INTERACTIVE EFFECTS OF PATHOGENS, PLANT DEFENSES AND PREDATORS ON HERBIVORE PERFORMANCE AND POPULATION DYNAMICS

A Dissertation

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

by

Mônica Frank Kersch Becker

August 2014

INTERACTIVE EFFECTS OF PATHOGENS, PLANT DEFENSES AND PREDATORS ON HERBIVORE PERFORMANCE AND POPULATION DYNAMICS

Mônica Frank Kersch Becker, Ph. D.

Cornell University 2014

Variation in plant defenses and predation has the potential to cause profound changes in herbivore performance and population dynamics. Evaluating the complex interactive effects of plant defenses and predators that lead to the exponential or density-dependent growth of herbivorous insects is critical for understanding their population dynamics. For my doctoral research I (i) investigated the effects of pathogen-induced plant defenses on herbivores, (ii) measured the effects of constitutive and induced plant defenses on the strength of herbivore density-dependent growth and (iii) evaluated the influence of plant resistance on predator-prey interactions. Chapter 1 evaluates how different strains of *Potato virus Y* differentially affect plant defensive pathways, showing that strains that strongly induce the salicylic acid pathway increase susceptibility to chewing herbivores, while not affecting the performance of its vector, a phloem feeding aphid. Chapter 2 demonstrates that plants with low constitutive levels of defense cause strong density-dependent population growth in aphids, whereas populations on plants with high levels of constitutive defense show density-independent growth. Building on chapter 2, chapters 3 and 4

focus on the critical role of plant defenses in predator-prey interactions. Chapter 3 shows that plant resistance affects not only the consumptive, but also the non-consumptive effects of predators on prey. I demonstrate that aphid prey could not exhibit predator-induced behavioral responses on plants with high levels of resistance. Chapter 4 explores the underlying mechanisms by which variation in plant defenses affect insect population: directly, through changes in herbivore population growth, or indirectly, by modifying predator impacts on prey populations. Chapter 4 shows that predators only cause prey density-dependent population growth when the herbivorous prey are feeding on low-resistance plants, which are still able to induce high levels of herbivore-associated cues. Collectively, these results highlight the strong effect that plant defenses have on herbivore population dynamics. This work has important implications; it suggests that induced plant responses to prey should be accounted for in biological control assessments, as these responses determine the efficiency of predators on target prey.

BIOGRAPHICAL SKETCH

Mônica Frank Kersch Becker was born and raised in São Leopoldo, in the State of Rio Grande do Sul, Southern Brazil. Mônica pursued her undergraduate degree in Biological Sciences at University of Vale do Rio dos Sinos in São Leopoldo, Southern Brazil.

It was under the excellent guidance and encouragement of Dr. Carlos Fonseca where Mônica began considering a career in Ecology. She spent four years investigating the effects of abiotic factors on the mutualistic interaction between ants and plants bearing extrafloral nectaries. After completing her undergraduate degree, Mônica moved to Campinas in the State of São Paulo where she attained her Master's degree in Ecology at State University of Campinas. Under the supervision of Dr. Thomas Lewinsohn, she spent two years investigating the effects of resprouting on the community of arthropods associated to an Asteraceae plant. Mônica has been interested in how plant traits affect herbivores and their natural enemies ever since.

From 2008 to 2014 she worked with Dr. Jennifer Thaler at Cornell University studying the effects of plant defenses on herbivores and predators. Her research interests now focus on the role of plant defenses in structuring species interactions. She used laboratory and field manipulation experiments to explore the mechanisms by which variation in plant defenses affect insect population dynamics and herbivore-predator interactions. Throughout her entire Ph.D, Mônica has counted with her husband's, Gui Becker, unconditional support, which improved not only her research,

but enriched her life.

Upon finishing her degree at Cornell University, Mônica will move to Brazil to pursue a post-doctoral position with Dr. Gustavo Romero, where she will study induced plant defenses in native Brazilian plants. Specifically, her research will focus on the importance of plant defenses in structuring food webs in natural systems.

Dedicated to

my mom, Dorotea Kersch, for supporting me along my entire life, and

my husband, Gui Becker, for his unconditional love and support

ACKNOWLEDGMENTS

I am deeply indebted to all my family, friends and mentors who supported and guided me through my Ph.D research. Having a great advisor is one of the most important factors for the success of a graduate student. I could not have wished for a better advisor. Jennifer Thaler is extraordinary; her knowledge, kindness, passion and mentorship motivated me throughout my entire Ph.D. I appreciate Jennifer's generosity with financial support, and facilitation of academic independence. I appreciate that she pushed me outside my comfort zone and for all the discussions about multiple issues faced by women in science. I am also thankful for her commitment in caring for her students' success, professionally and personally. I look up to her and I hope one day I can do for my students at least a little bit of what she has done for me. She is a role model, not only as person, but also as a mentor and a scientist. For all of that and much more I will forever be grateful.

I thank my committee members, Anurag Agrawal and Sunny Power for been an amazing source of scientific wisdom and for their continuous support. Anurag has always taken the time to meet with me to hear about the projects I was developing, providing important critical feedback. He pushed me to excel by encouraging critical thinking and challenging me to look at the big picture in my research. Sunny has laid the foundation for my understanding of aphid biology and disease ecology. She has provided invaluable guidance and encouragement throughout my dissertation. Katja Poveda, who I consider my informal committee member, has mentored me throughout

my entire dissertation. She helped me every time that I was struggling with data analyses, and she was always there for me when I needed some extra encouragement.

André Kessler has been an excellent source of thought-provoking discussions about plant defenses. I also thank Rayko Halitscke for teaching me every chemical assay I know. He helped me acquire a set of skills that will be invaluable for my scientific career. All my experiment work with plant virus was possible due to logistical and technical support provided by Dawn Smith and Stewart Gray. I am also grateful to have served as a graduate teaching assistant for Christy Goodale, Irby Lovette, Jennifer Thaler, Anurag Agrawal and Alex Flecker. Their engaging teaching philosophy has completely changed the way I teach.

I would like to thank my academic and scientific communities. In particular, thanks to all professors, post-docs, technicians, graduate and undergraduate students that are part of the Plant-Interactions Group (PIG) community. The PIG has been a valuable source for discussion of my research ideas and results; I also have learnt a lot about several aspects of plant and insect biology, ecology and evolution. I am grateful to be part of the supportive research community of the Department of Ecology and Evolutionary Biology and the Department of Entomology at Cornell University. I thank the current and former members of the Thaler Lab, particularly Suzi Claflin, Sara Ali, Scott McArt, Stuart Campbell, Ian Kaplan, Scott Nelson, Ordom Hout, Lucas Gonzalez, and Natasha Tigreros. I also would like to thank the Poveda Lab, in particular Chris Stieha and Heather Connelly. The members of the Thaler and Poveda Labs always provided helpful ideas and critical feedback. They have read all my manuscript and provided constructive feedback on my practice talks over the past six

years. Thanks to the research assistants who have worked with me in the field, installing cages and counting thousands of aphids, and in the lab, particularly Gui Becker, Suzi Claflin, Sara Ali, Marie Russel, Lucas Gonzalez, Ordom Hout, Josiane dos Santos, and Débora Becker.

Thanks to my wonderful friends who have been my family, making Ithaca my home. Suzi Claflin, Ana Longo, Nancy Chen, Margarita Lopes-Uribe, Karen Kiemnec-Tyburczy, Amos Belmaker, Dennise Belmaker and Fríða Jóhannesdóttir are incredible friends and made my life so much better. Thanks to many other friends, including Rayna Bell, Mia Park, Alberto Lopez-Torres, David Rodriguez, Scott McArt, Benjamin Freeman, Joseph Tyburczy, Christopher Dalton, Chris Stieha, Heather Connelly, Jared Ali, Stuart Campbel, Benjamin Dalziel, Luis Duque, Marjorie Weber, Morgan Mouchka, Renee Petipas and Janet Shortall. I "married into" the Zamudio Lab, and thanks to that I have met great people, including Kelly and Harry, who have supported and encouraged me throughout this six years. I consider Kelly my academic mother-in-law, she has always pushed me to do better, so that I could deserve her "son".

I would not have come to Cornell if it was not for my former undergraduate advisor, Carlos Fonseca and my masters advisor Thomas Levinsohn. I am very fortunate to have had these two amazing mentors who encouraged me to challenge myself. I would also like to acknowledge that my research was funded by many sources, including the Olin Fellowship, the Cornell University Graduate School, Fulbright/Capes, Toward Sustainability Foundation, the Tinker Foundation, Sigma Xi Cornell, Mario Einaudi Center for International Studies, the Andrew W. Mellon

Foundation, the Department of Ecology and Evolutionary Biology, and the Department of Entomology at Cornell.

I would also like to deeply thank my parents, Carlos and Dorotea, who always believed I could do anything if I worked hard. They have supported me every step along the way. I would not have made it through this process without my mom, who came to Ithaca several times to give me the unconditional love and support I needed. I thank my brother Guilherme and his wife, Daniela, for always having a kind word. I thank my aunt, Elisa Frank, who came to Ithaca and spent several weeks taking care of my little boy, as well as my in-laws, Evidio and Idalice. My uncle, Jorge Frank, his wife, Mariane, and his two children Ana and Joshua, for celebrating my achievements and talking me through the harder times.

My greatest thanks goes to my husband, Gui Becker, and my son, Samuel Becker, who are the core of my life. Gui has helped not only with fieldwork, data analyses and chapter drafts, but he contributed in innumerable ways to every step of this journey. I am blessed for having Gui in my life. His unconditional love has empowered me and together we have followed our dreams. Samuel is my daily reminder of how wonderful life is. Both have been the color of my life and my source of motivation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

VIRUS STRAINS DIFFERENTIALLY INDUCE PLANT SUSCEPTIBILITY TO APHID VECTORS AND CHEWING HERBIVORES

Published as: Mônica F. Kersch-Becker and Jennifer S. Thaler. 2013. Virus strains differentially induce plant susceptibility to aphid vectors and chewing herbivores. Oecologia 174:883–892.

Abstract

Plants are frequently attacked by both pathogens and insects and an attack from one can induce plant responses that affect resistance to the other. However, we currently lack a predictive framework for understanding how pathogens, their vectors, and other herbivores interact. To address this gap, we have investigated the effects of a viral infection in the host plant on both its aphid vector and non-vector herbivores. We tested whether the infection by three different strains of *Potato virus Y* (PVY^{NTN}, PVY^{NO} and PVY^{O}) on tomato plants affected: (i) the induced plant defense pathways; (ii) the abundance and fecundity of the aphid vector (*Macrosiphum euphorbiae*); and (iii) the performance of two non-vector species: a caterpillar (*Trichoplusia ni*) and a beetle (*Leptinotarsa decemlineata*). While infection by all three strains of PVY induced the salicylate pathway, PVY^{NTN} induced a stronger and longer response. Fecundity and density of aphids increased on all PVY-infected plants, suggesting that the aphid response is not negatively associated with salicylate induction. In contrast, the performance of non-vector herbivores correlated with the strength of salicylate induction. PVY^{NTN} infection decreased plant resistance to both non-vector herbivores, increasing their growth rates. We also demonstrated that the impact of host plant viral infection on the caterpillar results from host plant responses and not the effects of aphid vector feeding. We propose that pathogens chemically mediate insect-plant interactions by activating the salicylate pathway and decreasing plant resistance to chewing insects, which has implications for both disease transmission and insect community structure.

Introduction

Attack by vector-borne pathogens can alter host plant traits in ways that affect the community of organisms on the plant (Eigenbrode *et al.* 2002; Stout *et al.* 2006; Mauck *et al.* 2010a; Mauck *et al.* 2012). These pathogen-induced plant responses can have strong impacts not only on vectoring insects (Eigenbrode *et al*. 2002; Stout *et al.* 2006; Mauck *et al.* 2010a; Mauck *et al.* 2012) but also on myriad non-vectoring herbivores (Stout *et al.* 2006; Mauck *et al.* 2010b; Belliure *et al*. 2010). Identifying how pathogen-induced responses in the host plant impact both vectoring and non-vectoring herbivores will shed light on the mechanisms shaping insect-plant interactions (Stout *et al.* 2006).

The vast majority of plant viruses rely upon specific vector species for efficient transmission and dispersal (Ng and Perry 2004; Ng and Falk 2006). Aphids are responsible for the transmission of approximately 50% of insect-transmissible plant viruses, making them the most prevalent vector of this group of viruses (Nault 1997; Ng and Perry 2004). Therefore, plants are frequently attacked simultaneously by aphids and the viruses they carry, both of which can change the quality of the host plant. Viruses vary in the means and extent to which they alter plant quality and in their effects on vector species (Castle and Berger 1993; Eigenbrode *et al.* 2002; Belliure *et al.* 2005; Hodge and Powell 2008). Much of this variance is determined by the mechanism of viral transmission, either persistent or non-persistent. Persistent viruses are acquired and transmitted by aphids following prolonged and sustained feeding (hours to days) and are predicted to attract vectors and promote their long-term feeding by enhancing plant quality (Sylvester 1980; Eigenbrode *et al*. 2002; Alvarez *et*

al. 2007; Jiu *et al.* 2007; Mauck *et al.* 2012). In contrast, non-persistent viruses are rapidly acquired (seconds to minutes) and transmitted from plant to plant after brief probing. Aphids acquire non-persistent viruses by feeding on infected tissue; the virions bind to specific regions of the aphid stylet, but do not replicate within the aphid vector (Ng and Perry 2004; Ng and Falk 2006). Because of their rapid transmission rate, it has been suggested that non-persistent viruses will induce changes in the host plant that initially enhance vector attraction, but would subsequently reduce host plant quality to promote vector dispersal (Mauck *et al.* 2010a; Mauck *et al.* 2012).

Recent studies have demonstrated that certain pathogenic viruses are able to suppress the jasmonate response in plants and that this attenuation may positively affect the performance of insect vectors and potentially increase the rate of virus transmission (Preston *et al.* 1999; Jiu *et al.* 2007; Yang *et al.* 2008). However, viruses have variable effects on the performance of their vectors, ranging from positive (Castle and Berger 1993; Eigenbrode *et al.* 2002; Belliure *et al.* 2005; Belliure *et al.* 2008; Hodge and Powell 2010) to negative (Jensen 1959; Kluth *et al*. 2002; Hodge and Powell 2008; Mauck *et al.* 2010). The outcome depends on the virus species and strain (Castle and Berger 1993; Colvin *et al.* 2006; Ng and Falk 2006; Jiu *et al.* 2007, Mauck *et al.* 2012). Aphid performance on host plants infected with a persistent virus has been assessed in several studies (Belliure *et al*. 2005; Mowry and Ophus 2006; Alvarez *et al.* 2007, Hodge and Powell 2010). Although the majority of the costliest agricultural crop viruses are transmitted in a non-persistent manner (Ng and Perry 2004; Mauck *et al.* 2010a,b; Mauck *et al.* 2012), far less is known about the effect of non-persistent viruses on plant quality and vector performance. Multiple strains of a single virus may

also cause substantial variation in induced plant defenses, which may in turn affect vectors differently (Herbers *et al.* 2000; Verbeek *et al.* 2009; Kogovšek *et al*. 2010).

There is a growing body of literature demonstrating that pathogen infection has the potential to increase plant susceptibility to non-vectoring herbivores (Hare and Dodds 1987; Preston *et al.* 1999; Mayer *et al.* 2002; Cardoza *et al.* 2003; Stout *et al.* 2006; Belliure *et al.* 2010; Thaler *et al.* 2010). For example, plants are known to induce salicylic acid (SA), a phytohormone that is recognized as a critical signal for the expression of induced resistance to many pathogens and some herbivores (Delaney *et al. 1994*; Ryals *et al.* 1999; Fidantsef *et al.* 1999; Thaler *et al.* 1999). Leaf-chewing herbivores that infrequently induce the salicylate pathway themselves appear to be either positively or neutrally affected by SA induction (Inbar *et al.* 2001; Cui *et al.* 2002; Thaler *et al.* 2002). Vector-transmitted viruses can alter plant defenses and subsequent herbivore feeding through three pathways: 1. viral induction of plant responses, 2. aphid vector induction of plant responses, or 3. the virus and its vector have an interactive effect that modifies plant responses.

Little is known about how vector-borne viruses induce the jasmonate and salicylate defensive pathways (Kovač *et al.* 2009; Abe *et al.* 2012), as most of the evidence comes from studies with bacteria and directly transmitted viruses (Preston *et al.* 1999; Stout *et al.* 1999; Thaler *et al.* 1999; Cui *et al.* 2005; Thaler *et al.* 2010). Consequently, we still lack a mechanistic framework for understanding how vectored viruses will alter plant quality. This interaction is critical in predicting how defensive pathways induced by pathogens will affect vector and non-vector insect communities.

We studied the effects of three different strains of *Potato virus Y* (PVY) (Potyvirus: Potyviridae) on its tomato host plant (*Solanum lycopersicum* cv. Castlemart), its aphid vector *Macrosiphum euphorbiae*, and two co-occurring non-vector chewing herbivores: the cabbage looper (*Trichoplusia ni*) and the Colorado potato beetle (*Leptinotarsa decemlineata*). PVY exclusively infects plants in the Solanaceae family and can causes serious impact on important crops, such as potato, tobacco, pepper and tomato, around the world (Scholthof *et al.* 2011). This system is well-suited to distinguish between direct (feeding) and indirect (virus transmission) effects of aphids on subsequent herbivore feeding because plants can be mechanically inoculated with all three strains. PVY is transmitted in a non-persistent manner by at least 50 species of aphids, including *M. euphorbiae*, which can acquire PVY in 15 min of feeding (Harrington and Gibson 1989; Boquel *et al.* 2011a; Nanayakkara *et al.* 2012).

The three PVY strains (PVY^{NTN}, PVY^{NO} and PVY^{O}) used in this study infect tomato, but have different patterns of symptom development (Singh et al. 2008). PVY^{O} causes mosaic or vein clearing symptoms, whereas PVY^{NO} and PVY^{NTN} induce systemic vein necrosis (Singh et al. 2008). Strains of PVY are vectored differently by aphid species (i.e. retention and acquisition time differ) (Verbeek *et al.* 2010) and impact the host plant differently (i.e. may or may not cause necrosis or visual symptoms) (Singh *et al.* 2008). We hypothesized that the different virus strains would activate different levels of salicylic acid, and that higher levels of SA would intensify the inhibition of jasmonic acid, which would differentially affect vector and non-vector herbivores. We therefore predicted that different virus strains differentially affect the

performance of vectors and non-vectors, and that this variation is explained by differential activation of plant defense pathways.

Accordingly, we first tested for plant defense variation among strains by measuring the timing and strength of phytohormonal responses to PVY-infection in the tomato host plants. We then evaluated whether induced responses to infection were associated with the performance of aphid vectors and two non-vector chewing herbivores (*T. ni* and *L. decemlineata*). By using mechanically inoculated plants and manipulating the presence of aphids, we were able to discriminate the individual and interactive effects of the virus and aphids vectors on subsequent plant resistance to *T. ni* feeding.

Methods

Tomato Plants, Herbivores, Virus Isolates and Inoculation

Tomato plants (*Solanum lycopersicum* cv. Castlemart) used in these experiments were grown from seeds in 10 cm pots in a greenhouse and reared for 4 weeks (four-leaf stage) before being inoculated for use in phytohormonal or herbivore bioassays. Plants were grown in commercial potting soil, watered daily and received 85 g of fertilizer per week (Jack's professional® water-soluble fertilizer 21:5:20 N:P:K). Aphids, *Macrosiphum euphorbiae* (isolate WU-11-FR clone; Goggin *et al.* 2001), were reared on uninfected tomato plants in growth chambers (22 ºC, 16:8 h, L:D photoperiod). We obtained *T. ni* caterpillars as eggs from Benzon Research Inc. (Carlisle, PA, USA) and reared them exclusively on tomato before use. We collected *L.*

decemlineata beetles (hereafter CPB) as eggs from potato fields in Ithaca, NY, USA and we reared them on tomato plants prior to experiments.

We obtained the PVY isolates from PVY-infected tobacco plants provided by Stewart Gray (USDA-ARS, Cornell University). We mechanically inoculated tomato plants with inoculum from PVY strains as follows: we triturated leaf material from PVY-infected tobacco plants in 5 mL of PBS buffer. We individually applied PVY^{NTN}, PVY^{NO} and PVY^{O} inoculum to tomato plants by gently rubbing the entire upper leaflet surface with a cotton-tipped applicator saturated with the inoculum onto carborundum-dusted leaves (Mello et al. 2011). This method does not control for differences in virus titer from the source plants, which may affect plant and herbivore responses. We sham-inoculated control tomato plants following the same procedures with tissues from uninfected tobacco plants. To confirm inoculation, we used enzyme-linked immunosorbent assay (ELISA) to detect the presence of virus. We tested a subsample of 37 mechanically inoculated plants to confirm plant infection using ELISA. Of these 83% were confirmed positive (PVY^{NTN}, 13/13 = 100 %, PVY^{NO}, 12/13 = 92.5 % and PVY^{O},7/10 = 70 %). PVY-infected plants of all three strains were visually asymptomatic and infection did not affect host plant survival. To confirm that aphids were uninfected prior to inclusion in the bioassay, we caged 50 aphids on PVY-free plants and used ELISA to retest whether the plants were still PVY-free 10 days later. All 20 plants tested were PVY-free, therefore the aphids introduced to plants were not carrying PVY prior the experiments.

Phytohormonal Analyses

We assessed the differences in plant defense induction among PVY strains and uninfected controls by measuring concentrations of plant phytohormones (jasmonic acid, salicylic acid and abscisic acid). Plants were grown for three weeks in the greenhouse and were then inoculated with infected or uninfected plant sap as described above. We measured the temporal pattern of induction by quantifying the concentration of jasmonic acid and salicylic acid at three time points following infection (12, 24 and 48 hours). We used different plants for each time interval, and there were a different number of replicates for each treatment at each time point. At 12, 24 and 48 hours respectively, the numbers of plants tested were: Uninfected: 10, 17, 10; PVY^{O}: 7, 18, 8; PVY^{NO}: 10, 20, 9; and PVY^{NTN}: 10, 19, 10. For the analyses of abscisic acid concentration we only measured phytohormonal concentration after 24 hours, and the number of replicates per treatment differed (Control: 12, PVY^{O}: 15, PVY^{NO}: 15, PVY^{NTN}: 14). For each sample we collected and weighed (200-300 mg) the terminal leaflet of the youngest fully expanded leaf. We then transferred the tissue to 2-mL screw cap tubes containing 900 mg zirconia/silica beads (BioSpec, Bartelsville, OK, USA) and immediately immersed it in liquid nitrogen. We stored the samples at -80°C until chemical analysis. We extracted phytohormones from the tissue and analyzed them according the methods described in Thaler et al. (2010) and Pan et al. (2008). Briefly, we added 1mL of extraction buffer and 100 μL internal standard solution (d_4-SA, d_5-JA and d_6-ABA, CDN isotopes, Point-Claire, Canada). We homogenized the samples in a FastPrep (MP Biomedicals, Solon, OH, USA) at speed 6.5 for 45 seconds, and then in a centrifuge (Beckman Coulter, Allegra® X-22R, Fullerton, CA, USA) at 4°C for 20 minutes at 14000 rpm. We dissolved samples in 200

μL methanol after extraction with dichloromethane and solvent evaporation. We analyzed 15 μL on a triple-quadrupole LC-MS/MS system (Quantum access; Thermo Scientific, Waltham, MA, USA). We analyzed the concentration of phytohormones using one-way analysis of variance (ANOVA), including trial as a blocking effect. All statistical analyses were performed in JMP 9 (SAS Institute Inc. 2010). Even though our insect bioassay were performed 10 days post infection, we chose to measure phytohormones at 12, 24 and 48 hours after infection because it has been demonstrated that phytohormonal induction peaks hours after damage (Krečič -Stres et al. 2005, Thaler *et al.* 2010).

Aphid Bioassays

It takes a few days for the virus to spread systemically through the plant (Mehle et al. 2004), and induce the responses that might affect herbivores. Consequently we chose to carry out all insect bioassays 10 days after inoculation. We mechanically infected tomato plants with one of three different strains of PVY (PVY^{NTN}, PVY^{NO} and PVY^{O}) and transferred each plant to a field cage (1 m^3) 10 days after inoculation. These field cages were constructed of a pvc frame covered with a fine acrylic mesh (20x20 openings per 25.4 mm). These cages had a side zipper and were buried into the soil in the bottom to prevent insects of entering the cage. Plants were planted into the soil, and watered every other day, but they did not receive any fertilizer in the field. To assess the effect of plant infection on aphid density we placed 10 to 20 4^{th}-instar aphids on each plant. We recorded the number of aphids per plant after 2 weeks. We conducted the field experiment in an old field in Ithaca, NY in July of 2009 and 2010.

The number of replicates per treatment differed (Control: 29, PVY^{O}: 24, PVY^{NO}: 23, PVY^{NTN}: 23). To measure the effect of plant infection on the number of nymphs produced per aphid, we enclosed a single 4^{th}-instar aphid on the third leaf. Every five days, we counted and removed the nymphs produced until aphid death (ca. 20 days). We conducted this bioassay in the greenhouse over the summer of 2009 and 2010. The number of replicates per treatment differed (Control: 41, PVY^{O}: 44, PVY^{NO}: 42, PVY^{NTN}: 42).

We used analysis of covariance (ANCOVA) to analyze the effect of plant infection on the number of aphids, using initial number of aphids as covariate. We performed analysis of variance (ANOVA) to test the effect of plant infection on the number of nymphs produced by an aphid. We used trial as a blocking effect on both analyses, and when the results were significant we applied Fisher's LSD post-hoc test. The number of nymphs produced by an aphid was not normally distributed, therefore we square root transformed the data to meet parametric assumptions; homogeneity of variance was met after transformation.

Non-Vector Herbivore Bioassays

The caterpillar, *T. ni*, experiment followed a 4x2 factorial design: four plant infection levels (uninfected, PVY^{NTN}, PVY^{NO}, PVY^{O} –infected plants) crossed with two aphid infestation levels (no aphids and aphids added). Each plant was randomly assigned to one of the following treatments: uninfected control, uninfected + aphids, PVY^{NTN}, PVY^{NTN} + aphids, PVY^{NO}, PVY^{NO} + aphids, PVY^{O} or PVY^{O} + aphids. Plants with aphids received 50 individuals of mixed age. We added aphids and inoculated the

plants 10 days prior to bioassays. We placed three weighed second-instar caterpillars on each plant and allowed them to feed for 3 days, when we recorded the wet mass of each caterpillar and estimated its growth rate as ((final mass - initial mass) / initial mass) * 100 (Thaler *et al.* 2012). We excluded caterpillars that died before the final mass was measured and recorded the number of surviving caterpillars. We measured leaf consumption by recording the amount of leaf tissue removed for all leaves on the host plant. We used an acetate grid to score leaf tissue removal to the nearest mm^2. To account for herbivore mortality we divided the total damage per plant by the number of surviving caterpillars, and we analyzed leaf consumption per surviving individual. We conducted six trials in the greenhouse over the summer of 2009 and 2010. The number of replicates per treatment differed (Control: 47, Control+Aphids: 47, PVY^{O}: 20, PVY^{O}+Aphids: 19, PVY^{NO}: 19, PVY^{NO}+Aphids: 19, PVY^{NTN}: 19, PVY^{NTN}+Aphids: 19).

We performed two-way ANOVAs to test the effect of PVY infection, aphids and PVY infection-by-aphid interaction on caterpillar percent of survival and caterpillar relative growth rate. We used ANCOVA to determine the effects of PVY infection, aphids and PVY infection-by-aphid interaction on *T. ni* leaf consumption with initial mass as a covariate. We modeled the effects of both PVY infection and aphids on caterpillar conversion efficiency by including mass gain (final mass – initial mass) as the response variable and leaf consumption per caterpillar as the covariate (Raubenheimer and Simpson 1992). We included trial in all models as a blocking effect, and performed Fisher's LSD post-hoc contrasts. Survivorship was arcsine-transformed to equalize variance among treatments.

