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Costs of Insecticide Resistance on Fitness

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Costs of Insecticide Resistance on Fitness

A Thesis submitted in partial satisfaction
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by

Dylan James Brown

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ABSTRACT OF THE THESIS

Costs of Insecticide Resistance on Fitness

by

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The mechanisms behind insecticide resistance are commonly thought to induce fitness costs in the absence of insecticides in the environment. However, it is still largely unknown how these fitness costs present themselves and what factors may influence the variation in cost on fitness due to insecticide resistance. To investigate this, I conducted multiple meta-analyses' along with my own lab study on an insecticide resistant glassy-winged sharpshooter population. I first conducted a literature search and collected data on studies that tested for fitness costs associated with insecticide resistance. The data collected was then used to generate multiple meta-analyses' to determine the overall effects of resistance on insect fitness indicated through different life history traits. Fecundity, juvenile development time, juvenile survival percentage, and male longevity were significantly reduced due to insecticide resistance. Juvenile development time was prolonged in resistant individuals compared to susceptible individuals; however, female longevity was unaffected by resistance overall. Results from the meta-analyses' also indicated that there is no consistent trend between the magnitude of resistance and fitness cost. A lab study was also performed between field resistant and susceptible glassy-winged sharpshooter populations to detect any fitness costs associated with insecticide resistance. Glassy-winged sharpshooter fitness was significantly affected by neonicotinoid resistance. Individuals from the resistant sharpshooter population had a significantly reduced fecundity and adult lifespan compared to individuals from the susceptible population. Morphological features like tibia length and intraocular distance were larger in the resistant population compared to the susceptible population; however, there was no difference in wing vein length and dry weight between the two populations. This thesis provides evidence towards fitness costs associated with insecticide resistance that may be used to optimize existing resistance control strategies.

TABLE OF CONTENTS

List of Figures

Chapter II.

Chapter III.

List of Tables

Chapter II.

Chapter III.

Chapter I. Introduction

The development of insecticide resistance has resulted in pest control failures in the field and has become an increasing problem for the management of many pestiferous insects. Here, resistance is defined as ‘a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species’ (IRAC 2021). Insecticide resistance has led scientists to design alternative control techniques that involves multiple tactics including some chemical, biological, mechanical, and cultural to optimize pest control rather than just relying on insecticides (Stern et al. 1959). It has been repeatedly observed that resistance alleles may carry a fitness disadvantage in the absence of the insecticide and this can be exploited to assist in resistance management programs (Laxminarayan and Simpson 2002, Kliot and Ghanim 2012, Freeman et al.). This chapter will introduce a broad look at fitness costs associated with insecticide resistance throughout the field of entomology, and then narrow down the view towards an isolated example in the glassy-winged sharpshooter *Homalodisca vitripennis* Germar (Hemiptera:Cicadellidae).

For over four thousand years, insecticides have been utilized to manage pestiferous insects; although, mass production of synthetic insecticides did not occur until the early 1900s (Abubakar et al. 2020). The year 1939 marked the dawn of synthetic insecticides with the application of dichloro-diphenyl-trichloroethane (DDT) as an insecticide (Palumbi 2001). Since DDT, there are now over 288 insecticidal compounds and 32 different insecticides with different modes of action produced (Sparks and Nauen

2015, Sparks et al. 2021). Not only has the number of different insecticides increased, but the amount of insecticides applied around the world since 1939 has also increased. Approximately one billion pounds of insecticides are used annually throughout the world equating to $16 billion in crop protection costs (Grube et al. 2011). Crop productivity has significantly increased in the past half century without a significant increase in crop losses, a pattern largely attributed to pesticide use (Oerke 2006). Due to this reduction in pest damage, it is estimated that farmers save $4 for every $1 spent on insecticides (Pimentel et al. 1978). Pestiferous insects not only threaten global food security, but also cause over one million human deaths a year through biting, stinging, and transmission of diseases (Resnik 2014). From 2000-2015, it is estimated that insecticides helped prevent over 450 million cases of malaria in Sub-Saharan Africa (Bhatt et al. 2015). Many of these benefits from the use of insecticides, however, are at risk due to the development of insecticide resistance.

As reliance on insecticides for managing pest populations increased, the incidence of insecticide resistance also increased. There are now over 580 documented cases of insecticide resistance in arthropod pests, and that number is predicted to increase (Sparks et al. 2021). Indeed, an over reliance on insecticides has led to the coined term “The Pesticide Treadmill” (Van Den Bosch 1978). The pesticide treadmill responds to the presence of insecticide resistance in pest populations either by increasing the dose and frequency of the insecticide application (1st option) or by creating a new insecticide that works with a different mode of action (2nd option) (Popp et al. 2013). Increasing the dose and frequency of insecticide applications is more costly (Pimentel et al. 1992), with cost estimates of an additional $1.2 billion a year to re-treat fields in the United States due to resistance (Palumbi 2001). Re-treating fields is not only costly, but also may not even efficiently reduce the damage by already resistant pests. Recent studies found that growers can dramatically reduce their insecticide use by up to 60% and still achieve the same or even greater production through other control strategies (Lechenet et al. 2017, Krupke et al. 2017). The second option, creating a new insecticide with a different mode of action, can take a significant amount of time and money to develop more efficient and selective insecticides. Creating new insecticides with different modes of action can cost as much as $250 million to develop and up to 12 years to bring to market (Sparks 2013). For these reasons, growers have been urged to switch to a more sustainable integrated pest management strategy that relies on other management approaches alongside chemical (Barzman et al. 2015).

Fitness costs (significant disadvantage in survival and reproductive success compared to other individuals within the same species) are commonly associated with insecticide resistance due to the underlying resistance mechanisms. To date there are at least 4 different types of resistance mechanisms: metabolic, target site modification, behavioral, and penetration resistance (Dang et al. 2017). Metabolic resistance uses already existing enzymes (esterases, cytochrome P450 monooxygenases, and glutathione-S-transferases) to break down the insecticide to a less toxic compound before it reaches its target site (Li et al. 2007). This resistance mechanism may have a fitness cost due to a high energetic cost of over-producing metabolic enzymes (ffrench-Constant and Bass 2017). Target site resistance will also typically cause a fitness cost but works instead by modifying the ion channel subunits where the insecticide targets. The mutated ion channel may function less efficiently leading to a significant cost on the organism's fitness (ffrench-Constant and Bass 2017). Penetration resistance involves modifications in cuticle composition or cuticle thickening that reduces entry of insecticides into an insect (Balabanidou et al. 2018). There has been no study performed on fitness costs due to penetration resistance. Unlike the last three physiological resistance mechanisms, behavioral resistance involves a modification in behavior that reduces exposure to an insecticide (Lockwood et al. 1984). Behavioral resistance is a less documented resistant mechanism but there has been reports of fitness costs associated with behavioral resistance (Jensen et al. 2017, Silverman 1995). Fitness costs due to behavioral resistance, depicted through glucose aversion, may create an unbalanced diet in environments where glucose+insecticides are absent (Silverman 1995).

Fitness costs associated with insecticide resistance may be reflected in life-history traits, morphological, and behavioral traits. Some life-history tradeoffs associated with resistance may involve decreased longevity, fecundity, juvenile development time, and juvenile survival percentage (Gao et al. 2014, Martins et al. 2012, Ejaz et al. 2017). These reductions can be variable in magnitude and in some cases have no fitness costs at all (Kliot and Ghanim 2012, Ang and Lee 2011). The underlying causes for variability in costs may include insecticide classes, the genetic differences in insect populations, and magnitude of resistance. Morphological differences caused by resistance may also influence fitness. For example, the overall size of a female has been linked to her fecundity; smaller females typically produce fewer offspring than larger females

(Kozlowski 1992). Pyrethroid resistant mosquitoes exhibit reduced body sizes, which could be an explanation for the observed reduction in fecundity (Bourguet et al. 2004). Similarly, wing size and structure differences directly affect flying distance and dispersal (DeVries et al. 2010, Mikac et al. 2019). Resistant codling moth wings are structurally different compared to susceptible wings which led to poorer flight performance (Zivkovic et al. 2019). Additionally, behavioral changes seen in insecticide resistant insects may result in lower overall fitness. Resistant aphids with kdr site mutations were less likely to respond to alarm pheromones as quickly as susceptible individuals, which may lead to higher parasitism rates by parasitoids (Foster et al. 2003, Foster et al. 2007). Similarly, resistant house flies with kdr mutations failed to move to the optimal temperature as their susceptible counterparts did (Foster et al. 2003). This behavior may be maladaptive as flies have a lower fecundity and shorter lifespan in sub-optimal temperatures (Fletcher et al. 1990, Lysyk 1991).

Understanding if and how fitness costs present themselves in a pest population can assist in certain resistance management strategies to lower pest population density while controlling resistance development. The refuge strategy (incorporating small areas of untreated habitat within large, treated areas) has been one such strategy that has proven very effective in slowing down and treating insecticide resistant populations. Refuge strategies work best if fitness costs are present and if inheritance of resistance is recessive (Tabashnik et al. 2003). The rare resistant insects from the treated areas will mate with susceptible insects from untreated areas creating a heterozygous population that will not be able to survive in treated areas as long as inheritance is recessive. It is important that susceptible insects outnumber resistant insects in untreated areas. If there are fitness costs associated with resistance, then it is likely that susceptible insects will outcompete resistant insects in untreated areas. However, if there are no fitness costs then resistant individuals would become more common in untreated areas fixing the resistant allele in the population. Refuge strategies would then only be economically viable if there is a fitness cost associated with the insecticide resistance that will push the population back towards susceptibility and prevent the resistance allele from becoming fixed in the population (Laxminarayan and Simpson 2002, Takahashi et al. 2017).

Insecticide rotation strategies have been another strategy that manages insecticide resistance and works best if fitness costs are present in the resistant population. Insecticide rotation strategies implement the use of at least two different insecticide classes with different modes of action to prevent resistance development. It is critical that the insecticides used in the rotation have different modes of action as it is less likely to have cross-resistance with the original insecticide (Sparks and Nauen 2015). Fitness costs associated with insecticide resistance will make resistance unstable as natural selection will select against disadvantageous alleles (Alam et al. 2020, Shah and Shad 2020). Rotations of different insecticides with no cross-resistance will further select against the less fit individuals while also controlling populational densities. It is likely that the original insecticide may still be used in the rotation as the less fit, resistance allele will become rare in the population again (Barbosa et al. 2020). If there is no fitness cost, then the resistance allele will be stable and likely become fixed in the population (Kliot and Ghanim 2012). The original insecticide must be completely taken out of the rotation indefinitely in the absence of fitness costs associated with insecticide resistance (ffrench-Constant and Bass 2017). By incorporating the fitness costs associated with insecticide resistance into a pest management strategy, the likelihood of reducing the density of the pest population without increasing insecticide use is improved.

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae), is an important agricultural pest that has developed insecticide resistance in the field. The glassy-winged sharpshooter is an invasive agricultural pest in California introduced in the late 1980s on citrus rootstock (Sorensen and Gill 1996). There are two generations of this insect every year in the warmer spring and summer months followed by a reproductive diapause stage in the cooler fall and winter months (Castle et al. 2005). Their diet consists of the nutrient poor xylem of a wide range of host plants (https://www.cdfa.ca.gov/pdcp/Documents/HostListCommon.pdf). Glassy-winged sharpshooters are important vectors of *Xyllela fastidiosa* Wells, a major plant pathogen infecting the xylem of the host plant (Redak et al. 2004). Since the introduction of the glassy-winged sharpshooter to California, the increase in the incidences and outbreaks of Pierce’s disease have climbed significantly (Purcell and Feil 2001). Considering the potential of glassy-winged sharpshooters to transmit *X. fastidiosa* in Central and Southern California an area wide control program, relying heavily on insecticides, was established to lower sharpshooter population densities to economically viable levels (Wendel et al. 2002). With the geographically broad application of insecticides, a noticeable drop in population densities was noticed almost immediately. Unfortunately, densities have since rebounded most years in the past decade (Haviland et al. 2021). This rise in sharpshooter numbers can partially be explained by the development of insecticide resistance reported in sharpshooter populations (Andreason et al. 2018, Byrne and Redak 2021). Knowing if there are fitness costs associated with insecticide resistance in sharpshooter populations can assist in the development of the best resistance management strategy.

