Host acceptance behavior in the soybean aphid parasitoid *Binodoxys communis* (Hymenoptera: Braconidae) – The role of physiological state in biological control

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Introduction

Host choice behavior in insect parasitoids and herbivores has been predicted to vary dynamically in response to external and internal factors acting on a foraging female. Factors that have been identified to influence host choice behavior in females include physiological state (Odendaal & Rausher, 1990; Minkenberg et al., 1992; Heimpel & Collier, 1996; Mangel & Heimpel, 1998; Papaj, 2000), host experience and availability (Rosenheim & Rosen, 1991; Hegazi et al., 2007; Villagra et al., 2007), and mortality risk (Houston & McNamara, 1986; Roitberg et al., 1992; Roitberg et al., 1993; Fink & Völkl, 1995; Weisser et al., 1997; Papaj et al., 2007). Physiological state and egg load in particular have been shown to play a major role in host choice behavior (Minkenberg et al., 1992). Insects have been hypothesized to *rank* hosts according to their suitability and this ranking can be overthrown by their current physiological state (Courtney et al., 1989). In order to maximize lifetime fecundity, egg limited female insects that are characterized by low egg loads and long lifetimes are predicted to accept mainly high-quality hosts compared to time limited females that are characterized by high egg loads and short lifetimes (Minkenberg et al., 1992; Heimpel & Collier, 1996). Historically, research has been focused on the role of parasitoid physiological state on the rejection or acceptance of a single host species of varying quality (Minkenberg et al., 1992; Heimpel et al., 1996; Heimpel & Rosenheim, 1998; Papaj, 2000). Host quality parameters looked at in those studies have been host size or stage (Rosenheim & Rosen, 1991; Heimpel & Rosenheim, 1998) and previously parasitized vs. unparasitized host individuals (Roitberg et al., 1993; Fletcher et al., 1994; Ueno, 1999).

To my knowledge, there has been no study on how physiological state and the perceived risk of egg and time limitation influence the choice between different host species in parasitoid insects. However, theoretically the same predictions of egg and time limitation should apply to the acceptance of host species of varying quality. The concept of a dynamic host choice behavior that responds to interspecific variations in host quality has important implications for host range breadth and host specificity in insects, which in turn affect host-parasitoid interactions and community structures (Getz & Mills, 1996; Heimpel et al., 2003) as well as biological control efficacy and safety issues (Babendreier et al., 2005).

In my dissertation, I address the role of physiological state on host choice behavior in parasitoids with an emphasis on the effect of egg and time limitation on parasitoid host acceptance under field conditions. The parasitoid of choice was the classical biological control agent *Binodoxys communis* Matsumura that has been released in 2007 throughout the Midwestern USA as part of a classical biological control project against the invasive soybean aphid, *Aphis glycines* (Gahan). This project provided the unique opportunity of not only studying the underlying physiological mechanisms of parasitoid host choice behavior but also of putting it in the context of host risk assessment in biological control. In the following, I will discuss a) egg vs. time limitation and b) parasitoid host specificity and host range as it applies to my dissertation in order to better put my work into context.

Egg vs. time limitation

Egg limitation occurs when a female parasitoid is restricted by the number of mature eggs she has available for depositing into available hosts. Time limitation occurs when a female is restricted by the time she has for locating suitable hosts (Heimpel & Rosenheim, 1998). Thus, both egg and time limitation result in missed reproductive opportunities and ultimately limit a female's lifetime reproductive success (Iwasa et al., 1984; Rosenheim, 1996; Heimpel & Rosenheim, 1998; Rosenheim et al., 2008). The interplay between physiological variables (e.g. timing of egg maturation, egg maturation rates and oviposition rates, nutritional status, longevity), environmental variables (e.g. host availability, weather conditions), and oviposition behavior influences the risk of a female parasitoid becoming egg or time limited.

The timing of parasitoid egg maturation determines the severity egg or time limitation has on a parasitoid. Proovigenic species start their lives with their full egg complement already matured, and therefore egg limitation constitutes a permanent state in these species. Synovigenic species mature eggs throughout their life and egg limitation can therefore be restricted to transient periods throughout their lifetime (Casas et al., 2000; Jervis et al., 2001; Jervis & Ferns, 2004). Thus, in proovigenic species lifetime reproductive success is restricted by egg limitation itself and in synovgenic species by the rate at which eggs are matured. Synovigenic parasitoid species have been shown to minimize their risk of becoming egg or time limited by adjusting their egg maturation rates in response to ovarian status (Rivero-Lynch & Godfray, 1997; Wu & Heimpel, 2007) as well as host experience (Casas et al., 2009). In addition to the adjustment of egg

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maturation rates, parasitoids have also been shown to use a range of behavioral

adaptations as a means of minimizing negative effects of egg or time limitation. Such

behavioral responses include: adjustment of the host encounter rate as well as oviposition

rate in response to egg load and/or host availability (Collins & Dixon, 1986; Minkenberg

et al., 1992; Hughes et al., 1994; Michaud & Mackauer, 1995; Rosenheim et al., 2008),

switching to host feeding or foraging for food sources such as nectar or honeydew

(Heimpel & Collier, 1996), and acceptance of sub-optimal hosts (Minkenberg et al.,

1992; Hughes et al., 1994; Sirot et al., 1997). These physiological and behavioral

adaptations attest to an impressive potential for plasticity on the part of parasitoid insects;

and they further support hypotheses stating that parasitoids are capable of minimizing the

risks of egg and time limitation in order to maximize their lifetime reproductive success.

Host range and host specificity

The host range of an insect parasitoid or herbivore is commonly defined as a set of

species found to be attacked by an insect or herbivore (Askew & Shaw, 1986; Shaw,

1988). Typically, host ranges are understood from literature reviews and laboratory

studies and constitute the so called physiological (or fundamental) host range (Strand &

Obrycki, 1996; De Nardo & Hopper, 2004). Species within a physiological host range

usually vary in their suitability for larval development, and female insects are

hypothesized to rank hosts in accordance to suitability for offspring survival (Courtney et

al., 1989). Traditionally, the composition of host ranges is thought to be primarily

predicted by host taxonomy and host ecology (Futuyma & Moreno, 1988; Godfray, 1994;

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Strand & Obrycki, 1996). Host specificity refers to the number and taxonomic diversity

of species within a parasitoid's host range. Finally, physiological host ranges may differ

from host ranges observed in the field, i.e. ecological host ranges.

In the following paragraph, I will illustrate the concept of host range and host specificity

using Aphidiine parasitoids with a special emphasis on *Binodoxys communis*

(Hymenoptera: Braconidae). Aphidiinae are specialized in the use of aphids as hosts

(Gauld, 1988; Hagvar & Hofsvang, 1991; Wharton, 1993). They are all endoparasitic

koinobionts, i.e. the parasitoid females lay their eggs inside host individuals which

continue feeding until they are killed by the parasitoid larvae entering the pupal stage

(Gauld, 1988; Hagvar & Hofsvang, 1991). A parasitoid species that can successfully

develop on more than a single aphid species is considered oligo-, or polyphagous

depending on the breadth of its host range. Host specificity tests on *Binodoxys communis*

have determined that it is an oligophagous specialist stinging aphid species in the tribe

Aphidini and primarily in the genus *Aphis* (Fam. Aphididae, Subf. Aphidinae) (Desneux

et al., 2009). Host ecology has been shown to further restrict the host range of *B.*

communis. Wyckhuys et al. (2007) found that *Aphis monardae* Oestlund, an aphid

species native to North America and identified in laboratory host range tests to be at risk

from parasitism by *B. communis* (Desneux et al. 2009), aggregates in the flower heads of

its host plant, *Monarda fistulosa* L., providing a host refuge. Wyckhuys et al. (2007) also

found that *A. monardae* is attended by the ant species *Lasius neoniger* Emery and that

this attendance resulted in a significant reduction of parasitism by *B. communis* under

laboratory conditions. It was concluded that *A. monardae* is not likely to be attacked by

B. communis in the field (Wyckhuys et al. 2008). Thus, even though *A. monardae* has been identified as a suitable host species for *B. communis*, certain aspects of *A. monardae's* ecology have been shown to minimize its exposure to *B. communis* in the field. Apart from that, the display of defensive behavior on the part of a host species has been shown to minimize the risk of being attacked (Desneux et al., 2009).

As was already mentioned, physiological host ranges can differ from ecological host ranges that are observed in the field. Our understanding of the role that host taxonomy and ecology play in shaping parasitoid host ranges helps us explain observed host ranges to some extent. But there is mounting evidence of selectivity on part of the female insect that is not explained by taxonomy or ecology alone. In the field, female insects have been shown to attack host species that do not support development, to ignore species that do support development based on laboratory tests, or to focus only on the highest-quality host species (Janssen, 1989; Jaenike, 1990; Kraaijeveld & van Alphen, 1995; Morehead & Feener, 2000). It has been hypothesized that observed host ranges in the field reflect dynamic oviposition behavior by female insects in response to their risk of becoming egg or time limited (Janssen, 1989; Driessen & Hemerik, 1992). Time limited females might be more willing to accept a wider range of host species in the field as well as the laboratory compared to egg limited ones in order to maximize their lifetime reproductive success. However, empirical studies that explicitly address the influence of egg and time limitation on host specificity and host range breadth in parasitoids have not been conducted so far, in either the laboratory or the field to my knowledge. And researchers are becoming increasingly aware of this shortcoming (Heimpel & Casas, 2008).

A deeper understanding of the role of parasitoid physiology on host acceptance behavior is crucial in the context of host risk assessment of a (potential) biological control agent. Based on the above, host specificity in a synovigenic parasitoid biological control agent, like *B. communis,* might be predicted to change over time depending on a female's risk of becoming egg or time limited. This is, of course, under the assumption that dynamic behavior in parasitoids as it has been documented in response to intraspecific variation in host quality also applies to interspecific variation.

In my dissertation, I address the role of physiological state on parasitoid host choice behavior at the host species level with an emphasis on the effect of egg and time limitation on parasitoid host acceptance behavior under field conditions. Chapters 2 through 5 outline a series of laboratory and field studies. Chapter 2 describes a laboratory study that determined the risk of egg and time limitation in *Binodoxys communis*. A series of laboratory experiments were designed to assess egg load and the risk of egg limitation in female *B. communis* in response to temperature, sugar feeding, and host availability. These experiments showed that *B. communis* maintains a constant egg load by varying its egg maturation rate in response to oviposition events. I hypothesized that such a rapid egg maturation might help minimize the risk of egg limitation under field conditions. Therefore, in the summers of 2007 and 2008 we conducted a release-and-recapture study to assess the risk of egg and time limitation in the field. The results of this study are outlined in Chapter 3. I assessed egg load and nutritional state of field-collected *B. communis* and was able to show that the majority of female parasitoids maintained a relatively constant egg load under field conditions.

Furthermore, an analysis of body sugar levels indicated that *B. communis* is not prone to becoming time limited due to starvation, as the majority of females were well-fed. In the summer of 2009 then, I designed a no-choice test that explicitly addressed host choice behavior in field-collected *B. communis* (Chapter 4). Field-collected female parasitoids were exposed to a low- and high-quality host species in a sequential no-choice experimental set-up. Host acceptance behavior was analyzed in the light of physiological (egg load and nutritional status) and environmental (host availability and weather) variables. Overall, host specificity in *B. communis* was maintained in field-collected females, but low host availability and detrimental weather conditions increased the likelihood of females accepting the low-quality host species. Therefore, in 2010 I designed a field study that assessed the host choice behavior in *B. communis* in the context of egg and time limitation under non-constrained conditions in the field. That is, sentinel plants were used to estimate the likelihood a low-quality or a high-quality host species would be attacked under field conditions. My results strongly suggested that *B. communis* maintained a high level of host specificity. The results of this field study and their implications regarding host specificity in *B. communis* are discussed in Chapter 5.

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Chapter 2

Determinants of egg load in the soybean aphid parasitoid *Binodoxys communis*

Introduction

Egg load in insects is defined as the number of mature oocytes present in the ovaries, and it can play an important role in insect foraging and oviposition behavior (Minkenberg et al., 1992; Heimpel & Rosenheim, 1998). In parasitoids, egg load can also affect aspects of host-parasitoid population dynamics, including stability and the extent to which parasitoids are able to suppress host populations (Getz & Mills, 1996; Shea et al., 1996; Heimpel et al., 2003). The egg load at any given time is the result of a number of physiological, behavioral, and ecological variables including rates of pre- and post-eclosion egg maturation, oviposition, and egg resorption, as well as adult nutrition and temperature (Minkenberg et al., 1992; Heimpel & Rosenheim, 1998; Papaj, 2000; Jervis et al., 2008). Here, we investigate the influence of three such variables on egg load in the aphid parasitoid *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae): temperature within the first 24 h following adult female emergence, sugar feeding, and variable host availability. These variables were chosen based on their documented influence on insect egg load as well as their high variability in field settings. One of the main goals of this study was to determine conditions under which egg limitation of *B. communis* would be more or less likely to occur under field conditions.

Several studies over the past two decades have demonstrated effects of temperature, sugar meals, and host availability on parasitoid egg load. Temperature has a strong influence on insect physiological processes in general (May, 1979), and Rosenheim & Rosen (1991, 1992) found increased egg maturation rates in the armored scale parasitoid

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Aphytis lingnanensis Compere held at higher temperatures immediately upon adult

eclosion. Two soft scale parasitoid species in the genus *Metaphycus* also had higher egg

maturation rates when held at a constant temperature of 27 °C than when held under a

temperature regime that fluctuated between 27 and 18 °C (Kapranas & Luck, 2008).

Adult sugar meals can also affect parasitoid egg load but this effect appears to vary

among parasitoid species. Whereas some studies have found a positive effect of sugar

meals on egg load (Heimpel et al., 1997; Riddick, 2007), others have found either no

effect (Lee et al., 2004; Wang & Messing, 2003) or a negative one (Olson et al., 2000).

However, this relationship has shown some context-dependence, because sugar meals did

not influence egg load in the parasitoid *Diadegma insulare* (Cresson) in laboratory assays

(Lee et al., 2004), but the availability of nectar in field settings led to higher rates of egg

maturation in the same species (Lee & Heimpel, 2008).

Finally, host availability can stimulate egg maturation either via contact with hosts, host-

associated cues, or the act of oviposition (Michaud & Mackauer, 1995; Rivero-Lynch &

Godfray, 1997; Alonso-Pimentel et al., 1998; Papaj, 2000, 2005; Wang & Messing, 2003;

Wu & Heimpel, 2007; Casas et al., 2009). The act of oviposition can also lead to partial

or complete depletion of the ovaries and egg maturation rates may increase at lower egg

loads because of rapid replenishment of ovaries (Rivero-Lynch & Godfray, 1997). In

experiments in which host density is altered it can be difficult to separate the various

ways that host density can influence egg production, but in general, maturation rates

should increase with host density by any of these mechanisms. Host availability can also lead to increases in egg load as a result of nutrients gained through host feeding (Jervis & Kidd, 1986; Heimpel & Collier, 1996; Giron et al., 2004). But this only applies to species of parasitoids that engage in host feeding, which is not the case for *B. communis* or any other species of aphidiine braconid as far as we are aware (Stary, 1970; Desneux et al., 2009a; Wyckhuys et al., 2008b).

Binodoxys communis is a solitary koinobiont endoparasitoid of the soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), and some of its close relatives (Desneux et al., 2009b). This parasitoid is being introduced against the soybean aphid, a serious economic pest throughout soybean-growing areas in North America, as part of a classical biological control program (Heimpel et al., 2004, Ragsdale et al., 2011). *Binodoxys communis* attacks and is able to develop on all stages of *A. glycines* but has a preference for second and third instars (Wyckhuys et al., 2008b). Sugar feeding can increase the life span of *B. communis* from approximately 2 days to up to 20 days (Wyckhuys et al., 2008a). No studies on egg load or egg maturation have been done on *B. communis* prior to the work described here.

Materials and methods

Insect colonies

Our *A. glycines* colony was originally established using aphids collected from soybean

fields in St. Paul, MN, USA (44°56'39"N 93°5'9.87"W), in 2003. The aphids have been

reared on soybean plants (Syngenta NK S19-R5) in plant growth chambers (L16:D8, 60-

80% r.h., and 25 ± 5 °C).

Binodoxys communis were reared on *A. glycines*-infested soybean plants under controlled

conditions (L16:D8, 60-80% r.h., and 25 ± 5 °C). The parasitoid colony was established

from 102 mummies of a Chinese strain of *B. communis* in 2003 that had been collected

by K Hoelmer, K Chen, W Meikle, and LA Pin in Harbin, Heilongjiang province, China

(45°48'11.74"N 126°32'6.88"E), in 2002. Upon collection it had been maintained at the

USDA-ARS Beneficial Insect Introduction Research Laboratory in Newark, DE, USA,

before being moved to the Minnesota Department of Agriculture/Minnesota Agricultural

Experiment Station (MDA/MAES) Quarantine Facility in St. Paul, in 2003 (see also

Wyckhuys et al., 2008a,b). For this study, parasitoids were reared in individual rearing

containers which consisted of two aphid-infested soybean plants planted in a 15-cm-

diameter plastic pot and covered by a clear plastic cylinder (21 cm in height × 9.7 cm in

diameter) (Pioneer Plastics, Dixon, KY, USA) with eight mesh-covered holes. The soil

within the pots was covered with a layer of white plaster of Paris. Two females and four

males were added to each container, and 9 days after the containers were set up,

parasitoid mummies were collected by gently removing them from the plant tissue with a

fine brush and transferring them to a 100-mm-diameter plastic Petri dish containing a piece of moistened cotton. Petri dishes were checked four times per day and adult parasitoids were removed within 3 h of their emergence.

Experiment 1: Influence of temperature on egg load

Parasitoid mummies used for this experiment were harvested 7 days after the containers were set up and were isolated into clear gelatin caps (size 0; Solaray, Park City, UT, USA). Newly-emerged virgin female parasitoids were transferred into 0.65-ml microcentrifuge tubes containing a droplet of honey. Females were exposed to either 18 or 26 °C for either 8 or 24 h and a separate group of female parasitoids was frozen at -80 °C immediately upon emergence to assess egg load. Females in the other groups were similarly frozen at -80 °C prior to being dissected.

Egg loads were assessed by dissecting the females under a dissection microscope at 50× magnification (Leica Microsystems, Bannockburn, IL, USA). Individual insects were placed onto microscope slides within a 17-µl droplet of Ringer's Solution (0.075 g NaCl, 0.035 g KCl, and 0.021 g $CaCl_2$ in 100 ml distilled H_2O). The ovaries were gently removed from the abdomen using fine forceps and the oocytes then extracted with insect pins by tearing apart the membrane surrounding each ovariole. Mature eggs are clear and lemon-shaped, whereas immature eggs are of an opaque whitish color and round to oval-shaped. Also, both adult hind tibiae were removed and measured using an ocular micrometer to obtain a size estimate of the adult individual. An analysis of covariance

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(ANCOVA) was performed on the egg load with the explanatory variables being the hours since emergence (time), temperature, average length of hind tibiae (size), and a temperature*time interaction (JMP 7.0.2, SAS Institute, Cary, NC, USA).

