	AM, LL	LL, SP, AM
Experimental design and work	SP, AM, PL, IO	AM, LL, MR, PL	AM, LL, MR	AM, LL	SP, LL, AM, PL
Data analysis	AM, SP, PL, LL	MR	AM, MR	AM, LL	ST, JK, AM
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AM=Aigi Margus, LL=Leena Lindström, SP=Saija Piironen, PL=Philipp Lehmann, AG=Alessandro Grapputo, LG=Leona Gilbert, JK=Juha Karvanen, ST=Sanntu Tikka, MR=Miiia Rainio, YC=Yolanda Chen, MH=Marjo Helander, IO= Irina Ovčarenko.

ABBREVIATIONS

AChE	acetylcholinesterase enzyme
AZ	azinphos-methyl
AZoxon	azinphosmethyl-oxon
bi-PASA	bidirectional PCR amplification of specific alleles
CAR	carbaryl
CAT	catalase
CPB	Colorado potato beetle
G6PDH	glucose-6-phosphate dehydrogenase
GBH	glyphosate based herbicide
GP	glutathione peroxidase
GR	glutathione reductase
GSH: GSSG	glutathione reduced-oxidized
GST	glutathione S-transferase
OP	organophosphate
PCA	principal component analysis
RT-qPCR	real-time quantitative PCR
SOD	superoxide dismutase
tGSH	total glutathione reduced

Emale

1 INTRODUCTION

1.1 Species invasions

Humans have traded and transported different species outside their native range for millennia. The rate of introductions first peaked at the end of the Middle Ages, then at the beginning of the Industrial Revolution, and then again during the past 25 years with the highest rates of introductions (Hulme 2009). The recent increase in invasions has been especially evident among arthropods (Hulme 2009). However, of the many species that are introduced only a few have become successful invaders (Williamson and Fitter 1996). Invasive species are defined as alien species that are introduced deliberately or accidentally to new areas and whose introduction will likely have negative economic, ecological, or environmental impacts, or cause harm to human health and food security (Beck *et al.* 2008, Davis 2009, Paini *et al.* 2016). Nowadays, the spread of invasive species is easier than ever due to human influence and globalization (Perrings *et al.* 2005, Meyerson and Mooney 2007, Hulme 2009). Globalization is associated with increasing trade exchange, global movements, transport of supplies, demand for commodities, immigration, travelling, and human activities, all of which makes it easier for invasive species to “hitch-hike” to new areas where they would never reach without these human activities (Cox 2004). All these factors can contribute to the increased invasion risk, through increased rate and frequency of introductions and dispersal of invasive species. Moreover, the increasing rate of invasions is expected to continue in the future (Lodge *et al.* 2006).

The main factors contributing to the successful establishment of an invasive species have been suggested to be habitat matching, propagule pressure (i.e. a number of repeated dispersals during the invasion) and human affiliation (Jeschke and Strayer 2006, Hayes and Barry 2008). Species associated with humans and from human-influenced habitats are more likely to be introduced to the new areas (Jeschke and Strayer 2006) because most long distant transportation happens between human-induced habitats. Thus,

populations that are adapted to human-induced environments in their native range could be more successful invading human-induced environments in the introduced range (Hufbauer *et al.* 2012). The invasive species can become pre-adapted in their native habitat, which could promote invasions in a similar introduced habitat (Hufbauer *et al.* 2012). Propagule pressure is usually positively correlated with genetic variance (Bock *et al.* 2015) and genetically diverse populations are more likely to possess pre-adapted genotypes. Habitat matching is a prerequisite for invasion success, whereas increased propagule pressure can improve the likelihood of invasion success (Hayes and Barry 2008).

Besides habitat matching, adaptation to stress, in the form of high stress tolerance, can be an important factor contributing to invasion success (Lee and Gelembiuk 2008, Hufbauer *et al.* 2012). Stress can be defined as an environmental condition that lies outside the range of the organisms' optimal conditions and challenges its homeostasis maintenance and proper function (Ghalambor *et al.* 2007). Invasive species are introduced to new habitats, where they are exposed to various natural and anthropogenic stressors that may limit or slow down their establishment or further spread (Lee *et al.* 2003, Lee *et al.* 2009). Species that have a higher stress tolerance are expected to persist better until they become adapted to the introduced habitat. Individuals with broad stress tolerance could possess life history traits and/ or phenotypic plasticity that allows acclimation to a wide range of habitats and thus make them successful invaders (Sexton *et al.* 2002). The ability to physiologically tolerate and adapt to several stressors can determine the range limits of invasive species and invasion success (Addo-Bediako *et al.* 2000, Gilchrist *et al.* 2008).

1.2 Insecticide resistance

It is known that human activities can cause strong directional changes to many ecosystems and act as an evolutionary force, especially in commercially important species such as agricultural pests (Palumbi 2001). In agriculture, pesticides are widely used, and thus, form a major abiotic anthropogenic pressure for the invasive pest species. Continuous directional selection of insecticides has resulted in increased rates of evolution of insecticide resistance within different pest species, including invasive pests (Mota-Sanchez and Wise 2018, IRAC 2018). As a result, more than 580 species have become resistant to at least one insecticide and in total to 325 different insecticides (Sparks and Nauen 2015).

Insecticide resistance is defined as a heritable change in the sensitivity to insecticides that leads to failure of an insecticide application to control a pest population (IRAC 2018). Most current insecticides act on muscle and nerve targets, but they can also target the growth, respiration, and midgut (IRAC 2018). Insects can develop resistance to insecticides in many ways, including

metabolic changes, behavioural and physical changes, and most importantly changes in the target sites (Georghiou 1972, Roush and Tabashnik 2012, Sokhna *et al.* 2013). Metabolic resistance is the most common mechanism, and it protects the insect by destroying insecticide molecules or detoxifying insecticides into a less toxic form (Paini *et al.* 2016). There are three main enzymes involved in the detoxification of insecticides: cytochrome P450 monooxygenases, esterases, and glutathione S-transferases (Scott *et al.* 1999, Enayati *et al.* 2005, Paini *et al.* 2016). Behavioural resistance is defined as the ability to avoid or shorten the duration of insecticide exposure through changes in the insects' behaviour (Sparks *et al.* 1989, Sokhna *et al.* 2013). Physical changes, for example, decreased cuticle penetration, help to reduce insecticide absorption into the cells (IRAC 2018). Target site resistance is a result of modifications in the target site that make the insect less sensitive to insecticides. Insecticide resistance is often reported as changes in the target site (Ffrench-Constant *et al.* 1993, Zhu *et al.* 1996, Weill *et al.* 2004, Tmimi *et al.* 2018), but resistance can also be caused by many of the aforementioned mechanisms simultaneously (Metcalf 1989).

Insecticides are classified by their mode of action, which includes a specific molecular target site to which the insecticide binds. For example, pyrethroids bind to and disrupt voltage-gated sodium channels in the insect nervous system (Davies *et al.* 2007, Soderlund 2012). A high frequency of single mutations (L1014F) has been identified in many species with pyrethroid resistance (Rinkevich *et al.* 2013). Organophosphate and carbamate insecticides, on the other hand, inhibit acetylcholinesterase (AChE), which is an enzyme that catalyses the hydrolysis of the neurotransmitter acetylcholine. Both qualitative (e.g. mutations in the binding site) and quantitative (e.g. altered gene expression) changes in the target site can confer resistance to insecticides (Fournier *et al.* 1992). The quantitative changes involve point mutations in the two acetylcholinesterase genes (*ace1* and *ace2*) and various mutations have been identified that confer the resistance to OP and carbamate insecticides (Zhu *et al.* 1996, Weill *et al.* 2003, Weill *et al.* 2004, Menozzi *et al.* 2004, Li and Han 2004, Alout *et al.* 2007). The quantitative changes involve changes in gene amplification, for example, an overexpression of the target site genes (Fournier *et al.* 1992, Gao and Zhu 2002). The AChE1 encoded by the *ace1* gene is the main catalytic enzyme in many species (Kim and Lee 2013). Thus, changes in the *ace1* gene expression could contribute to the quantitative changes of the target site, and thus make individuals more resistant.

1.3 Effects of sublethal stress

The emphasis in resistance studies has been to investigate the lethal effects of insecticides (Fox *et al.* 2001, Ffrench-Constant *et al.* 2004). Although the aim of pesticide use is to kill all pests, not all individuals are exposed to lethal doses of insecticides. Pests can also be exposed to sublethal doses (Desneux *et al.* 2007),

for which there is no constant definition in literature. Typically, sublethal means less than lethal insecticide doses/concentrations that are under the median lethal dose (i.e. LD₅₀/LC₅₀) (Desneux *et al.* 2007, de França *et al.* 2017). However, target species are likely exposed to higher sublethal doses and non-target species to lower sublethal doses. Sublethal insecticide doses (i.e. killing less than 50 % of the population) can be caused by improper insecticide application, or insecticide degradation by rainfall or light, and occur at the edges of the fields, or due to changes in insect pest behaviour (Desneux *et al.* 2005).

Sublethal doses can affect individuals in various ways: by affecting the reproduction, survival and or the genetic constitution of subsequent generations (Moriarty 1969). Through these effects, sublethal stress can have several fitness consequences. Sublethal effects of insecticides can affect the number of eggs produced, oviposition period, development period, larval and pupal weight, adult emergency, fertility, and longevity but also the behaviour and physiology of insects (Lee 2000, Piironen *et al.* 2013b, de França *et al.* 2017). Because of their effects on fitness, these sublethal stressful conditions can also be extremely effective in shifting the means of the traits by imposing directional selection (Hoffmann and Hercus 2000). Sublethal insecticide stress can have different fitness consequences depending on the response to the stress.

Three different stress responses have been described previously: negative linear, threshold, and hormetic response (Fig. 1) (Costantini *et al.* 2010). The negative linear model assumes that fitness is decreasing with increasing levels of stress (Fig. 1a). Exposure to sublethal insecticide has been shown to have negative consequences on survival, fecundity, growth, and reproduction (Wang *et al.* 2008, Bao *et al.* 2009, Henry *et al.* 2012, Sandrock *et al.* 2014). The threshold model assumes that low sublethal doses have no effect on fitness until a certain threshold level of stress is met, after which it starts to decrease (Fig. 1b). Lastly, there are hormetic responses, where low sublethal doses induce stimulatory positive effects on fitness but are lethal at higher exposure levels (Costantini *et al.* 2010, Guedes and Cutler 2014). Within the same species, different insecticides can also induce different dose-responses on fecundity (Bao *et al.* 2009).