In this thesis, I investigate the fitness costs associated with insecticide resistance in glassy-winged sharpshooters (Chapter 2). Then, I present the first, to our knowledge, quantitative study examining patterns of fitness costs associated with insecticide resistance (Chapter 3). These studies increase our understanding of fitness costs associated with insecticide resistance that may lead to more efficacious control measures in the future.

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Chapter II. Fitness Costs Associated with Insecticide Resistance: A Meta-Analysis Approach

Abstract

Many studies have demonstrated a fitness cost associated with insecticide resistance; however, there is no quantitative review documenting this trade-off. Revealing patterns in the effect of insecticide resistance on fitness among insecticide classes, resistance levels and insect orders would improve our understanding of evolutionary costs of resistance and may optimize existing resistance management strategies. I performed a comprehensive literature search to identify studies that examined fitness costs associated with insecticide resistance. From these studies, I collected data on various life history traits influenced by insecticide resistance and subjected these data to multiple meta-analyses to determine overall effects of resistance. The emerging patterns suggest that (a) resistant females exhibited a lower fecundity compared to susceptible females within the orders Diptera, Hemiptera, and Lepidoptera; (b) resistant juveniles took longer to develop compared to susceptible juveniles within the order Diptera and Lepidoptera; (c) immature survival rate was much higher for susceptible immatures than resistant immatures in the orders Lepidoptera and Hemiptera; (d) female and male adult longevity were reduced for resistant Dipterans, and (e) there was no clear and consistent trend between the cost on fitness and magnitude of resistance.

Introduction

Insecticide resistance is a growing problem in controlling pestiferous insect populations. There have been over 580 reported cases of different insect species resistant to at least one type of insecticide (Sparks et al. 2021). Resistance is acquired through a continuous selection pressure (insecticide treatments) that select for resistant alleles within the population. This continuous selection process will ultimately lead to a resistant population of insects that are less affected by insecticide treatments. Although resistant alleles provide insects an advantage in the presence of insecticides, these alleles will typically result in a fitness disadvantage in the absence of the insecticides (ffrench-Constant and Bass 2017, Kliot and Ghanim 2012).

Fitness tradeoffs brought on by insecticide resistance are caused by altered biochemical processes whether its enzymes or ion channels. One such resistance mechanism, metabolic detoxification, can have a high energetic cost to the organism and lead to lower reproductive values or reduced lifespan (Tchouakui et al. 2020). Target site mutations can also result in lowered fitness due to mutational changes of enzymatic target sites that are critical to biological processes (ffrench-Constant and Bass 2017). Fitness costs associated with insecticide resistance can be evaluated in several ways including an examination of life-history traits, allele frequency, and competition assays (Ejaz and Shad 2017, Oliveira et al. 2007, Boivin et al. 2003). Life-history traits are effective indicators of an insect’s fitness and may include fecundity, juvenile development time, juvenile survival rate, and adult longevity to name a few. Reductions in fitness due to insecticide applications may indirectly affect not only insect herbivore damage, but also such disease transmission by insect vectors to both plants and animals.

Management strategies to limit or reduce insecticide resistance have been created that exploit fitness costs (Laxminarayan and Simpson 2002, Barbosa et al. 2020a). Insecticide rotation is one such strategy that combats resistance by rotating different classes of insecticides that exhibit little cross resistance to the original insecticide (Barbosa et al. 2020a, ffrench-Constant and Bass 2017). Natural selection will already select for susceptibility if there is a fitness cost associated with resistance. The use of non-cross-resistant insecticides will then help control populational densities while increasing the selection pressure on the less fit alleles. Reversion to susceptibility will not occur if there are no costs to fitness (Alam et al. 2020, Shah and Shad 2020).

Within the literature, there is a wide variation in the degree of insecticide induced costs in insect pests. It is unclear what might cause this high variation. Most insecticide classes have different modes of action; therefore, it is likely that different insecticide classes evolve different resistant mechanisms (Sparks et al. 2021). Additionally, different orders of insects possess significant variations in their genomes that may increase or decrease possible fitness tradeoffs due to insecticide resistance. It is unclear if insecticide resistance becomes more costly as resistance increases in the population. To understand if there are constant and predictable fitness costs associated with insecticide resistance, I performed a comprehensive literature search and multiple meta-analyses to determine if fitness costs vary with insecticide class, order of insect, resistance level and whether the type of development (complete or incomplete metamorphosis) affects these fitness costs.

Methods and Materials

Literature Search

A comprehensive literature search was conducted to find articles that evaluated the impact of insecticide resistance on life-history characteristics that have been demonstrated to be related to fitness. Literature searches were conducted using “Web of Science”, “Google Scholar”, “AGRICOLA”, and “Academic Search Complete”. Key words “insecticide resistance” and “fitness costs” were used in the search resulting in 919 studies. References from recent published articles were also examined until no additional articles were found. The later resulted in an additional 25 studies. The articles found through citations and the search engines ranged from the years 1966-2021. Any articles that failed to report treatment means, measures of variance (SD or SE), and sample sizes were excluded from the analysis. More details regarding articles that were included and excluded with reasons are detailed in Figure 2.1. A common approach throughout different studies were comparisons of multiple resistant populations to one susceptible population. If there were multiple resistant populations being compared to a single susceptible population then only the comparison with the largest difference was recorded. Studies with multiple independent comparisons between different susceptible and resistant population were treated as independent studies.

Meta-analysis

Meta-analyses were performed on five frequently reported life history traits commonly associated with fitness: female fecundity (eggs per female), female longevity (days), male longevity (days), juvenile development time (days), and juvenile survival (percent of first instar individuals that survive to adult eclosion). Each meta-analysis evaluated the effect of insecticide resistance on specific life history traits for resistant or susceptible populations. For each trait examined and where sample sizes allowed, four separate analyses were performed: the effects of insecticide resistance based on class of insecticide, order of insect, level of resistance (based on reported resistance ratios), and type of development (holometabolism, hemimetabolism). Resistance levels were partitioned into four different groups (1-100, 100-200, 200-500, and >500) depending on the reported resistance ratio (LC_{50} or LD_{50} value of resistant strain divided by LC_{50} or LD_{50} value of susceptible strain). For each study meeting the criteria, I extracted the means, measures of variance, and sample sizes for both resistant and susceptible populations being compared. I then calculated the effect size (Hedges g) and its variance (Var g) for each study. Hedges g was calculated by subtracting the mean of the control group (susceptible population) from the mean of the experimental group (resistant population) and dividing it by their pooled and weighted standard deviation. A negative effect size implies a higher value for the susceptible population.

Data Analysis

I used the “meta” package in R to perform all meta-analyses used in this study (Schwarzer 2007). All forest plots were generated using the R package “rmeta”

(Viechtbauer 2010). Forest plots were created using the mean effect size of each category ±95% confidence interval (CI). Each analysis was performed using a random effects model as it is unlikely that that the studies included in this meta-analysis have equal variance. The restricted maximum likelihood (REML) method with Hartung-Knapp-Jonkman (HKSJ) estimator was also used in the model to estimate heterogeneity variance (Q) and confidence intervals (CI) respectively. The REML method with HKSJ has been known to have considerably lower bias and less inflated confidence intervals compared to other methods (Langan et al. 2019). Effect sizes whose 95% CI did not include 0 were classified as significant.

Publication Bias

I evaluated publication bias using funnel plots and Egger's test in the METAFOR package in R (Rosenberg & Pitcairn 2002). Asymmetric distribution within the funnel plots indicates publication bias and would be detected through Egger's test. I also calculated Rosenthal's fail-safe statistic to determine how many non-significant, unpublished studies it would take to render the overall effect null.

Results

Fitness Costs

Fecundity

Insecticide resistance exhibited an overall negative effect on fecundity (grand means, **Figure 2.2a-d**). Females that were susceptible to insecticide classes consisting of pyrethroids, diamides, neonicotinoids, pyridalyl, and Bt laid more eggs on average than resistant females (**Figure 2.2a**). For all other insecticide classes, there were no significant differences in fecundity between resistant and susceptible females (**Figure 2.2a**). There was a significant difference detected between insecticide classes that partially explains the total heterogeneity in this analysis (**Table 2.1**). Susceptible females within the orders Diptera, Hemiptera, and Lepidoptera had a significantly higher fecundity than their resistant counterparts; however, all remaining insect orders showed no significant difference between resistant and susceptible populations (**Figure 2.2b**). Resistant individuals had significantly lower fecundity than susceptible individuals for all groups of resistance levels examined (**Figure 2.2c**). Resistant females also displayed a lower fecundity than susceptible females for both development types tested. Resistant Hemimetabolous insects had a lower fecundity on average than resistant Holometabolous insects (**Figure 2.2d)**. Between group variation for insect orders, resistance levels, and development type was non-significant (**Table 2.1**).

Juvenile Development Time

Insecticide resistance had a significant overall effect on juvenile development time (grand means, **Figure 2.3a-d**). Resistance to spinosyns prolonged development time, while resistance to all other insecticide classes showed no significant effect (**Figure 2.3a**). Resistant individuals within Diptera and Lepidoptera had a significantly longer development time than susceptible individuals (**Figure 2.3b**), while remaining orders exhibited no effect of insecticide resistance. Insects with resistance ratios greater than five hundred had a much slower development time than susceptible insects (**Figure 2.3c**). For all other resistant ratio categories, there were no significant effect observed on development time (**Figure 2.3c**). There was no significant difference in development time between resistant and susceptible hemimetabolous individuals, yet there was an increase in development time for holometabolous individuals (**Figure 2.3d**). Insecticide class, insect order, resistance level, and development type showed so significant variation between groups (**Table 2.1**)

Juvenile Survival

Overall, resistance to insecticides lowered juvenile survival across insecticide classes, insect orders, resistance levels, and development type (grand means, **Figure 2.4a-d**). None of the individual insecticide classes exhibited a response to insecticide resistance (**Figure 2.4a**). Resistant hemipteran and lepidopteran juveniles suffered significantly higher mortality throughout development compared to susceptible juveniles (**Figure 2.4b**). Insects with resistance ratios in-between 200-500 and 1-100 had significantly lower survival percentages than susceptible insects (**Figure 2.4c**). Resistant level groups consisting of 1-100, 200-500, and >500 had very similar effect sizes, while the group 100-200 effect size differed significantly from those groups (**Table 2.1**). Both resistant holometabolous and hemimetabolous insects have a significantly lower juvenile survival percentage than the susceptible populations (**Figure 2.4d**).

Adult Female Longevity

Insecticide resistance had no overall effect on female longevity regardless of insecticide class, insect order, resistance level or development type (grand means, **Figure 2.5a-d**). There was a significant amount heterogeneity among studies for which differences among insect order and resistance ratio groups accounted for a significant portion of this variance (**Table 2.1**). Resistance to neonicotinoids reduced female lifespan while resistance to all other insecticide classes had no effect (**Figure 2.5a**). Diptera was the only insect order where insecticide resistance reduced female lifespan (**Figure 2.5b**). There was no resistant level group with a significant effect size (**Figure 2.5c)**. Resistant female holometabolous insects had significantly shorter lifespans than susceptible females; however, there was no effect of resistance on hemimetabolous females (**Figure 2.5d**).

Adult Male Longevity

Overall, resistance to insecticides lowered adult male lifespan across insecticide classes, insect orders, resistance levels, and development type (grand means, **Figure 2.6a-d**). Males resistant to spinosyns lived shorter lives compared to susceptible males, while resistance to all other insecticides had no effect on male lifespan **(Figure 2.6a**). Resistant Dipteran males had reduced lifespans compared to their susceptible counterparts (**Figure 2.6b**). Males with resistant ratios between 1-100 had shorter lifespans while the male lifespans of all other resistant ratio groups did not differ with resistance (**Figure 2.6c**). Resistant holometabolous males also had reduced lifespans unlike resistant hemimetabolous males that suffered no significant effect from resistance (**Figure 2.6d**). There was a significant amount of heterogeneity in the effect of resistance on male longevity that isn't explained by the variation among insecticide class, insect orders, resistance levels, and development type (**Table 2.1**).

