INFLUENCES OF PLANT QUALITY AND MATERNAL ENVIRONMENT ON THE PERFORMANCE AND POPULATION DYNAMICS OF A PHLOEM-FEEDING INSECT HERBIVORE

by

CARALYN BETH ZEHNDER

(Under the Direction of Mark. D. Hunter)

ABSTRACT

An organism's population dynamics can be influenced by its current environment, as well as the environment experienced by previous generations. Maternal effects, the influence of maternal phenotype or environment on offspring phenotype, have been implicated in the generation of population cycles. For insect herbivores, plant quality is an obvious factor through which maternal effects might operate.

Aphis nerii, the milkweed-oleander aphid, is a phloem-feeding specialist of milkweed and oleander. Its host plants, species in the genus *Asclepias*, exhibit variation in many traits important to insect herbivores including foliar nitrogen concentrations, cardenolide concentrations and trichome densities.

I first describe a set of experiments examining the effects of plant quality, insect clone and insect density on aphid vital rates (birth, death & migration). The importance of these factors was systematically assessed in both the maternal and offspring generation. While maternal effects were present, within generation effects on vital rates were much stronger and therefore more likely to impact aphid population dynamics.

I next report an experimental examination of the effects of maternal age, density and host plant species on offspring vital rates. Of the three factors studied, maternal age had the largest influence on offspring vital rates. However, maternal age effects were not large in magnitude. Together, the first two studies indicate that while maternal effects are present in this system, it is unlikely that they strongly influence aphid population dynamics.

I then present a density-manipulation experiment examining the effects of simulated nitrogen deposition on the interaction between aphids and their host plant. Nitrogen deposition increased plant foliar nitrogen concentrations, plant biomass and aphid per capita population growth. Nitrogen deposition caused aphid R_{max} and K to increase proportionally, leading to no change in the strength of density-dependence.

Finally, I discuss the effects of variation in aphid density on induced plant responses. Aphid density influenced plant chemistry and biomass, and the effect varied among plant species. However, in no case was there an increased expression of plant defensive compounds at higher aphid densities.

INDEX WORDS: *Aphis nerii*, *Asclepias*, cardenolides, density-dependence, milkweeds, maternal effects, nitrogen deposition, plant quality

INFLUENCES OF PLANT QUALITY AND MATERNAL ENVIRONMENT ON THE PERFORMANCE AND POPULATION DYNAMICS OF A PHLOEM-FEEDING INSECT HERBIVORE

by

CARALYN BETH ZEHNDER

B.S., Pennsylvania State University, 1999

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2006

INFLUENCES OF PLANT QUALITY AND MATERNAL ENVIRONMENT ON THE PERFORMANCE AND POPULATION DYNAMICS OF A PHLOEM-FEEDING INSECT HERBIVORE

by

CARALYN BETH ZEHNDER

Major Professor: Mark Hunter

Committee: C. Ronald Carroll
Pejman Rohani
Kenneth Ross
Michael Strand

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2006

ACKNOWLEDGEMENTS

Many people have been integral to the research presented here. Mark Hunter has been an incredible advisor. He has been, and will continue to be, a role model for me. In addition to reading more drafts of this dissertation than I care to admit, Mark also ran all of the cardenolide analyses detailed in Chapter 5. My other committee members (present and past): Ron Carroll, MaryCarol Hunter, Becky Irwin, Pej Rohani, Ken Ross and Mike Strand have been extremely helpful and generous with their time, expertise and advice.

I have been lucky enough to work with bright and enthusiastic undergraduates here at the University of Georgia. Jeremy Castings, Paige Doty, David Egetter, Blake Joyce, Melissa Parris and Ashley Whittaker were all great to work with.

Tom Maddox and the Institute of Ecology Analytical Laboratory provided analytical support. The Institute of Ecology administrative staff was very helpful. Both Ron Carroll and Mark Bradford were generous with laboratory space and equipment. Ecograds, both past and present, made Athens and the Institute of Ecology a great place to be.

The Hunter lab of years past and the current orphaned Hunter lab have provided much advice, lab help and just made grad school a lot more fun than it would have been otherwise. Thank you: Becky Ball, Chris Frost, Carmen Hall, Sandy Spires and Kyle Wickings. Star Scott was a fabulous lab manager, and I really missed her during my final year.

My parents and family have been extremely supportive. Even if my research doesn't show it, I know that parental effects are very important and have long-lasting impacts.

Will Duncan in addition to reading manuscripts, discussing research ideas, keeping me sane and always being supportive – he even helped me count aphids.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

INTRODUCTION & LITERATURE REVIEW

For decades, population ecologists have attempted to tease apart the forces governing population change and to uncover which, of a myriad of possible choices, are most important in determining population dynamics, with the goal of eventually being able to predict future dynamics (Berryman 1999).

Maternal effects, the effect of a mother's (parent's) phenotype or environment on offspring phenotype (Mousseau and Fox 1998b), have the potential to influence the population dynamics of future generations (Benton et al. 2005) and, because they act on a time lag, to cause population cycles (Beckerman et al. 2002, Ginzburg and Colyvan 2004). While maternal effects are ubiquitous (Bernardo 1996, Mousseau and Fox 1998b), their persistence, strength, and dynamical consequences remain unclear (Berryman 1995, Turchin and Hanski 2001, Kendall et al. 2005, Beckerman et al. 2006, Plaistow et al. 2006).

For insect herbivores, variation in plant quality can influence both current and future generations (Denno and McClure 1983, Hunter and Price 1992, Underwood and Rausher 2000, McIntyre and Whitham 2003). Therefore, anthropogenic changes that influence plant quality are also likely to effect the population dynamics of insect herbivores (Hughes and Bazzaz 2001, Throop and Lerdau 2004, Hall et al. 2005).

This introductory chapter summarizes our current understanding of maternal effects on population dynamics and explains the motivation behind the hypotheses explored in subsequent chapters. I begin by reviewing the potential role of maternal effects in population dynamics and specifically in aphid biology. I then consider the effects of plant quality on insect herbivores and feedbacks from insects to plants via induced plant responses. Finally, I introduce my study system and present the specific hypotheses tested in subsequent chapters.

Maternal effects and population dynamics

Non-Mendelian parental effects occur when the phenotype or environment of the parent affects the phenotype of the offspring (Mousseau and Fox 1998b). Parents obviously provide a direct genetic contribution to their offspring, but maternal effects are the nongenetic transfer of information across generations. Maternal effects are ubiquitous, having been found in most taxa and for many characters for which they have been sought (Bernardo 1996, Mousseau and Fox 1998a, Mousseau and Fox 1998b).

Birth, **I**mmigration, **D**eath and **E**migration (BIDE) are the only four processes that can change the size of a population. I surveyed the insect literature (using ISI's Web of Science © , covering from 1945-2006, and searching approximately 50 journals) to locate examples of maternal effects that directly influence any of these four processes (Table 1.1). For example, gypsy moth daughters emerging from large eggs have higher fecundities than those from small eggs (Rossiter 1991b). Immigration and emigration were combined into one category in Table 1.1. Most of these examples refer to the production of dispersing morphs. The final category, death, contains examples of

maternal effects that were shown to influence survival. For example, maternal nutrition was found to influence offspring viability in the western tent caterpillar, *Malacosoma pluviale* (Wellington 1965).

Maternal effects can generate population cycles if two requirements are met: 1) they must influence one of the four population processes (birth, death, immigration, or emigration) and 2) their operation is density dependent. By connecting the parental environment or phenotype to the offspring phenotype, maternal effects introduce a time lag into population dynamics (Rossiter 1994, Beckerman et al. 2002). This time lag causes a delay in the density dependent response which can destabilize population dynamics and lead to cycles (Turchin 1990, Royama 1992, Berryman 1999). In other words, by influencing density dependent birth, death, immigration or emigration rates on delay, maternal effects have the potential to lead to population oscillations (Ginzburg and Taneyhill 1994, Ginzburg 1998, Inchausti and Ginzburg 1998, Benton et al. 2001, Ginzburg and Colyvan 2004, Benton et al. 2006).

A number of theoretical models have been developed that include maternal effects, though currently there is no consensus as to how this should be done (Berryman 1995, Benton et al. 2001, Beckerman et al. 2002, Kendall et al. 2005, Plaistow et al. 2006). Ginzburg and Taneyhill (1994) developed a model that includes offspring quality, (which in turn influences survival and fecundity) as a function of maternal quality. Their model produces cyclic population dynamics that fit the dynamics of six species of forest Lepidoptera better than delayed logistic models (Ginzburg and Taneyhill 1994). A similar model by Inchausti and Ginzburg (1998) was used to explore the population cycles of voles. In this model, changes in individual quality affect population growth

rates. Maternal effects are the method for the phenotypic transmissions of individual quality across breeding periods, which is a mechanism to generate delayed density dependence (Inchausti and Ginzburg 1998). However, it has been shown recently that a different model incorporating species interactions and not maternal effects fits those data better (Turchin and Hanski 2001). Ginzburg (1998) has shown how maternal effects can be a way to incorporate 'inertial growth' into population dynamics, and that perhaps it is better to measure changes in population growth rather than changes in abundance (Ginzburg 1998). This idea is further developed by Ginzburg and Colyvan (2004). Using physics and planetary orbits as a metaphor, they describe how the maternal environment affects the 'acceleration' (rate of change of the growth rate) of subsequent generations. They argue that population growth is a second-order dynamics process and that this second order process is due to maternal effects on the rate of change of population growth rate and not species (predator-prey) interactions (Ginzburg and Colyvan 2004).

Based upon her empirical work (Rossiter 1991a, b, Rossiter et al. 1993, Rossiter 1998), Rossiter (1994) developed a maternal effects hypothesis for herbivore outbreaks. She hypothesized that population quality in the parental generation is expressed on a time delay and influences population quality in subsequent generations via maternal effects. Maternal effects provide the means for a herbivore population to escape regulation by its predator leading to an outbreak. The density-dependent relationship between enemy and herbivore is temporarily lost leading to a disproportionately higher or lower than normal number of herbivores. The loss of the connection between an insect herbivore and its predator leads to outbreak dynamics (Rossiter 1994).

The strongest empirical evidence documenting the effects of maternal environment on population dynamics has been collected by Benton and colleagues working with the soil mite *Sancassania berlesei* (Benton et al. 2001, Beckerman et al. 2002, Plaistow et al. 2004, Benton et al. 2005, Beckerman et al. 2006, Plaistow et al. 2006). In their system, food availability in the maternal environment affects offspring age and size at maturity. Specifically, high maternal food availability reduces the slope of the reaction norm of age and size at maturity while low maternal food availability increases the slope (Plaistow et al. 2004). Additionally, maternal environment (food availability and age) influences subsequent population dynamics for up to three generations. The mechanism is the trade-off between female fecundity and per-egg provisioning of protein which depends on maternal food availability and age. This differential provisioning of offspring leads to differences in growth, maturation and fecundity which lead to differences in population dynamics (Benton et al. 2005). Maternal effects are context dependent and depend on the maternal and subsequent offspring environments (Beckerman et al. 2006, Plaistow et al. 2006). Low food availability in the maternal environment weakens the maternal effect because of a lack of variation in egg size. Conversely, high food in the maternal environment generates increasing variation in egg size which then leads to strong effects in later generations (Plaistow et al. 2006). Finally, the maternal environment, through its effects on maternal fecundity, changes the offspring competitive environment and these changes in offspring competitive environment have a stronger impact on offspring performance than changes in offspring quality (Beckerman et al. 2006).

Empirical evidence is equivocal concerning the influence of maternal effects on population dynamics in the field (Yerger and Rossiter 1996, Erelli and Elkinton 2000). Thus far, in only one system, the pine looper moth in Britain, has there been both empirical evidence supporting the maternal effects hypothesis (maternal body size affects offspring performance) and a dynamical model based on maternal effects that fits the field data better than a model based on plant quality or parasitoids (Kendall et al. 2005). Experimental research has documented the pervasiveness of maternal effects, and population dynamics research continues to emphasize the importance of delayed density dependence and population variability. However, it is currently unknown how often maternal effects exert a significant effect on population dynamics. In other words, are maternal effects pervasive factors affecting population dynamics or are they more or less frequent depending upon circumstances (Beckerman et al. 2002)?

Maternal effects in aphids

Aphids are ideal organisms which with to study maternal effects. Aphids have telescoping generations, which means that within a single aphid there are embryos within embryos (Figure 1.1). This large generational overlap, granddaughters are present within their grandmothers, could lead to strong maternal effects (Dixon 1998). Additionally, aphids reproduce parthenogenetically for much of their life cycle and some species never have sexual reproduction (Dixon 1998). Mothers are genetically identical to their daughters and sisters, and there is no genetic recombination (Hales et al. 2002). The lack of genetic variation across generations is ideal for focusing on non-genetic maternal effects. Furthermore, the lack of genetic variation prevents selection from taking place

during experiments. An additional benefit of working on aphids is their short generation time, which allows for a high potential correlation between maternal environment and offspring environment. Maternal effects are known to be important in aphids, especially in the production of different morphs (Dixon 1998). Typically, cues in the maternal environment such as crowding (Hall and Ehler 1980), low temperature, (Nunes and Hardie 1999), decline in plant quality (Wiktelius 1992), changing photoperiod (Hardie and Lees 1983), or the presence natural enemies (Dixon and Agarwala 1999) lead to the production of alates (winged aphids). Similar cues lead to the production of sexual or diapausing morphs in certain species (Lees 1963). Table 1.2 shows a wide variety of maternal effects that have been documented in many aphid species. The references were collected by searching through ISI's Web of Science © using the terms maternal effects, aphids, alate, sexuparae, and diapause, covering the years 1945-2006 and searching approximately 50 journals.

Plant-quality and insect herbivores

Plant quality is a broad term that encompasses any physical, chemical or biological traits of plants (e.g. size and structure, nutritional value, secondary compounds, and phenology) that influence herbivore preference or performance. Variation in host plant quality influences insect herbivore survival (Haggstrom and Larsson 1995, Lill and Marquis 2001, Ladner and Altizer 2005), development time (Haggstrom and Larsson 1995, Tsai and Wang 2001, Wheeler 2001, Ladner and Altizer 2005), fecundity (Rossiter 1988, Tsai and Wang 2001), propensity to diapause (Hunter and McNeil 1997), and the strength of density dependence (Agrawal 2004). Variation in

plant quality also influences herbivore population dynamics (Underwood and Rausher 2000, McIntyre and Whitham 2003, Underwood 2004, Helms and Hunter 2005). Additionally, variation in plant quality can also affect higher trophic levels which can then indirectly affect insect herbivores (Hunter and Price 1992, Hartvigsen et al. 1995, Lill and Marquis 2001, Helms et al. 2004, Fonseca et al. 2005). However, I am going to focus on direct effects of variation in plant quality on insect herbivores.

Typically, research that addresses the effects of variation in plant quality on insect herbivores follows one of two routes: (1) exploiting natural variation in plant quality within and among species (Underwood and Rausher 2000, McIntyre and Whitham 2003, Agrawal 2004, Agrawal et al. 2004, Helms et al. 2004) or (2) generating intra-specific variation by directly manipulating plant quality within a species (Nevo and Coll 2001, Perkins et al. 2004, Sudderth et al. 2005). Within the second group, there have been many reported effects of nitrogen fertilization on insect herbivores. Because most insect herbivores are nitrogen limited (White 1993) we might predict increases in herbivore densities with increasing levels of nitrogen. In a review of nitrogen fertilization experiments conducted by Kyto and colleagues, aphids were found to respond positively to nitrogen fertilization while other insect guilds often showed no response or a negative response (Kyto et al. 1996). Nitrogen fertilization has also been linked with increased insect density, shorter development time, higher survival rates, increased insect mass, higher fecundity and higher R_{max} (Mattson 1980, Cisneros and Godfrey 2001, Nevo and Coll 2001, Tsai and Wang 2001, Stiling and Moon 2005). However, other studies have found negative or no effects of nitrogen fertilization on insect abundance or performance (Bethke et al. 1998, Casey and Raupp 1999, Muller et al. 2005).

Insect herbivores and induced plant responses

Plant quality influences insect herbivores and insect herbivores influence plant quality. Induced plant responses to herbivory are broadly defined as any modification in the plant following damage, including changes in plant quality due to production of toxic or anti-nutritional compounds, protein or nutritional constituents, leaf toughness, thorns, spines or trichomes (Karban and Baldwin 1997). Following damage, plant nutritional quality may decrease or the concentration of secondary compounds or physical defenses, or both, may increase (Green and Ryan 1972, Karban and Baldwin 1997, Agrawal 1999, Abdala-Roberts and Parra-Tabla 2005). In many cases, these induced changes reduce subsequent herbivory and act as plant defenses (Carroll and Hoffman 1980, Karban and Baldwin 1997, Agrawal 2000). It is thought that induced defenses are less costly than constitutive defenses because the defenses are only activated after herbivory. Therefore, the plant does not need to pay any associated ecological or allocation cost in the absence of herbivory (Agrawal 1999, 2000, Strauss et al. 2002). Induced plant responses to herbivory have been demonstrated multiple times for leaf chewing insect herbivores (Green and Ryan 1972, Carroll and Hoffman 1980, Malcolm and Zalucki 1996, Karban and Baldwin 1997, Wold and Marquis 1997).

Less is known about the effects of phloem-feeding insect herbivores on plant induction. Because phloem-feeders do not remove leaf tissue *per se*, and in many cases cause no decrease in plant biomass or leaf area, it is unlikely that plant induction by phloem feeders will be the same as plant defense induction by leaf chewers (Martel and Malcolm 2004). Typically, phloem-feeders elicit induction of plant chemical defenses,

but this response varies among plant species (Martel and Malcolm 2004, Cardoza et al. 2005).

Current System of Study:

Asclepias

The genus *Asclepias* is composed of predominately herbaceous perennials with a wide geographic distribution including temperate to tropical North America, subtropical South America, and southern and eastern Africa (Woodson 1954). These species are known as milkweeds because almost all of them produce a milky, cardenolide-laden latex when damaged (Malcolm 1991). Cardenolides, or cardiac glycosides, are a group of cardiac-active steroids that are bitter tasting and toxic in high doses. Cardenolides have been shown to have many negative effects on species that consume them due to their emetic and toxic properties (Brower et al. 1984, Malcolm 1989, Malcolm and Brower 1989, Malcolm 1991). They act as a heart poison by blocking the Na^+/K^+/ATPase system which transports ions across cell membranes. It is their bitter taste combined with their potential toxicity that has implicated cardenolides in defensive use by plants against natural enemies, including herbivores, parasites and pathogens (Malcolm and Zalucki 1996, Agrawal and Malcolm 2002).

There is a large degree of variability in cardenolide concentrations within and among milkweed individuals and species (Malcolm 1991). For example, the roots of *Asclepias eriocarpa* have the lowest cardenolide concentration, which increases through leaves, stems, and latex (Nelson et al. 1981). Cardenolide concentrations also vary temporally with leaves and latex of *A. eriocarpa* showing a spring increase in

cardenolide, followed by a summer maximum and an autumn decline (Nelson et al. 1981). Additionally, analyses of population-level variation in cardenolide content have shown that cardenolides vary qualitatively and quantitatively in space (Malcolm and Brower 1989). Finally, there is considerable qualitative and quantitative variation among *Asclepias*. Most species can be distinguished by their distinctly different cardenolide 'fingerprint' (Malcolm et al. 1989, Malcolm 1991). Additionally, cardenolide concentrations range from undetectable in most *A. tuberosa*, through 14 μg cardenolide/0.1 g dry leaf tissue in *A. incarnata*, to 376 μg cardenolide/0.1 g dry leaf tissue in *A. viridis* (Malcolm et al. 1989, Martel and Malcolm 2004).

In addition to variation in cardenolide concentration, milkweed species also vary in carbon and nitrogen concentration, trichome density and plant size (Agrawal 2004). Some milkweed species also contain chemicals other than cardenolides. For example, pregnane glycosides have been isolated from the roots and leaves of *Asclepias tuberosa* and *A. incarnata* (Abe and Yamauchi 2000, Warashina and Noro 2000b, a).

Aphis nerii Boyer de Fonsclombe

Aphis nerii, the milkweed-oleander aphid, is an aposematic phloem feeding specialist of oleander (*Nerium oleander*) and species of *Asclepias*. The apterae are bright yellow with black siphunculi and cauda, and the antennae and legs are also dark. Alates are also yellow and black with dark wing veins. They often form dense colonies on younger stems of host plants (Hall and Ehler 1980). They are widely distributed through the Old and New World tropics and subtropics including many Pacific islands (Blackman and Eastop 2000). These multivoltine aphids undergo parthenogenesis in the warm

summer months. It was thought that *A. nerii* was obligately parathenogenetic (Blackman and Eastop 2000), however, sexual morphs (oviparae and males) are produced under short-day and cool temperature conditions (Takada and Miyazaki 1992, 1993). *A. nerii* have telescoping generations and are polymorphic with apterae (non-winged) and alate (winged) morphs produced under different conditions. They are viviparous and the aphids go through 4 instars before maturing to adults.

A. nerii feed on the internal phloem of host bicollateral vascular bundles (Botha et al. 1977) and have been shown to sequester cardenolides from their host plants (Rothschild et al. 1970, Malcolm 1990). Malcolm (1990) found that *A. nerii* sequestered almost all of the cardenolide forms present in the host plant, and so concluded that the cardenolide concentrations in the aphid will co-vary with the concentrations in the host plants. There does not appear to be a cost associated with feeding on high versus low cardenolide plants (Malcolm 1992). Malcolm (1992) found no difference in growth rates between aphid populations on plants with low and high concentrations of cardenolides in the absence of predators. However, population growth rates were lower on low cardenolide plants when predators were not excluded showing that the aphids sequester cardenolides and use them as a defense against predation (Malcolm 1986, 1990, Malcolm 1992).

In the following chapters, I further explore and test the following hypotheses:

- Chapter 2: A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid.
    - H1: Maternal density and host plant species affect offspring vital rates.
    - H2: Offspring experience within-generation density-dependent rates of survival, fecundity and alate formation that vary among host plant species.
    - H3: The expression of maternal effects depends upon the offspring environment.

- Chapter 3: Effects of maternal age and environment on offspring vital rates in *Aphis nerii*, the milkweed-oleander aphid.
    - H1: At low maternal densities, offspring survival and fecundity decrease with maternal age.
    - H2: At high maternal densities, older mothers produce offspring of equal quality to those of younger mothers.
    - H3: Host plant species influences the expression of maternal age effects.

- Chapter 4: Nitrogen deposition and variation in the population growth of insect herbivores.
    - H1: Nitrogen deposition influences environmental carrying capacity (K), aphid maximum per capita growth rate (R_{max}), and the strength of density dependence.

- Chapter 5: Declines in plant chemical defense induced by a phloem-feeding insect herbivore.
    - H1: Aphids induce quantitative and qualitative variation in *Asclepias* defenses.
    - H2: Species with high constitutive levels of cardenolides exhibit greater induction than species with little or no constitutive levels of cardenolides.

- Chapter 6: Conclusions and future directions.

REFERENCES

Abdala-Roberts, L., and V. Parra-Tabla. 2005. Artificial defoliation induces trichome production in the tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). Biotropica **37**:251-257.

Abe, F., and T. Yamauchi. 2000. Pregnane glycosides from the roots of *Asclepias tuberosa*. Chemical & Pharmaceutical Bulletin **48**:1017-1022.

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology **80**:1713-1723.

Agrawal, A. A. 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology **81**:1804-1813.

Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. American Naturalist **164**:113-120.

Agrawal, A. A., and S. B. Malcolm. 2002. Once upon a milkweed - In this complex community, one insects poison may be another's meal. Natural History **111**:48-53.

Agrawal, A. A., N. Underwood, and J. R. Stinchcombe. 2004. Intraspecific variation in the strength of density dependence in aphid populations. Ecological Entomology **29**:521-526.

Beckerman, A., T. G. Benton, E. Ranta, V. Kaitala, and P. Lundberg. 2002. Population dynamic consequences of delayed life-history effects. Trends In Ecology & Evolution **17**:263-269.

Beckerman, A. P., T. G. Benton, C. T. Lapsley, and N. Koesters. 2006. How effective are maternal effects at having effects? Proceedings Of The Royal Society B-Biological Sciences **273**:485-493.

Benton, T. G., S. J. Plaistow, A. P. Beckerman, C. T. Lapsley, and S. Littlejohns. 2005. Changes in maternal investment in eggs can affect population dynamics. Proceedings Of The Royal Society Of London Series B-Biological Sciences **272**:1351-1356.

Benton, T. G., S. J. Plaistow, and T. N. Coulson. 2006. Complex population dynamics and complex causation: devils, details and demography. Proceedings Of The Royal Society B-Biological Sciences **273**:1173-1181.

Benton, T. G., E. Ranta, V. Kaitala, and A. P. Beckerman. 2001. Maternal effects and the stability of population dynamics in noisy environments. Journal Of Animal Ecology **70**:590-599.

Bernardo, J. 1996. Maternal effects in Animal Ecology. American Zoologist **36**:83-105.

Berryman, A. A. 1995. Population cycles - A critique of the maternal and allometric hypotheses. Journal Of Animal Ecology **64**:290-293.

Berryman, A. A. 1999. Principles of population dynamics and their application. Stanley Thornes (Publishers) Ltd, Cheltenham.

Bethke, J. A., R. A. Redak, and U. K. Schuch. 1998. Melon aphid performance on chrysanthemum as mediated by cultivar, and differential levels of fertilization and irrigation. Entomologia Experimentalis Et Applicata **88**:41-47.

Blackman, R. L. 1975. Photoperiodic determination of male and female sexual morphs of *Myzus persicae*. Journal Of Insect Physiology **21**:435-453.

Blackman, R. L., and V. F. Eastop. 2000. Aphids on the world's crops: an identification and information guide. 2nd edition. Wiley, West Sussex, England.

Botha, C. E. J., S. E. W. Mabindisa, and R. F. Evert. 1977. Observations on penetration of phloem in young stems of *Nerium oleander* (Linn) by stylets of aphid *Aphis nerii*(B-De-F). South African Journal Of Science **73**:276-277.

Brower, L. P., J. N. Seiber, C. J. Nelson, S. P. Lynch, and M. M. Holland. 1984. Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus-plexippus* L (Lepidoptera, Danaidae) reared on milkweed plants in California .2. *Asclepias speciosa* (Apocynales, Asclepiadaceae). Journal Of Chemical Ecology **10**:601-639.

Cardoza, Y. J., J. Reidy-Crofts, and O. R. Edwards. 2005. Differential inter- and intra-specific defense induction in *Lupinus* by *Myzus persicae* feeding. Entomologia Experimentalis Et Applicata **117**:155-163.

Carriere, Y., C. Ellers-Kirk, Y. B. Liu, M. A. Sims, A. L. Patin, T. J. Dennehy, and B. E. Tabashnik. 2001. Fitness costs and maternal effects associated with resistance to transgenic cotton in the pink bollworm (Lepidoptera : Gelechiidae). Journal Of Economic Entomology **94**:1571-1576.

Carroll, C. R., and C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata*. Science **209**:414-416.

Casey, C. A., and M. J. Raupp. 1999. Supplemental nitrogen fertilization of containerized azalea does not affect performance of azalea lace bug (Heteroptera : Tingidae). Environmental Entomology **28**:998-1003.

Cisneros, J. J., and L. D. Godfrey. 2001. Midseason pest status of the cotton aphid (Homoptera : Aphididae) in California cotton: Is nitrogen a key factor? Environmental Entomology **30**:501-510.

Debarro, P. J. 1992. The role of temperature, photoperiod, crowding and plant-quality on the production of alate viviparous females of the bird cherry-oat aphid, *Rhopalosiphum-Padi*. Entomologia Experimentalis Et Applicata **65**:205-214.

Denno, R. F., and M. S. McClure, editors. 1983. Variable plants and herbivores in natural and managed ecosystems. Academic Press, New York, New York, USA.

Dewar, A. M. 1976. Effect of crowding on alate production and weight of apterous exules of apple-grass aphid, *Rhopalosiphum insertum*. Annals Of Applied Biology **82**:203-208.

Diss, A. L., J. G. Kunkel, M. E. Montgomery, and D. E. Leonard. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. Oecologia **106**:470-477.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. 2nd edition. Chapman and Hall, London.

Dixon, A. F. G., and B. K. Agarwala. 1999. Ladybird-induced life-history changes in aphids. Proceedings Of The Royal Society Of London Series B-Biological Sciences **266**:1549-1553.

Erelli, M. C., and J. S. Elkinton. 2000. Maternal effects on gypsy moth (Lepidoptera : Lymantriidae) population dynamics: A field experiment. Environmental Entomology **29**:476-488.

Fonseca, C. R., P. I. Prado, M. Almeida-Neto, U. Kubota, and T. M. Lewinsohn. 2005. Flower-heads, herbivores, and their parasitoids: food web structure along a fertility gradient. Ecol Entomol **30**:36-46.

Forrest, J. M. S. 1970. Effect of maternal and larval experience on morph determination in *Dysaphis devecta*. Journal Of Insect Physiology **16**:2281-&.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia **96**:139-146.

Fox, C. W. 2006. Colonization of a new host by a seed-feeding beetle: Genetic variation, maternal experience, and the effect of an alternate host. Annales Zoologici Fennici **43**:239-247.

Fox, C. W., J. A. Nilsson, and T. A. Mousseau. 1997. The ecology of diet expansion in a seed-feeding beetle: Pre-existing variation, rapid adaptation and maternal effects? Evolutionary Ecology **11**:183-194.

Fox, C. W., K. J. Waddell, and T. A. Mousseau. 1995. Parental host-plant affects offspring life-histories in a seed beetle. Ecology **76**:402-411.

Geden, C. J., L. Smith, S. J. Long, and D. A. Rutz. 1992. Rapid deterioration of searching behavior, host destruction, and fecundity of the parasitoid *Muscidifurax raptor* (Hymenoptera, Pteromalidae) in culture. Annals Of The Entomological Society Of America **85**:179-187.

Gildow, F. E. 1982. Influence Of Virus-Infection Of Host Plants On Aphid Vector Morphology. Phytopathology **72**:939-939.

Ginzburg, L. R. 1998. Inertial growth: Population dynamics based on maternal effects.*in* T. A. Mousseau and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Ginzburg, L. R., and M. Colyvan. 2004. Ecological orbits: how planets move and populations grow. Oxford University Press, New York, New York.

Ginzburg, L. R., and D. E. Taneyhill. 1994. Population-cycles of forest lepidoptera - a maternal effect hypothesis. Journal Of Animal Ecology **63**:79-92.

Green, T. R., and C. A. Ryan. 1972. Wound induced proteinase inhibitor in plant leaves - Possible defense mechanism against insects. Science **175**:776-&.

Haggstrom, H., and S. Larsson. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia **104**:308-315.

Hales, D. F., A. C. C. Wilson, M. A. Sloane, J. Christophesimon, J. F. Legallic, and P. Sunnucks. 2002. Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids. Genetical Research **79**:203-209.

Hall, M. C., P. Stiling, D. C. Moon, B. G. Drake, and M. D. Hunter. 2005. Effects of elevated CO2 on foliar quality and herbivore damage in a scrub oak ecosystem. Journal Of Chemical Ecology **31**:267-286.

Hall, R. W., and L. E. Ehler. 1980. Population ecology of *Aphis nerii* Homoptera, Aphididae on oleander. Environmental Entomology **9**:338-344.

Hardie, J. 1990. The photoperiodic counter, quantitative day-length effects and scotophase timing in the vetch aphid *Megoura viciae*. Journal Of Insect Physiology **36**:939-949.

Hardie, J., and A. D. Lees. 1983. Photoperiodic regulation of the development of winged gynoparae in the aphid, *Aphis fabae*. Physiological Entomology **8**:385-391.

Harrington, R. 1984. Photoperiodic control of sexual morph production by the currant-sowthistle aphid, *Hyperomyzus lactucae*. Entomologia Experimentalis Et Applicata **35**:169-175.

