WING INDUCTION IN THE SOYBEAN APHID, *APHIS GLYCINES* MATSUMURA (HEMIPTERA: APHIDIDAE): MECHANISMS AND TRADE-OFFS

By

Aldo Francisco Ríos Martínez

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Department of Entomology

University of Manitoba

Winnipeg, Manitoba

ABSTRACT

Alate morphs can benefit aphid populations by facilitating dispersal from deteriorating food sources and by escaping from natural enemies. Wing development, however, imposes constraints on fecundity. The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an important economic pest in the U.S. and Canada. I conducted a series of laboratory and field experiments to determine the environmental factors inducing wing development in this species, and to determine the effects of asexual alate individual production on an *A. glycines* population under predation. My results reveal that wing induction in *A. glycines* occurs in response to interactions between crowding and decreased plant quality cues, and that alate aphid production benefits an aphid population under predation by increasing prevalence at a temporary cost to fecundity. My results contribute to the growing knowledge on the production of asexual alate aphids and provide insight into the biology of *A. glycines* as an agricultural pest.

ACKNOWLEDGEMENTS

Dedicated to my parents and brother, who have taught me the meaning of hard work and self-improvement.

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CHAPTER 1: GENERAL INTRODUCTION

Polyphenism, the phenomenon by which one genotype produces multiple phenotypes (morphs), is a key component of aphid life cycles (Simpson *et al.* 2011). Allocating resources into different morphs allows aphids to maximize their fitness as a function of their changing environment (Dixon 1998). The production of alate (winged) individuals can benefit aphids by facilitating resource colonization, relieving population pressure, and decreasing risk of predation (Dixon 1998). However, energetically-costly traits often impose constraints on other functions (Zera and Harshman 2001), and wing development and flight result in reduced fecundity (Wratten 1977; Zhang *et al.* 2009). Because of this, the production of asexual alate individuals by aphids provides a useful model for studying the evolution and ecology of trade-offs.

The soybean aphid *Aphis glycines* Matsumura (Hemiptera: Aphididae) is a pest originally indigenous to Asia that currently occurs in 30 states of the U.S. and 3 Canadian provinces (Hodgson *et al.* 2005b; Mignault *et al.* 2006; Ragsdale *et al.* 2011). *Aphis glycines* causes injury to soybean plants, *Glycine max* (L.) Merrill (Fabaceae) (Macedo *et al.* 2003), leading to yield losses of up to 45% in North America (Ragsdale *et al.* 2007). The production of asexual alate individuals is a key component of the biology of this species as a pest, since it is likely to affect interactions with its host plants and its natural enemies (Harrison 1991; Clark and Perry 2002; Wang and Ghabrial 2002; Costamagna *et al.* 2013a). Despite *A. glycines* being an important pest, however, little is known about the ecology and biology of summer alate individuals in this species.

The aim of this thesis is to contribute to a growing body of knowledge on polyphenism, and to provide insight into the biology of *A. glycines*. Chapter 2 of this

thesis serves as a literature review on the ecology of aphids and the biology of *A. glycines* as an agricultural pest. Chapter 3 describes a series of laboratory and field experiments used to determine the effects of crowding and decreased host plant quality on wing induction in *A. glycines*. Chapter 4 describes a field experiment and a laboratory assay conducted to determine the role of asexual alate individuals in an *A. glycines* population under predation. Finally, chapter 5 integrates the different sections of this thesis into a general discussion.

CHAPTER 2: LITERATURE REVIEW

Aphid reproduction and life cycles

Aphids (Hemiptera: Aphididae) are sap-feeding insects of economic importance, as ca. 100 species feed on a broad range of agricultural crops (Dedryver *et al.* 2010). Aphids affect plants by removing sap chemicals, transmitting viruses, and excreting honeydew onto plant surfaces, resulting in the growth of moulds which contaminate the plant surfaces and affect photosynthesis (Minks and Harrewijn 1987b). The ability of aphids to exploit their food resources rapidly through population growth can result in large impacts on crop yield (Dedryver *et al.* 2010). Aphid outbreak events are highly variable depending on the occurrence of natural enemies and environmental factors like temperature and humidity (Dedryver *et al.* 2010), making the control of aphid populations in the field difficult. (Dedryver *et al.* 2010).

Most aphid species can complete their life cycle on a single host plant species or genus (autoecious life cycle), while ca. 10% of aphid species alternate between two different host plant species, which are often not closely related (heteroecious life cycle, Dixon, 1998). The life cycles of most aphid species involve an alternation between asexual and sexual reproduction (cyclical parthenogenesis, Dixon 1998). During the asexual phase, parthenogenetically-produced embryos often begin developing even before their parents are born (telescoping of generations), which results in particularly short generation times and high rates of increase (Blackman 1974; Dixon 1998). In many aphid species, transition to sexual reproduction from parthenogenesis is triggered by maternal exposure to changes in night duration and temperature, usually occurring by the end of summer (Forrest 1970; Lees 1973; Dean 1978). The parthenogenetic life stages are

not cold-hardy and sexual reproduction benefits aphid populations by allowing genetic recombination, and enabling the production of cold-hardy overwintering eggs in seasonal habitats (Blackman 1974; Dixon 1998).

Aphid – host plant interactions

As many phytophagous insects, aphids generally show strong host-specificity (Dixon 1998). Host plant location is first achieved through olfactory and visual cues (Kennedy *et al.* 1961; Nottingham and Hardie 1993; Visser and Piron 1995), followed by the location of nutritious plant tissues using gustatory and visual cues (Dixon 1998). Leaf surface is scanned by tactile receptors located at the tip of the proboscis, which allow aphids to identify preferred feeding areas (Wensler 1977). During leaf surface probing, aphids ingest small amounts of plant chemicals, which stimulate their gustatory epipharyngeal organs and elicit or hinder feeding behaviour (Srivastava *et al.* 1983). Finally, aphids extract and ingest the phloem sap of their host plants through their stylets (Dixon 1998).

Host plant quality involves several components with the potential to affect herbivore performance either positively or negatively (Awmack and Leather 2002). These components include basic chemical elements, nutrients, defensive compounds, and plant anatomical features (Agrawal 2000; Baldwin *et al.* 2001; Awmack and Leather 2002). Plant quality affects multiple components of herbivore biology including fecundity, survival, movement, competition rates, and mortality from natural enemies attracted by semiochemicals, (Dadd and Krieger 1968; Leckstein and Llewellyn 1974; Price *et al.* 1980; Weibull 1987; Stadler 1995; Denno *et al.* 2000; Lill *et al.* 2002).

Nitrogen acquisition is one of the principal challenges that aphids face (Douglas 1993; Nevo and Coll 2001). Nitrogen within the host plant moves mostly as free-amino

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acids (Wilkinson and Douglas 2003), and amino acid availability is of great importance for herbivore performance (Mittler 1958; Dadd and Krieger 1968; Weibull 1987; Weibull 1988; Telang *et al.* 1999). Despite symbiotic bacteria converting non-essential amino acids into essential ones (Dixon 1998; Douglas 1998; Wille and Hartman 2009), not all the amino acids required by aphids can be obtained from symbiotic bacteria (Awmack and Leather 2002). Nitrogen and amino acid availability also depend upon the relative concentration of other elements. The availability of C-based compounds like carbohydrates and lipids tends to impact herbivore performance negatively by diluting N (Awmack and Leather 2002). Thus, high (C:N) ratios may have adverse effects on insect herbivores. There is also a negative relationship between K and N concentrations. Potassium-deficiency disrupts protein synthesis, increasing amino-acid concentrations and aphid performance (Myers *et al.* 2005; Myers and Gratton 2006).

Within-plant quality is not constant across time. Plant phenology is linked to changes in amino acid composition that directly affect herbivore performance (Weibull 1987; Karley *et al.* 2002). Plant N and amino acid levels are typically highest in young and senescing plant tissues, and relatively low in mature ones (Watt 1979; Weibull 1987; Karley *et al.* 2002). Within-plant quality may also vary spatially at a given time. Nutrient and allelochemical composition can vary across the foliage of a plant, often determining the within-plant spatial distribution of aphids (Dixon 1976; Watt and Hales 1996; Awmack and Leather 2002).

Aphids and other herbivorous insects also interact with their host plants by enhancing plant quality to their advantage (Telang *et al.* 1999; Awmack and Leather 2002). Examples of these interactions include creating nutrient sinks, diverting

within-plant nutrient movement, inducing the formation of galls, and/or changing the amino acid composition of the plant (Prado and Tjallingii 1997; Eleftherianos *et al.* 2006; Goggin 2007; Nombela *et al.* 2009). Despite these potential positive short-term effects, heavy feeding activity by sap-feeders induces long-term reductions in plant quality (Olmstead *et al.* 1997; Denno *et al.* 2000).

Polyphenism in aphids

Polyphenism is defined as the production of two or more different phenotypes (morphs) by one genotype (Simpson *et al.* 2011). Polyphenism occurs in a variety of animals that produce offspring with behavioural and/or morphological adaptations to adverse conditions including increased risk of attack from natural enemies, increases in population density, and changes in photoperiod (Harvell 1990; Kingsolver 1995; Agrawal *et al.* 1999; Elliot *et al.* 2003). Polyphenism is an important component of the aphid life cycle, as different morphs are adapted to fulfill different ecological roles, allowing aphids to maximize the use of resources (Dixon 1998). Aphid life cycles involve a sequence of morphs influenced by environmental cues (Dixon 1998; Müller *et al.* 2001). Consequently, the investment in morphs by a clone (a parthenogenetic line or genotype, Blackman, 1974) is optimized as a function of environmental conditions including host plant quality and availability, changes in population densities, and interactions with natural enemies (Dixon 1977).

Because the life cycle of most aphids involves a sexual and an asexual phase, a distinction between sexually-reproducing and asexually-reproducing morphs is required. Sexually reproducing aphid morphs include females (denominated oviparae), which are usually apterous, and sexually-reproducing males (andioparae), which are usually alate.

Among viviparous females produced through parthenogenesis, two major morphs are distinguished. These two morphs produce either sexually-reproducing females or males, or parthenogenetically-reproducing-females, and are denominated gynoparae, androparae, and virginoparae, respectively. Within the virginoparae, two major morphs differ remarkably in their physiology and anatomy: apterous and alate morphs (Blackman 1974). Because of a particular interest in the ecological trade-offs between alate and apterous virginoparae, this thesis focuses mainly on these two morphs, which for simplicity are hereon referred to solely as apterous and alate individuals, except otherwise stated.

Wing dimorphism and its trade-offs

Life history theory predicts trade-offs between energetically-costly traits (Zera and Harshman 2001). Behavioural and morphological adaptations that reduce predation risk are often costly and result in trade-offs between elements of prey fitness (Lima 1998; Peacor *et al.* 2013). A trade-off between flight and reproduction is common in insects (Rankin and Burchsted 1992; Gatehouse 1994; Baguette and Schtickzelle 2006; Guerra and Pollack 2007). In wing-dimorphic aphid species, asexual alate individuals typically require longer developmental times, have reduced fecundity, and produce smaller progeny than asexual apterous individuals (Dixon 1972; Wratten 1977; Dixon 1998). Therefore, wing production and immediate reproduction are two alternative strategies in terms of both physiology and ecology. In addition to the energetic costs of wing development, flight activity can result in further reductions in fecundity (Zhang *et al.* 2009). A physiological response compensating for the trade-off between wing development and fecundity in insects is wing muscle autolysis (Zera and Harshman

2001), which occurs in some aphid species shorty after flight ceases, and increases nutrient availability for embryo development (Dixon 1998). In some aphid species, alate adults produce only or mostly apterous offspring (Lees 1967; Sutherland 1970; MacKay 1977; Dixon 1998), which may compensate for costs of wing development by allowing higher fecundity in the next generation.

In addition to costs to fecundity caused by wing development and flight, dispersal between host plants is risky, since numerous alate individuals are expected to fail to find a suitable host plant (Dixon 1998; Ward *et al.* 1998). Because all members of a particular clone share the same genotype, however, dispersal risks may be compensated for via benefits derived from kin selection, although this notion has been subject to debate (Dawkins 1976; Dixon 1998). Given the costs of wing development, the fitness of an aphid clone is expected to depend on a balanced investment between apterous and alate individuals as a function of its environment (Dixon 1977; Hodgson 2001). Flight is hypothesized to occur only in situations in which its absence would be deleterious (Rankin and Burchsted 1992). In accordance with this, the propensity of alate aphids to fly is often dependent upon particular environmental cues like population density and host-plant phenology (Walters and Dixon 1982; Dixon and Mercer 1983; Dixon 1998).

Despite its costs to fecundity and survival, the production of alate individuals is predicted to benefit an aphid clone under certain environmental conditions, since it allows it to exploit ephemeral resources more effectively (Waloff 1983; Dixon 1998). Alate individuals perform both long-distance and short-distance flights (Blackman 1974; Taylor *et al.* 1979; Irwin *et al.* 1988; Dixon 1998). The result of short-distance flights between host plants, or "trivial" flights, is reproduction within the same habitat (Walters

and Dixon 1983). Because host plants are temporary resources, continuous dispersal between host plants plays an important role in the population dynamics of aphids (Dixon 1998). Trivial flight allows aphid clones to exploit spatially-complex habitats more effectively. For example, the architectural complexity of trees makes it necessary for aphids to engage in short-distance flights, and arboreal aphid species tend to produce relatively higher proportions of winged individuals than non-arboreal species (Waloff 1983; Dixon 1998). Moreover, dispersal from highly infested host plants to uninfested ones can also relieve population pressure and delay negative density-dependent effects (Kidd 1990; Dixon 1998).

Aphids show numerous behavioural and physiological adaptations to predation including aposematism, dropping from the host plant, adopting defensive spatial distributions on host plants, releasing alarm pheromone, and producing soldier and alate morphs (Rothschild *et al.* 1970; Clegg and Barlow 1982; Itô 1989; Mondor *et al.* 2005; Francke *et al.* 2008; Duff and Mondor 2012). Some aphid species respond to predator cues by increasing their production of alate individuals, which is considered an important adaptation to reduce predation risk by allowing aphids to leave predators behind (Blackman 1974; Dixon 1998; Weisser *et al.* 1999). Jeffries and Lawton (1984) have defined enemy-free space as "ways of living that reduce or eliminate a species' vulnerability to one or more species of natural enemies". Predators are more likely to detect high-density aphid patches (i.e. plants or groups of plants), and to spend longer times foraging there than on low aphid-density patches (Kareiva and Odell 1987; Ives *et al.* 1993; Koch 2003). Additionally, predators show positive numerical responses to aphid populations through increased reproduction (Donaldson *et al.* 2007). Consequently, low-

density patches will potentially provide predator-free space, although this is a dynamic

refuge expected to disappear as aphid densities increase (Berryman and Hawkins 2006).

Dispersal by flight may also prove beneficial to a clone by increasing its number of

colonies (i.e. groups of aphids occupying a single plant or plant stem) and decreasing its

probability of local extinction (Lamb and MacKay 2010). Thus, continuous dispersal is

hypothesized to allow a prey species to coexist with its predators (Harrison 1991; Dixon

1998).

Environmental factors that induce wing development in aphids

Numerous environmental cues are known to induce the production of alate individuals in

wing-dimorphic aphid species. Environmental cues can have effects on morph

determination, which are species-specific and can act directly on an immature individual

(post-natal mechanism), indirectly through maternal stimuli (pre-natal mechanism), or

combinedly. Susceptibility to environmental cues is also known to vary among clones

within the same species (Lamb and Mackay 1979; MacKay *et al.* 1983). The

environmental factors influencing the production of asexual alate individuals in aphids

include crowding, host plant quality, the presence of natural enemies and/or natural

enemy cues, and temperature. Additionally, changes in photoperiod can induce the

production of both sexually- and asexually-reproducing alate morphs that differ

form virginoparae (Dixon and Glen 1971).