Experiment 2: Egg maturation in host-deprived Binodoxys communis *females*

Egg production traits as well as egg maturation patterns in host-deprived females were assessed in the presence and in the absence of a sugar source over the course of up to 10 days. Mummies for experiments were obtained as described above and emerged females were placed individually into 32-mm-diameter plastic Petri dishes modified to contain a hole in the lid covered with 0.25 mm no-see-um mesh (Quest Outfitters, Sarasota, FL, USA). A piece of moistened cotton was inserted as a water source through a 4-mm hole in the lid of the dish. To ensure mating, each female was held with one adult male of the same age for the first 8 h of the experiment. Females were kept under one of two nutritional regimes – water-only or sucrose-fed (50%-sucrose (wt/vol) solution; SIGMA-Aldrich, St. Louis, MO, USA) – for between 8 and 240 h. The sucrose solution was applied daily as a droplet on top of the mesh cover. The durations of the two treatments were based on a longevity study on *B. communis* by Wyckhuys et al. (2008a) that showed that *B. communis* females submitted to a water-only diet did not live beyond 72 h and had a survival chance of less than 25% after 48 h. On the other hand, females on a sucrose-and-water diet achieved a maximum life span of up to 14 days. Thus, for our study, females in the water-only treatment were held for 8, 16, 24, or 48 h. In contrast, females entering the sucrose-fed treatment were held for 8, 16, 24, 48, 72, 120, 168, or 240 h. For

logistic reasons, females that were assigned to be held for 8-48 h were no older than 3 h

at the beginning of the trials, whereas females held for 72 h or more were no older than

12 h at the beginning, irrespective of the nutritional regime. At the end of the designated

time period, females were frozen at -80 °C prior to being dissected as described above.

The immature eggs within ovaries were counted as well, to better interpret egg

maturation patterns and also to estimate an ovigeny index for this species. Lastly, the

lengths of both hind tibiae were recorded post-dissection as described above. Sample

sizes ranged between 19 and 27 per nutrition × time treatment combination. In addition,

27 females were frozen within 3 h of emergence to assess their egg load.

An ANCOVA was performed in which both the egg load and the number of immature

eggs were √x-transformed to meet the assumption of normality. The effects of the

independent variables of age, nutrition (starved or sucrose-fed), average length of the

hind tibiae (size), and their respective interaction terms, were tested on the dependent

variables egg load and immature eggs.

Experiment 3: Influence of host density on egg load of Binodoxys communis

The egg load of *B. communis* in response to the exposure to two host densities was

assessed. A single V1 soybean plant in a 9 × 9 cm plastic pot was covered with an

inverted 44 × 33.2 × 46.4 cm clear plastic cup (Solo Cup, Urbana, IL, USA) with the

bottom cut off and the opening covered with 0.25 mm no-see-um mesh. Plants were

infested with either 30 or 150 aphids of the late second or early third instar and then left

for 24 h to allow the aphids to settle. In a control treatment, no aphids were added onto the plants. Newly-emerged female parasitoids were obtained as described above, placed with a virgin, 1-day-old male for 1 h, and then each female was transferred for a total of 8 h into one of the prepared soybean plant pots. After this exposure time, a subsample of females was frozen immediately at -80 °C for dissection. The remaining females were transferred into 32-mm-diameter plastic Petri dishes (see experiment 2) and held for an additional 16 or 64 h, supplied with water and 50%-sucrose solution before being frozen at -80 °C. The aphid-infested plants were kept for another 9 days after the end of the assay to assess parasitism rates by counting the mummies. ANCOVA was used in analyses of the number of mummies produced as well as egg load, both of which were $\sqrt{x}$-transformed to meet the assumption of normality. The effects of host density and size on the number of mummies produced, as well as effects of time after host exposure, size, and host density on egg load were analyzed.

Results

Experiment 1: Influence of temperature on egg load

Egg load increased significantly over time and with increasing temperature ($F_{1,93}$ = 103.16 and 39.18, respectively; both P<0.0001). Also, egg loads were greater in larger females in the full model ($F_{1,93}$ = 10.42, P = 0.0017) (Fig. 2.1). There was no significant interaction between time and temperature ($F_{1,93}$ = 0.0005, P = 0.94).

Experiment 2: Egg maturation in host-deprived Binodoxys communis *females*

Binodoxys communis females had an average load of 40.7 ± 6.8 eggs upon emergence (n = 27; Fig. 2.2). Egg maturation was rapid within the first 24 h following emergence in both starved and sucrose-fed wasps and remained relatively constant between 24 and 48 h. Maximum egg loads were reached at 48 h for both groups, with sucrose-fed females exhibiting a slightly higher maximum egg load of 216.4 ± 20.1 (n = 20) compared to starved females with 177.9 ± 11.3 eggs (n = 24; Fig. 2.2). The observed difference in maximum egg loads corrected for female size, though, was not significant ($F_{1,43}$ = 1.09, P = 0.30). Egg load in sucrose-fed parasitoid females declined between 48 and 240 h, and was at a low of 82.6 ± 7.7 (n = 23) after 240 h (Fig. 2.2). Over the first 48 h, egg load significantly increased with size as well as with age ($F_{1,166}$ = 41.32 and 52.72, respectively; both P <0.0001) but was unaffected by nutrition as a main term in the full model ($F_{1,166}$ = 0.64, P = 0.43). Egg load decreased significantly in females age 48-240 h ($F_{1,103}$ = 81.36, P <0.0001; Fig. 2.2).

The number of immature eggs in females up to 48 h old decreased significantly over time and was higher in larger wasps ($F_{1,166}$ = 37.91 and 41.59, respectively; both P <0.0001; Fig. 2,2) and independent of the nutritional regime ($F_{1,166}$ = 0.83, P = 0.36). Between 48 and 240 h, the significant decrease in the number of immature eggs continued ($F_{1,103}$ = 26.40, P<0.0001) and the effect of body size remained ($F_{1,103}$ = 11.90, P = 0.0008).

An ovigeny index (OI) (sensu Jervis et al., 2001) was calculated by dividing the number of mature eggs at emergence (= 40.70) by the sum of mature and immature eggs (= 241.30) at emergence. This results in an OI of 0.14, which presumes that all of the eggs and oocytes were accounted for in our dissections. It is a conservative value (i.e., it potentially overestimates the actual OI) if some immature oocytes were not counted.

Experiment 3: Influence of host density on egg load of Binodoxys communis
Female *B. communis* parasitized significantly more aphids in the high host-density treatment than in the low host-density treatment ($F_{1,55}$ = 23.75, P<0.0001; Fig. 2.3). Also, the number of mummies significantly increased with female size indicating that larger females laid more viable eggs ($F_{1,55}$ = 12.57, P = 0.0008).

Females in the high-host-density treatment had lower egg loads than those in the other two treatments immediately after the host-exposure period, but this difference was not statistically significant ($F_{1,40}$ = 1.01, P = 0.37; Fig. 2.4). Within the first 16 h after exposure to hosts, there was a significant increase in egg load, but this was independent

of the host-density treatment in the full model ($F_{1,81} = 36.44$, P<0.0001 and $F_{2,81} = 0.17$, P = 0.85, respectively; Fig. 2.4). Individual regressions of egg load on time for the low and the high host-density as well as the control treatment between 0 and 16 h following host exposure revealed that each slope was significantly different from zero (low host density: t = 2.67, d.f. = 14, P = 0.018; high host density: t = 5.13, d.f. = 19, P<0.0001; control: t = 4.26, d.f. = 47, P < 0.0001). Females from the high host-density treatment matured 5.83 ± 1.2 eggs h^{-1} compared to 4.33 ± 1.6 eggs h^{-1} in the low aphid density treatment, and 3.93 ± 0.9 eggs h^{-1} in the control. This is consistent with the hypothesis that female *B. communis* adjust their egg maturation rates in response to the host density they had been exposed to, despite the non-significance of host density in the full model. Based on these findings, a comparison of more than two slopes including a Bonferroni correction was performed (Zar, 1984). Egg maturation rates in females among the three different treatments differed significantly ($F_{2,80} = 44.80$, 0.02<P<0.01). Pairwise comparisons of the slopes (Zar, 1984) showed that females in the high host-density treatment matured eggs faster compared to females in the low host-density treatment as well as the control (t = 2.32, d.f. = 33 and t = 2.43, d.f. = 66, respectively; both 0.025<P<0.05). However, egg maturation rates in females in the low host-density treatment were not faster than in the control (t = 0.08, d.f. = 61, P>0.2). The difference in the results between the ANCOVA and Zar's comparison of more than two slopes is probably due to a considerable loss of statistical power in the analysis of covariance (power = 0.32, β = 0.68).

Between 16 and 64 h following host exposure, the egg loads did not increase any further

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but rather stayed constant over time ($F_{1,66} = 1.78$, $P = 0.19$; Fig. 2.4). This result was independent of the aphid density treatment ($F_{1,66} = 0.17$, $P = 0.85$). Again, egg load was higher in larger individuals ($F_{1,66} = 39.46$, $P<0.0001$).

Discussion

Binodoxys communis females emerge with an initial load of approximately 40 eggs and eggs mature rapidly within the first 24 h, up to a maximum of approximately 200 eggs. We found a clear effect of post-eclosion temperature on egg load, but not of sugar meals. *Binodoxys communis* females exhibited a higher rate of egg maturation after being held with 150 rather than 30 or no aphids, but no difference was based upon host availability per se.

Post-eclosion temperature significantly influenced the rate at which eggs were matured. Females held for 24 h at 18 °C immediately following eclosion matured an average of 3.7 eggs h^{-1}, whereas females held at 26 °C matured an average of 5.2 eggs h^{-1}. All females used in this experiment were exposed to the same temperature during their larval and pupal development and only temperatures during early adulthood varied. Therefore, the observed difference in egg load and egg maturation rates is most likely the result of a reduced adult metabolic rate at lower temperatures (Carroll & Quiring, 1993) and it mirrors previous results on parasitoids of scale insects (Rosenheim & Rosen, 1991, 1992; Kapranas & Luck, 2008).

The lack of an effect of nutrition on egg load during the first 48 h after emergence suggests that females carry over enough resources from their larval stage to sustain maximum egg maturation in the absence of hosts. However, when females are given only access to water life span is significantly reduced compared to those of females given a

50% sucrose solution and other sources of sugar (Wyckhuys et al., 2008a). Thus, the absence of suitable food sources may still pose a considerable constraint on realized fecundity in this species under field conditions. Some parasitoids can experience fluctuating periods of egg- and time-limitation over the course of a single foraging day (Heimpel et al., 1998; Casas et al., 2000). However, the high maximum egg load and rapid egg maturation observed irrespective of sugar availability is more consistent with time limitation during the first 48 h of life for *B. communis*. Past this age though, a gradual reduction of the egg load was observed in the absence of hosts.

The decrease in egg load observed in sugar-fed females after 48 h could be explained by either oosorption or by deposition of eggs into the non-host environment ('egg dumping'). The recent observation of egg dumping by the ichneumonid parasitoid *Venturia canescens* (Gravenhorst) by Roberts & Schmidt (2004) has overturned previous assumptions that decreases in egg load must signify oosorption, and we are unable to distinguish between these hypotheses (see Asplen & Byrne, 2006, for demonstration of a method for the identification of oosorption in parasitoids). What the adaptive value of oosorption is in parasitoids and other insects is still not entirely clear. It may function on the one hand to recycle nutrients and therefore increase life span (Collier, 1995; Rosenheim et al., 2000) or it may be a mechanism to dispose of eggs that are no longer viable and are thus blocking the oviduct and preventing the deposition of younger, viable eggs (Rivero & Casas, 1999). Note that these hypotheses are not mutually exclusive, but also that egg dumping is only consistent with the latter hypothesis. An alternative non-

adaptive hypothesis is that egg maturation is continuous and that eggs are forced out of the ovipositor involuntarily as the oviduct fills (Roberts & Schmidt, 2004). The fact that the decline in egg load of *B. communis* was not seen in starved females that were supposedly nearing death is contrary to the nutrient recycling hypothesis and favors one or more of the other hypotheses. A pattern favoring the nutrient recycling hypothesis was observed in the aphelinid parasitoid *Aphytis melinus* DeBach, where egg loads declined much more rapidly in starved than in sugar-fed females (Heimpel et al., 1997). *Aphytis melinus* has large and nutrient-rich eggs, whereas the eggs of *B. communis* are small and presumably nutrient-poor. In another parasitoid with small eggs, the braconid *Macrocentrus grandii* (Goidanich), egg loads also decline in old, sugar-fed females but are actually *higher* in starved rather than sugar-fed females early in life (Olson et al., 2000). We interpret these results to suggest that oosorption of small eggs as found in *Binodoxys* and *Macrocentrus* is not used as a strategy of nutrient acquisition. A decline in egg load of sugar-fed females later in life seems more consistent either with the resorption of inviable eggs or with the jettisoning of eggs, either as an adaptive strategy to provide space in the ovaries for younger, healthier eggs, or as an involuntary response to continuous egg maturation and a full oviduct.

Egg maturation in *B. communis* was unaffected by exposure to a relatively low host density, but was accelerated by exposure to a higher host density. This suggests that host availability per se does not affect egg maturation. This is in contrast to other studies that have shown an increase in egg maturation spurred by the presence of hosts or host-related

cues (Luft, 1993; Michaud & Mackauer, 1995; Hopkins & Ekbom, 1996; Alonso-Pimentel et al., 1998; Papaj, 2000, 2005; Wang & Messing, 2003; Wu & Heimpel, 2007), including the exciting recent finding that even very brief antennal contact with a single host individual initiates a hormonal cascade leading to egg maturation in the eupelmid parasitoid *Eupelmus vuilleti* (Crawford) (Casas et al., 2009). However, *B. communis* females exhibited a higher egg maturation rate when provided a higher rather than a lower host density. As the higher host density treatment in our experiment led to higher rates of oviposition and concomitantly lower egg loads immediately after host exposure, we suggest that the most likely mechanism involves a response to partial ovary depletion with maturation rates inversely proportional to current egg load. Rivero-Lynch & Godfray (1997) observed a similar phenomenon in an encyrtid wasp whose egg maturation rate increased when exposed to high host densities compared to low host densities and this hypothesis could apply to their results as well.

In conclusion, egg maturation in *B. communis* varies with post-eclosion temperature and appears to increase as egg load is depleted through oviposition. The rapid maturation of eggs upon emergence as well as in the wake of oviposition would appear to be a means of avoiding or mitigating egg limitation in this species, which can be especially favorable in an environment with high variability in host availability (Rosenheim, 1996; Sevenster et al., 1998). Avoiding egg limitation under conditions of high host abundance should contribute to an increased ability of this parasitoid to suppress and stabilize populations of its host (Shea et al., 1996; Getz & Mills, 1996).