Hartvigsen, G., D. A. Wait, and J. S. Coleman. 1995. Tri-trophic interactions influenced by resource availability: Predator effects on plant performance depend on plant resources. Oikos **74**:463-468.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecological Entomology **29**:44-51.

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

Hercus, M. J., and A. A. Hoffmann. 2000. Maternal and grandmaternal age influence offspring fitness in Drosophila. Proceedings Of The Royal Society Of London Series B-Biological Sciences **267**:2105-2110.

Hockham, L. R., J. A. Graves, and M. G. Ritchie. 2001. Variable maternal control of facultative egg diapause in the bushcricket *Ephippiger ephippiger*. Ecological Entomology **26**:143-147.

Honek, A. 1980. Maternal regulation of wing polymorphism in *Pyrrhocoris apterus* - Effect of cold activation. Experientia **36**:418-419.

Hughes, L., and F. A. Bazzaz. 2001. Effects of elevated CO2 on five plant-aphid interactions. Entomologia Experimentalis Et Applicata **99**:87-96.

Hunter, M. D., and J. N. McNeil. 1997. Host-plant quality influences diapause and voltinism in a polyphagous insect herbivore. Ecology **78**:977-986.

Hunter, M. D., and P. W. Price. 1992. Playing chutes and ladders - Heterogeneity and the relative roles of bottom-up and top-down forces in natural communities. Ecology **73**:724-732.

Inchausti, P., and L. R. Ginzburg. 1998. Small mammals cycles in northern Europe: patterns and evidence for a maternal effect hypothesis. Journal Of Animal Ecology **67**:180-194.

Islam, M. S., P. Roessingh, S. J. Simpson, and A. R. McCaffery. 1994. Effects of population-density experienced by parents during mating and oviposition on the

phase of hatchling desert locusts, *Schistocerca gregaria*. Proceedings Of The Royal Society Of London Series B-Biological Sciences **257**:93-98.

Jann, P., and P. I. Ward. 1999. Maternal effects and their consequences for offspring fitness in the yellow dung fly. Functional Ecology **13**:51-58.

Kairo, M. T. K., and S. T. Murphy. 1999. Temperature and plant nutrient effects on the development, survival and reproduction of *Cinara* sp nov., an invasive pest of cypress trees in Africa. Entomologia Experimentalis Et Applicata **92**:147-156.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. Chicago University Press, Chicago.

Keena, M. A., T. M. Odell, and J. A. Tanner. 1998. Environmentally based maternal effects are the primary factor in determining the developmental response of gypsy moth (Lepidoptera : Lymantriidae) to dietary iron deficiency. Annals Of The Entomological Society Of America **91**:710-718.

Kendall, B. E., S. P. Ellner, E. McCauley, S. N. Wood, C. J. Briggs, W. M. Murdoch, and P. Turchin. 2005. Population cycles in the pine looper moth: Dynamical tests of mechanistic hypotheses. Ecological Monographs **75**:259-276.

Kern, S., M. Ackermann, S. C. Stearns, and T. J. Kawecki. 2001. Decline in offspring quality as a manifestation of aging in *Drosophila melanogaster*. Evolution **55**:1822-1831.

Kiritani, K., and K. Kimura. 1967. Effects of parental age on the life cycle of the southern green stink bug, *Nezara viridula*. Applied Entomology And Zoology **2**:69.

Kudo, S. 2001. Intraclutch egg-size variation in acanthosomatid bugs: adaptive allocation of maternal investment? Oikos **92**:208-214.

Kyneb, A., and S. Toft. 2006. Effects of maternal diet quality on offspring performance in the rove beetle *Tachyporus hypnorum*. Ecological Entomology **31**:322-330.

Kyto, M., P. Niemela, and S. Larsson. 1996. Insects on trees: Population and individual response to fertilization. Oikos **75**:148-159.

Ladner, D. T., and S. Altizer. 2005. Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. Entomologia Experimentalis Et Applicata **116**:9-20.

Lamb, K. P., and D. White. 1966. Effect Of Temperature Starvation And Crowding On Production Of Alate Young By Cabbage Aphid(Brevicoryne Brassicae). Entomologia Experimentalis Et Applicata **9**:179-&.

Lamb, R. J., and P. A. Mackay. 1979. Variability in migratory tendency within and among natural-populations of the pea aphid, *Acyrthosiphon pisum*. Oecologia **39**:289-299.

Lees, A. D. 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton .3. Further properties of the maternal switching mechanism in apterous aphids. Journal Of Insect Physiology **9**:153-164.

Lees, A. D. 1966. The control of polymorphism in aphids. Advances in Insect Physiology **19**:973-980.

Lees, A. D. 1967. Production of apterous and alate forms in *Aphid megoura V*iciae Buckton with special reference to role of crowding. Journal Of Insect Physiology **13**:289-&.

Li, S. Y., and R. Harmsen. 1993. Effects of maternal density and age on the daily fecundity and offspring sex ratio in *Tetranychus urticae*. The Canadian Entomologist **125**:633-635.

Lill, J. T., and R. J. Marquis. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia **126**:418-428.

Ludwig, D., and C. Fiore. 1960. Further studies on the relationship between parental age and the life cycle of the mealworm, *Tenebrio molitor*. Annals Of The Entomological Society Of America **53**:595-600.

Mackay, P. A. 1977. Alata production by an aphid - interval timer concept and maternal age effects. Journal Of Insect Physiology **23**:889-893.

Mackay, P. A., D. J. Reeleder, and R. J. Lamb. 1983. Sexual morph production by apterous and alate viviparous *Acyrthosiphon pisum* (Harris) (Homoptera, Aphididae). Canadian Journal of Zoology **61**:952-957.

Malcolm, S. B. 1986. Aposematism in a soft-bodied insect: a case for kin selection. Behavioral Ecology And Sociobiology **18**:387-393.

Malcolm, S. B. 1989. Disruption of web structure and predatory behavior of a spider by plant-derived chemical defenses of an aposematic aphid. Journal Of Chemical Ecology **15**:1699-1716.

Malcolm, S. B. 1990. Chemical defence in chewing & sucking insect herbivores: plant-derived cardenolides in the monarch butterfly and oleander aphid. Chemoecology **1**:12-21.

Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores.*in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: their interactions with secondary plant metabolites. Academic Press, San Diego.

Malcolm, S. B., editor. 1992. Prey defense and predator foraging. Blackwell Scientific, Oxford, UK.

Malcolm, S. B., and L. P. Brower. 1989. Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. Experientia **45**:284-294.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca*. Journal Of Chemical Ecology **15**:819-853.

Malcolm, S. B., and M. P. Zalucki. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. Entomologia Experimentalis Et Applicata **80**:193-196.

Mappes, J., T. Mappes, and T. Lappalainen. 1997. Unequal maternal investment in offspring quality in relation to predation risk. Evolutionary Ecology **11**:237-243.

Martel, J. W., and S. B. Malcolm. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. Journal Of Chemical Ecology **30**:545-561.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. Annual Review Of Ecology And Systematics **11**:119-161.

McIntyre, G. S., and R. H. Gooding. 2000. Effects of maternal age on larval competitiveness in house flies. Heredity **85**:480-489.

McIntyre, P. J., and T. G. Whitham. 2003. Plant genotype affects long-term herbivore population dynamics and extinction: Conservation implications. Ecology **84**:311-322.

McVean, R. I. K., and A. F. G. Dixon. 2001. The effect of plant drought-stress on populations of the pea aphid *Acyrthosiphon pisum*. Ecological Entomology **26**:440-443.

Messina, F. J., T. A. Jones, and D. C. Nielson. 1993. Performance of the Russian wheat aphid (Homoptera, Aphididae) on perennial range grasses - Effects of previous defoliation. Environmental Entomology **22**:1349-1354.

Mittler, T. E., and M. Matsuka. 1985. The production of different morphs by alate viviparae of the aphid *Myzus persicae* temporarily exposed to long scotophases. Entomologia Experimentalis Et Applicata **37**:175-182.

Mondor, E. B., J. A. Rosenheim, and J. F. Addicott. 2005. Predator-induced transgenerational phenotypic plasticity in the cotton aphid. Oecologia **142**:104-108.

Moran, N., J. Seminoff, and L. Johnstone. 1993. Induction of winged sexuparae in root-inhabiting colonies of the aphid *Pemphigus betae*. Physiological Entomology **18**:296-302.

Mousseau, T. A., and C. W. Fox. 1998a. The adaptive significance of maternal effects. Trends In Ecology & Evolution **13**:403-407.

Mousseau, T. A., and C. W. Fox. 1998b. Maternal Effects as Adaptations. Oxford University Press, New York, New York.

Muller, C. B., M. D. E. Fellowes, and H. C. J. Godfray. 2005. Relative importance of fertiliser addition to plants and exclusion of predators for aphid growth in the field. Oecologia **143**:419-427.

Muller, C. B., I. S. Williams, and J. Hardie. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology **26**:330-340.

Nelson, C. J., J. N. Seiber, and L. P. Brower. 1981. Seasonal and intraplant variation of cardenolide content in the California milkweed, *Asclepias eriocarpa*, and implications for plant defense. Journal Of Chemical Ecology **7**:981-1010.

Nevo, E., and M. Coll. 2001. Effect of nitrogen fertilization on *Aphis gossypii* (Homoptera : Aphididae): Variation in size, color and reproduction. Journal Of Economic Entomology **94**:27-32.

Nunes, M. V., and J. Hardie. 1999. The effect of temperature on the photoperiodic 'counters' for female morph and sex determination in two clones of the black bean aphid, *Aphis fabae*. Physiological Entomology **24**:339-345.

Perkins, M. C., H. A. Woods, J. F. Harrison, and J. J. Elser. 2004. Dietary phosphorus affects the growth of larval *Manduca sexta*. Archives Of Insect Biochemistry And Physiology **55**:153-168.

Phelan, J. P., and P. C. Frumhoff. 1991. Differences in the effects of parental age on offspring life-history between tropical and temperate populations of milkweed bugs (*Oncopeltus* Spp). Evolutionary Ecology **5**:160-172.

Phillips, S. W., J. S. Bale, and G. M. Tatchell. 1999. Escaping an ecological dead-end: asexual overwintering and morph determination in the lettuce root aphid *Pemphigus bursarius* L. Ecological Entomology **24**:336-344.

Plaistow, S. J., C. T. Lapsley, A. P. Beckerman, and T. G. Benton. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proceedings Of The Royal Society Of London Series B-Biological Sciences **271**:919-924.

Plaistow, S. J., C. T. Lapsley, and T. G. Benton. 2006. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics. American Naturalist **167**:206-215.

Priest, N. K., B. Mackowiak, and D. E. L. Promislow. 2002. The role of parental age effects on the evolution of aging. Evolution **56**:927-935.

Rasa, O. A. E. 1998. Biparental investment and reproductive success in a subsocial desert beetle: the role of maternal effort. Behavioral Ecology And Sociobiology **43**:105-113.

Raychaudhuri, A., and A. Butz. 1965. Effects of parental age on the life cycle of *Tribolium confuscum* (Coleoptera: Tenebrionidae). Annals Of The Entomological Society Of America **58**:535-540.

Rossiter, M. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology **69**:267-277.

Rossiter, M. 1994. Maternal effects hypothesis of herbivore outbreak. Bioscience **44**:752-763.

Rossiter, M., W. G. Yendol, and N. R. Dubois. 1990. Resistance to *Bacillus thuringiensis* in gypsy moth (Lepidoptera, Lymantriidae) - Genetic and environmental causes. Journal Of Economic Entomology **83**:2211-2218.

Rossiter, M. C. 1991a. Environmentally-based maternal effects - A hidden force in insect population-dynamics. Oecologia **87**:288-294.

Rossiter, M. C. 1991b. Maternal effects generate variation in life-history - Consequences of egg weight plasticity in the gypsy-moth. Functional Ecology **5**:386-393.

Rossiter, M. C. 1998. The role of environmental variation in parental effects expression.*in* T. A. Mousseau and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Rossiter, M. C., D. L. Coxfoster, and M. A. Briggs. 1993. Initiation of maternal effects in *Lymantria dispar* - Genetic and ecological components of egg provisioning. Journal Of Evolutionary Biology **6**:577-589.

Rothschild, M., J. V. Euw, and T. Reichste. 1970. Cardiac glycosides in the oleander aphid, *Aphis nerii*. Journal Of Insect Physiology **16**:1141-&.

Royama, T. 1992. Analytical population dynamics. Chapman & Hall, London.

Shibao, H. 1999. Reproductive schedule and factors affecting soldier production in the eusocial bamboo aphid *Pseudoregma bambucicola* (Homoptera, Aphididae). Insectes Sociaux **46**:378-386.

Spitzer, B. W. 2004. Maternal effects in the soft scale insect Saissetia coffeae (Hemiptera : Coccidae). Evolution **58**:2452-2461.

Stadler, B. 1990. Relationships between host plant-quality and reproductive investment in *Uroleucon Jaceae* (L) (Aphididae). Acta Phytopathologica Et Entomologica Hungarica **25**:177-183.

Stiling, P., and D. C. Moon. 2005. Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia **142**:413-420.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends In Ecology & Evolution **17**:278-285.

Sudderth, E. A., K. A. Stinson, and F. A. Bazzaz. 2005. Host-specific aphid population responses to elevated CO2 and increased N availability. Global Change Biology **11**:1997-2008.

Takada, H., and M. Miyazaki. 1992. Occurrence of sexuales of *Aphis nerii* B De F (Homoptera, Aphididae) in Japan. Applied Entomology And Zoology **27**:117-124.

Takada, H., and M. Miyazaki. 1993. Bisexual reproduction of a form of *Aphis nerii* B-De-F (Homoptera, Aphididae) from Hokkaido. Applied Entomology And Zoology **28**:199-205.

Throop, H. L., and M. T. Lerdau. 2004. Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. Ecosystems **7**:109-133.

Tsai, J. H., and J. J. Wang. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera : Aphididae). Environmental Entomology **30**:44-50.

Tschinkel, W. R. 1993. Crowding, maternal age, age at pupation, and life history of *Zophobas atratus* (Coleoptera: Tenebrionidae). Annals Of The Entomological Society Of America **86**:278-297.

Turchin, P. 1990. Rarity of density dependence or population regulation with lags. Nature **344**:660-663.

Turchin, P., and I. Hanski. 2001. Contrasting alternative hypotheses about rodent cycles by translating them into parameterized models. Ecology Letters **4**:267-276.

Underwood, N. 2004. Variance and skew of the distribution of plant quality influence herbivore population dynamics. Ecology **85**:686-693.

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

Via, S. 1992. Inducing the sexual forms and hatching the eggs of pea aphids. Entomologia Experimentalis Et Applicata **65**:119-127.

Wainhouse, D., R. Ashburner, and R. Boswell. 2001. Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. Ecological Entomology **26**:655-661.

Warashina, T., and T. Noro. 2000a. Cardenolide and oxypregnane glycosides from the root of *Asclepias incarnata* L. Chemical & Pharmaceutical Bulletin **48**:516-524.

Warashina, T., and T. Noro. 2000b. Steroidal glycosides from the aerial part of *Asclepias incarnata*. Phytochemistry **53**:485-498.

Wasserman, S. S., and T. Asami. 1985. The effect of maternal age upon fitness progeny in the southern cowpea weevil, *Callosobruchus maculatus*. Oikos **45**:191-196.

Wellington, W. G. 1965. Some maternal influences on progeny quality in western tent caterpillar *Malacosoma pluviale* (Dyar). Canadian Entomologist **97**:1-14.

Wheeler, G. S. 2001. Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. Biological Control **22**:256-264.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, New York.

Wiktelius, S. 1992. The induction of alatae in *Rhopalosiphum padi* (L) (Hom, Aphididae) in relation to crowding and plant-growth stage in spring sown barley. Journal Of Applied Entomology **114**:491-496.

Wohrmann, K., D. F. Hales, J. Tomiuk, E. M. Schmiedt, and G. Rettenmeier. 1991. Induction of sexual forms in the rose aphid *Macrosiphum rosae*. Entomologia Experimentalis Et Applicata **61**:17-24.

Wold, E. N., and R. J. Marquis. 1997. Induced defense in white oak: Effects on herbivores and consequences for the plant. Ecology **78**:1356-1369.

Woodson, R. E. 1954. The North American species of Asclepias L. Annals of the Missouri Botanical Garden **41**:1-211.

Yanagi, S.-I., and T. Miyatake. 2002. Effects of maternal age on reproductive traits and fitness components of the offspring in the bruchid beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Physiological Entomology **27**:261-266.

Yerger, E. H., and M. Rossiter. 1996. Natural causes and rates of early larval mortality in gypsy moths (Lepidoptera: Lymantriidae) sampled from field populations in different density states. Environmental Entomology **25**:1002-1011.

Table 1.1: Maternal effects and BIDE. This table lists examples of maternal effects on offspring birth (B), death (D) or immigration/emigration (I) in a variety of insect species. This table does not include maternal effects on alate production in aphids which are covered in Table 2.

Species	Maternal factor	Offspring response			Reference
		B	D	I/E	
Saissetia coffeae	fungi / density		X		(Spitzer 2004)
Tachyporus hypnorum	diet quality		X		(Kyneb and Toft 2006)
Ancanthosomatid bugs	variation in egg size & location		X		(Kudo 2001)
Callosobruchus chinensis	age		X		(Yanagi and Miyatake 2002)
Callosobruchus maculatus	age and # of matings		X		(Wasserman and Asami 1985, Fox 1993)
Drosophila melanogaster	age		X		(Kern et al. 2001, Priest et al. 2002)
Drosophila serrata	age		X		(Hercus and Hoffmann 2000)
Elasmucha ferrugata	variation in egg size & location		X		(Mappes et al. 1997)
Ephippiger ephippiger	age		X		(Hockham et al. 2001)
Hylobius abietis	size and nutrition		X		(Wainhouse et al. 2001)
Lymantria dispar	foliar quality, nutrition, mass, dietary iron,	X	X	X	(Rossiter et al. 1990, Rossiter 1991b, Rossiter 1994, Diss et al. 1996, Keena et al. 1998)
Malacosoma pluviale	nutrition		X		(Wellington 1965)
Megoura viviae	photoperiod			X	(Blackman 1975)
Musca domestica	age		X		(McIntyre and Gooding 2000)
Muscidfurax raptor	maternally transmitted pathogen	X			(Geden et al. 1992)
Nazara viridula	age		X		(Kiritani and Kimura 1967)
Oncopeltus fasciatus	age		X		(Phelan and Frumhoff 1991)
Parasitopus armaticeps	food availability		X		(Rasa 1998)
Pectinophora gossypiella	ingestion of Bt		X		(Carriere et al. 2001)

Pyrrhocoris apterus	photoperiod			X	(Honek 1980)
Scathophaga stercoraria	nutrition		X		(Jann and Ward 1999)
Schistocerca gregaria	density			X	(Islam et al. 1994)
Stator limbatus	nutrition, oviposition site, host experiment		X		(Fox et al. 1995, Fox et al. 1997, Fox 2006)
Tenebrio molitor	age		X		(Ludwig and Fiore 1960)
Tetranychus urticae	age	X			(Li and Harmsen 1993)
Tribolium confuscum	age		X		(Raychaudhuri and Butz 1965)
Zophobas atratus	age		X		(Tschinkel 1993)

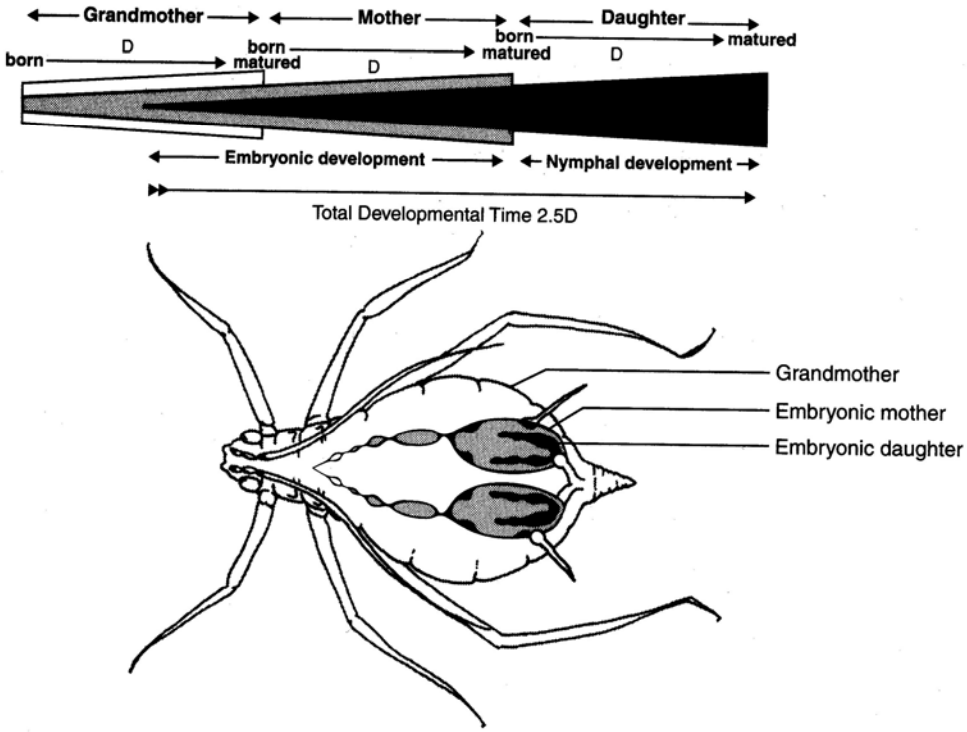
Table 1.2: The effects of maternal environment on production of alates (winged aphids), sexual morphs, soldier morphs in a variety of aphid species.

Species	Offspring response	Cue in maternal environment	Reference
Acyrthosiphon pisum	alate production	plant quality, density, age, morph, predator presence	(Mackay 1977, Lamb and Mackay 1979, Stadler 1990, Dixon and Agarwala 1999, McVean and Dixon 2001, Muller et al. 2001)
Acyrthosiphon pisum	sexual morph	photoperiod	(Mackay et al. 1983, Via 1992)
Aphis craccivora	alate production	density	(Muller et al. 2001)
Aphis fabae	alate production	photoperiod / temperature	(Nunes and Hardie 1999)
Aphis fabae	sexual morph	photoperiod / temperature	(Nunes and Hardie 1999)
Aphis gossypii	alate production	predator	(Mondor et al. 2005)
Aphis nerii	alate production	density	(Hall and Ehler 1980)
Brevicoryne brassicae	alate production	temperature / density / plant quality	(Lamb and White 1966, Muller et al. 2001)
Chaetosiphon fragaefolii	alate production	density	(Muller et al. 2001)
Cinara cupressi	alate production	density	(Kairo and Murphy 1999)
Diuraphsis nexia	alate production	density	(Messina et al. 1993)
Dysaphis devecta	alate production	density	(Forrest 1970)
Hyperomyzus lacturae	sexual morph	photoperiod	(Harrington 1984)
Macrosiphum euphorbiae	alate production	plant quality	(Muller et al. 2001)
Macrosiphum rosae	sexual morph	photoperiod / temperature	(Wohrmann et al. 1991)
Megoura viciae	alate production	density	(Lees 1967)
Megoura viciae	sexual morph	photoperiod	(Hardie 1990)
Myzus persicae	alate production	density	(Muller et al. 2001)
Myzus persicae	sexual morph	photoperiod	(Mittler and Matsuka 1985)
Pemphigus betae	sexual morph	temperature / density	(Moran et al. 1993)
Pemphigus bursarius	alate production	photoperiod / temperature	(Phillips et al. 1999)
Pseudoregma bambucicola	soldier production	density (colony size) / morph	(Shibao 1999)

Rhopalosiphum insertum	alate production	density	(Dewar 1976)
Rhopalosiphum padi	alate production	photoperiod / temp. / plant quality / density / virus infection of plant	(Gildow 1982, Debarro 1992, Wiktelius 1992)
Rhopalosiphum padi	sexual morph	photoperiod / temperature	(Lees 1966)
Sitobion avenae	alate production	density / virus infection of plant	(Gildow 1982, Muller et al. 2001)
Uroleucon jacaea	alate production	plant quality (water stress)	(Stadler 1990)

Figure 1.1: Telescoping generations in aphids (Dixon 1998), the embryonic grand-daughter is actually inside her grandmother.

Figure 1.1


Grandmother
Mother
Daughter
born
D
born
matured
D
born
matured
D
matured
Embryonic development
Nymphal development
Total Developmental Time 2.5D
Grandmother
Embryonic mother
Embryonic daughter

CHAPTER 2

A COMPARISON OF MATERNAL EFFECTS AND CURRENT ENVIRONMENT ON VITAL RATES OF *APHIS NERII*, THE MILKWEED-OLEANDER[1]

[1]Zehnder, C.B. and M.D. Hunter. Accepted by Ecological Entomology. Reprinted here with permission of publisher, 11/15/2006

ABSTRACT

Non-Mendelian maternal effects, the effects of maternal phenotype or environment on offspring phenotype, have been documented in numerous taxa. By affecting offspring vital rates (birth, death and movement), maternal effects have the potential to influence population dynamics. However, relatively few studies have directly linked maternal phenotype or environment to offspring vital rates. Additionally, even fewer studies have compared the magnitude of across generation effects (i.e. maternal effects) to within-generation effects.

Because of their telescoping generations aphids can be strongly influenced by maternal effects. The effects of maternal density and maternal host plant species on offspring survival, fecundity and alate formation were experimentally investigated in *Aphis nerii*, the milkweed-oleander aphid.

Additionally, the relative strength of maternal effects were compared with those operating within a generation. Therefore, in another set of experiments, the effects of current density and host plant species (within generation effects) on aphid vital rates were examined.

While maternal effects were present, within generation effects were much stronger and more strongly influenced aphid vital rates. Within a generation, aphids exhibited density-dependent survival, fecundity and alate formation and these effects varied among host plant species.

These results indicate that while maternal effects have the potential to affect population dynamics, this potential is not always met. Additionally, the current

environment, not the environment of previous generations, more strongly impacts population dynamics.

Keywords: *Aphis nerii*, density-dependence, maternal effects, plant-insect interactions, plant quality

INTRODUCTION

Non-Mendelian maternal effects describe the effect of a mother's phenotype or environment on offspring phenotype (Mousseau and Fox 1998, Agrawal et al. 1999, Beckerman et al. 2002). Cues in the maternal environment can affect offspring size (Wainhouse et al. 2001, Fischer et al. 2003, Plaistow et al. 2004), survival (Hockham et al. 2001, Wainhouse et al. 2001), development rate (Rossiter 1991a, Fox et al. 1995, Benton et al. 2005), fecundity (Rossiter 1991b, Hockham et al. 2001, Benton et al. 2005) , movement (Islam et al. 1994, Diss et al. 1996) and defenses (Agrawal 1999). While it is clear that maternal effects are pervasive, their potential ecological impacts and relative importance are not well understood and warrant further investigation (Beckerman et al. 2002).

Maternal effects have the potential to influence population dynamics (Rossiter 1991a, Ginzburg and Taneyhill 1994, Benton et al. 2001, Beckerman et al. 2002, Hunter 2002, Benton et al. 2005, Kendall et al. 2005). By connecting the maternal environment or phenotype to offspring phenotype, maternal effects introduce a time lag that can cause a delay in density-dependent processes (Rossiter 1994) which can lead to population cycles (Berryman 1999). Models with maternal effects as the mechanism underlying the time lag in density-dependence have shown that maternal effects generally cause increased population fluctuation (Benton et al. 2001).

Plant quality is a broad term that encompasses any physical, chemical or biological traits of plants (e.g. size and structure, nutritional value, secondary compounds, and phenology) that influence herbivore preference or performance. Variation in host plant quality influences insect herbivore survival (Haggstrom and

Larsson 1995, Lill and Marquis 2001, Ladner and Altizer 2005), development time (Haggstrom and Larsson 1995, Tsai and Wang 2001, Wheeler 2001, Ladner and Altizer 2005), fecundity (Rossiter 1988, Tsai and Wang 2001), and the strength of density dependence (Agrawal 2004).

For parthenogenetic herbivores, different clones may vary in their responses to different host plants. For example, studies of pea aphids on alfalfa and red clover have shown that clones collected from these two hosts prefer to feed on their own 'home' host (Via 1991) and that pea aphids recognize host specific chemical stimulants (Del Campo et al. 2003). In *Aphis nerii*, clonal variation in the proportion of alates produced has been correlated with the degree of habitat permanence (Groeters and Dingle 1989).

Aphids are ideal organisms with which to study maternal effects. Aphids have telescoping generations, which means that within a single aphid there are embryos within embryos. This large generational overlap, granddaughters are present within their grandmothers, could lead to strong maternal effects (Dixon 1998). Additionally, aphids reproduce parthenogenetically for much of their life cycle and some species never have sexual reproduction (Dixon 1998). Mothers are genetically identical to their daughters and sisters, and there is no genetic recombination (Hales et al. 2002). The lack of genetic variation across generations is ideal for focusing on non-genetic maternal effects. Furthermore, the lack of genetic variation prevents selection from taking place during experiments. An additional benefit of working on aphids is their short generation time, which allows for a high potential correlation between maternal environment and offspring environment. Maternal effects are known to be important in aphids, especially in the production of different morphs (Dixon 1998). Typically, cues in the maternal

environment such as crowding (Hall and Ehler 1980), low temperature, (Nunes and Hardie 1999), decline in plant quality (Wiktelius 1992), changing photoperiod (Hardie and Lees 1983), or the presence natural enemies (Dixon and Agarwala 1999) lead to the production of alates (winged aphids). Similar cues lead to the production of sexual or diapausing morphs in certain species (Lees 1963)

Variation in insect density, plant quality and insect clone can all potentially affect vital rates both across and within-generations. Therefore the question is: which of these ecological factors is most important and when do they operate? More specifically, it was hypothesised that maternal host plant species and maternal density would strongly influence offspring vital rates. Additionally, it was hypothesized that host plant species and aphid clone would affect within-generation density-dependent survival, fecundity and alate productive of *A. nerii*.

METHODS

Study system

Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae), the milkweed-oleander aphid, is an aposematic phloem feeding specialist of milkweed (*Asclepias* spp) and oleander, *Nerium oleander* L. (Apocynaceae) that reproduces parthenogenetically.

In August 2003, three *A. nerii* individuals were collected from geographically distinct locations (Atlanta, Georgia; Augusta, Georgia and Gainesville, Florida). Each of these is referred to as a separate clone (i.e. clone Emory, Augusta and Florida). An aphid colony was initiated from one individual from each location, leading to three lab colonies. Separate colonies of these aphid clones are kept at low densities on *Asclepias*

syriaca in the lab. The colonies are kept in the same growth chamber in which all the experiments were run (described below) and experience the same light and temperature regime. An explicit comparison of these clones was made only in experiment three (below). The genus *Asclepias* is composed of predominately herbaceous perennials that contain a class of compounds known as cardenolides or cardiac glycosides. There is a large degree of variation in cardenolide concentration among *Asclepias* species both qualitatively and quantitatively (Malcolm et al. 1989, Agrawal 2004).

All research was conducted in a temperature and light controlled walk-in growth chamber. Grow lights on timers (16 hours day: 8 hour night) provide heat and light, daytime and nighttime temperatures are 34 $\pm$ 2.4ºC and 24 $\pm$ 0.48 ºC, respectively. *Asclepias* seeds were obtained from a seed distributor (Butterfly Encounters), grown in Farfard 3B soil with Osmocote time-release fertilizer (14-14-14, N:P:K) and watered as needed. In all experiments, plants were randomly assigned to treatments and rotated daily to homogenize any environmental gradients within the growth chamber. Figure 2.1 summarizes the methods used in each experiment, described below.