There is also evidence that some environmental stressors can have beneficial effects at low levels of stress (Cutler *et al.* 2009, Calabrese and Blain 2011, Cutler 2013, Piironen *et al.* 2013b, Dickel *et al.* 2018). The hormetic effects have been commonly less than 2-fold fitness increase of the control group and can affect different traits, such as growth, metabolism, immune response, survival, and reproduction (Calabrese and Blain 2011). The size of the effects is constrained by the level of biological plasticity. Hormetic responses can be mediated via cell and/or receptor signalling pathways, changes in stress or antioxidant responses, DNA repair capacity, hormone systems or brain function, and epigenetic changes (Vaiserman 2011, Calabrese 2013). Hormetic effects might be stronger when organisms are exposed in their early life stages

as, for example, epigenetic changes can have life-long effects (Vaiserman 2011). Increased fitness due to hormetic effects can be adaptive because the genes coding for the hormetic effects will be selected during evolution (Forbes 2000). This could also mean that not only, insecticide selection but also hormetic effects, can facilitate insecticide resistance. As for already resistant individuals, sublethal doses might induce hormetic effects and thus these genotypes/phenotypes will be selected during evolution.

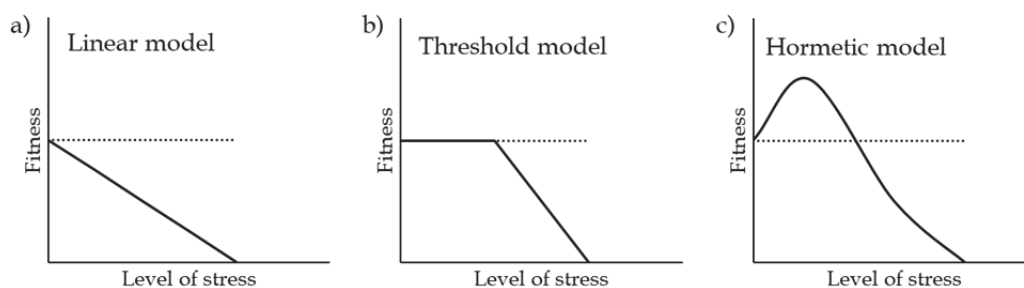


FIGURE 1 Examples of how increasing levels of stress can affect fitness (adapted from (Costantini *et al.* 2010). a) Linear model, where fitness is decreasing with increasing levels of stress compared to the control; b) threshold model, where fitness does not change until a threshold value after which fitness starts to decline with increasing levels of stress when compared to the control; and c) hormetic model, where low levels of stress increase fitness, while decreasing it at high levels when compared to the control. A dashed line represents the control (i.e. no stress exposure).

1.4 Multiple stressors

Although anthropogenic environments are often considered very simple due to the simplicity of biological interactions within them, pests can be exposed to multiple chemical stressors simultaneously. Besides insecticides, pest species can be exposed to other chemicals used in agriculture, such as herbicides and fungicides (Boyer *et al.* 2006, Lajmanovich *et al.* 2011, Hahn *et al.* 2014). Herbicides are applied up to 15 times more (i.e. in terms of tons of active ingredient) than insecticides (FAO 2016). Glyphosate is the most commonly used active ingredient in herbicides and has been claimed to be safe for animals and humans because it inhibits the shikimate pathway that is found in plants and microbes (Duke and Powles 2008, Duke 2018). However, recent evidence has raised doubts about its safety (Kishore and Shah 1988, Bentley and Haslam 1990, Helander *et al.* 2012). Many studies have shown that glyphosate and its metabolites can have various toxic effects on living organisms, such as changes in physiology, survival, behaviour, and cell functioning (Mesnage *et al.* 2015). Glyphosate is also known to alter oxidative biomarker status, by interfering with the antioxidant system or increasing the production of ROS (Webster and Santos 2015).

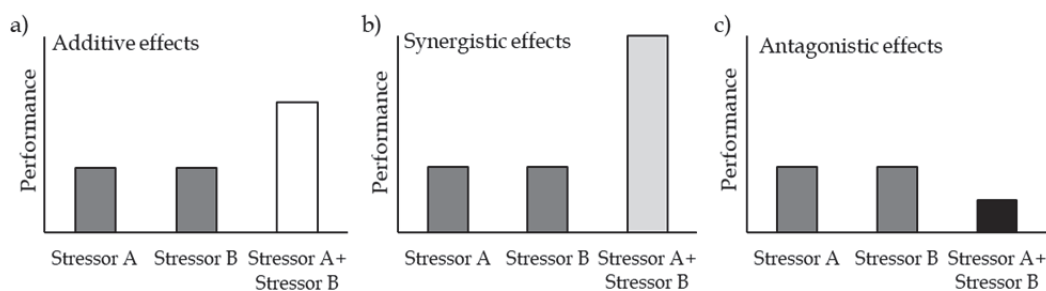


FIGURE 2 Examples of additive and interactive effects. a) Additive effect (white), b) synergistic effect (light grey), and c) antagonistic effect (black) of stressors A and B on performance (adapted from Todgham & Stillman (2013)).

Chemicals that are considered safe can also have interactive effects together with harmful chemicals (Cedergreen 2014). Exposure to multiple stressors can have one of three types of effects: additive, synergistic, or antagonistic (Fig. 2) (Todgham and Stillman 2013). Additive effects occur when exposure to two stressors has a similar effect as the sum of their individual effects (Fig. 2a). These effects occur when two stressors do not interact with each other. Synergistic effects occur when two stressors together have a greater effect than the sum of their individual effects (Fig. 2b) (Piggott *et al.* 2015). Synergistic effects are considered a worst-case scenario for ecosystem management, as even small changes in a single stress response can enhance an individual's performance in an unpredictable way (Todgham and Stillman 2013). Synergistic effects can occur, for example, when exposure to one stressor makes the individual more vulnerable to the other. To illustrate, multiple stressors interacting synergistically have been suggested to drive the ongoing bee colony losses (Potts *et al.* 2010, Goulson *et al.* 2015). An antagonistic effect occurs when two stressors together have a smaller effect than the sum of their individual effects (Fig. 2c) (Folt *et al.* 1999). Exposure to one stressor can mitigate the effect of the other stressor (Folt *et al.* 1999, Crain *et al.* 2008). Alternatively, antagonistic effects can be driven by the dominant stressor, if the effect of the stronger stressor overrides the effect of the weaker stressor (Folt *et al.* 1999, Sala *et al.* 2000). Interactive effects are difficult to predict from the research done with single stressors, due to their non-linear effects.

Extensive research on marine and freshwater systems has shown that interactive (i.e. antagonistic and synergistic) effects are more common than additive effects (Crain *et al.* 2008, Darling and Côté 2008, Piggott *et al.* 2015, Jackson *et al.* 2016). Also, different pesticides have been found to have interactive effects (Pilling and Jepson 1993, Schmuck *et al.* 2003, Anderson and Zhu 2004). For example, exposure to atrazine herbicide was not acutely toxic to the midge (*Chironomus tentans*), but had synergistic interactions with chlorpyrifos, diazinon, and methyl parathion insecticides and thus increased their toxicity (Pape-Lindström and Lydy 1997, Belden and Lydy 2000). Another study found that atrazine herbicide had synergistic effects together with several insecticides, which correlated with increased inhibition of AChE

activity in the midges (*Chironomus tentans*) (Anderson and Zhu 2004). In addition, exposure to atrazine herbicide and chlorpyrifos insecticide led to a decrease in antioxidant enzyme activities (Xing *et al.* 2012). Since interactive effects are difficult to predict from studies investigating single stressors, it is important to investigate possible interactive effects as they can contribute to invasion success. While negative synergistic effects are expected to decrease stress tolerance, antagonistic effects can be advantageous, as exposure to one stressor can make the individual more tolerant to the other stressor. Moreover, it is important to identify the possible mechanisms by which exposure to one stressor could modify the response to another stressor and thus have interactive effects (Todgham and Stillman 2013).

1.5 Transgenerational effects

Sublethal insecticide stress can induce changes in parents that can be transmitted to their offspring. It is well known that parental phenotype and genotype can affect offspring phenotype (Mousseau and Fox 1998, Bonduriansky and Day 2009). There could be several epigenetic mechanisms, also in addition to maternal effects, that could underlie these transgenerational effects (Mousseau and Fox 1998, Brevik *et al.* 2018). For example, epigenetic effects are heritable phenotypic changes that could involve changes in gene expression but also suppression and silencing of the genes (Davis 2009). Thus parental effects can directly stimulate the expression of advantageous phenotypes or lead to hormetic priming, and thus drive evolution (Brevik *et al.* 2018). Besides epigenetic effects, parents can provide a range of inputs that contribute to offspring fitness and development through direct and/or indirect resource provisioning. These inputs can affect egg nutrition, amount of yolk, enzymes, and mRNA (Mousseau and Fox 1998, Grindstaff *et al.* 2003, Bonduriansky and Day 2009, Badyaev and Uller 2009, Shea *et al.* 2011).

Parental effects can be adaptive and persist in a population for generations if they increase the fitness (i.e. survival or reproduction) of their offspring (Marshall and Uller 2007). Adaptive evolution requires that parents provide a source of information about the future that makes their offspring better suited to their environment (Shea *et al.* 2011). Insecticides are commonly used to control invasive pest species and thus transgenerational effects can be mediated via similar stressors in both generations. Exposure to sublethal insecticides could have both positive and negative within- and transgenerational effects. For example, parental exposure to sublethal (LC₂₅) doses of chlorantraniliprole (i.e. ryanoid) insecticide had negative within- generational effects on diamondback moths (*Plutella xylostella*) but also induced negative effects in their offspring that were never directly exposed to the insecticides (Guo *et al.* 2013). Such effects can have drastic effects on populations and invasion success. Exposure to sublethal concentrations of imidacloprid (i.e. neonicotinoid) insecticide-induced positive

transgenerational responses on the green peach aphid (*Myzus persicae*) and as a result total reproduction was doubled after four generations when compared to the control group (Ayyanath *et al.* 2013). Indeed, hormetic responses could act as a mechanism that allows organisms to cope better with subsequent exposure to a similar stressor (Calabrese *et al.* 2007). In addition, other studies have confirmed that stress-induced effects can often persist for many generations through parental inheritance and thus accelerate adaptive evolution (Badyaev 2005).

Environmental conditions can also differ between generations as parents and offspring can be exposed to different stressors. A reduction in parental environmental quality could lead to selfish maternal effects, anticipatory maternal effects, or bet-hedging maternal effects (Marshall and Uller 2007). In the case of selfish maternal effects, exposure to stress can lead to reductions in offspring quality and performance as maternal fitness is increased at the expense of offspring fitness (Marshall and Uller 2007). Anticipatory maternal effects suggest that mothers adjust the phenotype of their offspring according to local environmental conditions to maximise offspring fitness (Fox *et al.* 1997, Agrawal *et al.* 1999). A meta-analysis on that topic suggested there was overall weak evidence for anticipatory maternal effects, however, the effect was stronger when the environment matched between the parents and offspring (Uller *et al.* 2013). As a third option, mothers with a bet-hedging strategy produce offspring with a wide range of phenotypes, to increase the probability of offspring survival and thus maximise their own fitness (Kudo 2001; Marshall & Uller 2007). This strategy can be induced when the mother experiences varying or unpredictable environmental conditions (Crean and Marshall 2009).