Crowding

The relationship between crowding and wing development has been long known and

studied in aphids (Müller *et al.* 2001). Wing development by crowding is triggered

through tactile stimuli occurring between individual aphids (Lees 1967; Sutherland 1969). Higher aphid densities increase the frequency of tactile stimuli between individuals, although the production of alate progeny can be induced by as few as two adult aphids (Lees 1967; Sutherland 1969; Dixon 1998). The reception of tactile stimuli in aphids varies by species, and can involve antennal and/or other mechanoreceptors (Lees 1967; Kunert and Weisser 2005). Several aphid species are known to respond to pre- and/or post-natal crowding by inducing wing development (Müller *et al.* 2001). The production of alate individuals in response to crowding is considered an adaptation to mediate negative density-dependent effects resulting from plant deterioration (Blackman 1974).

Host plant quality

Pre-natal exposure to food sources of low nutritional quality induces wing development in the aphids *Acyrthosiphon pisum* (Harris) and *Aphis craccivora* Koch, (Johnson 1966; Mittler and Sutherland 1969; Sutherland and Mittler 1971). In the aphids *Dysaphis devecta* (Walker) and *Myzus persicae* (Sulzer), nymphs develop into alate adults when reared on N-deprived apple plants (Mittler and Dadd 1966; Forrest 1970). Exposure of adult aphids to plant precocenes (defensive secondary plant compounds) has also been found to induce the production of alate offspring in the aphids *Acyrthosiphon pisum* and *Macrosiphum euphorbiae* (Thomas) (Mackauer *et al.* 1979; Delisle *et al.* 1983; Hardie 1986). There is also a positive relationship between the presence of host plant pathogens and wing development in the aphids *Aphis gossypii* Glover, *Sitobion avenae* (Fabricius), and *Rhopalosiphum padi* (L.) (Gildow 1980; Blua and Perring 1992; Müller *et al.* 2001). This increase in the production of alate individuals in response to host plant viral

infections appears to be caused by changes in the concentration of certain nutrients caused by pathogens (Gildow 1980; Blua and Perring 1992).

Natural enemies

In populations of *Aphis gossypii*, the proportion of alate individuals increases with the abundance of natural enemies in cotton fields (Müller *et al.* 2001). In *Aphis gossypii* and *Acyrthosiphon pisum*, adult individuals exposed to coccinellid search tracks produce more alate offspring than those reared on plants without search tracks (Dixon and Agarwala 1999; Weisser *et al.* 1999; Mondor *et al.* 2005). Similarly, *Acyrthosiphon pisum* shows increased production of alate individuals in response to the presence of aphid-specific fungal pathogens and parasitoids (Müller *et al.* 2001; Sloggett and Weisser 2002; Hatano *et al.* 2012). *Sitobion avenae* responds to the presence of parasitized conspecifics (mummies) by decreasing wing development as an adaptation to decreased parasitoid pressure in previously-parasitized colonies (Fievet *et al.* 2009).

Temperature

There is a negative relationship between relatively high temperatures and wing development in aphids (Müller *et al.* 2001). In the aphids *Megoura viciae* Buckton and *Chaetosiphon fragaefolii* (Cockerell), adults developing under relatively higher temperatures produce a lower proportion of alate offspring (Lees 1967; Schaefers and Judge 1971). It has been hypothesized that this response allows aphids to compensate for increased mobility and more frequent tactile stimuli due to temperature increases (Müller *et al.* 2001).

Photoperiod

Changes in photoperiod result in the production of asexually- and sexually-reproducing alate morphs. In the aphid *Elatobium abietinum* (Walker), the production of viviparous alate migrants is induced by an increase in day-length (Fisher and Dixon 1986), and coincides with the burst of spruce buds and its associated decreases in quality (Fisher and Dixon 1986; Dixon 1998). In *R. padi*, a decrease in day-length induces the production of males and gynoparae (i.e. parthenogenetic females that produce sexual offspring, Dixon and Glen, 1971).

Combined environmental cues

Multiple environmental factors occur simultaneously in natural systems and can have mixed effects on aphid morph determination. Consequently, the ability to respond to multiple environmental cues is predicted to offer aphids a more effective way of tracking changes in the environment (Dixon 1998). Numerous interactions between environmental cues can influence wing development in aphids. In *S. avenae*, and *Myzus persicae*, host plant quality and crowding act together to mediate the production of alate offspring (Sutherland and Mittler 1971). In addition to the direct effects of predator cues on wing induction (Dixon and Agarwala 1999), the presence of natural enemies triggers the production of alarm pheromone in *Acyrthosiphon pisum*, which results in increased mobility and consequently increases the frequency of tactile stimuli among aphids (referred to as pseudo-crowding, Kunert *et al*., 2007). Similarly, *Myzus persicae* and *Megoura viciae* show increased mobility as a result of reduced host plant quality and crowding (Lees 1967; Williams *et al.* 1999). Physical contact occurring between aphids and their natural enemies is also hypothesized to trigger wing development through

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tactile stimuli, independent of any chemical cues (Müller *et al.* 2001). Environmental

factors occurring simultaneously may also antagonistically influence wing development.

In the aphids *Metopeurum fuscoviride* Stroyan and *Aphis fabae* Scop, the presence of

tending ants decreases the effects of other environmental factors like crowding and the

presence of predators on wing induction (Kleinjan and Mittler 1975; Mehrparvar *et al.*

2013). In *Myzus persicae* and *Aphis fabae*, previous host plant infestations by

conspecifics and heterospecifics affect host plant quality positively (Williams *et al.*

1998), thus, high aphid densities may not necessarily increase wing development.

Physiology of wing development in aphids

Histological studies suggest that metamorphosis of immature aphids into alate adults

occurs as the default developmental pathway, and that metamorphosis into apterous

adults represents an alternative pathway (Johnson and Birks 1960; Braendle *et al.* 2006).

Juvenile hormone plays a paramount role in the mediation of metamorphosis, growth, and

polyphenism in insects (Morgan 2010; Hartfelder and Emlen 2012), and relatively higher

concentrations of juvenile hormone in hemolymph can result in the retention of juvenile

characters (Blackman 1974). Even though the role of juvenile hormone in wing

development is somewhat controversial (Hardie 1986; Braendle *et al.* 2006), some lines

of evidence support the hypothesis that metamorphosis of immature aphids into apterous

adults occurs as a result of the retention of juvenile characters through high

concentrations of juvenile hormone (Johnson 1959; Kennedy and Stroyan 1959). In

accordance with this, topical application of juvenile hormone results in the suppression of

alatoid characteristics in nymphs of *M. viciae* (Lees 1980). Nymphs of the aphid

Brevicoryne brassicae (Linnaeus) treated with juvenile hormone are unable to undergo

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the cellular changes necessary for wing formation (White and Gregory 1972). Precocenes have been found to selectively destroy the cells of the corpus allatum, which is responsible for the production of juvenile hormone (Ohta and Bowers 1977, as cited by Braendle et al. 2006). In accordance with this, exposure of *A. pisum* nymphs to precocenes results in wing development (Mackauer *et al.* 1979). The production of juvenile hormone by the corpus allatum is mediated through neurosecretory pathways that can be influenced by environmental cues (Lees 1964; Steel and Lees 1977; Hales and Mittler 1983; Hales and Mittler 1987).

Aphis glycines as an agricultural pest

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a pest indigenous to Asia that was discovered in Australia, the United States, and Canada (Venette and Ragsdale 2004; Mignault *et al.* 2006). Since its arrival in 2000, *A. glycines* infestations have caused economic yield losses of up to 45% in soybean, *Glycine max* (L.) Merrill (Fabaceae) in North America (Ragsdale *et al.* 2007). By 2009, *A. glycines* had spread to over 30 states of the U.S. and 3 Canadian provinces (Ontario, Quebec and Manitoba), (Ragsdale *et al.* 2011). *Aphis glycines* causes reductions in *G. max* photosynthetic rates (Macedo *et al.* 2003), and is considered an important vector of alfalfa mosaic virus (AMV), soybean mosaic virus (SMV), and tobacco ringspot virus (TRS) (Clark and Perry 2002; Wang and Ghabrial 2002). Additionally, honeydew excreted by aphids is known to facilitate the production of sooty mould, which reduces photosynthetic capacity (Wu *et al.* 2004; Dedryver *et al.* 2010). Infestation symptoms of *G. max* plants by *A. glycines* include early defoliation, leaf deformation, decreased plant growth, pod size reduction, and losses to seed weight (Wu *et al.* 2004). Infestations of *A.*

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glycines seem to have varying effects on yield components depending on the developmental stage of *G. max* (Beckendorf *et al.* 2008).

The economic threshold for *A. glycines* on *G. max* (pest density at which control proves economically advantageous) has been established at 250 aphids per plant, and the economic injury level (pest density at which yield losses equal cost of pest management) has been established at 674 aphids per plant (Ragsdale *et al.* 2007). The magnitude of *A. glycines* outbreaks seems to be influenced by a variety factors including agricultural practices, natural enemy population dynamics, *G. max* phenology, and overwintering egg success on the primary host of *A. glycines* (Wu *et al.* 2004). Control of *A. glycines* populations in North America and Asia currently involves chemical management, cultural and biological control, and the use of resistant *G. max* varieties (Wu *et al.* 2004; Ragsdale *et al.* 2011).

Aphis glycines life cycle in North America

Aphis glycines has an heteroecious life cycle (Ragsdale *et al.*, 2004). In North America, two species of buckthorn act as primary hosts for *A. glycines* (*Rhamnus cathartica* L., and *Rhamnus alnifolia* L'Her), while *G. max* is the secondary host (Ragsdale *et al.* 2004; Voegtlin *et al.* 2004). *A. glycines* can also successfully reproduce on red clover (*Trifolium pratense* L.), and Carolina horsenettle (*Solanum carolinense* L., Ragsdale et al., 2004). Early in spring, parthenogenetic females (fundatrices) hatch from the overwintering eggs laid on buckthorn and produce several parthenogenetic generations (virginoparae). At the beginning of summer, alate virginoparae migrate to *G. max*, where they reproduce parthenogenetically throughout the *G. max* growing season, producing apterous and alate offspring. By the end of summer, alate parthenogenetic females (gynoparae) are produced

on *G. max* and migrate back to buckthorn, where they give birth to sexual females (oviparae). Later in the season, alate males (androparae) migrate from *G. max* to buckthorn and mate with the oviparae to produce overwintering eggs (Blackman 1974; Ragsdale *et al.* 2004; Rhainds *et al.* 2010). The life history of *A. glycines* consists of four immature instars and the adult stage (Hodgson *et al.* 2005b). The optimal growth and developmental temperature for *A. glycines* is between 20°C and 25°C, at which the pre-reproductive phase (i.e. time from birth to larvaposition) lasts between 5 and 7 days. At 30°C the pre-reproductive phase is prolonged, and at 35°C nymphs fail to complete development and die (McCornack *et al.* 2004).

Biology of *A. glycines* on *Glycine max*

Initial colonization of *G. max* by asexual alate individuals (usually in June) results in patchily-distributed colonies across fields, and these colonies consist mostly of early instar nymphs (Ragsdale *et al.* 2004). In parts of the North Central United States and Canada, colonization of *G. max* fields can occur after early July, in which cases, it is likely attributable to alate individuals immigrating from nearby *G. max* fields (Ragsdale *et al.* 2004). During the early vegetative stages of *G. max*, *A. glycines* individuals tend to aggregate on growing tissues, such as young trifoliate leaves, newly-developed petioles, and growing stems. Though the within-plant distribution of *A. glycines* changes throughout the season, most individuals are typically found on the upper nodes of *G. max* plants (Macedo *et al.* 2003; Wu *et al.* 2004; Costamagna and Landis 2006; McCornack *et al.* 2008; Costamagna *et al.* 2010). Field studies indicate that the production of asexual alate individuals increases along with population density (Hodgson *et al.* 2005b; Donaldson *et al.* 2007).

The production of asexual alate individuals by *A. glycines* plays an important role in the population dynamics of the species. Asexual alate *A. glycines* can fly for up to 11 hours and distances of over 16 km, as an alternative to investment in higher fecundity (Zhang *et al.* 2008; Zhang *et al.* 2009). Between-field dispersal potentially accounts for initial field colonizations (Ragsdale *et al.* 2004), and can potentially increase virus transmission (Wu *et al.* 2004; Zhu *et al.* 2006), as well as causing sudden population increases and outbreaks due to mass immigrations (Costamagna *et al.* 2013a). The production of asexual alate *A. glycines* individuals occurs continuously throughout the *G. max* growing season, and is known to peak along with population density increases, during *G. max* pod set stages (Hodgson *et al.* 2005a; Hodgson *et al.* 2005b; Donaldson *et al.* 2007; Bahlai *et al.* 2014).

Top-down control of *A. glycines* populations

Populations of *A. glycines* in Asia are often controlled by several natural enemies including predators, parasitoids, and pathogens (Wu *et al.* 2004). Parasitoids of *A. glycines* in China include *Lysiphlebus* sp. (Hymenoptera: Aphelinidae), *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae), and *Aphelinus* sp. (Hymenoptera: Aphelinidae) (Miao *et al.* 2007). Among the predatory coccinellids, *Harmonia axyridis* Pallas and *Hippodamia tredecimpunctata* L. seem to be particularly successful in supressing *A. glycines* populations in Asia (Wu *et al.* 2004). In North America, *G. max* had remained free of major pests prior to the detection of *A. glycines*, and natural enemies of *A. glycines* were consequently not abundant (Ragsdale *et al.* 2011). Approximately 10 species in the families Aphelinidae and Braconidae have been reported to parasitize *A. glycines* in North America (Ragsdale *et al.* 2011). The parasitoid *Aphelinus certus*

Yasnosh (Hymenoptera: Aphelinidae) is a useful biological control agent for *A. glycines* in Ontario and Eastern United States (Frewin *et al.* 2010; Heimpel *et al.* 2010; Xue *et al.* 2012; Frewin *et al.* 2014). The natural enemy complex of *A. glycines* in North America is mainly driven by generalist predators, among which coccinellids seem to be the most effective. Predators in the field can suppress *A. glycines* populations and cause a trophic cascade, protecting *G. max* biomass and yield (Costamagna *et al.* 2007; Rhainds *et al.* 2007). Two species of coccinellids, *Harmonia axyridis* and *Coccinella septempunctata* L., are considered particularly important predators of *A. glycines* due to their high abundance and per-capita predation rates, since both species show functional and numerical responses to *A. glycines* densities (Rutledge *et al.* 2004; Mignault *et al.* 2006; Costamagna and Landis 2007; Xue *et al.* 2009). Other coccinellids that feed on *A. glycines* in north America include *Coleomegilla maculata* De Geer, *Hippodamia variegata* (Goeze), and *Propylea quatuordecimpunctata* L. (Mignault *et al.* 2006; Costamagna and Landis 2007). Although not as effective as coccinellids in a per-capita consumption basis, the insidious flower bug, *Orius insidiosus* (Say) (Hemiptera: Anthocoridae) can play an important role in suppressing *A. glycines* depending on the time at which *G. max* fields are colonized, its abundance relative to that of *A. glycines*, and the presence of alternative prey (Rutledge and O'Neil 2005; Costamagna and Landis 2007; Yoo and O'Neil 2009). Other potential predators of *A. glycines* include members of the families Staphylinidae, Syrphidae, Chrysopidae, Cecidomyiidae, Nabidae, and Miridae, although their effects on *A. glycines* populations are not strong (Costamagna and Landis 2007). In general, the natural enemy complex shows a positive numerical response to *A. glycines* field densities (Donaldson *et al.* 2007). Some aphids can decrease

predation risk through dilution effects by aggregation (Cappuccino 1988; Turchin and

Kareiva 1989), however, high density patches of *A. glycines* at the within-field scale fail

to provide refuge from predation (Costamagna and Landis 2011). The effectiveness of

biological control agents for *A. glycines* can be potentially affected by intraguild

predation, and the introduction of exotic biological control agents needs

careful evaluation (Fox *et al.* 2004; Costamagna and Landis 2006; Costamagna *et al.*

2007; Gardiner and Landis 2007; Chacón *et al.* 2008; Chacón and Heimpel 2010; Xue *et

al.* 2012).