Experiment 1: Maternal host plant effects

This experiment was designed to determine if the host plant species of the maternal generation affects offspring vital rates; all variables except maternal host plant species were held constant. Because aphids have telescoping generations, it was thought that the maternal host plant effect would be stronger if multiple previous generations were on different host plant species. Therefore, aphids were reared on different host plant species for seven generations and then the eighth generation was on a common host

plant species. Seven *Asclepias* species were chosen: *A. exaltata*, *A. incarnata*, *A. speciosa*, *A. sullivantii*, *A. syriaca*, *A. tuberosa*, and *A. viridis*. These species encompass a range of variation in plant quality traits (Agrawal 2004). At the start of the experiment, January 2005, all seedlings were four weeks old and approximately the same size. There were seven replicates of each host plant species. A first instar aphid from the clone Florida colony was placed on each plant and the first generation developed. There was no movement of aphids among plants. When the first generation started to reproduce, all but one offspring was removed from the plant. Mass and age (in days) at maturity was recorded for each generation. This continued for seven generations. Finally, when the seventh generation started to reproduce, their first seven offspring were removed from the plant and each offspring was placed individually on an *A. syriaca* seedling. Daily observations were made of survival and fecundity in the offspring generation. Age, mass and aphid morph (alate or apterae) was recorded when the aphids reached maturity. Additionally, all offspring produced were removed (the offspring's offspring) after daily counts. These observations continued until all aphids died (approximately 30 days).

Experiment 2: Maternal Density Effects

This experiment was designed to determine if maternal density influences offspring vital rates; all variables were held constant except for maternal density. In February 2004, the maternal generation was established at four densities: 1, 25, 50 and 75 aphids per plant, with each density replicated seven times. These densities represent natural aphid abundances (Hall & Ehler, 1980). First instar clone Florida aphids were placed on four week old *A. syriaca* seedlings at the appropriate density, and each plant

was caged to prevent aphids in the high density treatments from walking off the plants. Cages were constructed from 710ml Ziploc containers and organza netting over wire frames. When the maternal generation started to reproduce, the first eight offspring were removed and placed individually on an *A. syriaca* seedling. There were a total of 224 aphids in the offspring generation (4 maternal densities x 7 replicates (mothers) per density x 8 offspring). Daily observations were made of survival and fecundity in the offspring generation. Aphid morph was recorded when the aphids reached maturity. As before, all offspring produced were removed (the offspring's offspring) to keep densities constant. These observations continued until all aphids died (approximately 30 days).

Experiment 3: Within Generation Effects

Laboratory experiments were performed to examine density dependent vital rates of *Aphis nerii* on four *Asclepias* species. These experiments investigated within-generation effects, not maternal effects. It was hypothesized that *A. nerii* would exhibit density dependent survival, fecundity and alate formation, and that these responses would vary among aphid clones and host plant species. Four *Asclepias* species (*A. tuberosa*, *A. viridis*, *A. incarnata* and *A. syriaca*) were chosen to represent a range of plant quality traits. The four species vary in foliar carbon:nitrogen ratios, trichome density (Agrawal, 2004) and cardenolide content (Agrawal, 2004; Malcolm, 1989)

The identical experimental design was repeated on the four host plant species (*A. tuberosa*: June 2004, *A. viridis*: July 2004, *A. incarnata*: October 2004 and *A. syriaca*: November 2004). For each experiment, three aphid densities (1, 25 and 50 aphids per plant) were established on 1 month old *Asclepias* seedlings. Initially, five adult aphids

were placed on all experimental plants and left to reproduce on the plants overnight. They were then removed and their offspring thinned out to the appropriate density. This was repeated for all three aphid clones, except for the experiment on *A. syriaca* for which only the Emory and Florida clones were used because there were not enough aphids from the Augusta clone at the start of the experiment. Each density was replicated seven times, for a total of 63 plants per experiment (3 densities x 3 clones x 7 replicates = 63 plants). Each plant was caged. Daily counts were made of the number of aphids surviving and number of offspring produced. All offspring were removed daily. This continued until all of the aphids died (approximately 30 days). When the aphids reached maturity, mass and proportion alate were recorded. Using a paintbrush, one aphid (on the low density treatment) or five aphids were removed and individually weighed on a Mettler Toledo balance (Mettler Toledo, Im Langacher, Switzerland, max 2.1g, d=0.1ug). Then the aphids were carefully placed back on the plant. Because of time and space limitations, we could not examine all host plants at the same time. However, photoperiod, temperature and other environmental conditions remained constant in the growth chamber over the course of all experiments.

Experiment 4: Variable Maternal and Offspring Density Effects

Results from the previous experiments led us to test the hypothesis that the expression of maternal effects depends on the offspring's environment. In April 2005, using aphids from a single clone (Florida), two densities were established in the maternal generation: one aphid per plant and 50 aphids per plant. There were 10 replicates of the high density treatment and 40 replicates of the low density treatment. Many more low

density plants were needed to insure that there were enough aphids to establish the high density treatment in the next generation. Their offspring were either put on plants at low density (L: 1 aphid per plant) or high density (H: 50 aphids per plant). This led to four possible combinations: $H_{maternal}H_{offspring}$, $H_{maternal}L_{offspring}$, $L_{maternal}H_{offspring}$, $L_{maternal}L_{offspring}$. For the low density offspring, one aphid was randomly chosen from the appropriate maternal density. For the high density offspring, 50 aphids were chosen from among multiple maternal plants. Each combination was replicated ten times for a total of forty experimental plants in the offspring generation. Each plant was caged. Daily counts were made of the number of aphids surviving and number of offspring produced. All offspring (the offspring's offspring) were removed daily. This continued until all of the aphids died. When the aphids reached maturity, mass and proportion alate was recorded. As before, when they reach maturity, aphids were removed, weighed and returned to their plant to continue reproducing.

Analyses

Data were analyzed using SAS 8.2 for Windows and the residuals of the ANOVA models were tested for normality. Data sets whose residuals failed to meet the assumptions of normality were transformed and reanalyzed. Proportion surviving and proportion alate data were arcsine square root transformed, and, when necessary, other data were log transformed.

Maternal effects data (experiments 1 and 2) were analyzed using a nested ANOVA (PROC GLM) with either host plant species or density as the treatment effect and maternal ID nested with species or density. Response variables were number of days

surviving, total fecundity, age at maturity and mass at maturity (for experiment 1 only). If there was a significant effect of maternal environment on offspring survival, fecundity or age at maturity, then aphid instantaneous rate of increase 'r' was calculated using Euler's equation. Specifically, daily survivorship and fecundity were used to calculate generation time and estimate 'r'. Then Euler's equation was iterated to produce a more exact estimate (Gotelli 2001).

Within-generation effects on survival and fecundity (experiment 3) were analyzed using the repeated measures framework of PROC MIXED with a Type1 autoregressive model (Littell et al. 1998). To investigate within-generation host plant effects (also experiment 3), all the within-generation effects experiments were combined in a single model. In this case, total fecundity, lifespan, mass, age at maturity and proportion alate data were analyzed using a 3-way ANOVA with host plant, density and clone as the treatment effects. A repeated measures framework was not used for this analysis because the response variables were all determined at the end of the experiment and not measured over time. Because the experiments with different host plants were conducted in different months (above), host plant effects are confounded with any uncontrolled environmental variation among experiments. Host plant comparisons in experiment 3 should therefore be viewed with caution.

Data from the final experiment were analyzed using a 2-way ANOVA with maternal density and offspring density as the two main effects and total fecundity, lifespan, mass, age at maturity and proportion alate as response variables.

RESULTS

Experiment 1

There was no effect of maternal host plant species on offspring survival (Nested ANOVA, $F_{6,38} = 0.52$, $P = 0.792$), fecundity (Nested ANOVA, $F_{6,38} = 0.56$, $P = 0.758$), mass at maturity (Nested ANOVA, $F_{6,38} = 1.66$ $P = 0.157$) or age at maturity (Nested ANOVA, $F_{6,38} = 1.43$, $P = 0.229$) (data not shown). No alates were produced

Experiment 2

Maternal density affected offspring development rate. Mothers in the lowest density treatment (1 aphid per plant) produced offspring that matured on average nine hours (6.31 $\pm$ 0.08 days) faster than those from mothers at higher densities (pooled mean for densities 25, 50 and 75: 6.9 $\pm$ 0.05 days; Nested ANOVA, $F_{3,24} = 4.88$, $P = 0.009$). Aphid instantaneous rate of increase 'r' decreased as maternal density increased (maternal density (aphids/plant) = r (aphids / aphids*day) : 1 = 0.67, 25 = 0.62, 50 = 0.613, 75 = 0.605. There was no effect of maternal density on offspring survival (Nested ANOVA, $F_{3,24} = 2.44$, $P = 0.089$) or total fecundity (Nested ANOVA, $F_{3,24} = 1.13$, $P = 0.357$) (data not shown). As in the first experiment, no alates were produced.

Experiment 3

Within-generation effects on *A. nerii* vital rates were strong. Aphids exhibited density-dependent survival, fecundity and alate formation. Rates of survival varied with density on all four host plant species. (Figure 2.2 A-D Proc Mixed; *A. tuberosa*: density*date $F_{54,1572} = 4.77$, $P < 0.0001$; *A. incarnata*: density*date $F_{52,1440} = 2.42$, $P <$

0.0001; *A. viridis* density*date $F_{64,1731} = 6.15$, $P < 0.0001$; *A. syriaca*: density*date $F_{54,990} = 2.64$ $P < 0.0001$). Additionally, density influenced patterns of fecundity over time on all host plant species (Figure 2.3 A-D: *A. tuberosa* density*date $F_{48,432} = 21.10$, $P < 0.0001$; *A. incarnata* density*date $F_{54,1494} = 2.66$, $P < 0.0001$; *A. viridis*: density*date $F_{52,1412} = 20.11$, $P < 0.0001$; *A. syriaca*: density*date $F_{42,783} = 3.5$, $P < 0.0001$). On *A. tuberosa*, clone Augusta reached its peak reproductive output earlier than the other aphid clones (data not shown: clone*date $F_{48,840} = 1.89$, $P < 0.0003$). The clone*date interaction was not significant on any of the other host plant species.

In a larger model which included all four host plant species, the expression of density dependence on aphid vital rates varied among *Asclepias* species. Density-dependent reductions fecundity were strongest on *A. viridis* (Figure 2.4a host*density: $F_{6,196} = 15.24$, $P < 0.0001$). Similarly, density-dependent reductions in aphid lifespan were strongest on *A. viridis* (Fig. 4b host*density: $F_{6,196} = 8.22$, $P < 0.0001$). As density increased aphid mass at maturity decreased on *A. viridis*, but not on the other two plant species (Figure 2.4c host*density: $F_{6,196} = 2.68$, $P = 0.034$). Mass at maturity on *A. tuberosa* could not be measured because the scale was not accessible at the time of that experiment. Age at maturity was constant over all three densities on *A. tuberosa*, but it decreased as density increased on the other three species (Figure 2.4d host*density: $F_{6,196} = 2.14$ $P = 0.05$). There were no alates produced at any density on *A. incarnata* and *A. syriaca*. However, the proportion alate increased with density on *A. tuberosa* and *A. viridis* (Figure 2.4e host*density: $F_{6,196} = 26.49$, $p < 0.0001$). There were alates produced at the highest density on *A. tuberosa* and on the intermediate and highest density on *A. viridis*. Total fecundity of clone Florida was 5.65% lower than that on the other clones

(data not shown: $F_{2,196} = 3.83$, $P = 0.023$). Aphid clone did not influence any other vital rates.

Experiment 4

When both maternal and offspring densities were varied consecutively, within-generation effects on *A. nerii* vital rates were stronger. In all cases except for proportion alate, the expression of maternal effects did not depend on the offspring environment. Total fecundity in the offspring generation was not influenced significantly by maternal density, offspring density or their interaction (Figure 2.5a: maternal $F_{1,33} = 3.80$, $P = 0.06$, offspring $F_{1,33} = 3.75$, $P = 0.061$, maternal*offspring $F_{1,33} = 2.19$, $P = 0.148$). High offspring density, but not maternal density or their interaction, reduced offspring survival (Figure 2.5b: maternal $F_{1,33} = 0.17$, $P = 0.682$; offspring: $F_{1,33} = 25.79$, $P < 0.0001$; maternal*offspring: $F_{1,33} = 0.12$, $P = 0.731$). Similar to experiments 2 and 3, age at maturity increased with density in the maternal generation, but decreased with density in the offspring generation (Figure 2.5c: maternal: $F_{1,33} = 5.74$, $P = 0.022$; offspring: $F_{1,33} = 32.36$, $P < 0.0001$, maternal*offspring: $F_{1,33} = 0.12$, $P = 0.733$). Alates were produced on only six of forty plants and five of these were $H_{maternal}H_{offspring}$ plants (Figure 2.5d: maternal: $F_{1,33} = 7.20$, $P = 0.011$; offspring: $F_{1,33} = 9.03$, $P = 0.005$; maternal*offspring: $F_{1,33} = 7.20$, $P = 0.011$). Neither maternal density, offspring density, nor their interaction explained any of the variation in aphid mass at maturity (data not shown; maternal: $F_{1,33} = 1.98$, $P = 0.169$; offspring $F_{1,33} = 0.15$, $P = 0.701$; maternal*offspring: $F_{1,33} = 0.001$, $P = 0.958$).

DISCUSSION

This research examined the effects of plant quality, insect clone and insect density on aphid vital rates. The importance of these factors was systematically assessed in both the maternal and offspring generation. While maternal effects were present, within generation effects on vital rates were much stronger and are therefore more likely to have an impact on aphid population dynamics.

Compared to within-generation effects, maternal effects were relatively weak and had little or no impact on aphid vital rates. Neither maternal density nor maternal host plant species influenced offspring survival, fecundity or alate formation. However, maternal density influenced the age at which offspring reached maturity. Offspring of mothers experiencing a low density reached maturity faster, approximately nine hours earlier, than offspring of high-density mothers. Whether a nine hour difference in development rate matters ecologically for an organism that lives on average for less than 20 days is unclear. Using daily survivorship and fecundity to calculate 'r' for each maternal density treatment showed that these small changes in development schedules influence 'r' and, therefore, have the potential to influence long term population dynamics. However, aphids only occur at these low densities (1 aphid per plant) when they are colonizing a new plant, and then their densities rapidly increase. Currently, it is unknown how these rapid changes in density would influence 'r'. Long term experiments would be required to asses the importance of this effect on the dynamics of *A. nerii*.

In many published cases, maternal effects are strong initially but then decline as offspring age (Mousseau and Fox 1998). Therefore, it was not surprising that maternal density influenced offspring age at maturity, but not offspring survival or fecundity. In a

review of environmental influences of diapause expression in insects, Mousseau and Dingle (1991) reported that most maternal effects link environmental cues experienced by mothers with phenotypic expression in embryos, and that plastic responses are generally strongest between adjacent developmental stages (Mousseau and Dingle 1991). Therefore, adult traits may not be influenced as strongly by the maternal environment as are embryonic or early developmental traits.

Maternal effects on offspring development rate are not uncommon. Maternal and paternal photoperiod influence offspring development rate in *Drosophila melanogaster* (Giesel 1988). Parents experiencing short-day conditions produce progeny with shorter development times than do long-day parents. The host plant species that a gypsy moth mother is feeding on influences offspring development rate. Offspring develop significantly faster when their mothers feed on black oak than they do on red oak (Rossiter 1991a).

Originally, we hypothesized that maternal effects would be a strong force in aphid population dynamics. This hypothesis was based on the fact that aphids have telescoping generations; therefore there is a wide degree of physiological overlap of generations. Additionally, maternal effects are already known to play an important role in determining whether aphids become alate or non-winged, or reproduce sexually or parthenogenetically (Dixon 1998). Given this evidence, it was thought likely that maternal effects would also influence aphid offspring vital rates. Why then is there an almost complete absence of maternal influence on offspring birth and death rates? Aphids can respond quickly to their environment. For example, some species use environmental cues perceived as first and second instars to determine wing development

(Muller et al. 2001) causing cues from the maternal environment to be unnecessary. Maternal effects may be stronger in organisms that are unable to respond as quickly to their environment and must rely on cues from previous generations.

Additionally, an aphid's environment is highly variable. Density of conspecifics can increase from zero to hundreds within two weeks (Helms et al. 2004) leading to concomitant changes in plant quality and food availability. Conversely, a strong rainstorm can cause rapid population decline. The environment that an aphid mother experiences may not be an accurate predictor of her offspring's environment. In contrast, maternal effects may be stronger when the maternal environment is a reliable indicator of the offspring's environment.

While across-generation effects appear weak in *A. nerii* population dynamics, within-generation effects are strong. Aphids exhibited density-dependent survival, fecundity and alate formation (Figures 2.3, 2.4 and 2.5). On all host plant species, aphids at low density had higher survivorship during the first half of the experiment (Figure 2.3). Low density aphids also had higher per capita fecundity (Figure 2.4). This is likely a result of reduced competition for food resources at low density. Density-dependent survival and fecundity has been documented in other experiments (Harrison and Cappuccino 1995), as well as in time series analysis. Agrawal (2004), detected negative density dependence of aphids on 17 of the 18 milkweed species he studied, though the strength of density-dependence varied across species.

In this study, production of alates increased with increasing aphid density (Figure 2.5e). This is a common response to high density in aphids and it has been documented in many species (Muller et al. 2001).

The comparisons of aphid vital rates among plant species in experiment 3 should be treated with caution because these experiments were in different months. Nonetheless, results varied among the different host plant species. On three of the four plant species (*A. incarnata*, *A. viridis* and *A. syriaca*), total fecundity decreased as density increased (Figure 2.4a). However, on *A. tuberosa* there was no significant difference in total fecundity between the high and low densities, and the intermediate density actually exhibited the highest total fecundity. The density-dependent reduction in fecundity was much stronger on *A. viridis* than on any of the other *Asclepias* species, with the largest difference in total fecundity between the low and high density treatments (Figure 2.4a).

Similarly, Agrawal (2004) demonstrated density dependent fecundity of *A. nerii* on different species of milkweed and the strength of this effect depended on host plant species. A number of plant quality traits, including cardenolide concentration and trichome density, were found to be correlated with the intrinsic rate of aphid population increase. Other studies have documented changes in herbivore population dynamics that are related to differences in plant quality. For example, bean beetle population dynamics vary depending on soybean genotype (Underwood and Rausher 2000). For spirea aphids, development time, survival and fecundity depend on host plant species (Tsai and Wang 2001).

In this study, variation in vital rates among aphid clones was only directly examined in one set of experiments (experiment 3). Clone Florida produced, on average, 5.65% fewer aphids than clone Augusta or Emory. Whether this translates into differences in population growth rates under field conditions is currently unknown. On only one host plant species, *A. tuberosa*, was there a significant clone effect on per capita

fecundity. While these clones were collected from geographically distinct locations, it is possible that they are identical genotypes, or that there is no clonal variation in survival and fecundity among the clones that we studied. Similarly, no clonal variation in survival and fecundity was found among 12 populations of the corn leaf aphid collected in Canada (Simon et al. 1995).

The offspring environment can interact with the maternal environment and influence the strength of maternal effects (Rossiter, 1998). In this study, the only instance where there was an interaction between the maternal and offspring environment was in the case of alate formation (Figure 2.5d). The proportion alate was much higher when both the mothers and offspring experienced high densities. When there was high density in one generation and low density the next, such as in experiment 2, then alates were not produced. This indicates that the cue for alate production may be compounded over generations. A similar phenomenon is seen in locusts (Islam et al. 1994).

In experiment 4, there was no effect of maternal density, offspring density or their interaction on total per capita fecundity (Figure 2.5a). Based on the earlier maternal density experiment (experiment 2), the lack of maternal density influence was expected. However, based on experiment 3, it was expected that offspring density would affect total fecundity. It is unknown why the results of the two experiments differed. In experiment 4 (as in experiment 3) aphids experiencing higher densities lived shorter lives irrespective of the maternal density. Likewise, age at maturity was influenced by across- and within-generation effects, similar to results of prior experiments. Offspring age at maturity decreased when their mothers were at low density or if offspring were at high density. Perhaps low density mothers have increased access to resources and produce offspring

that reach maturity more quickly and possibly experience a competitive advantage over later-maturing offspring. Maternal allocation decisions can directly influence offspring age/mass at maturity and also indirectly affect the competitive environment that the offspring experience (Benton et al. 2005). Additionally, offspring environment is predicted to influence offspring mass and age at maturity (Plaistow et al. 2004). Plaistow *et al*. (2004) found that offspring age at maturity decreased in a high food environment. Perhaps the high density of aphids created a phloem sink, which led to an increase in per capita food availability.

These results, like the few others that have compared both within- and across-generation effects on vital rates (Miao et al. 1991, Crone 1997), show that within-generation effects are stronger. Even though maternal effects have the potential to affect offspring vital rates and long-term population dynamics, this potential is not always met. Aphids can respond quickly to their highly variable environment. Therefore, in this system, cues in the maternal environment, at least those studied here, are not as important for vital rates as cues in the current environment. We conclude that conditions in the current environment should have greater impacts on population dynamics.

ACKNOWLEDGEMENTS

We would like to thank B. Ball, W. Duncan, C. Frost, M.C. Hall, and anonymous reviewers for their helpful comments on the manuscript. We thank P. Doty and S. Scott for laboratory assistance. This work was funded by NSF grant DEB-0342750 to MDH.

REFERNCES

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology **80**:1713-1723.

Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. American Naturalist **164**:113-120.

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defenses in animals and plants. Nature **401**.

Beckerman, A., T. G. Benton, E. Ranta, V. Kaitala, and P. Lundberg. 2002. Population dynamic consequences of delayed life-history effects. Trends In Ecology & Evolution **17**:263-269.

Benton, T. G., S. J. Plaistow, A. P. Beckerman, C. T. Lapsley, and S. Littlejohns. 2005. Changes in maternal investment in eggs can affect population dynamics. Proceedings Of The Royal Society Of London Series B-Biological Sciences **272**:1351-1356.

Benton, T. G., E. Ranta, V. Kaitala, and A. P. Beckerman. 2001. Maternal effects and the stability of population dynamics in noisy environments. Journal Of Animal Ecology **70**:590-599.

Berryman, A. A. 1999. Principles of population dynamics and their application. Stanley Thornes (Publishers) Ltd, Cheltenham.

Crone, E. E. 1997. Parental environmental effects and cyclical dynamics in plant populations. American Naturalist **150**:708-729.

Del Campo, M. L., S. Via, and M. C. Caillaud. 2003. Recognition of host-specific chemical stimulants in two sympatric host races of the pea aphid *Acyrthosiphon pisum*. Ecological Entomology **28**:405-412.

Diss, A. L., J. G. Kunkel, M. E. Montgomery, and D. E. Leonard. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. Oecologia **106**:470-477.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. 2nd edition. Chapman and Hall, London.

Dixon, A. F. G., and B. K. Agarwala. 1999. Ladybird-induced life-history changes in aphids. Proceedings Of The Royal Society Of London Series B-Biological Sciences **266**:1549-1553.

Fischer, K., P. M. Brakefield, and B. J. Zwaan. 2003. Plasticity in butterfly egg size: Why larger offspring at lower temperatures? Ecology **84**:3138-3147.

Fox, C. W., K. J. Waddell, and T. A. Mousseau. 1995. Parental Host-Plant Affects Offspring Life-Histories In A Seed Beetle. Ecology **76**:402-411.

Giesel, J. T. 1988. Effects of parental photoperiod on development time and density sensitivity of progeny in *Drosophila melanogaster*. Evolution **42**:1348-1350.

Ginzburg, L. R., and D. E. Taneyhill. 1994. Population-cycles of forest lepidoptera - a maternal effect hypothesis. Journal Of Animal Ecology **63**:79-92.

Gotelli, N. J. 2001. A Primer of Ecology. Sinauer Associates, Inc., Sunderland, MA.

Groeters, F. R., and H. Dingle. 1989. The cost of being able to fly in the milkweed oleander aphid, *Aphis nerii* (Homoptera, Aphididae). Evolutionary Ecology **3**:313-326.

Haggstrom, H., and S. Larsson. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia **104**:308-315.

Hales, D. F., A. C. C. Wilson, M. A. Sloane, J. Christophesimon, J. F. Legallic, and P. Sunnucks. 2002. Lack of detectable genetic recombination on the X chromosome during the parthenogenetic production of female and male aphids. Genetical Research **79**:203-209.

Hall, R. W., and L. E. Ehler. 1980. Population ecology of *Aphis nerii* Homoptera, Aphididae on oleander. Environmental Entomology **9**:338-344.

Hardie, J., and A. D. Lees. 1983. Photoperiodic regulation of the development of winged gynoparae in the aphid, *Aphis fabae*. Physiological Entomology **8**:385-391.

Harrison, S., and N. Cappuccino. 1995. Using density manipulation experiments to study population regulation.*in* N. Cappuccino and P. W. Price, editors. Population Dynamics. Academic Press, San Diego.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecological Entomology **29**:44-51.

Hockham, L. R., J. A. Graves, and M. G. Ritchie. 2001. Variable maternal control of facultative egg diapause in the bushcricket *Ephippiger ephippiger*. Ecological Entomology **26**:143-147.

Hunter, M. D. 2002. Maternal effects and the population dynamics of insects on plants. Agricultural and Forest Entomology **4**:1-9.

Islam, M. S., P. Roessingh, S. J. Simpson, and A. R. McCaffery. 1994. Effects of population-density experienced by parents during mating and oviposition on the phase of hatchling desert locusts, *Schistocerca gregaria*. Proceedings Of The Royal Society Of London Series B-Biological Sciences **257**:93-98.

Kendall, B. E., S. P. Ellner, E. McCauley, S. N. Wood, C. J. Briggs, W. M. Murdoch, and P. Turchin. 2005. Population cycles in the pine looper moth: Dynamical tests of mechanistic hypotheses. Ecological Monographs **75**:259-276.

Ladner, D. T., and S. Altizer. 2005. Oviposition preference and larval performance of North American monarch butterflies on four Asclepias species. Entomologia Experimentalis Et Applicata **116**:9-20.

Lees, A. D. 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton .3. Further properties of the maternal switching mechanism in apterous aphids. Journal Of Insect Physiology **9**:153-164.

Lill, J. T., and R. J. Marquis. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia **126**:418-428.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal Of Animal Science **76**:1216-1231.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca*. Journal Of Chemical Ecology **15**:819-853.

Miao, S. L., F. A. Bazzaz, and R. B. Primack. 1991. Effects Of Maternal Nutrient Pulse On Reproduction Of 2 Colonizing Plantago Species. Ecology **72**:586-596.

Mousseau, T. A., and H. Dingle. 1991. Maternal effects in insects: Examples, constrainsts, and geographic variation. Dioscorides Press, Portland, OR.

Mousseau, T. A., and C. W. Fox. 1998. Maternal Effects as Adaptations. Oxford University Press, New York, New York.

Muller, C. B., I. S. Williams, and J. Hardie. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology **26**:330-340.

Nunes, M. V., and J. Hardie. 1999. The effect of temperature on the photoperiodic 'counters' for female morph and sex determination in two clones of the black bean aphid, *Aphis fabae*. Physiological Entomology **24**:339-345.

Plaistow, S. J., C. T. Lapsley, A. P. Beckerman, and T. G. Benton. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proceedings Of The Royal Society Of London Series B-Biological Sciences **271**:919-924.

Rossiter, M. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology **69**:267-277.

Rossiter, M. 1994. Maternal effects hypothesis of herbivore outbreak. Bioscience **44**:752-763.

Rossiter, M. C. 1991a. Environmentally-based maternal effects - A hidden force in insect population-dynamics. Oecologia **87**:288-294.

Rossiter, M. C. 1991b. Maternal effects generate variation in life-history - Consequences of egg weight plasticity in the gypsy-moth. Functional Ecology **5**:386-393.

Simon, J. C., P. D. N. Hebert, C. Carillo, and R. Demelo. 1995. Lack Of Clonal Variation Among Canadian Populations Of The Corn Leaf Aphid, Rhopalosiphum-Maidis Fitch (Homoptera, Aphididae). Canadian Entomologist **127**:623-629.

Tsai, J. H., and J. J. Wang. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera : Aphididae). Environmental Entomology **30**:44-50.

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

Via, S. 1991. The Genetic-Structure Of Host Plant Adaptation In A Spatial Patchwork - Demographic Variability Among Reciprocally Transplanted Pea Aphid Clones. Evolution **45**:827-852.

Wainhouse, D., R. Ashburner, and R. Boswell. 2001. Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. Ecological Entomology **26**:655-661.

Wheeler, G. S. 2001. Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. Biological Control **22**:256-264.

Wiktelius, S. 1992. The induction of alatae in *Rhopalosiphum padi* (L) (Hom, Aphididae) in relation to crowding and plant-growth stage in ppring sown barley. Journal Of Applied Entomology-Zeitschrift Fur Angewandte Entomologie **114**:491-496.

Figure 2.1: Summary of experiments used to compare the relative importance of maternal and within-generation effects on the vital rates of *Aphis nerii*.

Figure 2.1: Experimental Designs

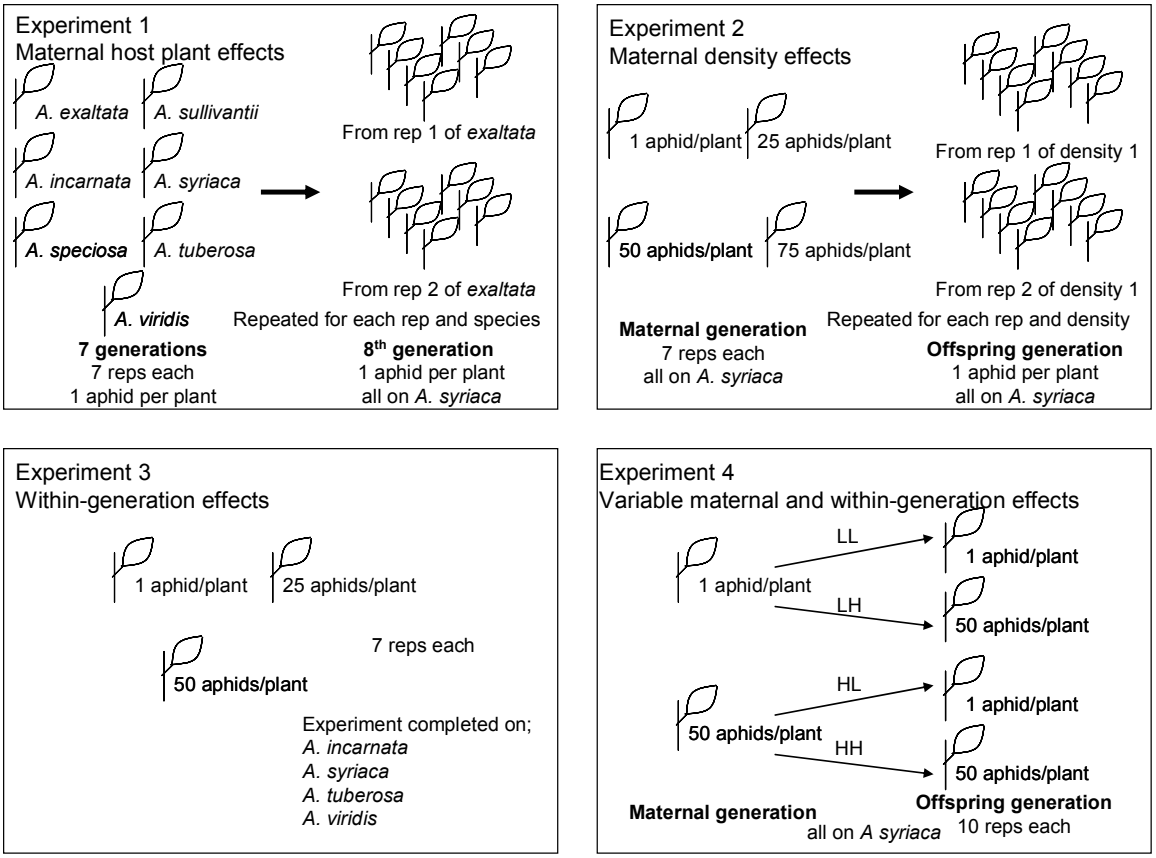


Figure 2.2: Effects of density (1, 25, 50 aphids per plant) on *Aphis nerii* survival on four *Asclepias* species (A-D). Because aphid clone did not influence survival, results from the three clones were combined. Each point is the mean of 21 replicates ± standard error.

