

**ASSESSING PRACTICAL RESISTANCE AND FITNESS COSTS OF
PYRETHROID RESISTANT SOYBEAN APHID**

A THESIS
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Dedication

To my mom, Margie

Thank you for your kind heart and unconditional love in raising me to be the person I am. I wish you would have had the chance to see this chapter of my life. I miss you every day.

To my dad, Ron

Thank you for encouraging me to peruse my own path and giving me the room to grow. Your love and support mean the world to me. I would not have been able to do this without you.

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Abstract

The soybean aphid remains the most economically important arthropod pest of soybean in the Upper Midwest Region even twenty years after its arrival in the United States. After years of sustained insecticidal pressures placed on the aphid, anecdotal reports of insecticides failing to control soybean aphid began to emerge, and resistance to the pyrethroid class of insecticides has since been documented in the laboratory. The reduction in the efficacy of field applications of pyrethroids against soybean aphid has not been thoroughly examined, nor the effects of this resistance on aphid fitness. A time-series was created of data from insecticide efficacy trials performed at two locations in Minnesota spanning 2005 – 2020 and changepoint-regression models were used to evaluate percent control over time. Also, the fitness of aphid populations displaying resistant and susceptible phenotypes to pyrethroid insecticides were compared across several experiments over three soybean growing seasons.

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Introduction

In 2021, soybean, *Glycine max* (L.) Merr., production in the United States climbed to over 35.2 million hectares planted with an estimated 4.34 billion bushels harvested, the third highest year of soybean production in United states history behind 2017 and 2018. In 2020, the overall production value for soybean in the US was over \$46 billion dollars (NASS, 2021). Most of the soybean production in the United States occurs in the North Central Region, which includes Minnesota and neighboring states. In 2020, Minnesota ranked third in US soybean production, behind Iowa and Illinois, with over 3 million hectares planted valued at nearly \$4 billion dollars (NASS, 2021).

The soybean aphid, *Aphis glycines* Matsumura, is a hemipteran insect whose native range includes much of Asia (Blackman and Eastop 2000). Within most of its native range, soybean aphid is typically only noted as an occasional pest; however, infestations in northern China may be more common (Wang et al 1962). The first soybean aphid collection in the United states was made in Wisconsin in 2000 (Alleman et al. 2002); however, it is likely that soybean aphid was present prior to this but remained undetected (Ragsdale et al. 2004). Three years after its original detection, infestations were noted in at least 21 US states and 3 Canadian providences (Venette and Ragsdale 2004), and by 2009 the aphid had spread to an additional 9 states encompassing a vast majority of the soybean growing region in the US (Ragsdale et al. 2011). Within a decade of its discovery, the soybean aphid had established itself as the primary insect pest of soybean in the Upper Midwest.

Soybean Aphid Biology

In the Upper Midwestern US, soybean aphid maintains a heteroecious (host-alternating) and holocyclic (generates sexual morphs that produce an overwintering egg) lifecycle, similar to that in its native range (Ragsdale et al. 2004). The invasive European buckthorn *Rhamnus*

cathartica, serves as the principal overwintering host (Voegtlin et al. 2004). In spring, overwintering eggs hatch and emerging aphids undergo 2-3 generations of parthenogenic reproduction before producing alate forms that migrate to the secondary host, soybean (Ragsdale et al 2004). On soybean, populations rapidly increase through parthenogenic live birth, typically completing >15 generations within a single growing season in the Upper Midwest (McCornack et al. 2004). This rapid parthenogenic reproduction is further accelerated through telescoping generations, wherein an aphid nymph is born with the next generation of young already developing inside of it. Under ideal conditions, populations can increase rapidly, and quickly reach infestation levels that are damaging to the crop (Ragsdale et al. 2007, Beckendorf et al. 2008).

In fall, soybean aphids respond to the seasonal change in weather, day length, and soybean senescence and begin the process of producing alate fall migrants. The female alates (gynoparae) leave soybean and return to buckthorn where they deposit wingless sexual females (oviparae). Male alates (androparae) are also produced on soybean and return to buckthorn where mating occurs. Oviparae then deposit their eggs along the buds of buckthorn, and next year's cycle is set to begin.

Feeding Injury and Damage

Soybean aphids use their stylet mouthparts to probe soybean and feed on the plant's phloem. Aphids can feed on any above ground part of the plant, robbing the plant of photosynthates, resulting in stunting and discoloration of the leaves (Ragsdale et al. 2007). The quality of the host plant is generally recognized as a significant rate limiting factor in determining aphid population growth (Myers et al. 2005). Throughout the early growing season, aphids are typically found on the newest growth where the highest concentration of mobile nutrients (i.e., Nitrogen) are concentrated. However, as aphid populations increase and pods begin to develop, the infestation may extend to all parts of the plant (McCornack et al. 2008).

Heavy aphid infestations can produce economically significant yield losses in soybean through a decrease in the number of pods per plant, seeds per pod, and a reduction in seed quality with respect to both size and oil concentration (Ragsdale et al. 2007, Beckendorf et al. 2008). Additionally, soybean aphid is known to vector plant viruses (e.g., Soybean mosaic virus and Alfalfa mosaic virus) (Hill et al. 2001), and their feeding exacerbates injury from other pests such as soybean cyst nematode, *Heterodera glycines* Ichinohe (McCarville et al. 2012). Furthermore, soybean aphid waste known as ‘honeydew’ accumulates on plant leaves and facilitates the growth of sooty mold which directly blocks sunlight to the plant, further interfering with photosynthesis (Hodgson et al. 2012).

Prior to the invasion of soybean aphid, soybean crops in the Upper Midwest rarely experienced significant losses due to insects. Soybean producers in the region may have treated an occasional caterpillar, grasshopper, beetle, or spider mite outbreak; but overall, insecticide use on soybean was rare. Prior to the arrival of soybean aphid, typically less than 0.1% of all soybean fields were treated with insecticide annually (Ragsdale et al. 2007). However, when left untreated, yield losses from soybean aphid infestations can reach 40% (Ragsdale et al. 2007), and it was estimated that the annual US economic loss could reach \$2.4 billion (Song et al 2006). As a result, within the first ten years of the aphid’s arrival, insecticide use had increased 130-fold (Ragsdale et al. 2011). At their peak, over half of the millions of hectares of soybeans planted in some states were being treated with insecticides annually (Ragsdale et al. 2011). Even though outbreaks have become less common in some areas since mid-2000’s (Bahlai et al 2015), soybean aphid remains a key insect pest in the Upper Midwest and persists in MN and neighboring states (Hurley and Mitchell 2014, Koch et al. 2018a).

Management

Soybean aphid management is approached through the lens of Integrated Pest Management (IPM). IPM is an ecological approach to pest management that looks to use

knowledge of the pest's lifecycle, population dynamics, behavior, and interactions within the environment in tandem with current economic conditions to manage pests (Pedigo and Rice 2009). Through this approach, the goal of IPM is to maintain the pest at tolerable levels for the crop with the least possible hazard to both people and the environment. IPM uses tactics that are both preventative and curative while trying to take advantage of appropriate control options at the appropriate time.

Preventative and cultural control tactics for soybean aphid management may include planting aphid-resistant varieties (i.e., host plant resistance) (Hesler et al. 2013), avoiding proximity to buckthorn (Bahlai et al. 2010), cover cropping (Koch et al. 2012, 2015), classical biological control (Heimpel et al. 2004), and the promotion of existing natural enemy populations (Rutledge et al. 2004). Host plant resistance is complicated by the presence of aphid biotypes able to overcome certain resistance genes (Kim et al. 2008, Hill et al. 2010), and the commercial adoption of soybean-aphid-resistant varieties has been minimal (Hanson et al. 2019). Local removal of buckthorn, while beneficial, cannot control all immigrating and dispersing populations as alate aphids can reach the summer jet streams and traveling long distances (Favret and Voegtlin 2001). While the Upper Midwest has a diverse community of natural enemies that can help prevent and suppress soybean aphid outbreaks (Rutledge et al. 2004), infestations still frequently overcome these measures and reach damaging levels where insecticides are required to suppress outbreaks (Costamagna and Landis 2006).

Foliar insecticides are the primary curative tactic deployed against the soybean aphid (Hodgson et al. 2012). Treatment recommendations for soybean aphid are based on multiple factors that form a relationship between damage to the crop and factors contributing to pest pressures (Ragsdale et al. 2007). Quantifiable yield loss doesn't happen until populations reach a specific level of infestation, referred to as the damage boundary. As pests surpass the damage boundary, they approach the economic injury level, the point at which economic yield losses start

to accumulate. The economic threshold is established at a level which provides a window of lead time allowing the grower to treat the infestation before it reaches the economic injury level (Pedigo and Rice 2009).

In soybean, the economic threshold for soybean aphid was established at 250 aphids per plant with more than 80% of the plants in a field infested, *and* with favorable conditions where aphid populations are expected to continue to rise (Ragsdale et al. 2007, Koch et al. 2016a). These conditions are set to avoid populations reaching the economic injury level of 674 aphids (Ragsdale et al. 2007). Song and Swinton (2009) projected that in the first fifteen years of its adoption, this threshold-based approach to insecticide would produce an estimated net economic benefit of \$1.3 billion.

While IPM strategies do stress the importance of scouting and judicious use of insecticides, scouting aphids is time intensive and threshold recommendations aren't always followed (Koch et al. 2018a, Hoidal and Koch 2021). Growers may be tempted to spray prophylactically or below threshold which can disrupt natural enemy populations leading to later resurging aphid populations or secondary pest infestations (Song et al. 2006). Also, aphid migratory movements and their high reproductive capacity can allow populations to recover and reinfest quickly (Meyers et al 2005), sometimes leading to fields being treated multiple times within a single growing season (Song et al. 2006). Although several groups of insecticides are approved for soybean aphid, the vast majority of those used in the Upper Midwest for the last two decades have been from just two groups; the organophosphates (1B) and pyrethroids (3A) (Hodgson et al. 2012). Chronic infestations and the failure to use proper scouting methods and established thresholds have contributed to repeated and sustained selection pressures placed on the soybean aphid by a limited number of insecticidal modes of action (Koch et al. 2018a). Over reliance on insecticides for pest management and repeated exposures can result in unintended effects such as the development of insecticide resistance (Pedigo and Rice 2009).

Resistance

Insecticide resistance in major agricultural pests has been noted for more than one hundred years, but it wasn't until the 1950s that most growers became familiar with pesticide resistance with the widespread use of DDT (IRAC, Resistance). In 1938, only a few species of mites and insects were known to have resistance to DDT however by the mid 1980's resistance was found in nearly 450 species across all principal classes of insecticides (NRC, 1986). Since then, more than 600 different arthropod species have developed resistance to one or more insecticides (Tabashnik et al. 2014). Insect populations have natural genetic variations that can affect their response to a toxin such as an insecticide; however, prior to exposure to a toxin, the frequency of alleles conferring resistance are typically rare (Roush and McKenzie 1987). Tabashnik (1994) defines field-evolved (or field-selected) resistance, as a genetically based decrease in susceptibility of a population that is caused by exposure of the population to the toxin in the field. The point at which this field-evolved resistance begins to effect management tactics in the field is defined as 'practical resistance' (Tabashnik et al. 2014).

Soybean growers and crop consultants began reporting failures of control when using pyrethroids in Minnesota in 2015, in both Minnesota and Iowa in 2016, and over the following years this expanded to South and North Dakota (Hanson et al. 2017, Koch et al. 2018b, Menger et al. 2020). In these fields, failures were often described as 'pockets of failure interspersed within fields having otherwise good control' and from fields undergoing multiple application methods at different dates, hinting that these were not isolated reports of product failure or product misapplication (Hanson et al. 2017). Hanson et al. (2017) confirmed these reports in the laboratory with leaf-dip and glass-vial bioassays showing evidence of a reduction in susceptibility to pyrethroids in field populations, reporting up to a 38-fold reduction in susceptibility at the LC50 when compared to a susceptible laboratory population. Resistance ratios greater than 10 are generally indicative of a genetic-based resistance; and large increases in the LC50, like those seen

in the Hanson et al. (2017) manuscript, indicate a severe level of field-evolved resistance (Tabashnik et al. 1994).

After this discovery of field-evolved resistance to pyrethroids in soybean aphid, efforts began to both monitor for resistant populations and evaluate the geographic extent of this resistance. The development a rapid-assessment bioassay using a single diagnostic-concentration for susceptibility of aphid populations, found pyrethroid resistance to be prevalent across the Upper Midwest (Menger et al. 2020). Mortality data for the commonly used pyrethroids bifenthrin and λ -cyhalothrin showed a reduction in susceptibility in both chemistries in both years of this study, with over 50% all populations examined in each year displaying resistance (Menger et al. 2020). Furthermore, the overwhelming majority of populations that were collected from reported field failures tested resistant, further corroborating that these failures did indeed occur as a result of a reduction in susceptibility as opposed to a fault of the product or application method.