Publication Bias

Funnel plots and Egger's test (not shown) for the fecundity and juvenile survival meta-analyses suggested a publication bias. This indicates that there was a potential for a lack of studies reporting no significant effect of insecticide resistance on fecundity. Regardless, Rosenberg's failsafe statistic suggest it would take a very large number of non-significant, unpublished studies to render the overall effect null for the fecundity along with survival percentage (**Table 2.1**).

Discussion

Many studies along with qualitative reviews have reported large reductions in fitness that are associated with insecticide resistance (Gassmann et al. 2009, Kliot and Ghanim 2012, Freeman et al. 2021). This meta-analysis has shown similar results to the previous qualitative reviews that insecticide resistance does typically carry a fitness cost indicated through life-history traits; however, it also provides some of the first explanations for the significant variation in fitness costs detected between studies.

There has been some variation in costs on fecundity with several studies reporting an increase in female fecundity (Mansoor et al. 2013, Huang et al. 2019, Ling et al. 2011) or a neutral outcome (Zhu et al. 2021, Horikoshi et al. 2016, Zhang et al. 2018b);

however, previous reviews (Freeman et al. 2021, Gassmann et al. 2009) along with results from this meta-analysis indicate that it is more common to see a reduction in fecundity due to resistance. It is beneficial to understand if resistance has a cost on fecundity, as fecundity is an important indicator of reproductive fitness and population dynamics (Bradshaw and McMahon 2008). A reduction in fecundity due to resistance may lead to lower population densities compared to susceptible populations under similar environmental conditions. As population density is usually directly involved in crop damage or disease outbreaks, lower fecundity may translate into less damage (Rivero et al. 2010, Sisterson 2009) caused by any remaining resistant insects.

This meta-analysis detected an overall increase in pre-adult development time for resistant populations. Populations resistant to Bt differed from this overall trend as Bt resistant populations showed no significant difference in development time compared to susceptible populations. Previous reviews on fitness costs associated with Bt resistance concluded that less than 50% of studies showed a significant effect on juvenile development time (Gassmann et al. 2009, Freeman et al. 2021) concluding no definite answer on whether Bt resistance affects juvenile development. Lepidopterans made up a majority (92%) of the study subjects used for the Bt insecticide class analysis, although Lepidopterans alone suffered a prolonged development time. It is possible the resistant mechanism(s) behind Bt resistance does not confer a cost on development time that apparently occurs with other insecticide classes. Since many agricultural pests, especially in the order Lepidoptera, are only pests as immatures, development costs on juveniles may be useful for the development of pest management strategies. Prolonged development time may provide more opportunities and time to consume crops; however, this prolonged development also exposes the larvae to natural enemies for longer periods (Dmitriew and Rowe 2005).

Many studies on juvenile survival have been performed resulting in a variation of results. Some results from juvenile survival studies showed significant reductions in juvenile survival to insecticide resistance (Wang and Wu 2014, Afzal and Shad 2016, Abbas et al. 2016b), while several others exhibited no significant difference at all (Zhu et al. 2021, Tang et al. 2002, Abbas et al. 2016a); however, no study was found displaying a higher juvenile survival rate for resistant populations compared to susceptible. Results from this meta-analysis indicated that insecticide resistance does decrease juvenile survival overall. A lower juvenile survival percentage may lower population densities and, in turn, translate to lower crop damage so resistance associated with this fitness cost may be beneficial to control strategies.

There has been mixed results regarding the impact of insecticide resistance on female longevity; multiple studies have indicated a cost (Huang et al. 2019, Lee et al. 1996, Shah et al. 2017), while others demonstrated little to no cost (Kuyucu and Caglar 2013, Banazeer et al. 2020, Okoye et al. 2007, Ang and Lee 2011). This meta-analysis may bring together previous contrasting results as most studies that showed significant resistance effects on female lifespan came from neonicotinoid resistance studies and those involving Diptera. It is important to note that there was only one study that was used for both neonicotinoid and dipteran analysis. It is possible the resistant mechanism(s) behind neonicotinoid resistance does impact female lifespan negatively. It is interesting to consider that approximately half of the studies (43%) that comprised Diptera in the female longevity meta-analysis consisted of mosquito species. Many mosquito-transmitted human pathogens require an incubation period within the mosquito to be transmissible (Macdonald 1957). A reduced female lifespan may reduce disease transmission by reducing the period available for pathogen incubation (Rivero et al. 2010).

The results here were the first to demonstrate a consistent reduction in adult male lifespan due to resistance. Resistant males within the order Diptera exhibited a shorter lifespan than susceptible males. All Dipteran adults, whether female or male, suffered a reduced lifespan and may be explained by differences in Dipteran genome compared to the other insect orders. Reductions in male lifespan may reduce the chances of mating and fertilization of females and consequently may lower the overall population density (Barbosa et al. 2020b).

It is still unclear if fitness costs tend to increase along with the magnitude of resistance; although, a few studies have detected an increase in fitness cost as the magnitude of resistance increased (Gassmann et al. 2009, Martins et al. 2012). Results from this analysis along with a recent review (Freeman et al. 2021) distinguished no clear and consistent correlation between the fitness cost and magnitude of resistance. A lack of robust sample size or the development of fitness modifiers in the resistant population are two possible explanations for the lack of correlation between fitness costs and resistance level. Some of the resistance level groups had smaller sample sizes ($k<10$) that can contribute to less powerful results and should be considered with caution. Fitness modifiers are compensatory genes that arise within the insect's genome that will alleviate the fitness costs associated with resistance (ffrench-Constant and Bass 2017, Pang et al. 2021). The sheep blowfly has evolved one such example of a fitness modifier. The diazinon resistant population of sheep blowflies originally conferred a fitness cost, but after 10 years of continuous selection pressure, the development of a fitness modifier alleviated the fitness costs (McKenzie et al. 1982). Clearly, fitness costs associated with resistance may gradually dissipate over time due to modifications in the genome (Raymond et al. 2001) and result in an inconsistent correlation between fitness cost and magnitude of resistance.

Fitness costs depicted through life-history traits may have possible indirect effects on the insects potential for crop damage or transmission of diseases. In some cases, depending on the life-history trait and insect, it might be more economically beneficial to continue applying insecticides and selecting for specific resistance that are accompanied with a fitness cost. Brown et al. (2013) has shown that fitness costs through certain life history traits can drastically change the economic management of resistant pests like *Anopheles* mosquitos. Having a better understanding of the fitness costs associated with insecticide resistance can further improve resistance control strategies. Many different factors that can also influence fitness such as host plant (Janmaat and Myers 2005), genetic background (Raymond et al. 2011), or even laboratory adaptation (Hoffmann and Ross 2018) should be controlled in future studies to accurately assess fitness costs due to insecticide resistance. Future research should continue to test for fitness costs depicted through life-history traits to help optimize control strategies of resistant pest populations.

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Table 2.1. Between-group heterogeneity with corresponding total heterogeneity (in parenthesis) of effect sizes in fecundity, immature development, immature survival, female longevity, and male longevity for susceptible versus resistant individuals. Failsafe N depicts Rosenthal's fail-safe statistic. * Depicts significant heterogeneity

Response	**Categorical Variable**	**d.f.**	**Q**	**P-Value**	**Failsafe N**
Fecundity	Pesticide Class	10(118)	54.15(1193.82)	<.001* (<.001)*	30275
	Insect Order	4(129)	6.43(1257.64)	.170 (<.001)*	41054
	Resistance Ratios	3(123)	5.62(1205.22)	.132 (<.001)*	36249
	Metamorphosis	1(135)	0.56(1354.03)	.478 (<.001)*	43407
Development Time (Days)	Pesticide Class	7(66)	6.62(1654.66)	.469 (<.001)*	3166
	Insect Order	4(71)	2.31(2124.54)	.680 (<.001)*	7910
	Resistance Ratios	3(70)	1.95(2101.72)	.583 (<.001)*	2961
	Metamorphosis	1(73)	0.93(2167.26)	.336 (<.001)*	7835
Immature Survival Rate	Pesticide Class	6(28)	9.50(74.85)	.147 (<.001)*	1651
	Insect Order	2(35)	0.14(82.92)	.933 (<.001)*	896
	Resistance Ratios	3(37)	10.10(88.94)	.018* (<.001)*	1712

	Metamorphosis	1(38)	0.13(89.12	.717 (<.001)*	1746
Female Longevity	Pesticide Class	5(37)	6.68(374.92)	.245 (<.001)*	448
	Insect Order	3(44)	9.26(400.15)	.026* (<.001)*	417
	Resistance Ratios	3(45)	8.11(352.46)	.044* (<.001)*	332
	Metamorphosis	1(48)	1.01(411.42)	.315 (<.001)*	599
Male Longevity	Pesticide Class	5(30)	7.66(342.17)	.176 (<.001)*	611
	Insect Order	2(36)	5.10(358.16)	.078 (<.001)*	447
	Resistance Ratios	3(38)	4.76(344.57)	.190 (<.001)*	569
	Metamorphosis	1(41)	0.71(368.66)	.400 (<.001)*	783