One bet-hedging strategy could be diversified bet hedging, for example, diapause duration. Diapause is an adaptation that allows individuals to skip environments they are poorly adapted to (Danks 1987). Moreover, prolonged diapause occurs when individuals skip one or more breeding seasons (see e.g. (Danks 1987, Hanski 1988). For those species that have prolonged diapause, up to 68 % of individuals can prolong their diapause, most often for 2 years (Danks 1987, Hanski 1988, Menu and Debouzie 1993). Prolonged diapause allows individuals to skip environmental conditions they are poorly adapted to and thus act as a buffer against stochastic environmental conditions or poor breeding seasons and ensure the survival of the marginal population (Takahashi 1977, Mahdjoub and Menu 2008, Salman *et al.* 2016). Definitions of diapause emphasize that diapause is programmed to arrest development and reproduction (Tauber and Tauber 1976, Danks 1987, Saunders 2002). Studies comparing diapausing to non-diapausing individuals have found that diapause has no or positive effects on reproduction and fertility (Jansson *et al.* 1989, Senanayake *et al.* 2000). Still, additional age-related effects, and effects on offspring fitness and stress tolerance caused by prolonged diapause have received less attention. Parental age has been shown to affect offspring fitness and viability. For example, maternal as well as grand-maternal age has a

negative effect on progeny viability in *Drosophila serrata* (Hercus and Hoffmann 2000). Similar maternal age effects have been found before, known as the 'Lansing effect', which means that an older mother produces short-lived offspring ((Priest *et al.* 2002) and references therein). Besides affecting offspring viability and longevity, maternal age can also affect offspring sensitivity to environmental stress. For example, older red flour beetles (*Tribolium castaneum*) produced offspring with lower starvation tolerance (Halle *et al.* 2015). Additionally, environmental stressors were shown to alleviate the effects of maternal age in the *Drosophila serrata* (Hercus and Hoffmann 2000). The authors suggested that increased larval development time in the stress environment, which allowed the offspring to obtain more resources and thus counter the negative effects of the maternal age.

Besides mothers, fathers can also affect offspring fitness - these are called paternal effects (Crean and Bonduriansky 2014). Fathers can affect offspring via many non-genetic factors (cytoplasmic or epigenetic), proteins or lipids transferred during ejaculation of semen (Avila *et al.* 2011, Kumar *et al.* 2013). However, paternal effects are usually suggested to be smaller than maternal effects, as sperm contributes to a smaller quantity of cytoplasm to the zygote than eggs (Priest *et al.* 2002). Also, paternal effects are often mediated via maternal effects (Crean and Bonduriansky 2014), which can possibly mitigate negative paternal effects. Few studies that have been investigating both maternal and paternal effects have concluded that maternal effects have a larger influence on offspring than paternal effects (Butz and Hayden 1962, Priest *et al.* 2002). There is also evidence that paternal effects can be stronger than maternal effects when exposed to increased temperatures because high temperatures can cause DNA damage and a reduction in fertilization (Guillaume *et al.* 2016) that mothers cannot mitigate.

1.6 Aims of the thesis

The objective of my thesis was to examine how exposure to anthropogenic stressors (i.e. insecticides and herbicides), multiple stressors and transgenerational effects the life-history, physiological, or genetic traits associated with fitness and stress tolerance in the Colorado potato beetle (*Leptinotarsa decemlineata*). The Colorado potato beetle (*Leptinotarsa decemlineata*, Say.) is an invasive pest of potato that originated from Mexico (Alyokhin *et al.* 2012), or from the USA (Izzo *et al.* 2017). Adaptation to cultivated Solanaceous plants has allowed the species to invade North America, Europe and Western Asia (EPPO 2006, CABI 2018) and with the help of global warming and host availability, the beetle is expected to invade further towards northern Europe, China, Australia, South Africa, and North America (Wang *et al.* 2017). As a pest species, the beetle has been under insecticide control and has currently developed resistance to more than 50 active ingredients used in insecticides

(Mota-Sanchez and Wise 2018). The first aim of my thesis was to understand the role of the organophosphate (OP) and carbamate insecticide target sites between two populations that differ in their resistance to these insecticides (study I). Insecticides are known to act on specific target sites and acetylcholinesterase is the target for two commonly used organophosphate and carbamate insecticide classes. I investigated the role of the acetylcholinesterase genes (*Ldace1* and *Ldace2*) by comparing two populations that differ in their resistance to carbamate and organophosphate (OP) insecticides. Besides comparing the two populations I also studied the effects of sublethal doses of these insecticides on the acetylcholinesterase genes. So far, resistance in the CPB has been based on non-synonymous point mutations (S291G, R30K) in the *Ldace2* gene (Zhu *et al.* 1996, Zhu and Clark 1997, Piironen *et al.* 2013a). However, the beetle also possesses a *Ldace1* gene, which was recently suggested to be the main target site of the OP insecticides (Revuelta *et al.* 2011). In many species, AChE1, encoded by the *ace1* gene has been described as the main catalytic enzyme and the main target site of the insecticides (Revuelta *et al.* 2011, Lu *et al.* 2012, Kim and Lee 2013).

Besides insecticides, pest species can be also exposed to other chemicals used in agriculture, for example, herbicides. (i.e. herbicides and fungicides). In the second study (II) I aimed to investigate the effects of direct glyphosate-based herbicide (GBH) exposure on survival and oxidative status biomarkers. Previous studies have found that glyphosate and GBH-s can have various toxic effects on non-target species (Gluszczak *et al.* 2006, Gluszczak *et al.* 2007, Samanta *et al.* 2014, Herbert *et al.* 2014). Besides single effects, herbicides could possibly have interactive effects together with insecticides (see Fig. 2). In the third study (III), I investigated whether exposure to indirect GBH via food has interactive effects together with an OP insecticide. I used a full factorial design, to study single, as well as possible interactive effects, of these two chemicals on survival, acetylcholinesterase gene expression, and oxidative status biomarkers. Previous studies have shown that atrazine herbicide can increase the toxicity of the insecticides while having no effect alone (Pape-Lindström and Lydy 1997, Belden and Lydy 2000).

The effects of insecticide stress can be complicated by the stressors and environmental conditions experienced by the previous generations (Parsons 1991, Piironen *et al.* 2013b). In the last two studies (studies IV, V) I aimed to investigate transgenerational effects on offspring fitness and stress tolerance. In particular, the aim of the fourth study (IV) was to investigate the effects of prolonged diapause on female and male fertility, and offspring fitness-related traits and stress tolerance. The previous studies comparing the diapausing CPBs to non-diapausing beetles have found that diapausing beetles show either increased fertility or no effects on fertility (Peferoen *et al.* 1981, Jansson *et al.* 1989). However, the transgenerational effects of prolonged diapause in this species are poorly understood. Prolonging diapause can be costly due to losses of metabolic resources, additional mortality and loss of reproductive

opportunities (Danks 1987, Matsuo 2006). Yet, prolonging diapause would allow species to skip poor environmental conditions they are poorly adapted to and can thus ensure the survival of the marginal population (Mahdjoub and Menu 2008, Salman *et al.* 2016).

The aim of the fifth study (V) was to investigate the transgenerational effects of insecticide exposure. Exposure to insecticide stress can lead to epigenetic and phenotypic modifications that can be transferred into the subsequent generation and may contribute to the rapid evolution and invasion success of the invasive species (Brevik *et al.* 2018). Exposure to stress can increase similarity between generations and make offspring better fitted to their environment and thus accelerate adaptive evolution (Badyaev 2005). On the other hand, exposure to stress can be costly for the parental generation and thus result in decreased offspring performance (Marshall and Uller 2007), which can also make the offspring more vulnerable to within-generational stress exposure.

2 MATERIALS AND METHODS

2.1 Study animal and rearing conditions

The study species used in this thesis is the invasive Colorado potato beetle, *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). This species is a notorious pest of potato (*Solanum tuberosum*) (Alyokhin *et al.* 2012). The Colorado potato beetle (henceforth CPB) is considered native to Mexico (but see (Izzo *et al.* 2017)) where it mainly feeds on buffalo bur (*Solanum rostratum*) (Hsiao 1981, Casagrande 1987). However, adaptation to potatoes has allowed this species to rapidly spread and increase its distribution, and the CPB is currently damaging potato crops all over Europe, North America, and is spreading towards Asia (Weber 2003, CABI 2018). Moreover, with global warming and host availability, the beetle is expected to increase its geographic range towards northern Europe, China, Australia, South Africa, and northern America (C. Wang *et al.* 2017).

The life cycle of the CPB consists of four stages, as in all insects with complete metamorphosis. The beetle has four larval instars after which they burrow into the soil to pupate (Boiteau and Le Blanc 1992). Adult beetles usually emerge from soil 1 - 2 weeks later (Boiteau and Le Blanc 1992). Whether the adults start to reproduction or prepare for diapause depends on the day length at adult emergence (Hsiao 1981, Lehmann *et al.* 2012, Lehmann *et al.* 2014). Reproduction is induced by long day photoperiods and diapause by the critical short photoperiod which varies with latitude (Senanayake *et al.* 2000, Lehmann *et al.* 2015). Diapause is a very important adaptation of the CPB as it allows it to skip harsh winter conditions. The CPB has facultative reproductive adult diapause (Alyokhin *et al.* 2012). The decision to enter diapause in the CPB is initiated by the critical short photoperiod but also by decreasing temperature, and changes in food quality (De Wilde *et al.* 1959, Voss *et al.* 1988, de Kort 1990, Lehmann *et al.* 2012). Diapause as mentioned above is terminated in the CPB when temperatures rise above 10 °C (de Kort 1990). However, individuals can

prolong their diapause for more than a year, and even up to 9 years (Tauber and Tauber 2002). Due to the long diapause occurrence, the CPB is suggested to be an excellent study species to study ageing (Peferoen *et al.* 1981). Beetles start oviposition within 4 - 6 days after diapause termination (Peferoen *et al.* 1981). Adult females are highly productive and can lay between 300 and 800 eggs ((Harcourt 1971), pers. obs.). Moreover, the species can be multivoltine in more southern latitudes (Boiteau and Le Blanc 1992). This complicated life-history together with its high reproduction rate makes the CPB a challenging pest to control (Alyokhin *et al.* 2008). Thus, the CPB has been under pesticide control (Alyokhin *et al.* 2008). Extensive use of pesticides and intensive pesticide selection pressure has led to the development of insecticide resistance. The CPB has currently developed resistance to more than 50 active ingredients used in insecticides (Mota-Sanchez and Wise 2018).