Resistance can take many forms, often divided into two major classes, behavioral and physiological. Behavioral resistance is usually defined in terms involving the evasion of insecticides (Gould 1984, Sparks et al. 1989). In behavioral resistance, an insect is said to detect the danger and avoid the toxin by stopping feeding or by leaving the area where spraying occurred. For example, the insect could move to the underside of a sprayed leaf, move deeper in the crop canopy where the insecticide doesn't penetrate as well, or simply fly away (IRAC, Mechanisms).

The larger category of resistance is physiological which includes modes like reduced penetration, metabolic, and target-site resistance. Penetration resistance often is simply a thickening of the insect cuticle that results in reduced or slowed penetration of the toxin which is likely to improve metabolic detoxification (Wood et al. 2010). Metabolic detoxification or sequestration is a common form of physiological resistance (reviewed by Li et al. 2007). *Myzus persicae*, the peach-potato aphid, is an aphid pest with many documented forms of resistance that

have been studied extensively (reviewed by Bass et al. 2014). In *M. persicae*, the over production of E4 and FE4 carboxyl-esterase genes were found to confer broad spectrum resistance to organophosphates, carbamates, and to a lesser extent pyrethroids (Bass et al. 2014). Bass et al. (2014) also showed that the level of esterase overproduction was highly correlated with the resistant phenotype, as the copy number of these genes increased it led to successively more and more resistant aphids. With respect to soybean aphid, a recent manuscript suggests that metabolic detoxification is also playing a role in soybean aphid resistance to pyrethroids (Paula et al. 2020). An interesting aspect of detoxification resistance is that it can be unstable leading to ‘revertant’ clones that display a sudden loss of both esterase gene expression and insecticide resistance (ffrench-Constant et al. 1988, Hick et al. 1996). The overproduction of esterases required for toxin detoxification is often believed to be energetically costly (ffrench-Constant and Bass 2017), and typically only confers a ‘modest’ resistance to pyrethroids in aphids (Bass et al 2014).

Target-site insensitivity, which is generally acquired through conserved point mutations, can reduce or outright abolish insecticide sensitivity by decreasing enzyme activity or diminishing their efficiency (ffrench-Constant et al. 2004). A specific form a target-site insensitivity common with respect to pyrethroid resistance is ‘knockdown resistance’ (KDR), and an enhanced allelic form named ‘super-kdr’, initially described in house flies (Sawicki 1978). Williamson et al. (1996) showed that these types of resistances were caused by mutations in the voltage-gated sodium ion channel genes that play a crucial role in the initiation and propagation of neuron action potentials. Later work done by Martinez-Torres et al. (1997, 1999) showed that KDR was a primary form of pyrethroid resistance occurring in *M. persicae* when they identified the L1014F mutation in several different clones and demonstrated that this mutation alone resulted in strong resistance to pyrethroids; furthermore, this resistance was enhanced even further in the presence of increased esterase production. Since then, several other mutations have been identified with respect to target site insensitivity in *M. persicae* and other insects (Dong et

al. 2014). Similarly, work has also begun on identifying target site polymorphisms associated with pyrethroid resistance in the soybean aphid, and several point mutations associated with KDR have been identified (Paula et al. 2021, Valmorbidia et al. 2021).

Fitness Costs

Although resistance to insecticides provides important benefits for pests in agricultural fields that are treated with insecticides, theory suggests that it may be associated with a reduction of fitness in environments that are insecticide free (Roush and McKenzie 1987, Carriere 1994). It follows that the higher the fitness cost, the longer it's likely to take for resistant individuals to spread within the population (Kliot and Ghanim 2012). A key postulate in evolutionary physiology is the 'allocation principle' (Sibly and Calow, 1986). Simply stated, when energy inputs are limited, individuals with higher maintenance costs will have less energy available for other life parameters such as growth, reproduction, development, and other aspects of performance, when in comparison to those with a lesser maintenance cost. A main prediction of this principle is that natural selection will maximize the available residual energy available to the insect for other life functions. When this is achieved, comparatively higher maintenance costs should be associated with a lower overall fitness, assuming there is no compensation taking place from another function to offset these effects (Castaneda et al. 2011). Enzyme overproduction is energetically costly (ffrench-Constant and Bass 2017). Target site mutations while preventing or reducing insecticide binding, can also make the target sub-optimal compared to its evolutionarily optimized 'wild-type' allele, and these mutations can lead to unwanted negative pleiotropic effects (ffrench-Constant and Bass 2017).

There are numerous examples of various fitness costs reported in the presence of insecticide resistance, which often include the reversion of resistance, lengthened pre-adult developmental times, and a reduction in reproduction (reviewed by Freeman et al. 2021). Other fitness costs reported specifically for pyrethroid resistance include shorter adult longevity for

Aedes aegypti (Alvarez-Gonzalez et al. 2017) and *Culex pipiens* (Li et al. 2002); reduced female adult size for *A. aegypti* (Plernsub et al. 2013, Jaramillo-O et al. 2014); and altered sex ratios for *Tetranychus urticae* (Bajda et al. 2018). *M. persicae* is an aphid species with documented resistance to several classes of insecticides (reviewed by Bass et al. 2014). Resistant *M. persicae* clones with increased levels of detoxification enzyme production have shown fitness costs such as: overwintering poorly (Foster et al. 1996), exhibiting behavioral changes associated with reduced movement in response to leaf deterioration (Foster et al. 1997), and having altered responses to the aphid alarm pheromone (Foster et al. 1999, 2003) resulting in higher rates of parasitism (Foster et al. 2007, 2011).

While adverse fitness costs due to insecticide resistance have been readily documented, they are not universally found. A recent review found that over 40% of studies evaluating pyrethroid resistance found either no effect of resistance on fitness, or occasionally fitness benefits (Freeman et al. 2021). Additionally, growing evidence of certain types of fitness modifiers such as the ‘adaptive walk’ where resistance is developed over a series of small steps as described in *Drosophila* with resistance to DDT (Schmidt et al 2010), and ‘permanent heterozygosis’ where a susceptible gene becomes duplicated, the original maintains the ‘wild-type’ allele function while the second copy confers resistance (Assogba et al 2016). Furthermore, point mutations associated with malathion resistance have been found in pinned blowflies that predate the introduction of organophosphorus insecticides (Hartley et al. 2006), and the leucine-to-phenylalanine replacement (L1014F) conveying KDR resistance in *M. persicae* was originally identified in several pyrethroid resistant clones that had been continuously reared in the laboratory for over two decades (Martinez-Torres et al. 1997, 1999) implying that these mutations represent balanced polymorphisms existing in populations prior to insecticide exposure (ffrench-Constant and Bass 2017).

Conclusion

For soybean aphid, multiple lines of evidence show pyrethroid resistance over several years covering a wide geographic area. Initial anecdotal reports of pyrethroid resistance from growers and crop consultants stem back to 2015; however, the decreased efficacy of field applications of pyrethroids has not been thoroughly examined. Similarly, work has begun to determine the mechanisms responsible for pyrethroid resistance in soybean aphid, but the effects this resistance imparts on soybean aphid fitness have yet to be examined. In the following chapters, the long-term efficacy of a common pyrethroid insecticide (i.e., λ -cyhalothrin) is examined by performing time-series changepoint analysis using data from insecticide efficacy trials conducted regularly over 15 years at two locations in Minnesota. These analyses permit evaluation of the onset and magnitude of practical resistance. Additionally, laboratory and greenhouse experiments were performed on phenotypically resistant and susceptible soybean aphid populations exploring effects of pyrethroid resistance on aspects of soybean aphid fitness.

Chapter I

**Change-point analysis of lambda-cyhalothrin efficacy against soybean aphid (Hemiptera:
Aphididae): Identifying practical resistance from field efficacy trials**

Summary

Soybean aphid, *Aphis glycines*, remains the most economically important arthropod pest of soybean in the Upper Midwest Region. Anecdotal reports of field applications of pyrethroid insecticides failing to control soybean aphid began to emerge from growers and consultants beginning in Minnesota in 2015. Since then, resistance been documented in the laboratory over multiple years, and encompassing a large geographic area of the Upper Midwest Region. Resistance detected in the laboratory does not always equate to reduction in the field efficacy, and the reduction in the efficacy of field applications of pyrethroid insecticides against soybean aphid has not been thoroughly examined. In this study a time-series was created using data from insecticide efficacy trials performed at two locations in Minnesota spanning 2005 – 2020. For each location, percent control relative to the untreated control was calculated for the common pyrethroid λ -cyhalothrin, an insecticide that soybean aphids have displayed resistance towards in laboratory bioassays. The novel approach of continuous two-phase changepoint-regression models was used to indicate if a change in percent control has occurred, and to provide an indication of when and to what degree the percent control has changed. At both sites examined in this study a significant change in percent control of λ -cyhalothrin was detected in 2014, thus marking the onset of practical resistance in the soybean aphid.

Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), persists as a significant pest in the Midwestern United States (US) (Hurley and Mitchell 2017) more than 20 years after its initial discovery in North America (Alleman et al. 2002). Despite extensive research into several alternative management tactics including host-plant resistance and biological

control (Ragsdale et al. 2011), threshold-based application of foliar insecticides has remained the most effective and economical strategy for managing soybean aphid (Johnson et al. 2009, Hodgson et al. 2012, Krupke et al. 2017). These foliar applications of insecticides on soybean have relied heavily on organophosphates (Group 1B) and pyrethroids (Group 3A) (Hodgson et al. 2012). Insecticide use on soybean in the Midwest increased dramatically after the invasion by soybean aphid (Ragsdale et al. 2011, Coupe and Capel 2016).

An over reliance on insecticides can lead to ecological backlash in the form of insecticide resistance. This evolutionary response is frequently associated with the repeated use of insecticides, and typically surfaces as a delayed response after years of seemingly good control (Pedigo and Rice 2009). The emergence of insecticide resistance in soybean aphid has posed a significant challenge to soybean production. Anecdotal reports of field applications of pyrethroid insecticides failing to control soybean aphid were made by growers and consultants in Minnesota in 2015 and in Minnesota and Iowa in 2016 (Hanson et al. 2017). In the following years, reports of such control failures continued in Minnesota and expanded to South Dakota and North Dakota (Koch et al. 2018b, Menger et al. 2020). In response to these reports, laboratory bioassays were performed and confirmed reduced susceptibility in some field-collected populations of soybean aphid from Minnesota, North Dakota, South Dakota, Iowa and Manitoba to the pyrethroids λ -cyhalothrin and bifenthrin, relative to a laboratory susceptible population (Hanson et al. 2017, Menger et al. 2020).

More recent work has begun to identify the mechanisms of resistance present in the soybean aphid. Phenotypically resistant populations of soybean aphid having both induced and constitutive overexpression of detoxifying enzyme genes have been reported (Paula et al. 2020). Furthermore, point mutations in the voltage-gated sodium channel genes associated with *skdr* and *kdr* have been identified in soybean aphid populations (Paula et al. 2021, Valmorbidia et al. 2021).

It is important to note that such documentation of resistance in the laboratory does not always equate to reduction in the field efficacy of insecticide applications against the pest (FAO, 1979). The potential differences in efficacy of insecticides under laboratory and field conditions have been documented elsewhere (Hafez et al. 2021, Mota-Sanchez et al. 2008, Yee and Alston 2006, Reissig 2003). Practical resistance is defined as “field-evolved resistance that reduces pesticide efficacy and has practical consequences for pest control” (Tabashnik et al. 2014). More specifically, the IRAC definition of resistance specifies “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, Resistance).

Reductions in the efficacy of field applications of pyrethroid insecticides over time in soybean aphid have not been thoroughly examined. However, data to perform such an examination is available from replicated and controlled field experiments (i.e., efficacy trials) performed by university researchers at multiple locations and over several years to evaluate the efficacy of various insecticides against soybean aphid. Time series analysis can be useful in elucidating underlying trends and systematic patterns in data over time. A class of regression models where predictors are associated with the outcome in a threshold-dependent manner by introducing a ‘change point’ provide a simple way to interpret certain kinds of nonlinear relationships (Fong et al. 2017). Change-point analysis is a distinct form of threshold analysis that is specifically concerned with finding structural changes within a ‘natural axis’ such as time (Fong et al. 2017). Data from these insecticide efficacy trials can provide insight into the onset and magnitude of practical resistance of soybean aphid to insecticides.

Here, a time series was created of data from insecticide efficacy trials performed at two locations in Minnesota spanning 2005 – 2020. For each location, percent control relative to the untreated control was calculated for a common pyrethroid (λ -cyhalothrin), an insecticide that soybean aphids have documented resistance towards in laboratory bioassays. In addition, for one

location, percent control was calculated for a common organophosphate (chlorpyrifos), an insecticide for which there have been no reports of resistance for soybean aphid. These analyses can indicate if a change point in percent control has occurred and provide an indication of when and to what degree the percent control has changed.