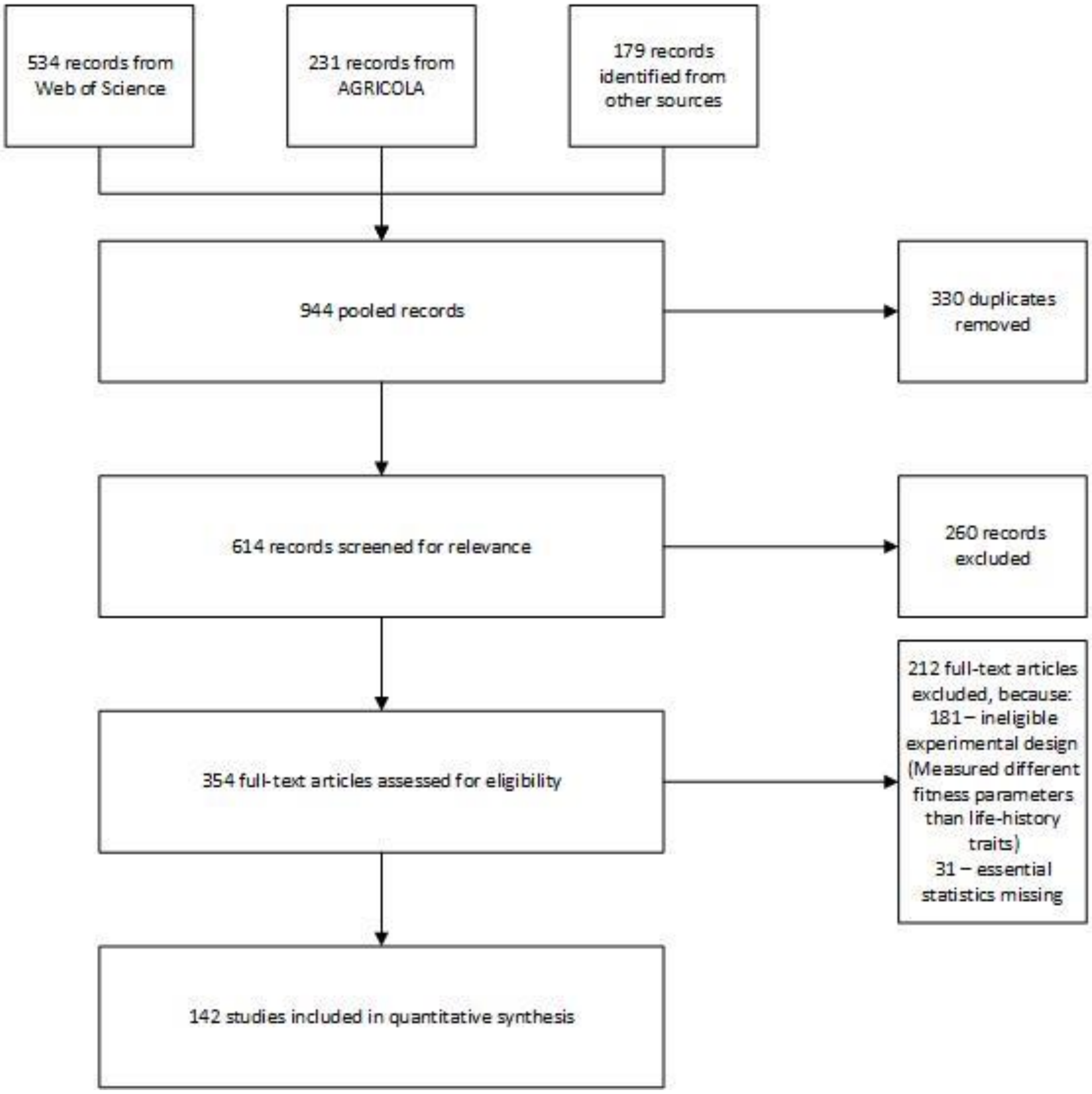

Figure 2.1. Outline of study selection

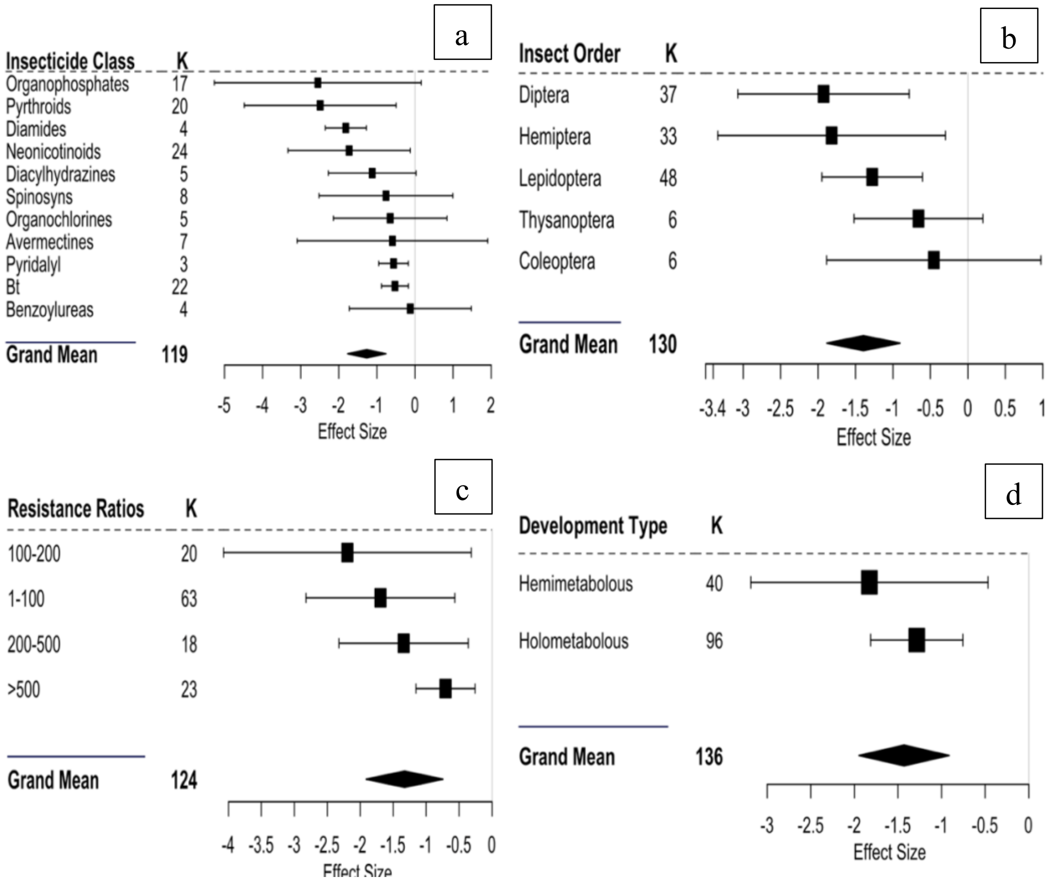

Figure 2.2. Effect of insecticide resistance (susceptible vs resistant) on fecundity. Values presented are effect size means ± 95% confidence intervals. Where confidence intervals overlap the origin indicate no significant difference between the two resistance levels. Values significantly to the left of the origin indicate that susceptible females laid more eggs on average than resistant females did. Fecundity effects (a) for insecticide class, (b) for insect order, (c) for resistance ratios, (d) for development type. K = Sample size

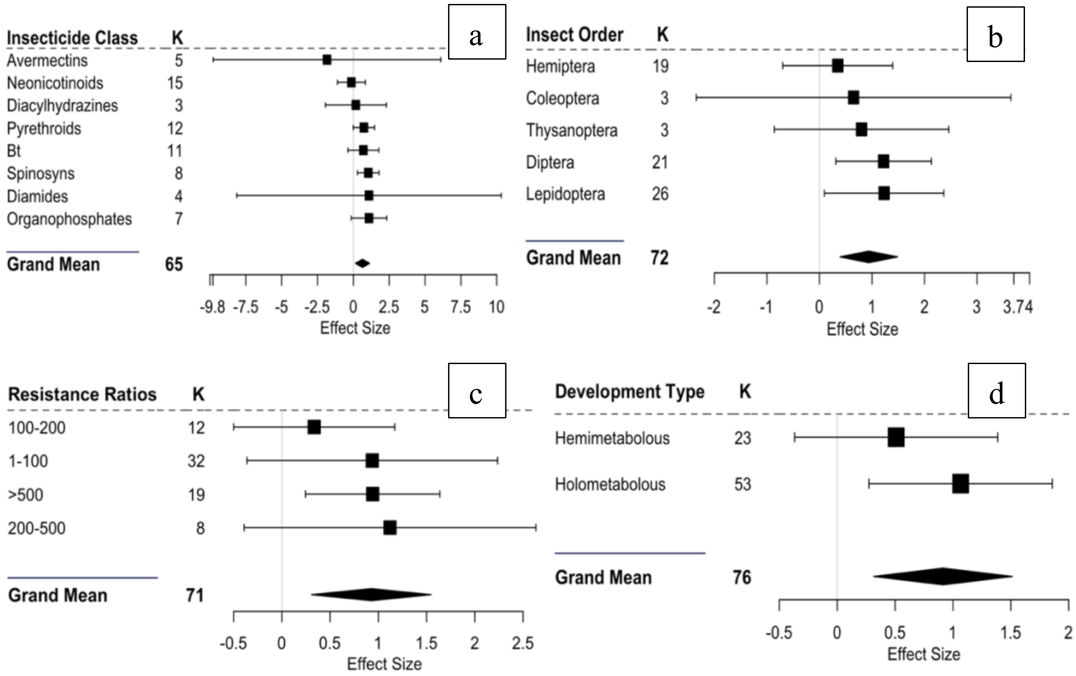

Figure 2.3. Effect of insecticide resistance (susceptible vs resistant) on immature development time (days). Values presented are effect means ± 95% confidence intervals. Where confidence intervals overlap the origin indicate no significant difference between the two resistance levels. Values significantly to the right of the origin indicate that resistant immatures took longer to develop into adults compared to susceptible immatures. Immature development effects (a) for insecticide class, (b) for insect order, (c) for resistance ratios, (d) for development type. K = Sample size

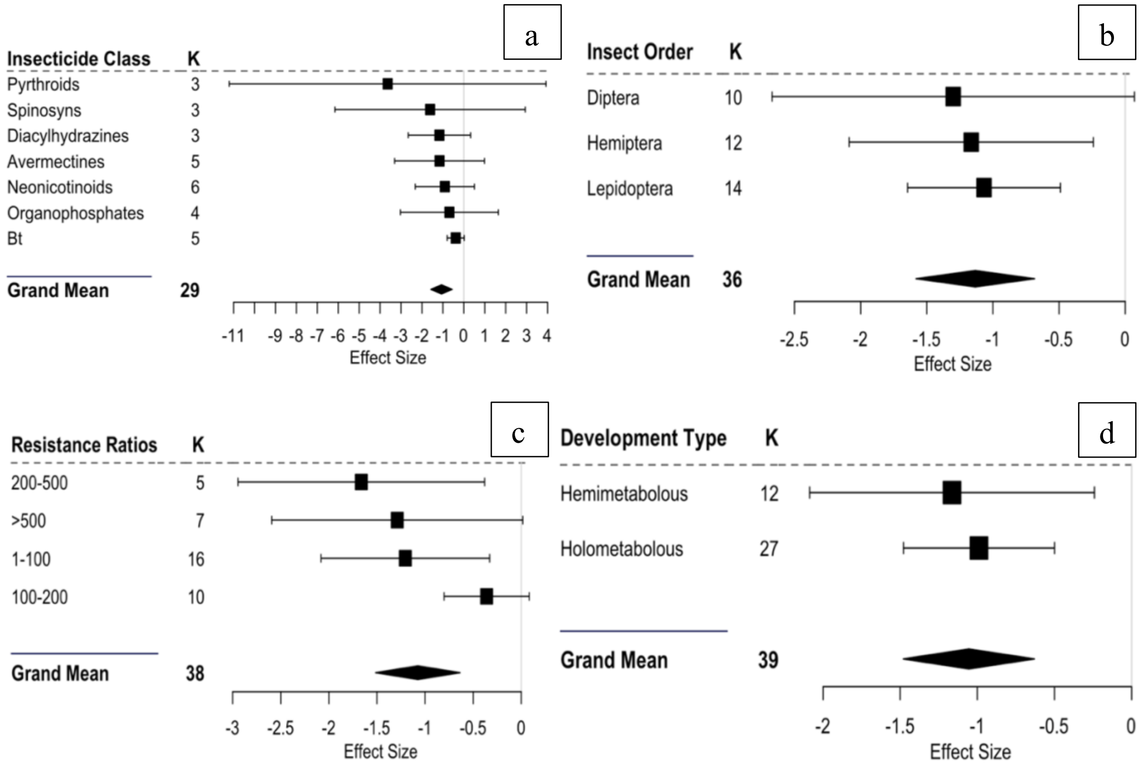

Figure 2.4. Effect of insecticide resistance (susceptible vs resistant) on immature survival rate. Values presented are effect means ± 95% confidence intervals. Where confidence intervals overlap the origin indicate no significant difference between the two resistance levels. Values significantly to the left of the origin indicate that susceptible immatures had a higher survival rate compared to resistant immatures. (a) for insecticide class, (b) for insect order, (c) for resistance ratios, (d) for development type. K = Sample size

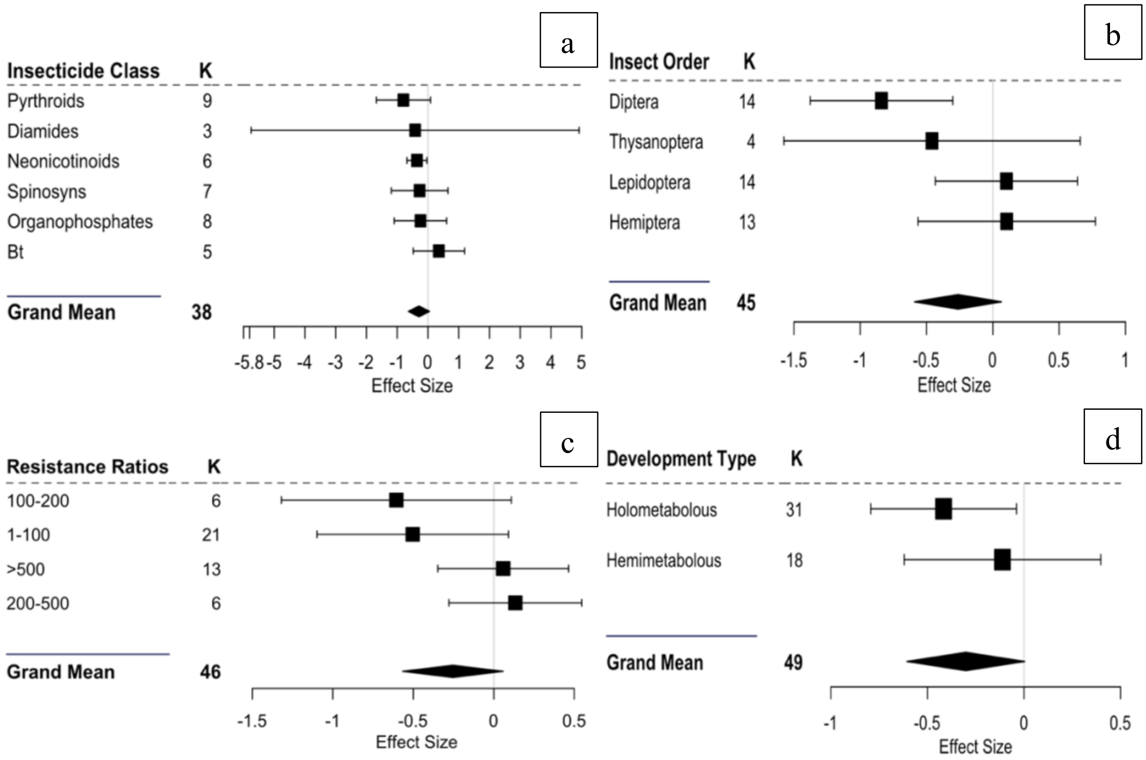

Figure 2.5. Effect of insecticide resistance (susceptible vs resistant) on female longevity. Values presented are effect means ± 95% confidence intervals. Where confidence intervals overlap the origin indicate no significant difference between the two resistance levels. Values significantly to the left of the origin indicate that susceptible females lived longer compared to resistant females. Female longevity effects (a) for insecticide class, (b) for insect order, (c) for resistance ratios, (d) for development type. K = Sample size