I used six different beetle populations collected in potato fields near Vermont (USA: 44° 43' N, 73° 20' W), Belchow (Poland: 52° 01' N, 20° 34' E), Padua (Italy: 45° 48' N, 12° 07' E), Emmen (Netherlands: 52° 54' N, 6° 51' E) in 2010, Ufa (Russia; 54° 44' N, 56° 00' E,) in 2009, and Petroskoi (Russia; 61° 49' N, 34° 10' E) in 2006. For the rearing of the lab population, unrelated beetles in each generation (overwintered generation) were mated randomly within the population and each pair, i.e. family, was reared separately in a Petri dish (Ø 92 mm, Sarstedt, Germany) lined with moisturized filter paper and fed daily with fresh potato leaves (*Solanum tuberosum* var. Van Gogh I, V and var. Challenger, II-IV). Pairs were checked daily for eggs and for egg-hatching, and larvae were reared in family groups until adulthood. Larvae (15 - 30 larvae per family) were reared on whole potato plants in the greenhouse at 23 °C or in climate chambers. Beetles were maintained at a constant temperature of 23 °C and under a long day regime of 18 h light and 6 h dark in the controlled climate chambers (Type B1300, Weiss technic, Reinkirchen-Lindenstruth, Germany until 2016 and from 2017 onwards Growth Chamber FH-1300, Hi-Point, Taiwan). Emerging adults were sexed (female, male) and weighed (\pm 0.1 mg, AM100, Mettler Toledo, USA), and were thereafter grown individually under short day conditions of 12 h light and 12 h dark to initiate diapause (Lehmann *et al.* 2012). After the adults stopped eating, at around 10 days old, they were transferred into jars containing moistened peat and overwintered individually. Overwintering was initiated and terminated by increasing or decreasing the temperatures gradually by 5 degrees in two-week intervals and finally at 5 °C dark in the climate chambers. Overwintering mortality in our laboratory populations has been around 30 %, which should minimize the overwintering selection of the beetles in the laboratory (Lehmann 2013).

2.2 Anthropogenic stressors

2.2.1 Insecticide bioassay (study I)

Bioassays were conducted to determine the population resistance to insecticides (see (Ovčarenko *et al.* 2014)). I wanted to find the population resistance levels for two commonly used insecticides azinphos-methyl (AZ, ChemService, West Chester, USA) and carbaryl (CAR, Sigma-Aldrich, Missouri, USA) to investigate the role of two acetylcholinesterase genes in the insecticide resistance of the CPB. AZ belongs to organophosphate- and CAR to a carbamate insecticide sub-group, nevertheless both are known as acetylcholinesterase (AChE) inhibitors (IRAC 2018). For the bioassays, I used six different CPB populations (Belchow, Emmen, Padua, Petroskoi, Ufa, and Vermont). To investigate the population sensitivity to insecticides, 95 - 260 larvae per population were divided and exposed to 4 - 8 different insecticide concentrations, ranging from 0.0075 - 30 µg for AZ and 0.06 - 90 µg for CAR. Based on the bioassay results, Vermont and Belchow populations were selected for further studies as they differed significantly in their resistance to both AZ and CAR insecticides.

2.2.2 Insecticide treatment

To study the effects of sublethal insecticides, late second or early third instar larvae were exposed to three different insecticides AZ (studies I, III-IV), CAR (study I) and Decis (study V). I used two different insecticide application techniques: topical application and filter paper technique. In the topical application technique (studies I, III, IV), 3 µl of insecticide was applied topically to the dorsal abdominal segment. The insecticides and concentrations used in the studies were 0.375 or 0.0075 µg of AZ and 1.5 or 0.15 µg of CAR for Vermont and Belchow population (study I), 0.75 µg, and 0.369 µg of AZ (study III and study IV). I used 3 µl of acetone as a control (studies I, III, IV) because insecticides were diluted in acetone.

In the filter paper technique, 1 ml of insecticide solution was pipetted onto filter paper (Ø 70 mm, grade 1002) on a Petri dish and allowed to absorb before the larvae in family groups were placed on the filter paper (study V). Larvae were exposed to either 1 ml of 1.59 µg Decis, pyrethroid insecticide solution (Decis, Aventis CropScience, Copenhagen, Denmark) or 1 ml of acetone as a control. The filter paper technique allowed the treatment of many larvae at the same time, while the topical application is a good technique when treating only a few larvae at a time because it allows the use of smaller amounts of insecticide.

2.2.3 Direct and indirect herbicide treatment

To study the effects of glyphosate-based herbicide (GBH) on a non-target species I used two different exposure treatments, direct and indirect exposure. The CPB can be exposed to direct GBH, for example via herbicide wind drift or when it is applied to the field nearby potato fields (Felix *et al.* 2011), or to indirect GBH via food, as the plants can uptake glyphosate from the soil (Helander pers. comm.). In case of direct GBH exposure (study II), I pipetted 3 μ l of the GBH (Roundup Bio, glyphosate concentration 360 g/l, Monsanto, USA) on the dorsal abdominal segment on second instar larvae. Larvae were divided equally between three different treatments and five time periods (2 h, 24 h, 48 h, 72 h, and 96 h). The three treatments were: 1) high herbicide concentration (100 %), 2) low concentration (1.5 %), and 3) control group (water). The 1.5 % herbicide concentration was chosen based on manufacturer recommendation as this is the concentration used in the fields for weed control.

In the indirect herbicide exposure group, 1- day old larvae were reared on potato (var. Van Gogh) grown in GBH exposed soil (Study III). For that, I sprayed the soil with GBH (Roundup Bio) following the manufacturer's instructions (4 l/ha). Eleven days after spraying I planted the potatoes into GBH exposed or control soil. Potatoes were grown in natural conditions in Jyväskylä, Finland (62° 13' N; 25° 44' E). Thus, larvae were exposed to GBH via food intake.

2.3 Life-history traits and physiological measurements

2.3.1 Life-history traits (Studies I, II, III, IV, V)

Life-history traits are traits that affect an organism's survival and reproduction. In my thesis, I studied the effects of insecticides on various traits. For example, I recorded survival in different life stages (larvae, pupa, and adult stage; studies I-V) and larvae-to-adult survival (study IV) after exposure to different insecticides. I calculated larvae-to-adult development time (in days; studies IV, V), and measured emergence body mass (mg; studies IV, V), body mass at the age of 7, 10 and 14 days (mg; studies IV, V), body mass before mating (mg; study IV), and recorded mating success (study IV). I also estimated offspring production by counting the number of egg batches, the number of eggs, and the number of larvae hatched (study IV). All these traits were chosen because they can be important for the fitness and invasion success of the CPB (Piiroinen *et al.* 2011, Piiroinen *et al.* 2013b, Piiroinen *et al.* 2014). Life-history traits such as the number of eggs laid and the number of larvae hatched affect reproduction directly, while body mass and development time (i.e. time to maturity) have been shown to affect reproduction, survival and overwintering survival

(Piiroinen *et al.* 2011; Honěk 1993). However, both of these can be important measures of fitness and thus affect the invasion success of the CPB.

2.3.2 Remaining AChE enzyme activity (Study I)

I measured the AChE enzyme activity to investigate the population sensitivities to insecticides. AChE enzyme activity was measured from individual larvae. For the measurement, a larva was put into an Eppendorf tube containing 200 μ l of ice-cold 1 x PBS buffer (pH 7.5) containing 0.5 % (v/v) Triton X-100. Samples were homogenized by crushing the samples for 2 - 3 minutes with the homogenizing stick and sonicated for 40 seconds, thereafter centrifuged at 13000 rpm for 20 minutes at 4 °C. Protein concentration was measured with NanoDrop ND - 1000 spectrophotometer (NanoDrop Technologies, USA). All samples were normalized to a protein concentration of 15 mg/ml. Normalized samples were used for the enzyme activity inhibition analysis. AChE enzyme activity (%) was determined against the control by using improved Ellman *et al.* (1961) method, following the instructions described in the QuantiChrom™ Acetylcholinesterase Assay Kit (DACE - 100; BioAssay Systems, USA). Both insecticides were obtained from Pestanal analytical standard from Sigma-Aldrich (Missouri, USA). Insecticide stock solutions (in ethanol ~100 %) were diluted in water to concentrations of 250 μ M and 12.9 μ M for AZoxon and CAR respectively so that the concentration of the ethanol in the final solution was < 1 %. Then the reaction mixture of 15 μ l supernatant and 2 μ l of the enzyme inhibitor was added to the 190 μ l of Working Reagent. This reaction mix was incubated at RT for 2 minutes. After that, I measured absorbance at 2 and 10 minutes at 405 nm with Victor X4 2030 multiplate reader (PerkinElmer, USA). Samples were measured in triplicates.

2.3.3 Oxidative stress biomarker status measurements (Studies II, III, IV)

I used larval homogenates to measure several oxidative status biomarkers, glutathione S-transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) homologs, catalase (CAT), glucose-6-phosphate dehydrogenase (G6PDH), total glutathione reduced (totGSH), glutathione reduced-oxidized (GSH:GSSG), superoxide dismutase (SOD), and oxidative damage (lipid hydroperoxides) in the CPB (studies II, III, IV). I studied the homologous enzymes for three markers GST, GPx, and GR since insects do not have these enzymes. However, since these markers seem to be very similar I measured the activities using the same kits as the original ones.

I used pooled larvae (studies II, III) and adult (study IV) samples for the oxidative status biomarker measurements. Pooled larvae samples contained larvae of the same age and were pooled within a family, treatment, and replicate. Adults were analysed individually (study IV). All oxidative status biomarkers were measured in triplicates so that the intra-assay coefficient of variability (i.e. CV %) was lower than 15 % using 96 - well (CAT, lipid

hydroperoxides) or 384 - well (G6PDH, GPx, GR, totGSH, GSH:GSSG, GST and SOD) plates with an EnVision microplate reader (PerkinElmer, Finland) as described previously by Rainio *et al.* (2013, 2015). For inter-assay precision, I included three control samples on each plate using the range of 0.8-1.2. Before measurement, all samples were homogenized in KF buffer (0.1 M K₂HPO₄ + 0.15 M KCl; pH 7.4). To minimize the sample volume, I used smaller reagent volumes than recommended in the manufacturer's instructions.

I measured G6PDH according to the protocol described by Noltmann *et al.* (1961). I measured GPx, SOD and GR, using the kits from Sigma (Sigma Chemicals, USA) by following the manufacturer's instructions. I measured the ratio of GSH: GSSG and totGSH, using ThioStar® Glutathione Fluorescent Detection Kit (K005-FI, Arbor Assays, USA). From all the samples I also measured the total protein concentration (mg/ml), using BCA (bicinchoninic acid) according to the Smith *et al.* (1985), with an EnVision microplate reader at an absorbance of 570 nm. Protein concentrations were estimated by using BSA (bovine serum albumin, Sigma Chemicals, USA) as a standard.