Materials and Methods

Data was compiled from insecticide efficacy trials conducted at the University of Minnesota's Southwest Research and Outreach Center (SWROC) in Lamberton, MN and the University of Minnesota Outreach, Research and Education Park (UMORE) in Rosemount, MN from 2005-2020. All the efficacy trials were performed using standard agronomic practices with aphid-susceptible soybean varieties (i.e., not containing *Rag* genes) adapted to the region (Table 1.1). Each efficacy trial was conducted as a randomized complete block design with four blocks and multiple insecticide treatments, including foliar application of the pyrethroid λ -cyhalothrin (i.e., Warrior or Warrior II, Syngenta Corp.) applied at a high label rate using standard application practices, and an untreated control treatment (Table 1.1). At SWROC, all trials were supervised by the same individual. At UMORE, two different individuals supervised trials, one before and one after 2012. In addition, several of the efficacy trials conducted at SWROC included the organophosphate chlorpyrifos (i.e., Lorsban 4e or Lorsban Advanced, Dow AgroSciences) applied at a high label rate (Table 1.1), which were also evaluated as a positive control for insecticide efficacy over time.

From each field trial, the efficacy of the targeted insecticides was estimated as percent control measured at approximately two weeks after insecticide application (Table 1.1). Percent control was calculated as: percent control = $100 \times (C-T)/C$ (Tabashnik et al. 2000, Burkness et al. 2001), where C represents the mean number of aphids per plant in the untreated control plots, and

The mean number of aphids per plant in the insecticide treated plots. The efficacy trials conducted at SWROC in 2004 and 2006 had two control treatments per block whose data were averaged prior to calculation of percent control. Additionally, the efficacy trial conducted at SWROC in 2018 had two control treatments and two λ -cyhalothrin treatments per block that were averaged prior to calculation of percent control. One efficacy trial was performed at each location per year, except in 2020 at SWROC and in 2014 at UMORE where two separate efficacy trials occurred. Percent control was calculated for each trial independently, and in instances of two trials within a single year at a location individual trial percent control was averaged to produce a single percent value for that location-year.

Data Analysis

The resulting time-series of percent control for λ -cyhalothrin at SWROC and UMORE, and for chlorpyrifos at SWROC, were analyzed in R version 4.0.3 (R Core Team, 2020) and R Studio version 1.3.1093 (Rstudio, Inc., 2020). Each time series was analyzed separately using the ‘chngpt’ package (code: chngptm) (Fong et al. 2017) with continuous two-phase regression models with percent control as the response and a main effect of year. In the models, years were weighted for the number of trials within a location. Models were selected by first fitting a ‘segmented’ model; however, in all cases, the slope before the threshold was not significantly different from 0, which allowed the use of a ‘hinge’ model. The use of a hinge model is preferred, as the model can be estimated with substantially higher precision than that of a segmented model (Fong et al., 2017; Elder and Fong, 2019). Model significance was evaluated using a likelihood ratio test (package: lmtest, code: lrtest) comparing the hinge model to the null (i.e., intercept only linear) model. Root mean square errors (RMSE) were manually calculated, and changepoint (i.e., breakpoint) significance was tested using the chngpt.test function. Estimation of slope after threshold were derived from the lincomb function.

Literature Search

To assess the novelty of using this approach to examine practical resistance, a literature search was conducted on 14 Nov 2021 using the search string ("piecewise regression" OR "threshold regression" OR "broken stick" OR "hockey stick" OR "changepoint" OR "change point") AND ("insecticide" OR "entomology" OR "insect" OR "resistance" OR "pesticide" OR "fungicide" OR "antibiotic" OR "herbicide") in Web of Science, CAB Abstracts, and Agricola searching for the use of similar statistical methods in evaluating field-resistance and the efficacy of pesticides.

Results

SWROC

The time series of percent control for λ -cyhalothrin at SWROC is presented in Figure 1.1. For λ -cyhalothrin, a significant changepoint in percent control was identified at 2014 ($\chi^2 = 26.40$, d.f. = 1, $p < 0.001$) with lower and upper bounds of 2010 and 2017 (RMSE = 4.27). The hinge model provided a better fit than the linear null model ($\chi^2 = 24.08$, d.f. = 2, $P < 0.001$). The pre-changepoint intercept (i.e., percent control) was 98.70% with lower and upper bounds of 96.12 and 100.50 (LB, UB), for the years 2005 – 2014. The slope after the changepoint was -4.27% per year (-25.70, -1.49). For chlorpyrifos at SWROC a candidate changepoint for percent of control was detected in 2011, however it was found to be nonsignificant ($\chi^2 = 2.12$, d.f. = 1, $p < 0.2692$), and the fit of the hinge model did not differ from that of the null model ($\chi^2 = 2.12$, d.f. = 2, $p = 0.3466$). Intercept only linear models revealed a percent control of 98.1% ± 1.46 SEM ($t = 64.7$, $df = 4$, $p < 0.001$).

UMORE

The time series of percent control for λ -cyhalothrin at UMORE is presented in Figure 1.2. For λ -cyhalothrin, a significant changepoint in percent control was identified at 2014 ($\chi^2 = 12.27$,

d.f. = 1, $p = 0.002$) with lower and upper bounds of 2009 and 2015 (RMSE = 18.90). The hinge model provided a better fit than the linear null model ($\chi^2 = 12.51$, d.f. = 2, $P = 0.002$). The pre-change point intercept (i.e., percent control) was 93.74% (85.68, 101.35) for the years 2005 – 2014, and the slope after the change point was -20.10% per year (-35.64, -5.90).

Literature Search

Our literature search resulted in 97, 47, and 27 publications from Web of Science, CAB Abstracts, and Agricola, respectively. Of the 171 total results, 120 unique publications were found. Within these unique publications 16 were related to entomology, and 21 involved the use of a pesticide; however, no publications were found evaluating pesticide resistance using a change point or similar analysis.

Discussion

For both locations examined in this study, a significant change point in percent control for λ -cyhalothrin was found at 2014; one year prior to the anecdotal reports of control failures and laboratory confirmation of reduced susceptibility (Hanson et al. 2017, Koch et al. 2018b). In this research, a retrospective time series analysis revealed the onset of practical resistance in the field, further validating and complimenting other lines of evidence demonstrating the presence pyrethroid resistance in soybean aphid.

Insecticides are designed to deliver high efficacy. Therefore, the use of hinge modeling (i.e., no initial slope) in this system would be expected; this is corroborated by the lack of pre-breakpoint slope indicated in the segmented models. The post-breakpoint slopes between the two locations examined in this study demonstrate the importance of repeated pesticide efficacy trials over time using consistent methods to provide such long-term data sets. At UMORE, the estimated slope of percent control after the breakpoint was approximately 5x that of SWROC,

although not statistically different due to wide lower and upper bounds, thus making precise estimates difficult. The continued evaluation of these insecticides at these sites may refine these estimates as trajectories of change near the terminus of a time series may be difficult to determine (Thogmartin et al. 2020).

Resistance to pesticides is an ever-increasing challenge to global agriculture with nearly a thousand species of pests, including ~ 600 arthropod species resistant to one of more pesticides (Tabashnik et al. 2014). Resistance to a pesticide can be directly documented by demonstrating a reduction in susceptibility over time within a population (Tabashnik 1994). However, the impact of resistance on the practical control of a pest can vary due to several factors including frequency of resistance, population density, and geographic distribution (Tabashnik et al. 2009, 2013).

The broader impact of field-evolved resistance on pest control can vary from insignificant to severe depending on the level of practical resistance realized in the field and the availability of alternative control measures (Tabashnik et al. 2014). The current state of pyrethroid resistance in the soybean aphid was likely accelerated through regularly deploying a limited number of insecticidal modes of action to manage the insect, and the lack of adoption of other IPM strategies such as host plant resistance and biological control (Koch et al. 2018a).

Within our literature search examples were found of changepoint and similar statistical methods used in entomology when modeling population dynamics in monarch butterflies (*Danaus plexippus*) (Thogmartin et al. 2020), pupation success and behavior of western flower thrips (*Frankliniella occidentalis*) (Steiner et al. 2011), and the residual efficacy of pesticide-impregnated ear tags for cattle (LySyk et al. 1996). Also, similar methods have been used when evaluating the dissipation of pesticides used as seed treatment in soils (Wayment et al. 2021), examining the behavior and environmental fate of glyphosate in water and sediments (Maqueda et al. 2017), and for determining the effects of defoliation on yield in field beans (Capinera et al. 1987). However, no examples were found of previous research using changepoint or similar types

of analyses to model time series data on insecticide efficacy related to resistance. This may be the first time this type of analysis has been used for retrospective assessment of the onset of practical resistance to insecticides in entomology.

Despite the practical resistance observed for the pyrethroids, the organophosphate chlorpyrifos has remained highly effective. However, on 18 August 2021, the US Environmental Protection Agency (EPA) released their Final Tolerance Rule for chlorpyrifos revoking all tolerances for the insecticide on food products nationwide (EPA, 2021), further limiting the products available for the control of soybean aphid. While some newer, more selective insecticides, such as sulfoxaflor and flupyradifurone (group 4C), and afidopyropen (group 9D) are labeled for and effective against soybean aphid (Tran et al. 2016, da Silva Queiroz et al. 2020, Koch et al. 2020, Aita et al. 2021), caution should be taken to preserve their efficacy through insecticide resistance management. Regular efficacy monitoring of these alternative chemistries both in the laboratory and in the field is necessary to warn of the onset of resistance and to avoid a similar fate as that of the pyrethroids for soybean aphid.

Table 1.1. Experimental details for insecticide efficacy trials^a against soybean aphid performed at Lamberton (SWROC) and Rosemount (UMORE), MN from 2005 to 2020.

Location	Year	Pre-treatment		Treatment date ^c	Post-treatment			Formulation	Rate (L/ha)	Volume (L/ha)	Pressure (kPa)	Reference ^f
		Sample date	Overall density ^b		Sample date	Control density ^d	Treated density ^e					
SWROC	2005	2 Aug	117	2 Aug	17 Aug	145	0	Warrior	0.23	187.08	275.8	*
	2006	28 Aug	146	29 Aug	12 Sep	233	0	Warrior	0.23	187.08	275.8	*
							0	Lorsban 4e	1.17			
	2007	9 Aug	284	9 Aug	24 Aug	300	1	Warrior	0.23	187.08	275.8	*
	2008	29 Jul	956	30 Jul	12 Aug	189	4	Warrior II	0.12	187.08	275.8	*
	2009	27 Jul	59	28 Jul	10 Aug	286	7	Warrior II	0.12	187.08	275.8	*
	2010	3 Aug	151	4 Aug	19 Aug	92	0	Warrior II	0.12	187.08	275.8	*
	2011	3 Aug	272	3 Aug	17 Aug	243	0	Warrior II	0.12	187.08	275.8	*
							0	Lorsban Adv.	1.17			
	2014	12 Aug	123	13 Aug	26 Aug	56	1	Warrior II	0.12	187.08	241.317	*
	2015	14 Aug	468	14 Aug	28 Aug	3742	502	Warrior II	0.12	187.08	241.317	*
							3	Lorsban Adv.	1.17			
	2016	8 Aug	59	10 Aug	25 Aug	462	30	Warrior II	0.12	140.31	241.317	*
	2017	23 Aug	284	24 Aug	6 Sep	153	12	Warrior II	0.14	140.31	241.317	*
							12	Lorsban Adv.	1.17			
2018	15 Aug	389	16 Aug	29 Aug	376	110	Warrior II	0.14	140.31	241.317	*	
						6	Lorsban Adv.	1.17				
2019	19 Aug	179	20 Aug	3 Sep	850	158	Warrior II	0.14	140.31	241.317	*	
2020	11 Aug	152	11 Aug	25 Aug	157	37	Warrior II	0.14	140.31	241.317	1	
2020	11 Aug	202	11Aug	25 Aug	163	48	Warrior II	0.12	140.31	241.317	1	
UMORE	2005	5 Jul	2	13 Jul	2 Aug	377	9	Warrior	0.23	†	†	2

2009	22 Jul	215	23 Jul	3 Aug	1784	39	Warrior	0.18	187.08	†	3
2013	12 Aug	382	13 Aug	27 Aug	744	26	Warrior II	0.12	187.08	275.8	4
2014	1 Aug	95	3 Aug	18 Aug	448	11	Warrior II	0.12	187.08	275.8	5
2014	8 Aug	328	12 Aug	26 Aug	526	21	Warrior II	0.12	187.08	275.8	4
2015	31 Jul	323	4 Aug	20 Aug	977	375	Warrior II	0.12	187.08	275.8	6
2018	16 Aug	181	17 Aug	30 Aug	83	102	Warrior II	0.12	140.31	206.8	7
2019	21 Aug	407	22 Aug	5 Sep	634	478	Warrior II	0.12	140.31	206.8	8

^a Experiments were performed as randomized complete block designs with four blocks and multiple treatments including foliar application of λ -cyhalothrin (Warrior or Warrior II), chlorpyrifos (Lorsban 4e or Lorsban Advanced), and an untreated control

^b Mean number of aphids per plant across the experimental plots

^c Date of foliar application of λ -cyhalothrin

^d Mean number of aphids per plant across the untreated control plots

^e Mean number of aphids per plant across the plots treated with λ -cyhalothrin

^f References: *= Unpublished data, B.D.P., 1= Potter and Vollmer 2020, 2= McCornack and Ragsdale 2006, 3= Heidel and Ragsdale 2012, 4= Tran et al. 2016, 5= Koch et al. 2016b, 6= Koch et al. 2016c, 7= da Silva Queiroz et al. 2019, 8= Aita et al. 2019