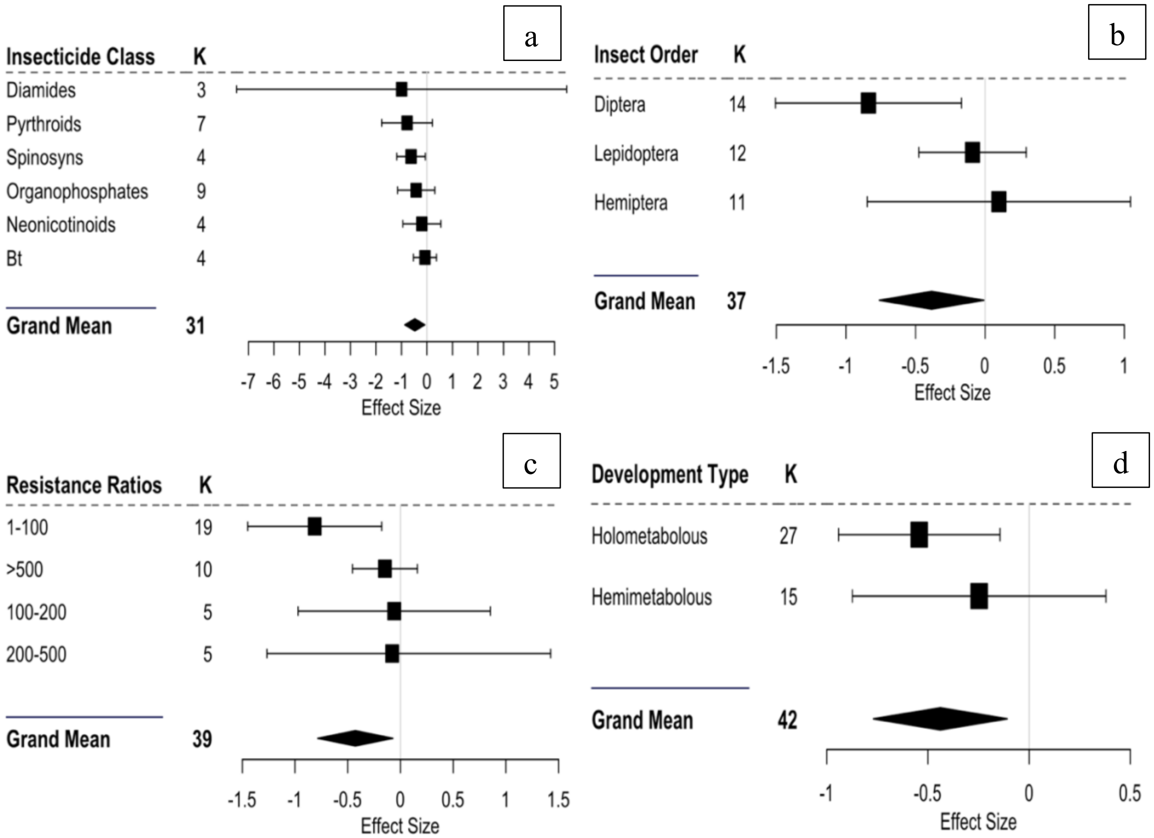

Figure 2.6. Effect of insecticide resistance (susceptible vs resistant) on male longevity. Values presented are effect means ± 95% confidence intervals. Values significantly to the left of the origin indicate that susceptible males lived longer compared to resistant males. Where confidence intervals overlap the origin indicate no significant difference between the two resistance levels. Male longevity effects (a) for insecticide class, (b) for insect order, (c) for resistance ratios, (d) for development type. K = Sample size

Chapter III. Fitness Costs Associated with Insecticide Resistance in California's Central Valley *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) Populations

Abstract

The glassy-winged sharpshooter *Homalodisca vitripennis* Germar (Hemiptera: Cicadellidae) is one of the more important vectors of Pierce's disease throughout the southern and southwestern portions of the United States and strong insect control measures are necessary to limit the spread of this disease. The purpose of this study was to determine the potential effects of insecticide resistance upon a variety of sharpshooter life history parameters associated with insect fitness. Susceptible and resistant field populations of glassy-winged sharpshooters were collected in Kern County California. Individuals from the susceptible population of glassy-winged sharpshooters exhibited significantly higher fecundity and longer adult lifespans than those from the resistant population. Of the adult size indicators measured, only tibia length and intraocular distance were slightly larger in the resistant population. This study provides a strong indication that there are fitness costs associated with insecticide use against this species.

Introduction

The glassy-winged sharpshooter *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) is a xylem-feeding insect pest that can transmit the plant pathogen *Xylella fastidiosa* Wells to a diversity of important agricultural crops: citrus, grapes, alfalfa, coffee, almond, and stone fruits (Redak et al. 2004). The glassy-winged sharpshooter invaded California in the early 1990's, most likely on nursery plants imported from Texas (Smith 2005, De Leon et al. 2004). Sharpshooter populations in California produce two generations a year in the field with adults overwintering in the cooler fall and winter months (Pilkington et al. 2014, Castle et al. 2005). Both adults and immatures feed on a wide variety of host plants topping over 340 plants reported to date (https://www.cdfa.ca.gov/pdcp/Documents/HostListCommon.pdf). The pathogenic bacterium causing Pierce's disease of grapes (a variant of X. *fastidiosa*), disrupts the water conducting elements in xylem leading ultimately to plant death (Sun et al. 2013, Purcell and Hopkins 1996). Since the establishment of the glassy-winged sharpshooter in California in the 1990's, the incidence of Pierce's disease has increased dramatically due to the high densities of the glassy-winged sharpshooter populations and the relatively efficient ability of this insect to transmit the disease (Blua et al. 1999).

In the early 2000's, area-wide programs across southern California implemented glassy-winged sharpshooter control strategies that relied on insecticides such as neonicotinoids (Wendel et al. 2002). These programs aimed to lower overall numbers of glassy-winged sharpshooters in citrus groves, vineyards, and plant nurseries through heavy insecticide use. Over the last decade, however, several glassy-winged sharpshooter populations have developed a resistance to acetamiprid, bifenthrin, and imidacloprid. As a result of the continuous use of these insecticides and consequential development of insecticide resistance, sharpshooter population numbers are increasing in vineyards and citrus groves in California's central valley (Byrne and Redak 2021). New and improved control strategies are needed to keep the population levels low to prevent outbreaks of Pierce's disease throughout Kern County.

With the development of insecticide resistance, there is often a cost to fitness (Carriere et al. 2001, Wang and Wu 2014, Kliot and Ghanim 2012). This cost can be brought on through metabolic resistance mechanisms that diverts a finite amount of energy in the insect to metabolize the harmful insecticide to more harmless byproducts (Tchouakui et al. 2020, ffrench-Constant and Bass 2017). A target site mutation may also lead to fitness costs as the mutated ion channel target site has a lower binding infinity and may function less efficiently compared to the original target site before the mutation (ffrench-Constant and Bass 2017, Stearns 1992). These trade-off costs have presented themselves in a multitude of biological and morphological traits including fecundity, longevity, survival rate, and size differences (Carriere et al. 2001, Wang and Wu 2014, Kliot and Ghanim 2012, Gassmann et al. 2009). Presently, there have been no studies that have examined potential fitness trade-offs associated with insecticide resistance in the glassy-winged sharpshooter.

Understanding if there is a fitness cost to insecticide resistance and how it may affect glassy-winged sharpshooter populations may lead to more efficacious control measures. When fitness costs are present with resistance then natural selection will select against the costly resistant alleles given that the original insecticide use is suspended, or if different non-cross-resistant insecticides are used in rotation (Barbosa et al. 2020). If fitness costs are absent, then the resistance allele will likely become fixed resulting in the complete removal of the original insecticide and any cross-resistance insecticides (Kliot and Ghanim 2012, ffrench-Constant and Bass 2017). This project will determine the potential effects of insecticide resistance on several life history characteristics correlated with overall individual fitness.

Materials and Methods

Insect Collection and Colonies

Glassy-winged sharpshooter populations were collected as adults in Kern County, CA in August 2019 (**Figure 3.1**). The resistant population was over 412 times more resistant to imidacloprid, a systemic insecticide, compared to the susceptible population (**Table 3.1**) (Byrne and Redak 2021). Insect populations were maintained in separate cages (60 x 60 x 60 cm, width x height x depth, Bioquip, Rancho Dominguez CA) and transported to UCR Agricultural Operations greenhouse with 14:10 light: dark photoperiod. Each cage contained a single basil, okra, sorghum plant, and two sunflower plants to ensure adequate food quality and availability (Mizell et al. 2008). Plants in cages were watered with approximately 250ml of water every other day and fertilized every two weeks with a 14.8 ml of Miracle-Gro® (24-8-16) infused with water. Plants in cages were replaced with fresh plants every two months.

Life-History Experiments

Individuals from the field collected populations were allowed to mate and lay eggs in their respected cages for two generations. The second generation of nymphs were used in the following fitness experiments because they had no previous exposure to insecticides and had acclimated to the greenhouse environment. One hundred resistant and susceptible first instar nymphs were collected and raised in two separate identical cages on sunflower plants. Insects in these cages were provided with six sunflowers plants; plants were replaced every three weeks. Upon adult emergences, newly emerged virgin males and females were paired together on an individual sunflower in mating cages (9”x4”x13” polypropylene bread bag over 3.5”sq x 3” pot). Mating cages were immediately placed in incubators (Percival Chamber I-30BLL, Percival Scientific INC, Perry IA) for the duration of the study. Twelve mating cages (six resistant pairs and six susceptible pairs), each with a single pair of sharpshooters were placed in each incubator. A total of five incubators were utilized: incubators were considered as replicate blocks and previous exposure to insecticides (resistant vs. susceptible) was considered as a main effect treatment in a randomized complete block design. All incubators were set at 27 ^{o}C with 14:10 light: dark period and ambient humidity. Each individual cage was checked daily to record fecundity and longevity of the paired individuals. For each mated pair, I determined the following: preoviposition time for adult females (time in days from adult emergence to first oviposition), fecundity (number of eggs produced per female), and adult longevity (time in days each male and female lived as adults). All dead insects were individually placed in vials with 2 ml of 100% ethanol and frozen at -20 ^{o}C until processed. For each individual, I measured the intraocular distance (distance between left inner eye to right inner eye, mm), tibia length (length of left metathoracic tibia, mm), and wing vein length (RA1 wing vein length from left metathoracic wing, mm) were measured after the adults died. All microscopic measurements were done using a stereomicroscope and micrometer lens (Wild M3 Series Stereomicroscope, Wild Leitz, Heerbrugg, Switzerland).

Dry Weight Experiment

All insects used in this experiment were isolated from the established resistant and susceptible colony populations. Susceptible (n=40) and resistant (n=40) first instar nymphs were collected and separated into two cages. Each cage contained six sunflower plants; plants were watered every other day and fertilized every other week. All plants were replaced every three weeks until adult emergences. From each population, 20 female and 20 male adults were collected within eight hours of emergence, and freeze-killed (-20 ^{o}C for 24 hours). Individual fresh masses (nearest 0.1mg) (NewClassic Balance MS Semi-Micro Model, Greifensee, Switzerland) were subsequently determined for each of the insects. To determine dry mass, insects were dried in a drying oven (Aloe Scientific Oven, St. Louis MO) for 72 hours at 65 ^{o}C. Dried specimens were allowed to cool in a desiccator equipped with dririte™ and then reweighed to nearest 0.1mg to determine dry mass.