We tested the effects of plant infection with four levels (uninfected, PVY^{NTN}, PVY^{NO}, PVY^{O} –infected plants) on Colorado potato beetle (CPB) performance. Because aphids did not strongly affect *T. ni* performance, we excluded the aphid treatment in the CPB bioassays. We placed a single weighed two-day-old beetle larva on the tomato plant and allowed it to feed for 3 days. The final mass of each beetle larva was measured to calculate growth rate, as with *T. ni*. We recorded the number of surviving beetles and measured leaf consumption per plant. We excluded beetles that died before final data collection, which caused the number of replicates per treatment to differ (Control: 47, PVY^{O}: 50, PVY^{NO}: 50, PVY^{NTN}: 51). We conducted four trials in a walk-in growth chamber (24ºC, 16:8, L:D photoperiod).

We used ANCOVA to determine the effects of PVY infection on leaf consumption of CPB, using initial mass as covariate. We ran a one-way ANOVA to test the effect of plant infection on beetle growth rate, and trial was entered as a blocking effect. We conducted Fisher's LSD post-hoc tests when the ANCOVA was significant. We performed a logistic regression to test the effect of PVY-infection on CPB survivorship.

Results

Phytohormonal Responses

The concentration of salicylic acid (SA) was affected differently by the PVY strains ($F_{[3,134]} = 26.549$, $P < 0.001$, Figure. 1.1a). Across the three time points, post-hoc analyses showed that PVY^{NTN}-infected plants had the highest concentration of SA; their SA content was 139% higher compared to uninfected control plants (Figure 1.1a).

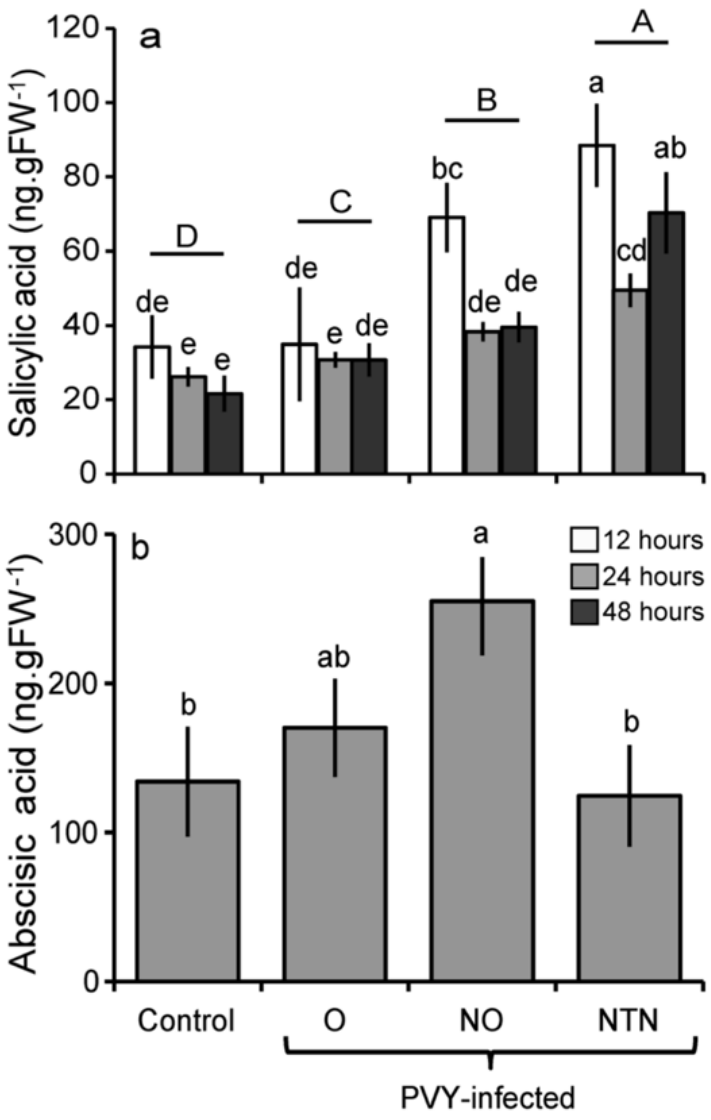

Figure 1.1: Effects of PVY infection on a) salicylic acid concentration (expressed in ng.g^{-1} fresh weight) at 12 (white bars), 24 (light gray bars) and 48 (dark gray) hours post-PVY inoculation and b) abscisic acid concentration (expressed in ng.g^{-1} fresh weight) after 24 hours of infection. Letters indicate significant differences at $P < 0.05$ following Fisher's LSD post-hoc test. On panel a, capital letters represent the differences among PVY infection treatment, while lowercase letters indicate the overall differences among all treatments. Treatments are coded as: uninfected controls and *Potato virus Y* strains PVY^{O}, PVY^{NO}, and PVY^{NTN}.

The concentration of SA in PVY^{NO} –infected plants was 71% higher than controls, and PVY^{O}-infected plants had concentrations only 16% higher than their uninfected counterparts. The induction of SA across all three strains was transient ($F_{[2,135]} = 3.491$, $P = 0.033$), peaking 12 hours after inoculation and decreasing by 48 hours after inoculation (Figure 1.1a).

Nonetheless, we found an interaction between time after infection and PVY strain ($F_{[6,135]} = 2.574$, $P = 0.022$, Figure 1.1a). Plants inoculated with the PVY^{NTN} strain showed the highest levels of SA induction relative to controls after 12 hours. Although SA levels in PVY^{NTN}-infected plants declined after 12 hours, SA levels at all time points were higher than those for the other treatments. The SA levels of PVY^{NO}–infected plants were strongly elevated relative to controls after 12 hours and decreased considerably 24 and 48 hours after inoculation. PVY^{O}-infected plants did not differ from controls at any specific time point.

The concentration of jasmonic acid (JA) was not altered by PVY-infection (Mean ± SE for Uninfected= 24.16 ± 12.63 ng/gFW; PVY^{O}= 25.10 ± 5.40 ng/gFW; PVY^{NO}= 33.55 ± 9.44 ng/gFW; PVY^{NTN}= 28.43 ± 8.03 ng/gFW; $F_{[3,133]} = 0.548$, $P = 0.650$) and it was similar at 12, 24 and 48 hours after infection (Mean ± SE for 12 hrs= 10.82 ± 2.24 ng/gFW; 24 hrs= 45.87 ± 8.68 ng/gFW; 48 hrs= 8.79 ± 2.23 ng/gFW; $F_{[2,133]} = 0.271$, $P = 0.763$). Infection by PVY^{NO} increased the concentration of abscisic acid (ABA) after 24 hours ($F_{[3,52]} = 2.927$, $P = 0.042$, Figure 1.1b). However, ABA concentration in plants infected with the two other strains did not differ from controls. In addition, 24 hours post infection ABA levels were strongly positively correlated

with SA levels ($r_{pearson}$=0.397, P<0.001) across all plants, suggesting a synergism between these two phytohormones.

Aphid Vectors

The number of aphids on plants was positively affected by PVY infection ($F_{[3,94]}$ = 3.001, P = 0.034, Figure 1.2a), though the strains did not differ from each other. We recorded 68% more aphids on PVY^{NTN} and 55% more on PVY^{NO} compared to uninfected plants. Aphid fecundity varied between virus strains, with more nymphs produced on PVY^{NO} than on PVY^{NTN} -infected plants ($F_{[3,160]}$ = 3.324, P = 0.021, Figure 1.2b).

Caterpillar and Beetle Non-Vectors

Plant infection and aphid presence differentially affected the measures of *T. ni* performance. *T. ni* survival was decreased by PVY-infection ($F_{[3,196]}$ = 6.589, P < 0.001), increased by the presence of aphids ($F_{[1,196]}$ = 3.942, P = 0.049), and there was a marginal PVY-infection-by-aphid interaction ($F_{[3,196]}$ = 2.173, P = 0.095). *T. ni* survival was 35% higher on uninfected plants compared to PVY–infected plants, and strains did not differ (Figure 1.3a). The proportion of survivors increased 15% when caterpillars fed on plants also hosting aphids, and this effect was strongest on PVY^{NO}-infected plants (Figure 1.3a). The negative effect of PVY infection on *T. ni* survival was counterbalanced by the positive effect of aphids on PVY^{NO}-infected plants, but not on PVY^{NTN} or PVY^{O}- infected plants.

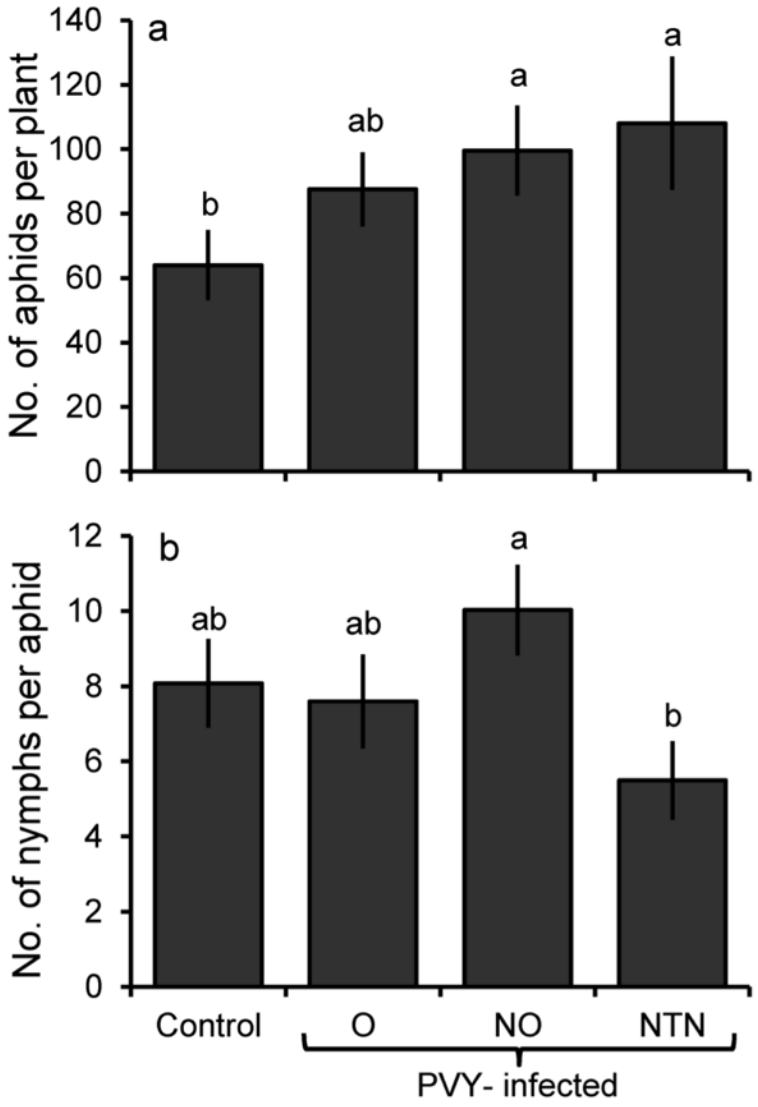

Figure 1.2: Aphid responses to uninfected controls and *Potato virus Y* strains PVY^{O}, PVY^{NO}, and PVY^{NTN}. a) Number of aphids per plant after 15 days, b) number of nymphs produced by a single aphid. Letters indicate significant differences at $P < 0.05$ following Fisher's LSD post-hoc test. Bars are mean ±SE.

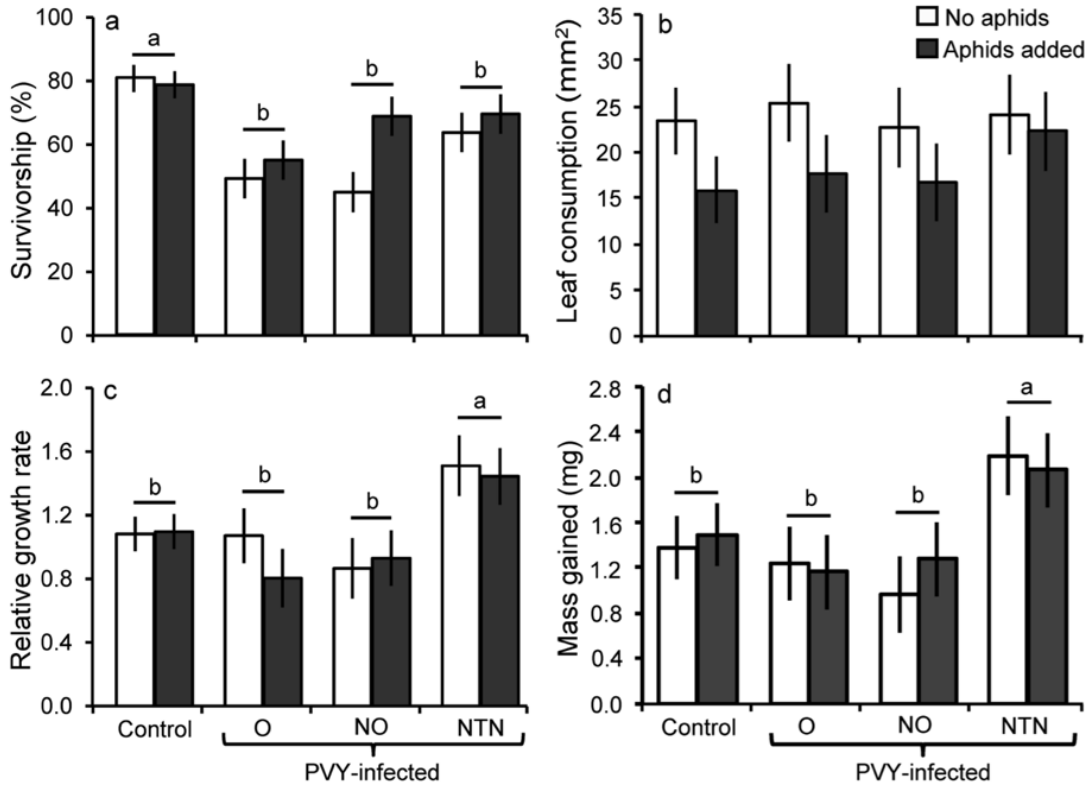

Figure 1.3: Effects of PVY infection and aphids on *T. ni* larval performance. a) Percentage of caterpillar survival, b) leaf consumption per caterpillar, c) *T. ni* growth rate, and d) consumption efficiency. Bars are mean ±SE for panels a and c, and LS-means ±SE for panel b and d. White bars represent plants with no aphids added and black bars represent plants with aphids added. Letters indicate significant differences at $P < 0.05$ following Fisher's LSD post-hoc test. . Treatments are coded as: uninfected controls and *Potato virus Y* strains PVY^{O}, PVY^{NO}, and PVY^{NTN}.

T. ni larvae consumed the same amount of leaf tissue on uninfected and PVY^{-} infected plants, and no difference among strains was found ($F_{[3,178]} = 0.897$, $P = 0.444$, Figure 1.3b). In contrast, caterpillar growth rate increased 28% on PVY^{NTN} -infected plants compared to control plants, while the growth rate of caterpillars fed on PVY^{NO} and PVY^{O}-infected plants did not differ from the controls ($F_{[3,178]} = 4.229$, $P = 0.007$, Figure 1.3c). Leaf consumption decreased 23% on plants with aphids ($F_{[1,178]\ aphids} = 10.514$, $P = 0.001$), but we did not detect a virus-by-aphid interaction ($F_{[3,178]\ virus*aphids} = 0.567$, $P = 0.637$). Relative growth rate was not affected by aphids or by the virus-by-aphid interaction ($F_{[1,178]\ aphids} = 0.2976$, $P = 0.586$; $F_{[3,178]\ aphid*virus} = 0.376$, $P = 0.770$). Although *T. ni* larvae consumed the same amount of leaf tissue on uninfected and PVY-infected plants, their mass gain on PVY^{NTN}-infected plants was higher. Therefore, caterpillars feeding upon PVY^{NTN}-infected plants showed a 66% higher conversion efficiency compare to caterpillars fed on uninfected, PVY^{O} and PVY^{NO}–infected plants ($F_{[3,178]} = 7.708$, $P < 0.001$, Figure 1.3d). We did not detect an effect of aphids or virus-by-aphid interaction on caterpillar conversion efficiency ($F_{[1,178]\ aphids} = 0.135$, $P = 0.714$; $F_{[3,178]\ aphid*virus} = 0.383$, $P = 0.765$).

The response of CPB larvae to PVY infection was similar to the response of caterpillars, with higher performance on PVY^{NTN}-infected plants, but PVY^{NTN} effects on CPB were stronger. Leaf consumption was on average 65% higher on PVY^{NTN}-infected plants than on uninfected, whereas PVY^{NO} and PVY^{O} –infected plants did not differ from the controls ($F_{[3,190]} = 10.919$, $P < 0.001$, Figure 1.4a). As a result, beetle larvae grew 50% more on PVY^{NTN} -infected plants compared to controls ($F_{[3,190]} =$

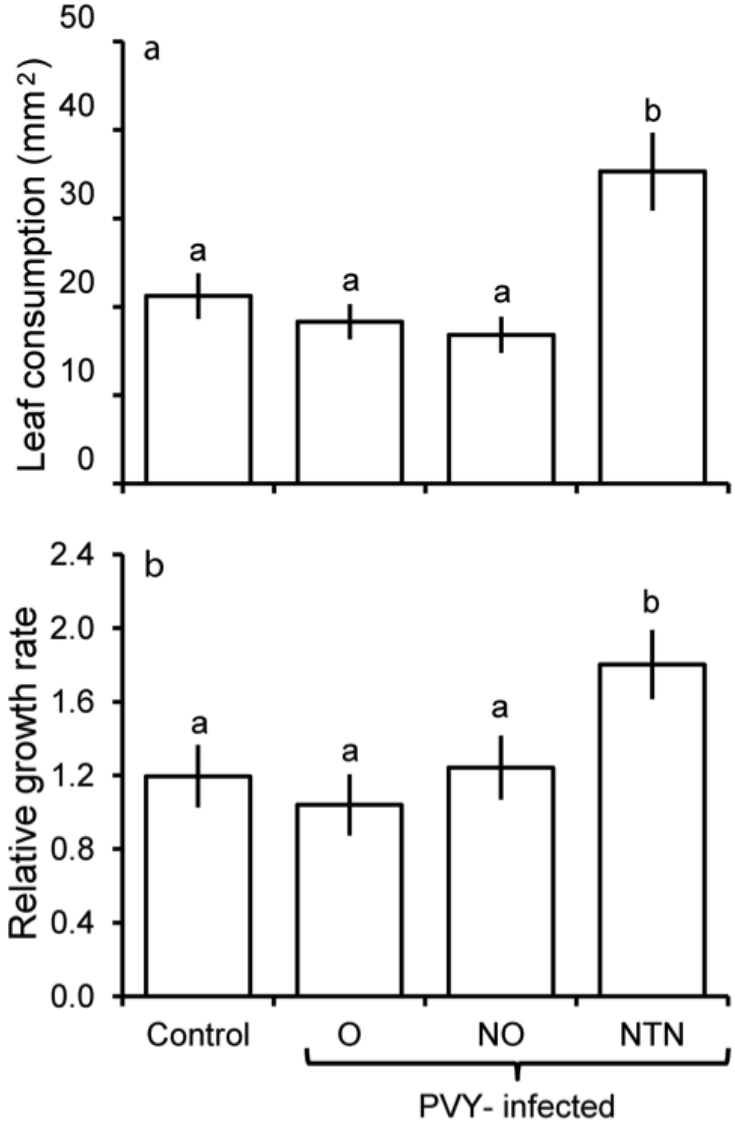

Figure 1.4: Colorado potato beetle responses to uninfected controls and *Potato virus Y* strains. a) Leaf consumption by beetle larva, and b) Beetle growth rate. Bars are mean ±SE; letters indicate significant differences at $P < 0.05$ following Fisher's LSD post-hoc test.

4.131, P = 0.007, Figure 1.4b). Beetle larval survivorship was not affected by PVY-infection (χ^2 = 1.419, df = 3, P = 0.701)

Discussion

Non-persistent plant viruses rely on herbivorous vectors for efficient transmission and dispersal. The deception hypothesis proposes a potential mechanism by which non-persistent viruses might influence vector dispersal and thus virus spread (Mauck *et al*. 2010a). It states that non-persistent viruses induce changes in the host plant to enhance vector attraction, but would then reduce host plant quality, decreasing vector performance and promoting rapid vector dispersal (Mauck *et al*. 2010a; Mauck *et al.* 2012). In contrast, some studies, including ours, have demonstrated that aphid vectors have higher population growth, fecundity and body mass on virus-infected plants compared to uninfected counterparts (Kennedy 1951; Castle and Berger 1993; Blua *et al.* 1994; Srinivasan & Alvarez 2007; Boquel *et al.* 2011b). Increasing host plant quality and aphid density causes crowding, which can increase aphid dispersal through the production and dispersal of winged aphids (Muller *et al*. 2001), and increased emigration by wingless aphids (Underwood *et al.* 2011). Increasing aphid dispersal by increasing aphid crowding is a slower mechanism than what is proposed by Mauck *et al*. (2010a).

Persistent viruses adopt a similar strategy, and it is hypothesized that they may have evolved mechanisms to suppress plant induced defenses against their vectors to promote rapid vector population growth, which will increase dispersal, leading to further virus spread (Belliure *et al.* 2005; Jiu *et al*. 2007). Because it takes 5 days for

the virus to spread systemically (Mehle *et al.* 2004) and probably longer to reach titers in the plant where it can be effectively transmitted, a slow mechanism may be sufficient. Even though non-persistent viruses are less dependent upon the vector for transmission than persistent viruses (Ng and Perry 1994), the positive effect of PVY infection on vectors also reveals a potential mutualistic relationship.

Although we predicted that PVY strains would differentially activate salicylic acid (SA) and correspondingly affect aphid fitness, we found that all three strains of PVY had a positive effect on aphid vector abundance even though SA was induced. Thus, in this work the performance of aphid vectors was not explained by differential induction of plant defense pathways. Aside from the activation and inhibition of defensive pathways, plant viral infections are also known to promote changes in host plant nutritional quality (Herbers *et al.* 2000; Shalitin and Wolf 2000), which could explained the increased performance of aphid vectors on PVY-infected plants.

Although all three strains of PVY induced the salicylate pathway, we found differences in the timing and extent of SA accumulation. PVY^{NTN} -infected plants showed the highest amount and longest duration of SA induction, followed by PVY^{NO} and PVY^{O}- infected plants. Other studies have demonstrated that PVY^{NTN} induces SA, although they did not compare its induction to other PVY strains (Krečič -Stres *et al.* 2005; Baebler *et al.* 2009; Kogovšek *et al.* 2010). The variation in phytohormonal induction by the different virus strains positively correlates with the performance of chewing herbivores. Plants infected by PVY^{NTN} were more susceptible to both non-vector herbivores, with both *T.ni* and CPB showing higher growth rates on PVY^{NTN}-infected plants. PVY^{NTN} was the strongest inducer of salicylic acid and the one that the

CPB and *T. ni* benefitted the most from. This is consistent with previous research showing that *T. ni* benefits from feeding on mutant plants with high levels of SA (Cui *et al.* 2002).

The induction of the salicylate pathway may affect insects both directly and indirectly. It has been demonstrated that the SA pathway can suppress the induction of the jasmonic acid (JA) pathway, which decreases plant resistance to herbivores. This suggests negative crosstalk between the pathways (Sano and Ohashi 1995; Felton *et al.* 1999; Thaler *et al.* 1999; Thaler *et al.* 2002; Zarate *et al.* 2007; Zhang *et al.* 2009). However, we did not find evidence of negative crosstalk as the concentrations of jasmonic acid in uninfected controls and PVY-infected tomato plants were similar. SA-JA crosstalk could still have occurred downstream of JA hormone induction, which we did not measure (Leon-Reyes *et al.* 2010). It is also reasonable to suggest that SA might be directly affecting herbivores or might be interacting antagonistically or synergistically with other phytohormones. It has been demonstrated that abscisic acid (ABA) can inhibit SA responses (Mauch-Mani and Mauch 2005); ABA-deficient tomato plants produced high levels of pathogenesis-related proteins, which were activated by the induction of the SA pathway (Thaler and Bostock 2004). The results of our work contradict these findings; we found a synergism between ABA and SA. In this study, high levels of ABA were strongly positively correlated with high SA levels ($r_{pearson}$=0.397, P<0.001), and therefore ABA did not suppress SA induction. We reported high levels of ABA in PVY^{NO}-infected plants, although higher levels of ABA were not linked with herbivore performance, contradicting previous findings (Thaler and Bostock 2004; Ton *et al.* 2009; Asselbergh *et al.* 2008; Kusajima *et al.* 2010).

In vector-transmitted pathosystems, both the virus and the vector can affect the host plant's induced responses. Accordingly, we discriminated between the individual and interactive effects of the virus and the aphid vectors on subsequent feeding by *T. ni* and demonstrated that the effect of PVY infection alone was stronger than the effect of the aphid vectors. Aphid feeding increased *T. ni* survival and decreased leaf consumption, but did not affect *T. ni* growth rate, or conversion efficiency. This suggests that in systems where the pathogen is transmitted non-persistently, such as PVY, the effect of viruses on non-vector herbivores may be mediated by host plant chemical defense responses to the virus, not to the feeding of the vector itself. These results contrast with research on persistent viruses, where the performance of non-vector herbivores is often higher on vector-damaged plants than on virus-infected plants with no damage (Belliure *et al.* 2010). The work of Belliure *et al.* (2010) suggests that in these systems the effects of host plant defensive responses on non-vector herbivores may be driven by the feeding of the vector and are not regulated by the changes induced by the virus.

Here we have shown that the response of aphid vectors to plant infection was positively affected by all three strains of PVY and was not associated with the induction of phytohormones. In contrast, the performance of chewing non-vector herbivores was associated with salicylate induction and was differentially affected by strains. Therefore, pathogens can chemically mediate insect-plant interactions by activating the salicylate pathway and decreasing plant resistance to chewing insects, and thus improve the mechanistic understanding of indirect community interactions.

Acknowledgements

We are greatly indebted to Dawn Smith and Stewart Gray's lab for kindly helping us with viral infection and proving the virus strains used in the experiments. We thank Rayko Halitschke for his valuable assistance in the phytohormone analyses; Ordom Huot for his help with lab work and Cesar Rodriguez-Saona and Isgouhi Kaloshian for providing the aphids. This study strongly benefited from comments and suggestions by Anurag Agrawal, Alison Power, Gui Becker, Suzi Claflin, Scott McArt, Ian Kaplan, the editor John Lill and two anonymous reviewers. This project received financial support from Sigma Xi (Cornell Chapter), the Department of Ecology and Evolutionary Biology and the Department of Entomology at Cornell. MFKB is recipient of the Olin fellowship and the CAPES/Fulbright fellowship (BEX 2234-08-4). The experiments comply with the current laws of the United States of America, in which the experiments were performed.

REFERENCES

Abe H *et al.* (2012) Antagonistic plant defense system regulated by phytohormones assists interactions among vector insect, thrips and a tospovirus. Plant and Cell Physiology 53:204-212.

Alvarez AE, Garzo E, Verbeek M, Vosman B, Dicke M, Tjallingii WF (2007) Infection of potato plants with *Potato leafroll virus* changes attraction and feeding behaviour of *Myzus persicae*. Entomologia Experimentalis Et Applicata 125:135–144.

Ament K, Kant MR, Sabelis MW, Haring MA, Schuurink RC (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiology 135:2025-2037.

Asselbergh B, Achuo A, Hofte M, Van Gijsegem F (2008) Abscisic acid deficiency leads to rapid activation of tomato defence responses upon infection with *Erwinia chrysanthemi*. Molecular Plant Pathology 9:11-24.

Baebler Š, Krečič-Stres H, Rotter A, Kogovšek P, Cankar K, Kok E J, Gruden K, Kovač M, Zel J, Pompe-Novak M, Ravnikar M (2009) PVY^{NTN} elicits a diverse gene expression response in different potato genotypes in the first 12 h after inoculation. Molecular Plant Pathology 10:263-275.

Belliure B, Janssen A, Maris PC, Peter D, Sabelis MW (2005) Herbivore arthropods benefit from vectoring plant viruses. Ecology Letters 8:70-79.

Belliure B, Janssen A, Sabelis MW (2008) Herbivore benefits from vectoring plant virus through reduction of period of vulnerability to predation. Oecologia 156:797-806.

Belliure B, Sabelis MW, Janssen A (2010) Vector and virus induce plant responses that benefit a non-vector herbivore. Basic and Applied Ecology 11:162-169.

Blua MJ, Perring TM, Madore MA (1994) Plant virus-induced changes in aphid population development and temporal fluctuations in plant nutrients. Journal of Chemical Ecology 20:691-707.