† Not reported in publication

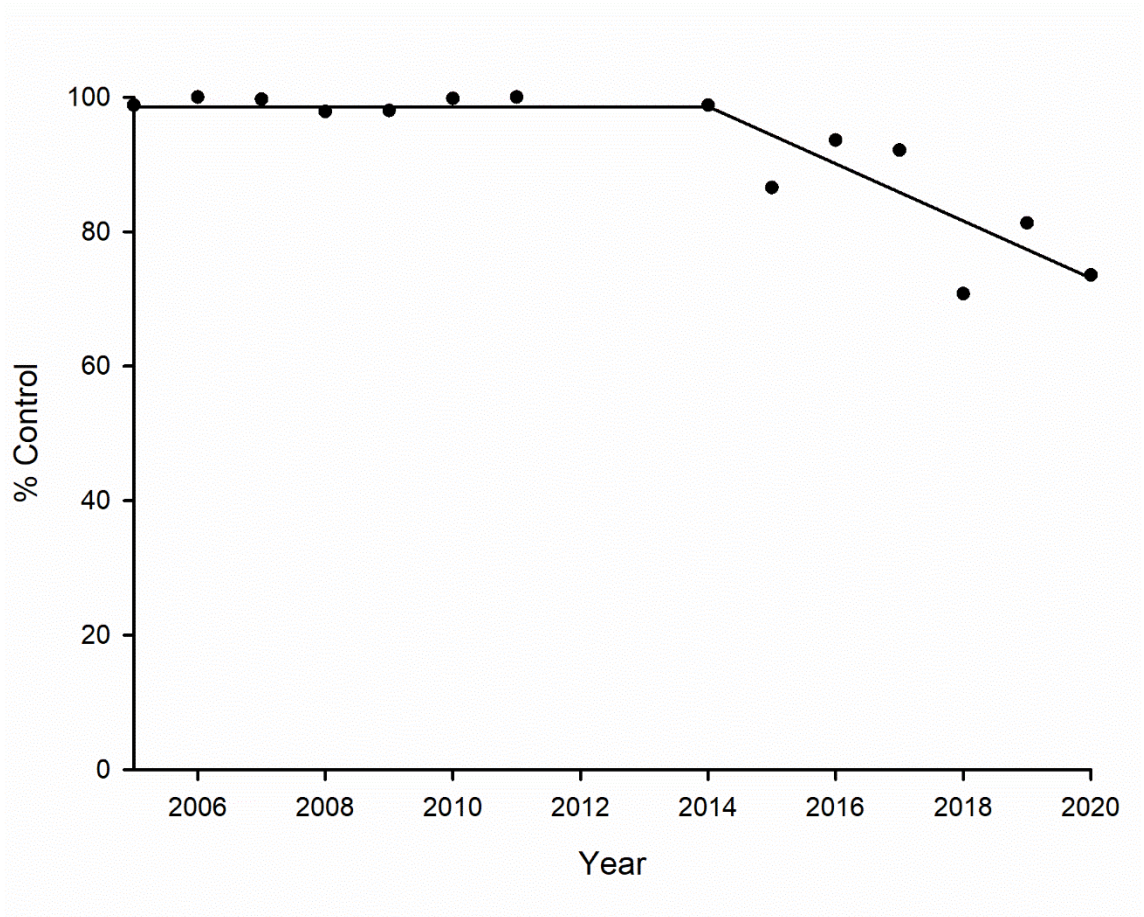


Fig. 1.1. Percent control of soybean aphid populations by foliar application of λ -cyhalothrin in insecticide efficacy field trials conducted at the University of Minnesota’s Southwest Research and Outreach Center (SWROC) in Lamberton, MN from 2005 -2020. Percent control was relative to the untreated control at approximately 2 weeks after insecticide application. Changepoint analyses identified 2014 as the breakpoint in λ -cyhalothrin efficacy at this location.

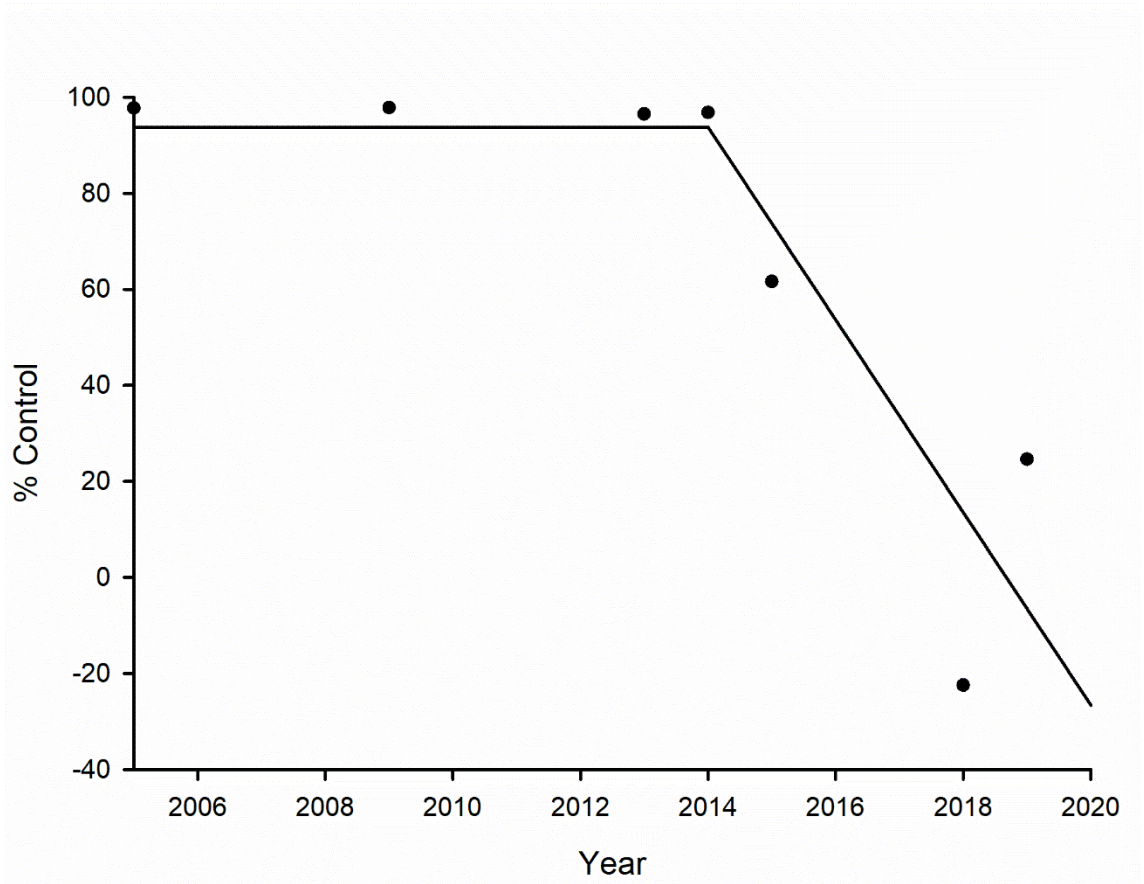


Fig. 1.2. Percent control of soybean aphid populations by foliar application of λ -cyhalothrin in insecticide efficacy field trials conducted at the University of Minnesota’s Outreach, Research and Education Park (UMORE) in Rosemount, MN from 2005 -2020. Percent control was relative to the untreated control at approximately 2 weeks after insecticide application. A negative percent control indicates a higher number of aphids in the treated plots versus untreated plots. Change-point analyses identified 2014 as the breakpoint in λ -cyhalothrin efficacy at this location.

Chapter 2

Lack of evidence for fitness costs in soybean aphid (Hemiptera: Aphididae) with resistance to pyrethroid insecticides in the Upper Midwestern United States

Summary

Twenty years after the arrival of soybean aphid, *Aphis glycines*, in the United States, it remains the most economically important arthropod pest of soybean in the Upper Midwest Region. After years of repeated and sustained insecticidal pressures placed on the aphid, resistance to the pyrethroid class of insecticides has been documented in multiple years over a large geographic area. In this study, the fitness of aphid populations displaying resistant and susceptible phenotypes to λ -cyhalothrin were compared within several experiments over three soybean growing seasons. The intrinsic rate of increase was calculated from leaf discs in the laboratory, rates of population increase were evaluated on whole plants in the greenhouse, aphid size and asymmetry were compared through tibial measurements, and stability of resistance was evaluated after one year. No evidence of a fitness cost associated with the resistant phenotype was in any of our experiments. In contrast, resistant populations occasionally supported significantly higher fitness values than susceptible populations, and there was a general numeric trend of greater fitness in resistant populations through most experiments and years. The lack of obvious reproductive fitness costs associated with the pyrethroid-resistant phenotype, and the lack of reversion of resistance observed in some populations raises concerns for longevity of pyrethroid use in soybean aphid management.

Introduction

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), a species native to Asia, is a pest of soybean, *Glycine max* (L.) Merr. (Blackman and Eastop 2000). The first soybean aphid collection in the United States (US) was made in Wisconsin in 2000 (Alleman et al. 2002). Within a few years, this pest spread to at least 30 US states and 3 Canadian provinces, encompassing a vast majority of the soybean-growing region in North America

(Ragsdale et al. 2011). Within its native range, soybean aphid is only a sporadic pest in some areas (Wang et al. 1994, Wu et al. 2004). However, in the US, soybean aphid quickly established itself as a primary insect pest of soybean, and after 20 years remains the most significant arthropod pest of soybean in the Upper Midwest Region (Hurley and Mitchell 2017).

Prior to the invasion of soybean aphid, soybean crops in the Upper Midwest rarely experienced region-wide insect pressure, wherein <0.1% of fields were treated with insecticide annually (Ragsdale et al. 2007). Since the establishment of this pest, the potential for significant economic losses has resulted in a dramatic increase in insecticide use on soybean (Ragsdale et al. 2011, Coupe and Capel 2016). Although neonicotinoid (Group 4A) seed treatments are widely used, cost-effective aphid control in the Upper Midwest is primarily conducted with foliar-applied insecticides (Krupke et al. 2017, Koch et al. 2018a). While several different insecticidal modes of action are approved for foliar use in soybean, aphid management has relied heavily on the pyrethroids (Group 3A) and organophosphates (Group 1B) (Koch et al. 2018a). Fall and spring migrations and high reproductive capacity (McCornack et al. 2004) can allow populations to reinfest fields quickly, sometimes leading to fields being treated multiple times within a single growing season (Song et al. 2006).

The overreliance on a limited number of insecticidal modes of action can result in field-evolved resistance (Tabashnik et al. 1994). Insecticide resistance can result from behavioral or physiological mechanisms. In behavioral resistance, which is typically defined in terms involving the evasion of insecticides (Nauen and Elbert 1997, Fray et al. 2014), an insect may leave the area being sprayed or move deeper into the canopy where insecticide coverage on the plants is reduced. Physiological resistance includes reduced insecticidal penetration from a thickened cuticle, increased activity of metabolic detoxification enzymes, increased toxin sequestration or excretion, and target-site modifications resulting in insecticide insensitivity (Kliot and Ghanim 2012, French-Constant and Bass 2017, Freeman et al. 2021).

Field evolved resistance to pyrethroids in soybean aphid was first documented in 2015 using laboratory bioassays (Hanson et al. 2017). Continued monitoring of soybean aphid populations in the Upper Midwest revealed pyrethroid resistance to be prevalent across a broad geography (Menger et al. 2020, Valmorbidia et al. 2021). While the mechanisms of insecticide resistance have been extensively studied in other hemipterans (e.g., *Myzus persicae* Sulzer (Hemiptera: Aphididae), reviewed by Bass et al. 2014), the mechanisms of pyrethroid resistance in soybean aphid have only begun to be examined. In China, a population of soybean aphid resistant to λ -cyhalothrin was developed in the laboratory and displayed metabolic detoxification, including elevated expression of CarE, E4 esterase and P450 enzymes (Xi et al. 2015, Bi et al. 2016). In the US, recent work suggests that several non-synonymous mutations in the voltage-gated sodium channel (e.g., knock down resistance - L1014F *kdr* and L1014+M918I *s-kdr*) (Paula et al. 2021, Valmorbidia et al. 2021) and metabolic detoxification (Paula et al. 2020) may be contributing to the pyrethroid-resistant phenotype in soybean aphid.

While insecticide resistance can provide important benefits for an insect pest in agricultural fields that are treated with insecticides, theory predicts that this resistance may impart fitness costs in the absence of insecticides (Roush and McKenzie 1987, Carrière 1994). Insect survival, reproductive rates, developmental times, and body size can be impacted by the development of insecticide resistance (Roush and McKenzie 1987). The overexpression of detoxification genes involved with metabolic resistance can be energetically costly, resulting in the reallocation of resources and energy at the expense of other life processes (Kliot and Ghanim 2012). Target-site mutations (i.e., *kdr* and *s-kdr*) result in the alteration or loss of function of the affected genes and may also extend pleiotropic effects that can impact additional phenotypic traits (ffrench-Constant and Bass 2017).