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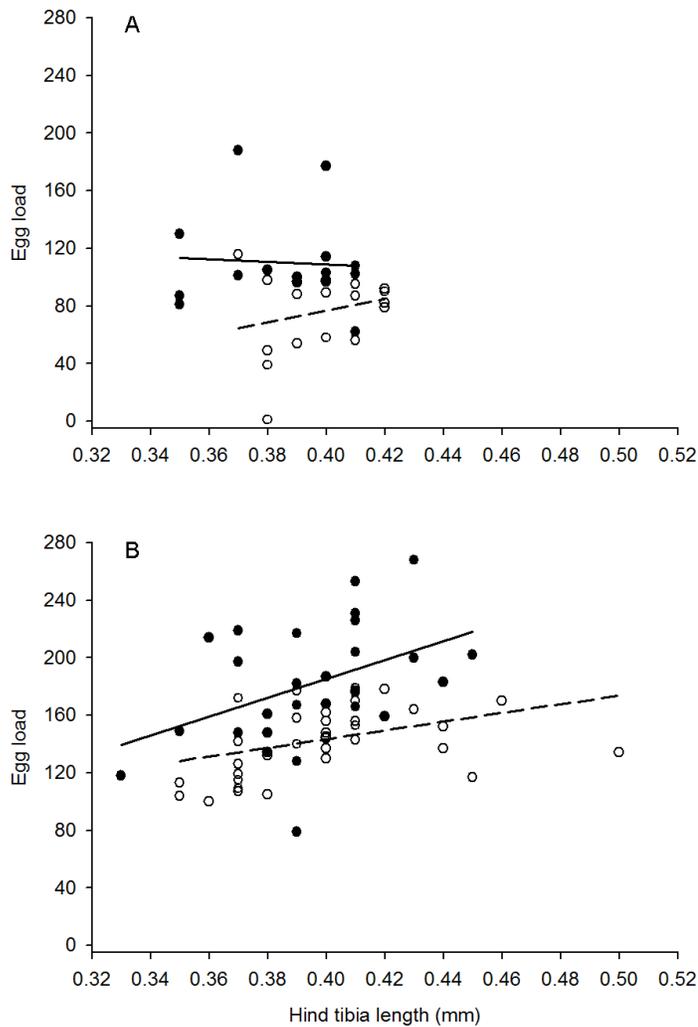
Figure 2.1: Effects of temperature and size on egg load in *Binodoxys communis* females that were held at a temperature of 18 °C (open circles) or 26 °C (closed circles) for 8 h (A) or 24 h (B). (A) Linear regression for the 18 °C group (n = 18, broken line): egg load = 410.39 × size – 87.5, r^2 = 0.0594, P = 0.33; for the 26 °C group (n = 15, solid line): egg load = -97.6 × size + 147.8, r^2 = 0.0043, P = 0.82; (B) Linear regression for the 18 °C group (n = 36, broken line): egg load = 303.5 × size + 22.01, r^2 = 0.153, P = 0.018; for the 26 °C group (n = 29, solid line): egg load = 655.6 × size – 76.9, r^2 = 0.189, P = 0.018.
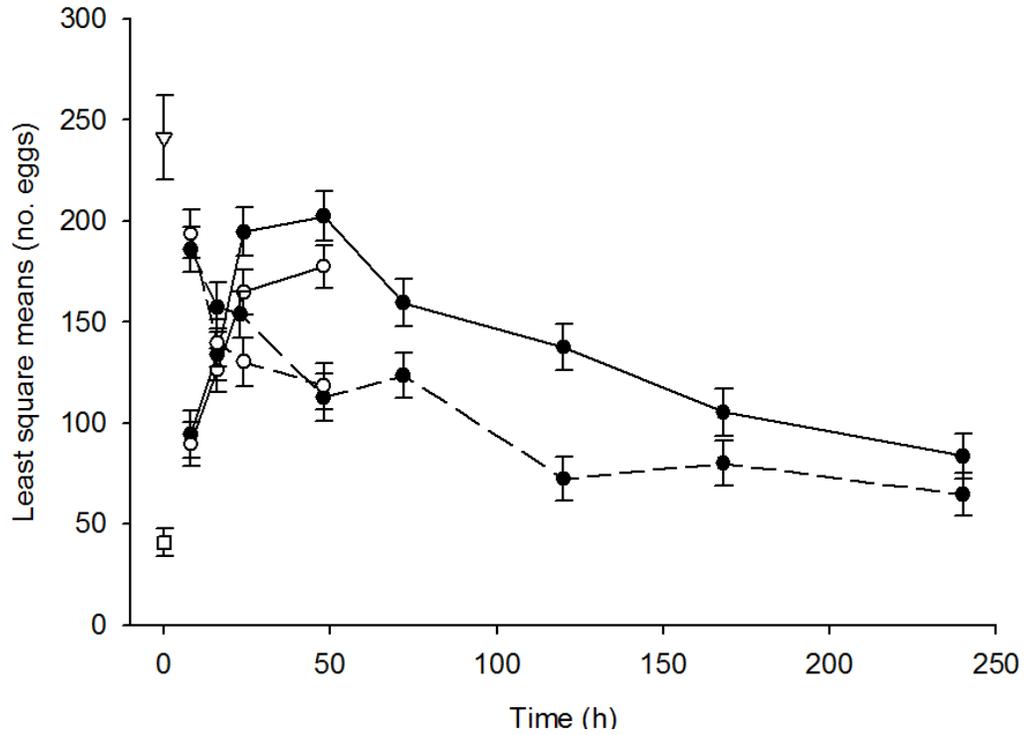
Figure 2.2: Number of mature and immature eggs in *Binodoxys communis* females over time separated by dietary treatments. Shown are the least square means corrected for parasitoid size (± SEM). — = egg load, - - - = immature eggs, ● = sugar-fed treatment, o = starved treatment, □ = egg load at emergence, ▽ = number of immature eggs at emergence.
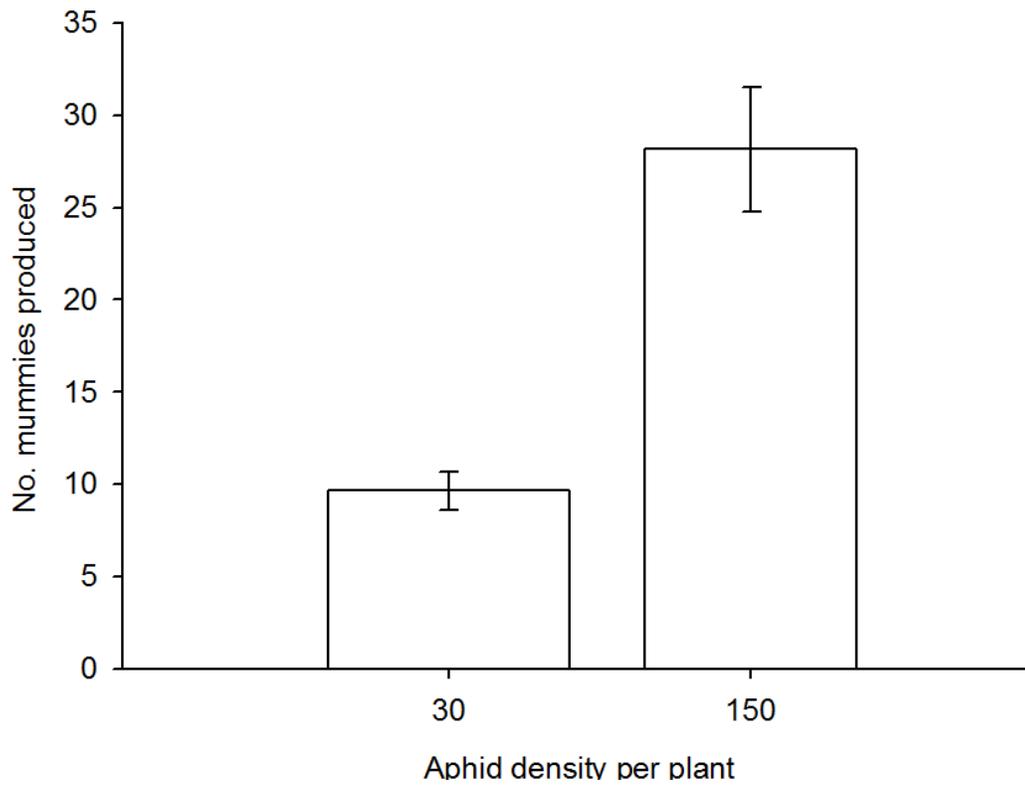
Figure 2.3: Mean number of mummies (± SEM) produced per aphid host density.
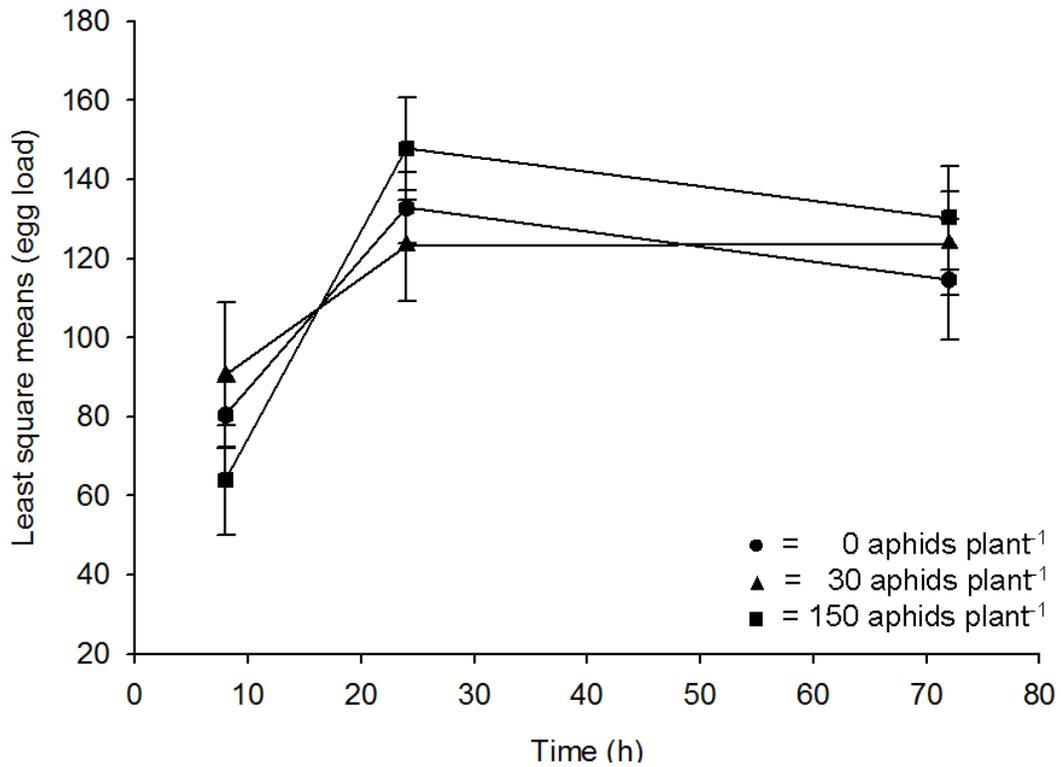
Figure 2.4: Mean egg load (± SEM) over time in female parasitoids that had access to soybean plants with varying host densities. Shown are the least square means of egg load corrected for body size. Sample sizes are: 0 aphids per plant at 8 h (n = 28), at 24 h (n = 22), and at 72 h (n = 8); 30 aphids per plant at 8 h (n = 7), at 24 h (n = 9), at 72 h (n = 10); and 150 aphids per plant at 8 h (n = 10), at 24 h (n = 11), and at 72 h (n = 12).

Chapter 3

Egg load dynamics and the risk of egg and time limitation of *Binodoxys communis*

(Hymenoptera: Braconidae) in the field

Introduction

Egg as well as time limitation can severely interfere with lifetime reproductive success in

insect parasitoids and herbivores as they both result in missed reproductive opportunities

(Rosenheim, 1996; Heimpel & Rosenheim, 1998; Heimpel et al., 1998; Rosenheim et al.,

2008). Missed opportunities arise from female parasitoids being limited by the number

of viable eggs that are available for immediate oviposition or by the time a parasitoid has

left to deplete her ovaries (Rosenheim, 1996; Sevenster et al., 1998). Thus, female

parasitoids are predicted to be selected for balancing the risk of becoming egg or time

limited to maximize lifetime fecundity (Godfray, 1994; Rosenheim, 1996). A body of

theoretical work has shown that dynamic behavior in insects can reduce the risk of egg

limitation (Iwasa et al., 1984; Mangel & Heimpel, 1998). Based on this work, a number

of behavioral adaptations have been identified that include switching from host searching

to host feeding (Heimpel & Collier, 1996) and reducing reproductive output (Collins &

Dixon, 1986; Rosenheim & Rosen, 1991; Michaud & Mackauer, 1995; Rosenheim,

1996) when eggs are limited, and accepting sub-optimal hosts (Iwasa et al., 1984; Sirot et

al., 1997) or increasing the oviposition rate (Rosenheim & Rosen, 1991; Minkenberg et

al., 1992; Hughes et al., 1994) when time is limited.

The timing of egg maturation of a parasitoid species has to be taken into account when

considering the impact of egg and time limitation. In proovigenic species that do not

mature eggs throughout their lifetime, egg limitation sets the boundaries for lifetime

reproduction. In synovigenic species egg limitation may be restricted to transient periods

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spread throughout a parasitoids lifetime (Shea et al., 1996; Heimpel et al., 1998; Casas et al., 2000; Rosenheim et al., 2000). The major constraint of egg limitation in synovigenic parasitoids is the rate at which eggs are matured. In order to minimize the risk of egg limitation, egg maturation rates should respond to external as well as to internal conditions affecting parasitoids. Casas et al. (2009) showed that host contact alone increased the ecdysone levels in the synovigenic parasitoid *Eupelmus vuilletti* which ultimately led to an increase in egg load. An increase in the egg maturation rate in response to low egg loads has been documented in several parasitoid genera (Rivero-Lynch & Godfray, 1997; Wu & Heimpel, 2007; Dieckhoff & Heimpel, 2010). Thus, dynamic egg maturation rates may provide a buffer to egg load, and thus lower the risk of egg limitation in parasitoids.

While parasitoid egg maturation rate may be the most important constraint for egg limitation, longevity plays a major restrictive role in the context of time limitation. Under field conditions, one component that influences a parasitoid's realized lifespan is the risk of starvation. In order to maximize longevity in the field, non-host feeding parasitoids require some form of a carbohydrate source like floral nectar, hemipteran honeydew, or pollen (Wäckers, 2005). Hemipteran honeydew is thought to be the dominant carbohydrate source for insects relying on non-prey food, especially in agricultural settings that constitute highly simplified ecosystems (Wäckers, 2005; Wäckers et al., 2008). However, availability and ingestion of hemipteran honeydew by an insect does not automatically translate into a prolonged lifespan. The suitability of

honeydew can be diminished by the presence of secondary plant metabolites and oligosaccharides as well as a tendency to crystallize quickly (Wäckers, 2001; Wäckers et al., 2008).

The purpose of this study was to assess egg load and nutritional state in the soybean aphid parasitoid *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae) under field conditions in order to estimate its risk of egg and time limitation in the field. This parasitoid species was introduced throughout the Midwestern USA as a classical biological control agent against the soybean aphid, *Aphis glycines* Matsumura, in 2007 (Heimpel et al., 2010; Ragsdale et al., 2011). It is a synovigenic parasitoid species that does not engage in host feeding (Wyckhuys et al., 2008a; Dieckhoff & Heimpel, 2010). We have previously shown that *B. communis* maintains a high egg load through rapid egg maturation in response to decreases in egg load in the laboratory. And we hypothesize that such a mechanism may mitigate negative impacts, i.e. missed reproductive opportunities, due to egg limitation in the field (Dieckhoff & Heimpel, 2010).

Materials & Methods

Insects

Binodoxys communis intended for release was reared on *Aphis glycines*-infested soybean plants in a greenhouse at the University of Minnesota, St. Paul, MN, USA. The *A. glycines* colony used was originally established from aphids collected from a soybean field in St. Paul, MN, USA, in 2003 and has been reared continuously on soybean plants (Syngenta NK S19-R5) in growth chambers (L16: D8; 60-80% r.h.; 25 ± 5 °C).

The *B. communis* colony originated from a strain collected in the Chinese province of Heilongjiang in 2002 (Wyckhuys et al., 2008a). Releases across the mid-western US have been conducted since 2007; however, at the time of this study there was no evidence that *B. communis* has established in North America (Asplen et al., in preparation; Heimpel et al., 2010). Parasitoids were reared in clear plastic, household storage boxes (Sterilite®, 62 × 45 × 18 cm) with ventilation windows cut into the long sides and the lid. Windows were covered with a coarse mesh (mesh size: 2mm) to prevent predators from entering the boxes in the field and a layer of no-see-um mesh (mesh size: 0.6 mm, Quest Outfitters, Sarasota, FL, USA) on top of the coarse mesh during parasitoid rearing. Each box contained 6-13 9 × 9 cm square plastic pots containing aphid-infested soybean plants and 16-26 female and twice as many male parasitoids.

Field plots and releases

This study was conducted in soybean fields at the University of Minnesota Agricultural

Experiment Station in St. Paul, MN, USA in the summers of 2007 and 2008. A single

field was used each year, and field sizes were 1.02 ha in 2007 and 0.73 ha in 2008.

Soybean varieties used were "cropLAN #RC1992" in 2007 and "Asgrow #AG1402RR"

in 2008. *Binodoxys communis* was mass-released weekly into flagged 4 × 4 m plots

embedded within a soybean field from 29 June - 31 August 2007 and 2 July - 27 August

2008. Each plot contained approximately 468 plants, was used for a single release only

with at least 30 m between individual plots, and approximately 30 m away from the field

edges. Prior to the first release in either year, each field was treated with the herbicide

Round-up (The Scotts Company LLC, Marysville, OH, USA).

Parasitoids were released over a 24-hr period starting between 9 and 10 am by placing

three rearing containers into the center of each plot on each release date. Adult

parasitoids were collected on two consecutive days immediately after the 24-hr release

period between 9 am and 4 pm or until no more parasitoids were recovered for 3 hours.

Soybean plants in each plot were manually searched for a minimum of 10 min and adult

B. communis on each plant were aspirated, transferred into a 0.65 ml microcentrifuge

tube (DOT Scientific, Inc., USA), and immediately put on ice to halt further egg

maturation. Hereafter, adult female *B. communis* caught between 9 and 11 am will be

referred to as "morning" and females caught between 11 am and 4 pm as "afternoon". In

addition, a subset of females ("caged") recaptured between 9 and 11 am was transferred

individually onto a single aphid-free soybean plant covered with a clear plastic cylinder with no-see-mesh-covered holes for ventilation (21 cm × 9.7 cm; Pioneer Plastics, Dixon, KY, USA). These "caged" females could mature eggs throughout the day in the absence of hosts and the difference in egg loads between "caged" and "morning" or "afternoon" females provided an estimate of the field egg maturation rate or oviposition rate, respectively. These "caged" parasitoids were placed among field soybean plants for 8 hours before they were frozen. All parasitoids were stored at -80 °C prior to being dissected.

Dissections and preparation for sugar analyses

Egg loads of recaptured as well as caged female parasitoids were assessed by dissecting each female under a dissecting microscope at 50x magnification. In both 2007 and 2008, ovaries were removed for egg load counts of field-collected parasitoids. Parasitoid size was assessed by measuring the hind tibia length of each adult. Prior to each dissection, a single hind leg was removed from the parasitoid body and the length of the hind tibia measured using a dissecting microscope (50x magnification). In addition, field-collected parasitoids in 2008 were used for a sugar analysis as described below. Therefore, after removing the ovaries the rest of the insect body was transferred into a microcentrifuge tube filled with a 40 µl aqueous solution of Ringer's solution and 100 % ethanol (1:1 ratio) and stored at room temperature until the analysis.

Effects of hind tibia length, average temperature (°C), and host availability (log10-

transformed) on egg load as well as day of collection, cohort, and the interaction thereof

on egg load were analyzed using multiple linear regression (JMP 8.0.1; SAS Institute,

Inc., Cary, NC, USA).

Calculation of field egg maturation and oviposition rates

Field egg maturation rates were calculated using linear regression of egg loads of

"morning" and "caged" parasitoids against time for each of the two days of collection

(Fig. 3.1). The regression line represents a hypothetical egg load, i.e. the number of eggs

a female parasitoid would mature over the course of a field day in the absence of

oviposition. In addition, the difference between the hypothetical egg load and the egg

load of each "afternoon" female provided and estimate of the number of eggs laid over

the course of a day in the field. Methods used here to estimate the number of eggs laid

are similar to the ones used by (Casas et al., 2000; Lee & Heimpel, 2008) The effects of

day of collection and hind tibia length on the number of eggs laid was assessed using an

ANCOVA (JMP 8.0.1; SAS Institute, Inc., Cary, NC, USA).

HPLC sugar analysis

Sugar analysis was performed on female parasitoids (n = 117) collected in the summer of

2008 only and their nutritional status was assessed. In preparation for the HPLC analysis

samples were transferred into individual 1.5 ml microcentrifuge tubes and 600 µl high

purity water (i.e. Milli-Q water, Milli-Q Integral system, Millipore, Billerica, MA, USA)

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was added to 49 samples and 400 µl Milli-Q water to 68 samples. The dilution factor

was adjusted after the first run of samples to make up for small sugar peaks. Each sample

was then thoroughly pestled and filtered through a chromacol syringe filter (PTFE, 0.2

µm membrane, 17 mm, Chromacol Ltd., Thermo Fisher Scientific, UK) into a 2 ml screw

top vial with an insert and a split cap top (Chromacol Ltd., Thermo Fisher Scientific,

UK). Sugar analyses were conducted as described by Wyckhuys et al. (2008b); 10 µl of

each sample were injected into a Dionex DX 500 HPLC-system (Dionex Corp.,

Sunnyvale, CA, USA) equipped with a GP 40 gradient pump, a Carbopac PA1 guard (4 ×

50 mm^2), an analytical column (4 × 250 mm), and an ED 40 electrochemical detector for

pulsed amperimetric detection (PAD) (Dionex, Leeds, UK). The column was eluted with

1 M NaOH and Milli-Q water (10:90 %, 1 ml min^{-1}) and kept at 20 °C for the analysis.

Calibration standards with concentrations of 2.5 ppm, 5.0 ppm, 7.5 ppm, and 10 ppm for

daily reference curves were prepared containing a total of 16 sugars each. These 16

sugars were: Sorbitol, Mannitol, Trehalose, Rhamnose, Galactose, Glucose, Sucrose,

Mannose, Fructose, Melezitose, Melibiose, Raffinose, Lactose, Stachyose, Erlose, and

Maltose. Individual sugar peaks were analyzed using PEAKNET Software Release 5.1

(DX-LAN module; Dionex, Leeds, UK).