Samples for lipid hydroperoxides measurement were homogenized in 125 µl methanol using 1 - 2 larvae per homogenate (depending on the weight of the larvae). Lipid hydroperoxides were measured using the FOX-2 method with some modifications (Nourooz-Zadeh *et al.* 1996, Bou *et al.* 2008). I used 45 µl of the sample and 5 µl 10 mM thiamine pyrophosphate (TPP), or methanol, and 950 µl of FOX reagent (Vuori *et al.* 2015). Before adding the FOX reagent, samples were incubated in TPP for 30 min and further incubated for 2h before measurement. A standard was prepared using cumene hydroperoxides at different concentrations (0/8/16/32/64/96/128/160 mM) (Sigma Chemicals, St. Louis, USA). Samples were measured with an EnVision plate reader in triplicate (CV < 10 %, in all cases). The results were normalized by the weight of the body mass of the used sample.

2.3.4 Relative lipid and water content measurements (Study V)

The amount of lipid reserves is the primary source of energy during diapause (Hahn and Denlinger 2007). The CPB should have acquired and stored a sufficient amount of lipid reserves by the age of 10 days (de Kort 1990, Piironen *et al.* 2011, Lehmann *et al.* 2012). Total lipid content and water content were measured from the adult beetles at the age of 14 days to investigate whether within- and/or transgenerational insecticide stress exposure has any effect on the energy reserves (study V). The Folch method (1957) was used to estimate the total lipid content, with modifications by Lehmann *et al.* (2012). I first weighed (fresh weight) the adult beetles, then dried them for 72 h at 55 °C (pre-extraction weight) and reweighed them again. Thereafter, lipids were extracted by placing the dried beetles into 20 ml glass vials filled with 10 ml of chloroform: methanol solution (ratio 2:1) for 72 h at the RT. After that beetles were dried for another 72 h at 55 °C (post-extraction weight). Relative lipid content (%) was calculated by subtracting post-extraction weight from pre-

extraction weight and dividing it by fresh weight. Relative water content was found by subtracting pre-extraction weight from fresh weight and dividing it by fresh weight. Dry mass was calculated by dividing the post-extraction weight by the fresh weight.

2.4 Genetic analyses

2.4.1 Population genetics (Study I)

The differences in the two acetylcholinesterase genes (*Ldace1* and *Ldace2*) were investigated in two populations: Vermont (USA) and Belchów (Poland). I adopted basic genetic methods. To begin this process, total RNA was extracted from 38 whole larvae ($N_{\text{Vermont}}=19$; $N_{\text{Belchów}}=19$). RNA was extracted using the TriReagent (Sigma, Aldrich, USA) and RNeasy Mini Kit (Qiagen, Germany). RNA extraction was followed by a DNase treatment, which is recommended for applications that are sensitive to small amounts of DNA (i.e. RT-qPCR). RNA purity and concentration were measured with a NanoDrop ND-1000 (NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, USA)). RNA quality and integrity were measured with an Agilent 2100 Bioanalyzer (Agilent, USA). Thereafter, all RNA samples were diluted to the same final concentrations 100 ng/ μl . Complementary DNA (i.e. cDNA) was synthesized from 100 ng of RNA using an iScript cDNA synthesis kit (Bio-Rad, USA) following the manufacturer's instructions. Isolated cDNA was used for sequencing. Primers were designed for the AChE genes based on sequence available in Genbank (Ace1: JF343436.1; Revuelta et al. 2011) (Ace2: L41180.1; Zhu & Clark, 1995) as described by Lehmann et al. (2014). Polymerase chain reactions (PCR) were performed in a 25 μl reaction, containing 4 μl of cDNA, 1 x Dream buffer (Thermo Fisher Scientific, USA), 1 μM of forward and reverse primer, 0.2 mM dNTPs, and 0.2 U of Dream Taq polymerase (Thermo Fisher Scientific, USA). Thermal cycling conditions were 94 °C for 3 min, then 35 cycles at 94 °C for 45 s, 56 °C for 1 min 30 s, and 72 °C for 1 min 30 s. PCR products were purified using exonuclease (Exonuclease I, Thermo Fisher Scientific, USA) and shrimp alkaline phosphatase (SAP, Thermo Fisher Scientific, USA), or by extracting PCR products from the 1 % agarose electrophoresis gel. Sequencing reactions were performed using a BigDye Terminator v3.1 Cycle sequencing kit (Thermo Fisher Scientific, USA). Sequencing reactions were performed in a 20 μl reaction, containing 3-10 μl of purified PCR product, 3.75 μl of 5 x BigDye Sequencing buffer, 0.5 μl of 2.5 x Ready Reaction Premix, and 1 μl of 3.2 μM primer. Sequencing reaction was purified with ethanol/EDTA/sodium acetate precipitation and diluted in formamide. Thereafter samples were run in the 16- capillary ABI Prism 3130xl genetic analyser (Thermo Fisher Scientific, USA).

2.4.2 Quantitative real-time PCR (Studies I, III)

The differences in the *Ldace1* and *Ldace2* genes between the two populations (I), GBH and insecticide treatment groups (III), were estimated using quantitative real-time PCR (qPCR). First, the total RNA was extracted from whole larvae as described earlier. The qRT-PCR reactions were performed using SYBR Green Supermix kit (Bio-Rad, USA). The qRT-PCR reactions were performed with a total volume of 20 μ l, by using 5 μ l of cDNA (diluted 1:4), 10 μ l of 2 x SYBR Green Supermix, 0.5 μ M of forward and 0.5 μ M of reverse primers, and 3 μ l of nuclease-free water in study I. The qPCR reactions were performed with a total volume of 20 μ l, by using 5 μ l of cDNA (diluted 1:4), 10 μ l of 2 x SYBR Green Supermix, 1 μ l of forward and 1 μ l of reverse primers, and 3 μ l of nuclease-free water in study III. The qPCR reactions were run with a Bio-Rad CFX96 instrument. Cycling conditions were the same for both studies 95 °C for 3 min, followed by 40 cycles of 95 °C for 10 s, 56 °C for 10 s and 72 °C for 30 s. The melt curve was measured from 65 °C to 95 °C to check the purity of the qPCR reaction. Ten (individual larvae) (study I) and eight biological replicates (study III) with three technical replicates were included for each population and/ or treatment group. Positive pooled samples were included for inter-run calibration. Amplification efficiencies were calculated using 2-fold serial dilutions of pooled cDNA samples.

Primers used for qPCR were designed based on sequences of an annotated transcriptome of CPB (Kumar *et al.* 2014) and sequences available in Genbank (*Ldace1*, JF343436.1; (Revuelta *et al.* 2011); *Ldace2*, L41180.1; (Zhu and Clark 1995)) using Primer 3 according to Lehmann *et al.* (2014).

2.4.3 Bi-PASA genotyping (Study III)

Bi-PASA (i.e. bi-directional PCR amplification of specific allele) is a genotyping method designed by Clark *et al.* (2001) to detect the S291G mutation in the CPB. The mutation results in a serine to glycine amino acid change in the *Ldace2* gene and is associated with increased AChE and with decreased insensitivity to OP and carbamate insecticide in the CPB (Zhu *et al.* 1996). Bi-PASA genotyping method is used to detect this mutation and it employs four different primers, two allele-specific primers at the mutation site and two outer primers that are allele-nonspecific. I obtained all the primers for the bi-PASA method from Clark *et al.* (2001). I extracted DNA from the adult legs with a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Germany) reagents and Kingfisher magnetic particle processor. PCR mixture and thermal cycle programs were prepared according to the procedure of Clark *et al.* (2001).

3 RESULTS AND DISCUSSION

3.1 The role of the two acetylcholinesterase genes in the insecticide resistance (study I)

For a long time, the CPB was considered to have only one acetylcholinesterase *Ldace2* gene, orthologous to the *Drosophila melanogaster* gene (Zhu and Clark 1995). Its resistance, to both OP and carbamate insecticides, was considered to arise from point mutations (S291G and R30K) in this *Ldace2* gene (Zhu and Clark 1995, Zhu and Clark 1997, Piironen *et al.* 2013a). However, recently it was reported that the CPB also has another acetylcholinesterase *Ldace1* gene, orthologous to *Anopheles gambiae* gene and paralogous to *Drosophila melanogaster* gene (Revuelta *et al.* 2011, Piironen *et al.* 2013a). Moreover, the *Ldace1* gene is more expressed than the *Ldace2* throughout all life stages (embryo to adult) and therefore it was suggested to be the main contributor to AChE activity and could act as a primary target for the OP insecticides (Revuelta *et al.* 2011). I aimed to investigate the role of these genes in two populations that differ in their resistance to OP and carbamate insecticides.

The bioassay results showed that the Vermont population is 107- to 20- times more resistant (i.e. the difference in their resistance levels based on the LD₅₀ dose) to AZ and CAR insecticides than the Belchow population (I). These differences could arise from the three non-synonymous point mutations in the *Ldace2* gene (R30K, Y54H, and S291G) that were previously associated with insecticide resistance (Zhu *et al.* 1996, Zhu and Clark 1997, Piironen *et al.* 2013a). I found that the Vermont population had a higher frequency of a S291G mutation (when both homozygous and heterozygous individuals are included) and it possessed a R30K mutation which was lacking from the Belchow population. These findings were consistent with that of Piironen *et al.* (2013a), who found a higher frequency of a S291G mutation in the USA population (94.7 %) when compared to European populations (71 %). The R30K mutation has been previously found only in the USA population and been suggested to

enhance AChE2 insensitivity in combination with a S291G mutation (Kim *et al.* 2007). I found a Y54H mutation in the Belchow population that was lacking from the Vermont population. The Y54H mutation has been previously found only in the European populations, but its role is unknown (Piiroinen *et al.* 2013a). However, it seemed to occur in the individuals carrying a S291G mutation, thus it could be associated with insecticide resistance.

In addition, I found two non-synonymous mutations G192S and F402Y in the *Ldace1* gene only from the Vermont population. These mutations have not been described for the CPB before. Different species (i.e. *Bemisia tabaci*, *Plutella xylostella*, *Chilo suppressalis*, *Culex tritaeniorhynchus*, *Myzus persicae*, *Culex pipiens*, *Anopheles gambiae*) that are resistant to OP or carbamate insecticides are known to possess similar point mutations in the *ace1* gene that were responsible for target site insensitivity, which makes the individuals more resistant to insecticides (Mutero *et al.* 1994, Walsh *et al.* 2001, Nabeshima *et al.* 2003, Russell *et al.* 2004, Weill *et al.* 2004, Baek *et al.* 2005, Alout *et al.* 2007, Alon *et al.* 2008, Jiang *et al.* 2009). Since both of these mutations occur in the more resistant Vermont population, and it could be hypothesized that these mutations also play a role in the resistance to OP and carbamate insecticides in the CPB. However, their role compared to mutations in the *Ldace2* gene in insecticide resistance needs to be studied further.