Fitness costs resulting from pyrethroid resistance have been examined for several insect species. *kdr* mutations have been associated with slowed larval development and a reduction in

fecundity in the yellow fever mosquito, *Aedes aegypti* L. (Diptera: Culicidae) (Brito et al. 2013), as well as a reduction in sensitivity to alarm pheromones and a reduction in overwintering survival in the peach-potato aphid, *M. persicae* (Foster et al. 1996, 1999, 2005). However, some studies have shown either neutral or advantageous effects of insecticide resistance. A recent manuscript by Valmorbidia et al. (2022), found no consistent relationship between pyrethroid resistance and declines in fitness within four iso-female soybean aphid populations with voltage gated sodium channel mutations. As well, Castañeda et al. (2011) evaluated *M. persicae* from Chile exhibiting insecticide resistance mutations and found no evidence of reproductive or energetic fitness costs associated with *kdr*, modified acetylcholinesterase, or total esterase activity when comparing metabolic rate and reproductive fitness energy. Furthermore, while examining the house fly, *Musca domestica* (Diptera: Muscidae), Rinkevich et al. (2013) showed no fitness costs for several *kdr* mutations, and the *kdr1* haplotype was found to carry a fitness advantage.

In this study, the fitness of several aphid populations with resistant and susceptible phenotypes to λ -cyhalothrin was evaluated using multiple experiments over three soybean growing seasons. Intrinsic rate of increase was evaluated on leaf discs in the laboratory, population increase was evaluated on whole plants in the greenhouse, aphid size and asymmetry were compared through tibial measurements, and stability of resistance was evaluated after one year.

Materials and Methods

Aphid Populations and Characterization

Biotype 1 soybean aphid served as a susceptible control (henceforth referred to as laboratory susceptible population) in all experiments in all years. This population of soybean aphid was received from the University of Illinois in June 2018 under the United States

Department of Agriculture (USDA) Animal and Health Inspection Service (APHIS) permit number P526P-18-02118 (Menger et al. 2020). This population of soybean aphid was originally collected in 2000, prior to any exposure to insecticides in North America, and has been maintained in the laboratory ever since.

Soybean aphid populations were collected from soybean fields prior to field application of foliar insecticides, unless otherwise noted, in Minnesota, Iowa, and North Dakota during the summer months of 2018, 2019 and 2020 (Table 2.1). Aphids were collected by gathering upper trifoliolate leaves from several plants within the field. Leaves were placed in a cooler with ice packs and returned to the laboratory at the University of Minnesota within 24h. Iowa and North Dakota aphid populations were collected similarly and shipped overnight to the University of Minnesota.

Apterous adult soybean aphids were transferred from field-collected leaves within 24 h of collection, unless otherwise noted (Table 2.1), and subjected to a diagnostic-concentration bioassay as described in Menger et al. (2020) to distinguish between susceptible and resistant phenotypes. In short, 20-ml glass scintillation vials were coated internally with 0.5 ml of technical-grade λ -cyhalothrin diluted in acetone to a concentration of 0.252 μg A.I./0.5 ml., the LC_{99} for the laboratory susceptible population. At the end of the bioassay, a single surviving adult aphid was then selected as a resistant representative from each location and was used to establish iso-female resistant aphid populations through parthenogenetic reproduction. At the end of each field season, all iso-female resistant aphid populations were again subjected to the diagnostic-concentration bioassay, and populations with the highest proportion of survivors were moved forward as resistant field populations for fitness experiments within their respective years. In all years, the time elapsed between initial field collections and the onset of fitness experiments was approximately 4 months. The 2018 resistant populations gathered from Hancock and Hastings were not characterized in an initial iso-female diagnostic assay.

In 2019 and 2020, additional field populations with near complete mortality in the initial diagnostic-concentration bioassay were screened further for the creation of susceptible field populations. This was done by transferring additional individual apterous adult aphids from the remaining leaves of initial field collections and creating individual iso-female populations. Once these iso-female populations had increased in number, they were subjected to the diagnostic-concentration bioassay. Iso-female populations with near total mortality were moved forward as susceptible field populations for fitness experiments within their respective years. In 2020, a laboratory resistant population was created from an individual aphid retained from the Rosemount, MN (2) population used in the 2019 experiments. This population was created by recovering an adult aphid after it survived over 4 h in a glass-vial bioassay treatment vial that had been coated with 29.87 μ g/vial λ -cyhalothrin (i.e., approx. 115 \times LC99).

After fitness experiments concluded within a field season, aphid populations were retained and maintained in the greenhouse under similar population maintenance protocol as described below. At the beginning of the next field season, these aphid populations were once again subjected to the diagnostic-concentration bioassay to measure the stability of phenotypic resistance present within each population after approximately one year of captive reproduction without exposure to insecticides.

The 2020 aphid populations were also evaluated with a glass-vial dose-response bioassay (Hanson et al. 2017) prior to fitness experiments. Treatment vials consisted of eleven serially diluted concentrations of λ -cyhalothrin diluted in acetone ranging from 99.58152 μ g/A.I. to 0.000588019 μ g/A.I. per vial, and a control vial treated with acetone. Ten apterous adults were used in each treatment vial, and four independent replications were completed for each population.

Population Rearing

Plants

The aphid-susceptible soybean variety 'IA 2104' was used in all aspects of this research. Plants were germinated in 8.9 x 8.9 x 8.9 cm square pots, using three seeds per pot in BM2 Soil Mix (Berger, Quebec, Canada) within an environmental growth chamber (Percival, Chagrin Falls, OH) held at 25 °C, with ~70% relative humidity, and under a photoperiod of 16:8 (L:D) (Hanson et al. 2017). At hypocotyl emergence, pots were thinned to two plants per pot, and maintained until the V1 growth stage (Fehr and Caviness 1977). These age-synchronized V1 soybean seedlings were used in all aspects of research.

Aphids

The above-mentioned aphid populations were maintained in a greenhouse at the University of Minnesota held at 25 °C with supplemental lighting to maintain a photoperiod of 16:8 (L:D). Individual populations were isolated within pop-up insect exclusion cages (34.29 x 34.29 x 60.96 cm, Bioquip, Rancho Dominguez, CA) on greenhouse benches. During the time from initial collection until 2 weeks prior to fitness cost experiments populations were maintained as mixed-age aphid colonies on eight pots of soybean seedlings with a closed canopy allowing the free movement of aphids between plants. Pots were watered with ~30-40 ml of water every 2-3 days on a Monday/Wednesday/Friday regimen.

Two weeks prior to fitness experiments, within each individual cage, soybean seedling pots reduced to a single plant per pot, and with four pots per cage. Individual plants within each cage were spatially isolated by the stiling of pots upon small, upturned trays within a larger flood tray containing ~5 cm of water with soap added as a surfactant. Aphids falling off the plant or otherwise attempting to leave their individually infested plant quickly succumb to the soap water mixture. Isolation of individual plants within each cage allowed the creation of age synchronized

cohorts upon individual plants akin to the Blackman Box design (Blackman 1971), but on whole live plants instead of individual excised leaves.

Upon entering the cage, individual V1 soybean plants were infested with a cohort of five to ten apterous adult aphids. This cohort of adults was removed from the plant after 2-3 days, leaving a cohort of first and second instar nymphs. These nymph cohorts were then maintained for an additional 6-7 days as described previously. At the end of the nine-day cycle, these nymphs had matured into cohorts of 2 to 4-day old adults. Individual plants were then removed from the cage, and these age-synchronized cohorts of adult apterous aphids were used in all aspects of research described below. This protocol was developed based on the soybean aphid life table parameters established by McCornack et al. (2004). In short, soybean aphids maintained at 25 °C undergo a pre-reproductive period of ~5 days, followed by peak adult reproduction lasting ~ 10 days, after which reproduction declines rapidly over several days (McCornack et al. 2004). In this design, cohorts of adult aphids exiting the cage for experimental use are within the early days of their peak reproductive output.

Within the greenhouse, individual cages were separated by a distance of >1 m that was interspersed with uncaged V1-V3 IA2104 soybean plants serving as trap plants. Trap plants were inspected every 2-3 days for aphid contamination. Regardless of aphid contamination, trap plants were removed and replaced every 2-5 days. Trap plants were originally intended to ensure population isolation in the event of escaping aphids; however, in practice, aphid escape was exceptionally rare. In turn, the primary role of the trap plants was realized as buffering the populations from outside contamination due to common greenhouse pests such as spider mites and thrips.

Assessments of Aphid Fitness

Aphid Population Growth

A population growth experiment was conducted in a University of Minnesota greenhouse maintained under conditions similar to those used for aphid rearing. The aforementioned pots containing first trifoliolate IA2104 soybean seedlings were reduced to one seedling per pot at the beginning of the experiment. Individual soybean plants were infested on the adaxial surface of the center leaflet of the V1 trifoliolate with five age-synchronized apterous adult soybean aphids exiting the cages described above. Infested soybean plants were individually caged within upturned circular 30.48 cm x 106.68 cm galvanized steel tomato cages with the legs removed and enclosed in No-See-Um mesh (Quest Outfitters, Sarasota, FL). These individually caged soybean plants were used as the experimental units. Within each cage, the potted soybean plant was stilted on a tray above a soap-water moat to further confine populations to their individual plants.

At 48 h post infestation, a visual inspection was made to determine the successful establishment of the adult aphid cohort. Successful infestations were maintained for 9 days according to the previously described regimen for the aphid population rearing. At the end of the 9-day experimental period, whole plants were cut at the soil level, placed in individual bags, and frozen for the later counting of total aphid numbers. The experiment was repeated over 2-3 temporal blocks within each year, and in total there were 13-14 successful replications for each population in 2018, 9-13 in 2019, and 9-11 in 2020.

Intrinsic Rate of Increase

A laboratory experiment was conducted on agar cups that were created following the methodology of the IRAC Susceptibility Test Method 019 (IRAC, Test Method). Leaf disks (3.8-cm diameter) were cut from the unifoliolate leaves of V1 soybean seedlings. The leaf disks were placed abaxial side up on a 1% w/w (agar (Fisher Scientific Molecular Genetics Granulated Agar, Thermo Fisher Scientific, Waltham, MA) /reverse osmosis (RO) water) agar bed in 29.6-ml soufflé cups (Solo Cup Company, Lake Forest, IL). The agar solution was boiled, and then allowed to cool to ~55 °C before being transferred to the soufflé cups. Just prior to the agar

congealing at ~35 °C, the leaf disks were gently pressed into the agar surface. Leaf disk adherence to the agar bed was maintained by rolling a drop of RO water around the interface of the leaf and agar daily. Aphids were carefully transferred to fresh leaf disks and agar cups approximately every 5 days over the duration of the experiment to maintain leaf quality.

A cohort of 5-10 age synchronized apterous adult aphids (as described above) were placed on individual leaf disk in agar cups and allowed to settle and reproduce. After 4 h, adult aphids and all progeny except for one nymph were removed from the cups. Cups were capped with a lid in which ~50 small holes were punched with a #2 insect mounting pin for ventilation and placed in an incubator (Percival 130VL, Perry, IA) maintained at similar conditions to the growth chamber and greenhouse (i.e., 25 °C, 16:8 L:D). Nymphs were monitored every 24 h until maturity, after which all newly born nymphs were removed daily. The intrinsic rate of increase (r_m) was calculated for each aphid as $r_m = 0.74 (\log_e M_d) / T_d$, where T_d is the developmental time of the aphid (i.e., birth to final molt), M_d is the number of nymphs produced in an equivalent amount of time, and 0.74 is the constant for aphids and mites (Wyatt and White 1977). This experiment was performed over 3-4 temporal blocks in each year with 11-13 replications in 2018, 9-11 in 2019, and 11-14 in 2020.

Size and Asymmetry

Aphid size and non-directional bilateral asymmetry were evaluated using measurements of the hind tibia. Cohorts of age synchronized adult aphids exiting the cages, up to twelve aphids per temporal block (i.e. plant), were slide mounted in glycerin. Left and right hind tibia of each aphid were measured using a Leica LAS Core (4.00x) Zoom Drive (Manual) 40.0x. Mean tibia length per aphid per population were examined, as well as non-directional differences between the left and right tibia as a measurement of asymmetry (Corrêa et al. 2006, Ribeiro et al. 2007). Within each population, a total of 46-48 aphids were measured in 2018, 51-60 in 2019, and 45-48 in 2020.

Data Analysis

All data were analyzed in R version 4.0.3 (R Core Team 2020) and R Studio version 1.3.1093 (RStudio Team, 2020). The greenhouse and laboratory aphid performance experiments, and tibia data were analyzed using ANOVA (package: car, code: Anova) through general linear models (package: stats, code: lm) with main effects of population and temporal block. Mean separation tests for the effect of population were performed using a Tukey HSD (package: agricolae, code: HSD.test). Assumptions of normality and homogeneity of variance were visually assessed via residual plots and verified using the Shapiro-Wilk test for normality. A square root transformation was used for analysis of tibia asymmetry data.