Baseline sugar spectra

Nutritional state and sugar feeding of field-collected parasitoids were quantified by

comparing total sugar levels (i.e. the sum of the concentration of all individual sugars) as

well as fructose levels to threshold levels based on a set of laboratory controls. Methods

applied follow the ones used by Wyckhuys et al. (2008b). Two sets of controls were

prepared that consisted of newly eclosed (< 3 hrs old) female parasitoids that were

transferred into 32-mm diameter Petri dishes with ad libitum access to water+honeydew

("honeydew-fed") or water only ("starved") for 15min. Afterwards, parasitoids were

either immediately frozen at -80 °C or kept with water only for another 4hrs in a growth

chamber at 25 ± 5 °C (L16: D8; 60-80% r.h.;) to allow imbibed sugars to be metabolized

before also being frozen at -80 °C.

Soybean aphid honeydew used in the control experiment was collected by covering an

aphid-infested soybean leaf with a piece of Parafilm and storing it in a growth chamber at

25 ± 5 °C (L16: D8; 60-80% r.h.) for 24 hours. The leaf was placed upside down on

moist cotton to prevent the leaf from drying out. Honeydew droplets ejected by the

aphids during the 24-hr period accumulated on the Parafilm and were used immediately

to prevent crystallization. This method of honeydew collection was adapted from

methods described in Burger et al. (2005) and Wyckhuys et al. (2008b).

In preparation for the HPLC analyses, the ovaries of each parasitoid were removed,

discarded, and the rest of the body transferred into a microcentrifuge tube filled with a 40

µl aqueous solution of Ringer's solution and 100 % ethanol (1:1 ratio) and stored at room

temperature. Ovaries of control parasitoids were removed to allow a comparison with the

equally treated field-caught parasitoids (see above). In addition, 5µl of pure honeydew

were stored in 40 µl Ringer's solution and 100% ethanol (1:1 ratio) and later analyzed

using HPLC to obtain the honeydew sugar spectra. Hemipteran honeydew is characterized by the presence of so-called honeydew signature sugars, mainly erlose and melezitose (Wäckers, 2001; Wyckhuys et al., 2008b). Presence and absence of those sugars in soybean aphid honeydew will help determine the occurrence of soybean aphid honeydew-feeding as field-collected parasitoids will test positive for these sugars if feeding occurred.

Feeding history and nutritional status of field-collected parasitoids

Sugar data, i.e. fructose as well as total sugar concentrations, for 0hrs and 4hrs starved and honeydew-fed controls were pooled as they were not significantly different from each other (Kruskal-Wallis H tests for fructose: $\chi^2 = 3.26$, d.f. = 3, P = 0.3532; and total sugar: $\chi^2 = 6.72$, d.f. = 3, P = 0.0813).

Feeding history of field-collected parasitoids was assessed based on fructose concentrations. Fructose is either not present or present in very low amounts in unfed insects (van Handel, 1984; Heimpel et al., 2004; Lee et al., 2004). In *B. communis*, no fructose was detected in starved control individuals, while honeydew-fed control parasitoids did test positive for fructose (0.0432 μg ± 0.13 SD). Thus, the presence of fructose in the sugar spectra of field-collected parasitoids was used to distinguish fed from unfed field-collected parasitoids.

Nutritional status of field-collected parasitoids was determined using total body sugar concentrations that provide a reliable indicator of an insect's energy reserves (Steppuhn & Wäckers, 2004). Total sugar contents of starved control parasitoids were significantly lower compared to honeydew-fed ones (0.39 ± 0.28 µg (± SE) vs. 2.54 ± 0.54 µg (± SE), $t = -2.84$, d.f. 23, $P = 0.0094$).

Total sugar levels as well as fructose levels were log10-transformed to meet the assumption of homogeneity. Total sugar levels as well as fructose levels between the three cohorts on the first day of collection were compared using an ANOVA followed by a Tukey-Kramer HSD test for multiple comparison of means. The effects of egg load, host availability, temperature, hind tibia length, and cohort on total sugar levels, fructose levels and log10-(erlose+1) levels in "morning" and "afternoon" parasitoids only were analyzed using multiple linear regression. Also, a linear regression taking hind tibia length into account was used to assess the effect of fructose and total sugar level on egg load in 2008 (JMP 8.0.1; SAS Institute, Inc., Cary, NC, USA).

Results

Egg load in 2007 and 2008

Egg load data of field-collected parasitoids were pooled for the two years of collection as there was no significant difference in parasitoid egg load between the two years of this study ($F_{1,185} = 0.38$, $P = 0.5345$), or between the two years when separated by day of collection (Year×Day of collection: $F_{1,185} = 1.87$, $P = 0.1731$) or cohort (Year×Cohort: $F_{2,185} = 0.83$, $P = 0.4377$).

Egg loads of field-collected female *Binodoxys communis* collected and immediately frozen between 9am and 4pm ranged from 1 to 193 eggs on day 1 (n = 132) and 6 to 264 eggs on day 2 (n = 30) (Fig. 3.2). The average egg load of field-collected parasitoids was 77.77 ± 3.77 eggs ($\pm$ SE) on day 1 and 81.70 ± 7.90 eggs ($\pm$ SE) on day 2 (t = 0.45, d.f. = 160, P = 0.6536). Egg loads were significantly different among the three cohorts with egg loads of "caged" parasitoids significantly higher than egg loads of "morning" and "afternoon" cohorts ($F_{2,191} = 14.15$, $P < 0.0001$; Fig. 3.3). Egg load in any of these cohorts was not significantly different between the two days of collection (Day of collection×Cohort: $F_{2,191} = 1.46$, $P = 0.2359$).

There was a significant positive effect of average daily temperature ($F_{1,156} = 7.27$, $P = 0.0078$; Fig..3.4) and a significant negative effect of host availability ($F_{1,156} = 6.07$, $P = 0.0149$; Fig. 3.5) on egg load in female *B. communis* captured in the field and placed

directly on ice. Egg load was not correlated with hind tibia length in this data set ($F_{1, 156}$ = 0.26, P = 0.6084).

Calculation of egg maturation rates using egg loads of "morning" and "caged" females at day 1 and 2 resulted in estimates of 4.36 ± 0.11 eggs hour^{-1} matured on day 1 and 8.67 ± 0.67 eggs hour^{-1} on day 2. Both slopes are significantly different from zero (day 1: $F_{1,61}$ = 7.4619, P = 0.008; day 2: $F_{1,20}$ = 12.11, P = 0.0024; Fig. 3.6), but a comparison of slopes (Zar, 1984) indicated that females did not mature significantly more eggs per hour on day 2 compared to day 1 (t = 1.29, df = 81, 0.1<P<0.2). Oviposition rates, i.e. the number of eggs laid per hour, were calculated from the difference between the hypothetical egg load, i.e. the estimate of egg load in the absence of oviposition, and egg loads of "afternoon" *B. communis* (Fig. 3.1). Female parasitoids laid on average 3.64 ± 0.85 eggs hour^{-1} on day 1 and 7.38 ± 2.15 eggs hour^{-1} on day 2. However, ovipositions rates were not significantly different between the two days of collection ($F_{1,157}$ = 0.239, P = 0.6256).

Nutritional status and feeding history in field-collected parasitoids in 2008
Total sugar levels of field-collected *B. communis* ranged from 2.03µg to 31.77µg compared to ranges of 8.4*10^{-7} µg through 5.95µg as well as 0.00024µg through 2.38µg in honeydew-fed as well as starved control parasitoids (Fig. 3.7a). Total sugar levels of field-collected parasitoids in the "morning", "afternoon", or "caged" cohort on the first day of collection were all significantly higher than total sugar levels of honeydew-fed and starved laboratory control parasitoids ($F_{4,121}$ = 29.80, P < 0.0001) (Fig. 3.8a). However,

total sugar levels between field-collected parasitoids were not significantly different from each other (Fig. 3.8a). Sample sizes of field-collected parasitoids on the second day of collection were overall too low to allow for reliable analyses. However, the majority of parasitoids collected in the afternoon of the second day also had total sugar levels well above total sugar levels of starved control females suggesting that energy was not a limiting factor on the second day in the field (Fig. 3.8b). Total sugar levels in "morning" and "afternoon" female parasitoids were positively correlated with hind tibia length ($F_{1,89}$ = 11.38, P = 0.0011) and host availability ($F_{1,89}$ = 9.74, P = 0.0024) (Fig. 3.9a). Total sugar levels were independent of egg load, average daily temperature, or parasitoid cohort ($F_{1,89}$ = 0.18, P = 0.6698; $F_{1,89}$ = 0.42, P = 0.5200; or $F_{1,89}$ = 0.19, P = 0.6636, respectively).

Using fructose levels to estimate the feeding history of field-collected *B. communis* showed that the majority of field-collected parasitoids tested positive for fructose (Fig. 3.7b). Only three field-collected parasitoids had a fructose level of 0µg. Mean levels in the "morning", "afternoon" and "caged" cohorts on the first day of collection were all significantly greater than total sugar levels of honeydew-fed ($F_{3,121}$ = 25.42, P < 0.0001) (Fig. 3.10a). Fructose levels in "morning" and "afternoon" parasitoids collected on the first day were significantly correlated with host availability ($F_{1,76}$ = 4.50, P = 0.0372) (Fig. 3.9b). However, there was no effect of hind tibia length ($F_{1,76}$ = 1.85, P = 0.1784), egg load ($F_{1,76}$ = 2.74, P = 0.1022), temperature ($F_{1,76}$ = 0.18, P = 0.6749), or cohort ($F_{1,76}$ = 2.31, P = 0.1326) on fructose concentrations in field-collected *B. communis*.

The positive effect of host availability on total sugar levels in *B. communis* suggests that soybean aphid honeydew was at least part of their diet in the field. The sugar spectrum of the pure soybean aphid honeydew sample confirmed the presence of erlose, a honeydew signature sugar, which constituted about 17.4 % of the total sugar content (see also Heimpel et al., 2004; Wäckers et al., 2006). Thus, presence of erlose in field-collected parasitoids can be used as an indicator of soybean aphid honeydew-feeding. In addition soybean aphid honeydew was composed of the following sugars: Fructose (43.3 %), Glucose (19.5 %), Stachyose (14.1 %), Sucrose (4.1 %), Trehalose (0.9 %), and Mannitol (0.8 %).

Soybean aphid honeydew feeding in the field was confirmed in 16 out of 85 field-collected parasitoids which tested positive for erlose in the HPLC. Erlose concentrations ranged from 0.576µg to 6.924µg (Fig. 3.7c). Host availability had also a significant effect on erlose ($F_{1,77} = 31.75$, $P < 0.0001$) (Fig. 3.9c) contrary to any of the other predictors (hind tibia length: $F_{1,77} = 3.51$, $P = 0.0649$; egg load: $F_{1,77} = 0.02$, $P = 0.8824$; temperature: $F_{1,77} = 0.14$, $P = 0.7063$; cohort: $F_{1,77} = 1.28$, $P = 0.2623$).

Discussion

The majority of female *Binodoxys communis* did not show signs of egg limitation under field conditions over the course of a single foraging day as well as over two consecutive days. *B. communis* maintained a remarkably constant egg load over time while also maturing an average of 4.36 ± 0.11 eggs hour^{-1} and 8.67 ± 0.67 eggs hour^{-1} on the first and second day of collection, respectively. Furthermore, egg load was inversely related to host availability suggesting that more eggs were laid in host-rich environments compared to host-poor ones. These findings strongly indicate that oviposition occurred in the field and that egg maturation rates were such that constant egg loads were maintained. There was an effect of temperature on egg load, but not on sugar feeding. The majority of field-collected parasitoids had generally high total body sugar concentrations indicating that sugar supply was not a limiting factor in the field. Furthermore, there was strong evidence for sugar feeding of *B. communis* in the field based on the presence and amount of fructose in field-collected parasitoids. Host availability and total sugar, fructose, and erlose levels were significantly correlated suggesting that the elevated sugar levels were at least partly due to soybean aphid honeydew-feeding in *B. communis*. The presence of erlose, a honeydew signature sugar, in 19% of the field-collected parasitoids further supports the hypothesis that *B. communis* fed on soybean aphid honeydew in the field.

Approximately 85% of the females collected on day 1 and 73% on day 2 had egg loads above the average egg load recorded at eclosion, i.e. ~40 eggs (Dieckhoff & Heimpel,

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2010). On average, *B. communis* maintained a constant egg load of about 80 eggs despite

an estimated egg maturation rate of approximately four and nine eggs per hour on the

first and second day of collection, respectively. Thus, there is an excess of eggs that are

unaccounted for in field-collected parasitoids and it is most likely that those eggs were

deposited into host aphids. However, there are alternative non-mutually exclusive

explanations as to where those eggs could have gone: oosorption or deposition into a non-

host environment. The function of oosorption is still under debate but egg resorption as a

means of recycling nutrients (Collier, 1995; Rosenheim et al., 2000) or removing inviable

eggs (Rivero-Lynch & Godfray, 1997) have been hypothesized (Asplen & Byrne, 2006).

B. communis is an unlikely candidate for the nutrient recycling hypothesis (Dieckhoff &

Heimpel, 2010); and see also Chapter 2). Oosorption consistent with the nutrient

recycling hypothesis has been documented in the scale parasitoid *Aphytis melinus* that has

large and nutrient-rich eggs (Heimpel et al., 1997). *Binodoxys communis,* however, has

small and supposedly nutrient-poor eggs. In a previous laboratory study, egg loads of

starved host-deprived *B. communis* that were presumably nearing death did not decline

prior to their deaths, while a decrease in egg loads was only observed in sugar-fed host-

deprived females that were older than 48 hours (Dieckhoff & Heimpel, 2010). The

deposition of eggs into a non-host environment, on the other hand, might occur when egg

maturation is continuous and host availability is too low to provide adequate oviposition

opportunities. A similar mechanism has been documented in *Venturia canescens*

(Roberts & Schmidt, 2004). We cannot, based on this study, exclude the possibility that

a proportion of field-collected *B. communis* deposited eggs into a non-host environment,

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voluntarily or involuntarily; especially early in the season when host availability is generally low in the field. However, we consider oviposition to be the far more likely explanation here. There is one other hypothesis that might explain the number of unaccounted for eggs in field-collected *B. communis* and that has its seeds in how the field egg maturation rate was estimated in the first place. The calculation of egg maturation rate in field-collected parasitoids was based on a comparison of egg loads between "morning" and "caged" females. There is the possibility that egg maturation of "caged" females was artificially high due to a greenhouse effect inside the cages. Those cages consisted of a single host-free soybean plant that was covered with a clear plastic cylinder with holes for ventilation and female parasitoids were kept for a total of eight hours inside those cages. Thus, it is possible that the temperature inside those cages was higher than the outside temperature which may have increased parasitoid egg maturation. We have previously shown that post-eclosion temperature has a significant positive effect on egg maturation in *B. communis* (Dieckhoff & Heimpel, 2010). Thus, if egg loads of "caged" female parasitoids were artificially high due to a design flaw this would have an effect on the egg load estimation by overestimating it. However, all cages had mesh-covered holes to provide constant ventilation and cages were kept in the shade during the eight hours of host-deprivation. Therefore, although we cannot dismiss this hypothesis we do not think it is a very likely scenario.

The maintenance of a constant egg load over time suggests that the risk of egg limitation in *B. communis* under field conditions is low, at least within the first two days post-

eclosion. That is, the oviposition rate in *B. communis* does not appear to be limited by the females' ability to mature eggs. We have previously shown that egg maturation in *B. communis* are inversely proportional to current egg load (Dieckhoff & Heimpel, 2010). In the laboratory, egg maturation rates in *B. communis* increased in a host-rich environment but host contact alone could not explain the observed increase in maturation rates. This suggested that egg maturation rates in *B. communis* respond to the ovarian status of a female by quickly replenishing eggs that have been oviposited (Dieckhoff & Heimpel, 2010). Such a purely physiological response may buffer egg load and therefore lower the risk of becoming egg limited. This study is consistent with the hypothesis that *Binodoxys communis* avoids becoming egg by buffering its egg load.

Egg load in field-collected *B. communis* was a negative function of host availability, i.e. egg load decreased with increasing availability of reproductive opportunities in a host-rich environment. To our knowledge, this is the first study that shows a correlation between egg load and host availability. The risk of becoming egg limited is predicted to be higher in host-rich environments compared to host-poor ones. The observed decline in egg load as a function of host availability is in accordance with Rosenheim's (1996) prediction that host density should be positively correlated with the proportion of parasitoids that become egg limited. Mangel & Heimpel (1998) expanded on Rosenheim's (1996) prediction applying a dynamic-state modeling approach that accounted for behavioral plasticity in the insect. The authors showed that the risk of egg limitation increases with host density but that an organism can minimize this risk by

displaying dynamic oviposition behavior. One of the few studies explicitly

characterizing egg limitation in field-collected parasitoids found no correlation between

egg load and host availability in the scale parasitoid *Aphytis aonidiae* (Heimpel &

Rosenheim, 1998). The authors argued that host choice behavior in *A. aonidiae* may be

dynamic as females with declining egg loads were more likely to oviposit into high-

quality hosts and host feed on low-quality ones. An effect of host availability on egg

load, as documented in this study here, implies that under conditions of high host

densities egg maturation rate may constraint parasitoid reproductive success (Rosenheim,

1996, 1999; Casas et al., 2000; Rosenheim et al., 2000). Temporal episodes of egg

limitation have been documented for several synovigenic parasitoid species in the field

(Heimpel et al., 1996; Heimpel & Rosenheim, 1998; Casas et al., 2000). Studies on field

collected *Aphytis aonidiae* and *A. melinus* showed that a proportion of a given population

is likely to experience periods of egg limitation in the field. As egg limitation implies

forgone oviposition opportunities it is speculated that it may also have destabilizing

properties for host-parasitoid population dynamics (Getz & Mills, 1996; Shea et al.,

1996; Casas et al., 2000). We cannot exclude that a small fraction of *B. communis* may

be subject to an increased risk of egg limitation, mainly in host-rich environments.

However, at least within the first two days post-eclosion we consider the risk of egg

limitation to be low in *B. communis*. This consideration.is based on the observation that

B. communis is capable of maintaining a constant egg load by adjusting its egg

maturation rate in response to its ovarian status (Dieckhoff & Heimpel, 2010). Thus,

such a dynamic physiological mechanism may be able to provide a buffer to egg load which would then minimize the risk of egg limitation.