Besides target site mutations, overexpression of the target sites can make individuals resistant to insecticides (Gao and Zhu 2002). I found that the *Ldace1* gene was significantly more expressed than the *Ldace2* gene. Second, the *Ldace1* gene is significantly more expressed in the Vermont population when compared to the Belchow population. In general, in insects that possess both AChE-s, the transcription level of AChE1 is 2- to 250- fold higher than of AChE2 in many species, such as *Plutella xylostella*, *Blattella germanica*, *Helicoverpa assulta*, *Pediculus* spp., and *Cimex lectularius* (Baek *et al.* 2005, Kim *et al.* 2006, Seong *et al.* 2012, Kim and Lee 2013). These results suggest that AChE1 encoded by the *ace1* gene might be more important than AChE2 in different species. However, I show for the first time that intensive insecticide use can also select for higher *ace1* expression within species, between different populations. Last, I found that the *Ldace2* gene was similarly expressed in the control group in both populations. However, the *Ldace2* gene is more sensitive to AZ insecticide in Belchow when compared to Vermont.

I found that the organophosphate insecticides can still inhibit AChE activity in both populations. AZoxon inhibited AChE activity more effectively in the Belchow population when compared to the Vermont population. No differences between in AChE inhibition between the populations were detected when inhibited with the CAR insecticide. This could be due to smaller differences between the populations in the resistance to CAR insecticide when compared to AZ or due to the insecticide doses I used. Alternatively, differences could be due to the difference in the insecticide inhibitory actions. OP-s are inhibiting the AChE in a non-reversible way, but carbamates split the enzyme by hydrolysis in a reversible way (Čolovic *et al.* 2013). Due to the

reversible AChE inactivation, carbamates are considered safer than OP (Gupta 2006).

Overall, my results suggest that populations can differ in their response to insecticide exposure in all levels tested. I found that the more resistant Vermont population possesses a higher frequency of a S291G mutation in the *Ldace2*, two non-synonymous mutations in the *Ldace1* gene and has higher baseline *Ldace1* gene expression than the less resistant Belchow population. Moreover, the Belchow population is less tolerant to sublethal insecticide stress as sublethal AZ exposure induced changes in the *Ldace2* gene expression and AChE activity. Given these population-level differences in the resistance and insecticide stress tolerance, it is not arbitrary to study the resistance status of pest populations that invade new areas, as resistant individuals that are also more tolerant could be more successful at invading.

3.2 Direct and indirect effects of herbicides (studies II, III)

In addition to insecticides, pest insects can be exposed directly or indirectly to other anthropogenic stressors like herbicides, for example, glyphosate-based herbicide (GBH). I found that the Belchow population was more sensitive to direct exposure to high concentrations of GBH, while no differences were found in the Vermont population (study II). Glyphosate is also an organophosphate and previous studies have shown that it can inhibit AChE activity (Glusczak *et al.* 2006, Lajmanovich *et al.* 2011) which is also the target site of OP insecticides. Thus, it is not unexpected that the population that is more sensitive to OP insecticides is also more sensitive to high concentrations of GBH. However, the beetles are not likely to be exposed to such high concentrations as used in the study. Low, field realistic concentrations had no effect on survival (study II). Similarly, indirect low GBH exposure had no effect on survival (study III). These results are in line with previous studies that have found no direct toxic effects of low GBH doses on invertebrates (Haughton *et al.* 2001, Michalková and Pekár 2009, Le Mer *et al.* 2013, Thompson *et al.* 2014, Baker *et al.* 2014, Salvio *et al.* 2016). However, many studies on invertebrates have also found sublethal and toxic effects of various glyphosate products (Castilla *et al.* 2008, Contardo-Jara *et al.* 2009, Schneider *et al.* 2009, Benamú *et al.* 2010, Castilla *et al.* 2010, Evans *et al.* 2010, Janssens and Stoks 2017). I followed the survival for up to 5 days, thus further research is still needed to assess the possible long-term effects of GBH exposure.

At the physiological level direct and indirect GBH exposure had no effect on glutathione metabolism (e.g. GPx, GR, GST, and tGSH activities), or antioxidant enzymes (e.g. CAT and SOD activities) in either of the studied populations (studies II, III). However, direct GBH exposure increased lipid hydroperoxides levels after 2 h, suggesting oxidative damage after exposure (study II). This effect was not visible after 24 h and 96 h, suggesting that larvae

with high hydroperoxides levels died, or that decreased hydroperoxides levels in the later time points are associated with a better detoxification system in the older larvae (at 96 h).

The Vermont population had overall slightly higher enzyme activities (CAT and SOD) and lower lipid hydroperoxides levels, suggesting that they have a more effective antioxidant defence than the Belchov population. CAT and SOD enzymes are related to ROS regulation, and lipid hydroperoxides with oxidative damage (Fridovich 1978, Ercal *et al.* 2001, Halliwell and Gutteridge 2015). This result suggests that intensive insecticide use can select for higher oxidative status biomarkers activity. Exposure to OP insecticides has been shown to induce changes in oxidative status biomarkers (Lukaszewicz-Hussain 2010). Previously, it has been shown that insecticide resistance in the CPB includes reduced penetration, enhanced metabolism, target site insensitivity and increased excretion and behavioural changes (Alyokhin 2009). In addition, an increased antioxidant defence could be related to increased insecticide tolerance, however, the effectiveness of the defence to insecticides should be investigated further (Lukaszewicz-Hussain 2010).

These results demonstrate that the less resistant Belchov population is more sensitive to high doses of GBH than the Vermont population. While the more resistant population is also more tolerant to other anthropogenic stressors. This could be due to higher resistance to OP insecticides, and/ or due to more effective antioxidant defence. Nevertheless, low field-realistic doses had no short-term effects on the survival and oxidative status biomarkers of the CBP. However, the long-term effects of GBH should be still studied to confirm its safety.

3.3 Herbicide and insecticide effects (study III)

The main concern of different authorities has been to investigate the interactive effects of harmful chemicals, while chemicals that are considered safe have received less attention (Cedergreen 2014). However, they can have interactive effects together and increase or decrease the effects of harmful chemicals (Anderson and Zhu 2004, Boyer *et al.* 2006) in a non-additive way (i.e. antagonistic, synergistic; Fig. 2) that can be difficult to predict. I aimed to investigate whether indirect GBH exposure via food ingestion can affect the response to an OP insecticide (study III). I identified two mechanisms 1) gene expression and 2) oxidative stress by which GBH could possibly modulate a response to the insecticide.

First, I found that GBH alone inhibited the *Ldace1* gene expression, while together with the insecticide this inhibitory effect disappeared. This suggests that the two chemicals have an antagonistic effect. An antagonistic effect can be driven by the dominant stressor (Folt *et al.* 1999, Sala *et al.* 2000), in this case, the insecticide, as the effect of the insecticide overrides the effect of GBH. In line

with this, I found that insecticide exposure selected for individuals carrying the S291G mutation in the *ace2* gene previously connected to insecticide resistance (Zhu *et al.* 1996), thus selecting for individuals with higher resistance to the insecticide and perhaps selecting against those with low *Ldace1* gene expression levels. Antagonistic effects, in general, are suggested to be challenging for pest management unless they are driven by the dominant stressor (Folt *et al.* 1999). If the insecticide stress is the dominant stressor, then the GBH will likely not interfere with pest management.

Second I found that exposure to both chemicals resulted in a decrease in the oxidative status biomarkers (e.g. GPx, GR, CAT) compared to exposure to the GBH and insecticide alone. This small effect could be a positive synergistic effect because the levels of oxidative status biomarkers were lower when exposed together. On the other hand, it could also mean that the exposure to both chemicals inhibited the oxidative status biomarkers activity. Negative synergistic effects can occur when exposure to one stressor selects for individuals that are more sensitive to the other stressor (Crain *et al.* 2008). Nevertheless, positive synergistic effects could occur when exposure to two stressors has a positive beneficial effect, in our case reducing oxidative stress. Positive synergistic effects are less common (Piggott *et al.* 2015). In general, negative synergistic effects are usually a big concern due to the cumulative effects of the two stressors (Coors and De Meester 2008). Moreover, positive interactive effects could be a big concern in pest management if the exposure to GBH makes individuals more resistant to insecticide stress. However, the observed effect was small and warrants more studies to form a stronger conclusion about the direction of synergistic effects.

Since the effects I found were rather small and did not affect the overall survival, as no interactive effects were found on survival, the effect of multiple stressors on the population level can be difficult to estimate. Survival was recorded for 3 hours after insecticide exposure, which might underestimate the effect on survival. In addition, I used a resistant population that is also more tolerant to sublethal insecticide exposure (study I, (Piiroinen *et al.* 2013a)), thus possible interactive effects might be underestimated as the insecticide has a smaller effect on the resistant population. In addition, I used a very low GBH concentration.

It seems that even though I managed to identify two possible mechanisms by which the GBH could modify the response to the insecticide, these do not seem to affect the overall survival. Therefore, even though the agrochemicals can have interactive effects, the fate of the population can be difficult to predict. Nevertheless, it is important to investigate the possible interactive effects of chemicals when aiming at an ecologically relevant risk assessment in a multivariate world.

3.4 Transgenerational effects (studies IV, V)

3.4.1 Prolonged diapause and sublethal insecticide (study IV)

Prolonged diapause could allow individuals to skip environmental conditions they are poorly adapted to and could, therefore, buffer against population declines (Mahdjoub and Menu 2008, Salman *et al.* 2016). However, it could bring additional age-related costs. This study set out to investigate the effects of prolonged diapause on fertility, offspring survival, offspring insecticide tolerance, and fitness. I found that prolonged diapause is costly for females, as older females are less fertile (i.e. they produce fewer egg-batches and fewer eggs with lower hatching rates) and their offspring suffered from higher larvae-to-adult mortality. Prolonged diapause in males had no such effects and old males produced a similar amount of offspring with similar larvae-to-adult survival as the ones that did not prolong their diapause. Studies on age effects have shown decreased egg size and hatching, in addition to decreased fertility with increasing age (Fox 1993, Fox and Dingle 1994, Hercus and Hoffmann 2000, Yanagi and Miyatake 2002, Fox *et al.* 2003). Furthermore, studies have shown that older mothers produce offspring with shorter lifespans or lower larvae-to-adult survival (the 'Lansing effect') (Hercus and Hoffmann 2000, Marshall *et al.* 2010, Lind *et al.* 2015). Thus, this study demonstrates that prolonged diapause in the CPB has similar effects to ageing. The age-related effects on offspring production and offspring larvae-to-adult survival have been suggested to be bigger in females than males, since producing eggs in females is more costly than producing sperm (Royer and McNeil 1993). In addition, paternal effects can be mediated via maternal effects and thus mothers can mitigate the possible negative effects of the older fathers (Crean and Bonduriansky 2014).