The stability of phenotypic resistance (i.e. survival through the diagnostic concentration bioassay before fitness experiments and after one year) was initially analyzed using ANOVA (package: car, code: Anova) through a generalized linear mixed model (package: lme4, code: glmer, family = binomial, link = logit) with main effects for timing of the diagnostic concentration bioassay (i.e., before fitness experiments and after one year), population, and their interaction. Population nested within year was also included as a random effect to account for repeated measures, however this effect was found to be near zero (i.e. 'isSingular' warning). Therefore, models were then evaluated with general linear models (package: stats, code: glm, family = binomial, link = logit) with main effects of diagnostic concentration bioassay survivorship, population, and their interaction. A mean bias-reduced adjustment was performed (Package: brglm2, method = brglmFit) to account for complete separation of the data. Mean separation tests for diagnostic concentration bioassay survivorship were performed on each population separately due to significant interaction effects using estimated marginal means (package: emmeans, code: emmeans). Residuals were visually assessed via a residual plot. Populations that did not have a complimentary set of diagnostic concentration bioassay were not

included in analysis. Additionally, the laboratory susceptible population was not included in this analysis due to complete mortality in all bioassays.

Dose-response data for the 2020 aphid populations were analyzed using the drc package (Ritz et al. 2015) using a two-parameter log-logistic function with lower limit fixed at 0 and upper limit fixed at 1 (code: `drm(LL.2)`,) for binomial data. Models were selected using goodness-of-fit criteria, and assumptions of normality and homogeneity of variance were visually assessed via residual plots. A likelihood ratio test (package: `stats`, code: `anova`) between the full model and the model assuming common slopes among populations revealed a nonsignificant difference between models, allowing the use of a common slope. Estimations of LC₅₀ values were derived from the ED (type = absolute) function. Standard errors and confidence intervals were established using the 'delta' method. LC₅₀s were compared among populations using one-sample t tests for the difference between each pairwise comparison (code: `compParm`), and the significance level was corrected with a Bonferroni adjustment to account for the multiple comparisons. Due to nonsignificant differences in slope values between populations, resistance ratios were able to be calculated based off LC₅₀ values.

Results

Aphid Phenotypes

A significant effect of temporal diagnostic concentration bioassay ($\chi^2 = 252.25$, $df = 1$, $p < 0.001$), population ($\chi^2 = 624.27$, $df = 11$, $p < 0.001$), and their interaction ($\chi^2 = 97.08$, $df = 11$, $p < 0.001$) was observed on aphid survivorship. Diagnostic concentration bioassay results for the populations prior to fitness experiments, and after one year of greenhouse reproduction are presented in Fig. 2.1.

Mean proportion survivorship of resistant populations in 2018 ranged from 0.93 to 0.45 prior to fitness experiments. After one year of retention in the greenhouse, the mean proportion survivorship of resistant populations ranged from 0.82 to 0.08. A significant reduction in phenotypic resistance frequency was observed in all 2018 resistant populations with a complimentary set of diagnostic concentration bioassays.

In 2019, mean proportion survivorship of resistant populations ranged from 1 to 0.98 prior to fitness experiments, and after one year mean proportion survivorship for resistant populations ranged from 1 to 0 (Fig. 2.1). After one year in the greenhouse, the resistant populations from Fairfax and Rosemount (1) had lost their phenotypic resistance, whereas the resistant population from Rosemount (2) retained a phenotypic resistance similar to that of the initial diagnostic assay. Mean proportion survivorship for 2019 field-collected susceptible populations were 0.18 for Sutherland and 0.08 for Lamberton prior to fitness experiments. After one year in the greenhouse, the mean proportion survivorship for both field-collected susceptible populations were 1.

In 2020, mean proportion survivorship of resistant populations ranged from 1 to 0.73 prior to fitness experiments, and after one year in the greenhouse ranged from 1 to 0.1 (Fig. 2.1). Unfortunately, the resistant population from Little Chicago (2) died during the time period after fitness experiments and the diagnostic assay after one year. The resistant population from Little Chicago (1) had a significant reduction in phenotypic resistance during the year in the greenhouse, conversely the resistant population from St. Paul and the laboratory resistant population retained similar levels of phenotypic resistance frequency. The 2020 field-collected susceptible population Richland had complete mortality in all diagnostic assays.

For the dose-response assay of the 2020 populations conducted prior to fitness experiments, the selected model fit the data ($X^2 = 97.373$, d.f. = 257, $P = 1$; p values <0.05 indicate a lack of fit (Hispano et al. 2016)) with a common slope 2.235 ± 0.131 SEM for all

populations. LC_{50} values ranged from 0.00616 to 18.536 $\mu\text{g A.I./vial}$ and differed significantly among all populations, except for St. Paul and Little Chicago (1) (Fig. 2.2, Table 2.2). Resistance ratios at the LC_{50} ranged from 0.318 for the susceptible population from Richland to 958.032 for the laboratory resistant population when compared against the laboratory susceptible population (Fig. 2.2, Table 2.2).

Aphid Population Growth

In 2018, mean counts of aphids at 9 days after infestation ranged from 612 to 682 aphids per plant (Fig. 2.3), but did not differ among populations ($F_{(5,74)} = 0.2212$, $p = 0.952$). Numerically, the laboratory susceptible population had the lowest number of aphids per plant when compared to the five resistant field populations. In 2019, mean counts ranged from 352 to 583 aphids per plant (Fig. 2.3) with significant differences among populations ($F_{(5,62)} = 2.3749$, $p = 0.049$). The resistant population from Fairfax had a greater number of aphids than the susceptible population from Lamberton. The remaining resistant and susceptible populations did not differ significantly from either of these populations. In 2020, mean counts ranged from 449 to 583 aphids per plant (Fig. 2.3), and did not differ among populations ($F_{(5,57)} = 1.0037$, $p = 0.4239$).

Intrinsic Rate of Increase

In 2018, the mean intrinsic rate of increase (r_m) ranged from 0.291 to 0.337 (Fig. 2.4), and varied significantly among populations ($F_{(5,62)} = 2.8477$, $p = 0.022$). The resistant population from New Ulm had a significantly higher r_m than the resistant population from Lamberton, while all other populations did not vary significantly from either. In 2019, mean r_m ranged from 0.340 to 0.389 (Fig. 2.4), and varied significantly among populations ($F_{(5, 51)} = 5.4170$, $p < 0.001$). The laboratory susceptible population had a lower mean r_m than the susceptible population from Sutherland and the resistant populations from Rosemount (1) and Fairfax. In 2020, mean r_m

ranged from 0.337 to 0.389 (Fig. 2.4), and varied significantly among populations ($F_{(5, 71)} = 6.9381, p < 0.001$). The laboratory susceptible population had a significantly lower r_m than all resistant field populations. Both susceptible populations (i.e., laboratory susceptible and Richland), had significantly lower mean r_m than the resistant population from St. Paul.

Size and Asymmetry

In 2018, mean hind tibia lengths ranged from 0.665 to 0.708 mm (Fig. 2.5), and varied significantly among populations ($F_{(5,268)} = 3.1865, p = 0.008$). The resistant populations from Mazeppa had lower mean tibia length than the resistant population from Hastings, and all other populations did not differ from either. In 2019, mean hind tibia lengths ranged from 0.703 mm to 0.745 mm (Fig. 2.5), and varied significantly among populations ($F_{(5,335)} = 3.5318, p = 0.004$). The resistant population Rosemount (2), and the susceptible populations Lamberton and the laboratory susceptible population, all had shorter mean tibia than the resistant population from Rosemount (1). In 2020, mean hind tibia lengths ranged from 0.650 mm to 0.689 mm (Fig. 2.5), and varied significantly among populations ($F_{(5,270)} = 4.0631, p = 0.001$). The resistant population from St. Paul had a longer mean tibia length than the resistant population from Little Chicago (2) and both susceptible populations (i.e., laboratory susceptible and Richland). Comparisons of mean non-directional asymmetry of hind tibia within all three years failed to show significant differences among populations. In 2018 mean asymmetry ranged from 0.011-0.017mm ($F_{(5,268)} = 1.2343, p = 0.293$); in 2019, 0.013-0.018mm ($F_{(5,335)} = 0.6886, p = 0.632$); in 2020, 0.012-0.015 ($F_{(5,270)} = 0.7335, p = 0.5989$).

Discussion

Fitness costs associated with insecticide resistance have not been universally identified. A recent review by Freeman et al. (2021) found over 40% of studies involving pyrethroids reported

either no effect or fitness benefits associated with insecticide resistance. In this work, field populations of soybean aphid were collected, challenged with a discriminating bioassay, and populations arising from surviving aphids were measured on their performance in an environment free of insecticides. Due to potential concerns pertaining to trait changes in the laboratory susceptible population after multiple years of laboratory rearing (Hoffman and Ross 2018), field-collected susceptible populations were included in two of the three years of these experiments. For the parameters in these experiments, no fitness costs associated with the pyrethroid resistant phenotypes of soybean aphid were found. On the contrary, in multiple experiments, some resistant populations outperformed susceptible populations, which perhaps indicates potential fitness benefits associated with a resistant phenotype in soybean aphid. This result is similar to recent work by Valmorbidia et al. (2022) wherein a soybean aphid with the *s-kdr* genotype supported higher overall fitness than the susceptible control and other resistant soybean aphid genotypes.

In the greenhouse experiment examining aphid population growth on potted plants, resistant aphid populations collected from the field in all years showed no significant differences in total number of aphids per plant at 9 DAI when compared to the laboratory susceptible population within their respective year. In 2019, the only year with significant differences among populations in this experiment, the resistant population from Fairfax had more aphids than the susceptible population from Lamberton. Interestingly, in each of the three years of this experiment, the populations with numerically higher means tended to be the field-collected resistant populations, and populations with numerically lower means tended to be laboratory and field-collected susceptible populations. Other researchers examining population growth on whole plants found a fitness costs for virulent of soybean aphids biotypes (i.e. aphid biotypes with the ability to overcome host plant resistance genes) on susceptible soybean varieties (Varenhorst et al. 2015). It is of note, that in the three years of this study, population densities for all populations

remained similar to those of the laboratory susceptible (i.e., avirulent Biotype 1) population. Recent research suggests that virulence of soybean aphid to *Rag*-genes decreases the susceptibility to λ -cyhalothrin (Valmorbida et al. 2020). Biotyping of soybean aphid populations was not performed in this work; however, the lack of differences between the laboratory susceptible (i.e., Biotype 1) and the field-collected populations may indicate a complex relationship between the factors of resistance and virulence.

The intrinsic rate of increase (r_m) values in this study are similar to those found by other researchers for *A. glycines* (0.32; Costamanga and Landis 2006, and 0.36 – 0.40; Valmorbida et al. 2022) and *M. persicae* (0.258 – 0.378; Castaneda et al. 2011). In this study, susceptible populations generally exhibited numerically lower r_m values than resistant populations, which is similar to the trend seen in population growth assessed in the greenhouse experiment. However, in 2018 the resistant population from Lamberton had the lowest r_m of all populations and it was, on average, ~14% lower than the r_m of the resistant population from New Ulm. Despite this, neither of these two resistant populations (Lamberton and New Ulm) differed statistically from the laboratory susceptible population. In both 2019 and 2020, several resistant populations had statistically higher r_m values than the laboratory susceptible population. Furthermore, in 2020 the resistant population from St. Paul had a higher r_m than all susceptible populations, which may indicate a subtle fitness benefit for this population.

Fitness costs on fecundity have commonly been found for pyrethroid-resistant mosquitos (i.e., *Aedes aegypti*, *Anopheles gambiae*, and *Culex pipiens*) (Reviewed by Freeman et al. 2021). In contrast, no significant effect of insecticide resistance or adaption to artificial rearing was found on the development time or r_m values of several insecticide-resistant clones of *M. persicae* (Erdos et al. 2021), and no differences were found in the onset of sexual reproduction in *Sitobion avenae* clones carrying *kdr* mutations (Walsh et al. 2021). Similarly, reproductive fitness costs were not found for the two-spotted spider mite, *Tetranychus urticae*, with a well-characterized

target-site mutation (L1024V) conveying resistance to bifenthrin (Bajda et al. 2018), a pyrethroid chemistry that soybean aphid has been shown to share cross resistance with (Hanson et al. 2017, Menger et al. 2020, Valmorbidia et al. 2021). Furthermore, recent work by Valmorbidia et al. (2022) on four resistant soybean aphid genotypes with voltage gated sodium channel mutations also failed to show a consistent relationship between levels of resistance and declines in fitness.

The inconsistency between the greenhouse experiment, which had a general lack of differences among populations, and the incubator experiment, which had more differences among populations, may be due to the more variable conditions of the greenhouse compared to the incubator. For example, the greenhouse environment, while maintained at 16:8 (L:D) with supplemental lighting and 25°C, was still subject to several varying external factors (e.g., ambient humidity, varying levels of solar radiation on sunny versus cloudy days), whereas conditions in the incubators were tightly controlled. If extrapolated to the larger and more variable setting of an agricultural field, the subtle differences detected among populations in the incubators of the laboratory experiment could easily be diminished or exacerbated in field populations where aphids of varying genotypic backgrounds are influenced by multiple biotic and abiotic factors (Kliot and Ghanim 2012, French-Constant and Bass 2017). A closer investigation into genotype by environment effects for soybean aphid may be needed.