Bottom-up control of *A. glycines* populations

Host plant quality influences aphid size, survival and fecundity (Leather and Dixon 1981;

Grayer *et al.* 1992; Awmack and Leather 2002; Karley *et al.* 2002; Myers and Gratton

2006). Reproduction in *A. glycines* is known to be positively correlated with *G. max* N

content (Walter and DiFonzo 2007). Up to 36% of sap N in *G. max* plants is in the form

of amino acids, of which asparagine constitutes more than 70% (McClure and Israel

1979). There is a negative relationship between N and K concentrations in *G. max* plants,

and K deficiency often results in an accumulation of asparagine (Yamada *et al.* 2002;

Walter and DiFonzo 2007). In accordance, *A. glycines* shows increased fecundity and

intrinsic rates of increase on K-deficient *G. max* plants (Myers *et al.* 2005; Myers and

Gratton 2006). Nonetheless, tillage regimes and fertilizer inputs do not affect *A.*

glycines populations (Costamagna and Landis 2006).

Changes in host plant physiology through time affect *A. glycines* populations

(Weibull 1987; Ragsdale *et al.* 2004). As *G. max* transitions from vegetative to

reproductive stages, pods, leaflets and stems suffer important reductions in N content

(Thibodeau and Jaworski 1975; Rainbird *et al.* 1984). Increases in dry weight throughout reproductive stages of *G. max* are mostly attributed to growing embryos in the seed, of which up to 60% N content is asparagine (Rainbird *et al.* 1984). During the pod filling stages of *G. max*, the highest concentrations of ureides (the main form of N in *G. max* plants) are contained in the seed coat, followed by the pod walls and stem (Rainbird *et al.* 1984). Plant quality can influence shifts between host species in aphids (Dixon and Glen 1971). Changes in concentrations of *G. max* sap N are thought to influence the migration of *A. glycines* to buckthorn, in addition to changes in photoperiod and temperature (Beckendorf *et al.* 2008).

In addition to changes in host plant quality through time, variations occur at a within-plant spatial scale. Non-uniform spatial N distributions allow plants to have higher C-exchange rates (Hirose and Werger 1987), and nutrient availability for aphids is typically not uniformly-distributed along the host plant. In the particular case of *G. max*, leaf-specific N concentrations are highest at the upper nodes and decrease towards the bottom of the plant (Muchow *et al.* 1986; Shiraiwa and Sinclair 1993).

Variations in host plant quality can also occur between plants due to herbivory. Infestations with *A. glycines* can lead to important decreases in photosynthetic rates and gas exchange in *G. max*, even at low aphid densities (Macedo *et al.* 2003). *Glycine max* plants infested with *A. glycines* show decreases in leaf-specific amino acid concentrations in comparison with those kept uninfested through treatment with insecticide (Chiozza *et al.*, 2010). Host plant responses induced by herbivory damage can alter the performance of conspecifics, heterospecifics, and their natural enemies (Rodriguez-Saona *et al.* 2005).

Host plant pre-infestation by herbivorous insects can induce plant resistance (Luo *et al.* 2016).

Multiple resistant *G. max* varieties have been developed and commercialized in North America, and present antixenosis (reduced attractiveness), antibiosis (induction of lethal effects), and/or tolerance (ability to overcome negative effects of infestations) against *A. glycines* (Ragsdale *et al.* 2011). Resistance to *A. glycines* has been shown to depend on single dominant genes (*Rag* genes – Resistance to *Aphis glycines*), which are expressed in a series of *G. max* varieties (Hill *et al.* 2004; Hill *et al.* 2006; Hesler *et al.* 2013). However, the effectiveness of resistant *G. max* varieties as biocontrol agents has been compromised, since three *A. glycines* biotypes can overcome resistance (*Rag* virulent *A. glycines* biotypes 2 - 4, Kim et al. 2008; Hill et al. 2009; Hill et al. 2010; Alt and Ryan-Mahmutagic 2013; Crossley and Hogg 2015). The optimal use of *Rag*-gene *G. max* varieties has failed due to a lack of knowledge on the geographic distribution of virulent *A. glycines* populations (Hesler *et al.* 2013). Additionally, host plant pre-infestation with virulent *A. glycines* biotypes can result in increased susceptibility to avirulent biotypes (i.e. induced susceptibility, Varenhorst *et al.*, 2015).

Overview and general research objectives

Despite an increasing body of research on the biology of *A. glycines*, there is a general knowledge gap regarding wing dimorphism in this species. The production of asexual alate individuals by *A. glycines* is likely to affect interactions between this species, its host plant, and its natural enemies, and is therefore an important biological component of this species as a pest. The objectives of this thesis are: 1) to determine the effects of crowding and host plant quality on the production of asexual alate individuals by *A.*

glycines, and 2) to investigate the effects of asexual alate individual production on *A. glycines* populations under predation in the field.

CHAPTER 3: EFFECTS OF CROWDING AND HOST PLANT QUALITY ON MORPH DETERMINATION IN THE SOYBEAN APHID, *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE)

Abstract

Polyphenism constitutes a key example of the adaptability of a genotype to a shifting environment. In aphids, the relative production of apterous and alate individuals is influenced by cues resulting from increases in population density, changes in host plant quality, and natural enemies. The ability to respond to multiple environmental cues offers aphids an effective way to optimize their fitness. Understanding the mechanisms behind alate morph production is particularly important in aphid species that are agricultural pests. I tested the effects of crowding and host plant quality cues on morph determination in *Aphis glycines* Matsumura (Hemiptera: Aphididae). I exposed *A. glycines* to pre-natal and post-natal crowding under laboratory conditions. In the field, I reared nymphs of *A. glycines* at high-density vs. low-density conditions in clip cages set on the top vs. bottom nodes of infested and uninfested soybean plants, *Glycine max* (L.) Merrill (Fabaceae). Apterous adult body size was used as an estimate of aphid performance, and the proportion of morphs developing under each condition was determined. Both pre- and post-natal crowding induced the production of asexual alate individuals by *A. glycines*. In the field, I observed additive negative effects of lower overall plant quality (infested vs. uninfested plants), within-plant differences in nutritional quality (bottom vs top nodes), and high vs. low levels of crowding on aphid size. These three factors also interacted to increase the proportion *A. glycines* nymphs that developed wings. My results suggest two levels in the production of asexual alate individuals by *A. glycines*: 1) a moderate level in response to aggregation, triggered by crowding cues, and, 2) a high level in response to

plant quality deterioration, triggered by both plant quality and crowding cues. These two processes ensure a continuous but flexible production of asexual alate individuals, which allows aphid colonies to rapidly adapt to changing environmental conditions.

Introduction

Polyphenism is the phenomenon by which two or more phenotypes (morphs) are produced by a single genotype, and constitutes a prime example of the adaptability of a genotype to a shifting environment (Simpson *et al.* 2011). The production of different morphs is an integral component in the life cycles of aphids (Dixon 1998). Polyphenism in species producing apterous (wingless) and alate (winged) morphs allows a more efficient exploitation of changing habitats, since different morphs are adapted to fulfill different ecological roles (Waloff 1983; Dixon 1998). The production of alate individuals can benefit an aphid clone (i.e. parthenogenetic line or genotype) by facilitating resource colonization, by permitting escape from natural enemies, and by reducing negative density-dependent population effects through dispersal (Dixon 1998). Nonetheless, reproductive costs associated with wing development (Mackay and Wellington 1975; Wratten 1977; Dixon 1998) suggest the need for an optimal allocation of morphs under diverse conditions, which requires the ability to track shifting environmental conditions.

In wing-dimorphic aphid species, the relative production of asexual apterous and alate individuals is influenced by cues resulting from population density increases (referred to as crowding), changes in host plant quality, and the presence of natural enemies (Müller *et al.* 2001). The effects of environmental cues on morph determination often vary among species, and can act directly on an aphid nymph (post-natal

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determination), indirectly through maternal stimuli (pre-natal determination) or through a combination of both mechanisms.

Crowding is the most studied environmental factor inducing wing development in aphids, and a positive response to crowding has been documented in 13 aphid species (Müller *et al.* 2001). Wing induction in response to crowding is triggered by tactile stimuli between individual aphids, and may occur in the absence of other environmental cues (Lees 1967; Sutherland 1969). Wing induction in response to crowding is considered an adaptation to reduce negative density-dependent effects leading to resource deterioration (Blackman 1974).

Exposure to low quality food sources can also induce wing development in some aphid species in the absence of crowding (Forrest 1970; Sutherland and Mittler 1971). Host plant quality affects the size, survival and fecundity of aphids (Leather and Dixon 1981; Grayer *et al.* 1992; Awmack and Leather 2002; Karley *et al.* 2002; Myers and Gratton 2006). Changes in host plant phenology result in changes in plant quality through time, affecting the performance of aphid populations (Dixon 1972; Weibull 1987; Awmack and Leather 2002; Karley *et al.* 2002). Similarly, previous host plant infestations by conspecifics can result in increases or reductions in quality for aphids and other sap-feeding insects (Denno *et al.* 2000; Awmack and Leather 2002; Chiozza *et al.* 2010; Varenhorst *et al.* 2015). Finally, spatial variations in within-plant quality can also affect the performance of aphids (Watt 1979; Costamagna *et al.* 2013b).

Since environmental factors in natural systems operate simultaneously, the ability to respond to multiple cues is considered of adaptive importance (Dixon 1998). In accord

with this, the production of asexual alate individuals in some aphid species is mediated both by crowding and host plant quality cues (Sutherland and Mittler 1971; Dixon 1972).

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an important economic pest in the North Central US and Southern Canada (Ragsdale *et al.* 2011), and can cause yield losses as high as 45% (Ragsdale *et al.* 2007). Throughout summer, *A. glycines* colonies on soybean plants (*Glycine max* (L.) Merrill) consist of a mixture of asexual parthenogenetic apterous and alate individuals. Though the production of asexual alate individuals has been observed to increase along with population density in *A. glycines* (Hodgson *et al.* 2005b; Bahlai *et al.* 2014), the roles of particular environmental factors on morph determination have not been examined in this species. The production of asexual alate individuals may play an important role in the ecology of *A. glycines* by contributing to population outbreaks (Costamagna *et al.* 2013a), by influencing its role as a plant virus vector (Wu *et al.* 2004; Zhu *et al.* 2006), and by increasing prevalence (Chapter 4).

The objectives of this study were: 1) to determine the pre-natal and post-natal effects of crowding on morph determination in *A. glycines*, and 2) to examine the combined effects of crowding and host plant quality cues on *A. glycines* under field conditions. I predicted that cues resulting from lower between-, within-plant quality, and crowding would interact to increase wing induction in *A. glycines*.

Materials and methods

Aphid colonies

A colony of *A. glycines* from a population originally collected in Guelph, Ontario, Canada was maintained at the University of Manitoba, Winnipeg, Manitoba, Canada

under controlled laboratory conditions (25 ± 1 °C; 16L:8D photoperiod) for ca. 5 years

before the experiments were conducted. Aphids in the colony were provided weekly with

a plastic tray (5 cm height, 25.5 cm width, 50.8 cm length) containing ca. 24 fresh VC –

V1-stage *G. max* plants (first node under development, Ritchie *et al*. 1985) grown in a

soil mix containing peat moss, perlite, nutrient charge, and limestone (Sunshine

Professional Growing Mix #4, Sungro Horticulture, Agawam, MA, U.S.A.). *Glycine max*

of the variety OAC Prudence (Shanawan Farms Ltd., Domain MB, Canada) was used for

all rearing purposes and experiments.

Clip cages

Clip cages were used in all laboratory and field experiments to confine aphids to specific

leaf tissues, and to control aphid densities. Clip cages were constructed with two rings of

transparent PVC pipe (1 cm length) mechanically bound together by stainless steel

duck-bill hair-clips. Foam circles were glued to the pipe rings to reduce leaf surface

damage due to pressure upon closing (Murdie 1969b). Each clip cage enclosed a 1.5 cm

diameter leaf area (1.77 cm^2) and was covered by semi-translucent fine plastic mesh

glued on with commercial cyanoacrylate (Krazy Glue, Elmers Products, High Point,

North Carolina, U.S.A.). Clip cages were tied to bamboo sticks to support their weight.

Pre-natal crowding under controlled conditions

A laboratory experiment was carried out to determine the pre-natal effects of crowding

on morph determination in *A. glycines*. Pairs of clip cages were mounted on individual

VC-stage potted *G. max* plants, one on each unifoliate leaf. Pots (15 cm diameter, 10.5

cm height) were watered every second day and kept under controlled laboratory

conditions (25 ± 1 °C, 16L:8D photoperiod, Model 1818 Microprocessor Controlled Low

Temperature Illuminated Incubator, Thermo Electron Corporation, Marietta, Ohio,

U.S.A.). Alate adults from the laboratory colony were haphazardly selected and

transferred in groups of four into each clip cage, where they were confined to the abaxial

leaf surface. Alate adults were allowed to produce progeny for 72 hours before being

removed. Alate adults were used because they are known to produce mostly apterous

progeny (Sutherland 1970; MacKay 1977). One of two nymph density treatments (high

vs low-density) was randomly assigned to each clip cage on the same plant. This

procedure minimized the effects of potential differences in plant quality among

individual plants on the density treatments. In the high-density treatment, nymph

densities were adjusted targeting 20 individuals/clip cage, but minimizing manipulations

resulted in slightly lower final numbers (19.9 ± 0.5, mean ± SD). All nymphs from the

low-density treatments produced within the same 72 hours were removed from the clip

cages and discarded, as they had already been exposed to crowding. Alate adults in those

clip cages were again allowed to produce nymphs for ca. 5 hours, after which, all nymphs

except one were removed from the clip cages along with the alate adults. This procedure

allowed the production of isolated nymphs wile minimizing manipulation of plants and

aphids. Nymphs inside clip cages were allowed to mature. Each adult aphid that had

developed in isolation (low-density treatment), and one haphazardly chosen adult from

the high-density treatment, were transferred to clip cages set on new VC-stage potted *G.

max* plants, where they were allowed to produce 5 nymphs before being removed. This

method allowed the production of a progeny sample consisting of 5 nymphs born from

apterous parents that were either reared under crowded conditions or in isolation. Potted

plants were watered every second day and kept under controlled laboratory conditions

until nymphs matured (ca. 1 week). The resulting morphs of the progeny were determined by the presence or absence of wings and wing buds, and were used to classify their parents either as alatae-producers or non-alatae-producers. An aphid was considered an alatae-producer if at least one individual from its progeny sample developed wings (Sutherland 1969; Mackauer *et al.* 1979; Lees 1980). Thirty-five replicates of each treatment were run simultaneously.