Figure 2.2

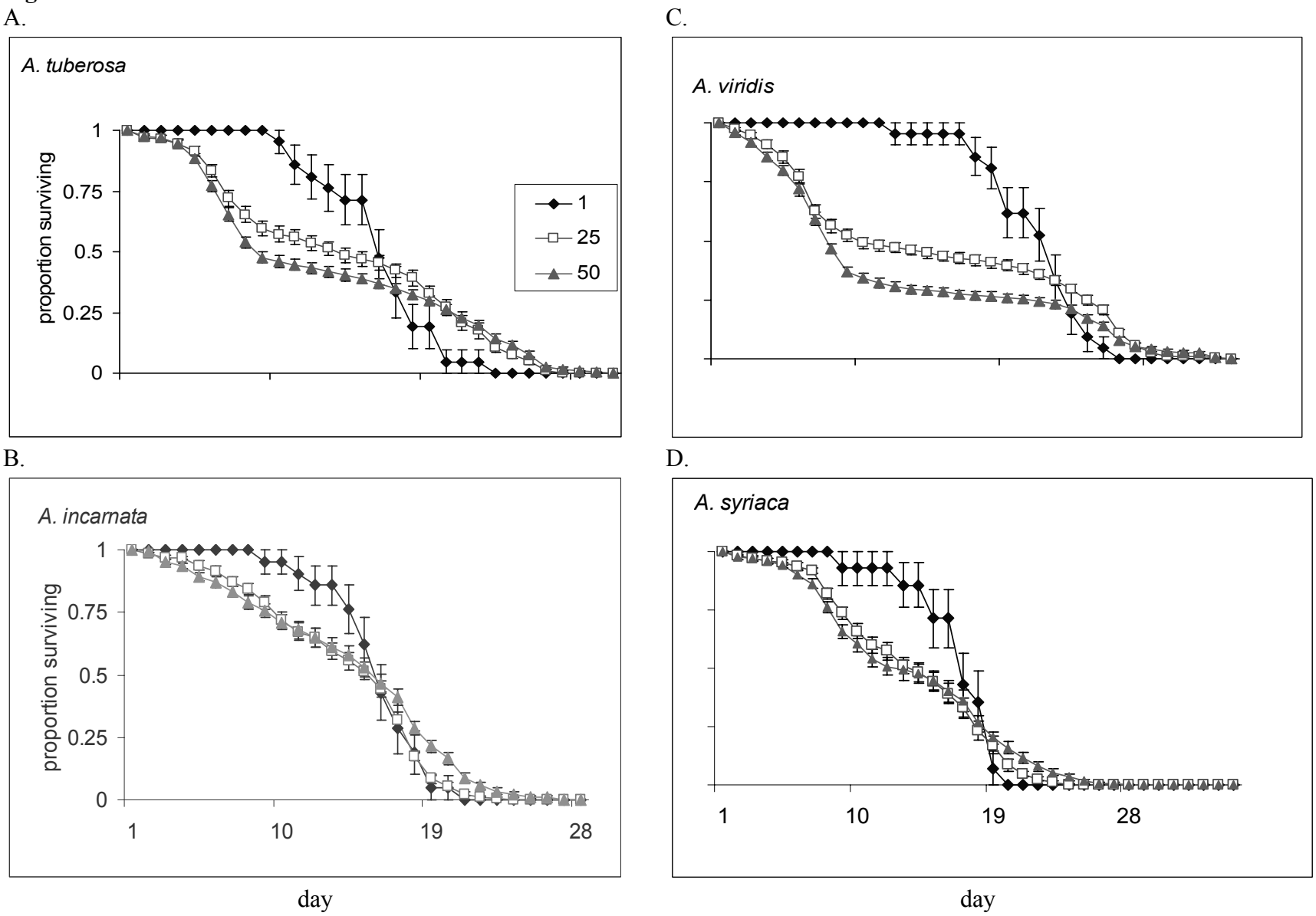

A.
A. tuberosa
proportion surviving
1
0.75
0.5
0.25
0
1
25
50
C.
A. viridis
B.
A. incarnata
proportion surviving
1
0.75
0.5
0.25
0
1
10
19
28
day
D.
A. syriaca
1
10
19
28
day

Figure 2.3: Effects of density (1, 25, 50 aphid per plant) on *Aphis nerii* fecundity on four species of *Asclepias* (A-D). Results from the three clones were combined. Each point is the mean of 21 replicates ± standard error.

Figure 2.3

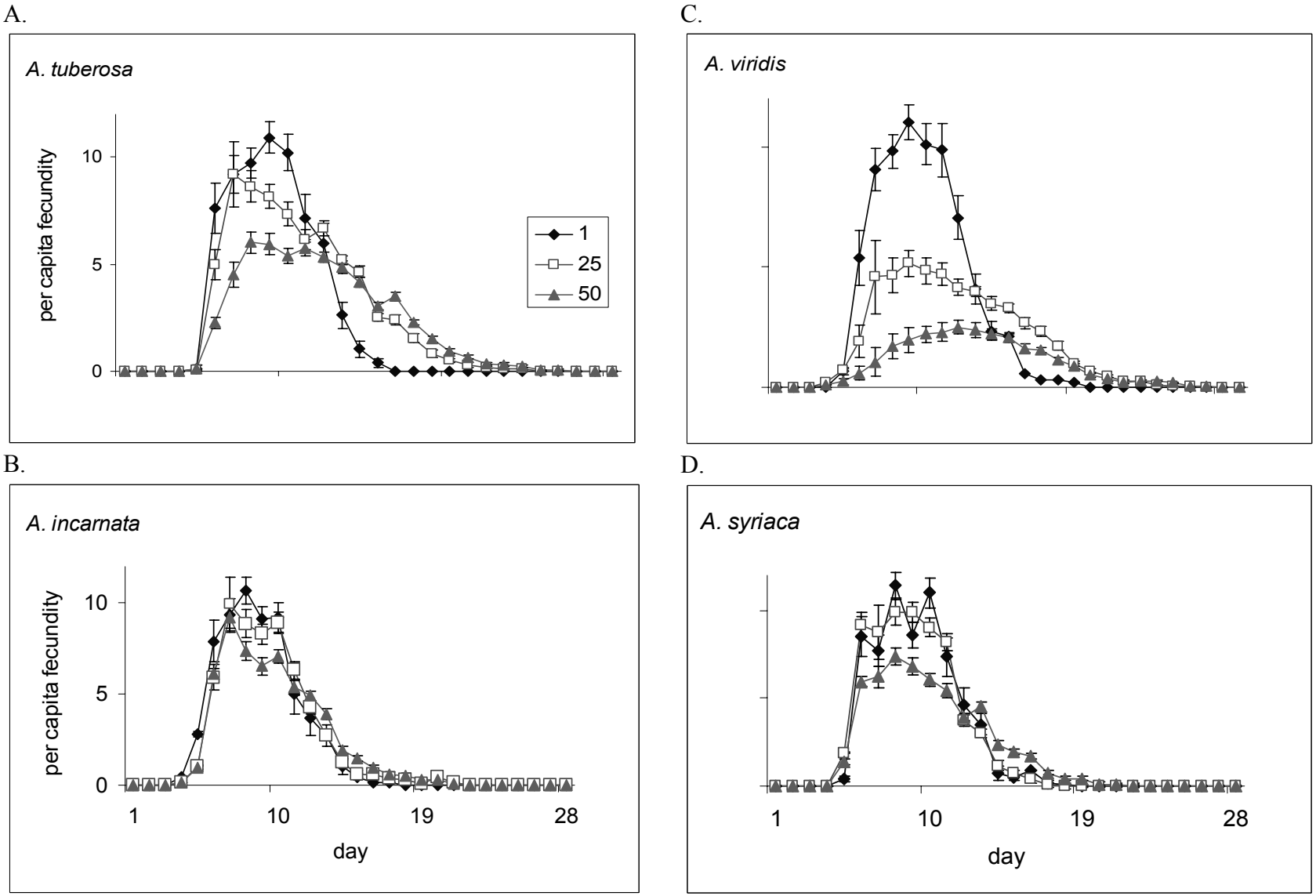

A.
A. tuberosa
per capita fecundity
10
5
0
1
25
50
C.
A. viridis
B.
A. incarnata
per capita fecundity
10
5
0
1
10
19
28
day
D.
A. syriaca
1
10
19
28
day

Figure 2.4: Effects of *Asclepias* species and aphid density on *Aphis nerii* fecundity (A), lifespan (B), mass at maturity (C), age at maturity (D) and proportion alate (E). Values are means of 21 replicates $\pm$ standard error.

Figure 2.4

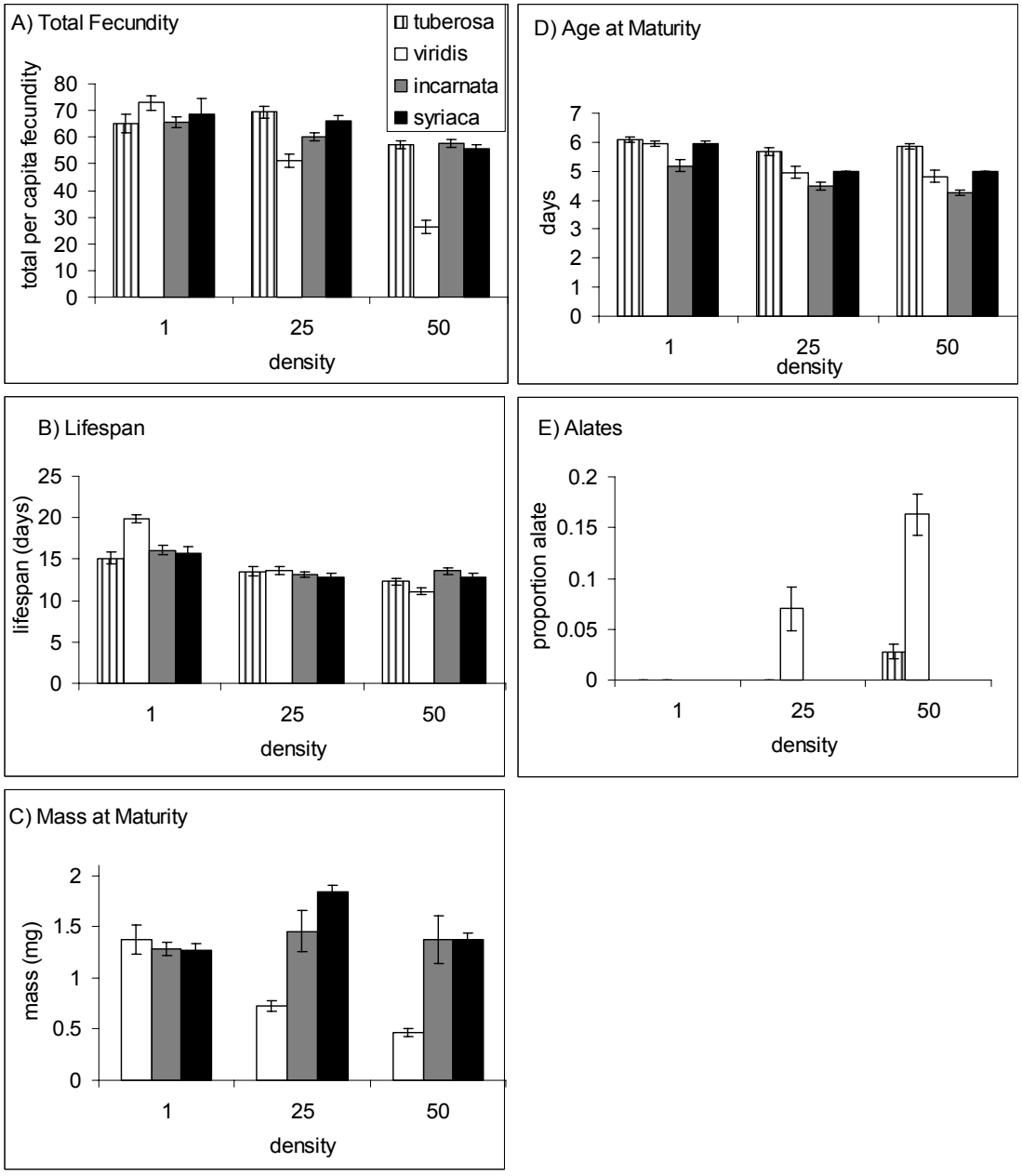
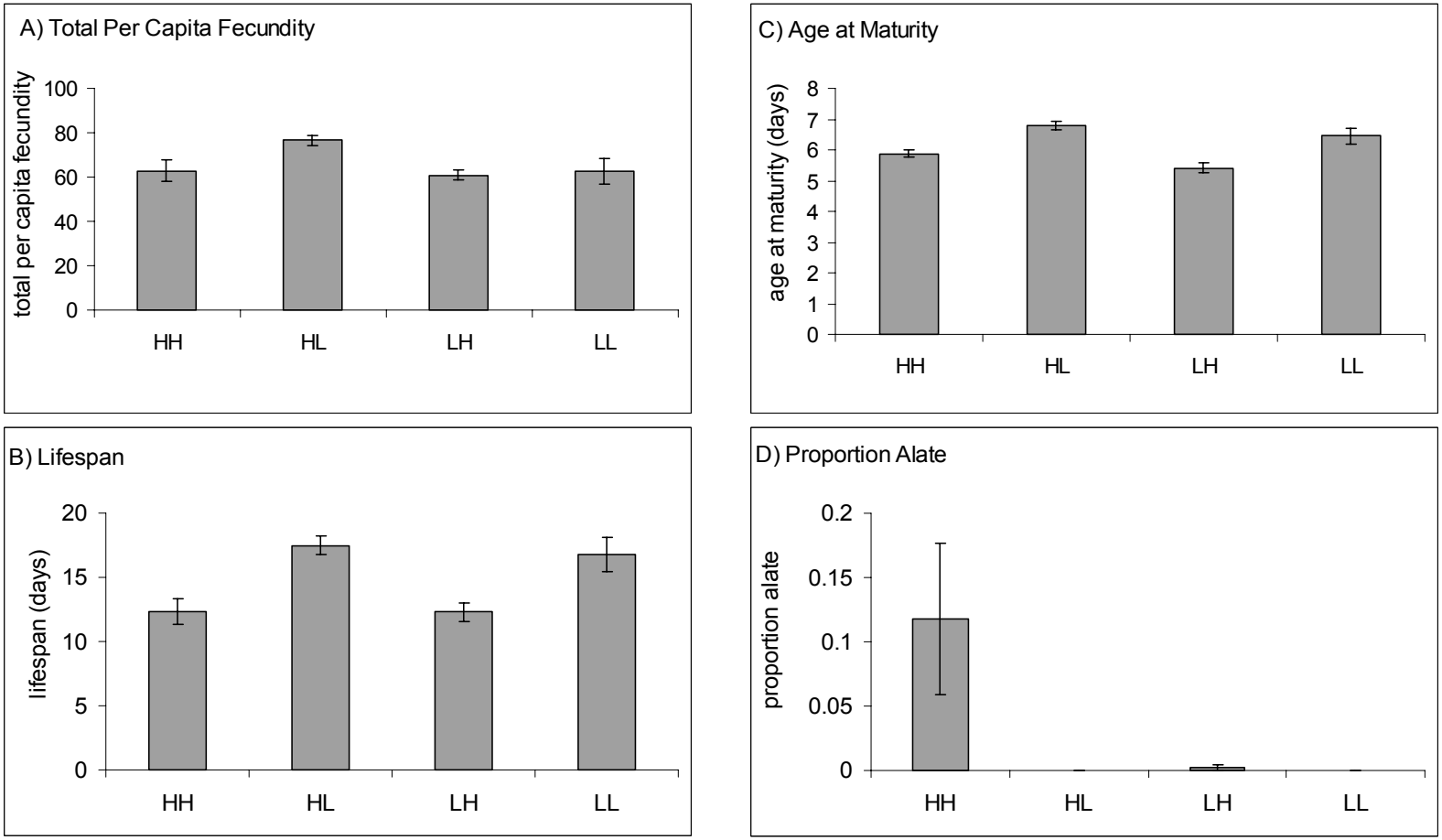

A) Total Fecundity
tuberosa
viridis
incarnata
syriaca
total per capita fecundity
80
70
60
50
40
30
20
10
0
1
25
50
density
D) Age at Maturity
days
7
6
5
4
3
2
1
0
1
25
50
density
B) Lifespan
lifespan (days)
25
20
15
10
5
0
1
25
50
density
E) Alates
proportion alate
0.2
0.15
0.1
0.05
0
1
25
50
density
C) Mass at Maturity
mass (mg)
2
1.5
1
0.5
0
1
25
50
density

Figure 2.5: Effects of maternal and offspring density on offspring fecundity (A), lifespan (B), age at maturity (C) and proportion alate (D) in *Aphis nerii*. For the x-axis labels, the first letter refers to the maternal density and the second letter refers to the offspring density (H = high = 50 aphids per plant; L = low = 1 aphid per plant). Values are means of 10 replicates $\pm$ standard error.

Figure 2.5


A) Total Per Capita Fecundity
total per capita fecundity
100
80
60
40
20
0
HH
HL
LH
LL
C) Age at Maturity
age at maturity (days)
8
7
6
5
4
3
2
1
0
HH
HL
LH
LL
B) Lifespan
lifespan (days)
20
15
10
5
0
HH
HL
LH
LL
D) Proportion Alate
proportion alate
0.2
0.15
0.1
0.05
0
HH
HL
LH
LL

CHAPTER 3

EFFECTS OF MATERNAL AGE AND ENVIRONMENT ON OFFSPRING VITAL RATES IN *APHIS NERII*, THE MILKWEED-OLEANDER APHID[1]

Zehnder, C.B, M.A. Parris and M.D. Hunter. Submitted to Oikos August, 2006

ABSTRACT

Maternal effects have the potential to affect population dynamics and evolution. In order to affect population dynamics, maternal effects must influence offspring vital rates (birth, death, or movement) in a density-dependent manner. Here, we explore the magnitude of non-genetic maternal influence on the vital rates of an insect herbivore, and explore predictability of maternal effects with reference to published studies. We experimentally investigated the effects of maternal age, host plant species (two *Asclepias* spp.) and density on offspring vital rates in *Aphis nerii*, the milkweed-oleander aphid. Older mothers produced offspring that lived shorter lives, consistent with the "Lansing Effect". Older mothers also produced offspring that matured at a younger age. As maternal age increased, offspring mass at maturity decreased when mothers were on *Asclepias syriaca*. However, offspring mass was highest from intermediate aged mothers on *A. viridis*. The absence of maternal density effects appears to exclude maternal density as a potential source of delayed density dependence in *A. nerii.* Our results indicate that maternal effects have some influence on *A. nerii* vital rates. However, references to published studies suggest that only the Lansing Effect is a predictable response to maternal age in insects. Moreover, the magnitude of observed effects was generally low.

Keywords: *Aphis nerii*, Lansing effect, maternal effects, plant quality, population dynamics

INTRODUCTION

Non-Mendelian maternal effects describe the influence of a mother's phenotype or environment on offspring phenotype (Mousseau and Fox 1998, Agrawal et al. 1999, Beckerman et al. 2002). Cues in the maternal environment can affect offspring size (Wainhouse et al. 2001, Fischer et al. 2003, Plaistow et al. 2004), survival (Hockham et al. 2001, Wainhouse et al. 2001), development rate (Rossiter 1991a, Fox et al. 1995, Benton et al. 2005), fecundity (Rossiter 1991b, Hockham et al. 2001, Benton et al. 2005), movement (Islam et al. 1994, Diss et al. 1996) and defenses (Agrawal 1999). Maternal effects also have the potential to influence population dynamics (Rossiter 1991a, Ginzburg and Taneyhill 1994, Benton et al. 2001, Beckerman et al. 2002, Hunter 2002, Benton et al. 2005, Fowler 2005, Kendall et al. 2005). By connecting the maternal environment or phenotype to the offspring phenotype, maternal effects introduce time lags that may cause delays in density-dependent responses of populations (Rossiter 1994) which can lead to population cycles (Berryman 1999). Models show that maternal effects generally cause increased population fluctuations (Benton et al. 2001). Maternal effects also have the potential to influence the evolution of life-history traits.

Many experiments investigating maternal effects use young mothers or mothers of unknown age. However, maternal age is a component of maternal phenotype and can influence offspring quality (Mousseau and Dingle 1991). Maternal age influences offspring survival (Lansing 1947, Ludwig and Fiore 1960, Raychaudhuri and Butz 1965, Kiritani and Kimura 1967, Wasserman and Asami 1985, Hercus and Hoffmann 2000, Kern et al. 2001, Priest et al. 2002), development time (Dixon et al. 1993, Mohaghegh et

al. 1998) and mass at birth and maturity (Dixon et al. 1993). However, other studies have found no effect of maternal age on offspring quality (Moore and Harris 2003).

A possible explanation for the lack of maternal age effects in some studies is that maternal age may interact with other aspects of the maternal environment. If this is the case, maternal age effects may be expressed in some environments and not in others. For insect herbivores, plant quality is an important component of the environment that could potentially interact with maternal age. Plant quality is a broad term that encompasses any physical, chemical or biological plant trait (e.g. size and structure, nutritional value, secondary compounds, phenology) that influences herbivore preference or performance. Variation in host plant quality influences insect herbivore survival (Haggstrom and Larsson 1995, Lill and Marquis 2001, Ladner and Altizer 2005), development time (Haggstrom and Larsson 1995, Tsai and Wang 2001, Wheeler 2001, Ladner and Altizer 2005), fecundity (Rossiter 1988, Tsai and Wang 2001), and the strength of density dependence (Agrawal 2004).

Aphids are ideal organisms with which to study maternal effects. Aphids have telescoping generations which means that, within a single aphid, there are embryos within embryos. This large generational overlap, granddaughters being present within their grandmothers, could lead to strong maternal effects (Dixon 1998). Additionally, aphids reproduce parthenogenetically for much of their life cycle and some species never exhibit sexual reproduction (Dixon 1998). Mothers are genetically identical to their offspring, making it easier to focus on effects of the maternal environment while controlling for genetic effects. Maternal effects are known to be important in aphids, especially in the production of different morphs. Typically, cues in the maternal environment such as

crowding (Hall and Ehler 1980), low temperatures, (Nunes and Hardie 1999), reductions in plant quality (Wiktelius 1992), changing photoperiods (Hardie and Lees 1983), or the presence of natural enemies (Dixon and Agarwala 1999) lead to the production of alates (winged aphids). Similar cues lead to the production of sexual or diapausing morphs in certain species (Lees 1963)

Previous research on *Aphis nerii* on *Asclepias* plants has shown that the current environment experienced by aphids has a stronger impact on aphid vital rates than does the maternal environment (Zehnder and Hunter 2007). In past experiments, aphids exhibited density-dependent fecundity and survival, and the strength of the density-dependent response varied among host plant species. Additionally, the effects of density on aphid survival and fecundity differed between the first and second half of an aphid's lifetime. In the first half, aphids experiencing low density conditions had higher survival and fecundity than those experiencing high density conditions; and these patterns reversed in the second half of the aphid's life (Zehnder and Hunter 2007). Moreover, the strength of this age effect varied among host plant species. Currently, we do not know the mechanism behind these patterns. However, these patterns led us to ask whether maternal age, host plant and density might interact to influence offspring vital rates (birth, death, & immigration). From those patterns, we hypothesized that as maternal age increased, offspring survival and fecundity would decrease, but only when the mothers were at low density. At high maternal density, we predicted that older mothers would produce offspring of equal quality to young mothers. Finally, we hypothesized that host plant species would influence the expression of the maternal age effects.

METHODS

Study system

Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae), the milkweed-oleander aphid, is an aposematic phloem feeding specialist of milkweed (*Asclepias* spp) and oleander (*Nerium oleander* L. (Apocynaceae)) that reproduces parthenogenetically. In August 2003, one *A. nerii* individual was collected near Gainesville, Florida. Our aphid colony (clone) was initiated from this one individual.

In the lab, aphids were kept on *Asclepias syriaca* at low densities. The genus *Asclepias* is composed of predominately herbaceous perennials that contain a class of compounds known as cardenolides or cardiac glycosides. There is a large degree of variation in cardenolide concentration among *Asclepias* species both qualitatively and quantitatively (Malcolm et al. 1989, Agrawal 2004). *Asclepias* seeds were obtained from a seed distributor (Butterfly Encounters), grown in Farfard 3B soil with Osmocote time-release fertilizer (14-14-14, N:P:K) and watered as needed.

Experimental methods:

All research was conducted in a temperature and light controlled walk-in growth chamber. The aphid colony is also kept in this growth chamber. Grow lights on timers (16 hours day: 8 hour night) provided heat and light; daytime and nighttime temperatures were 34$\pm$ 2.4ºC and 24 $\pm$ 0.48 ºC, respectively, well within the range of summer temperatures experienced in Florida, the source of our aphid clone. Figure 3.1 summarizes our experimental methods.

All experimental variation was in the maternal generation while all measurements were taken in the offspring generation. We chose two *Asclepias* species, *A. syriaca* and *A. viridis*, which differ in their cardenolide levels and foliar nitrogen concentrations. *A. viridis* typically has higher cardenolide levels and *A. syriaca* has slightly higher foliar nitrogen concentrations (Malcolm 1991, Agrawal 2004). At the start of the experiment, May 2005, all seedlings were 4 weeks old, approximately the same size (12-15 cm in height), and composed of a single stem bearing 6-10 leaves. To initiate the maternal generation, 3 adult apterous aphids were placed on each plant and left to reproduce overnight. The next morning, the adults were removed and the remaining juvenile aphids (which were all the same age: first instar) were thinned out to the appropriate densities. Maternal densities were set at 1, 25 and 50 aphids per seedling. These densities represent natural aphid densities (Hall and Ehler 1980); 50 aphids per seedling is approximately 250-500 aphids per gram dry weight of plant tissue, which is at the high end of natural aphid densities (Helms et al. 2004). There were seven replicates of each maternal density by host plant combination (3 maternal densities x 2 maternal host plant species x 7 replicates = 42 plants in the maternal generation). All maternal plants were individually placed in cages constructed from 710 ml Ziploc containers and organza netting over wire frames. On days 6, 10 and 14 (of the maternal generation = maternal age), 4 offspring were removed from each maternal plant. We were unable to mark individual aphids in the maternal generation; therefore, at densities greater than 1, we could not guarantee that offspring were sampled from the same individual each time. However, all maternal aphids were genetically identical and experienced identical conditions. Each offspring was placed individually on a 2 week old *A. syriaca* seedling.

All other offspring were removed from the maternal plants on a daily basis to keep the maternal generation densities constant. Moreover, removal of offspring is required to separate the effects of maternal density from potential effects of sibling density on offspring vital rates. Maintaining controlled maternal densities ensures that any density-dependent induction of cardenolides (Malcolm and Zalucki 1996, Martel and Malcolm 2004) is restricted to the parental generation and absent in the offspring generation.

There were 504 aphids (plants) in the offspring generation (3 maternal densities x 2 maternal host plant species x 3 maternal ages x 7 replicates x 4 offspring). Daily observations were made of survival and fecundity in the offspring generation. When the aphids reached maturity, age and mass were recorded. Using a paintbrush, each aphid was removed and individually weighed on a Mettler Toledo balance (Mettler Toledo, Im Langacher, Switzerland, max 2.1g, d=0.1ug). The aphid was then carefully placed back on its plant. The offspring's offspring were removed and counted daily to keep densities constant. These observations continued until all aphids died (approximately 15 days).

Analyses

Data were analyzed using SAS 8.2 for Windows and the residuals of the ANOVA models were tested for normality (Kery and Hatfield 2003). Offspring lifespan, fecundity, age at maturity and mass at maturity were subjected to repeated measures analysis using a mixed model test in SAS. Specifically, we used PROC MIXED with a type1 autoregressive model (Littell et al. 1998). This allows for the incorporation of both fixed and random effects within the model. Additionally, this procedure allows for unrestrictive assumptions about the structure of the variance-covariance matrices. In our

model, maternal age, density, host plant and all possible interactions were the fixed effects and offspring nested within maternal ID was the random effect. Tukey's honest significant difference tests were conducted to compare among treatment means (Littell et al. 2002).

RESULTS

Offspring from older mothers lived shorter lives than did offspring from younger mothers (Figure 3.2: age $F_{2,58} = 3.09$, $p = 0.0531$), with a 9.3% decrease in offspring lifespan between the youngest and oldest mothers. Neither maternal density nor host plant species (or their interaction) affected offspring lifespan (Table 3.1: density $F_{2,35} = 0.51$, $p = 0.6023$; Table 3.2: host $F_{1,58} = 0.01$, $p = 0.9366$).

Offspring from the oldest mothers matured more quickly than did offspring from mothers of intermediate age (Figire 3.3: age $F_{2,57} = 4.04$, $p = 0.0229$) by approximately 4.8 hours. Neither maternal density nor maternal host plant species affected offspring age at maturity (Table 3.1: density $F_{2,35} = 2.80$ $p = 0.0747$; Table 3.2 host $F_{1,57} = 0.64$, $p = 0.4269$). There were no significant interactions among any of the treatments.

When mothers were on *A. syriaca*, offspring mass at maturity decreased as maternal age increased. When mothers were on *A. viridis*, offspring mass at maturity was highest from the intermediate aged mothers (Figure 3,4: host*age $F_{2, 57} = 5.21$, $p = 0.0084$). Maternal density had no effect on offspring mass at maturity (Table 3.1: density $F_{2, 36} = 1.27$, $p = 0.2920$).

Offspring fecundity was unaffected by any of the maternal effects examined here (Table 3.1: density $F_{2, 35} = 0.56$, $p = 0.5767$; Table 3.2: host $F_{1,58} = 1.34$, $p = 0.2524$; age

$F_{2,58} = 1.37$, $p = 0.2626$ (data not shown for the effects of maternal age on offspring fecundity)).

DISCUSSION

We investigated whether variation in maternal age, density and host plant species affected offspring lifespan, fecundity, age at maturity and mass at maturity in *Aphis nerii*. Of the three factors studied, maternal age had the largest influence on offspring vital rates, affecting offspring lifespan, age at maturity and mass at maturity. Maternal host plant species was important as well. Offspring mass at maturity declined with maternal age on *Asclepias syriaca*, but peaked at intermediate maternal age on *A. viridis*. Maternal density did not influence any of the parameters we measured in the offspring generation.

Offspring lifespan decreased as maternal age increased (Figure 3.2). This pattern has been documented in many taxa including rotifers (Lansing 1947), stink bugs (Kiritani and Kimura 1967), fruit flies (Kern et al. 2001, Priest et al. 2002), flour beetles (Raychaudhuri and Butz 1965), mealworms (Ludwig and Fiore 1960), and weevils (Wasserman and Asami 1985), and is referred to as the Lansing effect (Lansing 1947). A potential proximate mechanism explaining why older mothers produce offspring with reduced lifespans is that wear and tear on oocytes over time produces less viable offspring from older mothers (Schatten et al. 1999). Another explanation is that older mothers invest fewer resources, or have fewer resources to invest, in older embryos compared to younger embryos. However, given that offspring fecundity did not decline with maternal age, it is not clear that late-born offspring of *A. nerii* are less fit or contribute less to subsequent population dynamics.

There was a small effect of maternal age on offspring age at maturity, though whether or not this effect is important to long term population dynamics is unclear. Offspring from the oldest mothers reached maturity slightly faster, by approximately 4.8 hours, than did offspring from intermediate age mothers, and there was no difference between offspring from the youngest mothers and those from older mothers (Figure 3.3).

Overall, as mothers aged, they produced offspring that reach maturity faster, lived shorter lives, and (on *A. syriaca*, Figure 3.4) matured at a smaller size. Small size, short lifespan and early maturity are all features of rapid population growth and r-selection (MacArthur & Wilson 1967). Field populations of *A. nerii* obviously include the offspring of both young and old mothers – whether they contribute differentially to future population growth is unclear and difficult to assess. However, *A, nerii* can exhibit unrestricted (exponential) population growth throughout the growing season until plants senesce in the fall (Helms et al. 2004). Even small differences in generation time may result in significant differences in future population size during 3 months of multivoltine exponential growth (Speight et al. 1999).