Broadway RM, Duffey SS, Pearce G, Ryan CA (1986) Plant proteinase-inhibitors - a defense against herbivorous insects. Entomologia Experimentalis Et Applicata 41:33-38.

Boquel S, Ameline A, Giordanengo P (2011a) Assessing aphids potato virus Y-transmission efficiency: A new approach. Journal of Virological Methods 178:63-67.

Boquel S, Giordanengo P, Ameline A (2011b) Divergent effects of PVY-infected potato plant on aphids. European Journal of Plant Pathology 129:507-510.

Cardoza YJ, Lait CG, Schmelz EA, Huang J, Tumlinson JH (2003) Fungus-induced biochemical changes in peanut plants and their effect on development of beet armyworm, *Spodoptera exigua* Hubner (Lepidoptera : Noctuidae) larvae. Environmental Entomology 32:220-228.

Castle SJ, Berger PH (1993) Rates of growth and increase of *Myzus persicae* on virus-infected potatoes according to type of virus-vector relationship. Entomologia Experimentalis et Applicata 69:51-60.

Colvin J *et al.* (2006) Host-plant viral infection effects on arthropod-vector population growth, development and behaviour: management and epidemiological implications. Advances in virus research 67:419-452.

Cooper WR, Goggin FL (2005) Effects of jasmonate-induced defenses in tomato on the potato aphid, *Macrosiphum euphorbiae*. Entomologia Experimentalis et Applicata 115:107-115.

Cui JP, Jander G, Racki LR, Kim PD, Pierce NE, Ausubel FM (2002) Signals involved in *Arabidopsis* resistance to *Trichoplusia ni* caterpillars induced by virulent and avirulent strains of the phytopathogen *Pseudomonas syringae*. Plant Physiology 129:551-564.

Cui J *et al.* (2005) *Pseudomonas syringa*e manipulates systemic plant defenses against pathogens and herbivores. Proceedings of the National Academy of Sciences of the United States of America 102:1791-1796.

De Vos M *et al.* (2005) Signal signature and transcriptome changes of Arabidopsis during pathogen and insect attack. Molecular Plant-Microbe interactions 18:923-937.

Delaney TP (1994) A central role of salicylic-acid in plant-disease resistance. Science 266:1793-1793.

Eigenbrode SD, Ding H, Shiel P, Berger PH (2002) Volatiles from potato plants infected with *Potato leafroll virus* attract and arrest the virus vector, *Myzus persicae* (Homoptera; Aphididae). Proceedings of the National Academy of Sciences of the United States of America 269:455-460.

Farmer EE, Johnson RR, Ryan CA (1992) Regulation of expression of proteinase-inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiology 98:995-1002.

Felton GW *et al.* (1999) Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. Current Biology 9:317-320.

Fidantsef AL, Stout MJ, Thaler JS, Duffey SS, Bostock RM (1999) Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. Physiological and Molecular Plant Pathology 54:97-114.

Goggin FL, Williamson VM, Ullman DE (2001) Variability in the Response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera: Aphididae) to the Tomato Resistance Gene *Mi*. Environmental Entomology 30:101-106.

Hare JD, Dodds JA (1987) Survival of the Colorado potato beetle on virus-Infected tomato in relation to plant nitrogen and alkaloid content. Entomologia Experimentalis et Applicata 44:31-35.

Harrington R, Gibson RW (1989) Transmission of *Potato virus Y* by aphids trapped in potato crops in southern England. Potato Research 32:167-174.

Heidel AJ, Baldwin IT (2004) Microarray analysis of salicylic acid and jasmonic acid-signalling in responses of *Nicotiana attenuate* to attack by insects from multiple feeding guilds. Plant, Cell and Environmental Entomology 27:1362–1373.

Herbers K, Takahata Y, Melzer M, Mock H-P, Hajirezaei M, Sonnewald U (2000) Regulation of carbohydrate partitioning during the interaction of *Potato virus Y* with tobacco. Molecular Plant Pathology 1:51-59.

Hodge S, Powell G (2008) Do plant viruses facilitate their aphid vectors by inducing symptoms that alter behavior and performance? Environmental Entomology 37:1573-1581.

Hodge S, Powell G (2010) Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant pathogen, *Pea enation mosaic virus*. Journal of Insect Science 10:155.

Inbar M, Doostdar H, Gerling D, Mayer RT (2001) Induction of systemic acquired resistance in cotton by BTH has a negligible effect on phytophagous insects. Entomologia Experimentalis et Applicata 99:65-70.

Jensen DD (1959) A plant virus lethal to its insect vector. Virology 8:164-175.

Jiu M, Zhou XP, Tong L, Xu J, Yang X, Wan FH, Liu SS (2007) Vector-virus mutualism accelerates population increase of an invasive whitefly. Plos One 1:e182.

Kennedy JS (1951) Benefits to aphids from feeding on galled and virus-infected leaves. Nature 168:825.

Kluth S, Kruess A, Tscharntke T (2002) Insects as vectors of plant pathogens: mutualistic and antagonistic interactions. Oecologia 133:193-199.

Kogovšek P, Pompe-Novak M, Baebler Š, Rotter A, Gow L, Gruden K, Foster GD, Boonham N, Ravnikar M (2010) Aggressive and mild *Potato virus Y* isolates trigger different specific responses in susceptible potato plants. Plant Pathology 59:1121-1132.

Kovač M, Müller A, Milovanovič Jarh D, Milavec M, Düchting P, Ravnikar M (2009) Multiple hormone analysis indicates involvement of jasmonate signalling in the

early defence of potato to *Potato virus* Y^{NTN}. Biologia Plantarum 53:195-199.

Krečič -Stres H, Vucak C, Ravnikar M, Kovač M (2005) Systemic *Potato virus* Y^{NTN} infection and levels of salicylic and gentisic acids in different potato genotypes. Plant Pathology 54:441-447.

Kusajima M, Yasuda M, Kawashima A Nojiri H, Yamane H, Nakajima M, Akutsu K, Nakashita H (2010) Suppressive effect of abscisic acid on systemic acquired resistance in tobacco plants. Journal of General Plant Pathology 76:161-167.

Lennon AM, Neuenschwander UH, Ribas-Carbo M, Giles L, Ryals JA, Siedow JN (1997) The effects of salicylic acid and tobacco mosaic virus infection on the alternative oxidase of tobacco. Plant Physiology 115:783-791.

Leon-Reyes A, Van der Does D, De Lange ES, Delker C, Wasternack C, Van Wees SCM, Ritsema T, Pieterse CMJ (2010) Salicylate-mediated suppression of jasmonate-responsive gene expression in *Arabidopsis* is targeted downstream of the jasmonate biosynthesis pathway. Planta 232:1423–1432.

Mauch-Mani B, Mauch F (2005) The role of abscisic acid in plant-pathogen interactions. Current Opinion in Plant Biology 8:409-414.

Mauck KE, De Moraes CM, Mescher MC (2010a) Deceptive chemical signals induced by a plant virus attract insect vectors to inferior hosts. Proceedings of the National Academy of Sciences of the United States of America 107:3600-3605.

Mauck KE, De Moraes CM, Mescher MC (2010b) Effects of Cucumber mosaic virus infection on vector and non-vector herbivores of squash. Communicative and Integrative Biology 3:579-582.

Mauck KE, Bosque-Pérez NA, Eigenbrode SD, De Moraes CM, Mescher MC (2012)

Transmission mechanisms shape pathogen effects on host–vector interactions: evidence from plant viruses. Functional Ecology 26:1162-1175.

Mayer RT *et al.* (2002) Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. Archives of Insect Biochemistry and Physiology 51:151-169.

Mehle N, Kovač M, Petrovič N, Novak, MP, Baebler Š, Stres HK, Gruden K, Ravnikar M (2004) Spread of potato virus Y^{NTN} in potato cultivars (*Solanum tuberosum* L.) with different levels of sensitivity. Physiological and Molecular Plant Pathology 64:293–300.

Mello AFS, Olarte RA, Gray, SM, Perry, KL (2011) Transmission efficiency of Potato virus Y strains PVY^{O} and $PVY^{N\text{-}Wi}$ by five aphid species. Plant Disease 95: 1279–1283.

Mowry TM, Ophus JD (2006) Influence of the *Potato leafroll virus* and virus infected plants on the arrestment of the aphid, *Myzus persicae.* Journal of Insect Science 6:22.

Muller CB, Williams IS, Hardie J (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology 26:330–340.

Nanayakkara UN, Nie X, Giguère M, Zhang J, Boquel S, Pelletier Y (2012) Aphid feeding behavior in relation to Potato virus Y (PVY) acquisition. Journal of Economic Entomology 105:1903-1908.

Nault LR (1997) Arthropod transmission of plant viruses: a new synthesis. Annals of the Entomological Society of America 90:521-541.

Ng JCK, Perry KL (2004) Transmission of plant viruses by aphid vectors. Molecular Plant Pathology 5:505–511.

Ng JCK, Falk BW (2006) Virus-vector interactions mediating nonpersistent and semipersistent transmission of plant viruses. Annual Review of Phytopathology 44:193-212.

Ohgushi T (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. Annual Review of Ecology Evolution and Systematics 36:81-105.

Preston CA, Lewandowski C, Enyedi AJ, Baldwin IT (1999) *Tobacco mosaic virus* inoculation inhibits wound-induced jasmonic acid-mediated responses within but not between plants. Planta 209:87-95.

Ryals J, Uknes S, Ward E (1994) Systemic acquired-resistance. Plant Physiology 104:1109-1112.

Raubenheimer D, Simpson SJ (1992) Analysis of covariance – an alternative to nutritional indexes. Entomologia Experimentalis et Applicata 62:221-231.

Sano H, Ohashi Y (1995) Involvement of small gtp-binding proteins in defense signal-transduction pathways of higher-plants. Proceedings of the National Academy of Sciences of the United States of America 92:4138-4144.

Scholthof KG *et al.* (2011) Top 10 plant viruses in molecular plant pathology. Molecular Plant Pathology. 12:938-954.

Shalitin D, Wolf S (2000) *Cucumber mosaic virus* infection affects sugar transport in melon plants. Plant Physiology 123:597-604.

Srinivasan R, Alvarez JM (2007). Effect of mixed viral infections (*Potato virus Y–Potato leafroll virus*) on biology and preference of vectors *Myzus persicae* and *Macrosiphum euphorbiae* (Hemiptera: Aphididae). Journal of Economic Entomology 100: 646–655.

Singh RP *et al.* (2008) Discussion paper: The naming of *Potato virus Y* strains infecting potato. Archives of Virology 153:1-13.

Stout MJ, Thaler JS, Thomma B (2006) Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. Annual Review of Entomology 51:663-689.

Sylvester ES (1980) Circulative and propagative virus transmission by aphids. Annual Review of Entomology 25:257-286.

Thaler JS, Fidantsef AL, Duffey SS, Bostock RM (1999) Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. Journal of Chemical Ecology 25:1597-1609.

Thaler JS, Agrawal AA, Halitschke R (2010) Salicylate-mediated interactions between pathogens and herbivores. Ecology 91:1075-1082.

Thaler JS, Bostock RM (2004) Interactions between abscisic-acid-mediated responses and plant resistance to pathogens and insects. Ecology 85:48-58.

Thaler JS, Fidantsef AL, Bostock RM (2002) Antagonism between jasmonate- and salicylate-mediated induced plant resistance: Effects of concentration and timing of elicitation on defense-related proteins, herbivore, and pathogen performance in tomato. Journal of Chemical Ecology 28:1131–1159.

Thaler JS, McArt SH, Kaplan I (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. Proceeedings of the National Academy of Sciences of the United States of America 109:12075-12080.

Thaler JS, Owen B, Higgins VJ (2004) The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiology 135:530-538.

Ton J, Flors V, Mauch-Mani B (2009) The multifaceted role of ABA in disease resistance. Trends in Plant Science 14:310-317.

Underwood N, Halpern S, Klein C (2011) Effect of host-plant genotype and neighboring plants on strawberry aphid movement in the greenhouse and field. American Midland Naturalist 165: 38–49.

Utsumi S (2011) Eco-evolutionary dynamics in herbivorous insect communities mediated by induced plant responses. Population Ecology 53:23-34.

Verbeek M, Piron PGM, Dullemans AM, Cuperus C, van der Vlugt RAA (2009) Determination of aphid transmission efficiencies for N, NTN and Wilga strains of *Potato virus Y*. Annals of Applied Biology 156:39-49.

Walling LL (2000) The myriad plant responses to herbivores. Journal of Plant Growth Regulation 19:195-216.

Willits MG, Ryals JA (1998) Determining the relationship between salicylic acid levels and systemic acquired resistance induction in tobacco. Molecular Plant Microbe interactions 11:795-800.

Yang JY, Iwasaki M, Machida C, Machida Y, Zhou XP, Chua NH (2008) beta C1, the

pathogenicity factor of TYLCCNV, interacts with AS1 to alter leaf development and suppress selective jasmonic acid responses. Genes Development 22:2564–2577.

Zarate SI, Kempema LA, Walling LL (2007) Silverleaf whitefly induces salicylic acid defenses and suppresses effectual jasmonic acid defenses. Plant Physiology 143:866-875.

Zhang P, Zheng SJ, van Loon JJA, Boland W, David A, Mumm R, Dicke M (2009) Whiteflies interfere with indirect plant defense against spider mites in Lima bean. Proceedings of the National Academy of Sciences of the United States of America 106: 21202-21207.

CHAPTER 2

PLANT RESISTANCE DETERMINES THE STRENGTH OF DENSITY-DEPENDENT PROCESSES IN APHID POPULATIONS

Abstract

Although density-dependent population growth has been well-studied, the mechanisms that generate the strength of density dependence are not well understood. In plant-herbivore systems, plant responses to herbivory can potentially strengthen density dependence because they can be incrementally induced as herbivore density increases. We investigated whether plant resistance affects the developmental time and fecundity of the aphid *Macrosiphum euphorbiae*. We also tested whether plant resistance levels influence aphid population dynamics and the strength of density dependence. Using genetically-modified tomato lines that vary in the expression of jasmonic acid, a phytohormone that mediates induced resistance, we assessed density dependence by density manipulation experiments. We found longer developmental time and lower aphid fecundity on high-resistance plants compared to low-resistance plants. Aphids feeding on low-resistance plants showed higher per capita growth rate which translated into higher aphid density after 15 days. In contrast, aphids showed 38% lower per capita population growth and 47% lower final densities on high-resistance plants. Aphid per capita population growth was strongly negatively density-dependent on low-resistance plants, still significant on plants with intermediate levels of resistance, and density-independent on high-resistant plants. Therefore, plant resistance can be an importance component in herbivore population dynamics by determining the strength of density dependence in aphid populations.

Introduction

Density-dependent population growth is fundamental in many ecological theories that attempt to explain the abundance and distribution of animal populations. Therefore, recognizing which factors can impose density-dependent population growth is crucial for both understanding of population dynamics and effective pest management (Hassell *et al*. 1989, Bonmarco *et al*. 2007). A negative feedback between density and per capita population growth rate is a necessary condition for the density-dependent regulation of populations (Harrison & Cappuccino 1995). This type of population growth is particular important for pest species, such as aphids (Myers *et al.* 2005, Alyokhin *et al*. 2005), as the short development time and clonal reproduction of aphids can lead to exponential population growth (Agrawal *et al.* 2004, Myers *et al.* 2005). Therefore, identifying density-dependent processes will aid in understanding why exponential growth may not occur. Although density dependence has been well-studied, the mechanisms that generate the strength of density dependence are not well understood.

By influencing the preference and performance of aphids and other herbivores, it is suggested that plant quality has potential to regulate herbivore population dynamics (Haukioja & Hakala 1975, Ylioja *et al.* 1999, Underwood & Rausher 2000, Helms & Hunter 2005). Induced plant defenses are plastic traits and are modified by insect feeding (Forrest 1971, Sandstrom *et al*. 2000, Walling 2000, Walling 2008),may reduce herbivore preference or performance, and depend on the density of attacking herbivores (Karban & Baldwin 1997). Feedbacks between herbivore density and plant

quality make harder to predict population dynamics, thus induced defenses have the potential to affect the strength of density dependence on inducible plants.

Induced resistance, for example, can intensify the density dependence of herbivores and thus control insect populations through a negative feedback (Underwood 1999, Underwood & Rausher 2002). Conversely, it has been suggested that constitutive resistance, which is the level of resistance an undamaged plant has, cannot limit herbivore population growth in a density-dependent manner and thus does not have the potential to regulate herbivore populations (Rhoades 1985, Hunter *et al*. 2000). Constitutive resistance, however, might be expected to affect other population attributes. It is predicted that higher levels of constitutive resistance might slow population growth or promote lower equilibrium population sizes (Hunter *et al.* 2000, Underwood & Rausher 2002). Although the impact of induced and constitutive resistance has been hypothesized to differently influence herbivore populations, few studies have attempted to experimentally test these predictions (Underwood & Rausher 2002).

It is broadly recognized that plants change their phenotype upon herbivore damage (Karban & Baldwin 1997). Aphid feeding, for example, triggers the induction of jasmonic acid (hereafter JA) and salicylic acid (hereafter SA) pathways; both of which can negatively affect aphids (Inbar *et al.* 1998, Thaler *et al.* 1999, Walling 2000, 2008). Therefore, we predict that induced plant responses might be affected by the number of aphids feeding on the plant, and thus it can potentially determine aphid density-dependent processes. Short-term density-manipulation experiments have been successfully employed to study density-dependent processes in different systems

(Underwood & Rauscher 2002, Rotem & Agrawal 2003, Agrawal 2004, Agrawal *et al.* 2004, Helms & Hunter 2005, Underwood 2010). Here we propose to understand the mechanisms by which induced plant resistance contributes to herbivore density-dependent population growth. By manipulating plant quality and herbivore density on plants we can evaluate how host plants respond to herbivore feeding and how these responses affect density-dependent population growth of herbivores.

We manipulated plant resistance using genetically-modified tomato lines that vary in the expression of jasmonic acid (JA) pathway, a phytohormone that mediates resistance to insects, and differ in their jasmonate inducibility: (i) Low resistance – a mutant tomato line (cv. *Jai-1;* Li *et al.* 2002) that does not perceive JA and hence does not induce the pathway, (ii) Intermediate resistance – wild-type tomato (cv. *Castlemart*), which can induce JA defenses upon damage, and (iii) High resistance – a transgenic line that overexpresses a component in the JA pathway (cv. *Prosystemin;* McGurl *et al.* 1994) and therefore has constitutively high levels of JA-dependent defenses. We investigated the effects of plant resistance on the aphid, *Macrosiphum euphorbiae*, performance in terms of fecundity, development time and density, and tested whether plant resistance levels influence aphid population growth rate and whether they affected the strength of density-dependence process at different aphid densities.

Methods

Study Organisms

Tomato plants (*Solanum lycopersicum* L.) used in this study were germinated in the lab and transplanted to four-inch pots in a greenhouse and grown for four weeks (four-leaf stage). Plants were grown in commercial potting soil, watered daily, and received 85 grams of fertilizer per week (Jack's professional® water-soluble fertilizer 21:5:20 N:P:K). Individual aphids came from a laboratory colony of aphids, *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae) (WU-11-FR clone, Goggin *et al.* 2001) that were reared on tomato plants (cv. Castlemart) in growth chambers (22ºC, 16:8, L:D photoperiod).

Effects of the Plant Resistance on Aphid Performance

To test whether plant resistance affects aphid performance, we measured aphid fecundity and developmental time on the different levels of plant resistance (low, intermediate and high). To measure aphid fecundity responses to plant resistance, we enclosed a single 4th instar aphid on the third leaf of each plant with a translucent spun polyester sleeve. We checked aphids every 5 days to remove and count the nymphs produced until aphid death. We carried out 3 trials in a greenhouse, totaling 28 replicates for low-resistance plants, 26 for intermediate-resistance, and 28 for high-resistance plants. To evaluate the effect of plant resistance on aphid developmental time, we enclosed a single one-day-old aphid on the third leaf of each plant with a translucent spun polyester sleeve. We checked the aphid daily until it produced its first nymph, which we considered as the aphid reaching the adult stage. Therefore, we measured aphid developmental time as the number of days taken to produce the first nymph. We carried out 2 trials in a greenhouse for a total of 11 replicates for low-

resistance plants, 9 for intermediate-resistance, and 7 for high-resistance plants. We performed one-way analyses of variance (ANOVA) to test for the effect of plant resistance on the number of nymphs produced by an aphid and aphid developmental time. For both ANOVAs, we included trial as a blocking factor. When plant resistance was significant we applied Tukey's post-hoc test to differentiate between the three plant resistance lines. The number of nymphs produced by an aphid was not normally distributed, therefore we square-root transformed the data to meet parametric assumptions; homogeneity of variance was met after transformation.

Effects of Plant Resistance on Aphid Density-Dependent Processes

To evaluate whether plant resistance drives aphid density-dependent processes, we manipulated initial aphid density and plant resistance. Initial aphid density ranged from 1 to 130 aphids per plant. We estimated aphid population growth by calculating the daily per capita growth rate of aphids (dN / Ndt) as $(\ln[N_2] - \ln[N_1]) / (t_2 - t_1)$, where N_2 and N_1 are the final and initial aphid densities, respectively, divided by the number of days elapsed between initial (t_1) and final (t_2) counting (ca. 15 days) (Agrawal *et al.* 2004, Vandermeer 2010). We carried out 4 trials in a greenhouse and the total number of replicates per treatment was: low-resistance = 60, intermediate-resistance = 69 and high-resistance = 66. Visual inspection of the data suggests linearity, therefore we used general linear models to analyze the effect of plant resistance and initial aphid density (ln-transformed) and their interaction on the aphid per capita growth rate and the final aphid density (ln-transformed). Density-dependent regulation in populations manifests itself as a relationship between per capita growth rate and initial density (Harrison &

Cappuccino 1995). A significant interaction between plant resistance level and initial aphid density indicates that the plant resistance modulates the strength of the density dependent population growth.

Results

Effects of Plant Resistance on Aphid Performance

Aphid developmental time was slower on high-resistance plants compared to low and intermediate-resistance plants ($F_{2,24}$ =3.74, P=0.039; Table 2.1; Figure 2.1a). Aphids feeding on high-resistance plants took 13 days (± 0.7) to mature, whereas aphids feeding on plants with intermediate- and low-resistance levels matured after 11 days (± 0.6). Aphids showed higher fecundity when feeding on low-resistance plants, producing 90% more nymphs compared to intermediate-resistance and 26% more compared to high-resistance plants ($F_{2,127}$ =3.14, P=0.047; Table 2.1; Figure 2.1b).

Effects of Plant Resistance on Aphid Density-Dependent Processes

We found a significant interaction between plant resistance level and initial aphid density on aphid per capita growth rate, which indicates that the strength of density-dependent population growth is affected by plant resistance ($F_{2,186}$=9.05, P<0.001; Table 2.1; Figure 2.2A). On low and intermediate-resistance plants, aphid population was negatively related with initial density, indicating density-dependent population growth ($F_{1,55}$=13.01, β=-0.02 P=0.001; $F_{1,64}$=12.23, β=-0.017, P=0.001, respectively). In contrast, aphids showed density-independent population growth on

Table 2.1: ANOVAs of development time (no. of days to first nymph), number of nymphs produced, final number of aphids and per capita population growth rate. Treatments are plant resistance (low, intermediate, high) and aphid initial density. Number of aphids and initial density were ln-transformed and development time was sqr-transformed. F-values and degrees of freedom (df) are shown.

Source of Variation	Development time	Fecundity	No. of aphids	Per capita growth rate
Trial	$0.021_{(1,24)}$	$1.676_{(2,127)}$	$44.098_{(2,186)}$***	$65.748_{(3,186)}$***
Plant resistance	$3.741*_{(2,24)}$	$3.140*_{(2,127)}$	$18.932_{(2,186)}$***	$18.114_{(2,186)}$***
Aphid Initial Density			$359.864_{(1,186)}$***	$11.379_{(1,186)}$***
Aphid Initial Density*Plant resistance			$7.922_{(2,186)}$***	$9.050_{(2,186)}$***

*P < 0.05, ***P < 0.0

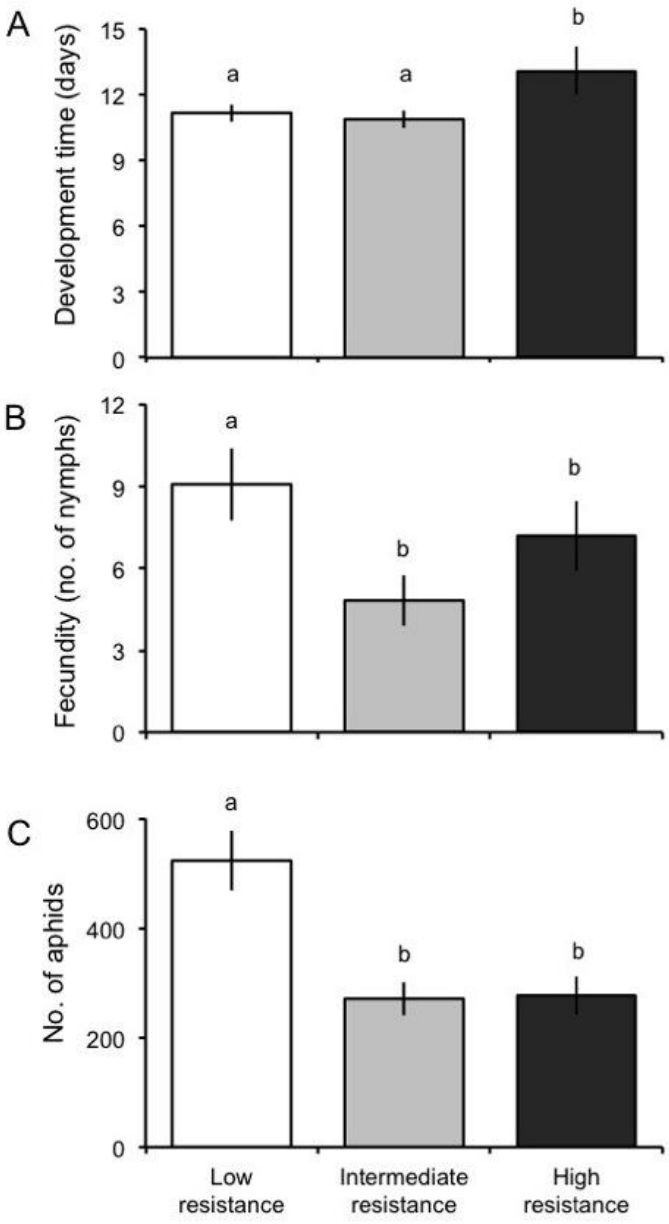

Figure 2.1: Aphid performance in response to tomato plants with varying levels of resistance. A) Aphid development time, measured as the number of days to production of first nymph. B) Aphid fecundity, measured as the number of nymphs over aphid lifetime. C) Final aphid density per cage after 15 days. Plant resistance treatments are: low resistance (unfilled bars), intermediate resistance (gray bars) and high resistance (black bars). Letters above bars indicate significant differences at $P < 0.05$ following Tukey's post-hoc test. Shown are means (±1SE).