Aphid size, as measured by hind tibia length, varied among populations in all years. The largest within-year tibia length variation (5.7%) between populations was observed in 2019. The general trend seen in the other experiments of numerically higher values for resistant populations compared to susceptible populations continued in this experiment, and a resistant population was statistically larger than the laboratory susceptible population in two of the three years studied. In other research, pyrethroid-resistant *C. pipiens* weighed more than susceptible individuals, despite deleterious effects of resistance on other fitness parameters (Li et al. 2002). Similarly, pyrethroid-resistant *Sitophilus zeamais* males weigh more than susceptible males, and these larger males

were preferred by females for mating, thus favoring the spread of the resistant phenotype in the population (Cordeiro et al. 2017).

Measures of non-directional asymmetry of soybean aphid were non-significant in all years. Asymmetry of physical traits can be used as a phenotypic indicator of developmental instability (Nattero et al. 2019). It is believed that the environmental stress imposed by insecticide applications, and the selection of associated genes to alleviate that stress, can cause the disruption of developmental processes that result in increased asymmetry (Corrêa et al. 2006). However, repeated selection can ameliorate this effect through the later selection of modifier genes that also reduce fluctuating asymmetry (Clarke et al. 2000). While it is possible that a resistant phenotype in soybean aphid does not convey significant pleiotropic effects which impact tibial asymmetry, it is also reasonable that after nearly two decades of repeated selection, resistance has settled into the genome and character instability has already been mitigated. Insecticide-resistant *S. zeamais* were found to have lower levels of asymmetry than susceptible individuals, which suggests that an extended period of insecticide selection had likely led to the evolution of modifier genes in the resistant genotypes (Corrêa et al. 2006 and Ribeiro et al. 2007).

At the conclusion of each season's fitness work, aphid populations were maintained in the greenhouse until the beginning of the subsequent field season, when they were once again subjected them to the diagnostic-concentration bioassay. Several of the resistant populations showed a significant reduction in the proportion of individuals surviving the assay. This may be indicative of metabolic mechanisms of resistance in these populations. Metabolic resistance conveyed by amplified E4 genes in *M. persicae* has been noted as unstable with clones suddenly losing both their gene expression and resistance (French-Constant et al. 1988). In contrast to this, the 2019 Rosemount (2) clone retained very high phenotypic resistance after one year. Later, this same aphid survived a 4 h glass-vial assay conducted at ~115× the rate of the diagnostic assay, striking such interest that it was decided to retain this surviving aphid for the 2020 fitness work

(i.e., the 2020 ‘Laboratory Resistant’ Population). The following year, when these reversion assessment assays were conducted on 2020 populations, both the resistant population from St. Paul as well as the retained 2019 Rosemount aphid (i.e., the 2020 ‘Laboratory Resistant’ Population) maintained a persistent resistant phenotype. This could be indicative of a more stable form of resistance in these populations, perhaps conveyed through mutations such as those associated with *kdr* and *s-kdr* mutations. There was no clear association between populations that retained or lost their respective resistance after one year and their performance in the fitness experiments evaluated herein.

The results presented here indicate that pyrethroid resistance in soybean aphid does not confer a large fitness cost related to reproductive performance and may actually confer subtle fitness benefits, most readily detected under tightly controlled laboratory conditions. These results have important implications for insecticide resistance management. However, the research presented here is not without limitations. The mechanisms of resistance present in the soybean aphid are yet to be fully understood, and the specific mechanisms at work in the aphid populations tested here remain unknown. Furthermore, the use of non-genetically related isolates makes population comparisons difficult (French-Constant and Bass 2017), but the creation of near-isogenic lines in insects remains extremely difficult and time-consuming (Bajda et al. 2018). In this initial examination of potential fitness costs related to pyrethroid resistance in soybean aphid, these limitations need to be considered. It is important to stress however, that soybean fields are not homogeneous with respect to aphid genotypes (Orantes et al. 2012), phenotypes (Bhusal et al. 2021), and pesticide exposure between fields (Hurley and Mitchell 2015) or within fields (Hoffmann et al., 2019). Therefore, the overall scope and variability of aphid populations presented in this work may reflect the variability seen within natural infestations in the field.

Table 2.1. Population locations, field collection dates, and field treatment histories for soybean aphid populations used in all experiments.

Experimental year	Population	Field collection date	Current-year treatment history prior to aphid collection
2018	Lab Susc.	N/A	N/A
	Hancock, MN	16 Aug 2018	Bifenthrin
	Hastings, MN	28 Aug 2018	λ -cyhalothrin
	Lamberton, MN	9 Aug 2018	*Untreated
	Mazeppa, MN	23 Aug 2018	λ -cyhalothrin
	New Ulm, MN	8 Aug 2018	Untreated
2019	Lab Susc.	N/A	N/A
	Lamberton, MN	21 Aug 2019	Untreated
	Sutherland, IA	4 Sep 2019	Untreated
	Rosemount, MN (1)	22 Aug 2019	Untreated
	Rosemount, MN (2)	30 Aug 2019	λ -cyhalothrin
	Fairfax, MN	15 Aug 2019	Untreated
2020	Lab Susc.	N/A	N/A
	[†] Richland, ND	12 Aug 2020	Untreated
	[‡] Lab Res.	N/A	N/A
	St. Paul, MN	19 Aug 2020	Untreated
	Little Chicago, MN (1)	18 Aug 2020	Untreated
	Little Chicago, MN (2)	18 Aug 2020	Untreated

* Population collected from untreated plots within a field where control failures involving λ -cyhalothrin occurred later.

[†] Population increased in the laboratory prior to initial D.C. bioassay.

[‡] Population created from a single soybean aphid from the Rosemount, MN (2) population used in 2019 experiments by re-capturing the adult aphid after it survived a glass vial bioassay treated with 29.87445607 $\mu\text{g}/\text{vial}$ λ -cyhalothrin (i.e. approx. 115x LC₉₉).

Table 2.2. Dose-response bioassay results for 2020 populations. Estimates of LC₅₀ µg A.I. λ-cyhalothrin per vial, standard error (SE), lower and upper 95% confidence limits, and resistance ratios (RR) compared to the laboratory susceptible (Lab Susc.) population, Biotype 1.

Population	LC50 ¹	SE	Lower	Upper	RR
Richland	0.0061559 A	0.00070808	0.0047681	0.0075437	0.318
Lab Susc.	0.019348 B	0.0022278	0.014982	0.023715	-
St. Paul	0.37907 C	0.044284	0.29227	0.46586	19.592
Little Chicago (1)	0.54721 C	0.063768	0.42223	0.67219	28.283
Little Chicago (2)	1.2257 D	0.14303	0.94539	1.5061	63.350
Lab Res.	18.536 E	2.1724	14.278	22.794	958.032

¹ Different letters indicate significant differences as determined by one-sample t tests. Significance level of pairwise comparisons was determined after a Bonferroni adjustment to account for multiple comparisons

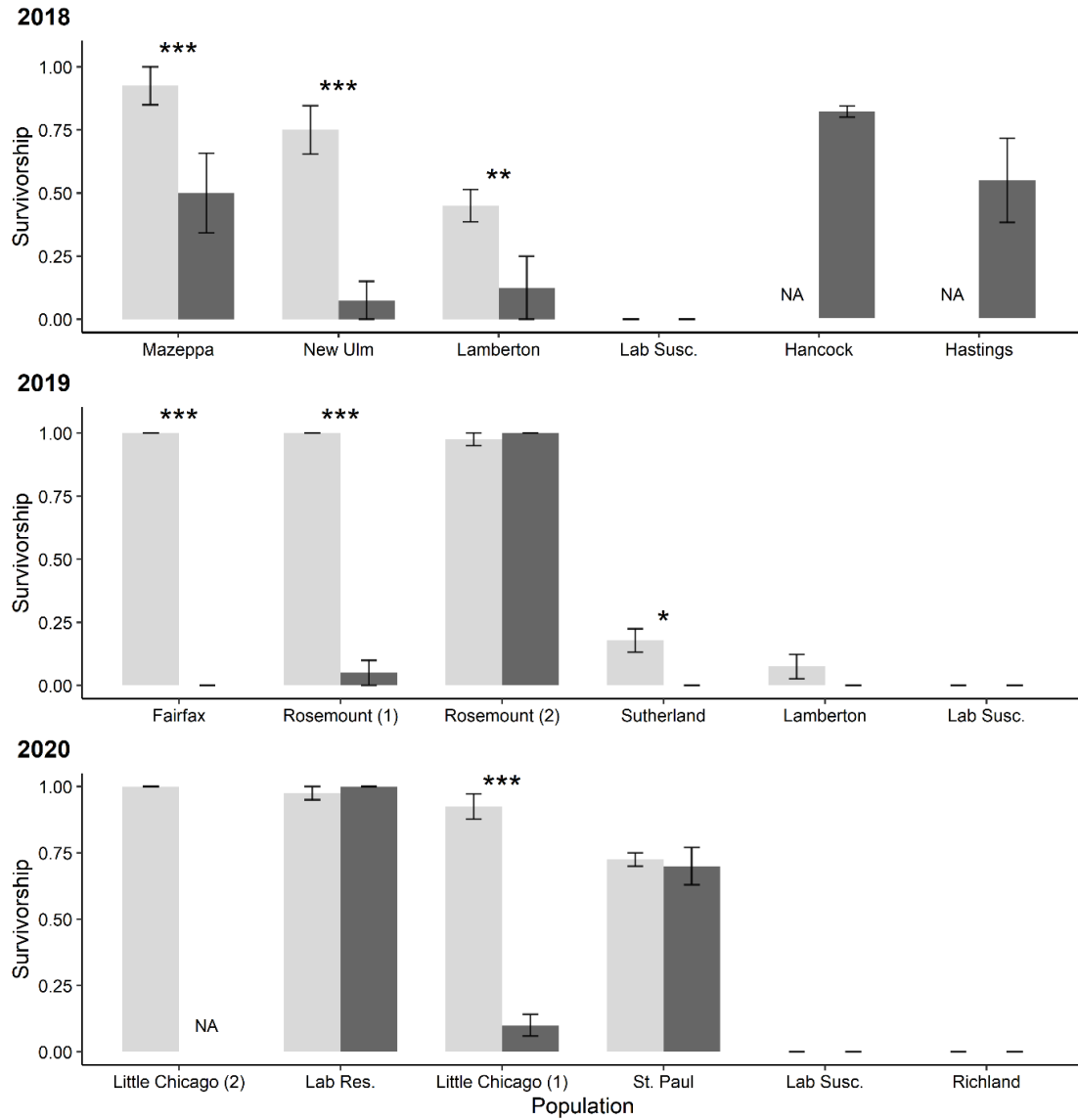


Fig. 2.1. Proportion survivorship of soybean aphid populations in diagnostic concentration bioassays (\pm SEM). Light grey bars represent bioassays conducted prior to fitness experiments; dark grey bars represent bioassays conducted after approximately one year of greenhouse reproduction without the exposure to insecticides. NA indicates missing data, *** indicates significant p values < 0.001 , ** indicates significant p values < 0.05 , and * indicates a marginal significance < 0.1 .

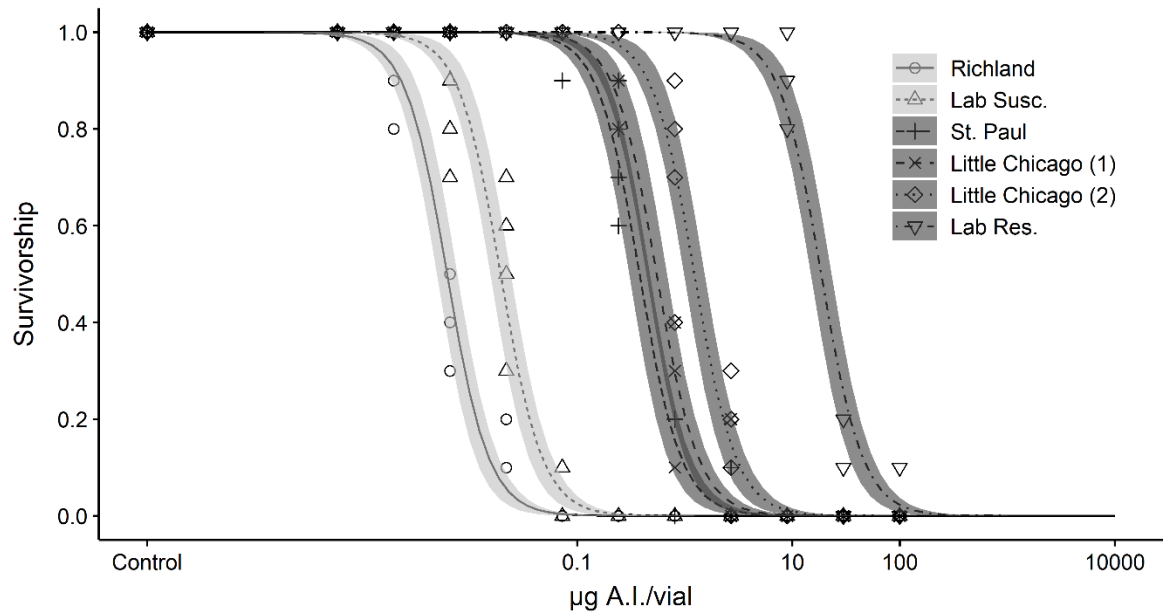


Fig. 2.2. Proportion survivorship of 2020 soybean aphid populations in a dose-response bioassay prior to fitness experiments. Light grey confidence interval bands indicate susceptible populations and dark grey confidence interval bands indicate resistant populations as determined based on survivorship after an initial diagnostic concentration bioassay.