The presence of fructose as well as the levels of both fructose and total sugar in field-collected *B. communis* strongly suggests that parasitoids commonly fed on an exogenous carbohydrate source in the field. Soybean aphid honeydew was most likely the main source accessed by *B. communis* in the field. Agricultural systems like soybean fields are highly simplified habitats with a limited diversity of plants that could provide floral nectar or pollen. Apart from the crop itself, the most common plant resources are weeds or flowering borders. Previous studies have shown that parasitoids can profit from the presence of floral or other resources (Casas et al., 2003; Lee & Heimpel, 2008; Winkler et al., 2009). In this study, however, all plots were placed at least 30 m from the field edge and had been treated with roundup herbicide the week prior to the study. Therefore, it is considered unlikely that females accessed a sugar source other than soybean aphid honeydew. In addition, about 20% of field-collected *B. communis* tested positive for the honeydew signature sugar erlose confirming that soybean aphid honeydew was available in the field and consumed by parasitoids. Finally, another strong indication that soybean aphid honeydew was a main resource is the positive correlation between host availability and total sugar, fructose, and erlose levels in field-collected *B. communis*.

Overall concentrations of total body sugars indicate that the majority of female parasitoids had a low risk of starvation in the field, and thus a reduced risk of becoming

time-limited due to an energy shortage. Time limitation can constraint parasitoid lifetime reproductive success by restricting the time a female has to deplete her egg complement. Parasitoid longevity can be used as an estimate of the risk of time limitation in the field. Longevity in *B. communis* is severely limited by an individual's access to exogenous carbohydrate sources (Wyckhuys et al., 2008b; Dieckhoff & Heimpel, 2010). In laboratory studies, female *B. communis* died within 48 hours without access to a sugar source but survived up to three weeks on a sugar diet like sucrose or honey (Wyckhuys et al., 2008b; Dieckhoff & Heimpel, 2010). Also, Wyckhuys et al. (2008b) showed that female *B. communis* given access to soybean aphid honeydew lived up to three times longer when also given access to water allowing female parasitoids to live up to nine days on a honeydew+water diet. The authors hypothesized that water increases saliva production in the parasitoid which in turn facilitates the parasitoid's access to easily crystallizing resources like honeydew (Stoffolano, 1995; Wyckhuys et al., 2008b). Soybean plants in the open field are often covered with dew early in the morning and small water pockets can potentially persist in the shadier parts of the canopy for the remains of the day forming easily accessible drinking fountains for insects (Dieckhoff, C. pers. obs.). Observed longevity of field-collected *B. communis*, here represented by the two consecutive days of collection, are in accordance with the study of Wyckhuys et al. (2008b). Thus, the assumption underlying the time-limitation-due-to-starvation argument is that female *B. communis* were actually starved or prone to starvation in the field due to unavailability or inaccessibility of suitable food sources. We found no evidence to support this assumption and therefore consider the risk of time-limitation due to

starvation for *B. communis* under field conditions to be low. However, other

environmental causes such as predation or adverse weather may also increase the risk of

time limitation in adult *B. communis* in the field. Insect predators are a common sight in

soybean fields and predator guilds are often dominated by generalist predators such as

coccinellids (Costamagna & Landis, 2007). Predation on larval parasitoids has been

documented in a number of studies, including predation on immature stages of *B.*

communis (Colfer & Rosenheim, 2001; Chacon et al., 2008; Chacon & Heimpel, 2010).

The risk of predation on adult *B. communis* in the field, though, has not been addressed,

but other studies have indicated high levels of predation on adult parasitoids (Heimpel et

al. 1997). Finally, rainfall has been shown to induce substantial mortality in insects

(Moran & Hoffmann, 1987; Roitberg et al., 1992; Weisser et al., 1997). Thus, both

predation and weather conditions have the potential to increase the risk of time limitation

in *B. communis* under field conditions.

In conclusion, the majority of field-collected female *B. communis* were not egg limited.

Female parasitoids maintained a constant egg load over time and we hypotethesize that

this process might provide a buffer to egg load, and thus reduce the risk of becoming egg

limited. Such a buffering mechanism may be adaptive in environments with variable host

densities (Sevenster et al., 1998) and may have the potential to result in higher pest

suppression (Getz & Mills, 1996). A small fraction of *B. communis*, however, may

become egg limited in host-rich environments based on a negative correlation between

egg load and host availability. Quanitification of body sugar contents showed that the

majority of field-collected *B. communis* had sugar levels that indicated a low risk of

starvation, at least within the first two days post-eclosion. Thus, the risk of time-

limitation due to starvation is considered to be low in *B. communis* in the field.

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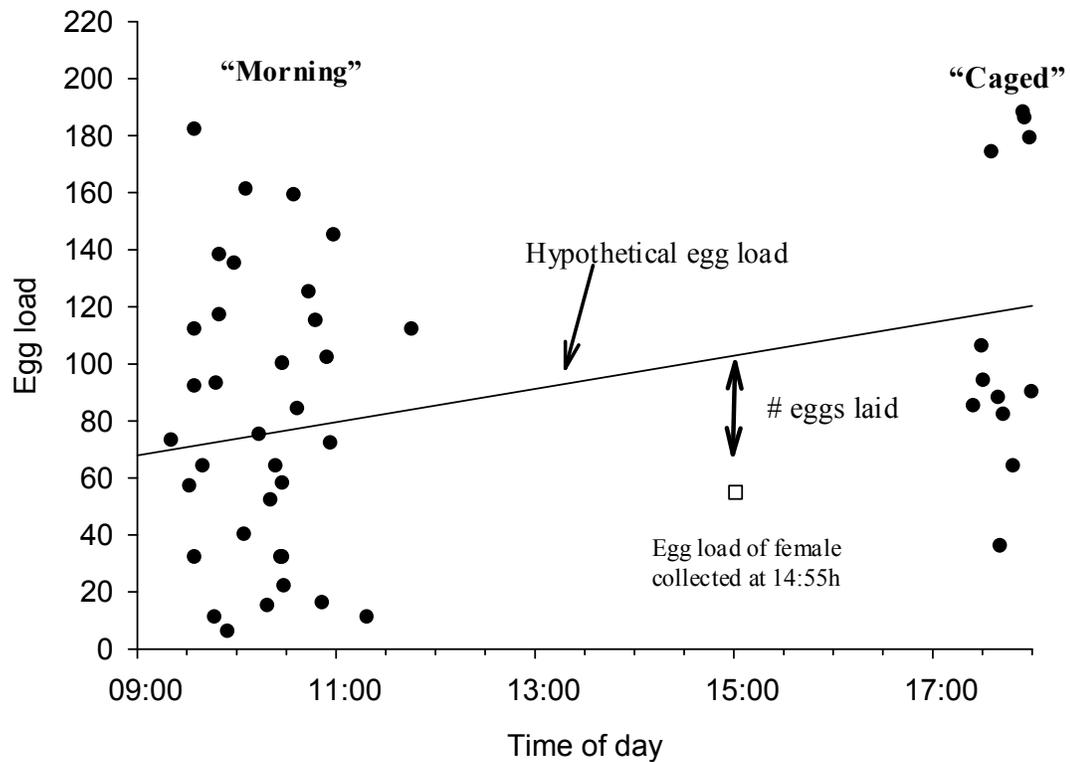
Figure 3.1: Estimation of field oviposition rates based on linear regression of egg loads of "morning" and "caged" female *B. communis,* illustrated on egg loads of females collected on day 1. Data points shown are egg loads of female *B. communis* collected between 9am and 11am and either frozen immediately ("morning") or kept for 8 hours in a host-free cage ("caged"). The linear regression line represents egg load development over the course of an afternoon in the absence of oviposition (i.e. hypothetical egg load). The estimate of oviposition rate is the difference between hypothetical egg load and egg load of an individual female collected in the afternoon. See also (Lee & Heimpel, 2008) for a detailed description of this egg load estimation procedure.
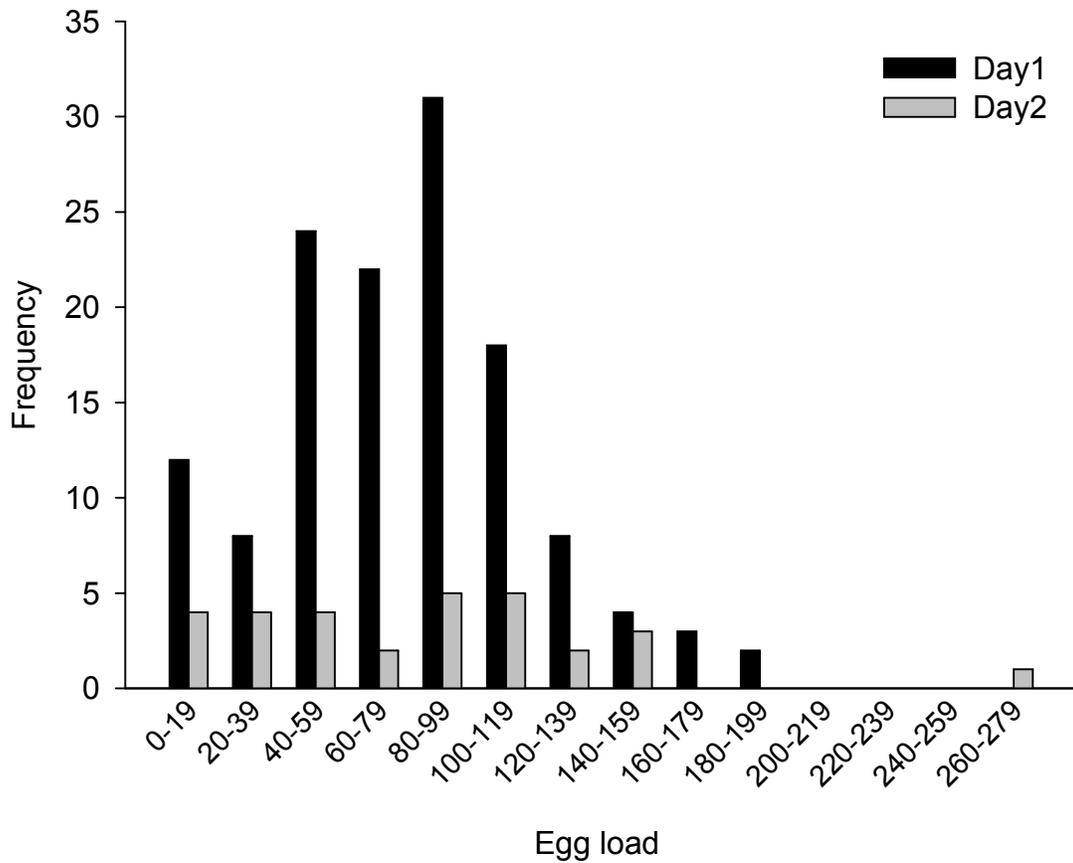
Figure 3.2: Egg load distribution of 162 *B. communis* females collected in soybean plots between 9am and 4pm ("morning" and "afternoon" females combined) in 2007-2008, separated by sampling day.
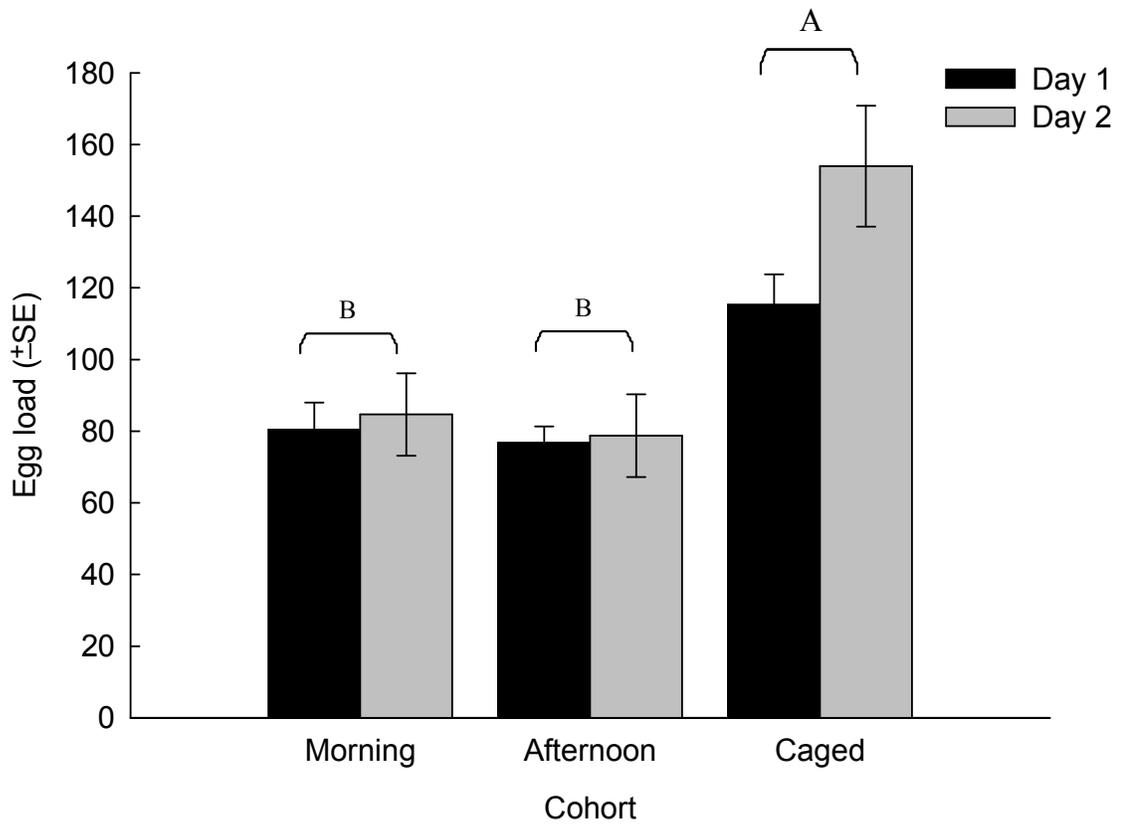
Figure 3.3: Egg load (mean ± SE) of female *B. communis* in the three cohorts, i.e. "morning", "afternoon", and "caged" separated by day. Bars not connected by the same letter are significantly different (Tukey-Kramer HSD for multiple comparisons, P < 0.05).
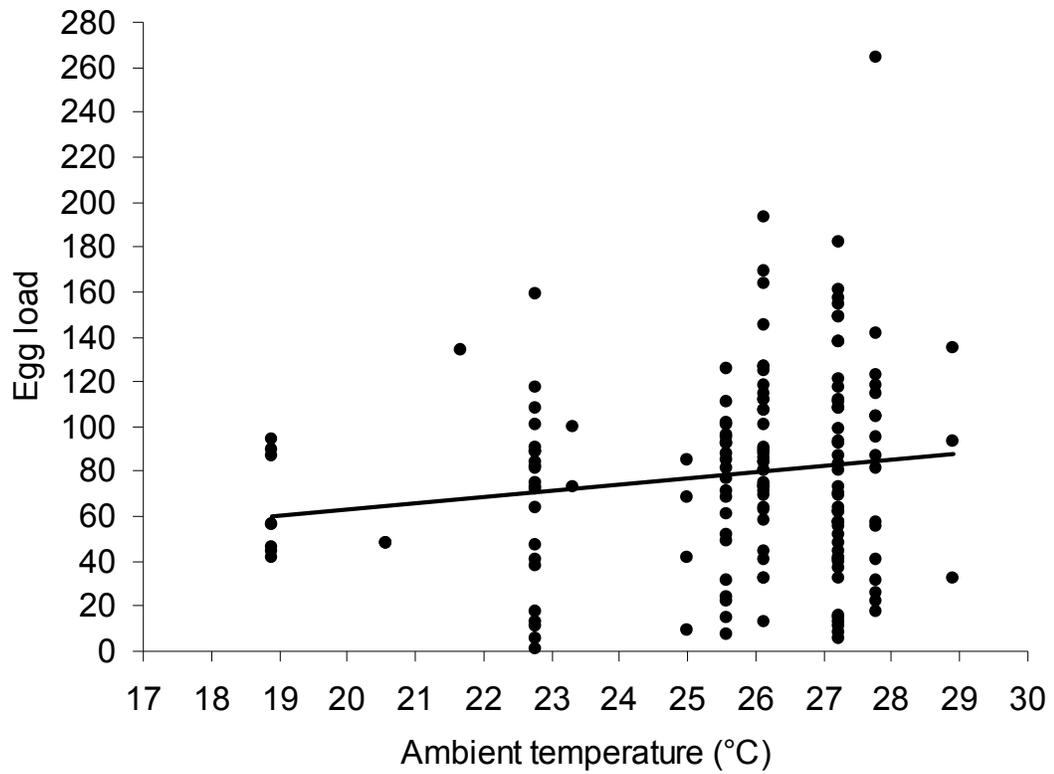
Figure 3.4: Scatterplot showing the correlation between egg load and ambient temperature. Linear regression: egg load = 2.708 * ambient temperature + 9.1769; $r^2 =$ 0.0208.
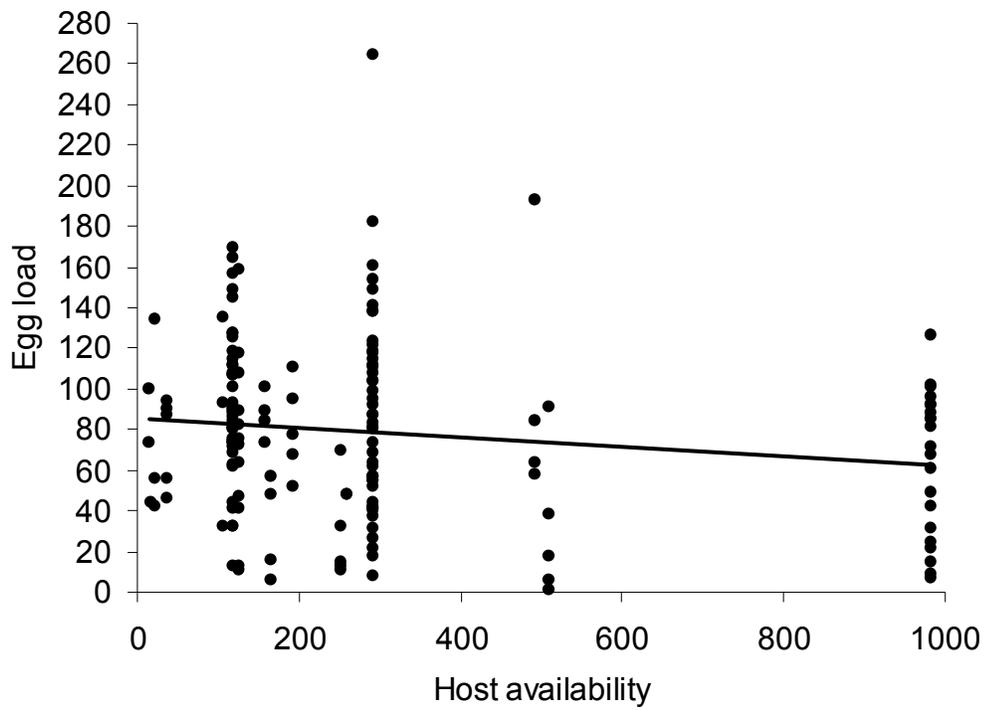
Figure 3.5: Scatterplot showing the correlation between egg load and host availability.