Post-natal crowding under controlled conditions

A second laboratory experiment was carried out to test the post-natal effects of crowding on morph determination in *A. glycines*. To produce aphids in the absence of crowding, multiple sets of two adult apterous aphids from the laboratory colony were allowed to produce progeny on individual VC - V1-stage *G. max* plants for 24 hours before being removed. Progeny densities were reduced to a maximum of five nymphs per plant and were allowed to mature under controlled laboratory conditions. Densities of no more than 5 nymphs per plant were used because preliminary trials revealed that they rarely induced the production of alate offspring. Once mature, adult apterous aphids were haphazardly selected and transferred in groups of four to clip cages set on the unifoliate leaves of VC - V1-stage potted *G. max* plants, where they were allowed to produce progeny for 24 hours before being removed. Clip cages were haphazardly assigned to one of two nymph density treatments (high vs low-density). Following the methods of the previous experiment, the two density treatments were set-up one on each of the unifoliates of individual potted plants. Nymph densities were adjusted targeting 30 and 6 individuals/clip cage in the high-density and low-density treatments respectively (actual final densities were 24.9 ± 6 and 6 ± 0.45, mean $\pm$ SD). Pots were watered every second

day and kept under controlled conditions (24 ± 1 °C, 16L:8D photoperiod). Nymphs were allowed to mature in order to determine the proportion of morphs in each clip cage. This method allowed the production of progeny reared at high or low densities, born from un-crowded apterous parents. Eleven replicates of each treatment were run simultaneously. Nymphs that died before morph determination (6 % in both treatments) were considered apterous individuals in my analysis, which prevented artificially inflating the proportion of alate individuals (Dixon and Glen 1971).

Field cage experiment

To determine the effects of post-natal crowding and host plant quality on wing induction in *A. glycines*, a field cage experiment was carried out in a *G. max* field located at the University of Manitoba, Winnipeg, MB, Canada (N49.8136 W97.1208), from 30 June through 29 July 2015. *Glycine max* was seeded on the last week of May with a ca. 18 cm row spacing. Twenty blocks with two plants each were established across the *G. max* field. Blocks were established to control for potential differences due to observers, but no other apparent gradient was observed in the field. All plants selected had a similar size and anatomy, and showed no signs of previous herbivory or mechanical damage. Plants were carefully inspected and any arthropods present were removed. Commercial tomato wire cages (41 cm diameter, 85 cm height) covered by a sleeve of white no-see-um mesh (0.24 mm^2) tied at the top of the cage and buried at soil level were used to enclose each plant individually. The top of the mesh was tied to a steel pole (1 m length) anchored to the ground. The bottom of the cage was secured to the ground using four commercial plastic camping pegs. Pitfall traps constructed with commercial plastic food containers (11 cm diameter, 7 cm height) were buried at soil level inside each cage to remove any

ground-dwelling arthropods present in the cages. Pitfall traps were kept inside the cages throughout the experiment.

To separate the effects of host plant quality cues (i.e. deterioration due to aphid infestations) from the effects of crowding cues (physical contact), the infestation level of the plants was first manipulated. Plants within each block were randomly assigned to one of two infestation treatments (infested vs uninfested plants). Plants assigned to the infested treatment were each inoculated with 15 adult apterous aphids from the laboratory colony, and populations were allowed to grow for 17 days. At the time of inoculation, caged plants were on their V3 - V4-stage (fully developed third and fourth node, respectively, Ritchie *et al*., 1985). A cohort of adult apterous aphids developing under low-density conditions and high plant quality was produced under controlled laboratory conditions for use in the field. Unifoliate leaves of *G. max* plants grown under laboratory conditions were excised and placed on moist paper towel circles kept inside plastic Petri dishes (9 cm diameter, 1 cm height, Fisherbrand, Ottawa, Ontario, Canada). Young *G. max* leaves were used due to their high nutritional quality, demonstrated by *A. glycines* aggregations on newly developed plant tissue (McCornack *et al.* 2008; Costamagna *et al.* 2013b). Adult apterous aphids were haphazardly selected from the laboratory colony and placed in groups of three on the excised leaves, where they were allowed to produce progeny for 24 hours before being removed. Nymph densities were then reduced to six individuals per Petri Dish and were allowed to develop under controlled conditions until maturity. Aphids developing into alate adults were discarded.

Clip cages were used to confine the aphids to specific parts of the plants in the field, and to control the densities of their progeny. Plants in the field were on

V9 / R4-stage (fully developed ninth node; full pod, Ritchie *et al*. 1985) at the time clip

cages were set. Each caged plant in the field held four clip cages, two of which were set

on the bottom part of the plant, and the other two on the top part of the plant (bottom vs

top treatments, respectively). Clip cages assigned to the bottom treatment were set on the

oldest and bottom-most expanded trifoliate of each plant (node 2), while those assigned

to the top treatment were set on the second newest expanded trifoliate of each plant

(nodes 7 - 8). In the case of infested plants, leaves on which clip cages would be mounted

were first cleared of all aphids, remaining exuviae, and honeydew drops using

paintbrushes moistened with water. Clip cages on each portion of the plant were

randomly assigned to one of two population density treatments (high- vs low-density

treatments). The high-density treatment was established by allowing adult aphids to

produce nymphs during three consecutive days, and was standardized to approximately

30 nymphs/clip cage (28.3 ± 3, mean ± SD). To avoid nymph crowding in the

low-density treatment, all nymphs produced during the first 24 hours were removed.

After another 24 hours, nymph densities were standardized to approximately 6

nymphs/clip cage (5.9 ± 0.6, mean ± SD) and adults were removed. Therefore, the

high-density treatment was established using nymphs from three consecutive days

whereas the low-density treatment was established with nymphs produced only during

the middle day of the period. Nymphs in each clip cage were allowed to develop until

maturity. The experiment had a total duration of 26 days. Day 0 was marked by the initial

inoculation of caged plants assigned to the infestation treatment. Clip cages containing

the aphids reared under controlled conditions were set on day 17. Nymphs from the high-

density treatment were produced during days 17 - 19, while nymphs from the low-density

treatment were produced during day 18. The positions of the clip cages were secured with

bamboo sticks. On day 26, leaves were excised along with the clip cages and were put

inside individual plastic zipper bags (16.5 x 8.2 cm), which were stored at -20 °C until

examination. Throughout the experiment, several plant height measurements and aphid

counts were performed to follow the population growth of the aphids infesting the cages

(days 4, 7, 11, 18, and 26). Aphids from the clip cages were observed under the

microscope for morph determination. Adult aphid length was measured from antennal

tubercles to the end of the cauda (Minks and Harrewijn 1987a) using an ocular

micrometer at a 40x magnification. Adult body size is often correlated with fecundity and

offspring size, and it constitutes a useful parameter for estimating aphid performance

(Murdie 1969a; Nevo and Coll 2001). Only size measurements from apterous adults were

analyzed, as not all clip cages contained alate adults and comparisons based upon

observations from alate adults lacked statistical power.

Data analysis

All analyses were performed using R version 3.1.2 (R Core Team 2014). The numbers of

alatae-producers and non-alatae-producers from the pre-natal crowding experiment were

compared using Fisher's exact test, which provides a robust test when sample sizes are

relatively small (Quinn and Keough 2002). Because in the post-natal crowding

experiment the proportion of alate aphids observed in the low-density treatment was

equal to zero, the proportion observed in the high-density treatment was tested as

significantly different from zero using a one-sample t-test (Quinn and Keough 2002). The

field cage experiment followed a split-split plot design testing three factors: a) plant

infestation level (whole plot factor), b) within-plant location (sub-plot factor), and, c)

population density inside each clip cage (sub-sub-plot factor). Aphid size data from the field cage experiment were tenth-root transformed (McCune *et al.* 2002) to fit model assumptions and analyzed using a mixed effects model, performed with the "lme4" package (Quinn and Keough 2002; Bates *et al.* 2015). The independent variables of this model were infestation level, within-plant location, clip cage density, and all their possible two-way interactions, in addition to their three-way interaction. The error term for each main effect in this analysis includes the random error term associated with block and its interactions with the three main effects. Data on the proportion of alate individuals from the clip cages set in the field were arcsine square-root transformed to fit model assumptions and analyzed using a mixed effect model like the one previously described. Contrasts were performed in this model to examine differences between treatments, using the "Phia" package in R (De Rosario-Martinez 2013). The significance levels of these contrasts were adjusted using the Holm-Bonferroni procedure for multiple comparisons (Rice 1989; Quinn and Keough 2002). Plant height data were square-root transformed to fit model assumptions and analyzed using a multivariate repeated-measures ANOVA model (O'Brien and Kaiser 1985), performed with the "Car" package (Fox *et al.* 2015). In this analysis, plant height data from each observation date were treated as separate dependent variables to avoid violation of the assumption of sphericity, which is often not met in biological studies. The independent variables of the model were plant infestation treatment, day, block, and their two-way interactions, except for a treatment-block interaction, which was not tested due to lack of treatment replication within block. Pre-planned contrasts were performed to examine differences between treatments across sampling days.

Results

Pre-natal crowding under laboratory conditions increased the production of alate offspring (Fisher's exact test, $P = 0.03$). When adult aphids were reared at high densities (n=28), 25.0% produced alate progeny, while none of the adult aphids reared at low densities (n=19) produced alate progeny. The final number of replicates between treatments differed because in the low-density treatment, the replicate was discarded if the isolated mother died or developed into an alate adult (the latter occurred in 14% of the low-density aphids). Proportional mortality was 0.33 ± 0.05 (mean $\pm$ SE) in the high-density treatment and 0.26 ± 0.05 in the low-density treatment (out of 5 nymphs/clip cage). Post-natal crowding also caused wing induction. When nymphs were reared at high densities (n=11), 16.2% ± 0.06 (mean $\pm$ SE) developed into alate adults, while none of the nymphs reared at low densities (n=11) developed into alate adults ($t_{(8)} = 2.60$, $P = 0.026$). Proportional mortality was 0.16 ± 0.03 (mean $\pm$ SE) in the high-density treatment and 0.03 ± 0.02 in the low-density treatment.

Plant infestation treatment, within-plant location, and aphid density treatment resulted in changes in aphid size reared in clip cages. By the time the clip cages were set on the plants (day 17 of the field experiment), infested plants had reached a density of 5425.33 ± 1647.85 (mean $\pm$ SD) aphids per plant (n=18), and by day 26, infested plants were 14.3% shorter than uninfested plants ($F_{5,12} = 51.05$, $P < 0.001$, Figure 1). Two plants were discarded due to cage damage from wildlife. Nymphs developing in clip cages on infested plants developed into smaller apterous adults than those developing on uninfested plants (Figure 2a and Table 1a). Nymphs developing on the bottom nodes of the plant (bottom treatment) developed into smaller apterous adults than those developing

on the top nodes of the plant (top treatment, Figure 2a and Table 1a). Finally, nymphs from the high-density treatment developed into smaller apterous adults than those from the low-density treatment (Figure 2a and Table 1a). Neither the two-way interactions nor the three-way interaction were significant.

Plant infestation treatment, within-plant location, and aphid density treatment interacted to influence the proportion of nymphs developing into alate adults (Figure 2b and Table 1b). When reared on top nodes, proportionally more nymphs developed wings at high aphid densities than at low densities. However, when reared on bottom nodes, no differences were observed between aphids exposed to high vs low densities. When reared on bottom nodes, proportionally more nymphs developed wings on infested plants than on uninfested plants. However, when reared on top nodes, no differences were observed between aphids from infested and uninfested plants. Within-plant location had no effect on wing development when nymphs were reared on uninfested plants. However, on infested plants, nymphs from low densities showed a notable increase in wing induction when reared on bottom nodes compared to top nodes.

Discussion

My results demonstrate that both pre- and post-natal crowding induce wing development in *A. glycines*, as is the case in several other aphid species including *Aphis craccivora* Koch, *Brevicoryne brassicae* (L.), *Myzus persicae* (Sulzer), *Sitobion avenae* F., *Rhopalosiphum padi* L., and *R. insertum* (Walker) (Müller *et al.* 2001). The ability to respond to crowding stimuli both pre- and post-natally is hypothesized to be of particular importance in species causing heavy deterioration of their host plants (Dixon and Glen 1971), such as that observed in my field experiment. In the pre-natal crowding laboratory

experiment, adult aphids from the low-density treatment were reared in complete

isolation from birth, and produced apterous progeny exclusively. It is possible, however,

that the production of 100% apterous progeny is less common in natural populations of *A.*

glycines, where development in isolation is unlikely to occur. The frequency of physical

contact between individual aphids is expected to be a function of their density, and two

adult aphids in close proximity are enough to elicit the production of alate progeny upon

physical contact in some aphid species (Johnson 1965; Dixon 1998). Nonetheless, my

post-natal crowding laboratory experiment indicates that densities lower than 3.4

nymphs/cm^2 are unlikely to induce wing development in *A. glycines*.

My results demonstrate that *A. glycines* adult body size decreases when

developing on heavily infested *G. max* plants. Adult body size is a good parameter for

estimating aphid performance, as it is often correlated with fecundity and offspring size

(Murdie 1969a). The reductions in body size observed in my field experiment are likely

attributable to a decrease in plant quality caused by previous feeding. Consistent with

other studies (Wu *et al.* 2004), I observed an important reduction in plant height as a

result of aphid infestations, indicating that infested plants were under significant stress.

One possible mechanism explaining the reduced performance of *A. glycines* on infested

plants is a potential change in amino acid concentrations. Although no chemical

measurements were taken in this experiment, a previous study showed that feeding by *A.*

glycines decreases the concentrations of aspartic acid and increases the concentrations of

isoleucine, tryptophan, and tyrosine in *G. max* plants (Chiozza *et al.* 2010). Dietary

deficiencies of some amino acids and low N concentrations can result in body size

reductions in aphids (Leckstein and Llewellyn 1973). Aspartic acid is an important

dietary component for *A. glycines*, while low concentrations of other amino acids such as isoleucine, tryptophan, and tyrosine in artificial diets appear to increase the performance of *A. glycines* (Wille and Hartman 2008). My study demonstrates that reductions in plant quality due to increasing aphid infestations result in smaller and potentially less fecund *A. glycines*, consistent with patterns of self-limitation population growth previously observed (Costamagna and Landis 2011).

I also saw a decrease in adult body size in aphids reared on the bottom nodes of *G. max* plants, compared to those reared on top nodes. *Aphis glycines* performance is positively correlated with *G. max* N content (Myers *et al.* 2005; Myers and Gratton 2006; Walter and DiFonzo 2007), which is highest in top nodes (Muchow *et al.* 1986; Shiraiwa and Sinclair 1993). Although the within-plant distribution of *A. glycines* changes throughout the season, most individuals in a colony are typically found feeding on the top nodes of *G. max* (Wu *et al.* 2004; McCornack *et al.* 2008; Costamagna and Landis 2011; Costamagna *et al.* 2013b). Thus, the within-plant spatial distribution of *A. glycines* likely constitutes a behavioral adaptation to within-plant quality variation, as is observed in other aphid species (Dixon 1976; Straw *et al.* 2006).

There was also a decrease in adult body size in aphids reared at high densities compared to those reared at low densities. This result is consistent with other aphid species (Murdie 1969b; Dixon 1998). *Acyrthosiphon pisum* nymphs reared at high densities develop into smaller apterous adults than those reared at low densities, and this effect has been attributed to disturbance and spatial restriction rather than leaf deterioration (Murdie 1969b). Because my results show an effect of crowding on body size independently of plant infestation level, I suspect that spatial restriction is a likely

explanation. In my post-natal crowding laboratory experiment, I observed a higher mortality in the high-density treatment than in the low-density one, as has been observed in other clip cage crowding experiments (Dixon and Glen 1971). If spatial restriction explains the reduction in body size observed, it could also be expected to increase mortality, as was observed in my results. Thus, crowding is shown to induce stress independently of plant deterioration.