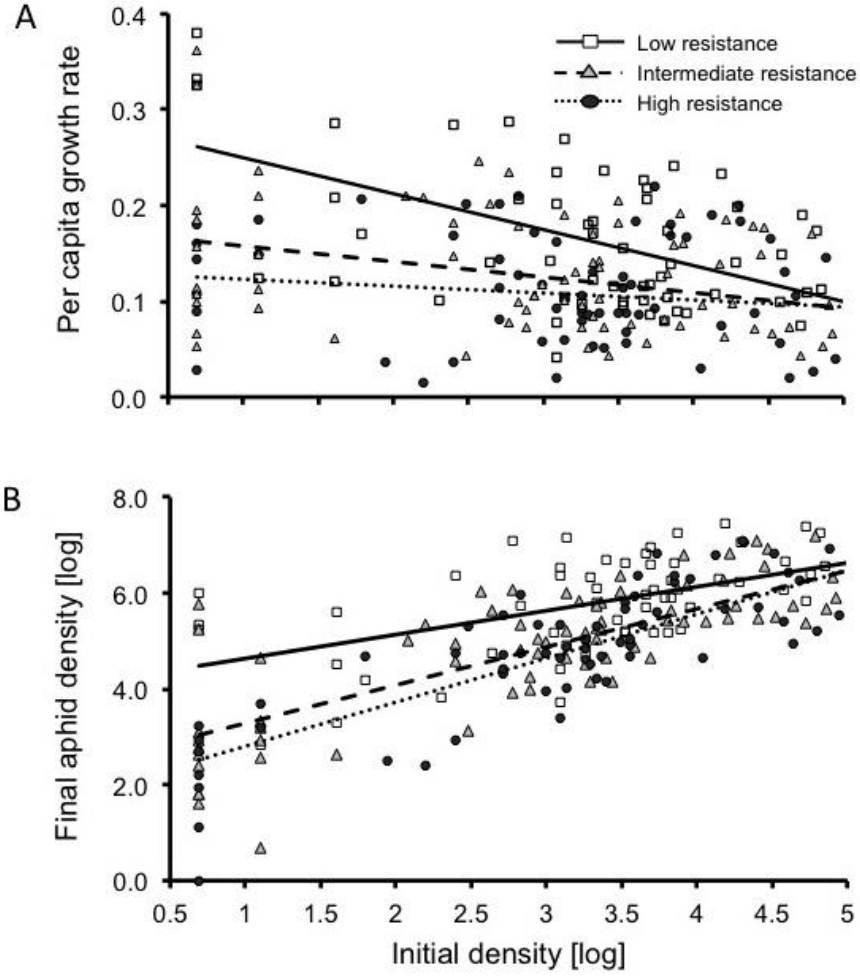

Figure 2.2: Effects of plant resistance and aphid density manipulation on per capita growth rates and final density. A) The strength of density dependence in the three plant resistance levels. B) Aphid final density in response to variation in plant resistance and aphid initial density. Plant resistance treatments are: low-resistance (unfilled squares, solid line), intermediate-resistance (gray triangles, dashed line) and high-resistance (black circles, dotted line).

high-resistance plants, as there was no relationship between per capita population growth and aphid initial density ($F_{1,61}$=2.05, β=0.007 P=0.158).

Aphid density increased 576% during the experiment, and plant resistance and aphid initial density influenced considerably to this increase (plant resistance: $F_{2,186}$=18.93, P<0.001; initial density: $F_{1,186}$=359.86, P<0.001; Table 2.1; Figure 2.2b). At lower initial densities, the final number of aphids was higher on low-resistance plants compared to high-resistance plants. At higher densities, all plant resistance levels converged to similar densities (plant resistance-by-initial density: $F_{2,186}$=7.92, P<0.001; Figure 2.2b), which corroborates our findings that per capita population growth at higher densities was similar among all plant lines.

Discussion

Using a density-manipulation experiment we simulated aphid population growth under high and low population density, which allowed us to assess the role of plant resistance on herbivore population dynamics and discuss the potential mechanisms involved in density-dependent population growth. We demonstrated that plant defenses can provide resistance against aphids and can drive density-dependent processes. Aphids took longer to grow, produced fewer nymphs, and consequently showed lower densities when feeding on high-resistance plants compared to low-resistance plants (Figure 2.1 and Figure 2.2B). In addition, increased plant resistance dampened aphid population growth. Aphids feeding on low and intermediate-resistance plants showed high population densities and displayed negative density-dependent population growth, while high-resistance plants dampened aphid population

densities and caused density-independent population growth. These results highlight the critical role of plant constitutive and inducible defenses in affecting the strength of density-dependent processes in herbivore populations, as we demonstrated that plants with low constitutive levels of defense (low-resistance) and inducible plants (intermediate-resistance) showed density-dependent population growth, in contrast high levels of constitutive defenses (high-resistance) showed density independent growth.

Previous studies have highlighted the importance of host plant quality on density dependence processes (Hunter *et al.* 2000, Underwood & Rausher 2000, 2002, Krebs 2002, Rotem & Agrawal 2003, Agrawal *et al.* 2004, Johnson 2008). By changing birth, death, emigration and immigration rates, plants will influence density dependence processes and thus population equilibrium. Density-dependent effects of variation in plant quality on insect populations of herbivores can occur through the limitation of food resources (Denno *et al.* 1995) and through the negative response associated with induced plant defenses (Underwood 1999). For example, spider mite (*Tetranychus urticae*) populations showed strong negative density-dependent growth on high-quality host plants (*Leonurus cardiaca*), whereas on low-quality host plants spider mite population grew in a density-independent manner and this result was attributed to potential differences in plant induced responses (Rotem & Agrawal 2003). In addition, the individual growth of herbivores (*Spodoptera exigua*) feeding on tomato plants was shown to be a function of initial density, which highlights the distinct potential effects of plant induced responses to population dynamics of herbivores (Underwood 2010). Using a density-manipulation experiment, Underwood

& Rausher (2002) demonstrated that induced resistance caused lower population growth and stronger density dependence on Mexican bean beetle populations compared to soybean varieties with no resistance.

Although plants have the potential to drive population dynamics of herbivores, few studies have explored which host plant traits have the potential to determine the strength of herbivore density dependence (Underwood & Rausher 2002, Agrawal 2004). High-resistance plants overexpress the jasmonic acid (JA) pathway, thus have high JA-dependent constitutive levels of resistance (McGurl *et al.* 1994). It has been hypothesized that high constitutive levels of resistance should not impose density-dependence (Rhoades 1985, Underwood 1999, Hunter *et al.* 2000), although there is some empirical evidence that constitutive resistance might affect herbivore density-dependent processes (Underwood & Rausher 2002). In theory, the level of induced resistance expressed by plants with high levels of constitutive resistance should be lower than those expressed by plants with low-levels of constitutive resistance (Karban & Baldwin 1997). This means that herbivores are always maximally suppressed on highly resistant plants and the negative feedback of herbivore density should be low or non-existent. Our results corroborate theory and showed that high constitutive levels of resistance caused low population growth regardless the initial density of aphids, and thus did not cause negative density dependence. Plants with higher levels of resistance are expected to impose their negative effects on herbivores at all times, thus not increasing resistance as densities rise. By lowering aphid fecundity and performance, high-resistance plants do not impose density-dependent growth and therefore maintain aphid populations at lower and stable level densities,

reducing overall damage to plants. Consequently, it is likely that jasmonate-linked defenses are involved in suppressing aphid population growth in our high-resistance plants.

In contrast, low constitutive levels of defense are mechanisms of density-dependent population growth of herbivores. For aphids on low-resistance plants, which did not induce the JA-dependent defenses and therefore showed low-constitutive defenses, sources other than the JA resistance may be playing a role influencing density dependence. Density-dependent effects on these plants may be intensified in response to depletion of edible resources (Denno *et al.* 1995) or induction of plant defenses (Underwood 1999). Because low-resistance plants do not induce JA-related responses it other defense pathways, such as the salicylic acid (SA) pathway may be induced. SA negatively affects aphids (Walling 2002) and may be induced more strongly on low-resistance plants because of signaling crosstalk between JA and SA pathways (Thaler *et al.* 2012) that occurs on plants with functional JA pathways. Low-resistance plants induce higher amounts of SA responses compared to high-resistance plants (chapter 4). In addition, on intermediate-resistance plants aphid population showed density-dependent growth, which might be a response to the induced resistance expressed by these plants. Regardless of the specific mechanism involved, here we showed that variation in plant resistance influences not only herbivore performance, but is a key factor driving herbivore population dynamics processes.

Acknowledgements

This study strongly benefited from comments and suggestions by Anurag Agrawal, Alison Power, Suzi Claflin, Chris Stieha and Gui Becker. This project received financial support from National Research Initiative of the USDA Grant 2006-35302-17431, the Department of Ecology and Evolutionary Biology, and the Department of Entomology at Cornell. MFKB was supported the CAPES/Fulbright fellowship (BEX 2234-08-4). Appropriate permits were obtained for the use of transgenic plants.

REFERENCES

Abbott, K.C., Morris, W.F. & Gross, K. (2008) Simultaneous effects of food limitation and inducible resistance on herbivore population dynamics. *Theoretical Population Biology*, **73**, 63–78.

Agrawal, A.A. (2004) Plant Defense and Density Dependence in the Population Growth of Herbivores. *The American Naturalist*, **164**, 113–120.

Agrawal, A.A., Underwood, N. & Stinchcombe, J.R. (2004) Intraspecific variation in the strength of density dependence in aphid populations. *Ecological Entomology*, **29**, 521–526.

Alyokhin, A., Drummond, F. a. & Sewell, G. (2005) Density-dependent regulation in populations of potato-colonizing aphids. *Population Ecology*, **47**, 257–266.

Bommarco, R., Wetterlind, S. & Sigvald, R. (2007) Cereal aphid populations in non-crop habitats show strong density dependence. *Journal of Applied Ecology*, **44**, 1013–1022.

Cappuccino, N. (1987) Comparative population dynamics of two goldenrod aphids: spatial patterns and temporal constancy. *Ecology*, **68**, 1634–1646.

Creel, S., Christianson, D.A. & Winnie, J.A. (2011) A survey of the effects of wolf predation risk on pregnancy rates and calf recruitment in elk. *Ecological Applications*, **21**, 2847–2853.

Forrest, J.M.S. (1971) The growth of *Aphis fabae* as an indicator of the nutritional advantage of galling to the apple aphid *Dysaphis devecta*. *Entomologia Experimentalis et Applicata*, **14**, 477–483.

Goggin, F.L. (2007) Plant-aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology*, **10**, 399–408.

Harrison, S.P. & Cappuccino, N. (1995) Using density manipulation experiments to study population regulation. *Population dynamics: new approaches and synthesis* (eds N. Cappuccino & P.W. Price), pp. 131–147. Academic press, San Diego.

Hassell, B.Y.M.P., Latto, J. & May, R.M. (1989) Seeing the wood for the trees: detecting density dependence from existing life-tables studies. *Journal of Animal Ecology*, **58**, 883–892.

Haukioja, E. & Hakala, T. (1975) Herbivore cycles and periodic outbreaks. Formation of a general hypothesis. *Reports of the Kevo Subartic Research Station*, **12**, 1–9.

Helms, S.E. & Hunter, M.D. (2005) Variation in plant quality and the population dynamics of herbivores: there is nothing average about aphids. *Oecologia*, **145**, 197–204.

Hunter, A.F. & Elkinton, J.S. (1999) Interaction between phenology and density effects on mortality from natural enemies. *Journal of Animal Ecology*, **68**, 1093–1100.

Inbar, M., Doostdar, H., Sonoda, R.M., Leibee, G.L., Mayer, R.T. & Ave, E.C. (1998) Elicitors of plant defensive systems reduce insect densities and disease incidence. *Journal of Chemical Ecology*, **24**, 135–149.

Karban, R. & Bladwin, I.T. (1997) *Induced Responses to Herbivory*. University of Chicago Press, Chicago.

Krebs, C.J. (2002) Two complementary paradigms for analysing population dynamics. *Philosophical transactions of the royal society of London . Series B - Biological Siences*, **357**, 1211–1219.

Li, L., Li, C., Lee, G.I. & Howe, G. a. (2002) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 6416–21.

McGurl, B., Orozco-Cardenas, M., Pearce, G. & Ryan, C. a. (1994) Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9799–802.

Myers, S.W., Gratton, C., Wolkowski, R.P., Hogg, D.B. & Wedberg, J.L. (2005) Effect of soil potassium availability on soybean aphid (Hemiptera: Aphididae) population dynamics and soybean yield. *Journal of Economic Entomology*, **98**, 113–20.

Nevo, E. & Coll, M. (2001) Effect of Nitrogen Fertilization on *Aphis gossypii* (Homoptera: Aphididae): Variation in Size, Color, and Reproduction. *Journal of Economic Entomology*, **94**, 27–32.

Rhoades, D.F. (1985) Offensive-defensive interactions between herbivores and plants:their relevance in herbivore population dynamics and ecological theory. *The American Naturalist*, **125**, 205–238.

Rotem, K.A. & Agrawal, A.A. (2003) Density dependent population growth of the two-spotted spider mite , *Tetranychus urticae* , on the host plant *Leonurus cardiaca*. *Oikos*, **3**, 559–565.

Sandström, J., Telang, a & Moran, N.A. (2000) Nutritional enhancement of host plants by aphids - a comparison of three aphid species on grasses. *Journal of insect Physiology*, **46**, 33–40.

Thaler, J.S., Agrawal, A. a & Halitschke, R. (2010) Salicylate-mediated interactions between pathogens and herbivores. *Ecology*, **91**, 1075–82.

Thaler, J.S., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1999) Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597–1609.

Underwood, N. (1999) The influence of plant and herbivore characteristics on the interaction between induced resistance and herbivore population dynamics. *The American Naturalist*, **153**, 282–294.

Underwood, N. (2000) Density dependence in induced plant resistance to herbivore damage: threshold, strength and genetic variation. *Oikos*, **89**, 295–300.

Underwood, N. (2007) Variation in and correlation between intrinsic rate of increase and carrying capacity. *The American Naturalist*, **169**, 136–141.

Underwood, N. (2009) Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. *Journal of Animal Ecology*, **78**, 839–847.

Underwood, N. (2010) Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. *Oikos*, **119**, 1993–1999.

Underwood, N. & Rausher, M.D. (2000) The effects of host-plant genotype on herbivore population dynamics. *Ecology*, **81**, 1565–1576.

Underwood, N. & Rausher, M. (2002) Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. *The American Naturalist*, **160**, 20–30.

Vandermeer, J. (2010) How populations grow: the exponential and logistic equations. *Nature Education Knowledge*, **3**, 15.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth and Regulation*, **19**, 195–216.

Walling, L.L. (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859–66.

Walters, D.R. & Fountaine, J.M. (2009) Practical application of induced resistance to plant diseases: an appraisal of effectiveness under field conditions. *The Journal of Agricultural Science*, **147**, 523.

Ylioja, T., Roininen, H., Ayres, M.P., Rousi, M. & Price, P.W. (1999) Host-driven population dynamics in an herbivorous insect. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 10735–40.

CHAPTER 3

PLANT RESISTANCE REDUCES THE STRENGTH OF CONSUMPTIVE AND NON-CONSUMPTIVE EFFECTS OF PREDATORS ON APHIDS

This version is currently in revision (accepted/major revisions) in the Journal of Animal Ecology as: Mônica F. Kersch-Becker and Jennifer S. Thaler. 2014. Plant Resistance Reduces the Strength of Consumptive and Non-consumptive Effects of Predators on Aphids.

Abstract

1. The impact of predators on prey has traditionally been attributed to the act of consumption. Prey responses to the presence of the predator (non-consumptive effects), however, can be as important as predation itself. While plant defenses are known to influence predator-prey interactions, their relative effects on consumptive versus non-consumptive effects are not well understood.
2. We evaluated the consequences of plant resistance and predators (*Hippodamia convergens)* on the mass, nymph production, population growth, density, and dispersal of aphids (*Macrosiphum euphorbiae*). We tested for the effects of plant resistance on non-consumptive and consumptive effects of predators on aphid performance and dispersal using a combination of path analysis and experimental manipulation of predation risk.
3. We manipulated plant resistance using genetically modified lines of tomato (*Solanum lycopersicum*) that vary incrementally in the expression of the jasmonate pathway, which mediates induced resistance to insects and manipulated aphid exposure to lethal and risk predators. Predation risk predators had mandibles impared to prevent killing.
4. Plant resistance reduced predation rate (consumptive effect) on high-resistance plants. As a consequence, predators had no impact on aphid density and population growth on high-resistance plants, whereas on low-resistance plants predators reduced aphid density by 35% and population growth by 86%. The results from the path analysis and direct manipulation of predation risk showed that predation risk rather than predation rate promoted aphid dispersal. Aphid

dispersal in response to predation risk was greater on low- compared to high-resistance plants. The predation risk experiment also showed that aphid numberof nymphs increased in the presence of risk predators.

5. In conclusion, predation risk accounts for most of the total effect of the predator on aphid dispersal and number of nymphs produced. Overall, there are stronger consumptive and non-consumptive effects of the predator on low-resistance plants.

Introduction

The impacts of predators on their prey arise not only through consumption, but also through non-consumptive effects (*i.e.*, perception of risk, "fear" or "intimidation") (Lima and Dill 1990; Peacor & Werner 2001; Preisser, Bolnick & Bernard 2005; McCauley, Rowe & Fortin 2011). Such non-consumptive effects occur when the predator's presence alone impacts the phenotype of the surviving prey, including effects on prey's behavior, physiology, development, and reproduction (Lima & Dill 1990; Peacor & Werner 2001; Preisser, Bolnick & Bernard 2005; Hawlena & Schmitz 2010; Kaplan & Thaler 2010; Thaler, McArt & Kaplan 2012). Remarkably, non-consumptive effects can be as important as consumptive effects, causing cascading changes in prey population dynamics, community structure and ecosystem functioning (Schmitz, Krivan & Ovadia 2004, Preisser, Bolnick & Bernard 2005; Schmitz, Hawlena & Trussel 2010; Finke 2011, Hawlena & Schmitz 2010).

The importance of plant traits for herbivore-predator interactions is well established (Price *et al.* 1980; Boethel & Eikenbary 1986; Forkner & Hunter 2000;

Poelman, van Loon & Dicke 2008). There is a lot of evidence for the importance of plant traits on predator consumption from agricultural systems, in which differential resistance traits among cultivars have been combined with biological control agents to reduce pest damage (Bottrell, Barbosa & Gould 1998; Hare 2002; Shannag & Obeidat 2008). It is often found that the combined effect of plant resistance and predation is more effective in reducing prey density than either strategy alone, suggesting a synergistic relationship between the two, although antagonisms also occur (Price *et al.* 1980; Hare 2002). Consumptive effect of predators (*i.e.* reduction in prey density), however, is only one component of the total effect of the predator (Lima & Dill 1990, Preisser, Bolnick & Bernard 2005). Despite the widespread recognition of the importance of non-consumptive effects, we still know relatively little about the impact of plant traits on the non-consumptive effects of predators on prey (Kaplan and Thaler 2010; 2012).

Both host plant quality and predators can influence the density, wing production, behavior, and performance of aphids (Dixon & Agarwala 1999; Müller, Williams & Hardie 2001; Aquilino, Cardinale & Ives 2005; Goggin 2007; Cabral, Soares & Garcia 2009; Kaplan & Thaler 2012). Dispersal is an important component of aphid population dynamics (Dixon 1998). Most aphid species exhibit a polyphenism whereby they can be either winged or wingless (Dixon 1998). Environmental conditions such as crowding, poor plant quality, or the presence of natural enemies can induce wing formation (Müller, Williams & Hardie 2001; Kunert & Weisser 2003). Although most aphids within a population are wingless and move by walking rather than flying, quantitative data on dispersal of wingless aphids is

limited (Schotzco & Smith 1991; Honek, *et al.* 1998; Underwood, Halpern & Klein 2011). Plant traits and predators can potentially influence the dispersal of wingless aphids (Underwood, Halpern & Klein 2011), resulting in a shift in the aphid population structure.

Here, we investigated the individual and interactive effects of plant resistance and the consumptive and non-consumptive effects of predators on aphid dispersal and performance. We manipulated plant resistance by using genetically modified lines of tomato (*Solanum lycopersicum*) that vary incrementally in the expression of the jasmonate pathway. Jasmonate-dependent induced responses are widely recognized to mediate resistance to a wide range of insects, including aphids (Walling 2000; Goggin, Williamson & Ullman 2001; Goggin 2007; Thaler *et al.* 2002). First, using manipulative experiments we determined (i) the influence of plant resistance on consumption of aphids by the ladybird beetle (*Hippodamia convergens)*, (ii) the consequences of plant resistance and predation on nymph production, population growth, density, and dispersal of aphids (*Macrosiphum euphorbiae)* and how plant resistance affects aphid mass. Second, we examined the impact of predation risk on aphids in two distinct ways to assess the importance of non-consumptive effects. We used path analysis to estimate the relative strength of the consumptive and non-consumptive effects of predators on aphid dispersal. This analysis also tested whether plant resistance directly or indirectly through changes in predation rate and aphid density, promotes aphid dispersal. We then performed an experiment explicitly manipulating predation risk to test the hypothesis that plant resistance influences the non-consumptive component of predation on aphid dispersal.

Methods

Study System and General Experimental Framework

We germinated tomato plants (*Solanum lycopersicum* L.) in the laboratory and transplanted them to four-inch pots in a greenhouse where we watered them daily and fertilized them weekly (21:5:20 N:P:K) for four weeks (four-leaf stage: 104 on BBCH scale). We maintained potato aphids, *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae) (WU-11-FR clone) (Goggin, Williamson & Ullman 2001) on tomato plants (cv. Castlemart) in growth chambers (22ºC, 16:8, L:D photoperiod). We used adults of the convergent ladybird beetle, *Hippodamia convergens* (Guérin-Méneville, 1842) (Coleoptera: Coccinellidae) (Rincon-Vitova Insectaries, Ventura California) as our predator. We did not separate males from females; however, ladybird beetles were randomly selected, which should control for potential differences in voracity and foraging between the sexes.

We used three tomato lines that vary in their expression of jasmonic acid: (i) Low resistance - a mutant tomato line (cv. *Jai-1;* Li *et al.* 2004) that does not react to jasmonic acid and does not express jasmonate-dependent defenses, (ii) Intermediate resistance - wild-type tomato (cv. *Castlemart*), which induces the jasmonate pathway upon herbivore feeding, and (iii) High resistance – a transgenic line that overexpresses the jasmonate pathway (cv. *Prosystemin;* McGurl *et al.* 1994) and therefore constitutively expresses jasmonate-dependent defenses. These three plant types differ in many traits regulated by the jasmonate pathway, including trichome density and secondary compounds, but have the similar growth rates (McGurl *et al.* 1994; Li *et al.*

2004; Kaplan & Thaler 2010). The jasmonate pathway confers resistance to potato aphids, reducing aphid abundance (Kaplan & Thaler 2012).

Using manipulative field and laboratory experiments we (1) determined the influence of plant resistance on aphid consumption by the ladybird beetle, and the consequences of plant resistance and predation on nymph production, population growth, density, and dispersal of aphids. (2) We also tested how plant resistance affects aphid mass. We then examined the impact of predation risk on aphids in two distinct ways. (3) We used path analysis to estimate the relative strength of the consumptive and non-consumptive effects of predators on aphid dispersal using data extracted from laboratory and field experiments. (4) Lastly, we manipulated predation risk by preventing aphid consumption by ladybird beetles to explicitly test the effects of predation risk on aphid performance and dispersal. Below we describe the specific methods and analyses addressing each question.

(1) Predation Rate, Aphid Performance and Dispersal in Response to Variation in Plant Resistance and Predator Exposure

To determine whether plant resistance and predators affect aphid performance and behavior, we manipulated plant jasmonate expression and aphid exposure to predators. In laboratory and field settings we tested the effects of plant resistance and predators by combining all three levels of plant resistance (low, intermediate and high resistance) with two levels of predators (no predators and predators). We measured the number of nymphs, aphid density, population growth, and dispersal in all six combinations of plant resistance-by-predator treatments. Because both field and

laboratory experiments showed similar results, both datasets were combined for analyses, and experimental setting was modeled as a blocking treatment in all analyses.

We conducted four field trials over the summer of 2009 and 2010 in an old field in Ithaca, NY, USA. We transplanted the plants into the soil and enclosed them with $1m^3$ cages made of PVC frame covered with fine acrylic mesh. These cages had side zippers that allowed us to access the plants and insects inside. We buried the bottom of the cages into the soil to exclude insects from entering and to prevent ladybird beetles and aphids from getting out. The number of replicates per treatment varied from 23 to 30 (see Table S3.1). We also conducted one laboratory trial in a walk-in growth chamber in 2010 (24ºC, 16:8, L:D photoperiod). In this set up, the experimental arena consisted of 60x30cm cages covered with translucent spun polyester sleeves tied closed on both sides. The number of replicates per treatment varied from 9 to 10 (Table S3.1) in the laboratory. All treatments in both field and laboratory trials were grouped in blocks to account for possible spatial variation in soil and light, respectively. Each block contained one replicate of each treatment.

Each cage received two plants from the same resistance type in both the field and laboratory trials. To all cages we added 100 wingless aphids of mixed ages to a bagged '*source plant'*, and the '*neighbor plant'* was aphid-free. After 1-2 days, the number of aphids on the *source plant* was counted and the bag was opened, thus allowing aphids to emigrate from the *source plant*. We added one ladybird beetle to every 100 aphids in all arenas that received predators (1 to 2 predators). We counted the number of aphids on the *source* and *neighbor* plants in each cage 4-5 days after

aphid release. Aphids that were in the soil or on the cage were also counted. We recorded whether aphids were adults, older nymphs (3^{rd} and 4^{th} instar) or younger nymphs (1^{st} and 2^{nd} instar) in the laboratory trial and in two of the four field trials. We visually identified the different instars. Assuming that all young nymphs were produced during the experiment in the response to the treatments, we used the number of young nymphs as an element of aphid reproduction.

Statistical Analyses

We estimated predation rate as the difference between the predicted aphid density in the absence of predators and the observed aphid density in the presence of predators (Predation rate = predicted aphid density – observed aphid density) for all replicates with predators. The predicted aphid density was calculated using the replicates (cages) without predators to predict how many aphids the replicates with predators would have in the absence of aphid consumption. The predicted aphid density was computed as $I + D*P$, where I is the initial aphid population of the replicate with predators, D is the duration of the experiment for the specific replicate (4 or 5 days), and P is the per diem population growth of the aphids in the absence of predators. P is specific for each tomato line and experiment. P in the field assays: Low-resistance = 85, Intermediate-resistance = 56, high-resistance = 39; P in the laboratory assays: Low-resistance = 100, Intermediate-resistance = 62, high-resistance = 44. We tested the effect of plant resistance on predation rate using mixed-effects models, modeling plant resistance as fixed effect. We modeled trial, block and experimental setting as random effects in all mixed-effects models.

We measured aphid population growth for all replicates by calculating daily per capita growth rate of aphids ($dN/(Ndt)$ as $(\ln N_2 - \ln N_1)/(t_2 - t_1)$ for each replicate (cage), where N_2 and N_1 are the final and initial aphid densities counted in the replicate, respectively, divided by the number of days elapsed between initial and final counting (4-5 days) (Gotelli 2001, Agrawal 2004). We used ln-transformed data to meet the assumptions of linear models. We used mixed-effect models to test for the effects of plant resistance and exposure to predators on daily per capita population growth rate and final aphid density (ln-transformed). We modeled plant resistance (3 levels), predators (2 levels), plant-by-predator interaction (6 levels) as fixed effects.

We measured the number of nymphs (first and second instars) as an element of aphid reproduction. We used mixed-effect models to test the effects of plant resistance and exposure to predators on aphid number of nymphs (Sqrt-transformed). We modeled plant resistance, predators, plant-by-predator interaction and density of adult aphids (ln-transformed) as fixed effects.

Since our results showed that aphids produced more nymphs in the presence of predators (see number of nymphs results) we performed an additional experiment to test the repeatability of this effect. We tested the effect of plant resistance (low, intermediate and high resistance) and predators (no predators and predators) on the number of nymphs produced by an aphid. We placed a single tomato leaflet in a Petri dish; each leaflet used in this experiment comes from an individual plant. Each Petri-dish received one of the six treatments and 10 randomly assigned 9-day old adult aphids (pre-reproduction individuals). We counted the number of nymphs after 12 h and 24 h. We analyzed these data as nymphs produced per adult aphid to account for

adults that were consumed during the assay. We replicated each of the 6 treatments 15 times for a total 90 samples. We used repeated-measures ANOVA to test the effects of plant resistance and predators (no predators and predators) on the number of nymphs produced by an aphid in a Petri dish.

We estimated dispersal rate by counting the number of aphids that moved away from the *source* plant. Aphid dispersal data were not normally distributed (count data); accordingly, we carried out a generalized linear model using a poisson distribution (GENMOD procedure in SAS). We tested the effect of plant resistance, predators (one-tailed), plant-by-predator interaction (one-tailed) on the number of aphids dispersing to the neighbor plants. We also included experimental setting and block in our model. Final density of aphids (ln-transformed) was included as an offset variable, which represents the denominators for our counts, so that a relative rate can be obtained. Our data exhibited over-dispersion, with a variance larger than the mean, which is a common phenomenon in data modeled with poisson distribution. To accommodate the excess of residual variation (over-dispersion), we estimated the dispersion parameter as the deviance divided by the degrees of freedom.

We performed Tukey's HSD post hoc comparison for all mixed-effects test results. We used SAS 9.3 (SAS Institute Inc, Cary, NC, USA 2012) to perform the aphid dispersal analyses, and JMP 10 (SAS Institute Inc, Cary, NC, USA 2012) for all the other analyses.