Statistical Analyses

All data analyses were conducted using the statistical software R (R Development Core Team 2020). To assess the effects of insecticide resistance upon preoviposition time (days), female fecundity (number of eggs deposited per female), adult male longevity (days), and adult female longevity (days), I used linear mixed models (‘lme4 package)(Bates et al. 2015), where resistance level (resistant or susceptible) was considered as a fixed effect at two levels and the rearing chambers (n=5) in which the cages were placed were considered a random block effect. All life-history trait models met the assumptions of normality and homoscedasticity. For each body size trait (ocular distance, tibial length, wing vein length, and dry weight), I conducted a separate two-way ANOVA with morphological trait and sex as fixed factors. All graphs were produced using R package GGPLOT2 (Wickham 2011).

Results

The preoviposition period for females did not differ between insecticide resistant and susceptible populations ($F_{1,46}$=0.003; P=0.9591). Susceptible females took an average of 24.20 $\pm$ 8.90 (mean $\pm SEM$) days to deposit their first egg mass while resistant females took 23.60 $\pm$ 5.40 days. There were 3 extreme outliers within this data set as one female in the resistant population and two females in the susceptible population took over 100 days to oviposit. When these three outliers are removed, the preoviposition period is significantly shorter for susceptible individuals as compared to resistant individuals ($F_{1,38.87}$=23.9; P<0.0001; 12.22 $\pm$ 0.83 days and 18.50 $\pm$ 1.52 days respectively)

The average number of eggs deposited per susceptible female was significantly greater than the mean number of eggs deposited by an individual from the resistant population ($F_{1,43}$=11.5; P=0.0015). Susceptible females laid an average of 370.84 $\pm$ 44.46 eggs while resistant females laid an average of 198.30 $\pm$ 31.65 eggs. 83% of the susceptible females were fertile (25 out of 30 individuals), while only 77% of the resistant females were fertile (23 out of 30). However, fertility between the susceptible and resistant population did not differ significantly (X^2_1=0.4, P=0.5186).

Longevity varied with resistance level for both sexes. Susceptible females lived an average of 96.5 $\pm$ 10.62 days while resistant females lived 59.93 $\pm$ 7.46 days

($F_{1,54}$=8.2; P=0.0059). Susceptible males lived an average of 129.97 ± 13.34 days while resistant males lived 80.07 ± 10.32 days ($F_{1,58}$=8.8; P=0.0045).

There was a significant difference in the intraocular distance between male eyes compared to female eyes whether they were from the resistant or susceptible populations ($F_{1,112}$=127.3, P<.0001). There was also a significant difference between resistant and susceptible populations when it came to intraocular distance ($F_{1,112}$=4.1, P=.0456). There was no interaction between sex and resistance ($F_{1,111}$=0.2, P=.6466). Susceptible females' eyes were an average of 2.22 ± .01 mm apart while resistant females' eyes were 2.25 ± .01 mm apart. Susceptible males' eyes were an average of 2.04 ± .01 mm apart while Resistant males' eyes were 2.08 ± .02 mm apart.

Regardless of resistance levels, males and females differed significantly when it came to tibia length ($F_{1,107}$=43.2, P<.0001). There was also a significant difference in tibia length between resistant and susceptible populations ($F_{1,107}$=4.78, P=.0310). There was no interaction between sex and resistance ($F_{1,106}$=0.6, P=.4495). Susceptible females' tibias were on average 4.01 ± 0.02 mm long while resistant females' tibias were 4.05± 0.03 mm long. Susceptible males' tibias were an average of 3.80 ± 0.03 mm long while resistant males' tibias were 3.88± 0.03 mm long.

The length of the RA1 vein in the metathoracic wing differed by sex ($F_{1,108}$=129.6, P<.0001); however, there was no significant difference in mean length of the R1 vein between resistant and susceptible populations ($F_{1,108}$=0.1, P=0.7336). There was no interaction between sex and resistance when it came to R1 vein length ($F_{1,107}$=0.4, P<.5268). The mean R1 vein length for susceptible females were 6.77 ± 0.03 mm while the mean R1 vein length 6.73 ± 0.05 mm for resistant females ($F_{1,57}$=0.5, P=.4804). The mean R1 vein length for susceptible males were 6.27 ± 0.05 mm while the mean R1 vein length 6.28 ± 0.04 mm for resistant males ($F_{1,50}$=0.05, P=.8261).

Dry mass between males and females differed significantly regardless of resistance levels ($F_{1,77}$=61.2, P<.0001). There was no effect of resistance level on dry mass ($F_{1,77}$=1.7, P=.191) nor an interaction between sex and resistance ($F_{1,76}$=0.3, P=.6046). Susceptible females had an average dry mass of 7.90 ± 0.46 mg while resistant females' average dry mass was 7.56 ± 0.32 mg. Susceptible males' dry weight was on average 6.36 ± 0.37 mg while resistant males' dry mass was 5.65 ± 0.37.

Discussion

Preoviposition period was not significantly different when outliers were present but was significantly different when outliers were excluded. The susceptible population had a significantly longer adult lifespan and higher fecundity compared to the resistant population. Shorter adult lifespans and reduced fecundity can drastically affect transmission rates of pathogens (Rivero et al. 2010). Given that transmission of the plant pathogen X. *fastidiosa* is dependent on its vector, any cost on the vector's fitness can significantly affect disease dynamics. For example, a shorter vector lifespan as seen with the resistant glassy-winged sharpshooter population compared its susceptible counterpart will result in less time and opportunities that the vectored pathogen will have to spread to new hosts (Lefevre and Thomas 2008). Also, the lower reproductive output marked by lower fecundity in resistant populations could directly relate to population density that is a key variable in the rate of vector transmission (Sisterson 2009). The susceptible glassy-winged sharpshooter has a greater potential to spread Pierces' disease due to a greater reproductive fitness affecting populational numbers and longer lifespans increasing the chances of picking up the pathogen and spreading it to susceptible grapevines.

Morphological trait differences have been observed between susceptible and resistant populations of whiteflies, codling moth and mosquitoes (Feng et al. 2009, Bourguet et al. 2004, Zivkovic et al. 2019). In addition, significant decreases in dry weight have been documented in chlordane-resistant German cockroaches (Grayson 1954). Results from this study indicate no difference in dry weight between susceptible and resistant populations. Assessing differences in morphological traits between susceptible and resistant populations can be an inexpensive way to denote resistance in field populations (Mikac et al. 2019). Here, I looked at four different size determining features including dry weight, ocular distance, tibia length, and wing vein length. The resistant population had a significantly wider intraocular distance and longer tibia length than the susceptible population; however, there was no significant difference between the two populations when it came to wing vein length or dry weight. A wider ocular distance along with a longer tibia may indicate a larger insect (Ameri et al. 2013). Previous studies on resistant codling moths indicated that larger insects may be more resistant and may explain why the resistant sharpshooters were slightly larger compared to susceptible (Reyes et al. 2015).

Fitness costs associated with insecticide resistance have been shown in a wide variety of pests (Gao et al. 2014, Abbas et al. 2015, Liu and Han 2006, Kliot and Ghanim 2012). Different resistant mechanisms, metabolic resistance or target site mutations, have been known to cause similar fitness costs as seen here (Tchouakui et al. 2020, Homem et al. 2020). The exact mechanism of insecticide resistance in glassy-winged sharpshooters is still unknown; however recent evidence suggests cytochrome P450 may be involved (Byrne and Redak 2021).

This study reported significant reductions in fecundity and adult longevity both for males and females that originated from populations of sharpshooters previously demonstrating insecticide resistance to neonicotinoids and pyrethroids in the field. These results indicate that there is a fitness cost associated with insecticide resistance in this species. It should be noted that the resistant and susceptible populations in Kern County CA were only being separated by ~12 miles. It is very likely that both populations were interbreeding as the susceptible population had resistance to imidacloprid without any known selection pressure used in the urban environment. It is likely that susceptibility could be renewed in field populations once the selection pressure is removed (Regev, Shalit and Gutierrez 1983). Insecticide resistance has been shown to be unstable in the absence of the selection pressure and the resistant population ultimately reverts to a more susceptible population within a couple of generations; however, resistance instability is only observed in populations that exhibit fitness costs (Banazeer, Shad and Afzal 2020, Shah and Shad 2020, Basit 2019). Additional research needs to be performed to confirm the stability of resistance in the absence of insecticide treatments in glassy-winged sharpshooter populations.

Understanding fitness costs associated with insecticide resistance in a pest system may influence its management since the removal of that insecticide may allow the affected population to revert to a more susceptible stage (Afzal et al. 2015). This reversion back to susceptibility can be sped up by insecticide rotations with different modes of action, as seen in control programs for pestiferous hemipterans (Chen et al. 2021). Resistance instability caused by fitness costs could be enhanced by use of a different insecticide for management to renew susceptibility to imidacloprid. This is the first report of a fitness cost associated with insecticide resistance in the glassy-winged sharpshooter to this date. More research is needed to identify the underlying mechanism(s) of resistance and confirm if resistance is unstable. IPM management should consider modifying insecticide applications to reduce maintenance of resistance.

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Table 3.1. Log-dose probit analysis of the contact toxicity of imidacloprid to glassy-winged sharpshooter adults in Kern County California. Data used from (Byrne and Redak 2021) and unpublished data from September 2019. General Beal resistant population was not included in probit analysis since resistance was too high to obtain an adequate dose-response.

Population	**Year**	**N**	**LD50 (ng/insect) (95% FL)**	**RR**	**Slope (±SE)**	**X^2**	**df**
Urban Site (Susceptible)	2019	140	12.12 (5.79-25.30)	1	1.29 ± 0.16	0.49	2
General Beal (Resistant)	2019	500	>5000	>412.54			

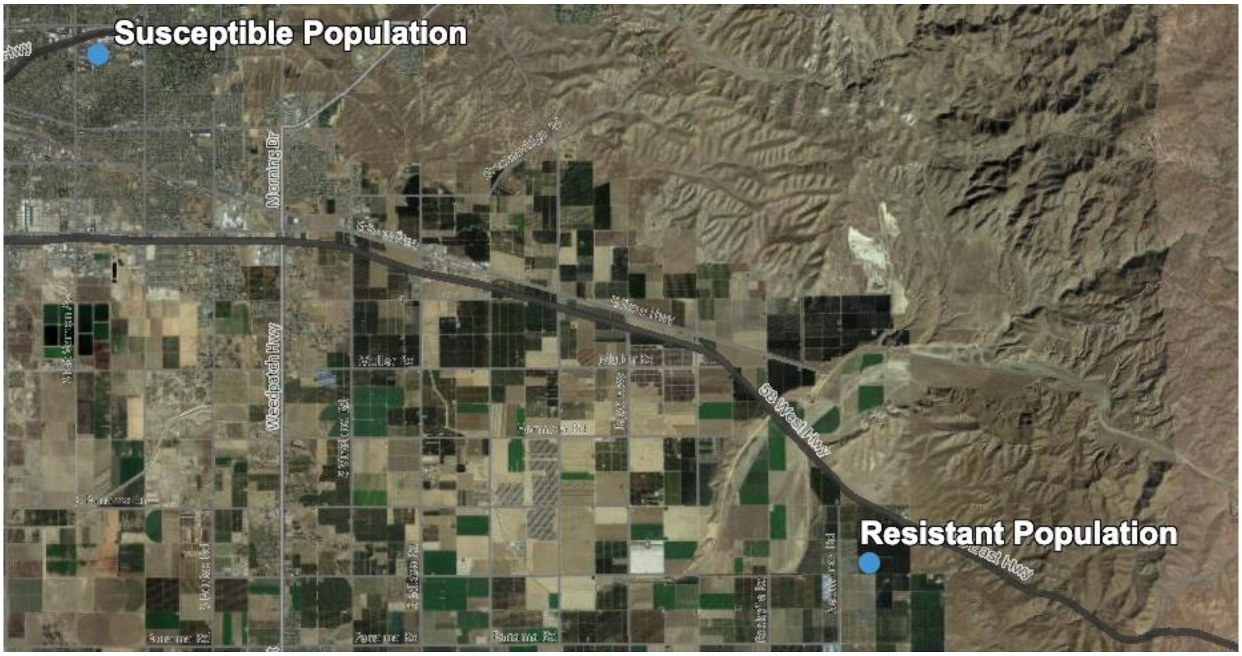

Figure 3.1. Map showing glassy-winged sharpshooter collection sites in Kern County California. Both populations were collected in September 2019 on the same day.