On *A. viridis,* the heaviest offspring came from mothers of intermediate age. *Asclepias* species are known to differ in their foliar nitrogen and cardenolide concentrations. *A. viridis* usually has higher cardenolide concentrations than *A. syriaca* while *A. syriaca* has higher foliar nitrogen concentrations (Agrawal 2004). It is possible that the different cardenolide concentrations affected the aphids differently over time or that cardenolide induction differed between the two plant species. In studies comparing *A. curassavica* and *A. incarnata*, *A. curassavica* exhibited density-dependent cardenolide induction caused by aphid feeding while there was no measurable induction in *A.*

incarnata (Martel and Malcolm 2004). At this time, we do not know what specific characteristics of the two plant species led to the different patterns of maternal age effects.

In our experiment, maternal density had no effect on offspring lifespan, fecundity, age at maturity or mass at maturity. The lack of maternal density effects on offspring lifespan, fecundity and mass at maturity is consistent with past research investigating maternal effects in *A. nerii* (Zehnder and Hunter 2007). However, in some of our previous experiments, maternal density was found to influence offspring age at maturity whereby offspring from low density mothers reached maturity about 9 hours earlier than did offspring from high density mothers. It appears that maternal density effects on offspring age at maturity are weak and expressed under a limited set of conditions in *Aphis nerii*. Models investigating the impact of maternal effects on long term population dynamics often assume that there will be a density-dependent maternal effect, and it is this delayed density-dependence that leads to population cycles (Ginzburg and Taneyhill 1994, Ginzburg 1998, Benton et al. 2001, Benton et al. 2005). The lack of a maternal density effect in our system means that it is unlikely that maternal effects could cause population cycles in *Aphis nerii*, and natural population of *A. nerii* do not normally exhibit cyclic behavior (Helms et al. 2004). However, maternal density effects have been found in other systems and these systems often exhibit cyclic behavior (Benton et al. 2005).

In some aphids, maternal effects are known to influence the production of winged or sexual morphs (Dixon 1998). Past work in this system has shown that high maternal density coupled with high offspring density leads to the production of alates (winged

aphids) in the offspring generation (Zehnder and Hunter 2007). In this experiment, no alates were produced, and this is most likely because of the low density conditions that the offspring experienced (1 aphid per seedling).

In this experiment, one possible explanation for the lack of strong maternal effects is that offspring experienced ideal conditions. The strength of maternal effects can vary depending on the offspring environment (Rossiter 1998). Therefore, maternal effects might be stronger in this system if the offspring generation experienced harsh conditions.

How predictable are the magnitude and direction of maternal age effects on offspring vital rates in insects? Predictability is key to any effort to forecast effects on insect population dynamics. Here, we focus on maternal age, the strongest maternal influence on offspring traits in our study. Table 3.3 lists experiments on maternal age conducted on a variety of insect taxa. References included in Table 3.3 were attained by conducting a Web of Science© search for 'maternal age' and then using those sources to find additional studies. Maternal age effects on longevity, the Lansing effect (Lansing 1947), are the most common and consistent. In our experiment, there was a 9.3% decrease in offspring longevity as mothers aged (Figure 3.2). In other experiments, maternal age effects range from no effect to a 53% decrease in offspring longevity. Three studies document an increase in offspring age at maturity with maternal age (Wasserman and Asami 1985, Fox 1993, Yanagi and Miyatake 2002), five studies, including this one, document a decrease (Ludwig and Fiore 1960, Phelan and Frumhoff 1991, Dixon et al. 1993, Mohaghegh et al. 1998), and three studies found no effect on offspring age at maturity (Kiritani and Kimura 1967, Phelan and Frumhoff 1991, McIntyre and Gooding 2000). Maternal age effects on offspring mass at maturity also

appear variable. Only three other studies have examined the effect of maternal age on offspring mass at maturity and all have found increases with maternal age; in contrast, we found varying results (Figure 3.4) depending upon host plant. Only five studies have examined maternal age effects on offspring fecundity. Three of these, including this one, found no effect on fecundity (Wasserman and Asami 1985, Moore and Harris 2003), while two studies documented a decrease (Kiritani and Kimura 1967, Li and Harmsen 1993). Our examination of the literature on maternal age effects on offspring vital rates suggests that only the Lansing effect is predictable.

The majority of studies described above are lab-based. Although a few field experiments of maternal effects in insects have been attempted, most are flawed because they use space-for-time substitution (Hunter 2002), an inappropriate design for trans-generational experiments on environmental factors. As a result, it is not clear at present how our results, or those of the studies in Table 3.3, relate to dynamics in the field. Nonetheless, there is a long and distinguished history of using laboratory experiments to study population dynamics (Birch 1953, Huffaker 1958, Ayala 1969). In fact, much of current population theory is based on such experiments (Speight et al. 1999). To our knowledge, only one population level experiment has adequately tied resources in the maternal environment to population dynamics in subsequent generations (Benton et al. 2005). At this time, evidence from laboratory studies provides the best empirical support for the view that maternal effects influence offspring vital rates and subsequent population dynamics. Here, we have shown that such effects are generally weak in *A. nerii* and often variable in magnitude and direction in other insect species.

ACKNOWLEDGEMENTS

We thank B. Ball, W. Duncan, K. Wickings, H. Wearing and two anonymous reviewers for their helpful comments and suggestions. We thank P. Doty and S. Scott for laboratory assistance.

REFERENCES

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology **80**:1713-1723.

Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. American Naturalist **164**:113-120.

Agrawal, A. A., C. Laforsch, and R. Tollrian. 1999. Transgenerational induction of defenses in animals and plants. Nature **401**.

Ayala, F. J. 1969. Experimental invalidation of principle of competitive exclusion. Nature **224**:1076-1078.

Beckerman, A., T. G. Benton, E. Ranta, V. Kaitala, and P. Lundberg. 2002. Population dynamic consequences of delayed life-history effects. Trends In Ecology & Evolution **17**:263-269.

Benton, T. G., S. J. Plaistow, A. P. Beckerman, C. T. Lapsley, and S. Littlejohns. 2005. Changes in maternal investment in eggs can affect population dynamics. Proceedings Of The Royal Society Of London Series B-Biological Sciences **272**:1351-1356.

Benton, T. G., E. Ranta, V. Kaitala, and A. P. Beckerman. 2001. Maternal effects and the stability of population dynamics in noisy environments. Journal Of Animal Ecology **70**:590-599.

Berryman, A. A. 1999. Principles of population dynamics and their application. Stanley Thornes (Publishers) Ltd, Cheltenham.

Birch, L. C. 1953. Experimental background to the study of the distribution and abundance of insects .3. The relation between innate capacity for increase and

survival of different species of beetles living together on the same food. Evolution **7**:136-144.

Diss, A. L., J. G. Kunkel, M. E. Montgomery, and D. E. Leonard. 1996. Effects of maternal nutrition and egg provisioning on parameters of larval hatch, survival and dispersal in the gypsy moth, *Lymantria dispar* L. Oecologia **106**:470-477.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. 2nd edition. Chapman and Hall, London.

Dixon, A. F. G., and B. K. Agarwala. 1999. Ladybird-induced life-history changes in aphids. Proceedings Of The Royal Society Of London Series B-Biological Sciences **266**:1549-1553.

Dixon, A. F. G., R. Kundu, and P. Kindlmann. 1993. Reproductive effort and maternal age in iteroparous insects using aphids as a model group. Functional Ecology **7**:267-272.

Fischer, K., P. M. Brakefield, and B. J. Zwaan. 2003. Plasticity in butterfly egg size: Why larger offspring at lower temperatures? Ecology **84**:3138-3147.

Fowler, M. S. 2005. Interactions between maternal effects and dispersal. Oikos **110**:81-90.

Fox, C. W. 1993. The influence of maternal age and mating frequency on egg size and offspring performance in *Callosobruchus maculatus* (Coleoptera: Bruchidae). Oecologia **96**:139-146.

Fox, C. W., K. J. Waddell, and T. A. Mousseau. 1995. Parental Host-Plant Affects Offspring Life-Histories In A Seed Beetle. Ecology **76**:402-411.

Ginzburg, L. R. 1998. Inertial growth: Population dynamics based on maternal effects.*in* T. A. Mousseau and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Ginzburg, L. R., and D. E. Taneyhill. 1994. Population-cycles of forest lepidoptera - a maternal effect hypothesis. Journal Of Animal Ecology **63**:79-92.

Haggstrom, H., and S. Larsson. 1995. Slow larval growth on a suboptimal willow results in high predation mortality in the leaf beetle *Galerucella lineola*. Oecologia **104**:308-315.

Hall, R. W., and L. E. Ehler. 1980. Population ecology of *Aphis nerii* Homoptera, Aphididae on oleander. Environmental Entomology **9**:338-344.

Hardie, J., and A. D. Lees. 1983. Photoperiodic regulation of the development of winged gynoparae in the aphid, *Aphis fabae*. Physiological Entomology **8**:385-391.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecological Entomology **29**:44-51.

Hercus, M. J., and A. A. Hoffmann. 2000. Maternal and grandmaternal age influence offspring fitness in Drosophila. Proceedings Of The Royal Society Of London Series B-Biological Sciences **267**:2105-2110.

Hockham, L. R., J. A. Graves, and M. G. Ritchie. 2001. Variable maternal control of facultative egg diapause in the bushcricket *Ephippiger ephippiger*. Ecological Entomology **26**:143-147.

Huffaker, C. B. 1958. Experimental studies on predation: dispersion factors and predator-prey oscillations. Hilgardia **27**:343-383.

Hunter, M. D. 2002. Maternal effects and the population dynamics of insects on plants. Agricultural and Forest Entomology **4**:1-9.

Islam, M. S., P. Roessingh, S. J. Simpson, and A. R. McCaffery. 1994. Effects of population-density experienced by parents during mating and oviposition on the phase of hatchling desert locusts, *Schistocerca gregaria*. Proceedings Of The Royal Society Of London Series B-Biological Sciences **257**:93-98.

Kendall, B. E., S. P. Ellner, E. McCauley, S. N. Wood, C. J. Briggs, W. M. Murdoch, and P. Turchin. 2005. Population cycles in the pine looper moth: Dynamical tests of mechanistic hypotheses. Ecological Monographs **75**:259-276.

Kern, S., M. Ackermann, S. C. Stearns, and T. J. Kawecki. 2001. Decline in offspring quality as a manifestation of aging in *Drosophila melanogaster*. Evolution **55**:1822-1831.

Kery, M., and J. S. Hatfield. 2003. Normality of raw data in general linear models: the most widespread myth in statistics. Bulletin of the Ecological Society of America **84**:92-94.

Kiritani, K., and K. Kimura. 1967. Effects of parental age on the life cycle of the southern green stink bug, *Nezara viridula*. Applied Entomology And Zoology **2**:69.

Ladner, D. T., and S. Altizer. 2005. Oviposition preference and larval performance of North American monarch butterflies on four *Asclepias* species. Entomologia Experimentalis Et Applicata **116**:9-20.

Lansing, A. I. 1947. A transmissible, cumulative, and reversible factor in aging. Journal of Gerontology **2**:228-239.

Lees, A. D. 1963. The role of photoperiod and temperature in the determination of parthenogenetic and sexual forms in the aphid *Megoura viciae* Buckton .3. Further properties of the maternal switching mechanism in apterous aphids. Journal Of Insect Physiology **9**:153-164.

Li, S. Y., and R. Harmsen. 1993. Effects of maternal density and age on the daily fecundity and offspring sex ratio in *Tetranychus urticae*. The Canadian Entomologist **125**:633-635.

Lill, J. T., and R. J. Marquis. 2001. The effects of leaf quality on herbivore performance and attack from natural enemies. Oecologia **126**:418-428.

Littell, R. C., P. R. Henry, and C. B. Ammerman. 1998. Statistical analysis of repeated measures data using SAS procedures. Journal Of Animal Science **76**:1216-1231.

Littell, R. C., W. W. Stroup, and R. J. Freund. 2002. SAS for Linear Models, Fourth Edition. SAS Institute Inc.,, Cary, NC.

Ludwig, D., and C. Fiore. 1960. Further studies on the relationship between parental age and the life cycle of the mealworm, *Tenebrio molitor*. Annals Of The Entomological Society Of America **53**:595-600.

Mackay, P. A. 1977. Alata production by an aphid - interval timer concept and maternal age effects. Journal Of Insect Physiology **23**:889-893.

Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores.*in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: their interactions with secondary plant metabolites. Academic Press, San Diego.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca*. Journal Of Chemical Ecology **15**:819-853.

Malcolm, S. B., and M. P. Zalucki. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. Entomologia Experimentalis Et Applicata **80**:193-196.

Martel, J. W., and S. B. Malcolm. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. Journal Of Chemical Ecology **30**:545-561.

McIntyre, G. S., and R. H. Gooding. 2000. Effects of maternal age on larval competitiveness in house flies. Heredity **85**:480-489.

Mohaghegh, J., P. De Clercq, and L. Tirry. 1998. Effects of maternal age and egg weight on developmental time and body weight of offspring of *Podisus maculiventris* (Heteroptera: Pentatomidae). Annals Of The Entomological Society Of America **91**:315-322.

Moore, P. J., and W. E. Harris. 2003. Is a decline in offspring quality a necessary consequence of maternal age? Proceedings Of The Royal Society Of London Series B-Biological Sciences **270**:192-194.

Mousseau, T. A., and H. Dingle. 1991. Maternal effects in insect life histories. Annual Review Of Entomology **36**:511-534.

Mousseau, T. A., and C. W. Fox. 1998. Maternal Effects as Adaptations. Oxford University Press, New York, New York.

Nunes, M. V., and J. Hardie. 1999. The effect of temperature on the photoperiodic 'counters' for female morph and sex determination in two clones of the black bean aphid, *Aphis fabae*. Physiological Entomology **24**:339-345.

Phelan, J. P., and P. C. Frumhoff. 1991. Differences in the effects of parental age on offspring life-history between tropical and temperate populations of milkweed bugs (*Oncopeltus* Spp). Evolutionary Ecology **5**:160-172.

Plaistow, S. J., C. T. Lapsley, A. P. Beckerman, and T. G. Benton. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proceedings Of The Royal Society Of London Series B-Biological Sciences **271**:919-924.

Priest, N. K., B. Mackowiak, and D. E. L. Promislow. 2002. The role of parental age effects on the evolution of aging. Evolution **56**:927-935.

Raychaudhuri, A., and A. Butz. 1965. Effects of parental age on the life cycle of *Tribolium confuscum* (Coleoptera: Tenebrionidae). Annals Of The Entomological Society Of America **58**:535-540.

Rossiter, M. 1988. Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology **69**:267-277.

Rossiter, M. 1994. Maternal effects hypothesis of herbivore outbreak. Bioscience **44**:752-763.

Rossiter, M. C. 1991a. Environmentally-based maternal effects - A hidden force in insect population-dynamics. Oecologia **87**:288-294.

Rossiter, M. C. 1991b. Maternal effects generate variation in life-history - Consequences of egg weight plasticity in the gypsy-moth. Functional Ecology **5**:386-393.

Rossiter, M. C. 1998. The role of environmental variation in parental effects expression.*in* T. A. Mousseau and C. W. Fox, editors. Maternal Effects as Adaptations. Oxford University Press, New York.

Schatten, H., A. Chakrabarti, and J. Hedrick. 1999. Centrosome and microtubule instability in aging Drosophila cells. Journal Of Cellular Biochemistry **74**:229-241.

Speight, M. R., M. D. Hunter, and A. D. Watt. 1999. The ecology of insects: concepts and applications. Blackwells Scientific, Oxford, UK.

Tsai, J. H., and J. J. Wang. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera : Aphididae). Environmental Entomology **30**:44-50.

Tschinkel, W. R. 1993. Crowding, maternal age, age at pupation, and life history of *Zophobas atratus* (Coleoptera: Tenebrionidae). Annals Of The Entomological Society Of America **86**:278-297.

Wainhouse, D., R. Ashburner, and R. Boswell. 2001. Reproductive development and maternal effects in the pine weevil *Hylobius abietis*. Ecological Entomology **26**:655-661.

Wasserman, S. S., and T. Asami. 1985. The effect of maternal age upon fitness progeny in the southern cowpea weevil, *Callosobruchus maculatus*. Oikos **45**:191-196.

Wheeler, G. S. 2001. Host plant quality factors that influence the growth and development of *Oxyops vitiosa*, a biological control agent of *Melaleuca quinquenervia*. Biological Control **22**:256-264.

Wiktelius, S. 1992. The induction of alatae in *Rhopalosiphum padi* (L) (Hom, Aphididae) in relation to crowding and plant-growth stage in spring sown barley. Journal Of Applied Entomology **114**:491-496.

Yanagi, S.-I., and T. Miyatake. 2002. Effects of maternal age on reproductive traits and fitness components of the offspring in the bruchid beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Physiological Entomology **27**:261-266.

Zehnder, C. B., and M. D. Hunter. 2007. A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid. Ecological Entomology **in press**.

Table 3.1: Effects of maternal density on offspring lifespan, age at maturity, mass at maturity and fecundity. All values are means (n = 168) ± standard error. There were no significant maternal density effects on offspring lifespan, age at maturity, mass at maturity or fecundity.

Maternal density (aphids/plant)	Offspring lifespan (days)	Offspring age at maturity (days)	Offspring mass at maturity (mg)	Offspring fecundity
1	15 ± 0.5	6 ± 0.06	0.94 ± 0.02	53 ± 2.26
25	15 ± 0.4	6 ± 0.04	0.93 ± 0.02	53 ± 1.99
50	14 ± 0.4	6 ± 0.06	0.86 ± 0.02	49 ± 2.09

Table 3.2: Effects of maternal host plant species on offspring lifespan, age at maturity and fecundity. All values are means (n = 252) ± standard error. There were no significant maternal host plant effects on offspring lifespan, age at maturity or fecundity.

Maternal host plant species	Offspring lifespan (days)	Offspring age at maturity (days)	Offspring fecundity
A. syriaca	15 ± 0.37	6 ± 0.05	50 ± 1.69
A. viridis	15 ± 0.38	6 ± 0.04	53 ± 1.75

Table 3.3: This table shows the effects of increasing maternal age on offspring quality in a variety of insect taxa. Within the offspring response category, any measures of survival, hatching viability, egg-adult viability, etc, are listed as longevity. Any measures of development time or rate are listed as age at maturity. The direction and magnitude of the offspring response was calculated directly from results and tables whenever possibly. However, in some cases it was necessary to refer to figures, which may lead to some slight inaccuracies in the calculations. Some of the studies listed examined other factors in addition to maternal age effects, and sometimes those other factors interacted with maternal age. However, in the interest of clarity, this table does not describe any of that additional detail. This table does not include maternal age effects on insect diapause or sex ratio (but see (Mousseau and Dingle 1991) for a review).

Species	**Offspring response**	**Direction & magnitude of response.**	**Reference**
Acyrthosiphon pisum	alate production	Increases and decreases	(Mackay 1977)
Aphis nerii	longevity	9.3% decrease	This study
	fecundity	No maternal age effect	
	age at maturity	3% decrease	
	mass at maturity	9% decrease	
Callosobruchus chinensis	age at maturity (female)	~9% increase	(Yanagi and Miyatake 2002)
	longevity	~16% decrease	
Callosobruchus maculatus	age at maturity	18% increase	(Fox 1993)
Callosobruchus maculatus	longevity	31% decrease	(Wasserman and Asami 1985)
	age at maturity	106% increase	
	fecundity	No maternal age effect	

Cavariella aegopodii	age at maturity	10% decrease	(Dixon et al. 1993)
	mass at maturity	9% increase	
Drosophila melanogaster	longevity	Decrease	(Kern et al. 2001)
Drosophila melanogaster	longevity	12% decrease	(Priest et al. 2002)
Drosophila serrata	longevity	37% decrease	(Hercus and Hoffmann 2000)
Ephippiger ephippiger	longevity	40% decrease	(Hockham et al. 2001)
Musca domestica	longevity	13% decrease	(McIntyre and Gooding 2000)
	age at maturity	No maternal age effect	
Nauphoeta cinera	longevity	No maternal age effect	(Moore and Harris 2003)
	fecundity	No maternal age effect	
Nazara viridula	longevity	18% decrease	(Kiritani and Kimura 1967)
	age at maturity	No maternal effect	
	fecundity	78% decrease	
	mass at maturity	11% increase	
Oncopeltus fasciatus	longevity	22% decrease	(Phelan and Frumhoff 1991)
	age at maturity	15% decrease	
	mass at maturity	7% increase	
Oncopeltus cingulifer	longevity	No maternal age effect	
	age at maturity	No maternal age effect	
	mass at maturity	No maternal age effect	
Podisus maculiventris	age at maturity	decrease	(Mohaghegh et al. 1998)
Tenebrio molitor	longevity	14% decrease	(Ludwig and Fiore 1960)
	age at maturity	9% decrease	
Tetranychus urticae	fecundity	50% decrease	(Li and Harmsen 1993)
Tribolium confuscum	longevity	53% decrease	(Raychaudhuri and Butz 1965)
Zophobas atratus	longevity	decrease	(Tschinkel 1993)

Figure 3.1: Experimental design used to study the effects of maternal age, density and host plant species on *Aphis nerii* vital rates.

Figure 3.1

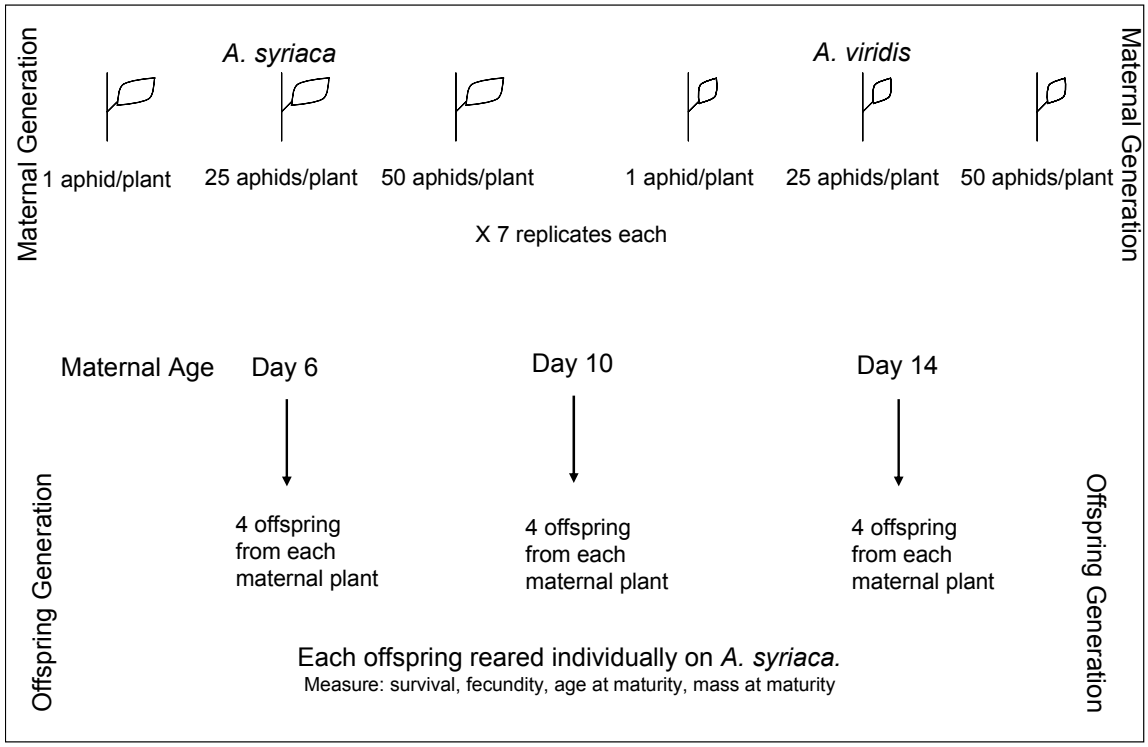

Maternal Generation
A. syriaca
A. viridis
Maternal Generation
1 aphid/plant
25 aphids/plant
50 aphids/plant
1 aphid/plant
25 aphids/plant
50 aphids/plant
X 7 replicates each
Maternal Age
Day 6
Day 10
Day 14
4 offspring from each maternal plant
4 offspring from each maternal plant
4 offspring from each maternal plant
Offspring Generation
Offspring Generation
Each offspring reared individually on A. syriaca.
Measure: survival, fecundity, age at maturity, mass at maturity

Figure 3.2: Effects of maternal age on offspring lifespan in *Aphis nerii*. Values are means (n = 168) ± standard error. Different letters above bars indicate significant differences among treatment means. There was no significant effect of maternal density or host plant on offspring lifespan, therefore we grouped these data and present averages for the three maternal ages.

Figure 3.2

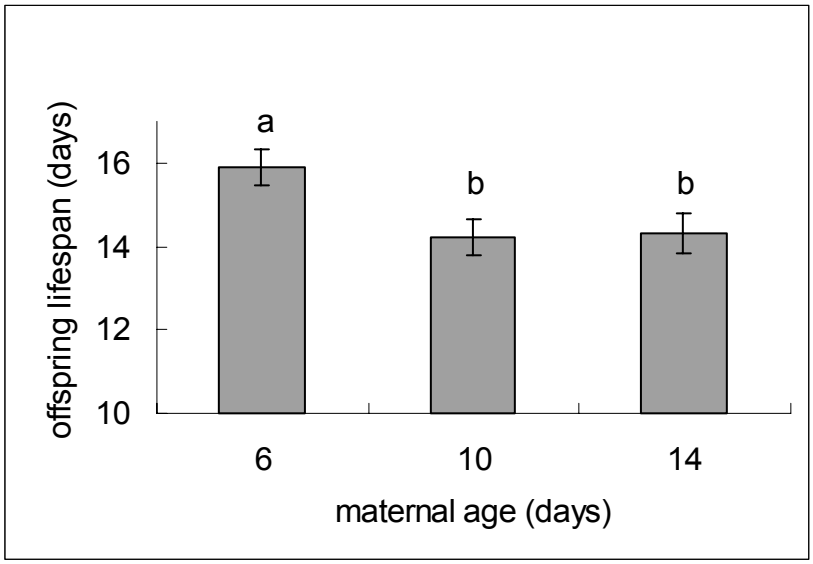

offspring lifespan (days)
16
14
12
10
a
b
b
6
10
14
maternal age (days)

Figure 3.3: Effects of maternal age on offspring age at maturity in *Aphis nerii*. Values are means (n = 168) $\pm$ standard error. Different letters above bars indicate significant differences among treatment means. There was no significant effect of maternal density or host plant on offspring age at maturity, therefore, we grouped these data and present averages for the three maternal ages.

Figure 3.3

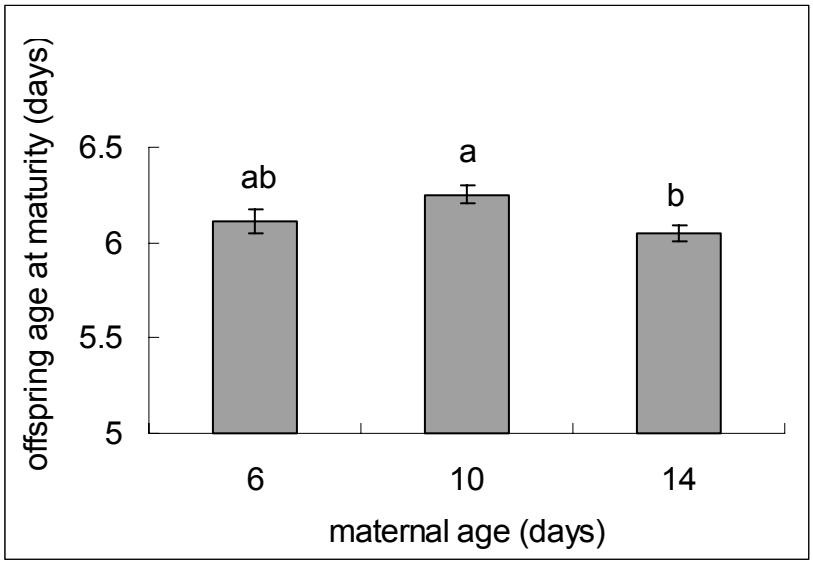
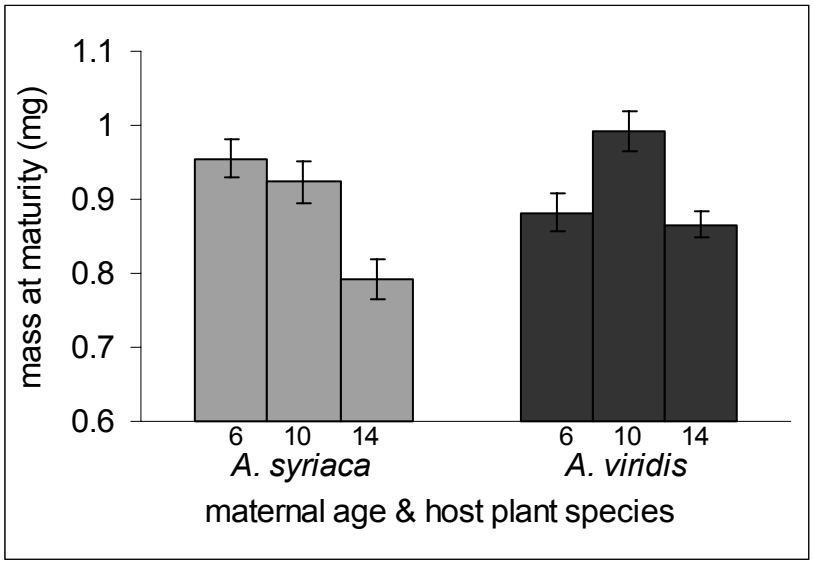

offspring age at maturity (days)
6.5
6
5.5
5
ab
a
b
6
10
14
maternal age (days)

Figure 3.4: Effects of maternal age and maternal host plant species (*Asclepias syriaca* light bars, *Asclepias viridis* dark bars) on offspring mass at maturity in *Aphis nerii*. Values are means (n = 84) ± standard error. There was no significant effect of maternal density on offspring mass at maturity, therefore, these data were pooled.

Figure 3.4


1.1
1
0.9
0.8
0.7
0.6
mass at maturity (mg)
6
10
14
6
10
14
A. syriaca
A. viridis
maternal age & host plant species

CHAPTER 4

NITROGEN DEPOSITION AND VARIATION IN THE POPULATION GROWTH OF INSECT HERBIVORES[1]

Zehnder, C.B., and M.D. Hunter. Submitted to Ecological Entomology July 2006

ABSTRACT

Anthropogenic increases in nitrogen deposition are causing changes to terrestrial ecosystems worldwide. While some of the direct ecosystem level effects of nitrogen deposition are understood, the effects of nitrogen deposition on plant-insect interactions and on herbivore population dynamics have received less attention. Nitrogen fertilization experiments typically lead to increases in both herbivore population growth and plant biomass. Subsequent effects on herbivore dynamics may depend upon the balance between these two responses.

There is no a priori reason to assume that rates of herbivore population growth and plant resource availability (herbivore carrying capacity) should respond in concert to nitrogen deposition. If increases in herbivore population growth outstrip increases in resource availability, we might predict increases in the strength of density dependence expressed within the herbivore population. Alternatively, if plant resources respond more vigorously or rapidly to nitrogen deposition than do herbivore populations, we might predict a decline in the strength of density dependence. No change in the strength of density dependence acting upon the herbivore population would suggest equivalent responses by herbivores and plants.

We performed a density manipulation experiment to examine the effect of nitrogen deposition on the interaction between a host plant, *Asclepias tuberosa*, and its herbivore, *Aphis nerii*. We estimated aphid maximum per capita growth rate (R_{max}), carrying capacity (K), and the strength of density-dependence under three nitrogen deposition treatments and asked whether nitrogen deposition changed the relationship among these three measures of insect population dynamics.