Linear regression: egg load = -0.0227 * host availability + 85.456; $r^2 = 0.0223$.
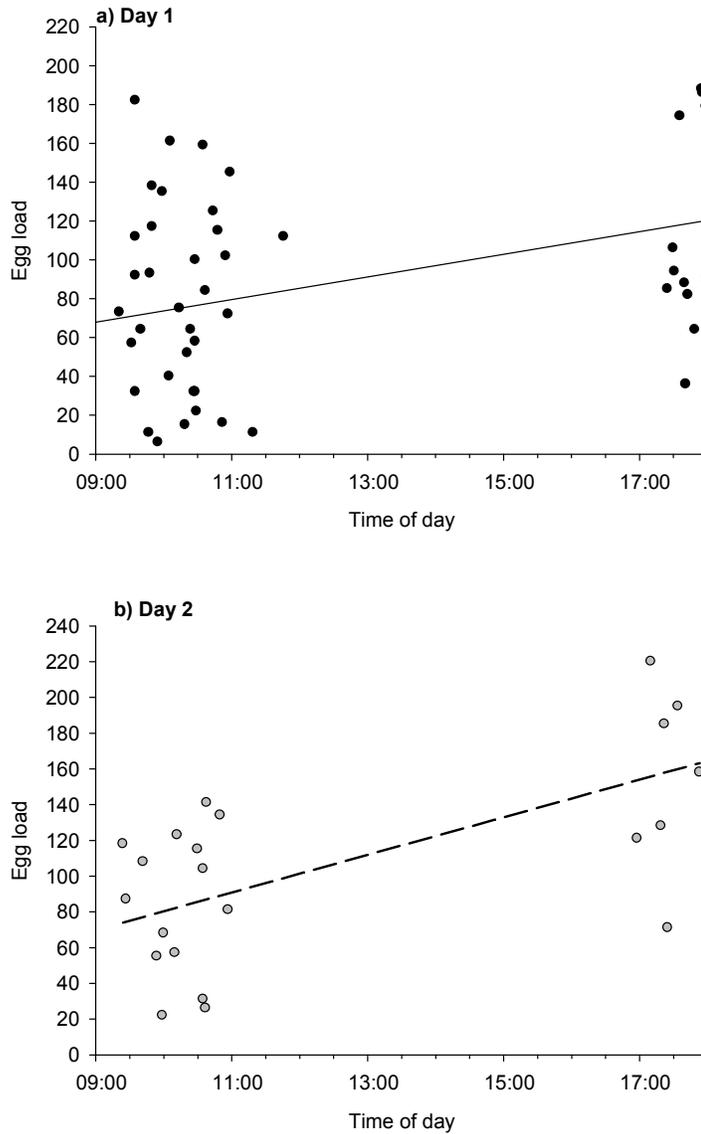
Figure 3.6: Egg maturation slopes and linear egg load estimation ("hypothetical egg load") based on egg loads of "morning" and "caged" female *B. communis* separated by day of collection. a) Linear egg load estimation of day 1 (solid line, "morning" n = 35, "caged" n = 28): egg load = 38.24231 + 0.0011608×Time, $F_{1,61}$ = 7.4619, P = 0.008, r^2 = 0.11. b) Linear egg load estimation of day 2 (broken line, "morning" n = 17, "caged" n = 7): egg load = -13.62 + 0.00267×Time, $F_{1,20}$ = 12.11, P = 0.0024, r^2 = 0.38.
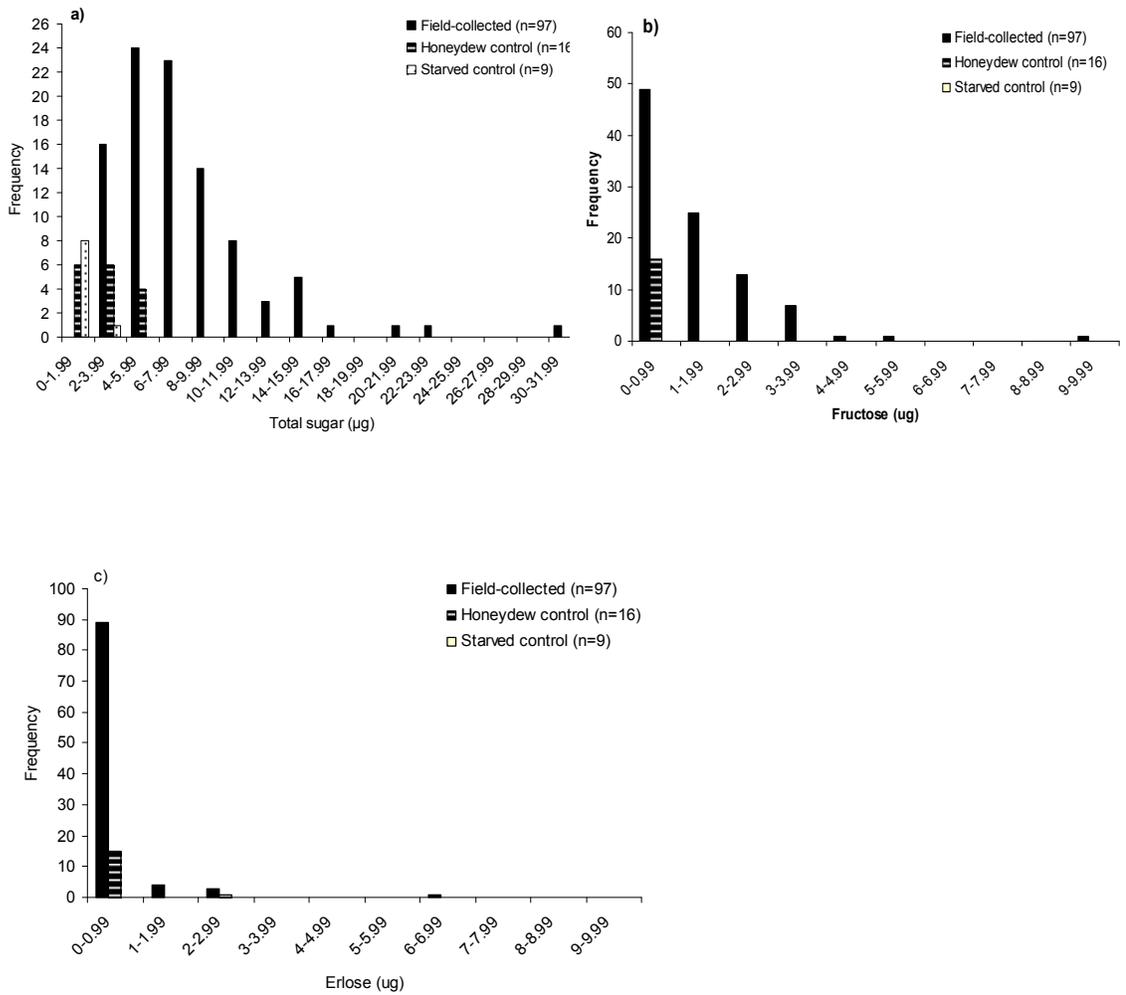
Figure 3.7: Frequency distributions of a) total sugar (µg), b) fructose (µg), and c) erlose (µg) in field-collected *B. communis* in 2008 and honeydew-fed and starved laboratory control parasitoids. Field-collected parasitoids comprise all parasitoids collected between 9am and 4pm on the first and second day of collection. "Caged" females are not included. Note different ranges on the x-axis.
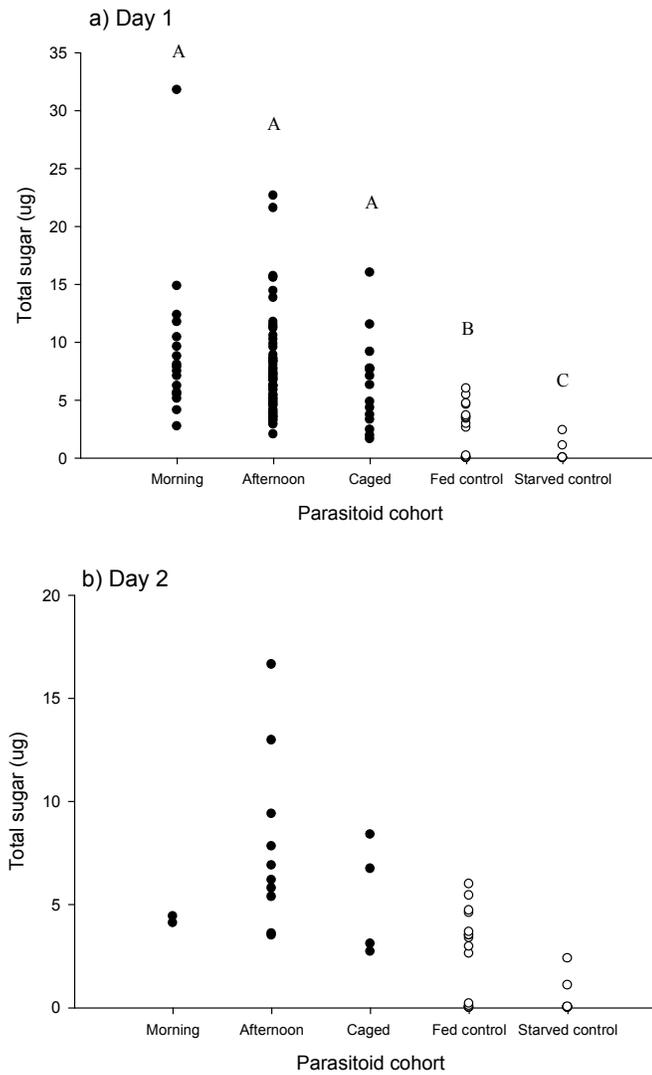
Figure 3.8: Scatterplot of total sugar levels (μg) in the three field-collected *B. communis* cohorts on (a) day 1 and (b) day 2 of collection (closed circles) as well as the honeydew-fed and starved laboratory controls (open circles). a) Day 1 of collection – morning: n = 17, afternoon; n= 68, caged: n = 16, honeydew-fed control: n = 16, starved control: n = 9. Plots not connected by the same letter are significantly different (Tukey HSD, $p < 0.05$). b) Day 2 of collection – morning: n = 2, afternoon: n = 10, caged: n = 4, honeydew-fed control: n = 16, starved control: n = 9.
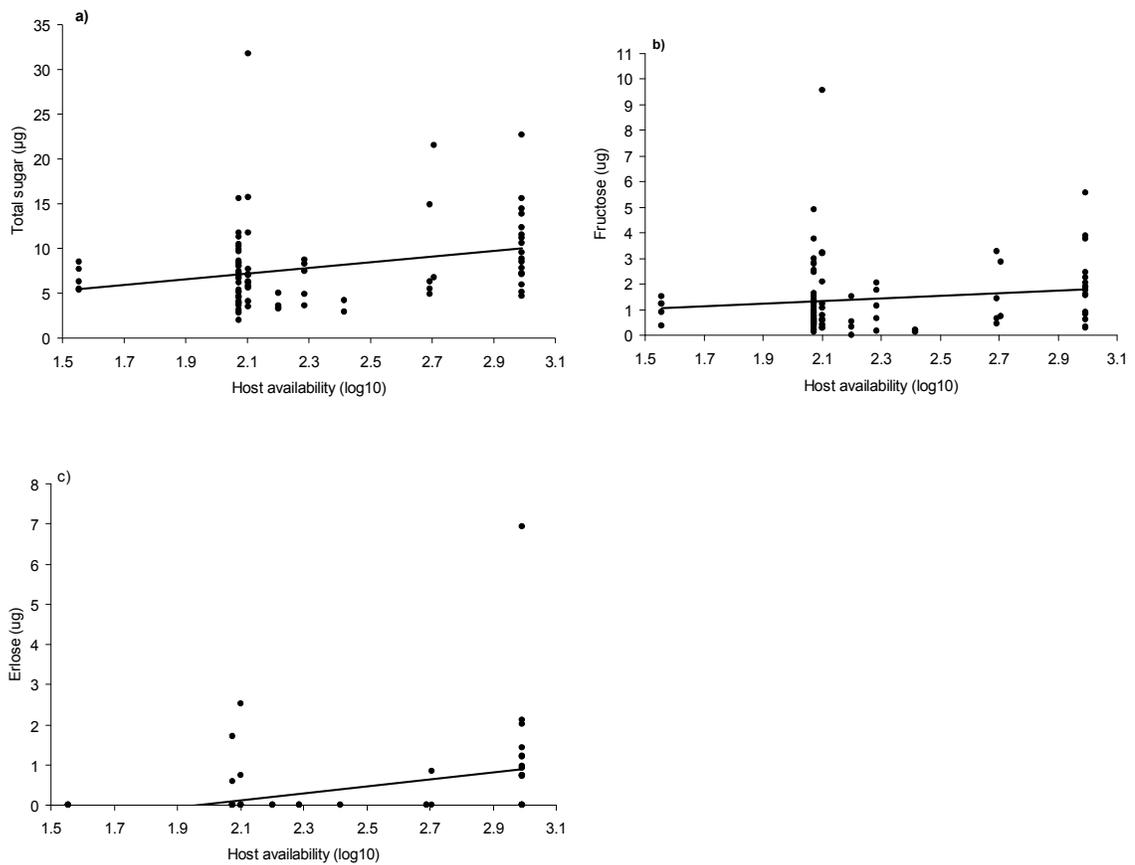
Figure 3.9: Scatterplots showing the correlation between total sugar (µg) (a), fructose (µg) (b) as well as erlose (µg) (c) and host availability (log10). a) Total sugar (µg): Linear regression: total sugar (µg) = 3.2058*(log10-host availability) + 0.4531, r^2 = 0.0769; b) Fructose (µg): fructose (µg) = 0.5213*(log10-host availability) + 0.2387, r^2 = 0.0219; c) Erlose (µg): Linear regression: erlose (µg) = 0.8697*(log10-host availability) - 1.7033, r^2 = 0.157).
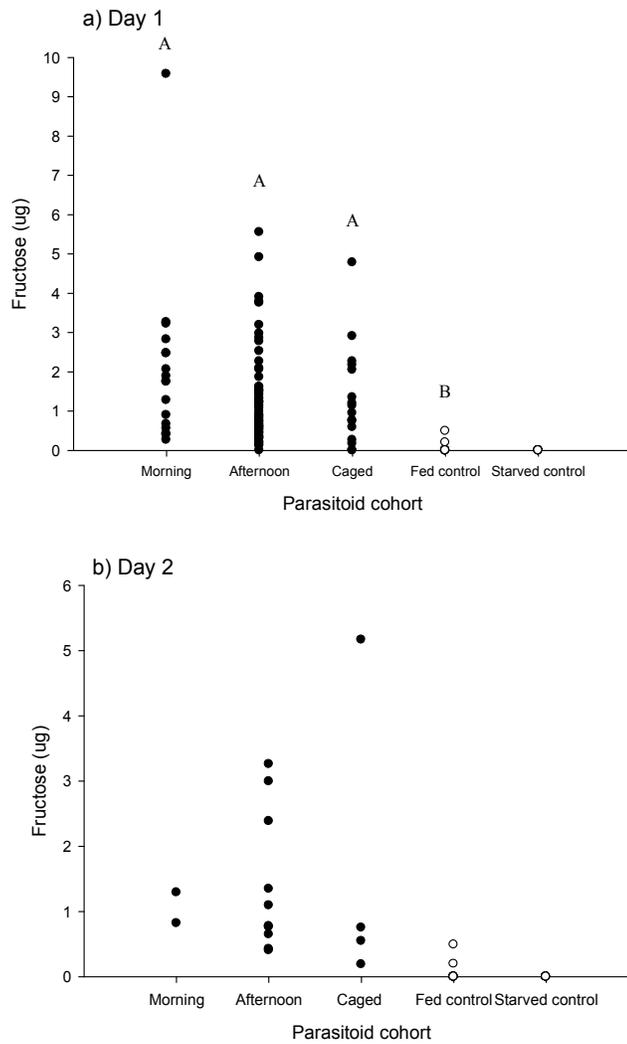
Figure 3.10: Scatterplot of total sugar levels (µg) in the three field-collected *B. communis* cohorts on (a) day 1 and (b) day 2 of collection (closed circles) and the honeydew-fed and starved laboratory controls (open circles). a) Day 1 of collection – morning: n = 17, afternoon: n = 68, caged: n = 16, honeydew-fed control: n = 16, starved control: n = 9. Plots not connected by the same letter are significantly different (Tukey HSD, $p < 0.05$).

b) Day 2 of collection – morning: n = 2, afternoon: n = 10, caged: n = 4, honeydew-fed control: n = 16, starved control: n = 9.

Chapter 4

Host choice behavior in field-collected *Binodoxys communis*

Introduction

Host use behavior in insects has been predicted to be dynamic as a means of balancing

the risk of egg and time limitation (Courtney et al., 1989; Mangel, 1989a; Mangel,

1989b). Female insects should focus on high-quality prey or hosts when they are egg

limited and accept a wider range of hosts when they are time limited (Iwasa et al., 1984;

Houston & McNamara, 1986; Charnov & Stephens, 1988; Courtney et al., 1989; Mangel,

1989a; Collier, 1995; Heimpel & Collier, 1996; Heimpel et al., 1996; Mangel & Heimpel,

1998). In parasitoids oviposition decisions have been shown to be flexible responding to

changes of external and internal state variables acting on an individual. These state

variables include both physiological (i.e. egg load, nutritional state, age) and

environmental factors (i.e. host availability, host quality, weather) and influence a

parasitoid's perceived risk of becoming egg or time limited. A plethora of theoretical and

empirical work has identified egg load as playing a central role in parasitoid host choice

and oviposition decisions (Iwasa et al., 1984; Courtney et al., 1989; Mangel, 1989a;

Minkenberg et al., 1992; Mangel & Heimpel, 1998)

Empirical studies have identified behavioral adaptations in parasitoid oviposition

behavior that maximize lifetime reproductive success by balancing the risk of egg and

time limitation. These behavioral adaptations include but are not limited to: acceptance

of low-quality hosts (Ikawa & Suzuki, 1982; Roitberg et al., 1992; Visser et al., 1992),

adjustment of host encounter rates (Donaldson & Walter, 1988; Rosenheim & Rosen,

1991), adjustments of clutch size per host (Rosenheim & Rosen, 1991), and use of hosts

for host feeding *vs.* oviposition (Collier, 1995b; Heimpel & Collier, 1996; Heimpel et al., 1996; McGregor, 1997). In *Microctonus pseudoplatani* a decrease in egg load was correlated with a reduction in its host encounter rate (Collins & Dixon, 1986). Female *Aphytis lingnanensis* laid smaller clutches per host (Rosenheim & Rosen, 1991) and *A. aonidiae* showed a higher probability of host feeding in contrast to oviposition (Heimpel et al., 1996) in response to decreasing egg loads. The occurrence of superparasitism, i.e. the acceptance of a previously parasitized and therefore suboptimal host, in *Venturia canescens* was correlated with high egg loads (Fletcher et al., 1994; Hughes et al., 1994).