(2) Aphid Mass in Response to Variation in Plant Resistance

To assess the effect of plant resistance on aphid attribute as prey we measured aphid mass in response to the different plant resistance levels. We enclosed one 1-day-old aphid on each plant (low, intermediate or high resistance) with translucent spun polyester sleeves. After 10 days, before they start reproducing, we weighed all the aphids. We replicated each of the 3 treatments 10 times for a total sample of 30. We performed ANOVA on aphid mass in response to the different plant resistance levels.

(3) Relative Strength of Plant Resistance, Consumptive and Non-Consumptive Effects of Predators on Aphid Dispersal

We used path analysis (*i.e.*, structural equation modeling) to examine the strength of the consumptive and non-consumptive effects of predators on aphid dispersal. We also measured the effects of plant resistance on aphid dispersal through a direct pathway and indirect pathways (via changes in aphid density and predation rate). The data used in the path analysis was extracted from the field and laboratory experiments describe in section 1. In our path model, we included plant resistance, non-consumptive effect (number of predators added) and consumptive (predation rate) effect of predators, aphid density (ln-transformed) and number of aphids that dispersed.

This analysis allows us to break down the effect of the predator into two components (consumptive and non-consumptive) and examine their relative importance on aphid dispersal. This approach also provides a more comprehensive understanding of the effects of plant resistance as it evaluates both direct and indirect

pathways to how plant can affect aphid dispersal. Path analysis may provide a realistic assessment of the different components of predators, but experimental manipulation of predation risk can easily separate the effect intimidation and consumption, but the predation risk manipulation is not perfect. We used Systat (Systat Software Inc, Chicago, IL, USA 2004) to run the path analysis.

(4) Plant Resistance and Experimental Manipulation of the Non-Consumptive Effects of Predators

To experimentally estimate the impact of plant resistance on non-consumptive effects of predators, we combined three plant resistance levels (low, intermediate and high) with four levels of predators (i) *Control*: 100 aphids, no predators, (ii) *Low predation risk*: predators that could hunt but not kill the prey, thus testing only the effect of the non-consumptive component of the predator (1 predator per 100 aphids was added), (iii) *High predation risk*: higher density of predators that could only hunt but not kill the prey (2 predators per 100 aphids was added), and (iv) *Lethal*: predators that could both hunt and kill the prey (consumptive + non-consumptive components) (1 predator per 100 aphids was added). *Predation risk* predators were impaired to prevent killing. Under CO_2, we glued their mandibles with a droplet of transparent nail polish. All lethal predators were also put under CO_2 and received a droplet of nail polish on one of their wings to control for possible effects of CO_2 and nail polish on predator behavior and prey perception of predators. All ladybird beetles were starved for 3 days and refrigerated for 24 hours prior experiments. For the '*high predation risk'* treatment we doubled the density of risk predators to test if the strength of the

non-consumptive effect is stronger at higher predator densities. Predator-induced prey responses can vary in a density-dependent manner, means that prey responses can be stronger under high densities of predators. While we did not measure the specific cue used by the aphids to detect the predators, other people have found a role for aphid alarm pheromone and general disturbance. This response is often density dependent, with high levels of predation causing a strong response by the aphids (Losey & Denno 1998; Kunert & Weisser 2003; Kunert *et al.* 2005).

We carried out an assay to evaluate the behavior of risk and lethal ladybird beetle predators. Both predators were observed foraging, grooming, and resting. Lethal predators were also seen consuming the aphids. Time budgets were analyzed by multivariate analysis of variance (MANOVA) on proportions spent engaged in each behavior (Clark & Messina 1998). The analysis showed that although risk and lethal predators engaged in similar behavioral activities, the time spent in each behavior is different. Lethal predators spend most of their time foraging (67%), while risk predators foraged for 29% of their time. Risk predators spent most of their time resting (41%), whereas lethal predators rested for 20% of their time. Lethal predators spent only 6% of their time grooming, but risk predators groomed themselves for 30% of their time. This suggests that our risk treatment is not a flawless manipulation and thus it is conservative on the true effects of the non-consumptive component of predators. It also suggests that the high-risk treatment (with 2 predators per 100 aphids) may be a better representation of the predator pressure aphids experience in the lethal treatment (1 predator per 100 aphids) since an individual risk predator foraged about half the time as an individual lethal predator.

We measured the number of aphid nymphs, daily per capita population growth rate, final aphid density, and aphid dispersal using the same methods and statistical analyses described above. Our predator treatment, however, had four levels (control, low-risk, high-risk and lethal), for a total of 12 treatment combinations. Experimental setting and trial were not included in the models because we carried out only one trial. The number of replicates per treatment varied from 8 to 10 in the laboratory (Table S3.1).

Results

(1) Predation Rate, Aphid Performance and Dispersal in Response to Variation in Plant Resistance and Predator Exposure

Ladybird beetle predators consumed 45% more aphids on low-resistance plants compared with intermediate-resistance and 892% more compared with high-resistance plants ($F_{[2,77]} = 51.11$, $P<0.001$, Figure 3.1). Plant resistance reduced the number of nymphs produced in high- and intermediate- resistance plants by 57% when compared with low-resistance plants ($F_{[2,134]} = 19.26$, $P < 0.001$, Figure 3.2A). Contrary to our expectations, predators increased the number of nymphs produced by aphids by 91% ($F_{[1,135]}= 29.59$, $P< 0.001$, Figure 3.2A). We did not find an effect of plant resistance-by-predator interaction number of nymphs produced ($F_{[2,130]} = 1.12$, $P=0.330$). In the Petri dish experiment, we found similar results. Plant resistance reduced the number of nymphs produced by an aphid ($F_{[2,78]} = 4.65$, $P=0.012$) and predators increased nymph production by 143% ($F_{[1,78]} = 4.48$, $P=0.037$). This result suggests that aphids might be

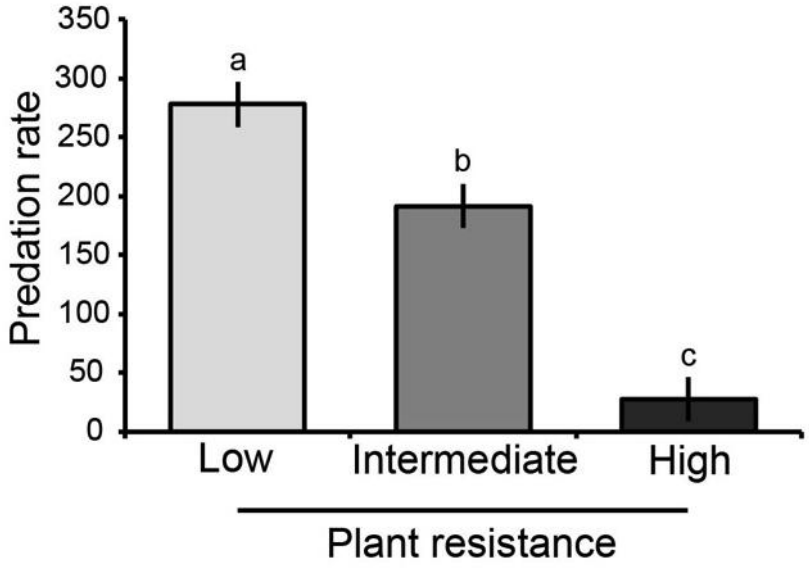

Figure 3.1: Estimate means (±1SE) of lady beetle predation rate (total number of aphids consumed) on tomato plants with different levels of resistance. Treatments are: plant resistance [low resistance (light gray bars), intermediate resistance (gray bars) and high resistance (dark gray bars)]. Letters above bars indicate significant differences at $P < 0.05$ following Tukey HSD post-hoc test.

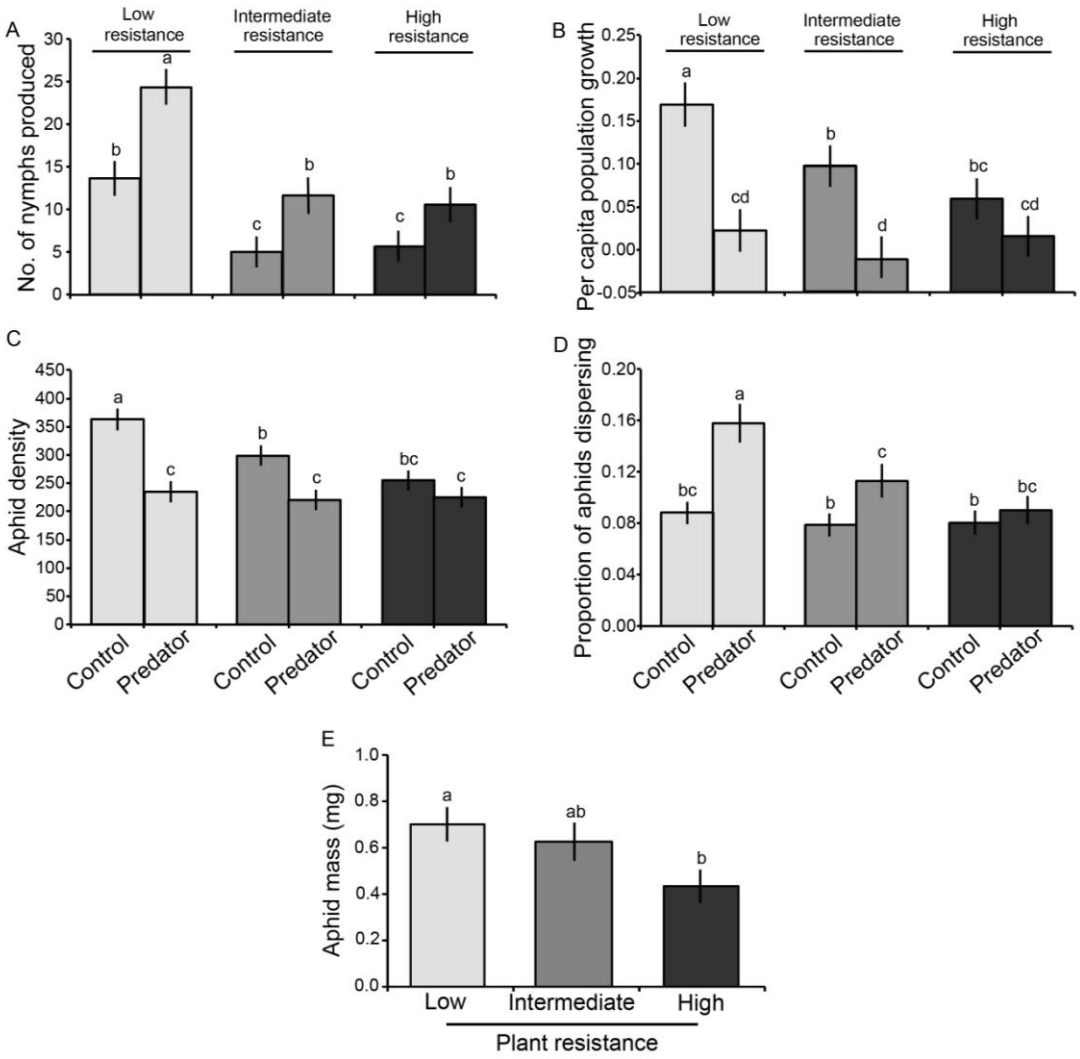

Figure 3.2: Least square-Means (±1SE) of aphid performance, dispersal and predation on tomato plants with varying levels of resistance. A) Number of nymphs produced by aphids over 5 days. B) Aphid per capita population growth rate per cage. C) Final aphid density per cage after 5 days. D) Proportion of aphids dispersing to the neighbor plant. E) Aphid mass (mg). Treatments are: plant resistance [low resistance (light gray bars), intermediate resistance (gray bars) and high resistance (dark gray bars)] and predators [control (no predators added), and predators]. Letters above bars indicate significant differences at $P < 0.05$ following Tukey HSD post-hoc test.

pushing their young out faster as a compensatory response to predators.

We found no plant resistance-by-predator interaction ($F_{[2,78]}$= 0.95, P=0.390). We found a plant resistance-by-predator interaction ($F_{[4,182]}$=3.08, P=0.048, Figure 3.2B) on the daily per capita population growth of aphids. On low and intermediate-resistance plants predators reduced aphid per capita population growth. In contrast, predators did not affect aphid per capita population growth on high-resistance plants. Plant resistance and predators also individually affected aphid daily per capita population growth rate ($F_{[2,185]}$ =4.38, P=0.014; $F_{[2,182]}$=33.71, P<0.001, Figure 3.2B, respectively).

The combined effect of plant resistance and predators affected final aphid density ($F_{[2,183]}$=4.05, P=0.019, Figure 3.2C). On low and intermediate-resistance plants, predators reduced aphid density, whereas on high-resistance plants, predators did not affect aphid density. Plant resistance and predators also individually affected final aphid density ($F_{[2,190]}$=6.55, P=0.002, $F_{[1,183]}$ =33.75, P<0.001, Figure 3.2C, respectively).

The effect of predators on aphid dispersal rate was contingent on plant resistance ($F_{[2,196]}$=2.90, P=0.029, Figure 3.2D). Predators increased aphid dispersal on low-resistance plants by 77% (Tukey HSD, Z=-4.83, P< 0.001) and by 38% on intermediate-resistance plants (Tukey HSD, Z=-2.43, P=0.015). In contrast, predators did not increase aphid dispersal on high-resistance plants (Tukey HSD, Z=-0.75, P=0.455, Figure 3.2D). This result indicates that host plant quality affects aphid anti-predator behavior, as aphids did not respond to predators on high-resistance plants.

Plant resistance and predators also individually affected aphid dispersal ($F_{[2,196]}$=5.71, P=0.004, $F_{[1,196]}$ =18.22, P<0.001, Figure 3.2D, respectively).

(2) Aphid Mass in Response to Variation in Plant Resistance

High-resistance plants reduced aphid mass by 39% compared to low-resistance plants ($F_{[2,30]}$=3.54, P=0.042; Figure 3.2E), which may affect predator consumption.

(3) Relative Strength of Plant Resistance, Consumptive and Non-Consumptive Effects of Predators on Aphid Dispersal

The path analysis and the mixed-effects model both showed that plant resistance and predators directly influenced aphid dispersal (Figure 3.3). Increased plant resistance directly decreased dispersal [spc=-0.216, 99.9% CI (-0.43, -0.01), Figure 3.3]. High plant resistance indirectly affected aphid dispersal by reducing predator consumption [standardized path coefficient (spc) =-0.294, 99.9% CI (-0.45, -0.14)] and decreasing aphid density [spc=-0.302, 99.9% CI (-0.51, -0.09)]. Density is one of the strongest drivers of aphid dispersal; higher densities resulted in higher dispersal [spc= 0.361, 99.9% CI (0.17, 0.55)].

By using this synthetic analysis we were able to discriminate whether the increase in aphid dispersal in response to predators was through the consumptive or non-consumptive effect of the predator. We removed the consumptive component of the predator by accounting for direct predation and inferred that the remaining effect of the predator was from the non-consumptive pathway. The consumptive and non-consumptive component affected aphid dispersal in different ways. Predation did not

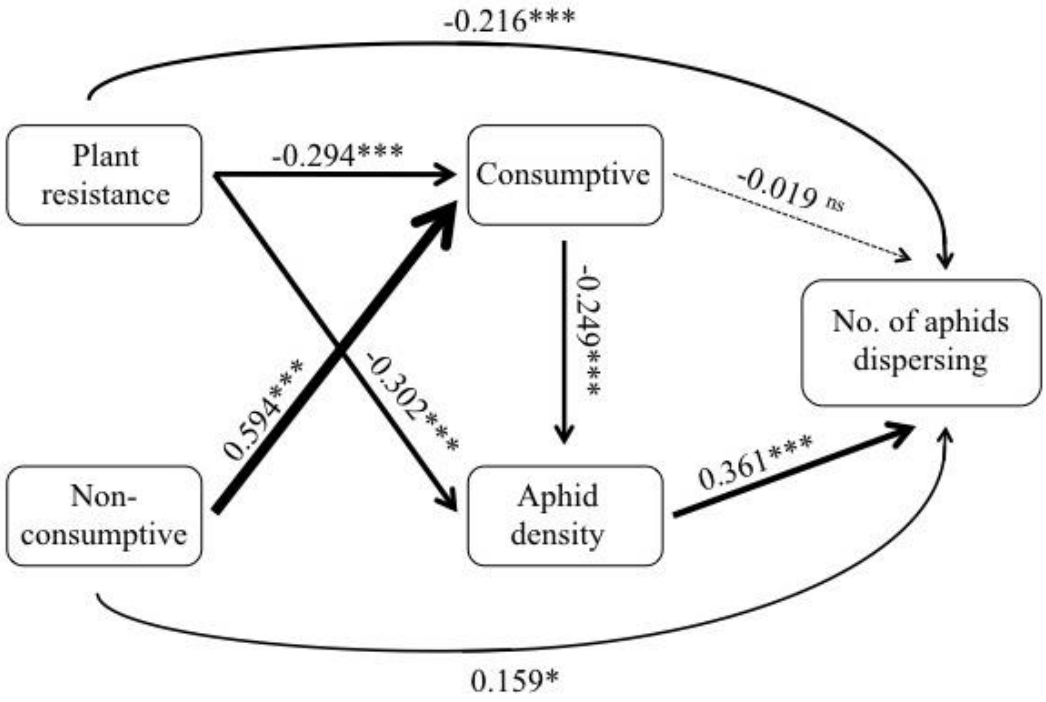

Figure 3.3: Path diagram for the model of the effect of plant resistance, consumptive (predation rate) and non-consumptive (number of predators) effects of predators, and aphid density (ln-transformed) on aphid dispersal. Solid lines denote significant effects, whereas dashed lines denote non-significant effects. Arrow thickness is scaled to illustrate the relative strength of effects. *P<0.05, ***P<0.001; ns=not significant

directly alter aphid dispersal [spc=-0.019, 95% CI (-0.28, 0.25)], but did indirectly reduce aphid dispersal by reducing aphid density [spc=-0.249, 99.9% CI (-0.46, -0.04)]. In contrast, the non-consumptive effect directly increased aphid dispersal. Our analysis indicates that the risk of predation, caused by the mere presence of predators is enough to promote aphid dispersal [spc=0.159, 95% CI (0.01, 0.30)]. This result suggests that predation risk accounts for a substantial part of the total effect of predators on aphid dispersal.

(4) Plant Resistance and Experimental Manipulation of the Non-Consumptive Effects of Predators

In our predation risk manipulation experiment high-density of risk predators increased the number of nymphs produced by aphids compared with controls ($F_{[1,42]}$=8.51, P=0.006, Figure 3.4A) and the low-density of risk predators did not ($F_{[2,42]}$= 2.13, P=0.152, Figure 3.4A). Even though risk predators enhanced nymph production they did not affect aphid per capita population growth rate ($F_{[2,69]}$=0.92, P=0.404) or aphid density ($F_{[2,68]}$=1.20, P=0.306) compared with controls.

Plant resistance influenced the effect of predation risk on aphid dispersal rate. On low-resistance plants with a high density of risk predators, aphids dispersed 112% more compared with controls (Tukey HSD, Z=-2.63, P=0.009), this effect was similar to lethal predators, which also increased aphid dispersal compared with controls (Tukey HSD, Z=-3.72, P<0.001). In contrast, aphid dispersal was similar between low density of risk predators and controls (Tukey HSD, Z=-0.79, P=0.427). On plants with intermediate and high levels of resistance, risk predator treatments were not different

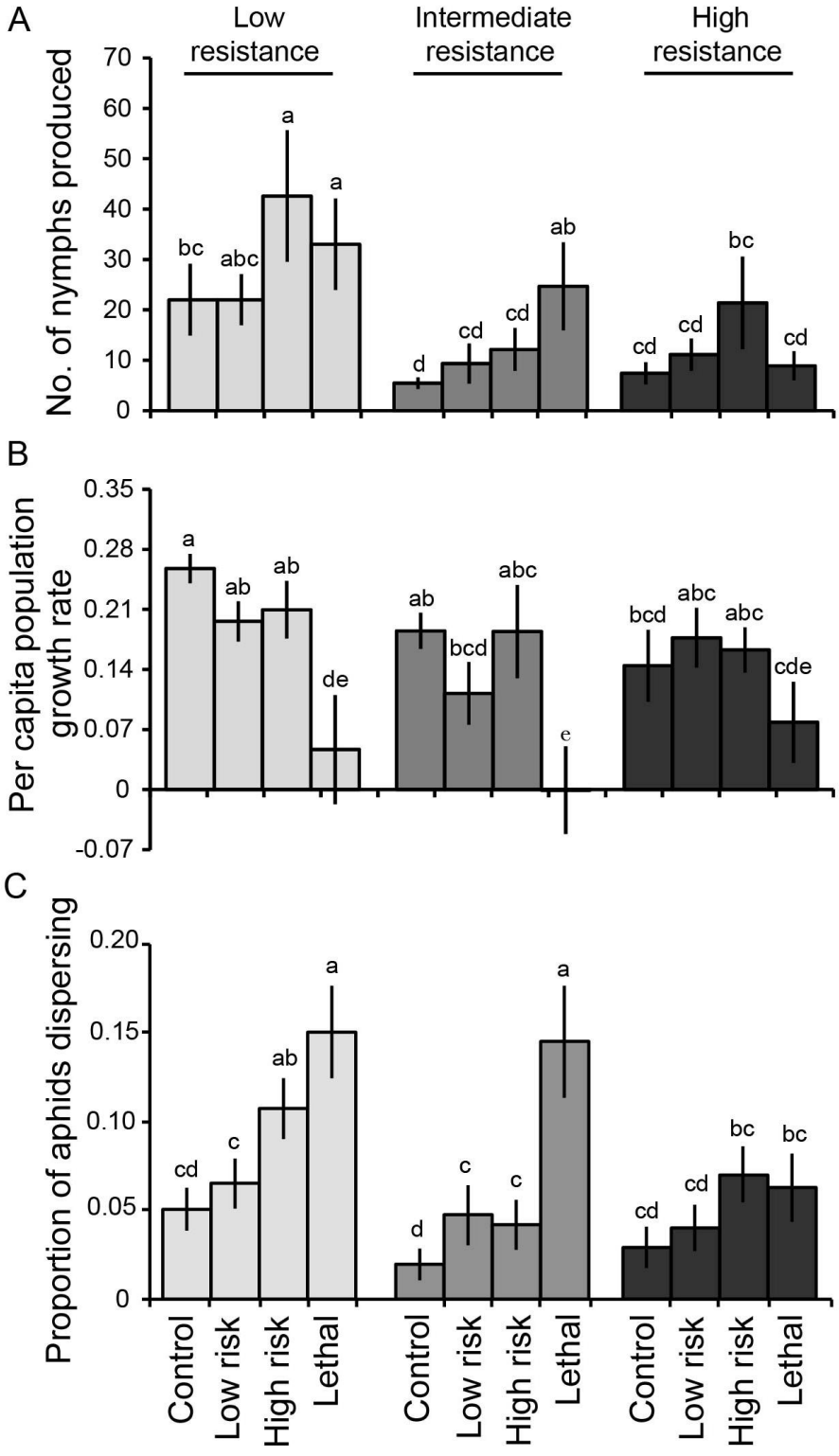
A
Low resistance
Intermediate resistance
High resistance
No. of nymphs produced
70
60
50
40
30
20
10
0
bc
abc
a
a
d
cd
cd
ab
cd
cd
bc
cd
B
Per capita population growth rate
0.35
0.28
0.21
0.14
0.07
0
-0.07
a
ab
ab
de
ab
bcd
abc
e
bcd
abc
abc
cde
C
Proportion of aphids dispersing
0.20
0.15
0.10
0.05
0
cd
c
ab
a
d
c
c
a
cd
cd
bc
bc
Control
Low risk
High risk
Lethal
Control
Low risk
High risk
Lethal
Control
Low risk
High risk
Lethal

Figure 3.4: Aphid number of nymphs produced and dispersal in response to predation risk. A) Number of nymphs produced over 5 days. B) Aphid per capita growth rate. C) Proportion of aphids dispersing to the neighbor plant. Treatments are: plant resistance [low resistance (light gray bars), intermediate resistance (gray bars) and high resistance (dark gray bars)] and predators [controls with no predators added, low risk predators and high risk predators and lethal predators]. Letters above bars indicate significant differences at $P < 0.05$ following Tukey HSD post-hoc test for all 12 comparisons. Shown are least square-means (±1SE).

from the control treatment. Predation risk alone affected aphid dispersal ($F_{[3,103]}$= 9.81, P< 0.001, Figure 3.4C). Compared with controls, high predation risk increased aphid dispersal, whereas low predation risk did not alter aphid dispersal.

Discussion

Wingless aphids respond to predators, by increasing both reproduction and dispersal (Figure 3.2A, D, Figure 3.3, Figure 3.4A, C). Our field and laboratory experiments, analyzed using two statistical approaches (*i.e.* GLM, path analysis), demonstrate that increased jasmonate plant defenses decreased both consumptive and non-consumptive effects of ladybird beetle predators on aphids (Figure 3.2B and Figure 3.4C). Based on our path analyses, the aphid dispersal response was more strongly driven by predation risk rather than by predation rate (Figure 3.3). Combined, our results suggest that the interactive effects of host plant resistance and predation risk largely determine prey behavioral responses to predators.

Plant Resistance Influences Predator Consumption

Our results indicate that jasmonate plant defenses can drastically reduce the impact of predators on prey, leading to plant-mediated differences in predator control of herbivores. Numerous mechanisms may underlie the differences in predator consumption of aphids on high and low resistance plants. Although none of these potential mechanisms are mutually exclusive, some are more likely to be linked to our results than others, including prey biomass, prey quality (nutritional or defensive) and prey-induced plant volatiles (Thaler 2002; Mallinger, Hogg & Gratton 2011;

Rodriguez-Saona *et al.* 2011). Here, we demonstrated that predation rate was reduced on high-resistance plants even though the aphids feeding on these plants were smaller. This result indicates that predators could be avoiding aphids feeding on high-resistance plants because they are somewhat low-quality food (lower biomass, unpalatable or low nutrition). In a no-choice predation experiment, however, we did not find lower consumption on high-resistance plants. What we found instead was evidence for a compensatory consumption response when aphids were feeding on high-resistance plants (Kersch-Becker, Kessler & Thaler *unpublished*). Accordingly, some studies have demonstrated that aphid consumption by predators increases when aphids are smaller, suggesting compensatory consumption to overcome reduced biomass (Latham & Mills 2010; Aqueel & Leather 2012). Thus, aphid mass alone cannot explain the low predation rate on high-resistance plants found in this study. Because predators were able to consume aphids that were feeding on high-resistance plants, it is possible that it may be difficult for predators to find the prey on those plants. We speculate that high resistance levels in plants may alter production of cues used by predators to find prey (herbivore-induced plant volatiles), which would increase searching time of predators, thus reducing overall consumption.

Plant Resistance Influences the Non-Consumptive Component of Predators

Both the path analysis and manipulative experiments showed that the risk of predation increased aphid dispersal rate and number of nymphs produced, and such responses were stronger on low-resistance plants compared with high-resistance plants. This result is consistent with recent studies that have reported the consequences

of plant defenses on the non-consumptive effects of predators, showing that the effect of the non-consumptive component of predators is stronger on plants with lower levels of defense (Kaplan & Thaler 2010, 2012, McArthur *et al.* 2012, Thaler, McArt & Kaplan 2012). For example, caterpillars feeding on plants with low-jasmonate levels consumed less leaf tissue under predation risk (Kaplan & Thaler 2010). Additionally, to lower the risk of predation, aphids may select lower quality host plants, when aphids perceive that predators have consumed prey on high quality host plants ("enemy-free space hypothesis"; Jeffries & Lawton 1984; Wilson & Leather 2012). The weaker non-consumptive effect on high-resistance plants may occur because predator impact on prey may be weaker on high-resistance plants. Consistent with this, lethal predators consumed fewer aphids on high-resistance plants, perhaps because predator might not want to forage on those plants or searching time might increase on high-resistance plants. Therefore, aphid predation rate alone does not predict aphid behavior. Plant resistance could affect aphid ability to perceive the risk of predation, so the cues exploited by aphids when under predation risk might be dependent on the host plant resistance level.