Chapter IV. Conclusion

Fitness costs are thought to accompany insecticide resistance in the absence of the insecticide in the environment. Studies determining if and how these fitness costs associated with insecticide resistance present themselves can improve our understanding of this concept and how it can be applied to resistance management. One way to identify fitness costs associated with resistance is a comparison of life history traits between resistant and susceptible pestiferous populations. The glassy-winged sharpshooter is an ideal model system to examine fitness costs associated with insecticide resistance as it has developed resistance in the field, and it is an economic pest of concern. Pooling together studies on life-history comparison between resistant and susceptible population through meta-analysis can then help identify overall patterns in these fitness costs due to insecticide resistance.

There is a large amount of literature on fitness costs associated with insecticide resistance that was quantitatively assessed to reveal overall patterns. I found that insecticide resistance had multiple impacts on life history characteristics overall, and resistance had different effects depending on different insect orders, insecticide classes, and magnitude of resistance. Overall fecundity, juvenile development time, juvenile survival rate, and male longevity were significantly affected by insecticide resistance; however, female longevity was not affected overall. Susceptible populations within Diptera, Hemiptera, and Lepidoptera had a significantly higher fecundity than resistant populations. Juvenile development time increased significantly for resistant dipteran and lepidopteran larvae; however, juvenile survival rate decreased only in resistant lepidopteran and hemipteran juveniles but not juveniles within Diptera. Only resistant insects within Diptera had a significantly reduced lifespan regardless of sex. Surprisingly, the meta-analysis did not detect a consistent correlation between magnitude of resistance and fitness costs.

Meta-analyses do have their limitations, most notably with small sample sizes. Any conclusions drawn from analyses with small sample sizes should be interpreted with caution. The results presented here highlight areas where future research should be conducted to improve our overall understanding of fitness costs and identify more predictable patterns. Resistance mechanisms may have partially explained the high heterogeneity observed in all the meta-analysis performed, but a lack of studies tying fitness costs to known resistance mechanism(s) prevented a categorical meta-analysis from being completed on mechanism. Future research should aim to uncover both the resistance mechanism(s) and the costs accompanied with that mechanism(s).

Insecticide resistance in glassy-winged sharpshooter had a significant effect on multiple life history parameters, which indicates an overall cost in fitness. Resistant populations exhibited 53.5% reduction in fecundity compared to the susceptible population in Kern County California. Adult longevity for both males and females of the resistant populations were 62.5% shorter than susceptible populations. Adult size indicators including intraocular distance and tibia length were larger for the resistant population compared to the susceptible population, but wing size and dry weight was no different between the two populations.

These lines of evidence suggest that the glassy-winged sharpshooter population in California's Central Valley does have a fitness cost associated with insecticide resistance. This is important to note, because insecticide resistance is commonly unstable if there are fitness costs associated with it (Alam et al. 2020, Banazeer et al. 2020, Shah and Shad 2020). Populations with unstable resistance will eventually revert to its original susceptible state. Future studies will be needed to test stability to see how long it will take for the resistant field populations to revert to its original susceptible state.

Here, the glassy-winged sharpshooter results (Chapter 2) corresponded well with the findings from the meta-analysis (Chapter 3). Resistance to neonicotinoids along with resistant individuals within the order Hemiptera had a significant reduction in the number of eggs laid per female. Similarly, the neonicotinoid resistant sharpshooter population had a significant reduction in fecundity (53.5%). Results from the female longevity meta-analysis showed that resistance to neonicotinoids significantly reduced female lifespan. Likewise, a large reduction in female lifespan was detected in the resistant glassy-winged sharpshooter population. The meta-analysis results indicated that neither resistant hemipteran males nor neonicotinoid resistant male lifespan was significantly different than susceptible individuals. Unlike the meta-analysis results, male sharpshooters resistant to neonicotinoids did exhibit a reduced lifespan. It is possible the resistant sharpshooter population possess a different resistance mechanism than those utilized in the meta-analysis. Two out of the three results from the meta-analysis agreed with the results from the glassy-winged sharpshooter study helping to support the idea that patterns in the meta-analysis may help predict fitness costs based off factors such as insect order, insecticide class, magnitude of resistance, and development type.

These studies provide further evidence for fitness costs associated with insecticide resistance while revealing some possible explanations for the variation in costs detected. Future research should aim to uncover both the resistance mechanism(s) and the costs accompanied with that mechanism in comparisons between resistant and susceptible populations. Resistant mechanisms may be an excellent explanatory moderator for the high heterogeneity observed in all the meta-analysis performed but many studies did not test for resistance mechanism. As molecular and genomic biology becomes more accessible to the field of entomology, future studies may employ techniques like CRISPR Cas9 to increase our understanding of fitness costs associated with insecticide resistance. CRISPR Cas9 can ensure isogenic lines with the only genetic difference being the inserted sequence conveying a known resistance mechanism (Douris et al. 2020). A great deal of research is still needed to cover knowledge gaps of fitness costs associated with insecticide resistance and how to exploit this concept in resistance control strategies.

With cases of insecticide resistance on the rise, new strategies will be needed to combat resistance and control pest populations. Resistance control strategies should incorporate information on fitness costs to slow down and possibly revert resistance in pest populations. Successful resistance control strategies that incorporate fitness costs due to insecticide resistance like insecticide rotations (Chen et al. 2021, Barbosa et al. 2020) and refuge strategies (Laxminarayan and Simpson 2002) are strongly recommended.

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Appendix A

Studies Used in Fecundity Meta-Analysis

Studies	Insecticide Class	Insect Order	Resistance Ratios	Development Type
(Abbas et al. 2015)	X	X	X	X
(Abbas et al. 2012)	X	X	X	X
(Cao and Han 2006)	X	X	X	X
(Boivin et al. 2001)*	X	X	X	X
(Boivin et al. 2001)*	X	X	X	X
(Wang et al. 2018b)	X	X	X	X
(Horikoshi et al. 2016)	X	X	X	X
(Shen et al. 2017)		X	X	X
(Shah et al. 2017)	X	X	X	X
(Martins et al. 2012)	X	X		X
(Jia et al. 2009)	X	X	X	X
(Fu et al. 2018)	X	X	X	X
(Fu et al. 2017)	X	X	X	X
(Abbas et al. 2016a)		X	X	X
(Haubruge and Arnaud 2001)	X	X	X	X
(Trisyono and Whalon 1997)	X	X	X	X
(Liu and Han 2006)	X	X	X	X
(Mansoor et al. 2013)	X		X	X
(Abbas et al. 2014a)	X		X	X
(Afzal et al. 2015a)	X	X	X	X
(Sayyed et al. 2008a)*	X	X	X	X
(Sayyed et al. 2008a)*		X	X	X
(Gao et al. 2014)	X	X	X	X
(Ejaz et al. 2017)	X	X	X	X

(Saingamsook et al. 2019)	X	X	X	X
(Shah et al. 2015)		X	X	X
(Wang et al. 2020a)	X	X	X	X
(Lee et al. 1996)			X	X
(Sayyed and Wright 2001)*	X	X	X	X
(Sayyed and Wright 2001)*	X	X	X	X
(Sayyed and Wright 2001)*	X	X	X	X
(Abbas et al. 2014b)	X	X	X	X
(Chen et al. 2020)	X	X	X	X
(Castellanos et al. 2019)	X	X	X	X
(Shi et al. 2020)	X	X	X	X
(Zhang et al. 2020)	X	X	X	X
(Ullah et al. 2020b)	X	X	X	X
(Gul et al. 2019)	X	X	X	X
(Ejaz and Shad 2017)		X	X	X
(Abbas et al. 2016b)	X	X	X	X
(Ling et al. 2011)	X	X	X	X
(Banazeer et al. 2020)	X	X	X	X
(Liu et al. 2021)	X	X	X	X
(Shah and Shad 2020)	X	X	X	X
(Ishtiaq et al. 2014)	X	X	X	X
(Wang and Wu 2014)	X	X	X	X
(Zaka et al. 2014)	X	X	X	X
(Zhang et al. 2018a)	X	X	X	X
(Cui et al. 2018)		X	X	X
(Huang et al. 2019)	X	X	X	X
(Gordon et al. 2015)*	X	X		X
(Gordon et al. 2015)*	X	X		X
(Gordon et al.	X	X		X

2015)*				
(Sun et al. 2012)		X	X	X
(Assogba et al. 2015)	X	X	X	X
(Yang et al. 2018)	X	X	X	X
(Zhang et al. 2015a)	X	X	X	X
(Zhang et al. 2018b)	X	X	X	X
(Wang et al. 2018a)	X	X	X	X
(Rowland 1991)*	X	X	X	X
(Rowland 1991)*	X	X	X	X
(Underhill and Merrell 1966)*	X	X	X	X
(Underhill and Merrell 1966)*	X	X	X	X
(Underhill and Merrell 1966)*	X	X	X	X
(Plernsub et al. 2013)	X	X	X	X
(Li et al. 2002)	X	X	X	X
(Hafeez et al. 2019)	X	X	X	X
(Saddiq et al. 2016)	X	X	X	X
(Amin and White 1984)	X	X	X	X
(Kuyucu and Caglar 2013)	X	X	X	X
(Spollen and Hoy 1992)			X	X
(Okoye et al. 2007)	X	X		X
(Barbosa et al. 2020b)		X	X	X
(Nkahe et al. 2020)	X	X		X
(Mahon and Olsen 2009)	X	X	X	X
(Zhang et al. 2015b)	X	X	X	X
(Zhang et al. 2015b)	X	X	X	X
(Ma et al. 2019)	X	X	X	X
(Ullah et al. 2020a)		X	X	X

(Wang et al. 1998)	X	X	X	X
(Yu-ping et al. 2010)	X	X	X	X
(Roush and Plapp 1982)	X	X		X
(Alam et al. 2020)		X	X	X
(Chen et al. 2015)	X	X	X	X
(Wang et al. 2010)	X	X	X	X
(Wang et al. 2020c)		X	X	X
(Ullah et al. 2021)	X	X	X	X
(Jin et al. 2021)	X	X	X	X
(Saeed et al. 2021)	X	X	X	X
(Naeem et al. 2021)		X	X	X
(Liang et al. 2008)*	X	X	X	X
(Liang et al. 2008)*	X	X	X	X
(Liang et al. 2008)*	X	X	X	X
(Santos-Amaya et al. 2017)*	X	X		X
(Santos-Amaya et al. 2017)*	X	X	X	X
(Cerda et al. 2003)*	X	X	X	X
(Cerda et al. 2003)*	X	X	X	X
(Zhu et al. 2016)	X	X	X	X
(Zhu et al. 2021)	X	X	X	X
(Wang et al. 2020b)	X	X	X	X
(Akbar et al. 2008)	X	X	X	X
(Liu et al. 2015)	X	X	X	X
(Sun et al. 2021)		X	X	X
(Rosen et al. 2021)	X	X	X	X
(Cai et al. 2020)	X		X	X
(Wang et al. 2020c)	X	X	X	X
(Fujii et al.	X	X	X	X