My results support the hypothesis that crowding and plant quality act together as interacting cues for aphids to track environmental shifts, and that wing induction in *A. glycines* occurs as an adaptive response to both. Nonetheless, as shown in my laboratory experiments, *A. glycines* is adapted to produce alate individuals under crowded conditions, even in the absence of plant deterioration cues. This suggests that crowding provides a reliable cue for aphids to detect future changes in plant quality and/or spatial restriction. Moreover, my field experiment shows that wing induction can also occur in response to decreased plant quality in the absence of crowding. In the aphid *Aphis fabae* Scop, wing induction occurs as a result of the omission of certain amino acids from its diet (Leckstein and Llewellyn 1973). My results also suggest that crowding and decreased plant quality cues act in an additive way when simultaneously present, as wing induction in nymphs reared at high densities was nearly double on infested plants than on uninfested plants. Based on these results I hypothesize that the production of asexual alate individuals by *A. glycines* occurs at two distinct points in the interaction among aphids and their host plant. First, *A. glycines* aggregates on the top nodes of *G. max* plants and crowding results in a moderate production of alate individuals. Second, as plant quality deteriorates through feeding, the production of alate individuals increases

significantly both due to crowding and plant quality. My results suggest that the ability to detect multiple cues allows *A. glycines* to increase its production of asexual alate individuals gradually, which constitutes an important adaptation to environmental changes.

The production of alate morphs in parthenogenetic summer *A. glycines* populations is a key component of the biology of this species as an agricultural pest. In many parts of the North Central United States and Canada, *G. max* fields are not colonized before early July, in which cases, the immigration of asexual alate *A. glycines* is hypothesized to occur from nearby *G. max* fields (Ragsdale *et al.* 2004). Between-field migrations of *A. glycines* might affect plant-pathogen interactions in *G. max*, since *A. glycines* is a vector of plant viruses (Wu *et al.* 2004; Zhu *et al.* 2006). Additionally, the production of asexual alate individuals by *A. glycines* might affect the interactions with its natural enemies. Immigrations of asexual alate *A. glycines* individuals to *G. max* fields can interfere with biological control by its predators (Costamagna *et al.* 2013a). Taking this into account, heavy infestations of *A. glycines* in the field may result in a higher production of asexual alate individuals through crowding and host plant deterioration, directly increasing the probability of mass immigrations and virus transmission.

In conclusion, my work provides the first empirical evidence of the effects of crowding and plant quality cues on morph determination in *A. glycines*. My findings provide further evidence of the effect of interacting cues on morph determination in aphids. Finally, the production of asexual alate *A. glycines* individuals through large aphid infestations might prove detrimental for the management of this pest.

Table 1. Split-split plot ANOVA tables for *Aphis glycines* reared in clip cages set on infested vs uninfested *Glycine max* plants, on

bottom vs top nodes, and high vs low aphid densities, (a) adult apterous aphid size and (b) proportion of individuals developing wings.

Factor	(a) Aphid size			(b) Proportion of alate aphids		
	Df	*F*	*P*	Df	*F*	*P*
Between-plant (infested vs uninfested)	1, 17.6	11.22	**0.004**	1, 18	21.15	**<0.001**
Within-plant (bottom vs top)	1, 34.3	34.88	**<0.001**	1, 34.4	3.67	0.06
Within-plant x Between-plant	1, 34.3	0.07	0.79	1, 34.4	6.11	**0.02**
Clip cage density (high vs low)	1, 59.6	12.02	**0.001**	1, 65.4	35.54	**<0.001**
Between-plant x Density	1, 59.7	0.01	0.93	1, 65.4	0.26	0.61
Within-plant x Density	1, 59.6	0.16	0.69	1, 65.4	10.52	**0.002**
Between-plant x Within-plant x Density	1, 59.6	0.12	0.73	1, 65.4	3.59	0.06
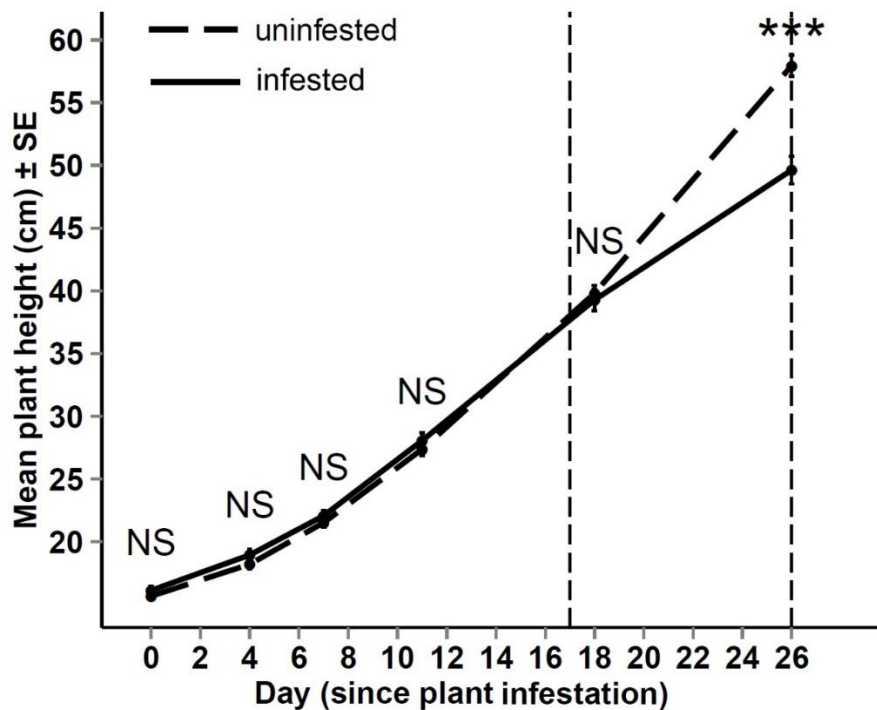
Figure 1. Changes in *Glycine max* plant height over time as a function of infestation with *Aphis glycines* in the field. Asterisks denote significance level of day-specific pre-planned contrasts: NS = not significant; *** $P < 0.001$. Dotted vertical lines indicate the period during which nymphs developed in the clip cages set on field plants.
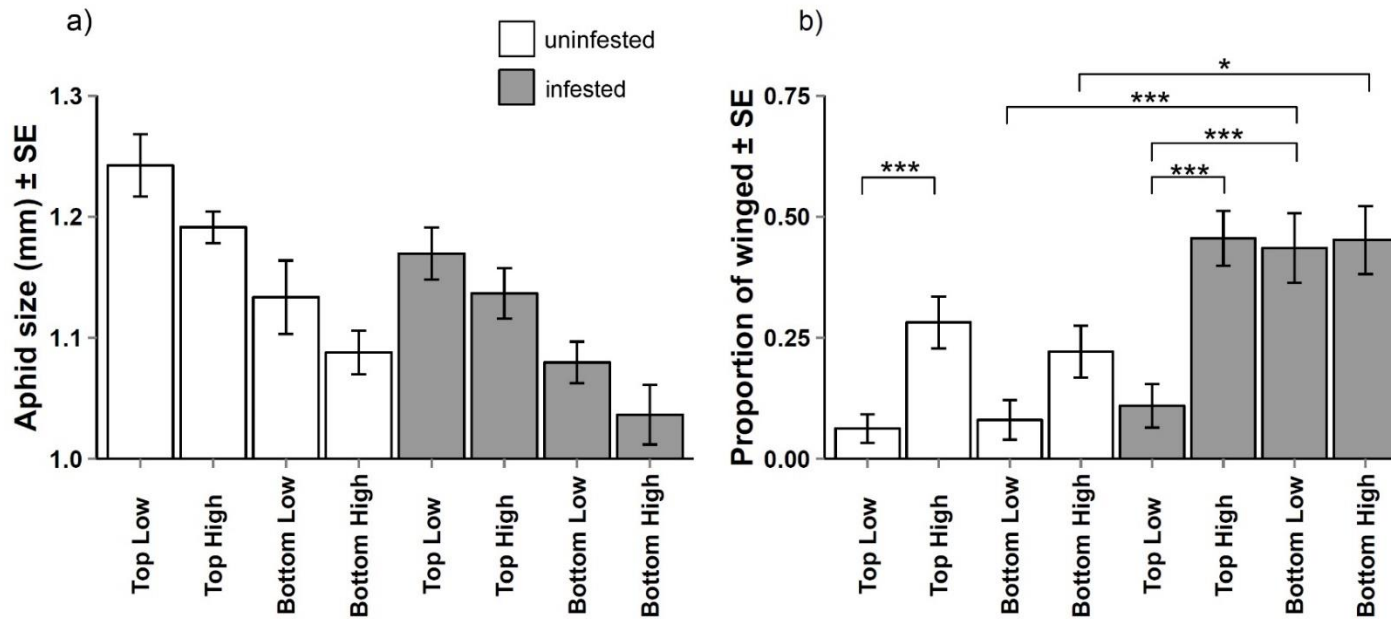
Figure 2. Effects of plant infestation (infested vs uninfested *Glycine max* plants), within-plant location (bottom vs top nodes), and clip cage density (crowding vs. non-crowding) on clip cage-reared *Aphis glycines* (a) apterous adult aphid size and (b) proportion of individuals developing wings. Only significant pairwise comparisons are shown. Asterisks denote significance level of day-specific contrasts after Holm-Bonferroni adjustment: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

CHAPTER 4: COLONIZATION OF PREDATOR-FREE SPACE BY ASEXUAL ALATE INDIVIDUALS OF THE SOYBEAN APHID, *APHIS GLYCINES* (HEMIPTERA: APHIDIDAE) AND ITS EFFECT ON POPULATIONS UNDER PREDATION

Abstract

Specialization in dispersal by alate aphids often imposes constraints on other functions, particularly a reduction in fecundity due to wing development. Short-distance flight from high population density patches to uninfested plants may provide temporary predator-free space, compensating for low fecundity. However, this theoretical prediction has not been explored experimentally. To test this hypothesis, I conducted a field cage experiment in which *Aphis glycines* Matsumura (Hemiptera: Aphididae) populations initiated with controlled proportions of apterous and alate individuals were exposed to predation, while predator-free space was accessible only through flight. I predicted that an investment in alate individuals would benefit a population under predation, regardless of associated costs to fecundity. As expected, I observed a strong trade-off between reproduction and wing development, reflected in a lower net fecundity in apterous versus alate individuals. However, populations initiated with a fixed proportion of alate and apterous individuals showed no reductions in final population size compared to populations initiated with apterous individuals exclusively. Moreover, the initial presence of alate individuals in the populations increased aphid prevalence (i.e. proportion of plants colonized). Similarly, I observed both increased population size and prevalence when predator-free space was accessible through flight, as opposed to when it was inaccessible. My results show that despite high costs to fecundity, an investment in alate individuals is neither beneficial nor

detrimental to population size when predator-free space is accessible, but increases aphid prevalence. I conclude that prevalence might provide an ecological advantage important enough to warrant the production of alate individuals under predation.

Introduction

Enemy-free space is defined as "ways of living that reduce or eliminate a species' vulnerability to one or more species of natural enemies" (Jeffries and Lawton 1984). Low population densities provide potential predator-free space, as patches with high prey densities are more easily detected by predators than low density ones, and predators often spend more time foraging in patches with higher prey densities (Kareiva and Odell 1987; Ives *et al.* 1993; Koch 2003). This temporary predator-free space is considered a dynamic refuge, since it is expected to disappear as population density increases (Berryman and Hawkins 2006). Species that produce morphs able to disperse by flight are hypothesized to benefit from exploiting this potential predator-free space (Weisser *et al.* 1999). This prediction, however, has not been tested empirically.

Most species of aphids reproduce parthenogenetically during summer, and can produce either apterous (wingless) or alate (winged) individuals (Müller *et al.* 2001). This wing polyphenism is an essential component of aphid life cycles, as different morphs perform different ecological roles (Blackman 1974). The level of allocation of resources to particular functions is likely to change the fitness of aphid clones (Dixon 1998). In addition to taking advantage of weather patterns and engaging in long-distance flights, alate aphids also perform short-distance flights (Blackman 1974; Taylor *et al.* 1979; Irwin *et al.* 1988; Dixon 1998). Dispersal between nearby host plants, referred to as "trivial flight" results in reproduction within the same habitat (Walters and Dixon 1983).

While flight clearly offers a benefit by enabling dispersal from dying host plants to more suitable ones, it has also been suggested to benefit aphids by allowing aphids to escape from predators (Blackman 1974). Moreover, exposure to predation results in an increased proportion of alate individuals in some aphid species (Weisser *et al.* 1999; Müller *et al.* 2001; Mondor *et al.* 2005). This suggests that predation might be an important force shaping aphid populations, and that flight from high-population density plants to uninfested plants within the same patch may increase the fitness of an aphid clone by reducing predation (i.e. a clone defined as a parthenogenetic line or genotype, Blackman, 1974).

Where refuges from predation are utilized by a species, however, trade-offs involving different elements of prey fitness are not uncommon (Lima 1998). Role specialization by alternative morphs often imposes constraints on other functions (Dixon 1985), and aphids, like other insects, often display reductions in fecundity as a consequence of wing development and flight (Walters and Dixon 1983; Rankin and Burchsted 1992; Zhang *et al.* 2009). Thus, flight constitutes an alternative strategy to immediate reproduction both physiologically and ecologically (Rankin and Burchsted 1992). Consequently, a successful clonal genotype is hypothesized to optimize its investment in morphs as a function of changes in host plant quality, high population densities, and/or the presence of predators (Dixon 1977).

Studies on aphid populations often focus solely on aphid abundance (average number of aphids per plant), while other population parameters such as prevalence (proportion of plants infested with aphids) have received less attention. Although traditionally used in parasitology research, prevalence is a concept applicable to other

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biological populations (Bush *et al.* 1997), and has been applied recently to gain insight into aphid ecology (Lamb and MacKay 2010). Focusing on prevalence may prove important to understand ecological characteristics of a population not necessarily reflected by abundance alone, such as population stability (Lamb and MacKay 2010). Moreover, a higher number of colonies (i.e. a colony defined as the aphids occupying a single plant or plant stem) might decrease the chance of local extinctions (Lamb and MacKay 2010). Thus, the production of alate individuals can be expected to increase the number of colonies and spread predation risk.

My species for this study is the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae). This species is an important economic pest in the North Central United States and Southern Canada (Ragsdale *et al.* 2011) and is responsible for yield losses of up to 45% on soybean plants, *Glycine max* (L.) Merrill (Ragsdale *et al.* 2007). The ability of coccinellids and other predators to suppress *A. glycines* populations in the field has been well documented (Costamagna and Landis 2006; Costamagna and Landis 2007; Gardiner *et al.* 2009; Costamagna and Landis 2011; Woltz *et al.* 2012).

The objectives of this study were 1) to determine the trade-offs associated with the production of asexual alate individuals, and 2) to investigate the effects that colonization of temporary predator-free space has on aphid population size and prevalence. I predicted that an investment in alate individuals would provide a net benefit to populations threatened by predation when predator-free space is available.

Materials and Methods

Field site

To determine the effects of colonization of predator-free space by asexual alate individuals on *A. glycines* populations under predation, I conducted an experiment in a *G. max* field located at the University of Manitoba, Winnipeg, Manitoba, Canada (N49.8136 W97.1208), from 22 July through 8 August 2014. *Glycine max* (OAC Prudence Soybean, Shanawan Farms Ltd., Domain, Manitoba, Canada) was seeded on the first week of June with a between-row spacing of 18 cm.