Parents that had prolonged diapause did not prime their offspring to increase their insecticide stress tolerance. Nevertheless, they also did not produce offspring with decreased stress tolerance. I found that both mothers and fathers that had prolonged their diapause produced offspring with higher adult emergence body mass. In general, older mothers are known to produce bigger offspring (Priest *et al.* 2002, Marshall *et al.* 2010) and can be subject to the progeny size-number trade-off. In addition, mothers with prolonged diapause might be able to compensate for their lower fertility by producing bigger offspring. However, since both parents produced bigger offspring, there could be specific effects of prolonged diapause behind the increased body mass of the offspring. These results suggested that prolonging diapause can be advantageous for the offspring because higher body mass has been associated with higher overall mating success, fertility, overwintering survival, and fitness (Stearns 1992, Kingsolver and Huey 2008, Lehmann *et al.* 2012).

Prolonged diapause allows individuals to skip environmental conditions, but has sex-specific effects on fertility and offspring viability. Parental

prolonged diapause has no disadvantage for the offspring insecticide stress tolerance, but it is advantageous as it increases their body mass. These results indicate that prolonged diapause can be one factor contributing to increased invasion risk as it can buffer against bad environments and thus contribute to the population dynamics of the invasive species.

3.4.2 Transgenerational sublethal insecticide exposure (study V)

In agriculture, pesticides are used repeatedly to manage pest populations. This extensive use of pesticides leads to pesticide resistance (Whalon *et al.* 2008, Sparks and Nauen 2015, Mota-Sanchez and Wise 2018). It has been suggested that insecticide-induced hormetic effects can also contribute to resistance by promoting the growth of the resistant population (Guedes *et al.* 2017). However, less is known about the potential transgenerational effects of sublethal insecticide exposure. I found out that stressful, sublethal pyrethroid insecticide exposure has both positive within- and transgenerational effects on the fitness-related traits of the CPB. Within the generation, the insecticide-stressed individuals had higher adult survival and females had higher body mass than those not exposed to stress. Insecticide-stressed mothers had positive transgenerational effects on their offspring. Offspring whose mothers were insecticide-stressed had higher larval survival and female offspring were heavier as adults when compared to those not exposed to stress. These findings suggest that females are more susceptible to stress than males and that maternal stress has a stronger effect on daughters. Other studies have also found that females are more sensitive to stress than males (Hercus *et al.* 2003, Sørensen *et al.* 2007, Piironen *et al.* 2013b, Tejeda *et al.* 2014). Sex-specific differences in sensitivity to stress may be due to sexual size dimorphism (Blanckenhorn 2005), hormetic effects that are induced partly by different mechanisms in females and males (Hercus *et al.* 2003), or sex-linked pyrethroid resistance mechanisms (Argentine *et al.* 1995).

Positive transgenerational effects could be mediated via hormetic effects. For example, exposure to sublethal pyrethroid insecticide of resistant maize weevils (*Sitophilus zeamais*) leads to an increase in the net reproductive rate, which could lead to population growth in the population at that dose (Guedes *et al.* 2010). Alternatively, an increase in maternal fitness in the first generation can lead to increased fitness of the offspring (Marshall and Uller 2007). Despite the exploratory nature, this study reveals that exposure to sublethal insecticide doses can lead to increased survival and fitness-related traits. Thus, these effects can contribute to the persistence of populations under stress-inducing environments (Räsänen and Kruuk 2007, Hufbauer *et al.* 2012). More importantly, high stress tolerance and/ or organismal flexibility of invasive species could contribute to population dynamics and thus facilitate invasions (Hufbauer *et al.* 2012). Here I show that exposure of the CPB to stressful sublethal pyrethroid insecticide can induce positive within- and transgenerational effects manifested as higher survival and higher female adult body mass, which may have implications for the invasion success of the species.

4 CONCLUSIONS

The aim of my thesis was to investigate the role of anthropogenic stressors on the stress tolerance and fitness-related traits of an invasive pest, the Colorado potato beetle. My aim was to investigate the consequences of insecticide and herbicide exposure on the OP and carbamate insecticide target sites, physiological stress, and various life-history traits. I focused on studying the effects of i) anthropogenic stressors (i.e. insecticides or herbicides) (study I-II), ii) multiple anthropogenic stressors (study III) and iii) the effects of prolonged diapause on the offspring stress tolerance (study IV), and iv) within- and transgenerational effects of the sublethal insecticide stress exposure (study V).

I found that with intensive insecticide use we have not only selected for higher insecticide resistance but also for higher stress tolerance to sublethal insecticides exposure and to high doses of GBH herbicides. The more resistant population possessed both qualitative and quantitative changes in the OP and carbamate insecticide target sites (study I), and higher antioxidant defence (study II). This demonstrates that exposure to anthropogenic stress seems to increase stress tolerance in the invasive species. Individuals with higher stress tolerance are expected to tolerate similar stressors in new environments or when they are introduced to a new area. This is not a trivial problem, as biological invasions are increasingly recognized (especially among arthropod species), and an increasing number of pests have become resistant to insecticides (Whalon *et al.* 2008, Hulme 2009, Sparks and Nauen 2015). Moreover, pest species are also likely exposed to insecticides in their introduced range. For the more resistant population, the used insecticide doses in the introduced area could be sublethal. Thus, higher stress tolerance of a population could mean increased invasion success and thus I propose that it would be important to investigate the resistance status of the invading population.

Insecticide resistance to insecticides is usually confirmed by the changes in the target site, however many other changes, such as an increase in metabolic resistance, cuticle thickening and changes in insecticide penetration can occur in the resistant population. These all can contribute to higher stress tolerance.

Changes in the target site can make individuals more resistant to insecticides that act on the same target site, also known as cross-resistance. While changes in the other resistance mechanisms could also contribute to the overall more effective xenobiotics metabolism or avoidance. For example, I found that more resistant population had higher baseline antioxidant defence, thus they might be better at tolerating oxidative stress. However, whether higher antioxidant defence comes from intensive insecticide use alone, needs to be confirmed, as well the role of oxidative stress biomarkers on insecticide tolerance.

Second, I found that exposure to multiple stressors can have various interactive effects. I found that two stressors can have different types of interactive effects, and thus their short-term effects at a population level can be difficult to predict. I studied the short-term effects because different stress responses are often induced quickly after being exposed to the stressor. However, in order to estimate their population-level effects, long-term monitoring would give a more accurate view of the overall effects (i.e. survival or reproduction) at the population level. However, during longer monitoring, changes in survival and reproduction could be also due to exposure to other stressors. Thus, the effects of low sublethal doses and other stressors might be difficult to disentangle. Alternatively, interactive effects could be studied by using a less resistant population. The effects of different stressors might occur faster in a population that is more vulnerable to the different stressors. In general, we should investigate the possible interactive effects of multiple stressors because studies on single stressor can underestimate their consequences in a multivariate world.

Third, prolonged diapause had sex-specific effects on fertility. However, parents with prolonged diapause produced offspring with equal insecticide stress tolerance, but with increased body mass. Prolonged diapause can be an important strategy for invasive species as it allows individuals to skip poor environmental conditions and thus decrease the risk of reproductive failure. The ability to spread the risk of failed reproductions could be an important strategy of invasive species in order to maintain high population densities, or as an alternative strategy for dispersal, even if individuals might suffer from higher mortality when prolonging their diapause. This might be important, especially for individuals in the introduced range, when they are insufficiently adapted (i.e. when invading towards northern latitudes) to unfamiliar environmental (for example colder summer temperatures) conditions which are likely to increase the risk of reproductive failure (see from (Sol *et al.* 2012)). This ability to spread the risks over multiple generations can contribute to the maintenance of the population and thus invasion success of the invasive species.

Fourth, sublethal insecticide doses have both positive within- and transgenerational effects, which could contribute to invasion success. This study demonstrated that low sublethal doses had small within generational effects. However, the maternal insecticide stress exposure significantly increased their

offspring survival, and lead to increased body mass of their female offspring. Positive sublethal effects can be induced by hormetic effects that have positive effects on fitness. Positive fitness effects can persist in the population for generations and can be extremely effective at imposing directional selection. These positive effects of insecticide stress can contribute to the rapid evolution and invasion success of the CPB when these involve epigenetic modifications that are stable and heritable (Brevik *et al.* 2018).

To conclude exposure to anthropogenic stressors can affect genetic, physiological and fitness-related traits that can contribute to the stress tolerance of the CPB. Increased human activity and pesticide resistance among invasive species confirm that we need to pay more attention when applying insecticides, not to lead to unwanted pesticide resistance and selection for higher stress tolerance and possibly invasion success.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Stressinsietokyky ja vieraslajin invaasio

Ihmisen levittämät vieraslajit ovat yksi merkittävimmistä uhkista ja kasvavista ongelmista luonnon monimuotoisuudelle. Vieraslajit voivat uhata alkuperäislajistoa monella tapaa, mm. kilpailemalla samoista resursseista, levittämällä tautteja ja loisia sekä muutoin muovaamalla niiden elinympäristöä. Hyvän kilpailukykyensä ja stressinsietokykyensä ansiosta monet vieraslajit valtaavatkin tilaa ja resursseja alkuperäislajistolta levitessään uusille alueille. Useat vieraslajit ovat myös tuholaisia, ja niitä vastaan käytetään erilaisia torjunta-aineita sekä niiden alkuperäisillä että myös uusilla levinneisyysalueilla. Toistuva torjunta-aineiden käyttö puolestaan johtaa monien tuholaislajien vastustuskyvyn ja stressinsiedon kasvamiseen. Levittäytyvät tuholaiset tarjoavat mahdollisuuden tutkia, kuinka paljon ihmistoiminnan muokkaamiin ympäristöihin sopeutuminen (mm. tuholaisstorjuntaan sopeutuminen) edistää vieraslajien leviämistä uusiin ympäristöihin.

Torjunta-aineiden käyttö tähtää tuholaispopulaatioiden hävittämiseen. Pitkään onkin tutkittu millaisia muutoksia torjunta-aineiden erilaiset käyttöpittoisuudet aikaansaavat tuholaisissa, ja miten nopeasti tuholaiset kehittyvät vastustuskykyisiksi torjunta-aineille. Käytännön toiminnassa torjunta-aineen pitoisuus voi kuitenkin vaihdella siten, että kaikki yksilöt eivät altistu suositelluille pitoisuuksille, vaan osa yksilöistä voi altistua paljon haluttua pienemmille pitoisuuksille (sublethal). Altistuminen näille ei-tappavalle pitoisuuksille voi johtaa kuitenkin stressireaktioon ja sitä kautta vaikuttaa yksilöihin monella tapaa. Tällaisen stressin vaikutukset eivät aina ole pelkästään negatiivisia. Positiiviset eli hormeettiset vaikutukset voivat näkyä yksilön suurempana kelpoisuutena, kuten parempana lisääntymismenestyksenä. Lisäksi ihmisen luomissa ympäristöissä tuholaiset voivat altistua useille kemiallisille stressinaiheuttajille samanaikaisesti.