Simulated nitrogen deposition increased aphid per capita population growth, increased plant foliar nitrogen concentrations and increased plant biomass. Nitrogen deposition caused R_{max} and K to increase proportionally, leading to no overall change in the strength of density-dependence. In our system, potential changes in the negative feedback processes operating on herbivore populations following nitrogen deposition appear to be buffered by concomitant changes in resource availability.

Keywords: *Aphis nerii*, *Asclepias tuberosa*, density-dependence, density manipulation experiment, global change, herbivory, nitrogen deposition, plant quality

INTRODUCTION

Anthropogenic increases in nitrogen deposition are important drivers of environmental change (Vitousek et al. 1997, Throop and Lerdau 2004). Sources of deposition are mainly transportation, power plants, industry and agriculture (Driscoll et al. 2003). Current levels of nitrogen deposition range from 0 – 40 kilograms (kg) per hectare (ha) per year, and in many areas downwind of major urban and agricultural centers, deposition levels approach 30 to 90 kg ha^{-1} yr^{-1} (Van der Eerden et al. 1998, Fenn et al. 2003b). As the human population continues to increase, it is predicted that nitrogen deposition will increase as well (Fenn et al. 2003b) with concomitant effects at multiple levels of biological organization including individuals, populations, communities and ecosystems (Jefferies and Maron 1997, Vitousek et al. 1997, Gotelli and Ellison 2002, Aber et al. 2003, Fenn et al. 2003a, Madritch and Hunter 2003, Throop and Lerdau 2004).

Nitrogen deposition may have positive, negative or no effects on insect herbivore population dynamics. Nitrogen deposition often leads to increases in foliar nitrogen concentrations and plant biomass (Aber et al. 2003, Fenn et al. 2003a, Throop and Lerdau 2004). Because most insect herbivores are nitrogen limited (White 1993) we might predict increases in herbivore densities with increasing levels of nitrogen deposition. This prediction assumes that nitrogen deposition does not change plant secondary compounds or alter amino acid profiles, both of which can lead to decreased herbivore performance (Throop and Lerdau 2004). Additionally, nitrogen deposition can affect insect herbivores by altering plant or insect community composition (Stevens et al. 2004).

Long term population dynamics depend, in part, on the strength of density dependence (Hunter 2001) which can vary in space and time (Cappuccino and Price 1995, Harrison and Cappuccino 1995). For insect herbivores, variation in the strength of density dependence has been attributed to plant size (Rotem and Agrawal 2003), plant genotype (Underwood and Rausher 2000), plant resistance traits (Underwood and Rausher 2002, Agrawal et al. 2004) and variation in herbivore maximum per capita population growth rate (Agrawal et al. 2004). However, we are still unable to predict when to expect strong or weak density-dependence, how anthropogenic environmental change will affect its strength, and subsequent effects on insect population dynamics.

Density dependence is often illustrated as the negative relationship between per capita growth rate and population density (Figure 4.1). On such diagrams, the y-intercept represents the maximum per capita growth rate (R_{max}), the x-intercept represents carrying capacity (K) and the slope of the line represents the strength of density dependence. How might nitrogen deposition influence the form of these relationships? Figure 4.1 illustrates three hypothetical scenarios, where the solid lines indicate zero anthropogenic nitrogen deposition and the dotted lines indicate conditions after nitrogen deposition. In all three cases we assume negative density dependence, meaning there is a decrease in per capita population growth as initial density increases (Gotelli 2001). In Figure 4.1.A, R_{max} (insect population growth) and K (environmental carrying capacity) increase proportionally and there is no change in the strength of density dependence after deposition (i.e. the slopes of the lines are the same). This is similar to Figure 4.1 (lines A and C) in (Agrawal et al. 2004). In Figure 4.1.B, R_{max} increases faster than K increases,

and the strength of density-dependence increases. In Figure 4.1.C, K increases faster than R_{max} increases, and the strength of density-dependence decreases.

While there are relatively few studies of the effects of nitrogen deposition *per se* on insect herbivores (but see (Throop and Lerdau 2004), there have been many reported effects of nitrogen fertilization on insect herbivores. Typically, nitrogen fertilization rates in such experiments far exceed any predicted nitrogen deposition rates. In a review of nitrogen fertilization experiments conducted by Kyto and colleagues, aphids were found to respond positively to nitrogen fertilization while other insect guilds showed no response or a negative response (Kyto et al. 1996). Nitrogen fertilization has also been linked with increased insect density, shorter development time, higher survival rates, increased insect mass, higher fecundity and higher R_{max} (Mattson 1980, Cisneros and Godfrey 2001, Nevo and Coll 2001, Tsai and Wang 2001, Stiling and Moon 2005). However, other studies have found negative or no effects of nitrogen fertilization on insect abundance or performance (Bethke et al. 1998, Casey and Raupp 1999, Muller et al. 2005).

As far as we are aware, there is no *a priori* reason to assume that insect population growth rate and environmental carrying capacity should respond in concert to environmental change. To explore this further, we performed a density manipulation experiment to examine the effect of nitrogen deposition on the interaction between a host plant, *Asclepias tuberosa*, and its herbivore, *Aphis nerii*. We estimated aphid maximum per capita growth rate (R_{max}), carrying capacity (K), and the strength of density-dependence under three nitrogen deposition treatments and asked whether nitrogen

deposition changed the relationship among these three measures of insect population dynamics.

METHODS

Study species

Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae), the milkweed-oleander aphid, is an aposematic phloem-feeding specialist of milkweed (*Asclepias* spp) and oleander (*Nerium oleander* L. (Apocynaceae)) that reproduces parthenogenetically (Rothschild et al. 1970, Hall and Ehler 1980). In August 2003, one *A. nerii* individual was collected near Gainesville, Florida. Our aphid colony (clone) was initiated from this one individual. In the lab, aphid colonies were kept on *Asclepias syriaca* at low densities. *Asclepias* seeds were obtained from a seed distributor (Butterfly Encounters).

Experiments were conducted in a temperature and light controlled walk-in growth chamber (September – October, 2005). Grow lights on timers (16 hours day: 8 hour night) provided heat and light; daytime and nighttime temperatures were 34$\pm$ 2.4ºC and 24 $\pm$ 0.48 ºC, respectively, well within the range normally experienced by both aphid and plant species. The host plant species used in this experiment was *Asclepias tuberosa*, a milkweed species native to Georgia. All seedlings were 4 weeks old and the same size at the start of the experiment (12-15 cm in height, 10-12 leaves per seedling). Nitrogen deposition levels and aphid densities were randomly assigned to plants. All plants were individually placed in cages constructed from 710 ml Ziploc containers and organza netting over wire frames. Plants were rotated and watered daily.

Nitrogen deposition

We applied three levels of nitrogen deposition: 0, 25 and 40 kg ha^{-1} yr^{-1}. These levels fall within the range of nitrogen deposition levels experienced in the United States (Ollinger et al. 1993, Throop and Lerdau 2004). Because this experiment was carried out in small pots (0.04 m radius), the amount of nitrogen in the form of ammonium nitrate NH_4NO_3 was calculated based on an area of 0.005 m^2. For the 3 nitrogen deposition levels: 0, 0.036 and 0.057 grams of NH_4NO_3 were needed, respectively. These were divided into four applications over the course of three weeks. For each application, 25% of the appropriate amount of NH_4NO_3 was dissolved in dioinized water and 20ml solution was applied to each plant with a pipette. Nitrogen deposition occurred on day1 (9/6/05), day8 (9/13/05), day15 (9/20/05) and day22 (9/27/05).

Aphid density manipulation

We established six aphid densities (0, 1, 5, 10, 15 and 20 aphids per plant), with 5 replicates of each nitrogen deposition (3 levels) by aphid density (6 levels) combination for a total of 90 experimental plants. On day14 (9/19/05), two adult apterous aphids were placed on each plant (except for those plants receiving the 0 aphid/plant treatment). The aphids were allowed to reproduce overnight, and the following day the adults were removed and the offspring thinned to the appropriate densities. Aphids were left on the plants for 14 days (until day28), spanning approximately 2-3 generations, when total population and number of alates (winged aphids) were counted for each plant. Because the aphid densities reached such high levels, we were unable to count all of the aphids on all plants in one day. Therefore, on day28 (10/3/05) half of the aphids were counted and

the rest were counted on day29 (10/4/05). Treatments were divided evenly between the two days of counting and population growth rates were adjusted accordingly for the extra day of growth (below). After aphids were counted, aboveground plant biomass was harvested. Each plant was clipped at the base of its stem and then rinsed to remove any aphid honeydew. Plants were dried, weighed and the top 5 leaves were ground into a fine powder using a ball mill grinder. For carbon and nitrogen analysis, ground samples were weighed into tin capsules with a Mettler UMT2 microbalance (Mettler Toledo, Im Langacher, Switzerland) and analyzed with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan Italy).

Statistical analyses

Aphid per capita growth rate was estimated on each plant by subtracting the natural log of the initial density from the natural log of the final density divided by the number of days that aphids were on the plants: $(\ln N_2 - \ln N_1)/(t_2-t_1)$. The strength of density dependence was determined for each nitrogen deposition level by finding the slope of the regression of per capita growth rate versus initial density. R_{max} (maximum population growth rate) for each nitrogen deposition level was found by determining the y-intercept of each regression equation, and K (carrying capacity) was found by determining the x-intercept for each equation. Although non-linear declines in per capita growth rate with population density have been reported (Sibly et al. 2005), non-linear parameters provided no improvement in models, confirming that linear density-dependence predominates in this aphid species (Agrawal et al. 2004).

Rates of aphid population growth were compared among deposition treatments using analysis of covariance (ANCOVA) with nitrogen deposition as a class variable and initial density as a covariate. ANCOVA is an appropriate method for comparing slopes and intercepts of regression lines (Sokal and Rohlf 1995), and therefore it allows us to compare R_{max}, K and the strength of density-dependence among the three nitrogen deposition treatments. Effects of nitrogen deposition on the strength of density dependence were assessed by the significance of the interaction term between nitrogen deposition and initial density. This functions as a comparison of slopes (Figure 4.1). Final plant biomass, foliar nitrogen concentrations and the proportion of alate aphids produced were compared among deposition treatments using ANCOVA as above, with nitrogen deposition as a class variable and initial aphid density as a covariate. Tukey's honest significant difference tests were conducted to compare among treatment means. All analyses were conducted using SAS version 8.2 for windows. Residuals were checked for normality and met assumptions of normality except in one case; proportion alate data were arcsine-square root transformed prior to analysis.

RESULTS

Aphid per capita population growth decreased as initial aphid density increased, confirming the presence of negative density dependence (Figure 4.2, Table 4.1). Nitrogen deposition led to increases in aphid R_{max}, K and per capita population growth (Figure 4.2, Tables 4.1 and 4.2). However, the strength of density dependence did not differ significantly among nitrogen deposition levels (Table 4.1: interaction term, Table 4.2). The proportion of alate aphids that were produced increased with initial aphid

density (Figure 4.3: density $F_{1,61} = 35.57$, $p<0.0001$). However, nitrogen deposition had no effect on the proportion of alates produced (Table 4.2: nitrogen $F_{2,61} = 0.44$, $p=0.6478$, nitrogen*density $F_{2,62} = 0.32$, $p=0.7275$).

A. tuberosa foliar nitrogen concentrations increased with nitrogen deposition and decreased with initial aphid density (Figure 4.4: nitrogen $F_{2,71}= 18.25$, $p<0.0001$, density $F_{1,71} = 17.21$, $p<0.0001$, nitrogen*density $F_{2,71}= 0.27$, $p=0.7665$).

Final biomass of *A. tuberosa* plants increased with nitrogen deposition whereas initial aphid density had no effect on final plant biomass (Figure 4.5: nitrogen $F_{2,74}= 19.14$, $p<0.0001$, density $F_{1,74}= 0.34$, $p=0.5643$, nitrogen*density $F_{2,74}= 2.58$, $p=0.0822$).

DISCUSSION

In this system, nitrogen deposition led to proportional increases in aphid per capita growth rate and carrying capacity (Figure 4.2), as well as increases in plant foliar nitrogen concentration and biomass (Figures 4.4 & 4.5). The proportional increase in aphid R_{max} and K results in no change in the strength of density-dependence, corresponding to the scenario illustrated in Figure 4.1.A. In this case, the increase in aphid population growth is balanced by the increase in environmental carrying capacity. Not surprisingly, under all experimental conditions, we saw negative density dependence (Figure 4.2). We have previously found decreases in aphid per capita survival and fecundity with increasing density on four milkweed species including *Asclepias tuberosa* (Zehnder and Hunter 2007). Likewise, Agrawal (2004) reported that *A. nerii* exhibited negative density dependence on 17 out of 18 milkweed species.

As Agrawal et al. (2004) have shown, R_{max} and K do not always vary in concert. In their experiments working with *Aphis nerii* on *Asclepias syriaca* and other milkweed species, they found no proportional change in K with variation in R_{max}. As a consequence, any increase in R_{max} was associated with an increase in the strength of density dependence, the scenario illustrated in Figure 4.1.B. Agrawal et al. (2004) did not explicitly manipulate plant quality within a single host plant species, as we did here. By manipulating plant quality via nitrogen deposition, we show that it's possible to increase R_{max} (insect population growth) and at the same time increase K (environmental carrying capacity). Therefore, under these conditions, R_{max} is not an accurate predictor of the strength of density-dependence.

In our experiment, nitrogen deposition led to increased aphid population growth and increased foliar nitrogen concentrations, confirming a link between foliar nitrogen content and *A. nerii* R_{max} (Agrawal 2004). Similarly, in a nitrogen fertilization experiment using cotton-melon aphids, *Aphis gossypii*, a positive relationship was found between foliar nitrogen concentrations and insect performance (Nevo and Coll 2001). Such relationships have been documented for many other insect herbivores (Mattson 1980) arguing for frequent nitrogen limitation among insect herbivores (White 1993). While nitrogen addition typically leads to an increase in aphid population growth rates, this is not always the case. In a field experiment examining the effects of fertilizer addition and predator exclusion on *Aphis jacobaeae*, an aphid-specialist of ragwort, there was no effect of fertilization on aphid density (Muller et al. 2005). However, foliar quality was not measured in this experiment; therefore it is possible that the fertilization treatment did not affect plant quality or that there was an increase in plant defensive

compounds. It is also possible that natural enemies had a larger impact on aphid population growth than did plant productivity (Muller et al. 2005).

In our study, nitrogen deposition increased both plant quality (Figure 4.4) and quantity (Figure 4.5) and similar results have been documented for a variety of plant species (Throop and Lerdau 2004). For example, nitrogen deposition was found to increase seedling nitrogen content and growth rate for five out of six annual species in a California grassland community (Cleland et al. 2006). In our experiment, as initial aphid density increased, foliar nitrogen concentrations decreased, but there was no effect of aphid density on plant biomass. This result suggests an imperfect, or complex, link between nitrogen availability and *Asclepias* biomass. In a comparable study examining the effects of nitrogen deposition and herbivory on common ragweed, simulated nitrogen deposition led to an increase in plant biomass and foliar nitrogen concentrations. Additionally, neither aphid nor beetle herbivory reduced plant shoot biomass; however there were negative effects of insect herbivory on root and seed biomass (Throop 2005).

In our experiment, nitrogen deposition caused an increase in insect herbivore densities. This has important implications for both crop and forest insect pests, especially if nitrogen deposition leads to an increase in the rate or severity of herbivore outbreaks. Recent work has suggested that nitrogen deposition is linked to increases in outbreak frequency of the monophagous heather beetle, *Lochmaea suturalis*, on *Calluna vulgaris* in the Netherlands (Bobbink et al. 1998). In the northeastern United States, there are hotspots of nitrogen deposition located in high elevation forests (Driscoll et al. 2003) with the potential to facilitate insect outbreaks.

In our system, nitrogen deposition increased the rate of insect population growth and increased plant biomass such that there was no change in the strength of density dependence. These results add to a growing body of literature examining the effects nitrogen deposition on plant-herbivore interactions (Throop and Lerdau 2004). However, there is still a lack of long term data examining the continuing impacts of nitrogen deposition on plant and herbivore dynamics. Moreover, it is important to consider higher trophic levels (Hunter 2001, Muller et al. 2005) and determine how predation and parasitism rates change under nitrogen deposition.

ACKNOWLEDGEMENTS

We thank M. Parris, S. Scott, and A. Whittaker for laboratory assistance. We thank B. Ball, S. Spires, K. Wickings, and H. Wearing for their helpful comments and suggestions.

REFERENCES

Aber, J. D., C. L. Goodale, S. Ollinger, M. L. Smith, A. H. Magill, M. E. Martin, R. A. Hallett, and J. L. Stoddard. 2003. Is nitrogen deposition altering the nitrogen status of northeastern forests? Bioscience **53**:375-389.

Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. American Naturalist **164**:113-120.

Agrawal, A. A., N. Underwood, and J. R. Stinchcombe. 2004. Intraspecific variation in the strength of density dependence in aphid populations. Ecological Entomology **29**:521-526.

Bethke, J. A., R. A. Redak, and U. K. Schuch. 1998. Melon aphid performance on chrysanthemum as mediated by cultivar, and differential levels of fertilization and irrigation. Entomologia Experimentalis Et Applicata **88**:41-47.

Bobbink, R., M. Hornung, and J. G. M. Roelofs. 1998. The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. Journal Of Ecology **86**:717-738.

Cappuccino, N., and P. W. Price, editors. 1995. Population Dynamics. Academic Press, San Diego.

Casey, C. A., and M. J. Raupp. 1999. Supplemental nitrogen fertilization of containerized azalea does not affect performance of azalea lace bug (Heteroptera : Tingidae). Environmental Entomology **28**:998-1003.

Cisneros, J. J., and L. D. Godfrey. 2001. Midseason pest status of the cotton aphid (Homoptera : Aphididae) in California cotton: Is nitrogen a key factor? Environmental Entomology **30**:501-510.

Cleland, E. E., H. A. Peters, H. A. Mooney, and C. B. Field. 2006. Gastropod herbivory in response to elevated CO2 and N addition impacts plant community composition. Ecology **87**:686-694.

Driscoll, C., T., D. Whitall, J. D. Aber, E. Boyer, M. Castro, C. Cronan, C. L. Goodale, P. Groffman, C. Hopkinson, K. Lambert, G. Lawrence, and S. Ollinger. 2003. Nitrogen pollution in the northeastern United States: sources, effects, and management options. Bioscience **53**:357-374.

Fenn, M. E., J. S. Baron, E. Allen, B., H. M. Rueth, K. R. Nydick, L. Geiser, W. D. Bowman, J. O. Sickman, T. Meixner, D. W. Johnson, and P. Neitlich. 2003a. Ecological effects of nitrogen deposition in the western United States. Bioscience **53**:404-420.

Fenn, M. E., R. Haeuber, G. S. Tonnesen, J. S. Baron, S. Grossman-Clarke, D. Hope, D. A. Jaffe, S. Copeland, L. Geiser, H. M. Rueth, and J. O. Sickman. 2003b. Nitrogen emissions, deposition, and monitoring in the western United States. Bioscience **53**:391-403.

Gotelli, N. J. 2001. A Primer of Ecology. Sinauer Associates, Inc., Sunderland, MA.

Gotelli, N. J., and A. M. Ellison. 2002. Nitrogen deposition and extinction risk in the northern pitcher plant, *Sarracenia purpurea*. Ecology **83**:2758-2765.

Hall, R. W., and L. E. Ehler. 1980. Population ecology of *Aphis nerii* Homoptera, Aphididae on oleander. Environmental Entomology **9**:338-344.

Harrison, S., and N. Cappuccino. 1995. Using density manipulation experiments to study population regulation.*in* N. Cappuccino and P. W. Price, editors. Population Dynamics. Academic Press, San Diego.

Hunter, M. D. 2001. Multiple approaches to estimating the relative importance of top-down and bottom-up forces on insect populations: Experiments, life tables, and time-series analysis. Basic And Applied Ecology **2**:295-309.

Jefferies, R. L., and J. L. Maron. 1997. The embarrassment of riches: Atmospheric deposition of nitrogen and community and ecosystem processes. Trends In Ecology & Evolution **12**:74-78.

Kyto, M., P. Niemela, and S. Larsson. 1996. Insects on trees: Population and individual response to fertilization. Oikos **75**:148-159.

Madritch, M. D., and M. D. Hunter. 2003. Intraspecific litter diversity and nitrogen deposition affect nutrient dynamics and soil respiration. Oecologia **136**:124-128.

Mattson, W. J. 1980. Herbivory in relation to plant nitrogen-content. Annual Review Of Ecology And Systematics **11**:119-161.

Muller, C. B., M. D. E. Fellowes, and H. C. J. Godfray. 2005. Relative importance of fertiliser addition to plants and exclusion of predators for aphid growth in the field. Oecologia **143**:419-427.

Nevo, E., and M. Coll. 2001. Effect of nitrogen fertilization on *Aphis gossypii* (Homoptera : Aphididae): Variation in size, color and reproduction. Journal Of Economic Entomology **94**:27-32.

Ollinger, S., J. D. Aber, G. M. Lovett, S. E. Millham, R. G. Lathrop, and J. M. Ellis. 1993. A spatial model of atmospheric deposition for the northeastern U.S. Ecological Applications **3**:459-472.

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

Rothschild, M., J. V. Euw, and T. Reichste. 1970. Cardiac glycosides in the oleander aphid, *Aphis nerii*. Journal Of Insect Physiology **16**:1141-&.

Sibly, R. M., D. Barker, M. C. Denham, J. Hone, and M. Pagel. 2005. On the Regulation of Populations of Mammals, Birds, Fish, and Insects. Science **309**:607-610.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry: the principles and practice of statistics in biological research. 3rd edition. W.H. Freeman & Company, New York.

Stevens, C. J., N. B. Dise, J. O. Mountford, and D. J. Gowing. 2004. Impact of nitrogen deposition on the species richness of grasslands. Science **303**:1876-1879.

Stiling, P., and D. C. Moon. 2005. Quality or quantity: the direct and indirect effects of host plants on herbivores and their natural enemies. Oecologia **142**:413-420.

Throop, H. L. 2005. Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. Oikos **111**:91-100.

Throop, H. L., and M. T. Lerdau. 2004. Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. Ecosystems **7**:109-133.

Tsai, J. H., and J. J. Wang. 2001. Effects of host plants on biology and life table parameters of *Aphis spiraecola* (Homoptera : Aphididae). Environmental Entomology **30**:44-50.

Underwood, N., and M. Rausher. 2002. Comparing the consequences of induced and constitutive plant resistance for herbivore population dynamics. American Naturalist **160**:20-30.

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

Van der Eerden, L., W. De Vries, and H. Van Dobben. 1998. Effects of ammonia deposition on forests in the Netherlands. Atmospheric Environment **32**:525-532.

Vitousek, P. M., J. D. Aber, R. W. Howarth, G. E. Likens, P. A. Matson, D. W. Schindler, W. H. Schlesinger, and D. G. Tilman. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications **7**:737-750.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, New York.

Zehnder, C. B., and M. D. Hunter. 2007. A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid. Ecological Entomology **in press**.

Table 4.1: The influence of nitrogen deposition and initial *Aphis nerii* density on per capita growth rate of the aphid population over 2-3 generations on *Asclepias tuberosa*. The non-significant interaction between initial aphid density and nitrogen deposition indicates that the strength of density-dependence did not differ among nitrogen deposition treatments.

	Type III sum of squares	df	MS	F	P
Nitrogen deposition	0.0224	2	0.0112	9.32	0.0003
Initial aphid density	0.2022	1	0.2022	168.24	<0.0001
Nitrogen deposition x initial density	0.0011	2	0.0006	0.48	0.6209
Error	0.0733	61	0.0012		

Table 4.2: The effect of nitrogen deposition on the maximum per capita population growth rate (R_{max}), carrying capacity (K), strength of density dependence (b) and proportion alate of *Aphis nerii* on its host plant *Asclepias tuberosa*. Data represent the means of 25 replicates followed by standard errors in brackets. Different letters within columns denote significantly different means under Tukey's test (R_{max}, K, proportion alate).

Nitrogen deposition (kg $ha^{-1}yr^{-1}$)	R_{max}	K	b	Proportion alate
0	0.2829 (0.012)[a]	29.504 (2.71)[a]	-0.00753 (0.001)	0.104 (0.02)[a]
25	0.3352 (0.011)[b]	35.124 (2.98)[b]	-0.00787 (0.001)	0.109 (0.02)[a]
40	0.3647 (0.017)[b]	32.407 (3.38)[b]	-0.00904 (0.001)	0.111 (0.02)[a]

Figure 4.1: Three hypothetical scenarios showing the effect of nitrogen deposition on the relationship between initial density and per capita population growth rate. The solid line indicates zero deposition conditions and the dotted line represents conditions after nitrogen deposition. The slope of the line is a measure of the strength of density-dependence and we assume negative density dependence in all 3 scenarios. A) The maximum population growth rate, R_{max} (the y-intercept), and the carrying capacity, K (the x-intercept), increase proportionally and there is no change in the strength of density-dependence. B) R_{max} increases fasters than K, and therefore the strength of density-dependence increases. C) K increases fasters than R_{max} and the strength of density-dependence decreases.

Figure 4.1

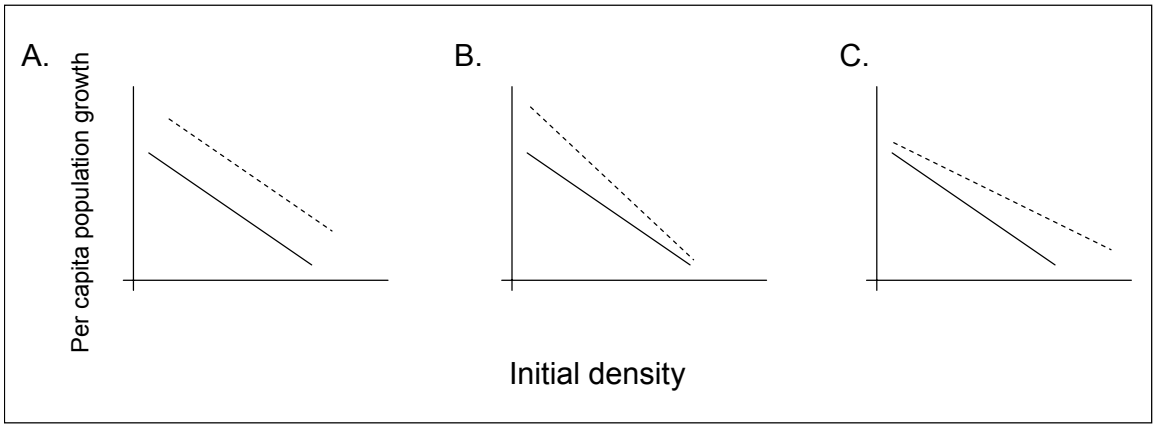


Figure 4.2: The effect of initial *Aphis nerii* density on *A. nerii* per capita population growth among 3 nitrogen deposition levels on *Asclepias tuberosa*. Each line represents the regression of initial aphid density on per capita population growth (n=25 for each nitrogen deposition level).

Figure 4.2

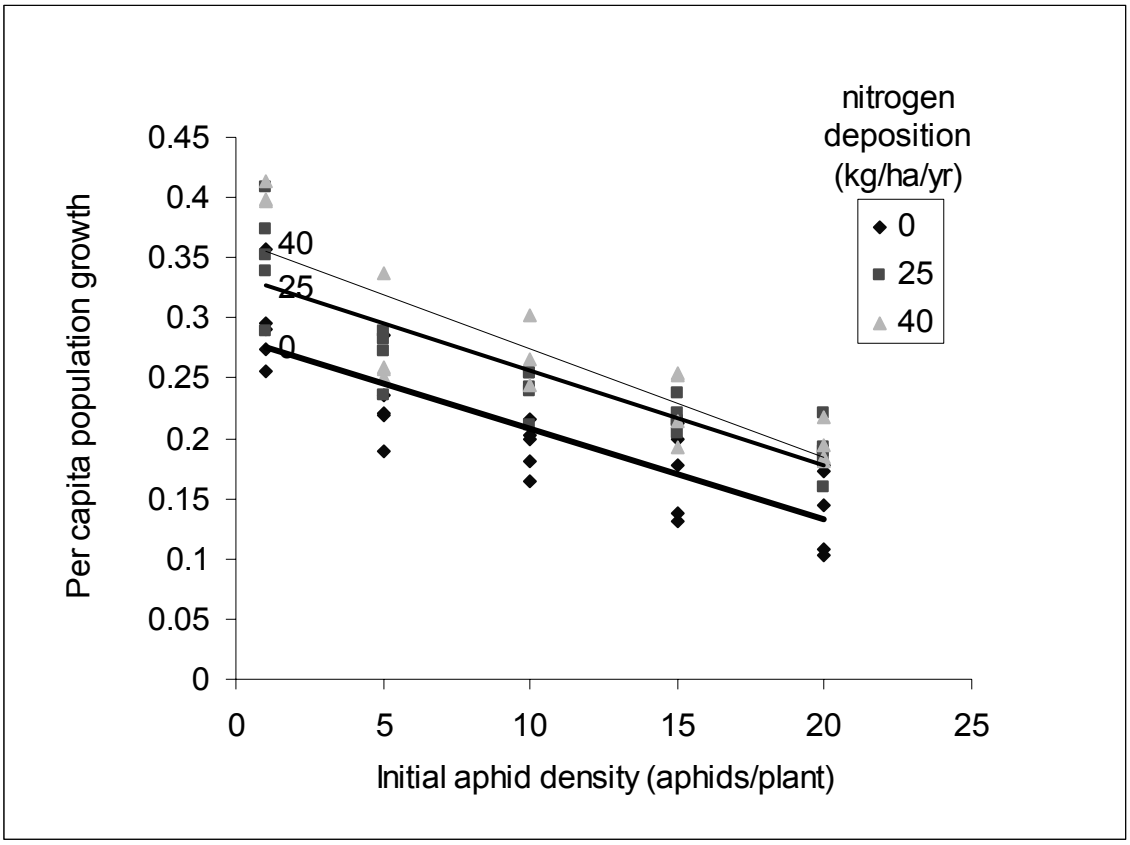

nitrogen deposition (kg/ha/yr)
0
25
40
Per capita population growth
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
40
25
0
0
5
10
15
20
25
Initial aphid density (aphids/plant)

Figure 4.3: The effect of initial *Aphis nerii* density on the proportion alate (winged aphids) present after 2 weeks on *Asclepias tuberosa* (bars are means of 15 $\pm$ 1 standard error).

Figure 4.3

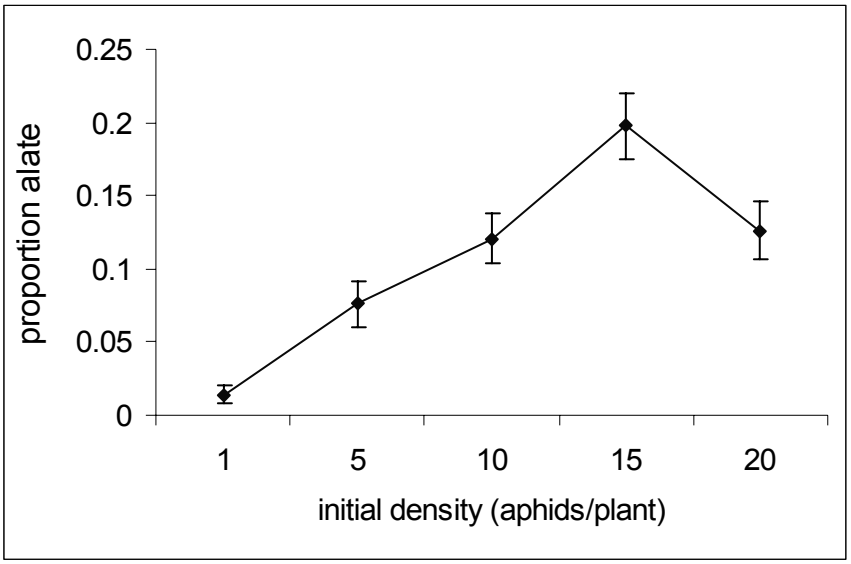

0.25
0.2
0.15
0.1
0.05
0
proportion alate
1
5
10
15
20
initial density (aphids/plant)

Figure 4.4: The effect of initial *Aphis nerii* density on *Asclepias tuberosa* percentage foliar nitrogen among 3 nitrogen deposition levels. Each line represents the regression of initial aphid density on percentage foliar nitrogen (n=30 for each nitrogen deposition level).