Insects

A colony of *A. glycines* from a population originally collected in Guelph, Ontario, Canada was maintained at the University of Manitoba under controlled laboratory conditions (25 ± 1 °C; 16L:8D photoperiod) for ca. 5 years before the experiments were conducted. Aphids in the colony were provided weekly with a plastic tray (5 cm height, 25.5 cm width, 50.8 cm length) of ca. 24 fresh VC – V1-stage *G. max* plants (first node under development, Ritchie *et al*. 1985) grown in a soil mix containing peat moss, perlite, nutrient charge, and limestone (Sunshine Professional Growing Mix #4, Sungro Horticulture, Agawam, MA, U.S.A.). *Glycine max* of the variety OAC Prudence was used for all rearing purposes and experiments.

To produce apterous aphids for the field cage experiment, groups of ca. 40 alate adults from the maintenance colonies were transferred with a paintbrush to V2-stage (second node under development, Ritchie *et al*. 1985) *G. max* plants grown in pots (15 cm diameter, 10.5 cm height) and were allowed to produce progeny for 24 hours before being removed. Alate adults were used because they are expected to produce few if any

alate progeny (Sutherland 1970; MacKay 1977). To produce alate aphids for the field

cage experiment, groups of ca. 70 adult apterous aphids were allowed to produce progeny

during 24 hours under the same conditions, as crowding induces wing development in *A.*

glycines (Chapter 3). Pots (15 cm diameter, 10.5 cm height) were covered by transparent

acrylic plastic cylinders (13 cm diameter, 20 cm height) with semi-translucent plastic

mesh on the top. Plants were watered every second day and kept under controlled

laboratory conditions (26 °C and 16L:8D photoperiod). Nymphs were allowed to develop

on the potted plants for five days to reach the fourth instar, and were then used to infest

field cages.

      Adult seven-spotted lady beetles (*Coccinella septempunctata* L.) (Coleoptera:

Coccinellidae) were used as predators in this experiment. Lady beetles were periodically

hand-collected from an alfalfa field located at the University of Manitoba's Glenlea

Research Station (N49.6475 W97.1308). All lady beetles used in my experiment were

starved for ca. 24 hours to elicit searching and feeding behavior inside the cages (Mondor

et al. 2005). Lady beetles were sexed based on abdominal apex morphology (Baungaard

1980) and sex ratios were standardized in all cages (2:0 and 2:1 female:male, when lady

beetles were introduced to cages in groups of two and three, respectively).

Cage design

To address the objectives of the study, three different cage types were used:

      1) Dispersal cage. This cage enclosed two separate arenas (hereafter referred to as

arena 1 and 2), and consisted of a 1m^3 polyethylene monofilament fiber mesh (0.34 mm^2

openings) cage supported by four steel poles running along the edges and anchored to the

ground (Figure 3). Internal separation of the cage into two arenas was achieved by a

physical barrier consisting of a combination of two mesh types. The first component of this barrier was a white no-see-um fabric mesh (0.24 mm^2 openings) square that divided the cage vertically into two halves. This square was sewn onto two parallel cage walls and extended vertically from the bottom of the cage (where it was buried at soil level) to ¾ of the way to the top of the cage. The function of this vertical barrier was to restrict the movement of apterous aphids between arenas. The second component of the barrier consisted of a commercial fiberglass mosquito mesh (1.2 x 1.4 mm openings), that extended as a ceiling covering arena 1. Preliminary experiments showed that this mosquito mesh allowed the dispersal of alate aphids to arena 2 by flight, while restricting all organisms to arena 1. Plastic zippers (75 cm length) were installed on the cage walls to allow the separate inspection of each arena.

2) The no-dispersal cage followed the same design as the dispersal cage, but the mosquito mesh component was substituted with no-see um fabric, restricting all organisms to arena 1 throughout the experiment (Figure 3).

3) The tomato cage consisted of a commercial tomato wire cage (41 cm diameter, 85 cm height) covered by a sleeve of white no-see-um mesh tied at the top of the cage and buried at soil level (Costamagna and Landis 2011). Tomato wire cages were anchored to the ground with four commercial plastic camping pegs passing through loops made at the bottom of the cage legs, and were further secured using a steel pole (1m length) tied to the top of the cage.

Dispersal and no-dispersal cages enclosed a row with five *G. max* plants in each arena, while tomato cages enclosed only two plants. Excess *G. max* plants and any weeds occurring inside the cages were removed by hand. Prior to setting up the cages, plants

were carefully inspected and any arthropods, including naturally occurring *A. glycines* were removed (< 10 aphids were found among all the inspected plants in the field). Pitfall traps were constructed with commercial plastic food containers (11 cm diameter, 7 cm height) and were buried at ground level in each arena 24 hours prior to the aphid infestations. All arthropods collected in the pitfalls were recorded and removed from the cages.

Experimental design

Six different treatments were used to test different hypotheses. These treatments differed from one another in the type of cage used, the combination of morphs used to initially infest the plants, and whether or not they were exposed to predation (Table 2). All insect manipulations occurred in arena 1, while arena 2 was only naturally colonized by alate aphids.

1) The dispersal/predation treatment was established using dispersal cages. In this treatment, arena 1 was initially infested with 10 fourth-instar apterous nymphs and 5 fourth-instar alatoid (undergoing wing development) nymphs per plant, which were exposed to predation by lady beetles. However, as in all treatments, arena 2 remained predator-free throughout the whole experiment. The dispersal/predation treatment was used to test the effect of predator-free space colonization by alate individuals in a population under predation.

2) The no-dispersal/predation treatment had the same initial conditions as the dispersal/predation treatment, but was set inside no-dispersal cages. This treatment acted as a control for predator-free space colonization by alate individuals.

3) The apterous/predation treatment was established inside dispersal cages and arena 1 was initially infested with 15 fourth-instar apterous nymphs per plant. Therefore, in this treatment, the predator-free space (i.e. arena 2) was naturally inaccessible to the first generation of aphids infesting the plants, but was accessible to alate individuals from later generations, which were naturally produced. This treatment allowed me to estimate an initial trade-off occurring in the first generation of aphids between increased apterous fecundity and the inability to colonize predator-free space.

4) The dispersal/no-predation treatment had the same initial conditions as the dispersal/predation treatment, but was never exposed to predation. This treatment was used to determine the negative effects of predation in the dispersal/predation treatment.

5) and 6) The alate and apterous controls were both established inside tomato cages. These two treatments were used to compare the increase over time of two populations initiated with alternate morphs. The alate control was infested with 15 fourth-instar alatoid nymphs per plant, while the apterous control was infested with 15 fourth-instar apterous nymphs per plant. Neither the alate control nor the apterous control were exposed to predation.

The field cage experiment followed a randomized block design with eight blocks across the field plot. Blocks were established to control for potential differences due to observers, but no other apparent gradient was observed in the field. Enclosed plants had a similar size and showed minimal to no previous damage from herbivory.

On day 0 of the experiment, each plant inside arena 1 of the dispersal and no-dispersal cages, and inside each tomato cage, was manually infested with fourth-instar aphids using a fine paintbrush (Table 2). Infestation of arena 2 was solely contingent

upon successful dispersal of alate individuals from arena 1. The fourth-instar aphids were allowed to settle on the plants for three days and develop to maturity before being exposed to predation in the dispersal/predation, the no-dispersal, and the apterous/predation treatments. On days 3 and 5 of the experiment, two adult lady beetles were introduced to arena 1 for 24 hours before being removed. Since aphid counts did not reveal a strong predation effect on population sizes, I increased the number of predators introduced and the duration of exposure to predation. This was achieved by introducing three lady beetles in arena 1 and removing them after three days, in a second wave of predation (day 7 - 9). This increase in predation pressure mimicked natural predator responses due to larger aphid populations (Ives *et al.* 1993). The predation schedule (no-predation - mild predation - intense predation) was repeated on day 10 to fit two predation cycles. To avoid undesired density-dependent effects, however, the experiment was finalized before the second wave of intense predation was completed, as aphid populations had reached large sizes. Cages were inspected daily and any lady beetle eggs found inside the cages were destroyed. Aphid counts were performed regularly (days 3, 7, 10, 14, and 17) in all cages and arenas.

During aphid counts, alate individuals were collected with aspirators and placed back on the plants before closing the cages to prevent them from escaping. Since population sizes reached very high numbers by day 14 and 17 of the experiment, a subsampling method was implemented for estimation on those two sampling dates. In this method, the number of aphids on one of the leaflets from each trifoliate leaf was counted, and this number was multiplied by three to estimate the number of aphids on each particular trifoliate leaf. Individual trifoliate leaves were identified by their position

within particular nodes, and the number of aphids on each trifoliate leaf was calculated independently. Similar methods have been successfully used to estimate *A. glycines* densities (McCornack *et al.* 2008; Costamagna *et al.* 2010; Meihls *et al.* 2010). Aphid prevalence was calculated as the proportion of infested plants (Lamb and MacKay 2010) inside a cage, including both arenas in the case of dispersal and no-dispersal cages. Even though the plants inside arena 2 of the no-dispersal cages were not available to aphids by design, these plants were considered in the calculations of prevalence to confirm the expected differences between the dispersal/predation and the no-dispersal/predation treatments resulting from colonization of arena 2. The proportion of adult alate individuals in each cage was also recorded in all sampling dates to track morph composition over time. This measurement did not consider immature aphids undergoing wing development, due to difficulties associated with careful aphid inspection in the field.

Laboratory assay

To compare the fecundity of apterous versus alate individuals under more controlled conditions, a laboratory assay was conducted at the University of Manitoba, Winnipeg, Manitoba, Canada, from September 06 through October 07, 2014. This experiment was conducted using clip cages on potted plants, and to achieve a broader level of inference, two different *A. glycines* populations were used. The first *A. glycines* population was the same one used in the field experiment (originally collected in Guelph) and the second one was originally collected in Emerson, Manitoba, Canada in 2014. Colonies of both populations were established and routinely maintained in the laboratory as previously described.

Clip cages were constructed using segments of transparent PVC tube (1 cm length) mechanically bound together by stainless steel duck-bill hair-clips. Rings of foam were glued to the tube segments to reduce leaf surface damage due to pressure upon closing (Murdie 1969b). Each clip cage enclosed a 1.5 cm diameter leaf area and was covered by semi-translucent fine plastic mesh, which was glued on with commercial cyanoacrylate to confine the insects (Krazy Glue, Elmers Products, High Point, North Carolina, U.S.A.). To standardize the age of *A. glycines* individuals within a single cohort, apterous adults from the colonies were haphazardly selected and transferred to individual potted V1-stage *G. max* plants, where they were allowed to produce progeny for 24 hours before being removed. Nymphs were allowed to develop under controlled conditions during five - six days to reach the fourth-instar, and were then haphazardly selected and isolated inside clip cages mounted on individual potted VC –V1-stage *G. max* plants. To minimize potential differences in nutritional quality among individual plants, pairs of clip cages enclosing each morph type were set on each individual potted plant. Thus, each potted plant contained one replicate for each treatment. Potted plants were kept under controlled laboratory conditions. The experiment consisted of four treatments in a full factorial design involving morph (apterous vs alate) and colony (Emerson vs Guelph). The experiment included 20 replicates for each treatment combination, which were run simultaneously. Clip cages were checked daily and the number of offspring produced by each aphid was recorded before being removed with a fine paintbrush. The experiment ran until the last adult aphid died, for a total duration of 32 days. Aphids were transferred weekly to new VC - V1-stage *G. max* plants to maintain

a constant host plant nutritional quality throughout the experiment. Total fecundity was determined for each aphid as cumulative offspring production.

Data analysis

All analyses were performed using R version 3.1.2 (R Core Team 2014). Population size data from the field cage experiment were tenth root-transformed (McCune *et al.* 2002) to achieve normality of the residuals and equality of variance between and within-subjects, and were analyzed using multivariate repeated measures ANOVA models (O'Brien and Kaiser 1985; Scheiner 1998) performed with the "Car" package of R (Fox *et al.* 2015). In this type of analysis, data from each day are treated as separate dependent variables to circumvent the assumption of sphericity (Quinn and Keough 2002), which is seldom met in natural systems. The independent variables in this model were treatment, block, day and their two-way interactions, with the exception of a treatment x block interaction, which was not tested due to lack of treatment replicates at the within-block scale. Three separate models were used to analyze aphid population size, one for the treatments in cages with two arenas (treatments 1 - 4), a second one for those same treatments, but including observations only from arena 1, and a third one to compare the control treatments in tomato cages (alate and apterous controls). Data on the proportion of alate adults in the cages were arcsine square root-transformed and analyzed as previously described. Aphid prevalence data from the dispersal and no-dispersal cages were square-root transformed and analyzed as previously described. Pre-planned contrasts were performed in all models to examine day-specific differences among treatments, using the "Phia" package in R (De Rosario-Martinez 2013).

Per-capita rates of increase (r) were calculated to examine aphid population growth in each treatment, using the formula $r = [\ln(N_t) – \ln(N_0)]/t$, where N_0 = initial number of aphids, N_t = number of aphids at time t, and t = duration of the experiment. Day 3 of the experiment was used as the initial period, and day 17 as the final period. Per-capita rates of increase were analyzed using a two-way ANOVA with treatment and block as the independent variables. Tukey's HSD was used to explore differences among treatments.

Fecundity data from the laboratory experiment were $\log(x + 1)$-transformed and analyzed with a fully crossed two factor ANOVA with morph, colony, and their interaction as the independent variables, followed by Tukey's HSD. Aphids that died within the first week of the experiment were excluded from the analysis to avoid spurious effects of artificial mortality by manipulation.

Results

Manipulations of predation, access to predator-free space, and initial morph composition resulted in significant changes in aphid population size and prevalence during the experiment (Table 3). Some of these effects changed over the span of the experiment, resulting in significant treatment x day interactions (Table 3). This was most likely due to the cyclic predation schedule and the production of alate individuals in the apterous/predation treatment. I also observed some significant block x day interactions (Tables 3 and 4), suggesting that changes in aphid population size and prevalence over time were not equal between blocks. Graphical exploration of the data suggests that this was most likely due to variability between replicate-specific trajectories across day-specific data, and did not affect the overall response to the treatments.

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Predation resulted in strong reductions in population size on the last four sampling days of the experiment (Table 3, Figure 4a, 4c, and 4e). By day 17, population sizes in the absence of predation were ca. 13 times higher than those under predation (i.e. dispersal/predation treatment). Similarly, I observed a strong negative effect of predation on aphid prevalence by day 14 (Table 3, Figure 4b, 4d, and 4f). Without predation, all plants available inside the cages were colonized by day 17 (Figure 4b), which was not the case in the dispersal/predation treatment, where prevalence was 19% lower. Thus, predation had a notable negative effect on aphid population size and prevalence.

Under predation, colonization of predator-free space by alate individuals resulted in an increase in population size when contrasted with the no-dispersal treatment (Table 3, Figure 4c). By day 17, population size when predator-free space was accessible was double that without predator-free space. Additionally, aphid prevalence was 31% higher in the treatment with access to predator-free space, indicating that alate individuals successfully colonized arena 2 in the cages (Table 3, Figure 4d). Several lines of evidence indicate that the increase in population size in the dispersal/predation treatment resulted from the colonization of predator-free space by alate individuals. First, contamination of arena 2 with aphids due to manipulation during counts or cage malfunction is unlikely to have occurred, as careful inspections of arena 2 in the no-dispersal treatment yielded zero values on all dates (data not shown). Furthermore, a follow-up repeated measures multivariate ANOVA comparing the number of aphids solely in arena 1 between the dispersal/predation and the no-dispersal/predation treatments showed no differences for any date, as determined by day-specific pre-planned contrasts (Table 3, $P > 0.05$ for all contrasts).