2020)*				
(Fujii et al. 2020)*	X	X	X	X
(Homem et al. 2020)	X	X	X	X
(Liao et al. 2019a)	X	X	X	X
(Khan 2018)	X	X	X	X
(Rahim et al. 2017)	X	X	X	X
(Steinbach et al. 2017)	X	X	X	X
(Li et al. 2017)	X	X	X	X
(Afzal and Shad 2017)	X	X	X	X
(Alam et al. 2017)	X	X	X	X
(Zhang et al. 2016)		X	X	X
(Afzal and Shad 2016)	X	X	X	X
(Afzal et al. 2015b)		X	X	X
(Tang et al. 2015)	X	X	X	X
(Germano and Picollo 2015)	X	X	X	X
(Ferreira et al. 2013)	X	X	X	X
(Belinato et al. 2012)	X	X	X	X
(Gulzar et al. 2012)	X	X	X	X
(Tang et al. 2011)	X	X	X	X
(Paris et al. 2011)	X	X	X	X
(Sayyed et al. 2008b)	X	X	X	X
(Liu et al. 2008)	X	X	X	X
(Bielza et al. 2008)	X	X	X	X
(Alyokhin and Ferro 1999)	X	X		X
(Baker et al. 1998)	X		X	X
(Belinato and Valle 2015)	X	X	X	X

(Belinato and Valle 2015)	*X*	*X*	*X*	*X*
(Diniz et al. 2014)	X	X		X

*= Same studies with separate resistant and susceptible populations being compared as individual studies

Studies Used in Immature Development Meta-Analysis

Studies	Insecticide Class	Insect Order	Resistance Ratios	Development Type
(Abbas et al. 2015)	X	X	X	X
(Feng et al. 2009)	X	X	X	X
(Boivin et al. 2001)	X	X	X	X
(Wang et al. 2018b)	X	X	X	X
(Shah et al. 2017)	X	X	X	X
(Jia et al. 2009)	X	X	X	X
(Fu et al. 2018)	X		X	X
(Abbas et al. 2016a)		X	X	X
(Trisyono and Whalon 1997)	X	X	X	X
(Shah and Shad 2020)	X	X	X	X
(Mansoor et al. 2013)	X		X	X
(Liao et al. 2019b)	X	X	X	X
(Abbas et al. 2014a)	X		X	X
(Gao et al. 2014)	X	X	X	X
(Ejaz et al. 2017)	X	X	X	X
(Yasoob et al. 2018)	X	X	X	X
(Shah et al. 2015)		X	X	X
(Lee et al. 1996)			X	X
(Ullah et al. 2020b)	X	X	X	X
(Ejaz and Shad 2017)		X	X	X
(Abbas et al. 2016b)	X	X	X	X
(Shah and Shad 2020)	X	X	X	X
(Ishtiaq et al. 2014)	X	X	X	X
(Wang and Wu 2014)	X	X	X	X
(Zaka et al.	X	X	X	X

2014)				
(Huang et al. 2019)		X	X	X
(Assogba et al. 2015)	X	X	X	X
(Wang et al. 2017)	X	X	X	X
(Wang et al. 2018a)	X	X	X	X
(Plernsub et al. 2013)	X	X	X	X
(Li et al. 2002)	X	X	X	X
(Hardstone et al. 2010)	X	X	X	X
(Hafeez et al. 2019)	X	X	X	X
(Saddiq et al. 2016)	X	X	X	X
(Okoye et al. 2007)	X	X		X
(Barbosa et al. 2020b)		X	X	X
(Nkahe et al. 2020)	X	X	X	X
(Mahon and Olsen 2009)	X	X	X	X
(Ma et al. 2019)	X	X	X	X
(Wang et al. 1998)	X	X	X	X
(Yu-ping et al. 2010)	X	X	X	X
(Alam et al. 2020)	X	X	X	X
(Chen et al. 2015)	X	X		X
(Ullah et al. 2021)	X	X		X
(Jin et al. 2021)	X	X	X	X
(Saeed et al. 2021)	X	X	X	X
(Naeem et al. 2021)		X	X	X
(Liang et al. 2008)*	X	X	X	X
(Liang et al. 2008)*	X	X	X	X
(Liang et al. 2008)*	X	X	X	X

(Crespo et al. 2010)	X	X	X	X
(Zhu et al. 2016)	X	X	X	X
(Zhu et al.)	X	X	X	X
(Wang et al. 2020b)		X	X	X
(Liu et al. 2015)	X	X	X	X
(Zhang et al.)	X	X	X	X
(Wang et al. 2020c)		X	X	X
(Fujii et al. 2020)	X	X	X	X
(Fujii et al. 2020)	X	X	X	X
(Wan et al. 2021)	X	X	X	X
(Guillem-Amat et al. 2020)	X	X	X	X
(Zhang and Yang 2019)	X		X	X
(Khan 2018)	X	X	X	X
(Tabbabi and Daaboub 2018)	X	X	X	X
(Alam et al. 2017)	X	X	X	X
(Afzal and Shad 2016)	X	X	X	X
(Afzal et al. 2015b)		X	X	X
(Tang et al. 2015)	X	X	X	X
(Ferreira et al. 2013)	X	X	X	X
(Gulzar et al. 2012)	X	X	X	X
(Tang et al. 2011)	X	X	X	X
(Sayyed et al. 2008b)	X	X	X	X
(Bielza et al. 2008)	X	X	X	X
(Li et al. 2007)	X	X	X	X
(Afzal and Shad 2017)	X	X	X	X
(Oppert et al. 2000)	X	X	X	X

(Huang et al. 2015)	*X*	*X*		*X*
(Anilkumar et al. 2008)	X	X		X

*= Same studies with separate resistant and susceptible populations being compared as individual studies

Studies Used in Immature Survival Rate Meta-Analysis

Studies	Insecticide Class	Insect Order	Resistance Ratios	Development Type
(Abbas et al. 2015)	X	X	X	X
(Abbas et al. 2012)	X	X	X	X
(Cao and Han 2006)	X	X	X	X
(Shah et al. 2017)	X	X	X	X
(Jia et al. 2009)	X	X	X	X
(Abbas et al. 2016a)		X	X	X
(Liu and Han 2006)	X	X	X	X
(Mansoor et al. 2013)	X		X	X
(Abbas et al. 2014a)	X		X	X
(Ejaz et al. 2017)	X	X	X	X
(Shah et al. 2015)		X	X	X
(Abbas et al. 2014b)	X	X	X	X
(Ejaz and Shad 2017)		X	X	X
(Abbas et al. 2016b)	X	X	X	X
(Ling et al. 2011)	X	X	X	X
(Shah and Shad 2020)		X	X	X
(Ishtiaq et al. 2014)	X	X	X	X
(Wang and Wu 2014)	X	X	X	X
(Yang et al. 2018)	X	X		X
(Zhang et al. 2018b)	X	X	X	X
(Saddiq et al. 2016)	X	X	X	X
(Castellanos et al. 2019)	X	X	X	X
(Alam et al. 2020)		X	X	X
(Liu et al. 2017)	X	X	X	X
(Liang et al.	X	X	X	X

2008)*				
(Liang et al. 2008)*	X	X	X	X
(Liang et al. 2008)*	X	X	X	X
(Zhu et al.)	X	X	X	X
(Wang et al. 2020b)		X	X	X
(Wang et al. 2020c)		X	X	X
(Khan 2018)	X	X	X	X
(Afzal and Shad 2017)	X	X	X	X
(Alam et al. 2017)	X	X	X	X
(Zhang et al. 2016)		X	X	X
(Afzal and Shad 2016)	X	X	X	X
(Afzal et al. 2015b)		X	X	X
(Tang et al. 2015)	X		X	X
(Tang et al. 2002)		X	X	X
(Oppert et al. 2000)	X	X	X	X

*= Same studies with separate resistant and susceptible populations being compared as individual studies

Studies Used in Female Longevity Meta-Analysis

Studies	Insecticide Class	Insect Order	Resistance Ratios	Development Type
(Horikoshi et al. 2016)	X	X	X	X
(Shen et al. 2017)		X	X	X
(Shah et al. 2017)		X	X	X
(Fu et al. 2018)*	X	X	X	X
(Fu et al. 2018)*	X	X	X	X
(Liao et al. 2019b)	X	X	X	X
(Ejaz et al. 2017)	X	X	X	X
(Lee et al. 1996)			X	X
(Chen et al. 2020)	X	X	X	X
(Zhang et al. 2020)	X	X	X	X
(Gul et al. 2019)	X	X	X	X
(Ejaz and Shad 2017)		X	X	X
(Abbas et al. 2016b)	X	X	X	X
(Banazeer et al. 2020)	X	X	X	X
(Liu et al. 2021)	X	X	X	X
(Zhang et al. 2018a)	X	X	X	X
(Huang et al. 2019)		X	X	X
(Sun et al. 2012)		X	X	X
(Wang et al. 2017)*	X	X	X	X
(Wang et al. 2017)*	X	X	X	X
(Chan and Zairi 2013)	X	X	X	X
(Li et al. 2002)	X	X	X	X
(Hafeez et al. 2019)	X	X	X	X
(Kuyucu and Caglar 2013)	X	X	X	X
(Okoye et al. 2007)	X	X		X
(Barbosa et al. 2020b)	X	X	X	X

(Nkahe et al. 2020)	X	X		X
(Mahon and Olsen 2009)	X	X	X	X
(Zhang et al. 2015b)*	X	X	X	X
(Zhang et al. 2015b)*	X	X	X	X
(Ullah et al. 2020a)		X	X	X
(Wang et al. 1998)	X	X	X	X
(Yu-ping et al. 2010)	X	X	X	X
(Chen et al. 2015)	X	X	X	X
(Naeem et al. 2021)		X	X	X
(Zhu et al. 2016)	X	X	X	X
(Zhu et al. 2021)		X	X	X
(Zhang et al. 2021)	X	X	X	X
(Wan et al. 2021)	X	X	X	X
(Zhang and Yang 2019)	X		X	X
(Steinbach et al. 2017)	X	X	X	X
(Afzal and Shad 2017)	X	X	X	X
(Afzal and Shad 2016)		X	X	X
(Afzal et al. 2015b)		X	X	X
(Tang et al. 2015)	X		X	X
(Ferreira et al. 2013)	X		X	X
(Bielza et al. 2008)	X	X	X	X
(Li et al. 2007)	X	X	X	X
(Diniz et al. 2014)	X	X		X

*= Same studies with separate resistant and susceptible populations being compared as individual studies

Studies Used in Male Longevity Meta-Analysis

Studies	Insecticide Class	Insect Order	Resistance Ratios	Development Type
(Horikoshi et al. 2016)	X	X	X	X
(Shen et al. 2017)		X	X	X
(Shah et al. 2017)		X	X	X
(Fu et al. 2018)	X		X	X
(Liao et al. 2019b)	X	X	X	X
(Ejaz et al. 2017)	X	X	X	X
(Lee et al. 1996)			X	X
(Gul et al. 2019)	X	X	X	X
(Ejaz and Shad 2017)		X	X	X
(Abbas et al. 2016b)	X	X	X	X
(Banazeer et al. 2020)	X	X	X	X
(Liu et al. 2021)	X	X	X	X
(Zhang et al. 2018a)	X	X	X	X
(Huang et al. 2019)		X	X	X
(Sun et al. 2012)		X	X	X
(Wang et al. 2017)*	X	X	X	X
(Wang et al. 2017)*	X	X	X	X
(Li et al. 2002)	X	X	X	X
(Hafeez et al. 2019)	X	X	X	X
(Amin and White 1984)	X	X	X	X
(Kuyucu and Caglar 2013)	X	X	X	X
(Okoye et al. 2007)	X	X		X
(Barbosa et al. 2020b)	X	X	X	X
(Nkahe et al. 2020)	X	X		X
(Zhang et al. 2015b)*	X	X	X	X

(Zhang et al. 2015b)*	X	X	X	X
(Ullah et al. 2020a)		X	X	X
(Wang et al. 1998)	X	X	X	X
(Yu-ping et al. 2010)	X	X	X	X
(Chen et al. 2015)	X	X	X	X
(Naeem et al. 2021)		X	X	X
(Zhu et al. 2016)	X	X	X	X
(Zhu et al.)		X	X	X
(Zhang et al.)	X	X	X	X
(Wan et al. 2021)	X		X	X
(Zhang and Yang 2019)	X		X	X
(Steinbach et al. 2017)	X	X	X	X
(Afzal and Shad 2016)		X	X	X
(Afzal and Shad 2017)	X	X	X	X
(Afzal et al. 2015b)		X	X	X
(Tang et al. 2015)	X		X	X
(Diniz et al. 2014)	X	X		X

*= Same studies with separate resistant and susceptible populations being compared as individual studies