Figure 4.4

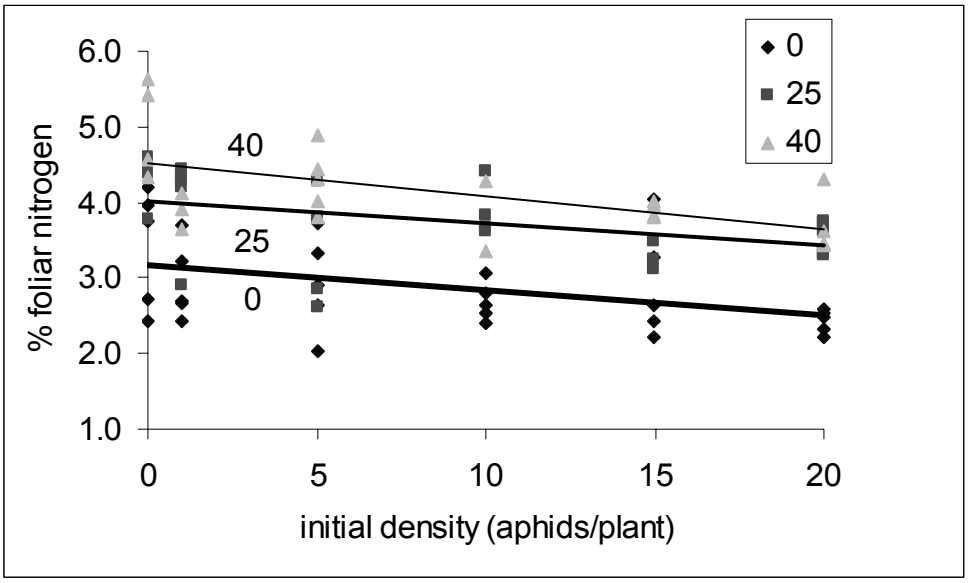
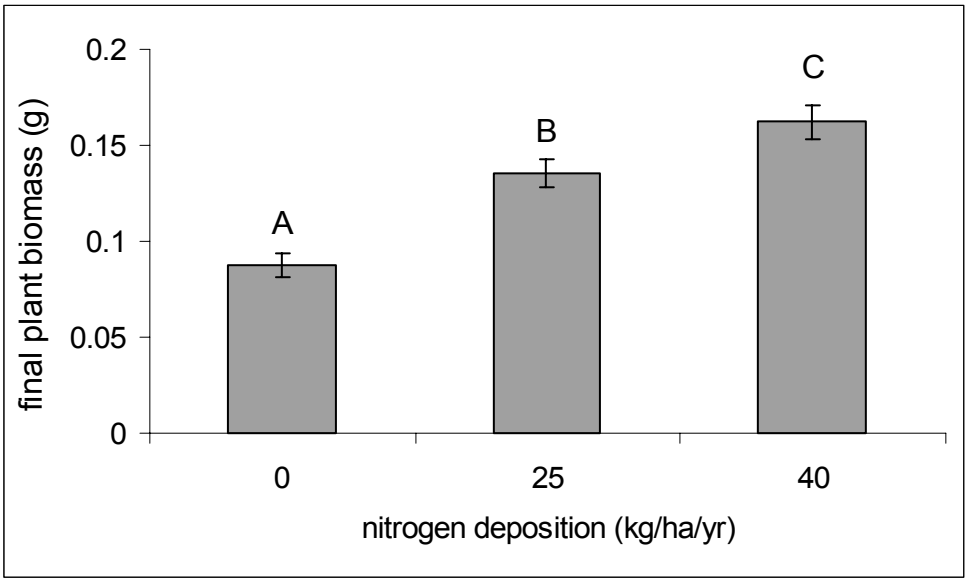

6.0
5.0
4.0
3.0
2.0
1.0
% foliar nitrogen
0
5
10
15
20
initial density (aphids/plant)
0
25
40
40
25
0

Figure 4.5: The effect of nitrogen deposition on *Asclepias tuberosa* final dry biomass (bars are means of 30 $\pm$ 1 standard error). Different letters above bars indicate significant differences among treatment means.

Figure 4.5


0.2
0.15
0.1
0.05
0
final plant biomass (g)
A
B
C
0
25
40
nitrogen deposition (kg/ha/yr)

CHAPTER 5

DECLINES IN PLANT CHEMICAL DEFENSE INDUCED BY A PHLOEM FEEDING INSECT HERBIVORE[1]

[1]Zehnder, C.B. and Hunter, M.D. To be submitted to the Journal of Chemical Ecology

ABSTRACT

Plant quality often changes following herbivory, and declines in quality may act as plant defenses. Induced plant responses to leaf chewing insects have been well studied, but considerably less is known about the effects of phloem-feedings insects on induction. In a set of laboratory experiments, we examined density-dependent induction by the milkweed-oleander aphid, *Aphis nerii*, of putative defenses in four milkweed species (*Asclepias incarnata*, *A. syriaca*, *A. tuberosa*, and *A. viridis*). We hypothesized that high aphid density would lead to increased cardenolide production in high cardenolide species (e.g. *A. viridis*), but that there would be no induction of cardenolides in low cardenolide species (e.g. *A. incarnata*). Contrary to our predictions, we observed feeding-induced declines in cardenolide concentrations in *A. viridis*. Cardenolide concentrations did not respond to aphid feeding in the other three milkweed species. Aphids also caused reductions in biomass accumulation by two of four *Asclepias* species. High aphid density led to increased foliar nitrogen and decreased foliar carbon concentrations in *A. incarnata*. However, aphid herbivory did not influence foliar nitrogen or carbon concentrations in any of other three milkweed species.

Keywords: *Aphis nerii*, *Asclepias*, cardenolide, density-dependence, induction, milkweed, plant defense, plant-insect interactions

INTRODUCTION

Induced plant responses to herbivory are broadly defined as any modification in the plant following damage, including changes in plant quality due to production of toxic or anti-nutritional compounds, protein or nutritional constituents, leaf toughness, thorns, spines or trichomes (Karban and Baldwin 1997). Following damage, plant nutritional quality may decrease or the concentration of secondary compounds, physical defenses, or volatile emission may increase (Green and Ryan 1972, Karban and Baldwin 1997, De Moraes et al. 1998, Agrawal 1999, Abdala-Roberts and Parra-Tabla 2005, Orians 2005). In many cases, these induced changes reduce subsequent herbivory and act as plant defenses (Carroll and Hoffman 1980, Karban and Baldwin 1997, Agrawal 2000). It is thought that induced defenses are less costly than constitutive defenses because the induced defenses are only activated after herbivory. Therefore, the plant does not pay any associated ecological or allocation cost in the absence of herbivory (Agrawal 1999, 2000, Strauss et al. 2002). Induced plant responses to herbivory have been demonstrated multiple times for leaf chewing insect herbivores (Green and Ryan 1972, Carroll and Hoffman 1980, Malcolm and Zalucki 1996, Karban and Baldwin 1997, Wold and Marquis 1997).

Less is known about the effects of phloem-feeding insect herbivores on plant induction. Because phloem-feeders do not remove leaf tissue *per se*, and in many cases cause no decrease in plant biomass or leaf area, defenses induced by phloem feeders may differ from those induced by leaf chewers (Martel and Malcolm 2004). Certainly phytochemical induction by phloem feeders varies among plant species (Martel and Malcolm 2004, Cardoza et al. 2005). For example, previous work within the *Asclepias*

genus found evidence for cardenolide induction in high cardenolide species, but no induction in species that lack constitutive cardenolide defenses (Martel and Malcolm 2004).

In a set of laboratory experiments, we measured changes in plant quality induced by varying densities of the milkweed-oleander aphid, *Aphis nerii*, on four milkweed species (*Asclepias incarnata*, *A. syriaca*, *A. tuberosa*, and *A. viridis*). Specifically, we measured changes in foliar nitrogen and carbon, defensive chemical concentrations (cardenolides, below), and plant biomass. Following Martel and Malcolm (2004), we hypothesized that the magnitude of induction responses would increase with increasing constitutive levels of cardenolides (lowest in *A. tuberosa*, highest in *A. viridis*).

METHODS

Study system

The genus *Asclepias* is composed of predominately herbaceous perennials with a wide geographic distribution (Woodson 1954). These species are known as milkweeds because most of them produce a milky, cardenolide-laden latex when damaged (Malcolm 1991). Cardenolides, or cardiac glycosides, are a group of cardiac-active steroids that are bitter tasting and toxic in high doses. Cardenolides have been shown to have many negative effects on species that consume them due to their emetic and toxic properties (Brower et al. 1984, Malcolm 1989, Malcolm and Brower 1989, Malcolm 1991). It is their bitter taste combined with their potential toxicity that has implicated cardenolides in defensive use by plants against natural enemies, including herbivores, parasites and pathogens (Malcolm and Zalucki 1996, Agrawal and Malcolm 2002).

There is a large degree of variability in cardenolide concentrations within and among milkweed individuals and species (Nelson et al. 1981, Malcolm 1991). Analyses of population-level variation in cardenolide content have shown that cardenolides vary qualitatively and quantitatively both spatially and temporally (Nelson et al. 1981, Malcolm and Brower 1989). Moreover, there is considerable qualitative and quantitative variation among *Asclepias* species, and most can be distinguished by their distinctly different cardenolide 'fingerprint' (Malcolm et al. 1989, Malcolm 1991). In addition to variation in cardenolide concentration, milkweed species also vary in carbon and nitrogen concentration, trichome density, specific leaf area and water content (Agrawal 2004).

Aphis nerii Boyer de Fonscolombe (Hemiptera: Aphididae), the milkweed-oleander aphid, is an aposematic phloem feeding specialist of milkweed, *Asclepias* spp, and oleander, *Nerium oleander* L. (Apocynaceae). *A. nerii* undergoes parthenogenetic reproduction for most of its life cycle, and sexually reproducing morphs are only produced when multiple generations are exposed to cool temperatures and short photoperiods (Takada and Miyazaki 1992, 1993).

Laboratory experiments

In August 2003, three *A. nerii* individuals were collected from geographically distinct locations (Atlanta, Georgia; Augusta, Georgia and Gainesville, Florida). Each of these is referred to as a separate clone (i.e. clone Emory, Augusta and Florida). An aphid colony was initiated from one individual from each location, leading to three lab colonies. Separate colonies of these aphid clones were kept at low densities on *Asclepias syriaca* in the lab. The colonies were kept in the same growth chamber in which all the

experiments were run (described below) and experienced the same light and temperature regime.

All experiments were conducted in a temperature and light controlled walk-in growth chamber. Grow lights on timers (16 hours day: 8 hour night) provided heat and light, daytime and nighttime temperatures were 34 $\pm$ 2.4ºC and 24 $\pm$ 0.48 ºC, respectively. *Asclepias* seeds were obtained from a seed distributor (Butterfly Encounters), grown in Farfard 3B soil with Osmocote time-release fertilizer (14-14-14, N:P:K) and watered as needed. In all experiments, plants were randomly assigned to treatments and rotated daily to homogenize any environmental gradients within the growth chamber. Four *Asclepias* species, *A. tuberosa*, *A. viridis*, *A. incarnata* and *A. syriaca*, were chosen to represent a range of plant quality traits. The four species vary in foliar carbon:nitrogen ratios, trichome density (Agrawal 2004) and cardenolide content (Malcolm et al. 1989, Agrawal 2004) The rank order from lowest to higher cardenolide contents are *A. tuberosa* (negligible), *A. incarnata* (low), *A. syriaca* (moderate) and *A. viridis* (high) (M.D. Hunter, unpublished data).

The identical experimental design was repeated on the four *Asclepias* species (*A. tuberosa*: June 2004, *A. viridis*: July 2004, *A. incarnata*: October 2004 and *A. syriaca*: November 2004). Because of time and space limitations, we could not examine all species concurrently. Consequently, any comparisons among plant species should be treated with caution because experimental period is confounded with plant species. However, photoperiod, temperature and other environmental conditions remained constant in the growth chamber over the course of all experiments. For each experiment, three aphid densities (1, 25 and 50 aphids per plant, and zero aphids on controls) were

established on 1 month old *Asclepias* seedlings. These densities represent natural aphid densities (Hall and Ehler 1980); 50 aphids per seedling is approximately 250-500 aphids per gram dry weight of plant tissue, which is at the high end of natural aphid densities (Helms et al. 2004). At the start of the experiment, all seedlings were 4 weeks old, approximately the same size (10-15 cm in height), and composed of a single stem bearing 6-10 leaves. Initially, five adult apterae aphids were placed on all experimental plants, except the controls, and left to reproduce on the plants overnight. The adult aphids were then removed and their offspring thinned to the assigned density. This was repeated for all three aphid clones, except for the experiment on *A. syriaca* for which only the Emory and Florida clones available. Each density by clone combination was replicated seven times and each plant was caged (including controls). All aphid offspring were removed daily to keep aphid densities constant. This continued until all aphids died (approximately 20 days).

For each plant species, seven individuals were harvested at the start of the experiment on the same day that the aphids were put on the plants (control A), and seven other plants served as controls throughout the experiment and received no aphids (control B). We chose these controls instead of measuring the same plants twice to avoid any induction caused by early leaf sampling.

Plants were harvested at the end of the experiment, after all aphids had died. Each plant was cut at the base of the stem and rinsed to remove any aphid honeydew. Plants were freeze-dried and weighed to determine above-ground plant biomass, then ground and homogenized. From each individual, a sub-sample (~2-3mg) was analyzed for carbon and nitrogen, and another sub-sample (~50mg) was analyzed for cardenolides

(methods below). We chose to analyze the entire above-ground plant because the aphids colonized both stems and leaves.

Plant chemistry

For carbon and nitrogen analysis, ground samples were weighed into tin capsules with a Mettler UMT2 microbalance (Mettler Toledo, Im Langacher, Switzerland) and analyzed with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan Italy).

Cardenolides were analyzed using methods modified from Malcolm and Zalucki (1996). Briefly, ground samples were extracted with methanol and analyzed for cardenolide content using high performance liquid chromatography (HPLC). Peaks were detected by diode array at 218nm and absorbance spectra from 200 – 200 nm with digitoxin as the standard. Peaks with symmetrical absorption maxima between 217-222 nm were recorded as cardenolides (Malcolm and Zalucki 1996).

Statistical analyses

Given that experiments were run sequentially, data from each plant species were analyzed separately. Total cardenolide concentration, plant biomass and plant foliar nitrogen and carbon were compared among treatments using 2-way ANOVAs with aphid density and clone as main effects. Residuals were checked for normality and data were transformed when necessary. *A. viridis* biomass and *A. syriaca* total cardenolide data were log transformed to meet the assumptions of normality. Tukey's honest significant difference tests were conducted to compare among treatment means.

RESULTS

Aphid density influenced total cardenolide concentration in only one plant species, while aphid clone had no effect on total cardenolide concentration in any of the plant species examined. As aphid density increased, the total cardenolide concentration in *A. viridis* decreased (Figure 5.1.A density $F_{2,59} = 9.04$, $p = 0.0004$, clone $F_{2,59} = 0.20$, $p = 0.8228$, density*clone $F_{4,59} = 1.93$, $p = 0.1202$). There was no effect of aphid density or clone on total cardenolide concentration in *A. syriaca* (Figure 5.1.B density $F_{2,27} = 0.44$, $p = 0.6516$, clone $F_{1,27} = 0.01$, $p = 0.9087$, clone*density $F_{2,27} = 0.29$, $p = 0.7542$). No cardenolides were detected in *A. tuberosa*, and cardenolides were detected in only one *A. incarnata* individual (receiving 50 aphids per plant from clone Florida).

The effect of aphid density on plant biomass varied among plant species (Figure 5.2 A-D), while aphid clone had no effect on plant biomass. As aphid density increased, the biomass of *A. incarnata* and *A. viridis* decreased (*A. incarnata*: Figure 5.2.A: density $F_{2,59} = 10.04$, $p = 0.0001$, clone $F_{2,59} = 1.32$, $p = 0.2750$, density*clone $F_{4,59} = 1.28$, $p = 0.2914$. *A. viridis*: Figure 5.2.D: density $F_{2,59} = 23.58$, $p<0.0001$, clone $F_{2,59} = 0.24$, $p=0.7868$, density*clone $F_{4,59} = 0.13$, $p = 0.9695$). However, there was no effect of aphid density on biomass of *A. syriaca* (Figure 5.2.B: density $F_{2,40} = 1.95$, $p = 0.1571$, clone $F_{1,40} = 0.05$, $p = 0.8222$, density*clone $F_{2,40} = 0.16$, $p=0.8502$) or *A. tuberosa* (Figure 5.2.C: density $F_{2,60} = 2.03$, $p = 0.1442$, clone $F_{2,60} = 0.57$, $p = 0.5688$, density*clone $F_{4,40} = 2.44$, $p = 0.0581$).

Foliar nitrogen increased at high aphid density on *A. incarnata* (Figure 5.3.B density $F_{2,62} = 2.96$, $p = 0.061$, clone $F_{2,62} = 0.58$, $p = 0.0562$, density*clone $F_{4,62} = 0.39$, $p = 0.8117$). Neither aphid density nor clone influenced plant foliar nitrogen in any of the

other species studied (Figure 5.3: *A. tuberosa* density $F_{2,58} = 0.39$, $p = 0.679$, clone $F_{2,58} = 0.19$, $p = 0.83$, density*clone $F_{4,58} = 0.27$, $p = 0.899$; *A. viridis* density $F_{2,62} = 1.18$, $p = 0.314$, clone $F_{2,62} = 0.11$, $p = 0.899$, density*clone $F_{4,62} = 0.65$, $p = 0.631$; *A. syriaca* density $F_{2,41} = 0.40$, $p = 0.6711$, clone $F_{1,41} = 0.25$, $p = 0.6201$, density*clone $F_{2,41} = 0.43$, $p = 0.6558$).

In *A. incarnata*, foliar carbon concentrations decreased at high aphid densities (Figure 5.4.B density $F_{2,62} = 4.97$, clone $F_{2,62} = 0.58$, $p = 0.5617$, density*clone $F_{4,62} = 0.86$, $p = 0.4952$). Neither aphid density nor clone influenced foliar carbon concentrations in any of the other three plant species (Figure 5.4 *A. tuberosa* density $F_{2,58} = 0.44$, $p = 0.646$, clone $F_{2,58} = 1.35$, $p = 0.270$, density*clone $F_{4,58} = 0.72$, $p = 0.580$; *A. viridis* density $F_{2,62} = 2.21$, $p = 0.119$, clone $F_{2,62} = 2.02$, $p = 0.123$, density*clone $F_{4,62} = 2.04$, $p = 0.102$; *A. syriaca* density $F_{2,41} = 1.78$, $p = 0.183$, clone $F_{1,41} = 1.08$, $p = 0.306$, density*clone $F_{2,41} = 0.91$, $p = 0.413$)

Table 1 summarized indicies of plant quality measured from control plants.

DISCUSSION

There was variation among the *Asclepias* species studied in their response to increasing aphid density. *A. viridis* was the only species to show a change in cardenolide concentration which declined with increasing aphid density (Figure 5.1.A). Both *A. viridis* and *A. incarnata* showed a decrease in final plant biomass with increasing aphid density (Figure 5.2). Previous research examining the effect of *Aphis nerii* density on cardenolide induction in two *Asclepias* species found that high aphid density induced cardenolide expression in *A. curassavica* but not *A. incarnata* which lacks both

constitutive and inducible cardenolide defenses (Martel and Malcolm 2004). Similarly, we found no effect of aphid density on *A. incarnata* cardenolide concentration, and in most cases were unable to detect any cardenolides in this species.

The results documented by Martel and Malcolm (2004) for *A. curassavica* (which also has high constitutive levels of cardenolides like *A. viridis*) are intriguing. Cardenolide concentration decreased at low aphid densities, and then increased as aphid density increased (Martel and Malcolm 2004). We observed no such increase in cardenolide concentrations at high aphid density on *A. viridis* (Figure 5.1.A). The densities used in our experiments fall within the range of natural aphid densities (Hall and Ehler 1980, Helms et al. 2004), so the observed reduction in *A. viridis* cardenolide concentration at high aphid densities is not an artifact of unrealistic population size. However, the plants used in our experiments were all young seedlings and previous work done by Martel and Malcolm (2004) used older plants. It is possible that aphid effects on cardenolide induction or suppression vary between young and old plants. In addition to reducing cardenolide concentrations, increasing aphid density also led to a reduction in plant biomass accumulation of *A. viridis*. Typically, aphids do not cause a decrease in plant biomass (Throop 2005). However, reductions in plant biomass due to aphid herbivory have been demonstrated for cotton (Rosenheim et al. 1997), and aphid infestation caused reduced stem elongation in alfalfa (Girousse et al. 2005). In our case, the densities were high enough that *A. viridis* exhibited reduced growth.

We also observed reduced growth of *A. incarnata* at high aphid density (Figure 5.3.B). That *A. incarnata* should exhibit stronger growth reductions than either *A. tuberosa* or *A. syriaca* is surprising. *A. incarnata* had the highest biomass of all the

species studied (Table 5.1), so it experienced the lowest aphid densities on a per gram of leaf tissue basis. It is currently unknown why aphid density influenced plant growth of some *Asclepias* species but not others.

In addition to causing a decrease in biomass accumulation, high aphid density led to an increase in foliar nitrogen and a decrease in foliar carbon concentrations in *A. incarnata* (Figures 5.3 & 5.4). These results imply an imperfect, or complex, link between nitrogen availability and *A. incarnata* growth. Currently, the mechanism behind the increased foliar nitrogen concentrations is unknown. One possible explanation is the formation of a phloem sink on *A. incarnata*, with high aphid densities forming nitrogen rich phloem sinks. Phloem-feeding insects, such as aphids, are able to manipulate host plant source-sink relationships (Burstein et al. 1994, Girousse et al. 2005, Thompson and Goggin 2006). Another possibility is that *A. incarnata* exhibited increased nitrogen concentrations as a mechanism to tolerate herbivory. After herbivory, tolerant plants may exhibit increased rates of photosynthesis (Houle and Simard 1996, Mabry and Wayne 1997, Strauss and Agrawal 1999), and photosynthesis increases linearly with leaf nitrogen concentration (Lambers et al. 1998). Photosynthetic rates were not measured on any of the plants used in this study. To determine if *A. incarnata* compensates for aphid herbivory, comparisons over a longer time period between damaged and undamaged plants would be necessary.

Previous research has found that mechanical damage to *A. syriaca* that mimics damage by *Danaus plexippus* induces cardenolide expression after 24 hr but that cardenolide concentrations drop to constitutive levels after 148 hr (~ 6 days) (Malcolm and Zalucki 1996). In our experiments, all plants were collected after all of the aphids

had died, approximately 20 days after each experiment started. If there was rapid induction of cardenolides due to aphid herbivory and a rapid subsequent decay, we would have been unable to detect this induction. It may also be that *A. syriaca* induces cardenolides in response to leaf chewer, but not phloem-feeder, damage.

A. nerii is a specialist of milkweed and oleander, and it is able to sequester cardenolides for defense against natural enemies (Rothschild et al. 1970, Malcolm 1986, 1989, 1990, 1991, Malcolm and Zalucki 1996, Agrawal and Malcolm 2002). Additionally, in the absence of natural enemies, there is no variation in *A. nerii* population growth rate on different milkweed species (Groeters 1993, Martel and Malcolm 2004). Therefore, it is unlikely that cardenolide induction would have a negative impact on *A. nerii*. From the plant's perspective, cardenolide induction in response to herbivory by *A. nerii* would be counterproductive because it would not decrease or deter herbivory by the agent causing the damage. In addition, cardenolide concentration could actually benefit the herbivore and harm the plant because higher cardenolides would allow the specialist herbivore to be better protected from natural enemies. This is known as the lethal plant paradox (Malcolm and Zalucki 1996). By not inducing cardenolides, all four *Asclepias* species studied avoid benefiting the herbivore at their own expense. Additionally, by reducing cardenolide concentration, *A. viridis* may prevent *A. nerii* from sequestering higher cardenolide concentrations and becoming better defended against natural enemies. Future work will compare sequestration of cardenolides at different aphid densities

In addition to there being variation in the response of these four plant species to increasing aphid density, there was also variation in plant quality traits. While we did not

statistically compare among the four plant species because our experiments were run at separate times, it is interesting to note some of the differences among the species. Previous research has documented that *A. viridis* is typically a high cardenolide species and that *A. incarnata* has few or no cardenolides expressed (Malcolm 1991, Martel and Malcolm 2004). Our results show the same patterns. Surprisingly, *A. incarnata* had cardenolides present at the very beginning of the experiment when the seedlings were approximately 1 month old (Table 5.1), but then these levels rapidly decreased to zero.

We have previously reported the results of aphid population growth during these laboratory experiments (Zehnder and Hunter 2007) and it is interesting to relate those dynamics to the plant quality traits measured here. *A. nerii* exhibited density-dependent survival, fecundity and alate production on all plant species. However, density-dependent responses were strongest on *A. viridis* and weakest on *A. incarnata*. On *A. incarnata*, foliar nitrogen concentrations increased at high aphid densities (Figure 3). Therefore, there might be reduced competition for nitrogen at high density which could lead to the weaker density-dependent responses documented on *A. incarnata* compared to the other plant species. *A. viridis* has the highest cardenolides concentration of all the species we examined, and it also has the lowest biomass. Competition for space, nutrients or cardenolides may have led to the strong density-dependent response by *A. nerii* on *A. viridis*. Because *A. nerii* sequesters cardenolides for defense against natural enemies, it has been hypothesized that intraspecific competition for cardenolides may limit aphid population growth more so than competition for primary plant metabolites (Malcolm 1986, 1989, 1991, Martel and Malcolm 2004).

Alate production by *A. nerii* was also density-dependent, and the response varied among plant species (Zehnder and Hunter 2007). Alate production is a common response to high density in aphids (Dixon 1998, Muller et al. 2001). In our previous work, alates were only produced on two plant species, *A. tuberosa* and *A. viridis*. These two species had the lowest biomass (Figure 2), which may have led to the most extreme crowding. Alate production can result from tactile stimulation as aphids bump into each other (Lees 1966, Debarro 1992), which would be more likely on smaller plants.

In summary, we found that changes in *A. nerii* density led to changes in plant chemistry and biomass, although not as we had predicted. In no case did increased aphid density lead to increased cardenolide expression. As aphid density increased, *A. viridis* cardenolide concentration and biomass decreased. For *A. incarnata*, increased aphid density led to a decrease in plant biomass and an increase in foliar nitrogen concentrations. Aphid density did not affect any measured trait of either *A. tuberosa* or *A. syriaca*. Our results suggest that congeneric plant species may respond very differently to the same levels of herbivore damage.

ACKNOWLEDGEMENTS: We would like to thank J. Castings, P. Doty, M. Fleming, T. Maddox and S. Scott for laboratory and field assistance. We thank B. Ball, W. Duncan, K. Wickings and two anonymous reviewers for helpful suggestions concerning this manuscript.

Table 5.1: Biomass, total cardenolide concentration, percent foliar nitrogen and percent foliar carbon for the control plants from the laboratory experiments. Control A plants were collected on the day that aphids were put on the plants, and Control B plants were collected at the end of the experiment but never had aphids on them. Values are means of 7 plants ± standard error.

	Biomass (g)		Total cardenolide concentration (mg/g)		Foliar Nitrogen (%)		Foliar Carbon (%)	
	Control A	Control B	Control A	Control B	Control A	Control B	Control A	Control B
A. tuberosa	0.051 ± 0.006	0.359 ± 0.05	0	0	5.541 ± 0.20	4.456 ± 0.17	43.58 ± 0.45	43.83 ± 0.70
A. incarnata	0.117 ± 0.018	1.398 ± 0.10	0.228 ± 0.03	0	5.973 ± 0.323	5.450 ± 0.04	43.28 ± 0.27	47.17 ± 0.43
A. viridis	0.019 ± 0.004	0.1616 ± 0.03	2.97 ± 0.56	5.02 ± 0.89	4.856 ± 0.22	4.836 ± 0.21	40.22 ± 0.55	41.83 ± 0.74
A. syriaca	0.125 ± 0.22	0.870 ± 0.78	0.502 ± 0.232	0.197 ± 0.06	6.156 ± 0.16	3.789 ± 0.28	44.24 ± 0.21	43.60 ± 0.50

REFERENCES

Abdala-Roberts, L., and V. Parra-Tabla. 2005. Artificial defoliation induces trichome production in the tropical shrub *Cnidoscolus aconitifolius* (Euphorbiaceae). Biotropica **37**:251-257.

Agrawal, A. A. 1999. Induced responses to herbivory in wild radish: Effects on several herbivores and plant fitness. Ecology **80**:1713-1723.

Agrawal, A. A. 2000. Benefits and costs of induced plant defense for *Lepidium virginicum* (Brassicaceae). Ecology **81**:1804-1813.

Agrawal, A. A. 2004. Plant defense and density dependence in the population growth of herbivores. American Naturalist **164**:113-120.

Agrawal, A. A., and S. B. Malcolm. 2002. Once upon a milkweed - In this complex community, one insects poison may be another's meal. Natural History **111**:48-53.

Brower, L. P., J. N. Seiber, C. J. Nelson, S. P. Lynch, and M. M. Holland. 1984. Plant-determined variation in the cardenolide content, thin-layer chromatography profiles, and emetic potency of monarch butterflies, *Danaus-plexippus* L (Lepidoptera, Danaidae) reared on milkweed plants in California .2. *Asclepias speciosa* (Apocynales, Asclepiadaceae). Journal Of Chemical Ecology **10**:601-639.

Burstein, M., D. Wool, and A. Eshel. 1994. Sink strength and clone size of sympatric, gall-forming aphids. European Journal Of Entomology **91**:57-61.

Cardoza, Y. J., J. Reidy-Crofts, and O. R. Edwards. 2005. Differential inter- and intra-specific defense induction in *Lupinus* by *Myzus persicae* feeding. Entomologia Experimentalis Et Applicata **117**:155-163.

Carroll, C. R., and C. A. Hoffman. 1980. Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata*. Science **209**:414-416.

De Moraes, C. M., W. J. Lewis, P. W. Pare, H. T. Alborn, and J. H. Tumlinson. 1998. Herbivore-infested plants selectively attract parasitoids. Nature **393**:570-573.

Debarro, P. J. 1992. The role of temperature, photoperiod, crowding and plant-quality on the production of alate viviparous females of the bird cherry-oat aphid, *Rhopalosiphum-Padi*. Entomologia Experimentalis Et Applicata **65**:205-214.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. 2nd edition. Chapman and Hall, London.

Girousse, C., B. Moulia, W. Silk, and J. L. Bonnemain. 2005. Aphid infestation causes different changes in carbon and nitrogen allocation in alfalfa stems as well as different inhibitions of longitudinal and radial expansion. Plant Physiology **137**:1474-1484.

Green, T. R., and C. A. Ryan. 1972. Wound induced proteinase inhibitor in plant leaves - Possible defense mechanism against insects. Science **175**:776-&.

Groeters, F. R. 1993. Tests for host-associated fitness trade-offs in the milkweed-oleander aphid. Oecologia **93**:406-411.

Hall, R. W., and L. E. Ehler. 1980. Population ecology of *Aphis nerii* Homoptera, Aphididae on oleander. Environmental Entomology **9**:338-344.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecological Entomology **29**:44-51.

Houle, G., and G. Simard. 1996. Additive effects of genotype, nutrient availability and type of tissue damage on the compensatory response of *Salix planifolia* ssp *planifolia* to simulated herbivory. Oecologia **107**:373-378.