Populations initiated with 1/3 alate individuals (dispersal/predation treatment) showed only a temporary, short term negative effect on population size when compared to the treatment initiated exclusively with apterous individuals (Table 3, Figure 4e). Colonization of arena 2 in the dispersal/predation treatment occurred by day 7, most likely as a result of trivial flights of the initial alate individuals used for cage infestation (colonization data not shown). By contrast, in the apterous/predation treatment, no aphids were observed in arena 2 until day 10. Colonization of arena 2 in the apterous/predation treatment was attributed to the trivial flights performed by the alate progeny of the initial apterous generation. Thus, in the dispersal/predation treatment, predator-free space colonization occurred one generation earlier than in the apterous/predation treatment. Despite this earlier utilization of predator-free space, population size in the dispersal/predation treatment was lower than in the apterous/predation treatment during days 7 and 10. However, the differences in population size between these treatments were diluted by day 14 (Figure 4e). By contrast, having earlier access to predator-free space due to alate individuals resulted in a higher final aphid prevalence than in the apterous/predation treatment (Table 3, Figure 4f). Thus, populations initiated with alate and apterous individuals showed no reductions in final population size compared to populations initiated only with apterous individuals, but showed a notable increase in prevalence.

Differences in the proportion of alate adults between the dispersal/predation and apterous/predation treatments occurred only at the beginning of the experiment, as a product of initial morph composition being a treatment component (Table 3, Figure 5c). However, by day 7, this difference disappeared as nymph production occurred naturally

and the proportion of alate adults relative to that of immature aphids decreased in both treatments to equally low treatments. The dispersal/predation treatment, the dispersal/no-predation treatment, and the no-dispersal/predation treatment were initiated with the same morph composition, and their proportion of alate adults did not differ on any date, with the exception of a higher proportion of alate adults in the dispersal/no-predation treatment by day 17 (Figure 5a).

The control treatments established in tomato cages showed a strong effect of initial morph composition on population size over time (Table 4, Figure 4g). By the end of the experiment, populations started exclusively with apterous individuals (apterous control) showed population sizes three times larger than those initiated exclusively with alate individuals (alate control). Because populations of the two control treatments were started with completely opposite morph compositions, differences in their proportions of adult alate individuals were initially very pronounced and were not diluted until day 17 (Table 4, Figure 5d).

Per-capita rates of increase were up to six times higher in treatments without predation than in those with predation (F $_{5,35}$ = 45.21, P < 0.001, Figure 6). Per-capita rates of increase did not differ among those treatments exposed to predation, although there appeared to be a non-significant trend toward a lower rate of increase when predator-free space was not accessible (no-dispersal/predation treatment, Figure 6). Surprisingly, per-capita rates of increase did not differ between colonies initiated exclusively with alate individuals and those initiated exclusively with apterous individuals, despite the notable differences in population sizes throughout the experiment.

Results from the laboratory clip cage assay confirmed the results from the field cage experiment, with a 30% lower net fecundity in alate than in apterous individuals ($F_{1,55} = 31.87$, $P < 0.001$, Figure 7). This result was consistent across the two *A. glycines* populations sampled ($F_{1,55} = 2.06$, $P = 0.16$), and there was no significant morph x population interaction ($F_{1,55} = 0.546$, $P = 0.46$).

Discussion

Aphid polyphenism provides a useful system to test the potential benefits of predator-free space in the presence of a reproductive trade-off. To my knowledge, this is the first empirical test of this trade-off in an aphid population under predation. My results show that when accessible, predator-free space is likely to increase both the size and prevalence of an *A. glycines* population under predation. Moreover, my results suggest that despite a strong reproductive trade-off associated with wing development, the ability of alate individuals to colonize predator-free space can increase aphid prevalence while maintaining population size.

Although the negative effects of predation on population size and prevalence observed in my field experiment were not surprising, predation pressure was a condition necessary to ascertain the benefits of access to predator-free space. While the numbers of predators introduced in each cage were arbitrary and their confinement within a cage somewhat artificial, the ability of coccinellids and other predators to suppress *A. glycines* populations has been well documented under natural field conditions (Costamagna and Landis 2006; Costamagna and Landis 2007; Costamagna *et al.* 2007; Costamagna and Landis 2011). Moreover, per-capita rates of increase observed in my cages are within the range previously reported in field studies (Costamagna and Landis 2011; Costamagna *et*

al. 2013a). The short duration of my field experiment, designed to accommodate two predation cycles, mimics a short temporary persistence of predator-free space, since predation increases as a function of prey density (Ives *et al.* 1993). Thus, colonies developing in predator-free space would become more susceptible to predation after reaching a certain size.

Fecundity constraints associated with wing development have been well identified in other aphid species (Mackay and Wellington 1975; Campbell and Mackauer 1977; Wratten 1977). In accordance with this, my laboratory experiment revealed considerably lower reproductive output in alate than in apterous individuals. This difference was consistent across two populations of *A. glycines* from different geographic regions, and demonstrates an important trade-off between wing development and fecundity in this species. Additionally, I observed large differences in population size between the control treatment initiated exclusively with alate individuals versus the one initiated exclusively with apterous individuals in my field cage experiment. I attribute this result to a difference in initial fecundity between the two populations as a consequence of morph composition, as their per-capita rates of increase did not differ.

The primary objective of my field cage experiment was to investigate the effects of a partial investment in alate morphs, as opposed to a full investment in apterous morphs, in an *A. glycines* population under predation. This condition was met in my field cage experiment, since differences in morph composition between these two treatments were present only at the outset of our trials and later converged to similar proportions of alate adults. In populations initiated with a portion of alate individuals, the potential benefits of predator-free space colonization were available one generation earlier than in

apterous populations, at a cost to immediate fecundity. However, the fact that the

differences in population size between these two treatments disappeared by day 14,

suggest that this cost to immediate fecundity resulting from an investment in alate

individuals can be later compensated for by the benefits of predator-free space

colonization. Additionally, it has been documented that alate aphids seldom produce alate

offspring (Sutherland 1970; MacKay 1977), which may represent an important

physiological adaptation to compensate for fecundity costs.

In species with polyphenism, no phenotype is expected to provide a higher fitness

in all circumstances. Instead, trade-offs involving the relative fitness contributions by

different phenotypes are expected to depend on particular environmental conditions

(Dixon 1985; Braendle *et al.* 2006). Under predation, an initial investment in alate

individuals did not prove beneficial to the population in terms of its size through the

duration of my experiment, however, it did not appear to be detrimental either.

Nonetheless, because the second arena inside the cage was accessible to alate individuals

over a short distance, my experiment may have underestimated the energetic costs of

flight on fecundity associated with longer flying distances in this species (Zhang *et al.*

2009). However, the number of alate individuals able to colonize predator-free space

might be higher in a natural system lacking a physical barrier like the one imposed by

my cages.

The production of alate morphs in other aphid species has been hypothesized to

benefit a population by counteracting density-dependent effects (Kidd 1990).

Additionally, evidence of crowding and changes in host plant quality inducing wing

development have been well documented (Müller *et al.* 2001). In my field cage

experiment, I observed a large increase in the proportion of alate adults in the dispersal/no-predation treatment by day 17. I suspect that this might have been caused by crowding and possible decreases in plant quality (Chapter 3), since the population densities in this treatment had become quite high by then.

Given the costs of wing development and flight to fecundity, dispersal by flight is hypothesized to occur only under circumstances in which its absence would prove detrimental (Rankin and Burchsted 1992). My results suggest that investment in alate individuals is not likely to increase, but it would also not decrease aphid population size under predation, when predator-free space is available. Moreover, this investment will result in higher aphid prevalence if successful colonization of predator-free space is achieved. Although my cage design artificially controls the number of plants available for aphids and might not recreate prevalence in a natural scenario, it allows for a relative comparison of resource colonization across my different treatments. Regional persistence of a species relies critically on colonization when its populations are subject to local extinction (Harrison 1991). Thus, increased aphid prevalence may result in enhanced persistence of a population. For the aphid *Uroleucon rudbeckiae* (Fitch), a higher number of colonies (i.e. higher number of plants colonized by aphids) might decrease the chance of local extinctions (Lamb and MacKay 2010). High dispersal can cause patches to become united into a more uniform population with a reduced probability for local extinction, potentially facilitating the coexistence of a prey species with its predators (Harrison 1991).

In summary, my results provide the first empirical evidence suggesting a compensation for immediate fecundity costs by the colonization of predator-free space in

alate aphids. My results suggest that prevalence might provide an ecological advantage important enough to explain the production of alate individuals in aphid populations under predation. Moreover, the lack of a long-term cost to population size involved in the production of alate individuals under predation may serve as a key factor promoting selection for wing development among aphid clones.

Table 2. Summary of treatments used in a field cage experiment to determine the effects of predator-free space colonization by alate individuals on *Aphis glycines* population size and prevalence under predation in a *Glycine max* field. See chapter 4 for a detailed description of the experiment).

Treatment name	Initial nymphs per plant		Predation	Access to predator-free space	Cage type
	apterous	alatoid			
1: Dispersal/predation	10	5	YES	YES	Dispersal cage
2: No-dispersal/predation	10	5	YES	NO	No-dispersal cage
3: Apterous/predation	15	0	YES	YES	Dispersal cage
4: Dispersal/no-predation	10	5	NO	YES	Dispersal cage
5: Alate control	0	15	NO	-	Tomato cage
6: Apterous control	15	0	NO	-	Tomato cage

Table 3. Multivariate repeated measures ANOVAs on the effects of manipulations of predation, access to predator-free space, and initial morph composition on *Aphis glycines* population size, prevalence and morph composition over time under predation in a *Glycine max* field. Pillai's trace statistics are reported. See chapter 4 for a detailed description of the experiment.

	Df	Population size (whole cage)		Aphid prevalence (whole cage)		Proportion of alate adults (whole cage)		Total aphids in arena 1	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Treatment	3, 21	54.08	**<0.001**	15.70	**<0.001**	11.80	**<0.001**	48.03	**<0.001**
Block	7, 21	0.89	0.53	0.83	0.57	1.11	0.39	0.64	0.72
Day	4, 18	18.68	**<0.001**	13.84	**<0.001**	3.65	**0.03**	15.40	**<0.001**
Treatment X Day	12, 60	3.64	**<0.001**	3.43	**<0.001**	3.29	**0.001**	2.79	**0.004**
Block X Day	28, 84	4.03	**<0.001**	2.24	**<0.001**	0.98	0.51	4.5	**<0.001**

Table 4. Multivariate repeated measures ANOVAs on the effects of manipulations of initial morph composition on *A. glycines* population size and morph composition over time on caged *Glycine max* field plants. Pillai's trace statistics are reported. See chapter 4 for a detailed description of the experiment.

	Df	Population size		Proportion of alate adults	
		F	*P*	*F*	*P*
Treatment	1, 7	43.9	**<0.001**	138.06	**<0.001**
Block	7, 7	0.90	0.56	2.03	0.19
Day	4, 4	35.16	**0.02**	11.59	**0.02**
Treatment X Day	4, 4	0.58	0.69	19.55	**0.01**
Block X Day	28, 28	2.12	**0.03**	1.16	0.36
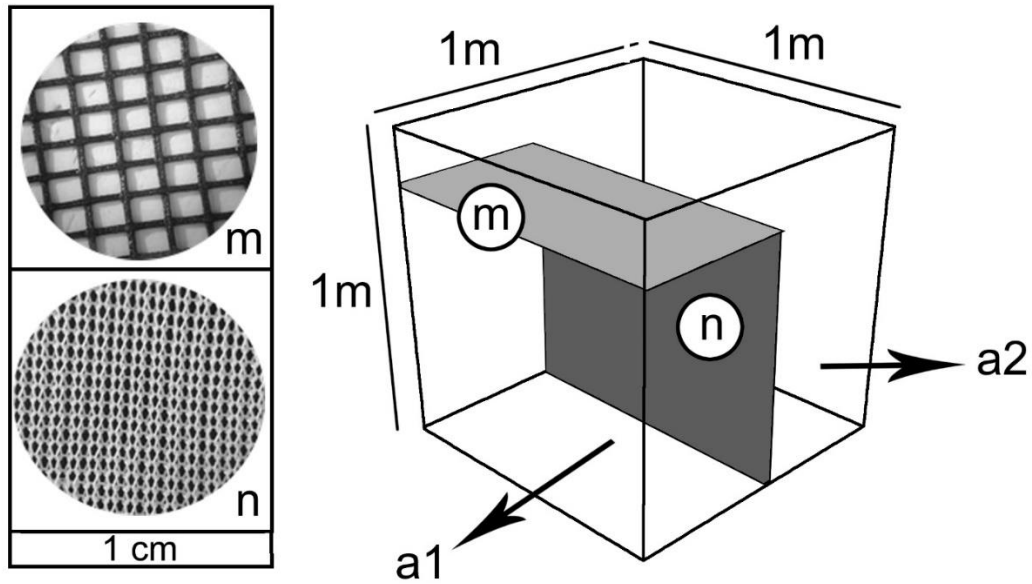
Figure 3. Dispersal cage design. Cage is divided into two arenas (a1 and a2) by a barrier component made of mosquito mesh (m, 1.2 x 1.4 mm openings) and no-see-um mesh (n, 0.24 mm^2 openings). Arena 1 (a1) was used to release different combinations of apterous and alate individuals and predators. Arena 2 (a2) was maintained as a predator-free space accessible only by alate individuals. Mosquito mesh was substituted with no-see-um mesh in the no-dispersal cage design.
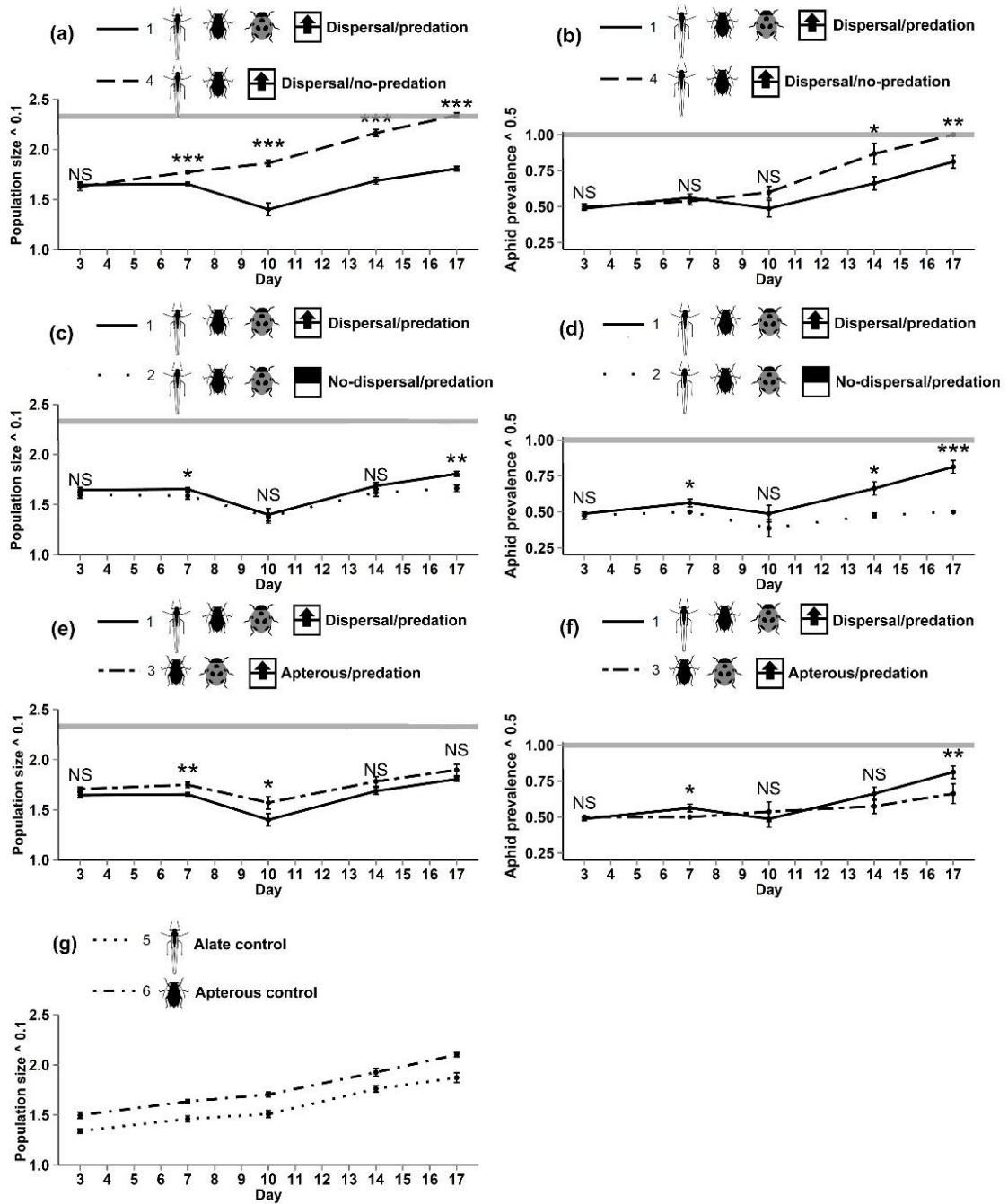
Figure 4. *Aphis glycines* population size (tenth-root transformed, mean ± SE), and

prevalence (proportion of plants colonized per cage, square-root transformed mean ± SE).