Useat tutkimukset ovat osoittaneet, että turvallisiksi ajatellut kemikaalit voivat lisätä haitallisempien kemikaalien vaikutusta. Nämä vaikutukset voivat näkyä altistuneiden yksilöiden jälkeläisissä. Esimerkiksi ei-tappavan tuholaisstorjunta-aineen aiheuttama stressi voi aiheuttaa perinnöllisiä epigeneettisiä muutoksia. Tällaiset muutokset voivat johtaa geenin ilmentymisen muutoksien lisäksi myös geenien toiminnan estämiseen tai vaimenemiseen. Kelpoisuutta lisäävät epigeneettiset muutokset voivat siirtyä vanhemmilta jälkeläisille, mikä taas voi tukea hyödyllisten fenotyyppien ilmentymistä ja siten ohjata evoluutiota.

Tässä väitöskirjassa selvitin antropogeenisten stressinaiheuttajien roolia stressinsietokyvyssä ja vieraslajien kelpoisuuteen liittyvissä ominaisuuksissa koloradonkuoriaisella. Koloradonkuoriainen on perunan paha tuholainen, joka on erittäin kestävä monia tuholaisstorjunta-aineita vastaan. Se ei ole vielä kuitenkaan levinnyt Suomeen. Lisäksi tutkin altistumisen vaikutuksia fysiologiseen stressiin ja useisiin elinkierron vaiheisiin. Tutkimuksessani keskityin tar-

kastelemaan i) antropogeenisten stressinaiheuttajien (tuholais- ja rikkakasvien torjunta-aineiden) (tutkimukset I-II) ja ii) useiden antropogeenisten stressinaiheuttajien (tutkimus III) vaikutuksia sekä iii) pitkittyneen diapaussin vaikutuksia jälkeläisten stressinsietokykyyn (tutkimus IV) ja haitallisen tuholaistorjunta-aineen altistumisen aiheuttamia sukupolvien sisäisiä ja välisiä vaikutuksia (tutkimus V).

Verratessani eri tavalla vastustuskykyisiä populaatioita toisiinsa havaitsin, että intensiivinen torjunta-aineiden käyttö ei ole ainoastaan aiheuttanut korkeampaa vastustuskykyä torjunta-aineille, vaan johtanut myös korkeampaan stressinsietokykyyn. Vastustuskykyisemmällä populaatiolla oli sekä laadullisia (pistemutaatiot) että määrällisiä (geeniekspressio) muutoksia organofosfaatti- ja karbamaattitorjunta-aineiden kohdealueilla *ACHe1* ja *ACHe2* asetyylikoliiniesteraasia koodaavissa geeneissä (tutkimus I) sekä korkeampi antioksidanttipuolustus (tutkimus II). Näin ollen ne voisivat paremmin sietää oksidatiivista stressiä. Altistuminen ihmisen aiheuttamalle stressille näyttääkin lisäävän torjuntaaineresistenssin lisäksi vieraslajien stressinsietokykyä.

Yksilöiden, joilla on korkeampi stressinsietokyky, oletetaan sietävän samankaltaisia stressinaiheuttajia myös levittäytyessään uusiin ympäristöihin joko omatoimisesti tai ihmisen avustuksella. Tämä on merkittävä ongelma, sillä biologiset levittäytymiset ovat lisääntyneet maailmanlaajuisesti (myös niveljaltaisilla), ja kasvava määrä tuholaislajeja on kehittynyt vastustuskykyisiksi torjunta-aineille. Lisäksi tuholaislajit ovat todennäköisesti altistuneet samankaltaisille torjunta-aineille levittäytymisalueellaan. Vastustuskykyisemmille populaatioille niiden levittäytymisalueilla käytetyt torjunta-aineannokset eivät välttämättä ole kuolettavia. Näin ollen populaation korkeampi stressinsietokyky voi mahdollistaa paremman levittäytymismenestyksen. Tästä syystä olisikin tärkeää tutkia levittäytyvän populaation vastustuskykyä.

Altistuminen useille stressinaiheuttajille voi aiheuttaa monenlaisia yhdysvaikutuksia. Tutkimuksessani selvisi, että rikkakasvien torjunta-aineiden käytöllä voi olla erilaisia yhdysvaikutuksia tuholaistorjunta-aineiden kanssa (tutkimus IV). Kuoriaiset, joita altistettiin ensin rikkakasvien torjunta-aineille ja sen jälkeen tuholaistorjunta-aineille havaittiin antagonistisia vaikutuksia torjunta-aineiden kohdealueiden geeniekspressiossa mutta synergisiä vaikutuksia oksidatiivisissa biomarkkereissa verrattuna tilanteeseen, jossa kuoriaisia altistettiin vain yhdelle stressinaiheuttajalle. Vaikka tutkimuksessa tutkittiin lyhyen altistusajan (3 h) vaikutuksia, viittaa kahden tai useamman stressitekijän tulokset siihen, että niiden vaikutuksia populaatiotasolla voi olla hankala ennustaa. Tutkin lyhytaikaisia vaikutuksia, koska erilaiset vasteet voidaan saada aikaan nopeasti stressinaiheuttajalle altistumisen jälkeen. Kuitenkin arvioitaessa niiden populaatiotasoa vaikutuksia, pitkäaikaisella tarkastelulla saataisiin tarkempi arvio kokonaisvaikutuksista (esim. selviytyminen ja lisääntyminen).

Ihmistoiminta voi johtaa myös elinkiertostrategioiden muutoksiin. Pitkittynyt diapaussi voisi olla vieraslajeille tärkeä strategia, koska sen avulla ne voivat siirtää lisääntymistä yli huonojen ympäristöolojen ja näin ollen pienentää riskiä epäonnistua lisääntymisessä. Neljännessä osatyössä havaitsin, että pitkittynyt diapaussi voisi olla vieraslajeille tärkeä strategia, koska sen avulla ne voivat siirtää lisääntymistä yli huonojen ympäristöolojen ja näin ollen pienentää riskiä epäonnistua lisääntymisessä. Neljännessä osatyössä havaitsin, että pitkittynyt diapaussi voisi olla vieraslajeille tärkeä strategia, koska sen avulla ne voivat siirtää lisääntymistä yli huonojen ympäristöolojen ja näin ollen pienentää riskiä epäonnistua lisääntymisessä.

tyneellä diapaussilla oli naaraille ja koiraille erilaisia vaikutuksia lisääntymiskykyyn. Siinä missä naaraiden lisääntymispotentiaali pieneni pitkittyneestä diapaussista, koirilla samoja kustannuksia ei havaittu. Toisaalta pitkän diapaussin läpikäyneiden vanhempien jälkeläisillä oli yhtä hyvä stressinsietokyky torjunta-aineelle, mutta ne olivat isompia. Isosta koosta voi olla monia etuja mm. lisääntymisessä ja talvehtimisessa, ja se voi osittain kompensoida pienentyneen lisääntymispotentiaalin. Kyky jakaa riskejä useille sukupolville voi pienentää lisääntymisen epätodennäköisyyden riskiä ja siten edistää vieraslajien levittäytymismenestystä.

Viimeisessä osatyössä osoitin, että pienillä ei-tappavilla torjunta-aineannoksilla oli sekä positiivisia sukupolvien sisäisiä että positiivisia sukupolvien välisiä vaikutuksia. Matalat torjunta-ainepitoisuudet lisäsivät toukkien kuolleisuutta kaksi prosenttia, mutta lopulta ne kasvoivat isoimmiksi ja selvisivät aikuisiksi todennäköisemmin kuin ne toukat, joita ei altistettu torjunta-aineelle. Kun altistettujen ja ei-altistettujen yksilöiden annettiin lisääntyä, lopputuloksena oli, että äidin altistuminen stressille lisäsi merkittävästi niiden jälkeläisten selviytymistä (17 %), ja johti korkeampaan ruumiinpainoon naarasjälkeläisillä. Pienillä torjunta-ainepitoisuuksilla voikin olla ei-toivottuja positiivisia, hormoneettisia vaikutuksia, jotka edelleen lisäävät yksilöiden kelpoisuutta. Torjunta-ainestressin aiheuttamat positiiviset vaikutukset voivat edistää koloradonkuoriaisen nopeaa evoluutiota ja levittäytymismenestystä, kun näihin yhdistyy pysyviä ja perinnöllisiä epigeneettisiä muutoksia. Nämä positiiviset kelpoisuusvaikutukset voivat säilyä populaatiossa useiden sukupolvien ajan ja olla erittäin tehokkaita helpottaessaan levittäytymisiä.

Yhteenvedona totean, että altistuminen antropogeenisille stressinaiheuttajille vaikuttaa geneettisiin, fysiologisiin ja kelpoisuuteen liittyviin ominaisuuksiin, jotka voivat edistää koloradonkuoriaisen stressinsietokykyä. Ihmisten lisääntynyt aktiivisuus ja vieraslajien vastustuskyky torjunta-aineita vastaan tarkoittavat, että meidän tulee kiinnittää yhä enemmän huomiota torjunta-aineiden käyttöön, jos haluamme välttyä vieraslajien ei-toivotulta vastustuskyvyltä, suuremmalta stressinsietokyvyltä sekä mahdolliselta kasvavalta levittäytymismenestykseltä.

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ORIGINAL PAPERS

I

ROLES OF ACETYLCHOLINESTERASE GENES IN ORGANOPHOSPHATE AND CARBAMATE RESISTANCE IN *LEPTINOTARSA DECEMLINEATA*

by

Aigi Margus, Saija Piironen, Philipp Lehmann, Alessandro Grapputo, Yolanda
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II

**EFFECTS OF A GLYPHOSATE-BASED HERBICIDE ON
SURVIVAL AND OXIDATIVE STATUS OF A NON-TARGET
HERBIVORE, THE COLORADO POTATO BEETLE
(*LEPTINOTARSA DECEMLINEATA*)**

by

Miia Rainio, Aigi Margus, Philipp Lehmann, Marjo Helander, Leena Lindström

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III

MULTIPLE STRESSORS: CAN INDIRECT HERBICIDE EXPOSURE MODIFY THE RESPONSE TO ORGANOPHOSPHATE INSECTICIDE IN AN INVASIVE PEST?

by

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IV

AGE DOES MATTER: PROLONGED DIAPAUSE HAS SEX-SPECIFIC FERTILITY AND FITNESS COSTS IN THE COLORADO POTATO BEETLE

by

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Submitted manuscript

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**SUBLETHAL PYRETHROID INSECTICIDE EXPOSURE
CARRIES POSITIVE FITNESS EFFECTS OVER GENERATIONS
IN AN INVASIVE PEST**

by

Aigi Margus, Saija Piiroinen, Philipp Lehmann, Santtu Tikka, Juha Karvanen,
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