In addition to egg load and host availability, the time a parasitoid has for foraging (i.e. its life expectancy) and its risk of mortality are also predicted to affect host choice decisions. Adult parasitoid life expectancy can vary with a female's age as well as with nutritional status, short-term weather events as well as seasonal changes, and predation pressure (Roitberg et al., 1992; Roitberg et al., 1993; Fink & Völkl, 1995; Heimpel et al., 1997a; Weisser et al., 1997; Wyckhuys et al., 2008a). Parasitoids with an increased risk of becoming time limited are predicted to have a higher likelihood of accepting low-quality hosts in order to maximize lifetime reproductive success (Iwasa et al., 1984; Houston & McNamara, 1986; Mangel, 1989a; Godfray, 1994). This has been shown in *Leptopilina heterotoma*, a parasitoid of *Drosophila* spp., for example. Female parasitoids that perceived an increased risk of mortality based on a rapid deterioration of their environment had an increased acceptance rate of sub-optimal hosts (Roitberg et al., 1993).

All the studies outlined so far have focused on female insects responding to intraspecific variances in host quality. To our knowledge, no studies have been conducted that specifically addressed dynamic behavior in parasitoids in response to interspecific host quality differences. The concept of a dynamic host choice behavior that responds to interspecific variations in host quality has important implications for host range width and host specificity in insects, which in turn affect host-parasitoid interactions and community structures (Getz & Mills, 1996; Heimpel et al., 2003) as well as biological control efficacy and safety issues (Babendreier et al., 2005)

There is a dichotomy in the definition of host ranges in insect parasitoids and herbivores, i.e. physiological vs. ecological host range. Physiological host ranges are thought of as the whole of host species that have been found to be attacked by a female insect. They are typically the result of laboratory studies and literature reviews (Askew & Shaw, 1986; Shaw, 1988; Onstad & McManus, 1996; De Nardo & Hopper, 2004). The ecological host range, on the other hand, includes only those host species that have been found to be under attack in the field (Onstad & McManus, 1996; Strand & Obrycki, 1996; de Nardo & Hopper, 2004). The difference between these two host ranges can partly be explained by the existence of temporal or spatial refuges (Askew, 1994; Strand & Obrycki, 1996; Van Driesche et al., 2004; Wyckhuys et al., 2009), host defensive behavior (Desneux et al., 2009), or host plant characteristics that interfere with the foraging process (Hua et al., 1987; Babendreier et al., 2003). However, the influence of physiological state variables on the acceptance and rejection of host species in the field is understudied. It has been

hypothesized that host choice behavior as has been observed for several parasitoid species in the field might be the result of egg or time limitation on part of the females (Driessen & Hemerik, 1992; Janssen, 1989). For example, time limited females might be more prone to accepting a wider range of host species in the field (as well as the laboratory) compared to egg limited ones in order to maximize their lifetime reproductive success. However, empirical studies that explicitly address the influence of egg and time limitation on host specificity in parasitoids have not been conducted so far, in either the laboratory or the field. And researchers are becoming increasingly aware of this shortcoming (Heimpel & Casas, 2008).

In this study, we tested the influence of physiological (egg load, age, and nutritional status) as well as environmental variables (host availability and weather) on the host acceptance behavior in the soybean aphid parasitoid *Binodoxys communis* (Gahan) (Hymenoptera: Braconidae) under semi-field conditions. The focus in this study was on the role of egg and time limitation on the acceptance or rejection of host species of varying quality in terms of parasitoid larval survival. Furthermore, a floral nectar source was added to every other field plot in an attempt to vary the risk for time limitation due to starvation through parasitoid nutritional status. Sweet alyssum, *Lobularia maritima* (L.) Desv. (Brassicaceae, tribe Alysseae), was chosen as the floral nectar source based on its documented positive effect on parasitoid longevity (Araj et al., 2008).

Material & Methods

Host-parasitoid system

Binodoxys communis is an oligophagous specialist parasitoid that sting aphids in the tribe Aphidini and primarily in the genus *Aphis* (Fam. Aphididae, Subf. Aphidinae) (Desneux et al. 2009). Field-collected female *B. communis* were exposed to either a high-quality host (*A. glycines* Matsumura) or a low-quality host (*Aphis craccivora* Koch) of known size. The high-quality host species of choice was the soybean aphid, *A. glycines*, which *B. communis* has been released against in North America in 2007. *Aphis craccivora* has been shown to be a low-quality host species to *B. communis* in terms of acceptability as well as parasitoid larval survival (Desneux et al., 2009). In the laboratory, female *B. communis* detected individual *A. craccivora* in over 90% of the times that they came in contact with this aphid in no-choice tests, and in about half of these encounters female parasitoids successfully stung the host individual. However, less than 5% of these ovipositions resulted in a viable adult parasitoid (Desneux et al., 2009). Therefore, we found this aphid species to be an appropriate low-quality host species.

Insect rearing

Aphis glycines, i.e. the high-quality host species, used in this experiment was originally established from aphids collected from a soybean field in St. Paul, MN, USA, in 2003. Since then aphids have been reared on soybean plants (Syngenta NK S19-R5) in growth chambers (L16: D8; 60-80% r.h.; 25 ± 5 °C).

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The colony of *Aphis craccivora*, i.e. the low-quality host, was reared on *Vicia fabae* L. in growth chambers (L16: D8; 60-80% r.h.; 25 ± 5 °C). It was originally established from individuals collected from soybean crops at the Minnesota Agricultural Experiment Station located in St. Paul, MN, USA.

Binodoxys communis intended for mass-releases was reared on *Aphis glycines*-infested soybean plants. The parasitoid colony used in this study originated from a strain collected in the Chinese province of Heilongjiang in 2002 (Wyckhuys et al., 2008a). In the summer of 2009, *B. communis* was not established in Minnesota and therefore we established local populations of free-foraging parasitoids by releasing laboratory-reared parasitoids into experimental plots. (Asplen et al., in preparation; Heimpel et al., 2010). *Binodoxys communis* were reared in commercial insect rearing cages (BugDorm-44545, MegaView Science Co., Ltd., Taiwan, 47.5 × 47.5 × 47.5 cm) in an environmentally-controlled greenhouse located at the Minnesota Agricultural Experiment Station at the University of Minnesota, St. Paul, USA. Each cage contained 14 9 × 9 cm plastic pots with aphid-infested soybean plants (Syngenta NK S19-R5) and at least 20 females and as many male parasitoids. Parasitoids were released into the experimental plots on the morning of their eclosion. On the evening before each release, soybean plants covered with parasitoid 'mummies' (i.e. parasitoid pupal stage) of 12 soybean plant pots were cut down and transferred into another rearing cage containing clean soybean plants only to allow non-parasitized *A. glycines* to relocate. The following morning, the aphid-free plant cuttings were equally distributed among eight storage crates (39 × 35 × 10 cm,

Sterilite® Co., Townsend, MA) used as release containers. The crates were modified by covering their insides with nylon white net fabric (100% nylon, 183 cm × 36 m) which allowed parasitoids to exit the crates but excluded predators. In addition, during transport of the crates from the greenhouse to the field plots each crate was tightly covered with no-see-um mesh (Quest Outfitters, Sarasota, FL, USA).

Field plots

The goal of our study was to test host choice behavior in field-collected female *B. communis*. The study was conducted in a single soybean field (0.43 ha, soybean variety: Pioneer T91Y70) that was located at the University of Minnesota Agricultural Experiment Station, St. Paul, MN, USA. From 8 July through 7 September 2009, *Binodoxys communis* was mass-released into two flagged 3 × 4 m plots embedded within the soybean field on each of 8 separate release dates. Each plot contained about 351 soybean plants, was used for a single release only with at least 30 m between individual plots, and approximately 30 m away from a field edge. Prior to the first release each field was treated with the herbicide Round-up (The Scotts Company LLC, Marysville, OH, USA).

To each plot 12 13 × 16.5cm plastic pots with *Lobularia maritima* (Livingston Seed Co., Columbus, Ohio) were added; one plot received *L. maritima* at the flowering stage and the other one where flower buds were not open yet. On the morning of each release, *L. maritima* pots were evenly spaced in pairs between rows 2-3 and 4-5 of each plot and buried into the soil up to the pot rim.

Between 9 am and 10 am, release containers were placed among the same rows as the paired *L. maritima* pots with two containers per row. Collection of parasitoids started at 1pm on the day of each release and between 10am and 4pm on the following three days. Soybean plants were manually searched for a minimum of 10 min per single plant and each recovered adult parasitoid was aspirated, sexed and transferred into a 0.65 ml microcentrifuge tube (DOT Scientific, Inc., USA). Female *B. communis* were used immediately in the bioassay.

Bioassay

Host choice behavior in *B. communis* was studied under semi-field conditions using a no-choice experimental set-up. Adjacent to the soybean field, a field-based "bioassay station" (Fig. 4.1) was established to conduct bioassays with minimal delay between parasitoid collection and behavioral observation. The bioassay station consisted of a portable 0.7 m×1.3 m×1.5 m folding garden work bench, a dissecting microscope, a laptop for behavioral recordings, and a windbreak consisting of a 0.6 m×1.8 m white sheet. The observational arena used on the bioassay station consisted of a 100 × 15mm Petri dish filled with a thin layer of 2% agar (Fisher Scientific, Pittsburgh, PA, USA) and a single leaf (soybean for *A. glycines* and fava bean for *A. craccivora*) with the stem placed in a 1.5 ml microcentrifuge tube. Prior to being observed within this arena, each field-caught *B. communis* was transferred into a clear plastic dome (d = 1cm, h = 0.65cm) and allowed to settle for 5min. The dome with the parasitoid was then placed over the single host aphid and observation began when the parasitoid set foot on the leaf. The

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observational arena was placed under a dissection microscope at 10× magnification. Various aspects of parasitoid behavior were recorded using a laptop with the free software JWatcher V1.0 (Macquarie University, Sidney & UCLA).

Parasitoid behaviors were coded as "contact" (physical contact between host and parasitoid but no antennal examination on the part of the parasitoid), "antennation" (antennal examination of the host by the parasitoid), "acceptance" (bending of the parasitoid abdomen towards the aphid), and "oviposition" (insertion of the ovipositor into the aphid for >3 s) (Desneux et al., 2009). In addition, "handling time" as well as "search intensity" were calculated from those observations in which the first host was accepted for oviposition. "Handling time" was defined as the total time from "contact" through "oviposition". "Search time" was defined as the time from the start of an observation until first "contact" that resulted in an oviposition.

Host acceptance behavior in field-collected parasitoids was tested using a sequential no-choice test. Female parasitoids were randomly assigned to one of two no-choice test sequences each consisting of two consecutive single aphid hosts on a leaf of its respective host plant. The first host was either *Aphis craccivora* Koch (on: *Vicia fabae*), i.e. the low-quality host species (LQH), or *Aphis glycines* (on: *Glycine max* L.), i.e. the high-quality host species (HQH). The second host was always an HQH and was added to control for physiological constraints on part of the parasitoid. Only parasitoids that successfully stung the second host were included in the analyses but behavior recorded on the second host-parasitoid interaction was not part of the statistical analyses.

Observations lasted until an oviposition event, for a maximum of 5min, or until a host was antennated but not used for oviposition (i.e. rejected). Preliminary studies using the same observational design have shown that female *B. communis* reject or accept a host for oviposition as a result of the first antennation event. Therefore, the latter cut-off point was included to avoid obtaining false positives resulting from repeated host-parasitoid run-ins. A potential influence of aphid size on host choice was accounted for by pairing 3rd instar *A. glycines* (i.e. the host instar preferred by *B. communis*, (Wyckhuys et al., 2008b)) with *A. craccivora* individuals that were of equal size in each test sequence. Following the end of the second host-parasitoid exposure, female parasitoids were frozen at -80°C for later dissections and egg load counts.

Dissections and preparation for sugar analyses

Egg loads of recaptured as well as caged female parasitoids were assessed by dissecting each female under a dissecting microscope (50x magnification). For a detailed description of the dissection methods refer to (Dieckhoff & Heimpel, 2010). Parasitoid size was assessed by measuring the hind tibia length of each adult. Prior to each dissection, a single hind leg was removed from the parasitoid body and the length of the hind tibia measured using a dissecting microscope (50x magnification). In addition, parasitoid gut sugar contents were analyzed with HPLC (high-performance liquid chromatography). After removal of the parasitoid ovaries the remainder of the insect body was transferred into a 0.5ml microcentrifuge tube filled with a 40 µl aqueous

solution of Ringer's solution and 100 % ethanol (1:1 ratio) and stored at room

temperature until the analysis.

Sugar analysis

Parasitoids were supplied individually in 0.5ml microcentrifuge tubes each containing a

40µl aqueous solution of 1:1 Ringer's solution:100% ethanol. In a first step of preparing

the samples for the HPLC, each sample was transferred into 160µl of 100% ethanol, and

then evaporated under vacuum in a centrifuge at 45°C. 20µl of 70% ethanol and 180µl of

ultrapure water were added to a total volume of 200µl per sample. The samples were

then thoroughly macerated with a pestle within the microcentrifuge tubes, transferred into

a 2ml centrifugal filter device (Ultrafree – MC with a 0.22µm PVDF Durapore

membrane, Millipore Corporation, MA), centrifuged for 2min at 13,000rpm, and stored

overnight at -20°C. The filtrate was pipetted into a 2ml screw top vial with an insert and

a split cap top (Chromacol Ltd., Thermo Fisher Scientific, UK) and then filled into an

HPLC auto-sampler tray set to a constant temperature of 10°C. Samples that could not

be analyzed immediately were stored at -20°C. Sugar analyses were conducted as

described by Wyckhuys et al. (2008a); 10 µl of each sample were injected into a Dionex

DX 500 HPLC-system (Dionex Corp., Sunnyvale, CA, USA) equipped with a GP 40

gradient pump, a Carbopac PA1 guard (4×50 mm^2), an analytical column (4×250 mm),

and an ED 40 electrochemical detector for pulsed amperimetric detection (PAD) (Dionex,

Leeds, UK). The column was eluted with 1 M NaOH and Milli-Q water (10:90 %, 1 ml

min^{-1}) and kept at 20 °C for the analysis. Calibration standards with concentrations of

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2.5ppm, 5.0ppm, 7.5ppm, and 10ppm for daily reference curves were prepared containing

the following 16 sugars: Sorbitol, Mannitol, Trehalose, Rhamnose, Galactose, Glucose,

Sucrose, Mannose, Fructose, Melezitose, Melibiose, Raffinose, Lactose, Stachyose,

Erlose, and Maltose. Individual sugar peaks were analyzed using PEAKNET Software

Release 5.1 (DX-LAN module; Dionex, Leeds, UK).

In addition to the field-collected parasitoids, ~5µl of pure *L. maritima* nectar was

collected, stored in 40 µl Ringer's solution and 100% ethanol (1:1 ratio), and its sugar

composition analyzed using HPLC. The HPLC analysis showed that *L. maritima* nectar

was mainly comprised of the monosaccharide mannose (~68%), followed by sucrose

(~17%) and glucose (~13%) which is in accordance with at least one prior study (Davis et

al., 1998). In addition, trace amounts of erlose (~1.5%) and galactose (~0.1%) were also

detected. Neither mannose nor galactose have been documented in soybean aphid

honeydew contrary to erlose (Heimpel et al., 2004; Wyckhuys et al., 2008a) (and see also

Chapter 3). Thus, the presence of both mannose and galactose were used to assess nectar

feeding in field-collected parasitoids. Total sugar concentrations of individual parasitoids

were obtained by calculating the sum of the concentrations of all 16 sugars. The effects

of log10-host and log10+1-nectar presence-absence, hind tibia length, day of collection,

time of day on log10-total sugar as well as log10-fructose concentrations were analyzed

using multiple linear regression.

Statistical analysis

Egg load was square-root transformed to meet the assumption of homogeneity. The influence of day of collection and time of day on √Egg load was analyzed using an ANOVA, followed by a Tukey HSD for multiple comparisons of means. Multiple linear regression was used to analyze the effects of hind tibia length, day of collection, flower presence-absence, host availability, log10-total sugar concentrations and host quality treatment on √egg load.

Both search time and handling time were log10-transformed to meet the assumption of homogeneity. Search as well as handling time between females that accepted or rejected a host individual was compared using t-test. Variables influencing search intensity and handling time were analyzed using stepwise linear regression. Variables were host species, day of recapture, time of day, log10-total sugar concentration, log10-host availability, temperature, hind tibia length, √egg load, waiting time (i.e. the time between collection and the bioassay), and flower presence-absence.

The probability of acceptance of a host species (dependent variable) in *Binodoxys communis* was analyzed using forward stepwise logistic regression. Independent variables were day of recapture, time of day, log10-total sugar concentration, log10-host availability, barometric pressure (mm Hg), hind tibia length, √egg load, waiting time (i.e. the time between collection and the bioassay), flower presence-absence, host quality, and the interaction terms host quality×√egg load and day of collection×egg load.

Results

Egg load dynamics in field-collected parasitoids

Egg load in field-collected *B. communis* ranged from a minimum of 2 eggs on day 1 of

collection to a maximum of 297 eggs on day 2 of collection (Fig. 4.2). Egg load varied

significantly by day of collection ($F_{3,135} = 4.35$, $P = 0.0058$) but not over time of the day

($F_{1,135} = 0.02$, $P = 0.8833$) (Fig. 4.3). There was a significant positive effect of total body

sugar concentration on egg load ($F_{1,128} = 4.10$, $P = 0.0449$). This is the first time that a

significant effect of body sugar concentration on egg load for *B. communis* has been

documented (Dieckhoff and Heimpel, 2010) (see also Chapter 3). There was no

significant difference in egg loads between females assigned to the high-quality or the

low-quality host treatment ($F_{1,128} = 3.05$, $P = 0.0831$). Egg load was also independent t of

hind tibia length ($F_{1,128} = 1.05$, $P = 0.3086$), host availability ($F_{1,128} = 0.61$, $p = 0.4358$),

and flower presence-absence ($F_{1,128} = 2.22$, $p = 0.1384$, respectively).

Nutritional status and feeding history

Total sugar concentrations in field-collected *Binodoxys communis* ranged from 0 µg to

3.40 µg (Fig. 4.4). Host availability had a significant positive effect on total sugar

concentration ($F_{1,129} = 5.87$, $P = 0.0168$) while flower presence-absence did not ($F_{1,129} = $

0.79, $P = 0.3758$). Also, there was a significant positive correlation between hind tibia

length and total sugar concentration ($F_{1,129} = 6.60$, $P = 0.0113$). Total body sugar

concentrations were not significantly different over the course of the day ($F_{1,129} = 1.26$, P

$= 0.2634$) or among the four days of collection ($F_{1,129} = 0.01$, $P = 0.9414$) (Fig. 4.5).