Aphids responded to predation risk by producing more nymphs on the low-resistance plants. Risk of predation has been shown to reduce prey reproduction (Walzer & Schausberger 2009; Choh, Uefune & Takabayashi 2010; Clinchy, Sheriff & Zanette 2013), but some studies suggested that females with low life expectancy may increase reproduction (Roitberg *et al.* 1983; Fletcher, Hughes & Harvey 1994; Javoiš & Tammaru 2004). In support for the latter hypothesis, here we showed in independent experiments that the number of nymphs produced by aphids increased in

the presence of predators, and this result was consistent across all three plant lines. Immobile stages of prey, such as eggs and pupae may be more vulnerable to predation than mobile prey. Thus, in some systems, a reduction in oviposition may be a predator avoidance behavior, and such phenomenon has been observed in several oviparous insect species (Faraji, Janssen & Sabelis 2001; Agarwala, Yasuda & Kajita 2003; Nomikou, Janssen & Sabelis 2003; Škaloudová, Zemek & Křivan 2007; Choh, Uefune & Takabashi 2010). Because aphids are parthenogenic, the maternal environment has a strong influence on the phenotype of the offspring; adapting the offspring to the current environment (i.e. increasing wing production in offspring). Aphids have live birth, their offspring are immediately mobile and capable of escaping predators. Therefore, we suggest that increased number of nymphs in the presence of predators could be attributed to its life-history reproductive traits. Because our experiment lasted 5 days we can only infer that aphids were pushing their young out faster in response to predators. The number of embryos per aphids could not have been affected and true multiplication could not have occurred in this timeframe (Ward, Wellings & Dixon 1983). Long-term experiments are needed to further explore whether aphid overall fecundity is also increased or possible decreased. It is also important to highlight that because we did not measure nymph survivorship following exposure to predators, we do not know whether by pushing their young out faster they may be producing weaker nymphs.

The aphid alarm pheromone, (*E*)-ß-farnesene, triggers several predator-induced behavioral responses including increased walking, dropping off the plant, withdrawal of the stylets, and increased the number of winged morphs, all of which may reduce

predation risk of aphids (Dixon & Agarwala 1999; Minoretti & Weisser 2000; Kunert *et al.* 2005; Kunert, Trautsch & Weisser 2007). Exposing aphids to their alarm pheromone has been shown to have no effect on aphid nymph production (Kunert *et al.* 2005; Kunert, Trautsch & Weisser 2007). Thus, it is possible that in our study other cues used by aphids to perceive predation risk (*i.e*. disturbance or predator chemical cues) may be playing a role in aphid increased nymph production in the presence of predators. This suggests that the aphid alarm pheromone alone does not induce all prey responses to predators.

Aphid Dispersal Response to Variation in Plant Resistance and Exposure to Predators and Predation Risk

Although winged aphids can disperse over great distances, most of the movement within and between plants is undertaken by wingless morphs (Harrington & Taylor 1990; Honek *et al.* 1998; Underwood, Halpern & Klein 2011). Aphids can exhibit higher dispersal rates mainly in response to (1) poor quality food (Wilson & Leather 2012), (2) increased physical contact between aphids, "crowding effect" (Kunert *et al.* 2005), (3) alarm pheromone emitted by prey aphids to warn other aphids about potential risk of predation (Kunert *et al.* 2005), (4) aphid feeding disruption by predator touch (Losey & Denno 1998; Minoretti & Weisser 2000), and (4) visual or chemical cues used by aphids to assess predation risk (Grostal & Dicke 1999,Wilson & Leather 2012). When herbivores move, they may also suffer performance costs due to energy expended, lost feeding time, or increased vulnerability to predators (Bergelson & Lawton 1988). Aphid dispersal rates were higher in the presence of

predators and the magnitude of this response was contingent on plant resistance. On low-resistance plants, predation was high leading to higher aphid dispersal. In contrast, on high-resistance plants, predation and aphid dispersal were low. Others have observed similar responses (Hannunen & Ekbom 2002; Underwood, Halpern & Klein 2011). For instance, wingless strawberry aphids (*Chaetosiphon fragaefolii*) responded to differences in quality across strawberry genotypes by increasing movement rate on high quality genotypes (Underwood, Halpern & Klein 2011).

Aphids may increase dispersal on low-resistance plants to escape the higher levels of predation on those plants. By using path analysis we were able to discriminate the relative strength of the effects of plant resistance and predators on aphid dispersal. Indeed, this analysis revealed that plant resistance had both a direct and an indirect impact on aphid dispersal. The indirect impact arose from a reduction in predation rate and aphid density on high-resistance plants. Also, this analysis showed that the non-consumptive effect drives the predator impact on aphid dispersal. If aphids were to respond to the consumptive component of the predator then we would detect a positive and significant effect of predation rate on aphid dispersal, which we did not find. What we found instead was that the mere presence of predators, revealed by the direct path between number of predators and aphid dispersal, is sufficient to induce aphid movement. We do not know, however, whether aphids are responding to the direct disturbance caused by predators or other specific cues.

It is especially interesting that even nonlethal predators promoted aphid dispersal, demonstrating that the risk of predation alone has the potential to provoke

predator-induced responses in aphids. These impacts, however, were stronger under higher densities of risk predators. The number of predators can determine the impact that predators cause on prey (Bowler, Yano & Amano 2013). The risk of predation perceived by prey might not be a dichotomy between presence and absence, but a density-dependent response, where the impact of predators arises from frequent encounter (touching and disrupting aphids) or higher predator-derived chemical cues (Dicke & Grostal 2001; Bowler, Yano & Amano 2013). In spider mites, density-dependent anti-predator behavior emerges from predator chemical cues left on a host plant leaf patch (Bowler, Yano & Amano 2013). In addition, plant resistance also dampened aphids from responding to risk predators. As a result, when feeding on high resistance plants aphids did not engage in anti-predator behaviors. While we do not know whether aphids cannot perceive the risk of predation or simply cannot respond to predators when feeding on low quality/highly resistant plants; here we showed that plant resistance and predators interact in complex ways to determine herbivore population structure and behavior.

Acknowledgments

We thank Cesar Rodriguez-Saona and Isgouhi Kaloshian for providing the aphids, and Gui Becker and Ordom Hout for their help in the field. This study strongly benefited from comments and suggestions by Anurag Agrawal, Alison Power, Suzi Claflin, Sara Hermann, Chris Stieha, Gui Becker and three anonymous reviewers. This project received financial support from National Research Initiative of the USDA Grant 2006-35302-17431, the Department of Ecology and Evolutionary Biology, and

the Department of Entomology at Cornell. MFKB was supported by the Cornell Olin Fellowship and the CAPES/Fulbright fellowship (BEX 2234-08-4).

REFERENCES

Agarwala, B.K., Yasuda, H. & Kajita, Y. (2003) Effect of conspecific and heterospecific feces on foraging and oviposition of two predatory ladybirds: role of fecal cues in predator avoidance. *Journal of Chemical Ecology*, **29**, 357–76.

Aqueel, M. a. & Leather, S.R. (2012) Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. *Annals of Applied Biology*, **160**, 6–15.

Aquilino, K.M., Cardinale, B.J. & Ives, A.R. (2005) Reciprocal effects of host plant and natural enemy diversity on herbivore suppression : an empirical study of a model tritrophic system. *Oikos*, **2**, 275–282.

Bergelson, J.M. & Lawton, J.H. (1988) Does foliage damage influence predation on the insect herbivores of birch? *Ecology*, **69**, 434–445.

Boethel, D.J. & Eikenbary, R.D. (1986) *Interactions of Host Plant Resistance and Parasitoids and Predators of Insects*. Halsted Press.

Bottrell, D.G., Barbosa, P. & Gould, F. (1998) Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology*, **43**, 347–367.

Bowler, D.E., Yano, S. & Amano, H. (2013) The non-consumptive effects of a predator on spider mites depend on predator density (ed R Knell). *Journal of Zoology*, **289**, 52–59.

Cabral, S., Soares, A.O. & Garcia, P. (2009) Predation by *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): Effect of prey density. *Biological Control*, **50**, 25–29.

Choh, Y., Uefune, M. & Takabayashi, J. (2010) Predation-related odours reduce oviposition in a herbivorous mite. *Experimental & Applied Acarology*, **50**, 1–8.

Clark, T.L. & Messina, F.J. (1998) Plant architecture and the foraging success of ladybird beetles attacking the Russian wheat aphid. *Entomologia Experimentalis et Applicata*, **86**, 153–161.

Clinchy, M., Sheriff, M.J. & Zanette, L.Y. (2013) Predator-induced stress and the ecology of fear (ed R Boonstra). *Functional Ecology*, **27**, 56–65.

Dicke, M. & Grostal, P. (2001) Chemical detection of natural enemies by arthropods : an ecological perspective. *Annual Review of Ecology and Systematics*, **32**, 1–23.

Dixon, A.F.G. (1998) *Aphid Ecology: An Optimization Approach*, 2nd edition. Chapman & Hall, London, UK.

Dixon, A.F.G. & Agarwala, B.K. (1999) Ladybird-induced life-history changes in aphids. *Proceedings of the Royal Society of London (Series B)*, **266**, 1549–1553.

Faraji, F., Janssen, a & Sabelis, M.W. (2001) Predatory mites avoid ovipositing near counterattacking prey. *Experimental & Applied Acarology*, **25**, 613–23.

Finke, D.L. (2012) Contrasting the consumptive and non-consumptive cascading effects of natural enemies on vector-borne pathogens. *Entomologia Experimentalis et Applicata*, **144**, 45–55.

Fletcher, J.P., Hughes, J.P. & Harvey, I.F. (1994) Life expectancy and egg load affect oviposition decisions of a solitary parasitoid. *Proceedings of the National Academy of Sciences of the United States of America*, **258**, 163–167.

Forkner, R.E. & Hunter, M.D. (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. *Ecology*, **81**, 1588–1600.

Goggin, F.L. (2007) Plant-aphid interactions: molecular and ecological perspectives. *Current Opinion in Plant Biology*, **10**, 399–408.

Goggin, F.L., Williamson, V.M. & Ullman, D.E. (2001) Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera : Aphididae) to the tomato resistance gene *Mi*. *Environmental Entomology*, **30**, 101–106.

Gotelli, N.J. (2001) *A Primer of Ecology*, 3rd ed. Sinauer, Sunderland, Massachussets.

Grostal, P. & Dicke, M. (1999) Direct and indirect cues of predation risk influence behavior and reproduction of prey: a case for acarine interactions. *Behavioral Ecology*, **10**, 422–427.

Hannunen, S. & Ekbom, B. (2002) Within species variation in host plant quality and movement behavior of *Lygus rugulipennis* nymphs. *Entomologia Experimentalis et Applicata*, **104**, 95–101.

Hare, D.J. (2002) Plant genetic variation in tritrophic interactions. *Multitrophic level interactions* (eds T. Tscharntke & B.A. Hawkins), pp. 8–43. Cambridge University Press, Cambridge, UK.

Harrington, R. & Taylor, L.R. (1990) Migration for survival : Fine-scale population redistribution in an aphid. *Journal of Animal Ecology*, **59**, 1177–1193.

Hawlena, D. & Schmitz, O.J. (2010) Herbivore physiological response to predation risk and implications for ecosystem nutrient dynamics. *Proceedings of the National Academy of Sciences of the United States of America*, **107**, 15503–7.

Honek, A., Jarosik, V., Lapchin, L. & Rabasse, J.M. (1998) The effect of parasitism by *Aphelinus abdominalis* and drought on the walking movement of aphids. *Entomologia Experimentalis et Applicata*, **87**, 191–200.

Javoiš, J. & Tammaru, T. (2004) Reproductive decisions are sensitive to cues of life expectancy: the case of a moth. *Animal Behaviour*, **68**, 249–255.

Jeffries, M.J. & Lawton, J.H. (1984) Enemy free space and the structure of ecological communities. *Biological Journal of the Linnean Society*, **23**, 269–286.

Kaplan, I. & Thaler, J.S. (2010) Plant resistance attenuates the consumptive and non-consumptive impacts of predators on prey. *Oikos*, **119**, 1105–1113.

Kaplan, I. & Thaler, J.S. (2012) Phytohormone-mediated plant resistance and predation risk act independently on the population growth and wing formation of potato aphids, *Macrosiphum euphorbiae*. *Arthropod-Plant Interactions*, **6**, 181–186.

Kunert, G., Otto, S., Röse, U.S.R., Gershenzon, J. & Weisser, W.W. (2005) Alarm pheromone mediates production of winged dispersal morphs in aphids. *Ecology Letters*, **8**, 596–603.

Kunert, G., Trautsch, J. & Weisser, W.W. (2007) Density dependence of the alarm pheromone effect in pea aphids, *Acyrthosiphon pisum* (Sternorrhyncha: Aphididae). *European Journal of Entomology*, **104**, 47–50.

Kunert, G. & Weisser, W.W. (2003) The interplay between density- and trait-mediated effects in predator-prey interactions: a case study in aphid wing polymorphism. *Oecologia*, **135**, 304–12.

Latham, D.R. & Mills, N.J. (2010) Quantifying aphid predation: the mealy plum aphid *Hyalopterus pruni* in California as a case study. *Journal of Applied Ecology*, **47**, 200–208.

Li, L., Zhao, Y., Mccaig, B.C., Wingerd, B.A., Wang, J. & Mark, E. (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation , jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, **16**, 126–143.

Lima, S.L. & Dill, L.M. (1990) Behavioral decisions made under the risk of predation: a review and prospectus. *Canadian Journal of Zoology*, **68**, 619–640.

Losey, J.E. & Denno, R.F. (1998) The escape response of pea aphids to foliar-foraging predators: factors affecting dropping behaviour. *Ecological Entomology*, **23**, 53–61.

Mallinger, R.E., Hogg, D.B. & Gratton, C. (2011) Methyl salicylate attracts natural enemies and reduces populations of soybean aphids (Hemiptera: Aphididae) in soybean agroecosystems. *Journal of Economic Entomology*, **104**, 115–124.

McArthur, C., Orlando, P., Banks, P.B. & Brown, J.S. (2012) The foraging tightrope between predation risk and plant toxins: a matter of concentration. *Functional Ecology*, **26**, 74–83.

McCauley, S.J., Rowe, L. & Fortin, M.J. (2011) The deadly effects of "'nonlethal'" predators. *Ecology*, **92**, 2043–2048.

McGurl, B., Orozco-Cardenas, M., Pearce, G. & Ryan, C. A. (1994) Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9799–802.

Minoretti, N. & Weisser, W.W. (2000) The impact of individual ladybirds (*Coccinella septempunctata*, Coleoptera: Coccinellidae) on aphids colonies. *European Journal of Entomology*, **97**, 475–479.

Müller, C.B., Williams, I.S. & Hardie, J. (2001) The role of nutrition, crowding and interspecific interactions in the development of winged aphids. *Ecological Entomology*, **26**, 330–340.

Nomikou, M., Janssen, A. & Sabelis, M.W. (2003) Herbivore host plant selection: whitefly learns to avoid host plants that harbour predators of her offspring. *Oecologia*, **136**, 484–8.

Peacor, S.D. & Werner, E.E. (2001) The contribution of trait-mediated indirect effects to the net effects of a predator. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 3904–3908.

Poelman, E.H., van Loon, J.J. a & Dicke, M. (2008) Consequences of variation in plant defense for biodiversity at higher trophic levels. *Trends in Plant Science*, **13**, 534–41.

Preisser, E.L., Bolnick, D.I. & Benard, M.E. (2005) Scared to death ? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, **86**, 501–509.

Price, P.W., Bouton, C.E., Gross, P., Bruce, A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annual Review of Ecology and Systematics*, **11**, 41–65.

Rodriguez-Saona, C., Kaplan, I., Braasch, J., Chinnasamy, D. & Williams, L. (2011) Field responses of predaceous arthropods to methyl salicylate: A meta-analysis and case study in cranberries. *Biological Control*, **59**, 294–303.

Roitberg, B.D., Sircom, J., Roitberg, C.A., van Alphen, J.J. & Mangel, M. (1993) Life expectancy and reproduction. *Nature*, **364**, 108.

Schmitz, O.J. (2004) Perturbation and abrupt shift in trophic control of biodiversity and productivity. *Ecology Letters*, **7**, 403–409.

Schmitz, O.J., Hawlena, D. & Trussell, G.C. (2010) Predator control of ecosystem nutrient dynamics. *Ecology Letters*, **13**, 1199–209.

Schmitz, O.J., Krivan, V. & Ovadia, O. (2004) Trophic cascades: the primacy of trait-mediated indirect interactions. *Ecology Letters*, **7**, 153–163.

Schotzko, D.J. & Smith, C.M. (1991) Effects of host plant on the between-plant spatial distribution of the Russian wheat aphid (Homoptera : Aphididae). *Journal of Economic Entomology*, **84**, 1725–1734.

Shannag, H.K. & Obeidat, W.M. (2008) Interaction between plant resistance and predation of *Aphis fabae* (Homoptera: Aphididae) by *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Annals of Applied Biology*, **152**, 331–337.

Škaloudová, B., Zemek, R. & Křivan, V. (2007) The effect of predation risk on an acarine system. *Animal Behaviour*, **74**, 813–821.

Thaler, J.S. (2002) Effect of jasmonate-induced plant responses on the natural enemies of herbivores. *Journal of Animal Ecology*, **71**, 141–150.

Thaler, J., Karban, R., Ullman, D., Boege, K. & Bostock, R. (2002) Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. *Oecologia*, **131**, 227–235.

Thaler, J.S., McArt, S.H. & Kaplan, I. (2012) Compensatory mechanisms for ameliorating the fundamental trade-off between predator avoidance and foraging. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 12075–80.

Underwood, N., Halpern, S. & Klein, C. (2011) EEffect of host-plant genotype and neighboring plants on strawberry aphid movement in the greenhouse and field. *American Midland Naturalist*, **165**, 38–49.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth and Regulation*, **19**, 195–216.

Walzer, A. & Schausberger, P. (2009) Non-consumptive effects of predatory mites on thrips and its host plant. *Oikos*, **118**, 934–940.

Ward, S.A., Wellings, P.W. & Dixon, A.F.G. (1983) The effects od reproductive investment on pre-reproductive mortality in aphids. *Journal of Animal Ecology*, **52**, 305–313.

Wilson, M.R. & Leather, S.R. (2012) The effect of past natural enemy activity on host-plant preference of two aphid species. *Entomologia Experimentalis et Applicata*, **144**, 216–222.

CHAPTER 4

PLANT DEFENSES DRIVE POPULATION DYNAMICS OF A HERBIVORE BY CHANGING PREDATOR-PREY INTERACTIONS

Abstract

Plants can affect insect populations directly, through a negative feedback associated with induced-plant defenses in a density-dependent manner, or indirectly, by changing predator-prey interactions. It is commonly assumed that increased plant defenses and predators act synergistically in reducing herbivore populations, however this might not be the case if plant defenses reduce the numerical and functional responses of predators on prey. We conducted a manipulative field experiment to evaluate the 1) effect of prey density and plant resistance on predator abundance, richness and consumption and 2) relative strength of plant resistance and predators on population dynamics of aphids (*Macrosiphum euphorbiae*). We then tested hypotheses for how plant defenses and prey density affect predators by measuring aphid honeydew production and plant volatile organic compounds (VOCs). We manipulated predators by enclosing aphid populations to prevent predator access and used genetically modified lines of tomato (*Solanum lycopersicum*) that vary incrementally in the expression of the jasmonate pathway, which mediates induced resistance to insects. On low-resistance (jasmonate-insensitive) plants, predator abundance, richness and consumption increased in response to aphid density, whereas this effect was weaker on high-resistance (jasmonate-overexpressing) plants. Consistent with jasmonate-salicylate antagonism, we found that aphid feeding increased methyl salicylate volatile emissions on jasmonate-insensitive but not on jasmonate-overexpressing plants. Because methyl salicylate is well-known to be a predator attractant, it may be the underlying mechanism driving the predator response to the increased density of aphids on low-resistance plants. Using path analysis, we showed

that increased plant resistance had a strong negative and indirect effect on herbivore population growth by reducing predator impacts on prey abundance. Neither predator abundance or richness explained the net effect of predators on prey. By factorially manipulating plant resistance, aphid density and predator exposure, we have demonstrated that predators can only cause density-dependent population growth in herbivorous prey when they are feeding on high-quality plants that can induce resistance.

Introduction

By reducing population growth rates as densities rise, density-dependent processes may regulate population growth (Hassell *et al.* 1980, Harrison & Cappuccino 1995). It has been hypothesized and experimentally demonstrated that intraspecific variation in host plant quality may be a critical factor regulating the population growth of herbivorous insects (Underwood & Rausher 2002, Helms & Hunter 2005, Johnson 2008, Underwood 2009). In particular, variation in plant defensive traits, which influence herbivore performance (Walling 2000), have been shown to influence the population dynamics of herbivores (Underwood & Rausher 2002, Rotem & Agrawal 2003). Indeed, variation in plant quality can affect herbivore population dynamics directly, through a negative feedback associated with induced plant defenses in a density-dependent manner, or indirectly, by changing the impact of predators on herbivorous prey.

Moving up the food chain, prey species have the potential to drive the abundance, richness, and consumption of predators. The ability of a predator to

respond numerically by aggregating in areas of high prey density and, thus imposing density-dependent prey mortality, is an important attribute that makes them capable of suppressing prey populations (Murdoch *et al.* 1985, Schellhorn & Andow 2005, Donaldson *et al.* 2007). Predators use numerous cues to locate and kill their insect prey, and while some are more widely recognized than others, they are typically not mutually exclusive (Price *et al.* 1980, Vet & Dicke 1992, Dicke & van Loon 2000, Bahlai *et al.* 2008). These include prey density and quality (Donaldson *et al.* 2007), herbivore chemical cues (e.g. alarm pheromones and excretia) (Purandare *et al.* 2012), plants architecture and structure (Marquis & Whelan 1996, Yang 2000, Kennedy 2003, Styrsky *et al.* 2006), and herbivore-induced plant compounds (Vet & Dicke 1992, Rodriguez-Saona *et al.* 2011, Kaplan 2012). For example, upon damage by herbivores, plants can emit quantitatively and qualitatively higher volatile organic compounds, including predator attractants (Paré & Tumlinson 1997, Kessler & Baldwin 2001, Zhu & Park 2005, Rodriguez-Saona *et al.* 2011). However, it is unclear whether these cues vary in a density-dependent manner and to what extent they are influenced by plant quality.

The ultimate impact that predators inflict on prey populations will depend on sundry tritrophic effects, including the quality of the plant the herbivorous prey is feeding on. Variation in host plant defenses has the potential to modify the impact of predators on prey by directly affecting feeding behavior, or indirectly through changes in prey quality or density. Although it has been widely demonstrated that plants can alter predators' impact on individual prey (Bottrell *et al.* 1998, Shannag & Obeidat 2008), less is known on how plant quality can influence the effect of predators on

herbivore population dynamics (Hare 2002). Empirical studies have demonstrated that the strength of density-dependent processes in aphid populations can vary with host plant quality (Underwood & Rausher 2002, Rotem & Agrawal 2003, Agrawal *et al.* 2004, Underwood 2010, chapter 2). Determining which factors promote strong or weak density dependence offers a mechanistic basis for the differences between exponential and density-dependent growth. It is commonly assumed that plants and predators act synergistically, both negatively affecting herbivores (Bottrell *et al.* 1998, Hare 2002), but one could also expect that plants and predators have opposing effects on herbivores, such as when plants reduce the numerical and functional responses of predators on prey.

We addressed how plant resistance mediates predator-prey interactions by conducting a factorial manipulative field experiment to (1) evaluate the effects of plant resistance and prey density on predators, and (2) investigate the interactive effects of plant resistance and predators on population dynamics of aphids (*Macrosiphum euphorbiae*). We manipulated predators by enclosing aphid populations to prevent predator access and used variation in the expression of the jasmonate pathway as our manipulation of plant quality. The phytohormone jasmonic acid (JA) regulates induced responses against a variety of organisms, including aphids (Inbar *et al.* 1998, Thaler *et al.* 1999, Walling 2000, 2008). In a previous study, we demonstrated that JA-insensitive plants provide low resistance to aphids, while JA-overexpressing plants confer resistance to aphids (chapter 2). Here, we specifically evaluated whether prey density and plant resistance affected predator abundance, richness, community composition, and consumption. We then tested hypotheses for how plant defenses and

prey density affect predators by measuring aphid honeydew production and plant volatile organic compounds (VOCs). Lastly, using path analysis, we determined whether plant resistance drives herbivore population dynamics directly through changes in population growth or indirectly by changing the impact of predators on prey.

Methods

To determine whether plant resistance, predators and aphid density affect aphid density-dependent population growth and final density, we manipulated plant jasmonate expression, aphid exposure to predators, and aphid initial densities. In a field experiment, we fully crossed all three levels of plant resistance (low, intermediate and high resistance), two levels of predators (natural levels and predators excluded), and five initial aphid densities ranging from (5-100 per plant). Field experiments were carried out at the Homer Thompson research farm in Freeville, New York, USA in June, July and August 2011.

We used three tomato (*Solanum lycopersicum* L.) lines that vary in their expression of jasmonic acid (JA): (i) Low resistance - a mutant tomato line (cv. *Jai-1;* Li *et al.* 2004) that does not induce the JA pathway, (ii) Intermediate resistance - wild-type tomato (cv. *Castlemart*), which induces the JA pathway upon herbivore feeding, and (iii) High resistance – a transgenic line that overexpresses the JA pathway (cv. *Prosystemin;* McGurl *et al*. 1994) and therefore is constitutively induced. These three plant types differ in traits regulated by the jasmonate pathway, including trichome density and secondary compounds, yet show similar growth (McGurl *et al.* 1994, Li *et*

al. 2004, Kaplan & Thaler 2010). Jasmonate overexpression greatly reduced aphid abundance and performance (Kaplan & Thaler 2012, Kersch-Becker & Thaler unpublished).

We germinated all tomato plants in the lab and transplanted them to four-inch pots in a greenhouse where they were watered daily and fertilized weekly (21:5:20 N:P:K) for four weeks (four-leaf stage). We maintained potato aphids, *Macrosiphum euphorbiae* (Thomas, 1878) (Hemiptera: Aphididae) (WU-11-FR clone) (Goggin *et al*. 2001) on tomato plants (cv. *Castlemart*) in growth chambers (22ºC, 16:8, L:D photoperiod). At the four-leaf stage we transplanted the plants to a tilled field. All plants were bagged with a spun polyester sleeve and were randomly assigned to receive different aphid densities. This method has been successfully employed by different authors to study population dynamics (Underwood & Rausher 2000, 2002, Agrawal 2004, Agrawal *et al*. 2004). We initially added 5, 25, 50, 75, 100 aphids per plant. Following aphid infestation, we allowed the aphids to settle and feed for 3 days. After 3 days, we then removed the bag and counted the number of aphids per plant. The number of aphids recorded was used as our initial density treatment and densities varied between 1 and 370 aphids per plant. When plants were transplanted to the field they were also randomly assigned to one of the following predator treatments: natural levels of predators and predators excluded. Plants assigned to "predators excluded" were planted inside 0.25 m^2 cages made with aphid proof cloth (spun polyester sleeve). Plants receiving "natural predators" grew inside identical but open-sided cages, allowing natural colonization of predators. These plants received the same aphid proof cloth on top of the cage to control for difference in sunlight and rainfall

received by the "predators excluded" group. We carried out three separate trials of this experiment over the summer of 2011 and the total number of replicates per treatment ranged from 55-63.

Predator Responses to Variation in Plant Resistance and Aphid Density

We recorded the number of aphids on each plant after 5 and 15 days. In addition, we performed visual censuses twice a week for 15 days (total 4 censuses) to record the abundance and identity of all predators on the "natural predators" treatment. Ladybird beetles were identified to the level of species, whereas the other taxonomic groups were identified to the level of order. We used ANOVA to test the effect of plant resistance, initial aphid density, and plant resistance-by- initial aphid density interaction on predator abundance, richness and evenness, modeling trial as a blocking effect.

To evaluate whether predator community composition varied among the different plant types, we conducted permutation multivariate analysis of variance (perMANOVA) using distance matrices (adonis) and the Bray-Curtis dissimilarity coefficient. We used Monte Carlo permutation (10000) to test the significance of the results. The perMANOVA was performed using quantitative data for each species group. We conducted this analysis using R software (R development Core Team 2008) and the Vegan Package (Oksanen *et al.* 2008).