Karban, R., and I. T. Baldwin. 1997. Induced responses to herbivory. Chicago University Press, Chicago.

Lambers, H., S. F. Chapin, and T. L. Pons. 1998. Plant physiological ecology. Springer-Verlag, New York.

Lees, A. D. 1966. The control of polymorphism in aphids. Advances in Insect Physiology **19**:973-980.

Mabry, C. M., and P. W. Wayne. 1997. Defoliation of the annual herb *Abutilon theophrasti*: Mechanisms underlying reproductive compensation. Oecologia **111**:225-232.

Malcolm, S. B. 1986. Aposematism in a soft-bodied insect: a case for kin selection. Behavioral Ecology And Sociobiology **18**:387-393.

Malcolm, S. B. 1989. Disruption of web structure and predatory behavior of a spider by plant-derived chemical defenses of an aposematic aphid. Journal Of Chemical Ecology **15**:1699-1716.

Malcolm, S. B. 1990. Chemical defence in chewing & sucking insect herbivores: plant-derived cardenolides in the monarch butterfly and oleander aphid. Chemoecology **1**:12-21.

Malcolm, S. B. 1991. Cardenolide-mediated interactions between plants and herbivores.*in* G. A. Rosenthal and M. R. Berenbaum, editors. Herbivores: their interactions with secondary plant metabolites. Academic Press, San Diego.

Malcolm, S. B., and L. P. Brower. 1989. Evolutionary and ecological implications of cardenolide sequestration in the monarch butterfly. Experientia **45**:284-294.

Malcolm, S. B., B. J. Cockrell, and L. P. Brower. 1989. Cardenolide fingerprint of monarch butterflies reared on common milkweed, *Asclepias syriaca*. Journal Of Chemical Ecology **15**:819-853.

Malcolm, S. B., and M. P. Zalucki. 1996. Milkweed latex and cardenolide induction may resolve the lethal plant defence paradox. Entomologia Experimentalis Et Applicata **80**:193-196.

Martel, J. W., and S. B. Malcolm. 2004. Density-dependent reduction and induction of milkweed cardenolides by a sucking insect herbivore. Journal Of Chemical Ecology **30**:545-561.

Muller, C. B., I. S. Williams, and J. Hardie. 2001. The role of nutrition, crowding and interspecific interactions in the development of winged aphids. Ecological Entomology **26**:330-340.

Nelson, C. J., J. N. Seiber, and L. P. Brower. 1981. Seasonal and intraplant variation of cardenolide content in the California milkweed, *Asclepias eriocarpa*, and implications for plant defense. Journal Of Chemical Ecology **7**:981-1010.

Orians, C. 2005. Herbivores, vascular pathways, and systemic induction: Facts and artifacts. Journal Of Chemical Ecology **31**:2231-2242.

Rosenheim, J. A., L. R. Wilhoit, P. B. Goodell, E. E. GraftonCardwell, and T. F. Leigh. 1997. Plant compensation, natural biological control, and herbivory by Aphis gossypii on pre-reproductive cotton: the anatomy of a non-pest. Entomologia Experimentalis Et Applicata **85**:45-63.

Rothschild, M., J. V. Euw, and T. Reichste. 1970. Cardiac glycosides in the oleander aphid, *Aphis nerii*. Journal Of Insect Physiology **16**:1141-&.

Strauss, S. Y., and A. A. Agrawal. 1999. The ecology and evolution of plant tolerance to herbivory. Trends In Ecology & Evolution **14**:179-185.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. Direct and ecological costs of resistance to herbivory. Trends In Ecology & Evolution **17**:278-285.

Takada, H., and M. Miyazaki. 1992. Occurrence of sexuales of *Aphis nerii* B De F (Homoptera, Aphididae) in Japan. Applied Entomology And Zoology **27**:117-124.

Takada, H., and M. Miyazaki. 1993. Bisexual reproduction of a form of *Aphis nerii* B-De-F (Homoptera, Aphididae) from Hokkaido. Applied Entomology And Zoology **28**:199-205.

Thompson, G. A., and F. L. Goggin. 2006. Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. Journal of Experimental Botany **57**:755-766.

Throop, H. L. 2005. Nitrogen deposition and herbivory affect biomass production and allocation in an annual plant. Oikos **111**:91-100.

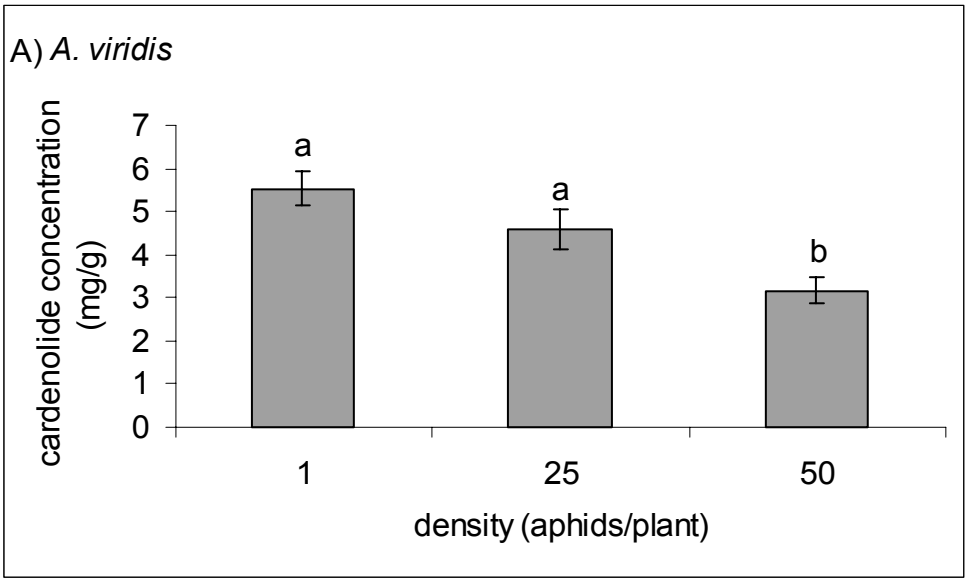
Wold, E. N., and R. J. Marquis. 1997. Induced defense in white oak: Effects on herbivores and consequences for the plant. Ecology **78**:1356-1369.

Woodson, R. E. 1954. The North American species of Asclepias L. Annals of the Missouri Botanical Garden **41**:1-211.

Zehnder, C. B., and M. D. Hunter. 2007. A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid. Ecological Entomology **in press**.

Figure 5.1: The effect of increasing *A. nerii* density on total cardenolide concentration in A) *A. viridis* and B) *A. syriaca*. Data from three aphid clones were pooled because there was no significant effect of aphid clone on total cardenolide concentration. Bars are means of 21 plants (14 for *A. syriaca*) $\pm$ standard error. Different letters above bars represent significant differences among treatment means.

Figure 5.1


A) A. viridis
cardenolide concentration (mg/g)
7
6
5
4
3
2
1
0
a
a
b
1
25
50
density (aphids/plant)

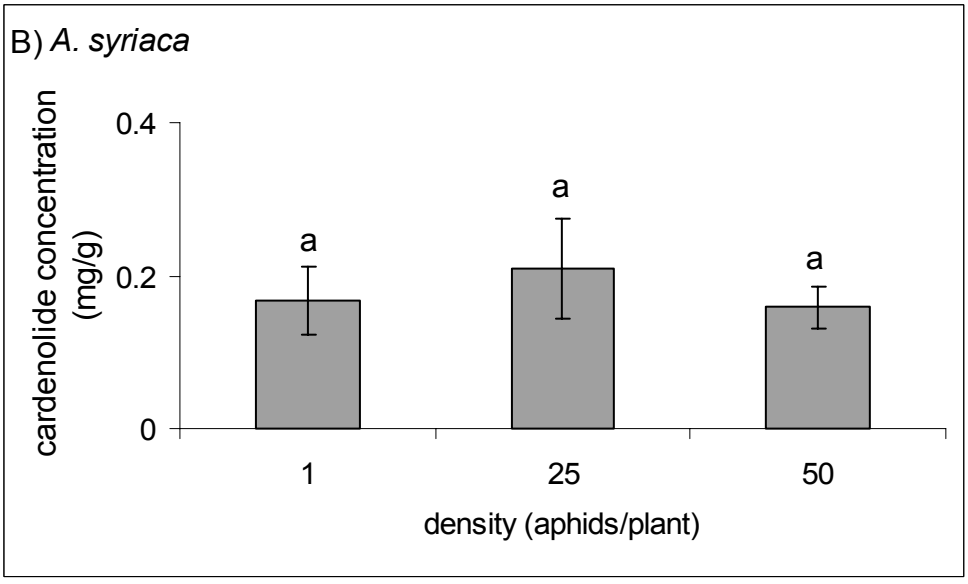

B) A. syriaca
cardenolide concentration (mg/g)
0.4
0.2
0
a
a
a
1
25
50
density (aphids/plant)

Figure 5.2: The effect of increasing *A. nerii* density on dry biomass of four *Asclepias* species A) *A. tuberosa*, B) *A. incarnata*, C) *A. viridis* and D) *A. syriaca*. Data from three aphid clones were pooled because there was no effect of aphid clone on plant biomass for any plant species. Bars are means of 21 plants (14 for *A. syriaca*) $\pm$ standard error. Different letters above bars represent significant differences among treatment means.

Figure 5.2

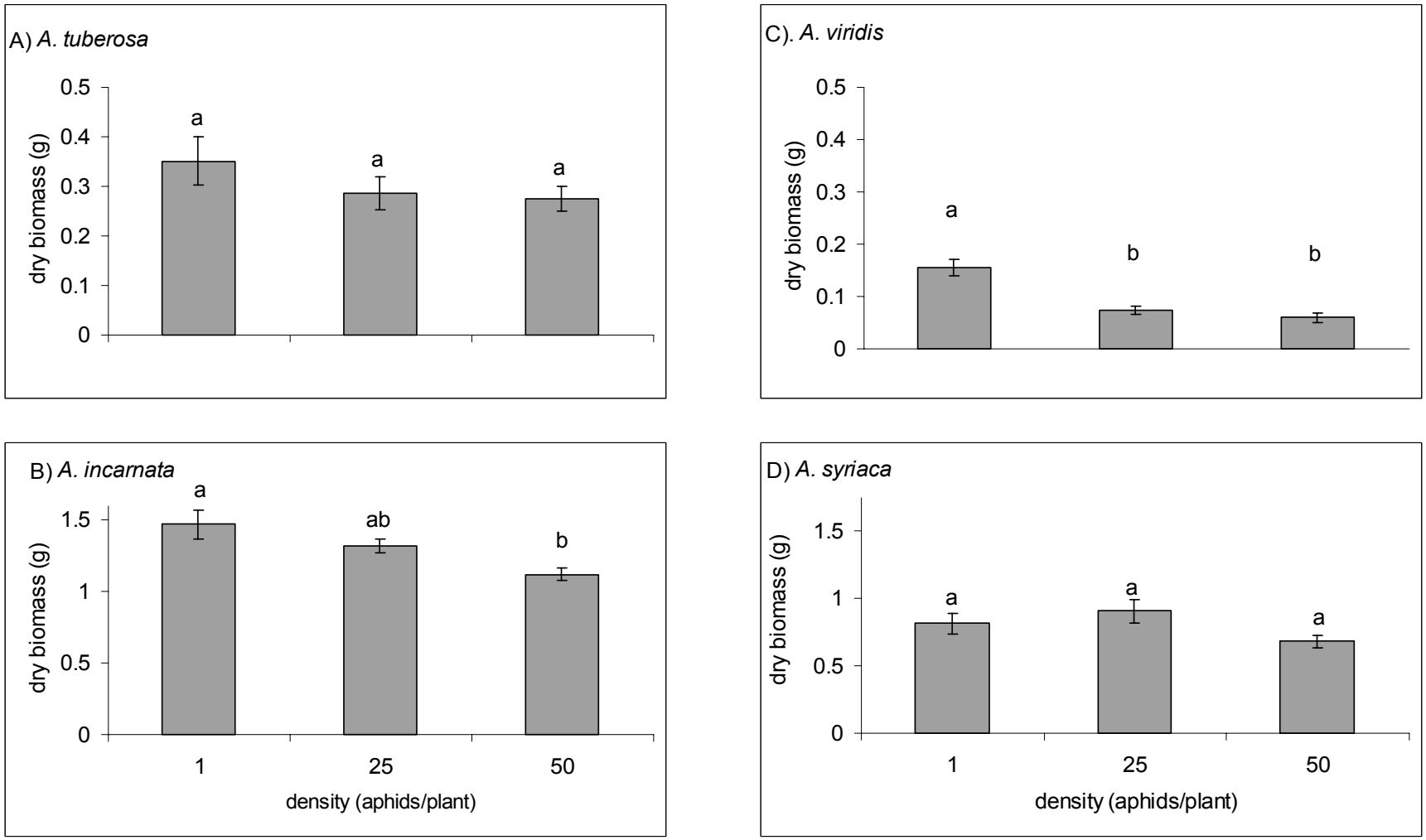

A) A. tuberosa
dry biomass (g)
0.5
0.4
0.3
0.2
0.1
0
a
a
a
C). A. viridis
dry biomass (g)
0.5
0.4
0.3
0.2
0.1
0
a
b
b
B) A. incarnata
dry biomass (g)
1.5
1
0.5
0
a
ab
b
1
25
50
density (aphids/plant)
D) A. syriaca
dry biomass (g)
1.5
1
0.5
0
a
a
a
1
25
50
density (aphids/plant)

Figure 5.3:The effect of variation in A. nerii density on foliar nitrogen of four *Asclepias* species A) *A. tuberosa*, B) *A. incarnata*, C) *A. viridis* and D) *A. syriaca*. Data from three aphid clones were pooled because there was no effect of aphid clone on foliar carbon concentrations for any plant species. Bars are means of 21 plants (14 for *A. syriaca*) + standard error. Different letters above bars represent significant differences among treatment means.

Figure 5.3

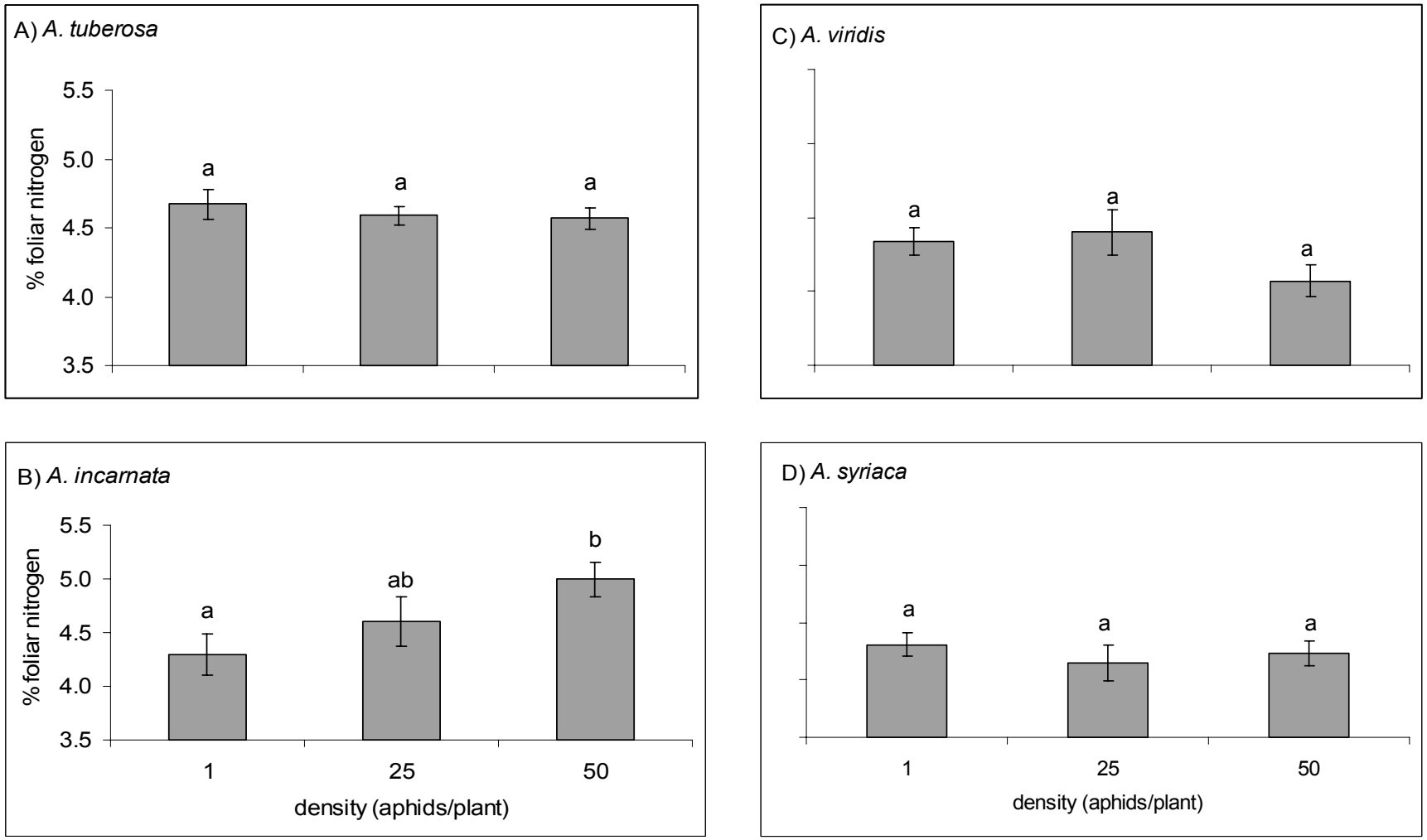
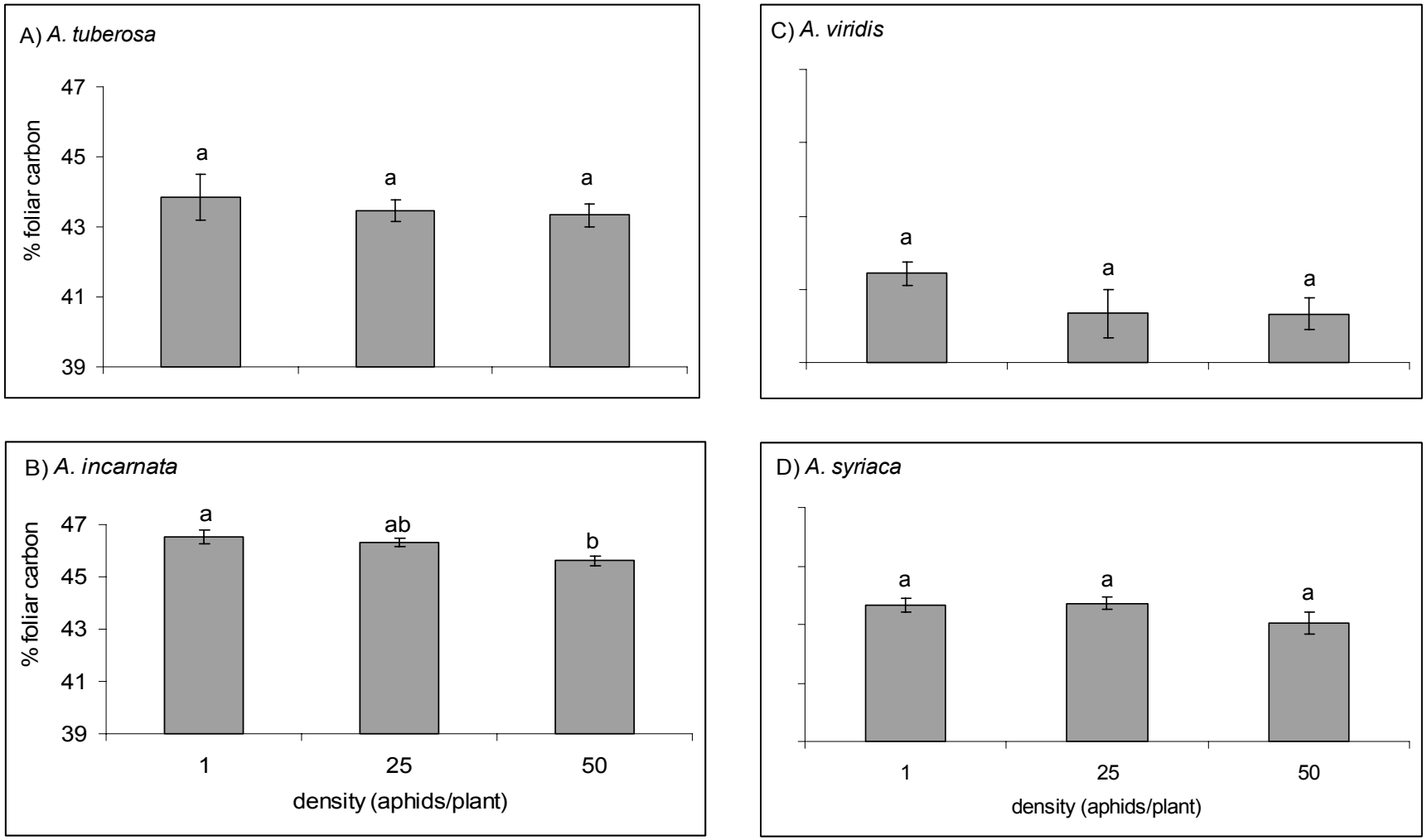

A) A. tuberosa
% foliar nitrogen
5.5
5.0
4.5
4.0
3.5
a
a
a
C) A. viridis
a
a
a
B) A. incarnata
% foliar nitrogen
5.5
5.0
4.5
4.0
3.5
a
ab
b
1
25
50
density (aphids/plant)
D) A. syriaca
a
a
a
1
25
50
density (aphids/plant)

Figure 5.4: The effect of variation in *A. nerii* density on foliar carbon of four *Asclepias* species A) *A. tuberosa*, B) *A. incarnata*, C) *A. viridis* and D) *A. syriaca*. Data from three aphid clones were pooled because there was no effect of aphid clone on foliar carbon concentrations for any plant species. Bars are means of 21 plants (14 for *A. syriaca*) $\pm$ standard error. Different letters above bars represent significant differences among treatment means.

Figure 5.4


A) A. tuberosa
% foliar carbon
47
45
43
41
39
a
a
a
C) A. viridis
a
a
a
B) A. incarnata
% foliar carbon
47
45
43
41
39
a
ab
b
1
25
50
density (aphids/plant)
D) A. syriaca
a
a
a
1
25
50
density (aphids/plant)

CHAPTER 6

CONCLUSIONS & FUTURE DIRECTIONS

In general, the results of my experiments show that variation in plant quality and aphid density in the current environment influence aphid performance, but variation in the maternal environment has less of an influence on aphid vital rates. I examined a number of variables in the maternal environment including maternal density, host plant species, age, and also interactions among these variables. While maternal effects are known to impact population dynamics in certain systems (Benton et al. 2005, Plaistow et al. 2006), .the *A. nerii – Asclepias* system does not seem to be strongly influenced by the maternal effects studied here. In hindsight, this may be the case for many aphid species. Aphids have the ability to respond quickly to their current environment, and the maternal environment may not be a good predictor of the offspring environment. Phase polymorphism, production of different morphs like alate or apterae, in aphids is well documented (Dixon 1998). Looking back over Table 1.2 (Chapter 1), it becomes clear that all documented examples of maternal effects and aphids involve phase polymorphism. As far as I can tell, there are no published examples in which maternal effects influence offspring survival or fecundity.

Maternal effects seem to be most important when conditions in the maternal environment induce variation in egg or offspring size (Plaistow et al. 2004, Benton et al. 2005, Beckerman et al. 2006). In many cases, offspring size translates into some index of

offspring quality (Bernardo 1996). Perhaps there is a lack of plasticity in offspring size at birth for aphids. Or perhaps the maternal contribution to offspring quality is quickly swamped by the offspring current environment.

Both inter and intra – specific variation in plant quality were found to be important to *A. nerii* vital rates and population dynamics. Many insect herbivores are nitrogen limited (White 1993), so variation in host plant foliar nitrogen concentrations often has an impact on insect herbivores (Kyto et al. 1996, Throop and Lerdau 2004). Additionally, variation in quality among the milkweed species that I studied influenced *A. nerii* vital rates. Specifically, there was variation in aphid density-dependent survival, fecundity and alate formation. Moreover, changes in plant quality induced by aphid feeding also varied among *Asclepias* species.

Results from Chapter 2 show that while maternal effects were present, within generation effects on vital rates were much stronger and therefore more likely to impact aphid population dynamics. For example, offspring density had a stronger impact on vital rates than did maternal density. When I focused on the aphid's current environment, I found that aphid per capita survival, fecundity and alate production were density-dependent on all host plant species studied. However, the strength of these density-dependent responses varied among species. While these results did not support all of our original predictions, they did lead to the generation of the hypotheses tested in Chapters 3, 4 and 5.

For example, in Chapter 2 I showed that aphids experiencing high densities had higher survival and fecundity in the second half of their lives than did aphids experiencing low densities (Figures 2.2 and 2.3). I became interested in whether

maternal age might influence maternal effects. Therefore, in Chapter 3 I examined the effects of maternal age, density and host plant species on offspring vital rates. Of the three factors studied, maternal age had the largest influence on offspring vital rates. Older mothers produced offspring that lived shorter lives. This is consistent with the Lansing Effect (Lansing 1947). However, maternal age effects were not large in magnitude. In a review of the literature, maternal age effects in insects were found to be generally weak and unlikely to have a big impact on population dynamics (Table 3.3). Together, Chapters 2 and 3 indicate that while maternal effects are present in this system, it is unlikely that they have a strong influence on aphid population dynamics.

Results from the first set of experiments (Chapter 2) showed that density-dependent processes influence aphid vital rates and that variation in plant quality affects these density-dependent processes. Based upon these results, I then chose to focus on variation in the strength of density dependence and how intra-specific variation in plant quality may affect this. This led to the density-manipulation experiment examining the effects of simulated nitrogen deposition on the interaction between aphids and their host plants which is reported in Chapter 4. Results show that nitrogen deposition increased plant foliar nitrogen concentrations, plant biomass and aphid per capita population growth. Nitrogen deposition caused aphid R_{max} and K to increase proportionally, leading to no change in the strength of density-dependence. Previous work by Agrawal and colleagues (2004) on *A. nerii* and multiple *Asclepias* species found variation in the strength of density-dependence. They reported an increase in R_{max} on high quality plants but no proportional increase in K. Therefore, they concluded that R_{max} is a predictor of the strength of density-dependence (Agrawal et al. 2004). My results show that R_{max} and

K can increase proportionally on high quality plants, so an increase in R_{max} does not necessarily correlate to an increase in the strength of density-dependence.

The effects of nitrogen deposition on plant-insect interactions remains topical, given predicted anthropogenic increases in nitrogen availability. I conducted one small, short-term laboratory experiment which suggests that insect populations may rise as nitrogen deposition increases. However, future work needs to explore long term effects of nitrogen deposition on natural systems, and also the interactive effects of nitrogen deposition on plant defenses and natural enemies (Throop and Lerdau 2004).

Chapter 5 again makes use of experimental work in Chapter 2, but this time it's from the plant's perspective. Using plants collected at the end of experiment 3 in Chapter 2, I examined the effects of variation in aphid density on induced plant defenses of four *Asclepias* species. Aphid density influenced plant chemistry and biomass, and the effect varied among plant species. However, in no case was there an increased expression of plant defensive compounds at higher aphid densities. Cardenolide concentration was found to decline at high aphid density on *A. viridis*, and cardenolide concentration did not respond to aphid feeding in the three other milkweed species. These results, combined with results documenting interspecific variation in cardenolide induction in *Asclepias* species by Martel and Malcolm (2004), demonstrate that closely related plant species may respond very differently to similar levels of herbivore damage.

In summary, the environment that an aphid currently experiences is more important to aphid vital rates than the environment of the maternal generation. Variation in plant quality influences aphid population growth rates and vital rates. Additionally, nitrogen deposition affects both plants and herbivores, leading to no apparent change in

the strength of density-dependence. Finally, congeneric plant species respond differently to the same levels of herbivore damage and plants may not induce defenses against specialist herbivores.

All of the research covered in this dissertation was laboratory based and did not include higher trophic levels. Therefore, given a chance to continue in this system, I would investigate the interactions among *Asclepias* spp, *Aphis nerii* and natural enemies of *A. nerii*. I would examine the effects of variation in aphid density and host plant species on aphid cardenolide sequestration, and determine how this interaction influences higher trophic levels, specifically parasitism by *Lysiphlebus testaceipes*. Based upon Chapter 5, I would hypothesize that aphid cardenolide concentration would decrease at high aphid densities. It is currently unknown how aphid cardenolide concentrations affect parasitism by *L. testaceipes*. However, parasitism rates are known to vary among *Asclepias* species (Helms et al. 2004).

Additionally, I am interested in examining how strong maternal effects must be in order for them to have an effect on subsequent population dynamics. For example, maternal effects were found to be less important than the offspring's competitive environment at influencing offspring performance in a soil mite system (Beckerman et al. 2006). For *A. nerii*, maternal effects were weak compared to the effects of the current environment and, therefore, unlikely to influence on population dynamics (Zehnder and Hunter 2007). How strong must maternal effects be compared to the effects of the current environment in order for them to cause cycles? I think a modeling approach would be the best way to examine this question (which for me means collaboration). Additionally, a modeling approach could investigate if small changes in development

time, like a 9 hour decrease in age at maturity, could lead to changes in long term dynamics.

Finally, further research in this system would include fieldwork. Do natural populations of *A. nerii* experience density-dependent survival, fecundity and alate production and is there variation in these responses among host plant species? Additionally, how does nitrogen deposition affect rates of parasitism and predation, and how does nitrogen deposition affect other members of the *Asclepias* arthropod community?

REFERENCES

Agrawal, A. A., N. Underwood, and J. R. Stinchcombe. 2004. Intraspecific variation in the strength of density dependence in aphid populations. Ecological Entomology **29**:521-526.

Beckerman, A. P., T. G. Benton, C. T. Lapsley, and N. Koesters. 2006. How effective are maternal effects at having effects? Proceedings Of The Royal Society B-Biological Sciences **273**:485-493.

Benton, T. G., S. J. Plaistow, A. P. Beckerman, C. T. Lapsley, and S. Littlejohns. 2005. Changes in maternal investment in eggs can affect population dynamics. Proceedings Of The Royal Society Of London Series B-Biological Sciences **272**:1351-1356.

Bernardo, J. 1996. The particular maternal effect of propagule size, especially egg size: patterns, models, quality of evidence and interpretations. American Zoologist **36**:216-236.

Dixon, A. F. G. 1998. Aphid ecology: an optimization approach. 2nd edition. Chapman and Hall, London.

Helms, S. E., S. J. Connelly, and M. D. Hunter. 2004. Effects of variation among plant species on the interaction between a herbivore and its parasitoid. Ecological Entomology **29**:44-51.

Kyto, M., P. Niemela, and S. Larsson. 1996. Insects on trees: Population and individual response to fertilization. Oikos **75**:148-159.

Lansing, A. I. 1947. A transmissible, cumulative, and reversible factor in aging. Journal of Gerontology **2**:228-239.

Plaistow, S. J., C. T. Lapsley, A. P. Beckerman, and T. G. Benton. 2004. Age and size at maturity: sex, environmental variability and developmental thresholds. Proceedings Of The Royal Society Of London Series B-Biological Sciences **271**:919-924.

Plaistow, S. J., C. T. Lapsley, and T. G. Benton. 2006. Context-dependent intergenerational effects: The interaction between past and present environments and its effect on population dynamics. American Naturalist **167**:206-215.

Throop, H. L., and M. T. Lerdau. 2004. Effects of nitrogen deposition on insect herbivory: Implications for community and ecosystem processes. Ecosystems **7**:109-133.

White, T. C. R. 1993. The inadequate environment: nitrogen and the abundance of animals. Springer-Verlag, New York.

Zehnder, C. B., and M. D. Hunter. 2007. A comparison of maternal effects and current environment on vital rates of *Aphis nerii*, the milkweed-oleander aphid. Ecological Entomology **in press**.