To better illustrate pre-planned contrast results, the dispersal/predation treatment is

compared to manipulations of each separate factor. Effect of predation on (a) population

size and (b) aphid prevalence. Effect of preventing dispersal on (c) population size and

(d) aphid prevalence. Effect of initial morph composition on (e) population size and

(f) aphid prevalence; Effect of initial morph composition in control treatments on

(g) population size. Solid horizontal lines depict maximum population size and

prevalence reached without predation. Asterisks denote significance level of day-specific

pre-planned contrasts: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. See chapter 4 for a

detailed description of the experiment.
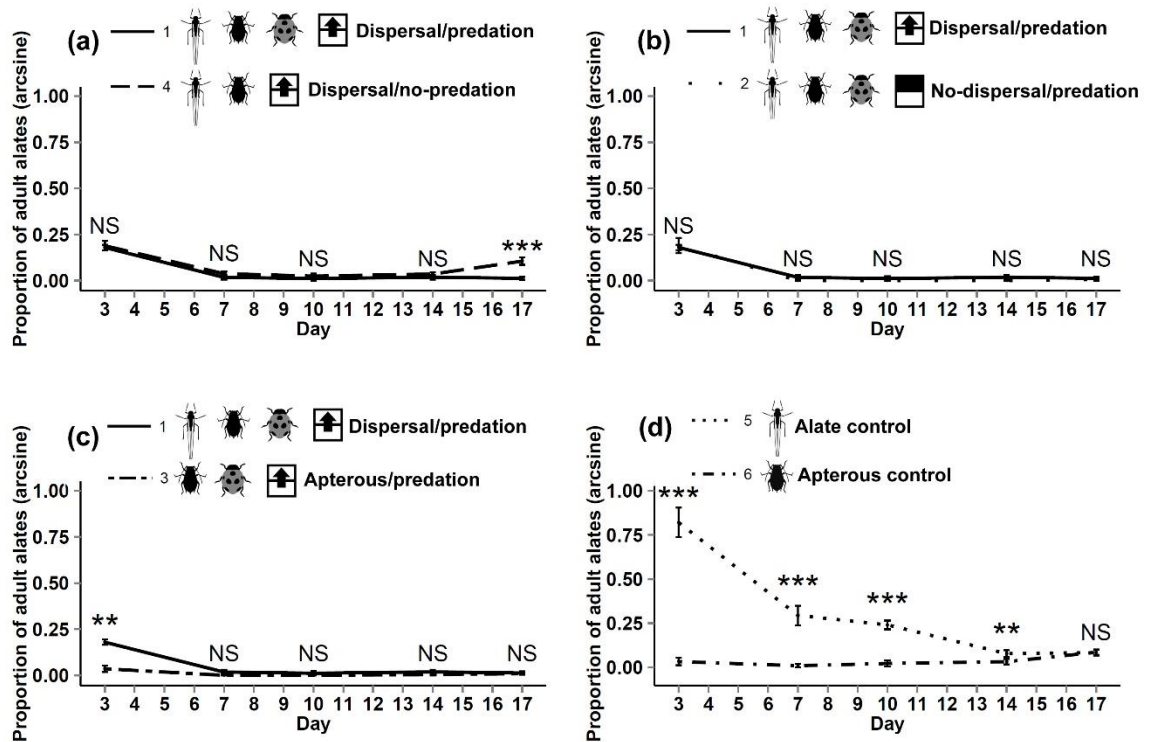
Figure 5. Proportion of alate *Aphis glycines* adults (arcsine transformed, mean ± SE) in the dispersal/predation treatment versus (a) the dispersal/no-predation treatment, (b) the no-dispersal/predation treatment, and (c) the apterous/predation treatment. (d) Proportion of alate adults in the alate versus the apterous controls; Asterisks denote significance level of day-specific pre-planned contrasts: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. See chapter 4 for a detailed description of the experiment.
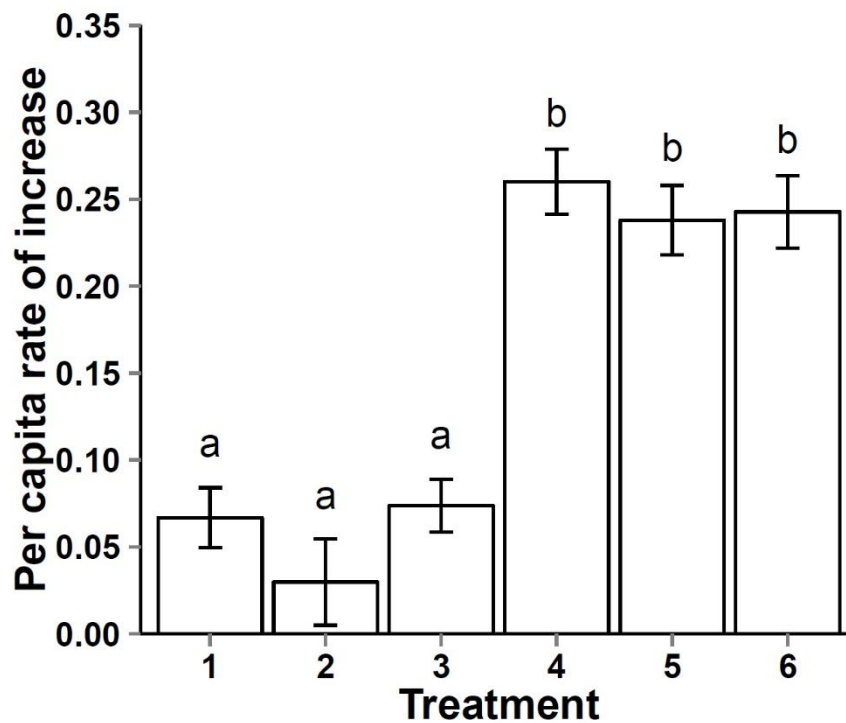
Figure 6. *Aphis glycines* per-capita rates of population increase as a function of

manipulations of predation, access to predator-free space, and initial morph composition

in a *Glycine max* field. 1) Dispersal/predation; 2) No dispersal/predation;

3) Apterous/predation; 4) Dispersal/no-predation; 5) Alate control; 6) Apterous control.

See chapter 4 for a detailed description of the experiment.
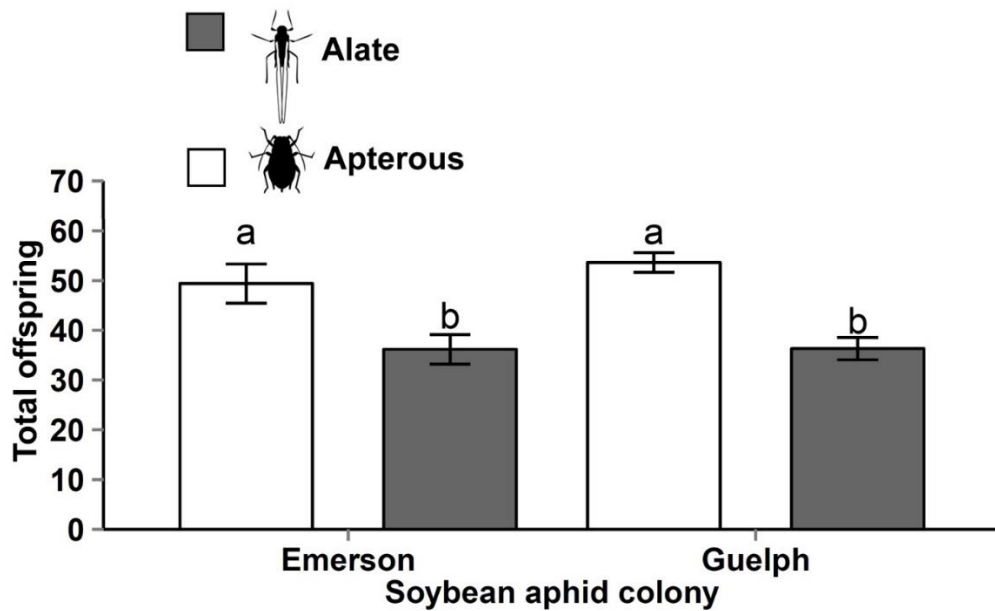
Figure 7. Net fecundity (total offspring produced ± SE) of apterous versus alate *Aphis glycines* adults on potted *Glycine max* plants under controlled laboratory conditions. An *Aphis glycines* laboratory-maintained colony originally collected in Emerson, Manitoba, Canada (2014) is compared to another collected in Guelph, Ontario, Canada (2009).

CHAPTER 5: GENERAL DISCUSSION

Aphids show numerous physiological, morphological and behavioural traits which are often plastic, and allow them to maximize their fitness as a function of their environment (reviewed in Chapter 2). Among these adaptations, polyphenism (the environmentally-induced production of different phenotypes by one genotype) is one of the most characteristic aphid traits (Dixon 1998; Simpson *et al.* 2011). The aim of this thesis is to gain a better understanding of the production of asexual alate individuals in *Aphis glycines* Matsumura (Hemiptera: Aphididae). My first objective was to investigate the factors causing the production of asexual alate individuals in this species. For my second objective, I examined the effects of the production of asexual alate individuals by an *A. glycines* population under predation.

Though there is a relatively large body of research investigating the role of environmental cues on the production of asexual alate individuals in aphids (Chapter 2), a general pattern has not been identified, and the importance of different cues is species-specific (Müller *et al.* 2001). The cues involved in the production of asexual alate individuals in *A. glycines* had not been previously studied. In Chapter 3, I examined the effects of two of the most widely studied environmental cues inducing the production of asexual alate individuals in aphids: crowding and low host plant quality. These two cues often occur simultaneously and have interacting effects on the overall production of asexual alate individuals. For example, crowding and host plant quality interact to influence the production of alate individuals in the aphids *Acyrthosiphon pisum* (Harris), *Drepanosiphum dixoni* H.R.L., and *Myzus persicae* (Sulzer) (Sutherland 1967; Sutherland and Mittler 1971; Dixon 1972). The experimental design of the field cage study on the effects of crowding and plant quality on wing induction (Chapter 3) is novel

in that it allows the disentanglement of the effects of crowding, within-plant quality, and between-plant quality on morph determination in a factorial design. My results provide the first empirical evidence that *A. glycines* increases its production of asexual alate individuals in response to crowding and decreased plant quality, and that these cues can act both independently and in conjunction with each other. The interaction between these cues allows *A. glycines* to increase its production of asexual alate individuals in a gradual manner, which likely constitutes an adaptation to changes in the environment. My findings on the effects crowding are confirmed by results in the laboratory and field experiments.

The occurrence of negative density-dependent population effects in aphids is well documented (e.g. Agrawal et al. 2004; Donaldson et al. 2007). In aphids and other clonal populations, dispersal is considered a means to mitigate negative density-dependent population effects by reducing competition and predation risk (Dixon 1977; Plantegenest and Kindlmann 1999). Though this assumption is reasonably well accepted, the benefits of producing asexual alate individuals under predation has not been rigorously studied. In Chapter 4 of this thesis, I provide experimental evidence that dispersal to predator-free space by asexual alate individuals proves beneficial to an aphid population under predation. These results can potentially be applied to other biological populations with spatially-heterogeneous distributions. Colonization and re-colonization of resources plays a paramount role in the resistance of aphids to local extinction (Harrison 1991; Zheng *et al.* 2009). Within this context, recent work by Lamb and MacKay (2010) has successfully integrated prevalence and intensity to aphid ecology, concepts which are traditionally used in parasitology but apply to other organisms (Bush *et al.* 1997). In this thesis, I

adopt this approach to aphid ecology and consider prevalence as a metric of dispersal on an aphid population. Results from the field experiment on aphid populations under predation (Chapter 4) suggest that prevalence might be important enough to justify temporary declines in fecundity, since it may facilitate coexistence with natural enemies (Harrison 1991; Lamb and MacKay 2010).

In addition to contributing to a growing body of knowledge on aphid polyphenism, trade-off theory, and population dynamics of aphids, this thesis provides insight into the biology of *A. glycines*, which remains an important invasive pest in North America. The results presented in this thesis suggest that asexual alate individuals play an important role in host plant colonization by *A. glycines*, and possibly influence its interactions with natural enemies. The production of asexual alate individuals by *A. glycines* can have important effects on the management of this pest in agricultural systems. In addition to increasing prevalence, mass immigrations of alate individuals to a *G. max* field have been observed to disrupt biological control by natural enemies (Costamagna *et al.* 2013a). Additionally, dispersal by asexual alate individuals can increase plant disease transmission, since *A. glycines* is a vector of plant viruses (Gildow 1980; Wu *et al.* 2004; Zhu *et al.* 2006).

In conclusion, this thesis provides a contribution to the understanding of aphid population dynamics in regard to dispersal by flight, and describes an important component of the biology of *A. glycines*. The results presented in this thesis will prove useful in future studies on evolutionary ecology and pest management.

Future research

An outstanding question is whether *A. glycines* increases its production of asexual alate individuals in response to the presence of natural enemies, as occurs in other aphid species (Dixon and Agarwala 1999; Weisser *et al.* 1999; Müller *et al.* 2001; Mondor *et al.* 2005). If dispersal represents an advantage important enough for the survival of a clone under predation, it is possible that selective pressures have resulted in the production of asexual alate individuals by *A. glycines* in response to the presence of predators. My results show that the production of asexual alate individuals in *A. glycines* can occur in response to multiple cues, in a gradual manner. It is possible that the presence of natural enemies further increases the proportion of asexual alate individuals in conjunction with other cues in this species. Nonetheless, despite the evident relationship between the production of asexual alate individuals and predation, future research is needed to address this question.

Though prevalence is not a common concept in aphid ecology, previous research indicates that it is a useful parameter for the description of aphid populations (Lamb and MacKay 2010). Understanding the consequences of dispersal might be particularly important in agricultural pests, and prevalence proves useful as a measure of that. My results suggest that prevalence should continue to be considered in aphid ecology studies to provide a better understanding of its relationship with predation.

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