We estimated predation rate as the difference between the predicted aphid density in the absence of predators and the observed aphid density in the presence of predators (chapter 3). The predicted aphid density in the absence of predators was

computed as $I + D*P$, where I is the initial aphid population of the replicate, D is the duration of the experiment for the specific replicate (8-12 days), and P is the *per diem* population growth rate of the aphids in the absence of predators. P is specific for each tomato line, trial and density. We used ANOVA to test the effect of plant resistance, initial aphid density, and plant resistance-by-aphid initial density interaction on predation rate, modeling trial as a blocking effect. We also included a quadratic term (initial aphid-by-initial aphid) to account for the non-linear relationship between predation rate and aphid initial density.

Plant Volatile Organic Compound (VOC) Emissions and Aphid Honeydew Production in Response to Plant Resistance and Aphid Density

To evaluate whether our manipulation of plant resistance affected volatile signaling in response to aphid feeding, we collected volatile organic compound (VOC) emissions induced by all three plants under three different aphid densities in a separate experiment. We again inoculated aphid densities (0, 10 or 100) to the second leaf of low, intermediate and high-resistance tomato plants grown for four-weeks. All leaves were bagged with a spun-polyester sleeve to enclose aphids. Aphids were allowed to feed for 64 hours before we collected the VOCs. We collected VOCs from the entire second leaf using an open-flow dynamic headspace trapping design described in Kessler & Baldwin (2001). Leaves were carefully put inside 16oz plastic cups, which were connected to a pump. Collections were taken over 8 hours in a greenhouse. Each treatment was replicated 5 times for a total of 45 plants.

To assess whether plant resistance affects aphid feeding we collected aphid honeydew in this same experiment. We added previously weighed aluminum foil to each plastic cup used for VOC collection. Aphid honeydew per capita production was then measured by weighing the aluminum foil after 72 hours of aphids feeding and dividing by the number of aphids. We used two-way ANOVA to test for the effect of plant resistance and aphid density on aphid honeydew production.

We used multivariate analysis of variance (MANOVA, Roy's greatest root) to analyze the effects of plant resistance and aphid density (0, 10, 100) on the 25 VOCs collected. Because the MANOVA was significant, we then performed protected ANOVA's (Scheiner 2001) on the individual compounds known to be attractive to predators (methyl salicylate and farnesene). Both methyl salicylate (a phenolic) and farnesene (a sesquiterpene) have been identified in the headspace of several herbivore-infested plant species (Vet & Dicke 1992, de Boer *et al.* 2004, Francis *et al.* 2005, Verheggen *et al.* 2007) and have been demonstrated as predator attractants (3-4 cites). We used two-way ANOVA to test the effect of plant resistance and aphid density on the diversity (Shannon index) of volatile compounds, amount of methyl salicylate and amount of farnesene emitted by the plants, and we included aphid honeydew production as a covariate to control for possible differences in aphid feeding in response to the treatments. When ANOVAs yielded a significant result, we carried out pairwise comparisons between treatments by comparing treatment means with Tukey's HSD tests.

No-Choice Consumption Experiment

We performed a no-choice feeding assay with ladybug predators to directly test whether predators consume different amounts of aphids in response to the plants they were reared on (natal plants) or placed on (receiver plants). We reared aphids on the three plants lines for several generations (3 months). We then offered 10 10-day old aphids to 1 ladybug (*Hippodamia convergens*) in a Petri-dish. We counted the number of aphids in each Petri-dish after 24 h. Therefore, this experiment follows a 3x3 experimental design, where we manipulated the natal (low, intermediate or high-resistance) plants and the receiver (low, intermediate or high-resistance). We performed an ANOVA on the proportion of aphids consumed to test whether predators eat more aphids on the natal, receiver or natal-by-receiver plants.

Aphid Population Growth and Final Density in Response to Plant Resistance and Predator Exposure Treatments

We recorded the number of aphids on each plant after 15 days. We estimated aphid population growth by calculating the daily per capita growth rate of aphids (dN/Ndt) as (ln[N2] –ln[N1])/(t2- t1), where N2 and N1 are the final and initial aphid densities, respectively, divided by the number of days elapsed between initial and final counting (15 days) (Gotelli 2001). We used mixed-effect models to test for the effects of plant resistance and exposure to predators on daily per capita population growth rate. We modeled trial as random effect and initial aphid density (continuous variable), plant resistance (3 levels), predators (2 levels), plant resistance-by-predator, plant resistance-by-initial aphid density, predator-by-initial aphid density and plant

resistance-by-predator-by-initial aphid density as fixed effects. We also performed the same mixed-effect model to test the effects of treatments on aphid final density (ln-transformed). We performed Tukey's HSD post hoc comparison for all mixed-effects test results. We used JMP 10 (SAS Institute Inc, Cary, NC, USA 2012) for all mixed effects models.

Relative Strength of Plant Resistance, Predators and Initial Density on Aphid Population Growth

We used path analysis (i.e., structural equation modeling) to explore our prediction that plant resistance has direct and/or indirect (via changes in predator impact) effects on aphid population growth rate. We examined the strength of each pathway and assessed the importance of increased plant resistance and aphid initial density in driving predator abundance, richness and consumption. When we included predator abundance and richness in the same model it caused multicolinearity because both variables were cross-correlated. Thus, we decided to run two separate models, one with richness and one including abundance. Because the results were similar we only report the results of the model including predator richness. In our path model, we included plant resistance, initial aphid density (ln-transformed), predator richness (square root-transformed), predation rate and aphid population growth rate. We used Systat (Systat Software Inc, Chicago, IL, USA 2004) to run the path analysis.

Results

Predator Responses to Variation in Plant Resistance and Aphid Density

We collected 183 individuals from at least 9 species, including 5 species of ladybugs: *Coleomegilla maculata* (N= 45), *Hippodamia variegata* (N= 36), *Harmonia axyridis* (N= 16), *Propylea quatuordecimpunctata* (N= 10) and *Coccinella septempunctata* (N= 2), which combined accounted for 60% of the total number of predators observed. The other four groups of predators included spiders (N=23), ants (N=2), lacewings (N=2) and unidentified ladybug larvae (N=47). As expected, we found higher abundance and higher richness of predators on high aphid density plants ($F_{1,175}$=8.63, P=0.004; $F_{1,175}$=10.54, P=0.001; Figure 4.1A, C; respectively). We recorded 20% more predators and 77% more species of predators on low-resistance plants than on the two other plant types ($F_{2,175}$=7.68, P<0.001; $F_{2,175}$=3.76; P=0.025; Figure 4.1B, D; respectively). Predator abundance and richness increased with aphid density on low and intermediate resistance plants, but did not respond to aphid density on high-resistance plants ($F_{2,175}$=3.13; P=0.046, $F_{2,175}$=3.36; P=0.037; Figure 4.1A, C). PerMANOVA indicated that plant resistance did not alter the community structure of predators ($F_{2,8}$=0.32, P= 0.279).

Predators consumed 113% more aphids on low-resistance plants compared to intermediate-resistance and 161% more compared to high-resistance plants ($F_{2,174}$ = 22.17, P<0.001, Figure 4.2). Predation rate increased in response to aphid density across all plant types ($F_{2,174}$ = 595.26, P<0.001, Figure 4.2). The relationship between predation rate and aphid density was stronger on low-resistance plants compared to intermediate and high-resistance plants, but it was similar between intermediate and high-resistance plants (Tukey HSD, P<0.05, Figure 4.2).

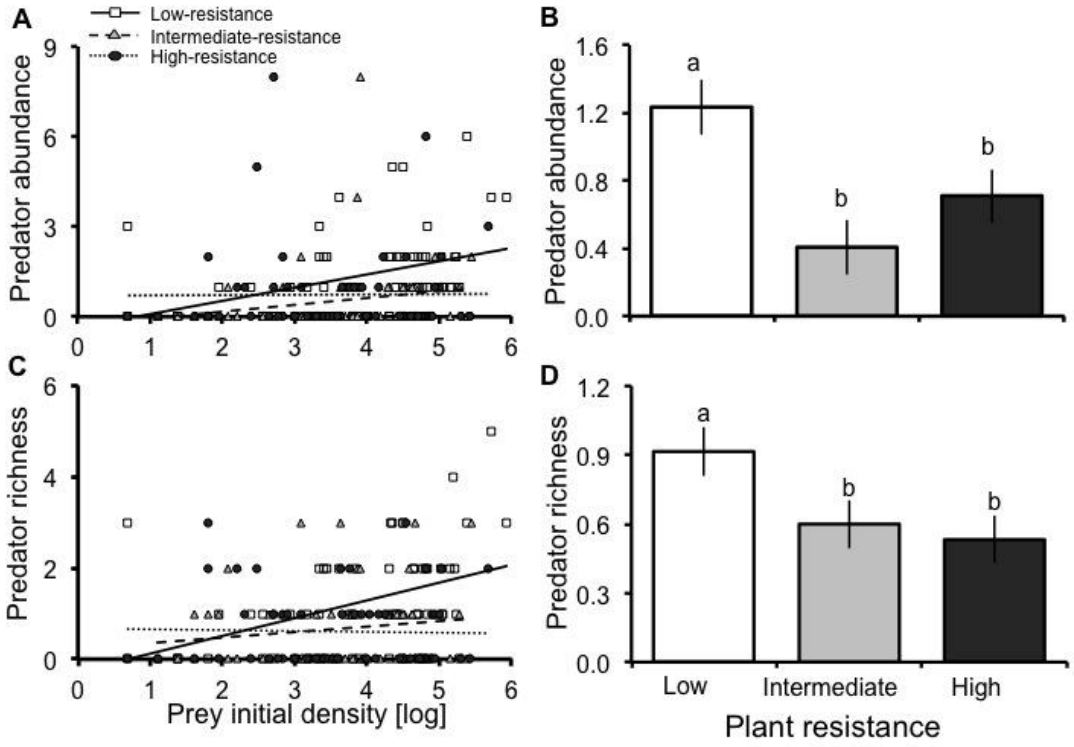

Figure 4.1: Predator responses to aphid density and plant resistance. A) Abundance of predators in response to aphid initial density and plant resistance. B) Predator abundance per plant resistance. C) Predator species richness in response to aphid initial density and plant resistance. B) Predator species richness per plant resistance. Plant resistance levels are: low (solid line and white squares), intermediate (dashed line and gray triangles), and high (dotted line and black circles). Letters above bars indicate significant differences at $P < 0.05$ following Tukey's post-hoc test. Shown are LS-means (±1SE).

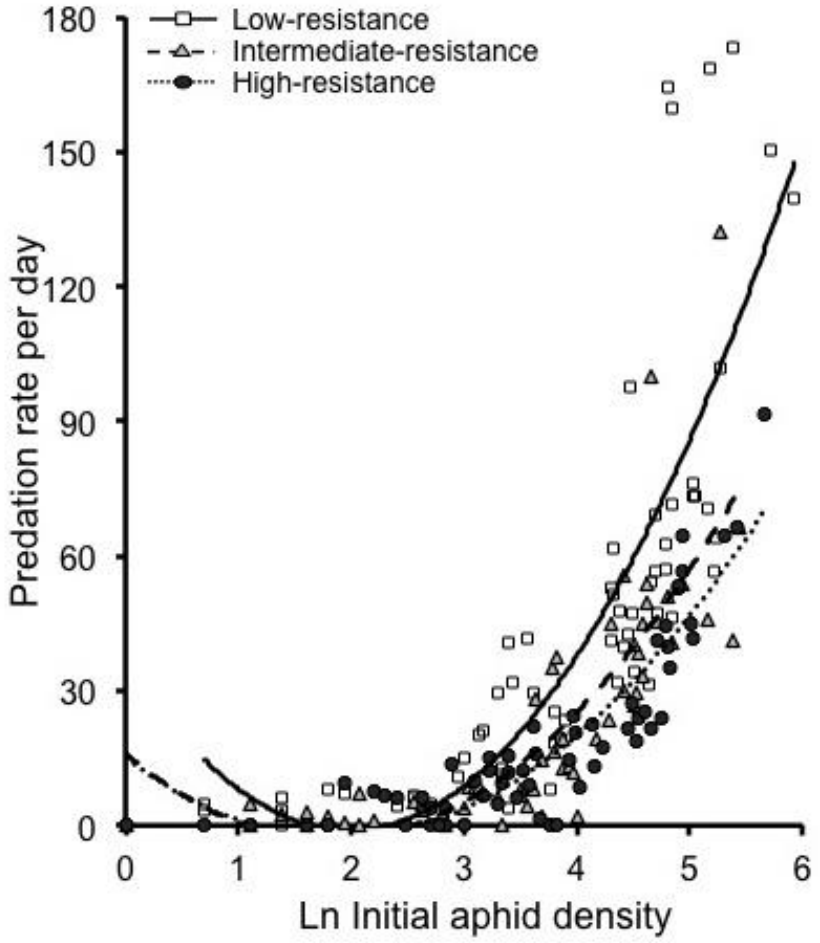

Figure 4.2: Number of aphids consumed by predators per day in response to aphid initial density and plant resistance levels. Plant resistance levels are: low (solid line and white squares), intermediate (dashed line and gray triangles), and high (dotted line and black circles).

Predator No-Choice Consumption Experiment

Predators eat more aphid that were reared on high-resistance natal plants regardless which line is the receiver plants ($F_{2,84}$=4.02, P=0.022, Figure 4.3). Predators did not discriminate aphids from either one of the receiver plants ($F_{2,84}$=0.17, P=0.841). We did not find a natal-by-receiver difference on predators consumption ($F_{2,84}$=0.43, P=0.788). This result demonstrates that predators showed a compensatory consumption, since aphids feeding on high-resistance plants are smaller (chapter 3).

Plant Volatile Organic Compound (VOC) Emissions and Aphid Honeydew Production in Response to Plant Resistance and Aphid Density

The number of VOC emitted was higher at higher aphid density ($F_{1,39}$= 6.46, P=0.015), while plant resistance ($F_{2,39}$= 0.11, P= 0.894) and plant resistance-by-aphid density ($F_{2,39}$ = 4.15, P=0.744) did not affect total VOC emissions (Figure 4.4A). The diversity of VOCs (Shannon index) decreased with aphid density ($F_{1,38}$ = 7.18, P= 0.011), but it was not altered by plant resistance ($F_{2,39}$ = 2.34, P= 0.11), or plant resistance-by-aphid density interaction ($F_{2,38}$ = 0.43, P= 0.655, Figure 4.4B). MANOVA indicated differences among plant resistance lines ($F_{25,13}$ = 9.91, P<0.001), aphid density ($F_{25,13}$ =5.62, P=0.001), and aphid-by-plant resistance ($F_{25,15}$ =4.15, P=0.003) on total VOC emissions.

Methyl salicylate (MeSA) emission increased in response to aphid density ($F_{1,38}$=11.50, P=0.002, Figure 4.4C), was reduced in response to increased plant resistance ($F_{2,38}$ =4.75, P=0.014) but differentially responded to aphid density on the

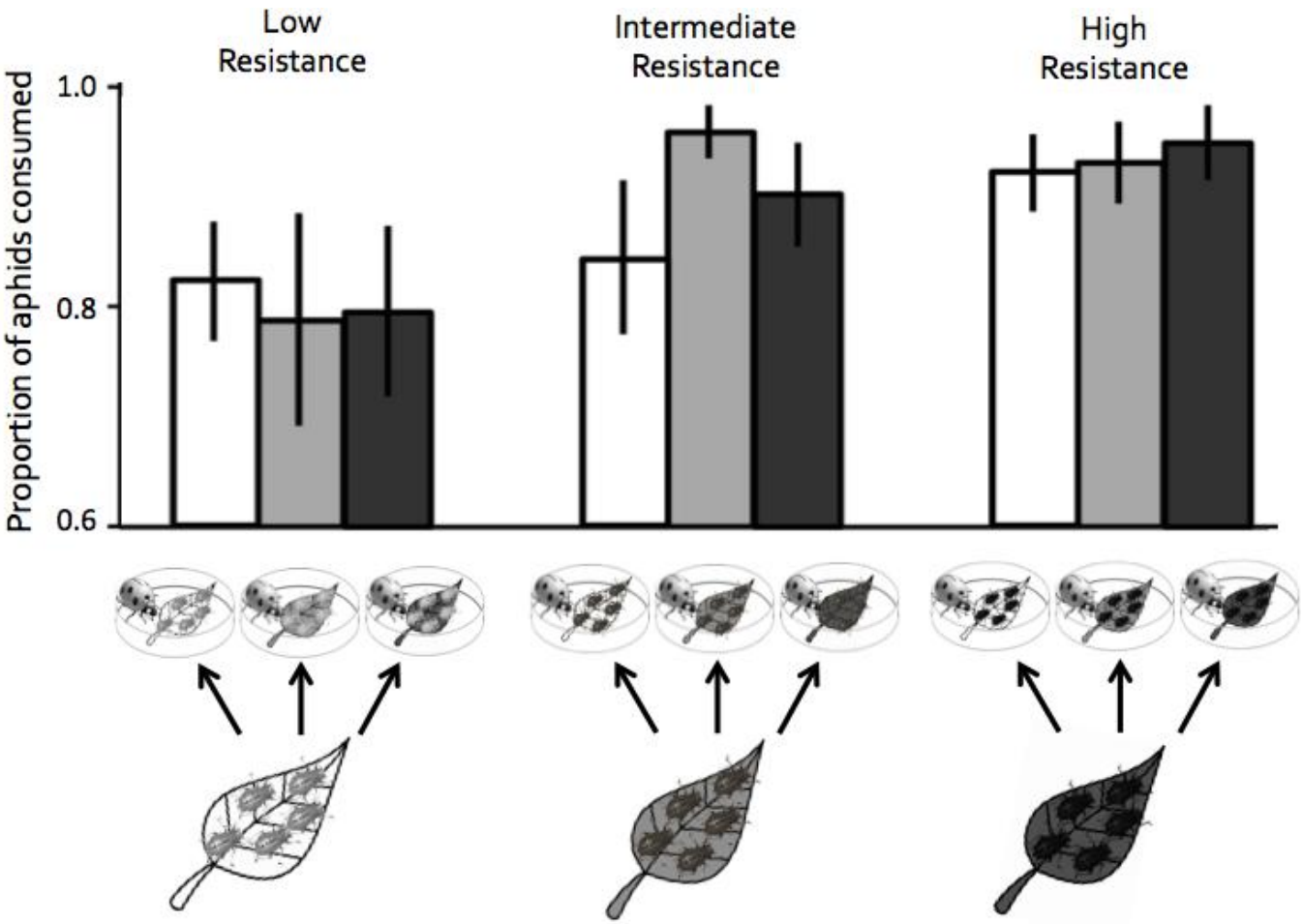

Figure 4.3: Proportion of aphid consumed in a no-choice experiment. Aphids reared on the natal plants (bottom leaves) were offered on the receiver plants (leaves on Petri-dish). Plant resistance levels are: low (white bars), intermediate (gray bars), and high (black bars). Shown are means ±1SE.

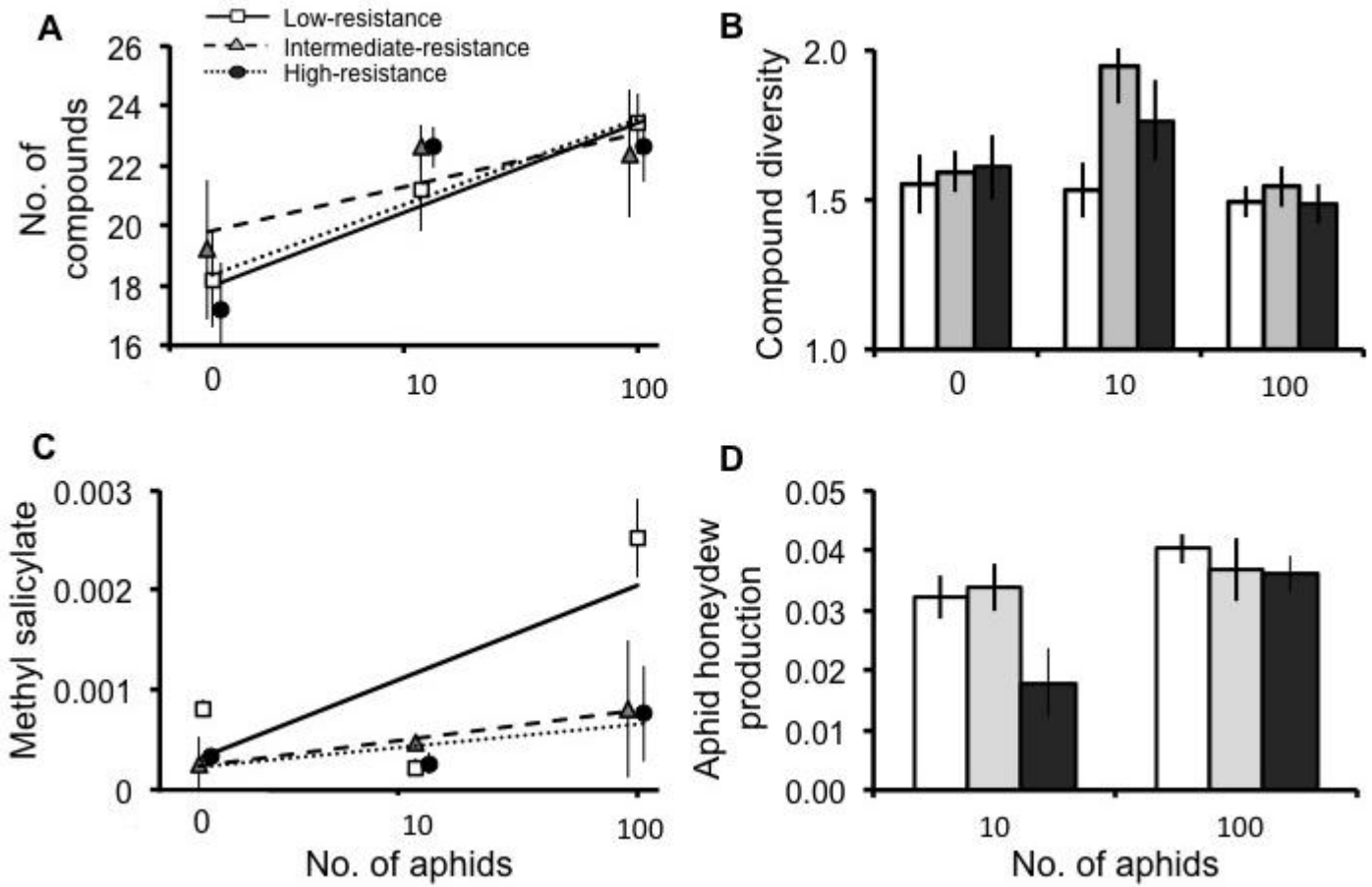

Figure 4.4: Plant volatile organic compound emission and aphid honeydew production in response to variation in plant resistance and aphid density. A) Number of compounds emitted. B) Diversity of compounds emitted (Shannon Index). C) Methyl salicylate induction. D) Aphid per capita honeydew production. Plant resistance levels are: low (solid line and white squares), intermediate (dashed line and gray triangles), and high (dotted line and black circles). Shown are means ±1SE.

different plant lines ($F_{2,38}$ =4.60, P=0.016). Aphids induced higher levels of MeSA on low-resistance plants ($F_{1,12}$ =10.24, P=0.008), while aphids did not alter MeSA emission on intermediate ($F_{1,12}$ =1.53, P=0.239) or high-resistance plants ($F_{1,12}$ =0.43, P=0.526, Figure 4.4C). Aphids induced farnesene ($F_{1,38}$=14.86, P<0.001), which was completely absent in plants without aphids, but this induction was not affected by plant resistance (plant resistance: $F_{2,38}$=0.28, P=0.755; plant resistance-by-aphid density: $F_{2,38}$=0.51, P=0.607).Aphid density increased the total amount of honeydew on all plant-types ($F_{1,24}$=8.162, P=0.009, Figure 4.4D). Total amount of aphid honeydew production was slightly higher on plants with low and intermediate levels of resistance compared to high-resistance plants ($F_{1,24}$=2.965, P=0.071, Figure 4.4D). The effect of aphid density was similar across all plant lines ($F_{1,39}$=1.7362, P=0.198, Figure 4.4D). Although increased aphid density increased the total amount of honeydew, higher densities reduced per capita honeydew production ($F_{1,24}$=41.835, P<0.001). Aphid per capita honeydew production differentially responded to aphid density on the different plant lines ($F_{1,24}$=3.944, P=0.033). The effect of aphid density was stronger on low and intermediate levels of resistance, which suggests that aphids ingest more phloem on those two plant types.

Aphid Population Growth and Final Density in Response to Variation in Plant Resistance and Predator Exposure

Predators reduced aphid population growth rate by 120% and explained 33% of the total variation in population growth rate ($F_{1,336}$=177.35, P<0.001; Figure 4.5A,

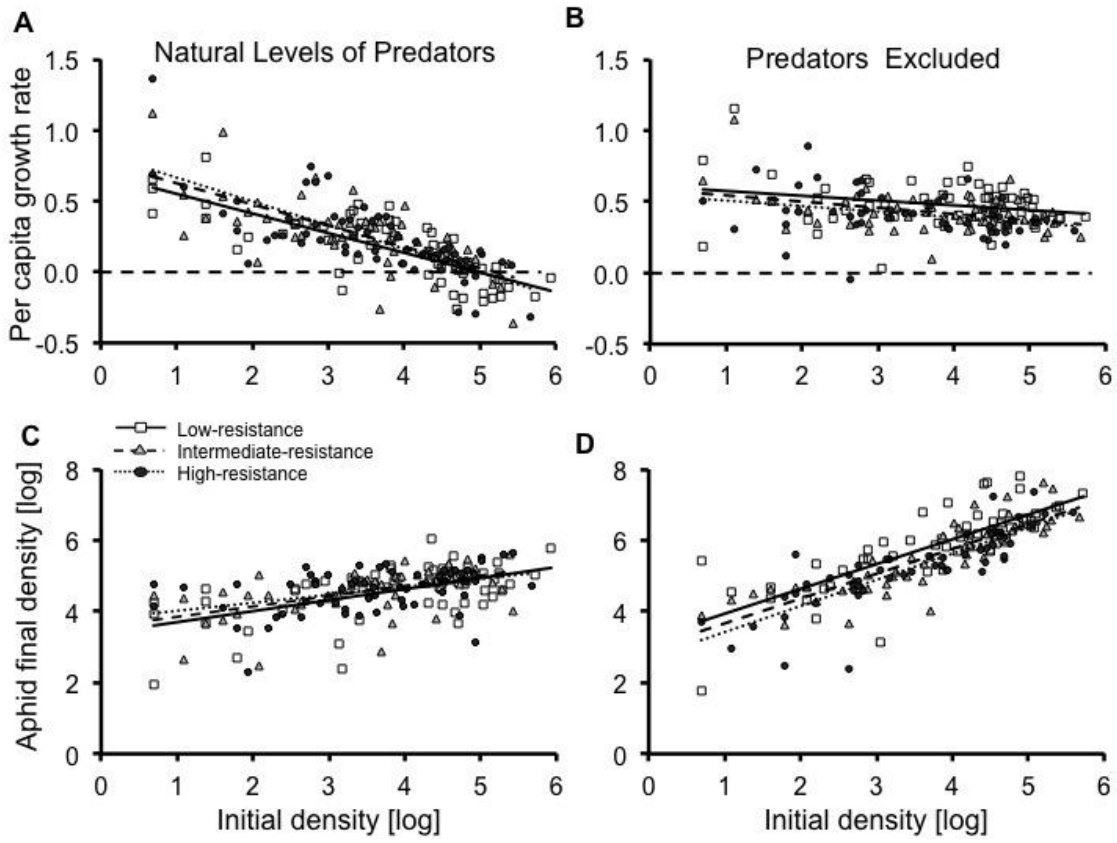

Figure 4.5: Aphid per capita population growth rate and final density in response to predators, plant resistance and initial aphid density. A) Aphid per capita population growth response to initial aphid density and plant resistance when predators were present. B) Aphid population growth response to initial aphid density and plant resistance when predators were excluded. C) Aphid final density in response to initial aphid density and plant resistance when predators were present. D) Aphid final density in response to initial aphid density and plant resistance when predators were excluded. Plant resistance levels are: low (solid line and white squares), intermediate (dashed line and gray triangles), and high (dotted line and black circles).