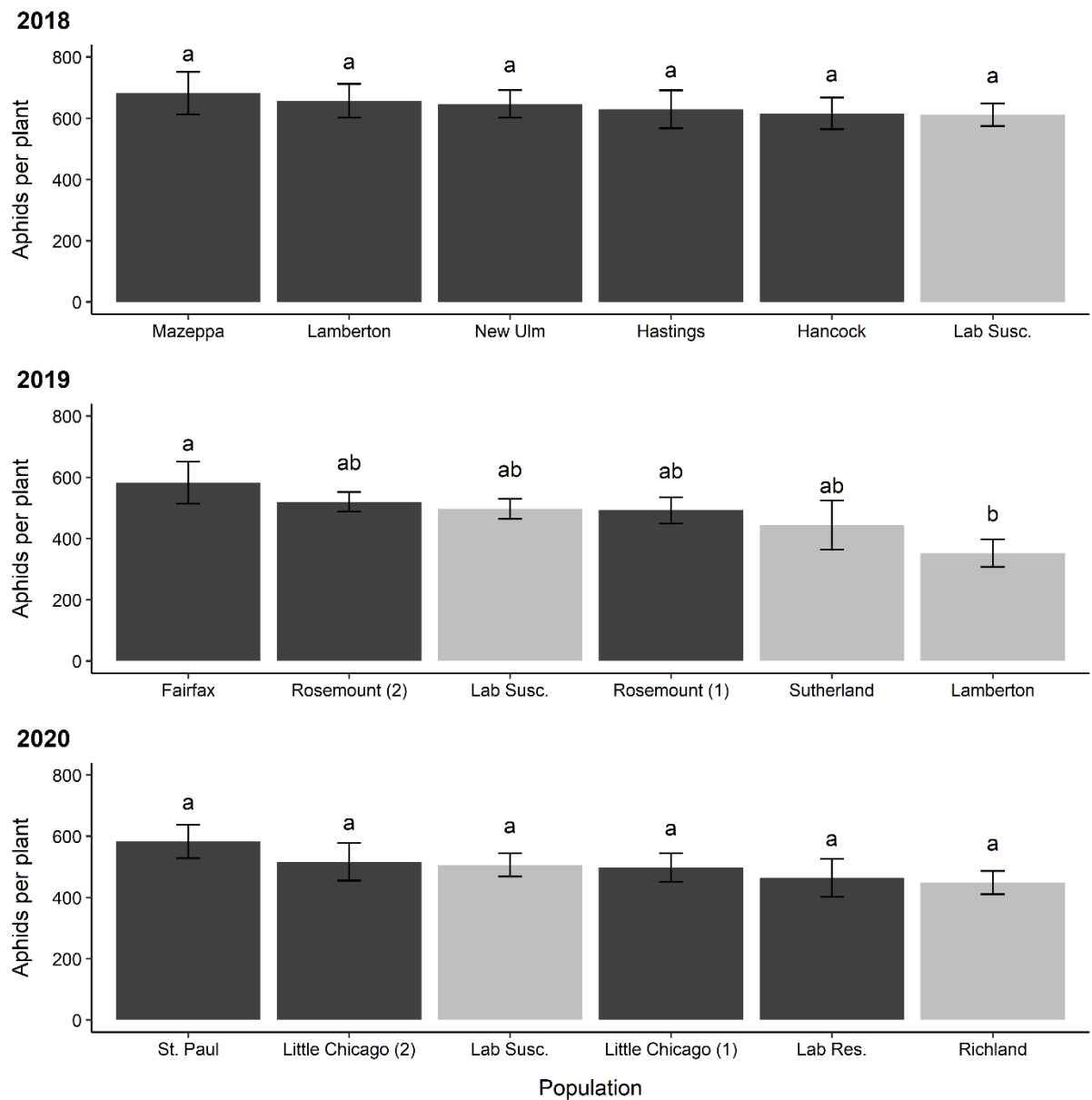


Fig. 2.3. Mean number of aphids per plant (\pm SEM) nine days after infestation of each plant with five apterous adult aphids with resistant or susceptible phenotypes to pyrethroid insecticides in a greenhouse experiment. Light grey bars indicate susceptible populations, and dark grey bars indicate resistant populations as determined based on survivorship after a diagnostic concentration bioassay. Means are not significantly different from the laboratory susceptible population with the same letter at $\alpha = 0.05$.

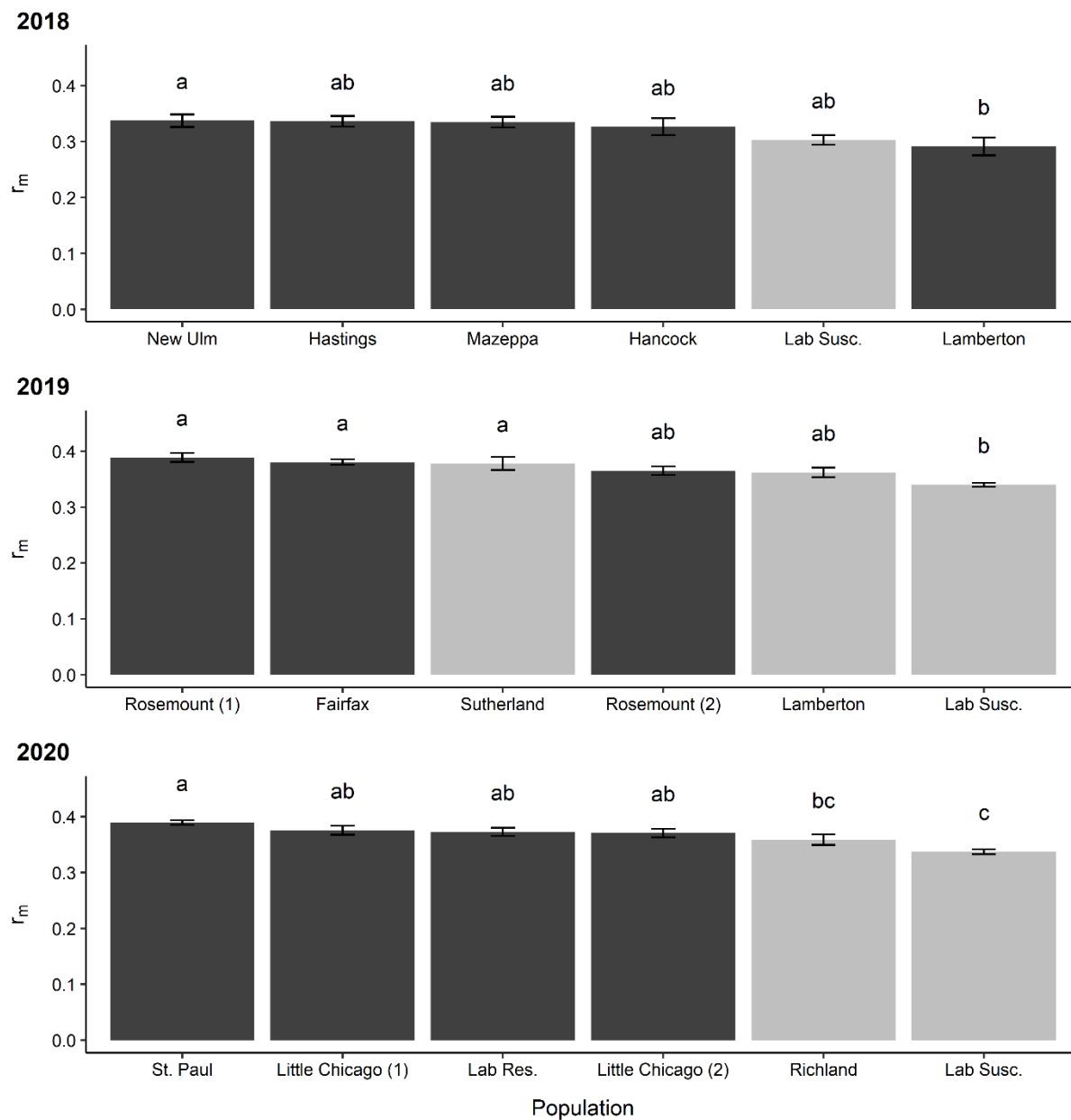


Fig. 2.4. Intrinsic rate of population increase (r_m) (\pm SEM) for soybean aphid populations with resistant or susceptible phenotypes to pyrethroid insecticides in a laboratory experiment. Light grey bars indicate susceptible populations and dark grey bars indicate resistant populations as determined based on survivorship after a diagnostic concentration bioassay. Means are not significantly different from the laboratory susceptible population with the same letter at $\alpha = 0.05$.

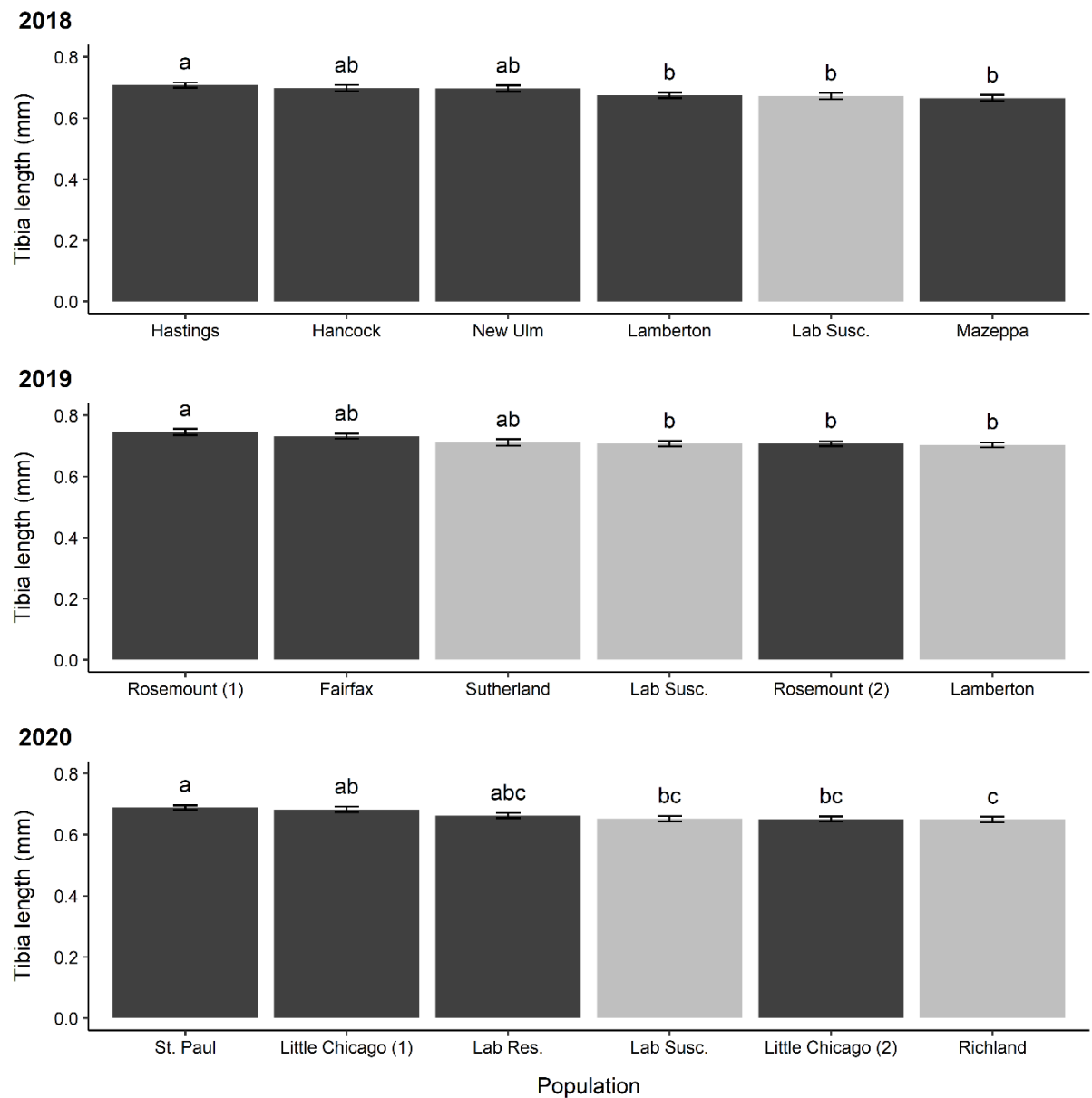


Fig. 2.5. Mean tibia length (\pm SEM) for soybean aphid populations with resistant or susceptible phenotypes to pyrethroid insecticides. Light grey bars indicate susceptible populations and dark grey bars indicate resistant populations as determined based on survivorship after a diagnostic concentration bioassay. Means are not significantly different from the laboratory susceptible population with the same letter at $\alpha = 0.05$.

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