B). The presence or absence of predators combined with initial aphid density determined whether aphid populations experienced strong or weak density-dependent population growth. In the presence of predators, the strength of the relationship between aphid population growth and initial density was 238% stronger compared to when predators were excluded (Predators-by-initial density interaction: $F_{1,336}$=65.25, P<0.001; Figure 4.5A, 4B). Thus, when aphids were exposed to natural levels of predators they showed strong negative density-dependent population growth (β=-0.186, $F_{1,179}$=208.96, P<0.001, Figure 4.5A). In contrast, in the absence of predators, there was a weak, but still significant, relationship between per capita growth rate and initial density (β =-0.054, $F_{1,159}$=35.67, P<0.001, Figure 4.5B). Plant resistance alone did not affect aphid per capita population growth ($F_{2,336}$=0.31, P=0.737) but we found a plant resistance-by-predator interaction ($F_{2,336}$=6.91, P=0.001), which indicates that high levels of plant resistance reduced aphid population growth rate when predators were excluded. In other words, predators did not control aphid population growth on high-resistance plants.

Aphid population size increased 288% during the experiment, and initial aphid density and predators had a stronger effect on aphid final density than plant resistance (Figure 4.5C, D). Aphid initial density and predators combined to explai 84% of total variation in aphid final density. When predators were excluded, the final abundance of aphids was 260% higher compared to when predators were present ($F_{1,336}$=214.84, P<0.001). Lower initial aphid densities reduced final aphid density when predators were excluded, but aphid initial density had no effect on final aphid density when predators were present (initial density-by-predators: $F_{1,336}$=66.13, P<0.001). This

result demonstrates that predators have a stronger impact on low-resistance plants, reducing the number of aphids on those plants, whereas when predators were excluded, aphids thrived on low-resistance plants (plant resistance-by-predators: $F_{2,336}$=7.236, P<0.001).

Relative Strength of Plant Resistance, Predators and Initial Density on Aphid Population Growth

We used path analysis to test the relative strength of plant resistance, aphid initial density and predators (richness and predation rate) in determining aphid population growth (Figure 4.6). Predator richness did not affect aphid population growth (standardized path coefficient (spc) =-0.032, 95% CI [-0.139, 0.075]), or predation rate (spc=0.073, 95% CI [-0.028, 0.173]). In contrast, predation rate, which was another component of the predator effect included in our model, strongly reduced aphid population growth (spc=-0.233, 99% CI [-0.433, -0.033]).

By using this synthetic analysis we were able test whether plant resistance causes a direct or indirect (through changes in predator impact) on aphid population growth. We found that plant resistance did not directly influence aphid population growth rate (spc=0.028, 95% CI [-0.084, 0.139]), however, plant resistance indirectly affected aphid population growth by reducing predation rate (spc=-0.270, 99.9% CI [-0.434, -0.105]). Aphid initial density directly reduced aphid population growth (spc=-0.519, 99.9% CI [-0.746, -0.292]) and indirectly influenced population growth by increasing predation rate (spc=0.654, 99.9% CI [0.520, 0.789]). Therefore, the

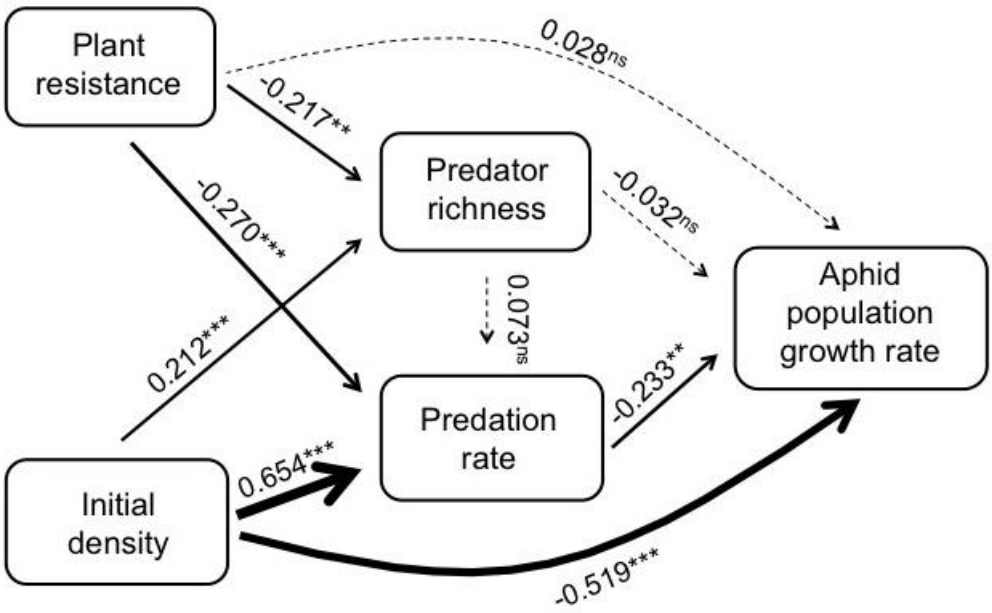

Figure 4.6: Path diagram for the model of the effect of plant resistance, aphid initial density (ln-transformed) on predators (richness and predation rate) and aphid population growth. Solid lines denote significant effects, whereas dashed lines denote non-significant effects. Arrow thickness is scaled to illustrate the relative strength of effects. *P<0.05, **P<0.01, ***P<0.001; ns=not significant.

combined effects of plant resistance, predators and initial density determined aphid population growth.

Discussion

There has been a great deal of interest in understanding the consequences of top-down and bottom-up forces on the population dynamics of herbivores (Price *et al.* 1980, Hunter *et al.* 2000, Johnson 2008). We performed an experiment combining variation in plant resistance, the exclusion of predators, and herbivore density-manipulations to examine the relative strength of their individual and interactive effects on aphid population dynamics. Three results are particularly noteworthy. First, variation in plant resistance and aphid density affected predator abundance and richness; predators showed a density-dependent response to aphid populations on low-resistance plants, but a density-independent response to aphids on high-resistance plants (Figure 4.1 and 4.2). Second, we found that methyl salicylate emission in response to aphid feeding has the potential to be the mechanism for predator increased responses on low-resistance plants and reduced impact on high-resistance plants (Figure 4.4). Lastly, plant resistance indirectly imposed density-dependent population growth on herbivores by influencing predator richness and consumption (Figure 4.5, 4.6). These results show that plant defenses indirectly drive herbivore population dynamics by altering the impact of predators on herbivorous prey.

Predator Responses to Variation in Plant Resistance and Aphid Density

Aphid density and plant resistance level may interact to affect predators in

several ways and below we evaluate several hypotheses for how this may occur. Numerical interactions (i.e. correlated densities) between predators and their prey have been documented in several studies (Murdoch *et al.* 1985, Ponsonby & Copland 2007, Cabral *et al.* 2009, Middleton *et al.* 2013). Aphid density is recognized as major factor driving ladybug predator abundance, richness and consumption (Evans & Dixon 1986, Schellhorn &Andow 2005, de Valpine & Rosenheim 2008, Latham & Mills 2010). Correspondingly, we collected more predators and more predator species on plants with higher aphid densities. However, plant quality mediated this relationship. On low-resistance plants we documented a strong, positive relationship between aphid density and predators, whereas predators did not respond to aphid density on high-resistance plants. Aphid density was controlled experimentally and so is unlikely to be a factor in this study. Variation in plant resistance may also alter production of cues used by predators to find prey.

Adult coccinellids utilize visual and chemical cues to select their prey (Evans & Dixon 1986, Bahlai *et al.* 2008). They not only discriminate prey-infested from non-infested plants (Ninkovic *et al.* 2001, Zhu & Park 2005, Pettersson *et al.* 2008), but they are also able to distinguish the variation among host plant genotypes (Glinwood *et al.* 2011). Numerous mechanisms may underlie the difference in predator response to variation in plant resistance. Although none of these potential mechanisms are mutually exclusive, some are more likely to be linked to our results than others, including herbivore chemical cues (e.g. alarm pheromone and honeydew), structural plants traits, aphid quality, and herbivore-induced chemical cues.

(E)-β-farnesene is a major component of the aphid alarm pheromone (Francis *et al.* 2005, Ahlmedi *et al.* 2010), and it also has the potential to be a predator kaironome. Farnesene was only detected on plants with aphids. The source of emission, the aphids or the plants, remains unclear, but as plant resistance did not affect farnesene emissions it suggests that farnesene emissions are an unlikely mechanism for our findings. Aphid predators have been reported to stay for longer time on plants with aphid excretion (honeydew) (Ide *et al.* 2007, Leroy *et al.* 2010, Leroy *et al.* 2011, Purandare *et al*. 2012). We found a higher amount of honeydew on plants with high aphid density, which was not affected by plant resistance levels. Because this we did not find a aphid density-by-plant resistance interaction, it is likely that honeydew production does not explain our findings. While it is possible the plants are indirectly affecting predators by changing the honeydew quality, the pattern does not match that observed in the study, so it remains an unlikely mechanism.

In a previous study, we showed that these plant lines altered aphid mass; aphids were smaller when feeding on high-resistance plants (Kersch-Becker & Thaler unpublished). Larger body mass, however, does not directly translate into higher predator consumption (Francis *et al.* 2001, Latham & Mills 2010; Aqueel & Leather 2012). Because in our no-choice experiment we found that ladybugs consume more aphids from high-resistance plants we eliminate aphid mass and quality as a mechanism explaining results. If they were to explain our results we would have found higher predator consumption on aphids that were reared on low-resistance plants and lower consumption on high-resistance plants. Predatory insects use herbivore-induced plant volatile cues to locate and kill their prey (Vet & Dicke 1992, Dicke & Van Loon

2000, de Boer *et al.* 2004, Takabayashi *et al.* 2005). Methyl salicylate (MeSA) is considered one of the most important compounds induced by herbivores, and it has been identified in the volatile blends of several plants (Kessler & Baldwin 2001, de Boer et al. 2004, Rodriguez-Saona *et al.* 2011), including tomatoes. A large number of studies have demonstrated that this phenolic compound is used as a kairomone by numerous predatory species (James 2003a, 2003b, James 2005). Because of this predator attraction potential, there has been a dramatic increase in the number of studies that investigate its role in pest management as augmentative biological control (Rodriguez-Saona *et al.* 2011, Kaplan 2012). Here we showed that MeSA is strongly induced by aphids on low-resistance plants. Therefore, higher predator abundance, richness and prey consumption on low-resistance plants with higher aphid densities is likely to be a response of high herbivore-induced chemical signal emitted by those plants.

Relative Strength of Plant Resistance, Predators and Initial Density on Aphid Population Growth

Although we expected that predators would reduce aphid numbers, we were unsure whether they would suppress population growth in such a way that they could generate density dependence. Generalist predators, such as those found in this study, can feed on a variety of prey, which may or may not be spatially or temporally synchronized with the target herbivore species. Because of this behavior they may lack the ability to rapidly respond to increasing herbivore populations (Murdoch *et al.* 1985). Our results do not support this argument as they indicate that generalist

predators can promote herbivore density dependence, which is a critical factor for the regulation of populations. We demonstrated that the strength of the density-dependent aphid population growth was, remarkably, stronger in the presence of predators compared to when predators were excluded.

Using a path analysis, we showed that predator impacts on the prey population were strongly affected by plant resistance levels. Predators caused aphid density-dependent population growth only on low-resistance plants, which induced higher levels of prey-related plant cues as densities rise. When plant resistance levels were high, predators did not reduce aphid densities, and thus, not cause density-dependent population growth. Plants and predators can only act synergistically on regulating aphid population when herbivore-induced plant cues correlate positively with herbivore densities. When induced plant responses are low or non-existent the ability of predators to find prey is reduced and so is their impact on prey numbers.

Variation in plant resistance can have direct and indirect ecological effects on predator-prey interactions, when differences in resistance affect the abundance, richness and prey consumption of predators. These relationships are particularly important for agricultural systems, in which plant resistance and biocontrol agents are frequently combined to reduce pests. Our results suggest that plant-induced responses to prey should be accounted for in biological control assessments, as it determines the efficiency of biocontrol agents on target prey. By factorially manipulating plant resistance, aphid density and predator exposure, we showed that predators can only control insect populations when herbivorous prey feed on high quality plants that can induce high herbivore-associated volatile cues.

Acknowledgements

We thank Rayko Halitschke for his valuable assistance in the volatile organic compound analyses; Sara Ali, Gui Becker and Marie Russell for their help with field and laboratory work. This study strongly benefited from comments and suggestions by Anurag Agrawal, Alison Power, Gui Becker and Suzi Claflin. This project received financial support from the USDA Grant 2006-35302-17431, the Department of Ecology and Evolutionary Biology and the Department of Entomology at Cornell. MFKB is recipient of the CAPES/Fulbright fellowship (BEX 2234-08-4). The experiments comply with the current laws of the United States of America, in which the experiments were performed. Appropriate permits were obtained for the use of transgenic plants.

REFERENCES

Agrawal, A.A., Underwood, N. & Stinchcombe, J.R. (2004) Intraspecific variation in the strength of density dependence in aphid populations. *Ecological Entomology*, **29**, 521–526.

Alhmedi, A.M., Haubruge, E.R.I.C. & Francis, F.R. (2010) Identification of limonene as a potential kairomone of the harlequin ladybird *Harmonia axyridis* (Coleoptera : Coccinellidae). *European Journal of Entomology*, **107**, 541–548.

Alyokhin, A., Drummond, F. a. & Sewell, G. (2005) Density-dependent regulation in populations of potato-colonizing aphids. *Population Ecology*, **47**, 257–266.

Aqueel, M. a. & Leather, S.R. (2012) Nitrogen fertiliser affects the functional response and prey consumption of *Harmonia axyridis* (Coleoptera: Coccinellidae) feeding on cereal aphids. *Annals of Applied Biology*, **160**, 6–15.

Bahlai, A.C.A., Welsman, J.A., Macleod, E.C., Schaafsma, A.W. & Sears, M.K. (2008) Role of visual and olfactory cues from agricultural hedgerows in the orientation behavior of multicolored Asian lady beetle (Coleoptera : Coccinellidae). *Environmental Entomology*, **37**, 973–979.

De Boer, J.G., Posthumus, M. a & Dicke, M. (2004) Identification of volatiles that are used in discrimination between plants infested with prey or nonprey herbivores by a predatory mite. *Journal of Chemical Ecology*, **30**, 2215–30.

Bottrell, D.G., Barbosa, P. & Gould, F. (1998) Manipulating natural enemies by plant variety selection and modification: a realistic strategy? *Annual Review of Entomology*, **43**, 347–367.

Cabral, S., Soares, A.O. & Garcia, P. (2009) Predation by *Coccinella undecimpunctata* L. (Coleoptera: Coccinellidae) on *Myzus persicae* Sulzer (Homoptera: Aphididae): Effect of prey density. *Biological Control*, **50**, 25–29.

Dicke, M. & van Loon, J.J. a. (2000) Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context. *Entomologia Experimentalis et Applicata*, **97**, 237–249.

Donaldson, J.R., Myers, S.W. & Gratton, C. (2007) Density-dependent responses of soybean aphid (*Aphis glycines* Matsumura) populations to generalist predators in mid to late season soybean fields. *Biological Control*, **43**, 111–118.

Doumbia, M., Hemptinne, J.-L. & Dixon, A.F.G. (1998) Assessment of patch quality by ladybirds : role of larval tracks. *Oecologia*, **113**, 197–202.

Evans, E.W. & Dixon, A.F.G. (1986) Cues for oviposition by ladybird beetles (Coccinellidae): response to aphids. *Journal of Animal Ecology*, **55**, 1027–1034.

Francis, F., Haubruge, E., Hastir, P. & Gaspar, C. (2001) Effect of aphid host plant on development and reproduction of the third trophic level, the predator *Adalia bipunctata* (Coleoptera: Coccinellidae). *Environmental Entomology*, **30**, 947–952.

Francis, F., Martin, T., Lognay, G. & Haubruge, E. (2005) Role of (E)-B-farnesene in systematic aphid prey location by *Episyrphus balteatus* larvae (Diptera: Syrphidae). *European Journal of Entomology*, **102**, 431–436.

Glinwood, R., Ahmed, E., Qvarfordt, E. & Ninkovic, V. (2011) Olfactory learning of plant genotypes by a polyphagous insect predator. *Oecologia*, **166**, 637–47.

Gotelli, N.J. (2001) *A Primer of Ecology*, 3rd ed. Sinauer, Sunderland, Massachussets.

Hare, D.J. (2002) Plant genetic variation in tritrophic interactions. *Multitrophic level interactions* (eds T. Tscharntke & B.A. Hawkins), pp. 8–43. Cambridge University Press, Cambridge, UK.

Harrison, S.P. & Cappuccino, N. (1995) Using density manipulation experiments to study population regulation. *Population dynamics: new approaches and synthesis* (eds N. Cappuccino & P.W. Price), pp. 131–147. Academic press, San Diego.

Hassell, B.Y.M.P., Latto, J. & May, R.M. (1989) Seeing the wood for the trees: detecting density dependence from existing life-tables studies. *Journal of Animal Ecology*, **58**, 883–892.

Hemptinne, J., Doumbia, M. & Dixon, A.F.G. (2000) Assessment of patch quality by ladybirds: role of aphid and plant phenology. *Journal of Insect Behavior*, **13**, 353–359.

Ide, T., Suzuki, N. & Katayama, N. (2007) The use of honeydew in foraging for aphids by larvae of the ladybird beetle, *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *Ecological Entomology*, **32**, 455–460.

Inbar, M., Doostdar, H., Sonoda, R.M., Leibee, G.L., Mayer, R.T. & Ave, E.C. (1998) Elicitors of plant defensive systems reduce insect densities and disease incidence. *Journal of Chemical Ecology*, **24**, 135–149.

James, D.G. (2003a) Synthetic herbivore-induced plant volatiles as field attractants for Beneficial insects synthetic herbivore-induced plant volatiles as field attractants for beneficial insects. *Environmental Entomology*, **32**, 977–982.

James, D.G. (2003b) Field evaluation of herbivore-induced plant volatiles as attractants for beneficial insects: methyl salicylate and the green lacewing, Chrysopa nigricornis. *Journal of Chemical Ecology*, **29**, 1601–9.

James, D.G. (2005) Further field evaluation of synthetic herbivore-induced plan volatiles as attractants for beneficial insects. *Journal of Chemical Ecology*, **31**, 481–495.

Johnson, M.T.J. (2008) Bottom-up effects of plant genotype on aphids, ants, and predators. *Ecology*, **89**, 145–54.

Kaplan, I. (2012) Attracting carnivorous arthropods with plant volatiles: The future of biocontrol or playing with fire? *Biological Control*, **60**, 77–89.

Kaplan, I. & Thaler, J.S. (2012) Phytohormone-mediated plant resistance and predation risk act independently on the population growth and wing formation of potato aphids, *Macrosiphum euphorbiae*. *Arthropod-Plant Interactions*, **6**, 181–186.

Kessler, A. & Baldwin, I.T. (2001) Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, **291**, 2141–2144.

Latham, D.R. & Mills, N.J. (2010) Quantifying aphid predation: the mealy plum aphid *Hyalopterus pruni in* California as a case study. *Journal of Applied Ecology*, **47**, 200–208.

Leroy, P.D., Heuskin, S., Sabri, A., Verheggen, F.J., Farmakidis, J., Lognay, G., Thonart, P., Wathelet, J.-P., Brostaux, Y. & Haubruge, E. (2012) Honeydew volatile emission acts as a kairomonal message for the Asian lady beetle *Harmonia axyridis* (Coleoptera: Coccinellidae). *Insect Science*, **19**, 498–506.

Leroy, P.D., Sabri, A., Heuskin, S., Thonart, P., Lognay, G., Verheggen, F.J., Francis, F., Brostaux, Y., Felton, G.W. & Haubruge, E. (2011) Microorganisms from aphid honeydew attract and enhance the efficacy of natural enemies. *Nature Communications*, **2**, 1–7.

Li, L., Li, C., Lee, G.I. & Howe, G. a. (2002) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. *Proceedings of the National Academy of Sciences of the United States of America*, **99**, 6416–21.

Li, L., Zhao, Y., Mccaig, B.C., Wingerd, B.A., Wang, J. & Mark, E. (2004) The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation , jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell*, **16**, 126–143.

Marquis, R.J. & Whelan, C. (1996) Plant morphology and recruitment of thrid trophic level: subtle and little-recognized defenses? *Oikos*, **75**, 330–334.

Mashanova, A., Gange, A.C. & Jansen, V. a. a. (2008) Density-dependent dispersal may explain the mid-season crash in some aphid populations. *Population Ecology*, **50**, 285–292.

McGurl, B., Orozco-Cardenas, M., Pearce, G. & Ryan, C. a. (1994) Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that

constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the United States of America*, **91**, 9799–802.

Middleton, A.D., Kauffman, M.J., McWhirter, D.E., Jimenez, M.D., Cook, R.C., Cook, J.G., Albeke, S.E., Sawyer, H. & White, P.J. (2013) Linking anti-predator behaviour to prey demography reveals limited risk effects of an actively hunting large carnivore. *Ecology Letters*, **16**, 1023–30.

Murdoch, W.W., Chesson, J. & Chesson, P.L. (1985) Biological control in theory and practice. *American Naturalist*, **125**, 344–366.

Ninkovic, V., Al Abassi, S., Ahmed, E., Glinwood, R. & Pettersson, J. (2011) Effect of within-species plant genotype mixing on habitat preference of a polyphagous insect predator. *Oecologia*, **166**, 391–400.

Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G.L., Solymos, P., Stevens, M.H.H. & Wagner, H. (2008) Vegan: community ecology package. *R Foundation for Statistical Computing* Vienna, Austria.

Paré, P.W. & Tumlinson, J.H. (1996) De novo biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant physiology*, **114**, 1161–1167.

Pettersson, J., Ninkovic, V., Glinwood, R., Al Abassi, S., Birkett, M., Pickett, J. & Wadhans, L. (2008) Chemical stimuli supporting foraging behaviour of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae): volatiles and allelobiosis. *Applied entomology and zoology*, **43**, 315–321.

Pettersson, J., Ninkovic, V., Glinwood, R., Birkett, M.A. & Pickett, J.A. (2005) Foraging in a complex environment – semiochemicals support searching

behaviour of the seven spot ladybird. *European Journal of Entomology*, **102**, 365–370.

Ponsonby, D.J. & Copland, M.J.W. (2007) Influence of host density and population structure on egg production in the coccidophagous ladybird, *Chilocorus nigritus* F. (Coleoptera: Coccinellidae). *Agricultural and Forest Entomology*, **9**, 287–296.

Preisser, E.L., Orrock, J.L. & Schmitz, O.J. (2007) Predator hunting mode and habitat domain alter nonconsumptive effects in predator-prey interactions. *Ecology*, **88**, 2744–51.

Price, P.W., Bouton, C.E., Gross, P., McPheron, B.A., Thompson, J.N. & Weis, A.E. (1980) Interactions among three trophic levels:influence of plant interactions between insect herbivores and natural enemies. *Annual Review Of Ecology And Systematics*, **11**, 41–65.

Purandare, S.R. & Tenhumberg, B. (2012) Influence of aphid honeydew on the foraging behaviour of *Hippodamia convergens* larvae. *Ecological Entomology*, **37**, 184–192.

Rhoades, D.F. (1985) Offensive-defensive interactions between herbivores and plants:their relevance in herbivore population dynamics and ecological theory. *The American Naturalist*, **125**, 205–238.

Rodriguez-Saona, C., Kaplan, I., Braasch, J., Chinnasamy, D. & Williams, L. (2011) Field responses of predaceous arthropods to methyl salicylate: A meta-analysis and case study in cranberries. *Biological Control*, **59**, 294–303.

Rotem, K.A. & Agrawal, A.A. (2003) Density dependent population growth of the two-spotted spider mite , *Tetranychus urticae* , on the host plant *Leonurus cardiaca*. *Oikos*, **103**, 559–565.

Scheiner, S.M. (2001) MANOVA: Multiple response variables and multispecies interactions. *Design and analysis of ecological Experiments*, 2nd ed (eds S.M. Scheiner & J. Gurevitch), pp. 99–115. Oxford University Press, Inc, New York.

Schellhorn, N. a. & Andow, D. a. (2005) Response of coccinellids to their aphid prey at different spatial scales. *Population Ecology*, **47**, 71–76.

Shannag, H.K. & Obeidat, W.M. (2008) Interaction between plant resistance and predation of *Aphis fabae* (Homoptera: Aphididae) by *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Annals of Applied Biology*, **152**, 331–337.

Styrsky, J.D., Kaplan, I. & Eubanks, M.D. (2006) Plant trichomes indirectly enhance tritrophic interactions involving a generalist predator, the red imported fire ant. *Biological Control*, **36**, 375–384.

Takabayashi, J., Sabelis, M.W., Janssen, A., Shiojiri, K. & Wijk, M. (2005) Can plants betray the presence of multiple herbivore species to predators and parasitoids? The role of learning in phytochemical information networks. *Ecological Research*, **21**, 3–8.

Team, R. development core. (2008) R: A language and environment for statistical computing.

Thaler, J.S., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1999) Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597–1609.

Underwood, N. (2009) Effect of genetic variance in plant quality on the population dynamics of a herbivorous insect. *Journal of Animal Ecology*, **78**, 839–847.

Underwood, N. (2010) Density dependence in insect performance within individual plants: induced resistance to *Spodoptera exigua* in tomato. *Oikos*, **119**, 1993–1999.

Underwood, N. & Rausher, M.D. (2000) The effects of host-plant genotype on herbivore population dynamics. *Ecology*, **81**, 1565–1576.

Underwood, N. & Rausher, M. (2002) Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. *The American Naturalist*, **160**, 20–30.

De Valpine, P. & Rosenheim, J. a. (2008) Field-scale roles of density, temperature, nitrogen, and predation on aphid population dynamics. *Ecology*, **89**, 532–41.

Verheggen, F.J., Fagel, Q., Heuskin, S., Lognay, G., Francis, F. & Haubruge, E. (2007) Electrophysiological and behavioral responses of the multicolored Asian lady beetle, *Harmonia axyridis pallas*, to sesquiterpene semiochemicals. *Journal of Chemical Ecology*, **33**, 2148–2155.

Vet, L.E.M. & Dicke, M. (1992) Ecology of info chemical use by natural enemies in a tritrophic context. *Annual Review of Entomology*, **37**, 141–172.

Walling, L.L. (2000) The myriad plant responses to herbivores. *Journal of Plant Growth and Regulation*, **19**, 195–216.

Walling, L.L. (2008) Avoiding effective defenses: strategies employed by phloem-feeding insects. *Plant Physiology*, **146**, 859–66.

Yang, L.H. (2000) Effects of body size and plant structure on the movement ability of a predaceous stinkbug, *Podisus maculiventris* (Heteroptera: Pentatomidae). *Oecologia*, **125**, 85–90.

Yu, H., Zhang, Y., Wu, K., Gao, X.W. & Guo, Y.Y. (2008) Field-testing of synthetic herbivore-induced plant volatiles as attractants for beneficial insects. *Environmental Entomology*, **37**, 1410–1415.

Zhu, J. & Park, K.-C. (2005) Methyl salicylate, a soybean aphid-induced plant volatile attractive to the predator *Coccinella septempunctata*. *Journal of Chemical Ecology*, **31**, 1733–1746.

APPENDIX

SUPPLEMENTARY INFORMATION

Table S3.1: Specific number of replicates per treatment are shown. The number of replicates for the number of nymphs produced in the field differed from the other variables and are shown separated.

Plant resistance and predation in the field	N
Low resistance + no predators	23
Low resistance + predators	26
Intermediate resistance + no predators	30
Intermediate resistance + predators	30
High resistance + no predators	29
High resistance + predators	30
Number of nymphs produced in the field	
Low resistance + no predators	15
Low resistance + predators	17
Intermediate resistance + no predators	18
Intermediate resistance + predators	18
High resistance + no predators	18
High resistance + predators	18
Plant resistance and predation in the laboratory	
Low resistance + no predators	10
Low resistance + predators	10
Intermediate resistance + no predators	9
Intermediate resistance + predators	9
High resistance + no predators	10
High resistance + predators	10
Plant resistance and predation risk in the laboratory	N
Low resistance + no predators	10
Low resistance + high predation risk	10
Low resistance + low predation risk	10
Low resistance + predators	10
Intermediate resistance + no predators	9
Intermediate resistance + low predation risk	9
Intermediate resistance + high predation risk	8
Intermediate resistance + predators	9
High resistance + no predators	10
High resistance + low predation risk	9
High resistance + high predation risk	11
High resistance + predators	10