Fructose concentrations in field-collected parasitoids ranged from 0µg to 1.92 µg (Fig. 4.4). A total of 63 (out of 140) females did not test positive for fructose suggesting that a large proportion of field-collected *B. communis* in this study had not fed on sugar in the field or had digested the sugar before they were collected. Fructose concentrations were significantly correlated with host availability ($F_{1,131}$ = 7.78, P = 0.0061) but not with any of the other predictors (day of collection: $F_{1,131}$ = 0.29, P = 0.5913; time of day: $F_{1,131}$ = 0.35, P = 0.5529; nectar presence-absence: $F_{1,131}$ = 0.00, P = 0.9445; hind tibia length: $F_{1,131}$ = 1.33, P = 0.2516).

There was no indication that nectar feeding was a common occurrence in field-collected parasitoids. Only two (out of 140) field-collected *B. communis* tested positive for mannose and not a single female for galactose and both females that tested positive for mannose were collected from plots that had no nectar provided. In addition, even though both fructose and total sugar concentrations were significantly correlated with host availability, i.e. soybean aphid density, only a single female parasitoid tested positive for the honeydew signature sugar erlose. This indicates that field-collected *B. communis* in this study did either not feed on soybean aphid honeydew in the field or that honeydew signature sugars were digested before parasitoids were collected.

Host choice decisions

Out of the 140 female *B. communis* that were subjected to the bioassay, 19 females did not detect the aphid they were exposed to (Fig. 4.6). 60% of the the *A. craccivora* offered to female *B. communis* were rejected and about 20% were either accepted or not

detected (Fig. 4.6). In contrast, 92% of *A. glycines* were accepted (i.e. 92%), while 6% and 2% were rejected or not detected, respectively. Egg loads were not significantly different in parasitoids that rejected, accepted, or did not discover *A. craccivora* ($F_{2,88}$ = 2.53, p = 0.0857). Out of the 49 female *B. communis* that were given *A. glycines,* only a single female did not discover the host and only three rejected it. Therefore, we analyzed the data set in two ways: 1) we analyzed the data set including all 121 female *B. communis* subjected to the bioassay and accounted for host species by including it as an independent variable; 2) we included only parasitoids that were given *A. craccivora* (the low-quality host species) in the analysis.

Female *B. communis* had a significantly higher probability of accepting the high-quality host, *A. glycines*, for oviposition compared to the low-quality host, *A. craccivora* (Table 4.1). Day of collection had a significant negative effect on the probability of host acceptance in female parasitoids. That is, the more time a female parasitoid spent in the field before it was collected and the older it was, the less likely it was to accept a given host of either species (Fig. 4.7a). Only 3 out of a total of 48 *A. glycines* hosts were rejected in contrast to 55 out of 73 *A. craccivora* host individuals; thus, female *B. communis* became essentially more choosy towards the low-quality host over the four days. Finally, host acceptance decreased with increasing barometric pressure (Fig. 4.8). An increase in barometric pressure was correlated with fair weather, i.e. sunshine, reduced wind, and no rain. As mostly low-quality hosts were rejected, this indicates that females were more likely to reject a low-quality host on a fair day compared to a day when the weather was changing.

When considering only the host acceptance behavior towards *A. craccivora*, day of collection still had a significant influence (Table 4.2a, Fig. 4.7b). In addition, the time of day had a marginally significant influence on acceptance. That is, the probability that *A. craccivora* decreased slightly over the day. Barometric pressure had only a marginal effect on host choice when taking only observations with low-quality hosts into account. Waiting time had an influence on acceptance; the more time a female had to spent waiting in the observation dome prior to the bioassay itself the more likely she was to accept *A. craccivora*. Waiting times in field-collected parasitoids ranged from 5min to 18min. Therefore, we ran the analysis on low-quality hosts again this time excluding female parasitoids that had to wait more than 10min prior to the bioassay to see if the influence of other biologically more relevant variables were obscured by this range of waiting times. Barometric pressure as well as host availability had a significant influence on the probability of acceptance of a low-quality host confirming our suspicion that waiting time overshadowed their relative influence on host choice behavior (Table 4.2b).

Search time

Female *Binodoxys communis* took significantly longer to locate *A. craccivora* (the high-quality host species) than *A. glycines* (the low-quality host species) (Table 4.3a). Temperature had a significant positive influence on how long it took a female to locate a host, i.e. hosts were located faster with increasing temperature. Also, search time increased with increasing host availability. This indicates that female parasitoids that had presumably had more host experience prior to the bioassay took longer to locate the host.

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Time to locate a host was not correlated with the amount of total body sugars of a parasitoid. Only three female *B. communis* rejected a high-quality host which precludes a comparison of search times between high-quality hosts that were accepted or rejected. Average search times for *A. craccivora* were not significantly different between female *B. communis* that rejected or accepted the host (t = 1.52, d.f. = 66, P = 0.1334). And only host availability had a significant influence on search time of field-collected parasitoids that were offered *A. craccivora* (Table 4.3b).

Handling time

Female *B. communis* that accepted a host for oviposition had a significantly longer handling time compared to females that rejected a host regardless of species (t = -11.03, d.f. = 119, P <0.00001). Handling time in *B. communis* was significantly influenced by host species (Table. 4.4a). Female parasitoids offered *A. glycines* had a significantly longer (13.46 ± 1.74s (mean ± SE)) handling time than parasitoids offered *A. craccivora* (6.2 ± 1.02 s (mean ± SE)). A comparison of handling times between females that rejected or accepted *A. craccivora* confirmed that handling times were longer when the host was accepted (t = -7.60, d.f. = 71, P < 0.0001). Also, there was a significant negative correlation between handling time and host availability. Females collected from a host-poor environment and with presumably less prior host experience had a significantly longer handling time compared to more experienced females. Furthermore, female parasitoids with low egg loads had a significantly longer handling time compared to females with higher egg loads (Fig. 4.9; Table 4.4a). Again, the low sample size of

high-quality hosts that were rejected by *B. communis* made a comparison of handling

times between host species unreliable. A multiple regression model including only

parasitoids that were given a low-quality host identified host availability and egg load to

have a significant influence on handling time (Table 4.4b). In addition, waiting time had

a significant positive influence on handling time in females offered *A. craccivora*.

Discussion

As expected, *B. communis* showed a preference for the high-quality host species *A. glycines*. Environmental factors (low host availability and barometric pressure) increased acceptance of *A. craccivora*, the low-quality host species. These factors may have increased the parasitoid's perceived risk of time limitation leading to the acceptance of a poor quality host species. Host handling time alone was influenced by egg load in *B. communis*. The probability to accept the low-quality host species decreased over the course of the four days of collection. That is, older parasitoids with a potentially higher level of host experience were more likely to reject a low-quality host species. Overall body sugar concentrations in field-collected parasitoids were low which might explain that we did not find an influence of nutritional status on host choice behavior.

Egg load by itself had no influence on host acceptance in *B. communis*. This is contrary to a body of theoretical and empirical work that has suggested that egg load has a strong influence on host acceptance in insects (Courtney et al., 1989; Mangel, 1989b; Rosenheim & Rosen, 1991; Heimpel et al., 1996; Mangel & Heimpel, 1998). Host availability was a stronger indicator for the risk of egg or time limitation in this species. The underlying assumption here is that host availability is an indicator of parasitoid host experience, i.e. females should have a higher host encounter rate (i.e. experience) when host availability is high compared to when it is low. However, one problem with field studies is that egg load and experience are fundamentally confounded as both are affected by host availability (Rosenheim & Rosen, 1991; Collier, 1995a; Heimpel et al., 1996).

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Each host encounter or the lack thereof leads to a change in egg load as well as the level of host experience which in turn influences a parasitoid's perceived risk of becoming egg or time limited (Heimpel et al., 1996). In host-rich environments, for example, parasitoids are predicted to have a low egg load combined with a high level of host experience. Thus, host choice decisions in these field-collected *B. communis* have to be seen in the context of both parasitoid egg load and experience (Heimpel et al., 1996). That is, any observed host choice behavior in the field is the result of the combined influence of egg load and experience acting on an individual. However, the observed precedence of host availability over parasitoid egg load might not come as quite such a surprise given that *B. communi*s maintains a constant egg load presumably as a means of balancing the risk of becoming egg or time limited (Dieckhoff & Heimpel, 2010) (see also Chapter 3).

The influence of host availability on search time in field-collected *B. communis* fits the predictions of egg and time limitation. High host availability was correlated with an increased search time as well as a decreased handling time. Thus, females with an increased risk of becoming egg limited in a host-rich environment had a potentially lower host encounter rate compared to females from a host-poor environment. Similar patterns regarding search time have been found in several parasitoids (Donaldson & Walter, 1988; Rosenheim & Rosen, 1991) as well as phytophagous insects (Odendaal & Rausher, 1990). It has been hypothesized that such a reduction in host encounter rates minimizes a

female's risk of becoming egg limited (Odendaal & Rausher, 1990; Rosenheim & Rosen, 1991).

Handling time was the only aspect of host acceptance behavior in field-collected *B. communis* that was affected by both host availability and egg load. Handling times decreased as a function of increasing host availability as well as egg load. Thus, females with a higher egg load as well as a presumably higher level of experience spent significantly less time handling a host. There was a strong indication that this decrease in handling time was not based on a higher proportion of females rejecting the host but rather on females reducing the oviposition time. There are three possible non-mutually explanations for this observation: a) females in a host-rich environment are more efficient at oviposition compared to naïve females, b) females in host-rich environments increase their host encounter rate by minimizing the time spent per host, or c) females in host-rich environments minimize their mortality risk by spending less time on each host. A decrease in handling time as a function of host encounter rates has been shown in the genus *Trichogramma* (Gross et al., 1981; Wajnberg, 1989). Alternatively, a decrease in host handling time in host-rich environments may alter the functional response of female parasitoids potentially resulting in higher pest suppression when host availability is high (Rosenheim, 1990). Finally, (Iwasa et al., 1984) hypothesized that the interplay between parasitoid mortality during host handling and egg load should influence host acceptance. The authors theorized that females with a high egg load should minimize their risk of mortality in order to maximize the number of eggs laid. Our observation that handling

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times decreased as a function of high parasitoid egg load as well as high host availability support Iwasa et al.'s (1984) prediction. Predator densities have been shown to be positively correlated with soybean aphid densities in the field (Costamagna & Landis, 2006; 2007), and predation pressure is predicted to have a strong impact on parasitoid mortality in the field (Heimpel et al., 1997b). Thus, a reduction in handling time at high host densities may be adaptive in order to minimize the risk of mortality and therefore time limitation in the field.

Acceptance of a low-quality host species in field-collected *B. communis* was negatively correlated with parasitoid age as well as host availability. Female *B. communis* collected on the first and second day were more likely to accept a low-quality host compared to females collected on the third and fourth day. Again if we assume that host encounter rates increase over time, the level of host experience in female parasitoids should increase as a function of time. Similarly, female parasitoids collected from a host-rich environment with presumably higher host encounter rates showed a lower probability to accept the low-quality host compared to females from a host-poor environment. Visser et al. (1992) found that the level of superparasitism, i.e. the acceptance of low-quality hosts, was influenced by the pre-patch experience of female *Leptopilina heterotoma*. Females were more willing to superparasitize when they had previously been exposed to low-quality habitats compared to females that had been exposed to high-quality habitats. However, host availability may be more strongly confounded with egg load when an increased host encounter rate leads to a decrease in egg load which in turn is predicted to

increase parasitoid choosiness towards host quality (Mangel, 1989; Odendaal & Rausher, 1990; Rosenheim & Rosen, 1991; Visser, 1991; Visser et al., 1992; Heimpel et al., 1996; Heimpel & Rosenheim, 1998). Thus, so far we have shown that experience appears to trump the risk of time limitation in *B. communis*.

Host acceptance behavior in field-collected *B. communis* was also influenced by barometric pressure. Females responded to changes in barometric pressure by increasing their acceptance of low-quality hosts when pressure was dropping. A decrease in pressure generally indicates a deterioration of environmental conditions due to storms and rainfalls. Such severe weather events have been shown to have the potential to significantly decrease lifetime expectancy in insects (Roitberg et al., 1992, 1993; Weisser et al., 1997). Roitberg et al. (1993) showed that *Leptopilina heterotoma* responded to changes in barometric pressure with increased host encounter rates, longer patch residence time, and a higher frequency of superparasitism. The observations on *L. heterotoma* by Roitberg et al. (1993) as well as on *B. communis* here are consistent with the prediction that parasitoids will alter their host acceptance behavior in order to increase lifetime reproductive success (Iwasa et al., 1984; Houston & McNamara, 1986; Mangel, 1989a)

Total body sugar concentrations in *B. communis* in this study were overall low but remained constant over time. Sugar concentrations were positively correlated with host availability which strongly suggests honeydew feeding by *B. communis*. This notion is

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supported by a previous analysis of sugar spectra in field-collected parasitoids which showed soybean aphid honeydew feeding in *B. communis* (see Chapter 3). This study further suggests that even low levels of body sugar concentrations or less frequent feeding events are sufficient to support survival of parasitoids under field conditions based on parasitoids being collected for up to four days. Furthermore, sugar concentrations in field-collected parasitoids were apparently high enough to not increase a female's perceived risk of time limitation due to starvation or else we would have expected to see an influence of nutritional status on host choice decisions.

There was no indication that female *B. communis* fed on the provided nectar source in form of flowering *Lobularia maritima* plants in the field. So, either *B. communis* was physically not able to access the nectar in the flower heads or nectar does not elicit a feeding response in this parasitoid species. (Patt et al., 1997) showed that two eulophid parasitoids comparable in size to *B. communis* were not able to access nectar provided by *L. maritima* as the parasitoid head width was wider than the gaps between the flowers' stamens and petals. And the same mismatch between parasitoid and flower physiology was suggested to be responsible for the lack of nectar feeding on *L. maritima* by *Microctonus hyperodae* (Vattala et al., 2006). Thus, it is possible that such a physical constraint may have prevented *B. communis* as well from accessing the nectaries successfully. On the other hand, floral nectar may not have elicited a strong feeding response in *B. communis*. For aphid parasitoids like *B. communis* aphid honeydew is a readily accessible carbohydrate source (Vollhardt et al., 2010). Therefore, it may not be

adaptive for an aphidophagous insect to spent time searching for an alternative food source. Finally, simulation models on nectar use in cereal aphid parasitoids indicated that nectar use may be dependent upon aphid densities in the field (Vollhardt et al., 2010). Nectar feeding was predicted to be negligible under high-host densities when enough aphid honeydew was present for parasitoid consumption (Vollhardt et al., 2010). Thus, over the course of this study soybean aphid densities may not have been low enough to encourage nectar feeding in *B. communis*.

In conclusion, host availability was the dominant factor influencing host choice behavior in *B. communis,* while egg load may have indirectly affected host choice decisions through experience. *B. communis* tendency to maintain a constant egg load over time presumably minimized the risk of time limitation. Low host availability and changes in the environment which indicate a decline of favorable conditions increased the probability to accept low-quality hosts in female *B. communis,* presumably as a result of an increase in the perceived risk of time limitation by the parasitoids. Overall though, host specificity in *B. communis* was maintained even under conditions that have been shown to maximize the risk of time limitation and the acceptance of low-quality hosts in other parasitoid species.

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Table 4.1: Stepwise linear regression of variables influencing host choice in *Binodoxys communis*. n = 121. Variables are listed in the order they were incorporated into the model.

Step number	Entered independent variables	Estimate	χ^2	*p*
1	Host quality	2.58	21.49	<0.0001
2	Waiting time (min)	0.13	2.06	0.1512
3	Day of collection	-1.52	5.67	0.0172
4	Flower presence-absence	0.57	2.95	0.0856
5	Barometric pressure (mmHg)	-0.33	4.71	0.0300
6	Host availability	-0.52	0.71	0.4007
7	Hind tibia length	-15.23	2.71	0.0994
8	Time of day	0.00	1.87	0.1711

Table 4.2: Stepwise linear regression of variables influencing the probability to accept a low-quality host in field-collected *Binodoxys communis*. a) Included here are all females given a low-quality host (n = 73); b) included are females given a low-quality host and with a waiting time of <10min. Variables are listed in the order they were incorporated into the model.

a)

Step number	Entered independent variables	Estimate	χ^2	P
1	Flower presence-absence	0.70	2.93	0.0870
2	Day of collection	-2.70	6.21	0.0127
3	Time of day	0.00	3.97	0.0464
4	Waiting time (min)	0.28	4.15	0.0416
5	Host availability	-1.38	2.42	0.1198
6	Hind tibia length	-15.39	1.51	0.2195
7	Barometric pressure (mmHg)	-0.35	3.67	0.0554

b)

Step number	Entered independent variables	Estimate	χ^2	P
1	Day of collection	-5.10	5.82	0.0159
2	Time of day	0.00	3.95	0.0468
3	Barometric pressure (mm Hg)	-0.57	4.94	0.0263
4	Host availability	-2.56	4.64	0.0312
5	Waiting time (min)	0.71	3.95	0.0468
6	Flower presence-absence	1.19	3.46	0.0628

Table 4.3: Stepwise linear regression of variables influencing search time in *Binodoxys communis*. Variables are listed in the order they were included into the model.

a)

Step number	Entered independent variables	Estimate	*F* to enter	P
1	Host quality	-0.25	10.72	0.0014
2	Temperature (°C)	-0.13	9.11	0.0032
3	Host availability	0.41	7.42	0.0075
4	Total sugar conc.	-0.38	2.63	0.1081

b)

Step number	Entered independent variable	Estimate	*F* to enter	*p*
1	Host availability	0.52	9.23	0.0035
2	Temperature (°C)	-0.06	1.43	0.2360

Table 4.4: Stepwise linear regression of variables influencing handling time in *Binodoxys communis*. a) Included are females that were given either a low-quality host or a high-quality host (n = 121); b) Included are only females given a low-quality host (n = 73). Variables are listed in the order they were incorporated into the model.

a)

Step number	Entered independent variables	Estimate	*F* to enter	P
1	Host quality	-0.2656	43.02	< 0.0001
2	Host availability	-0.3792	23.91	< 0.0001
3	Egg load	-0.0330	7.38	0.0077
4	Time of day	0.0000	3.00	0.0861
5	Temperature (°C)	0.0282	1.72	0.1924

b)

Step number	Entered independent variables	Estimate	*F* to enter	P
1	Host availability	-0.41	19.60	<0.0001
2	Egg load	-0.04	5.91	0.0179
3	Waiting time	0.04	6.28	0.0148
4	Time of day	0.00	1.99	0.1635

Figure 4.1: The bioassay station used to conduct behavioral observations; set up adjacent to the field plots.
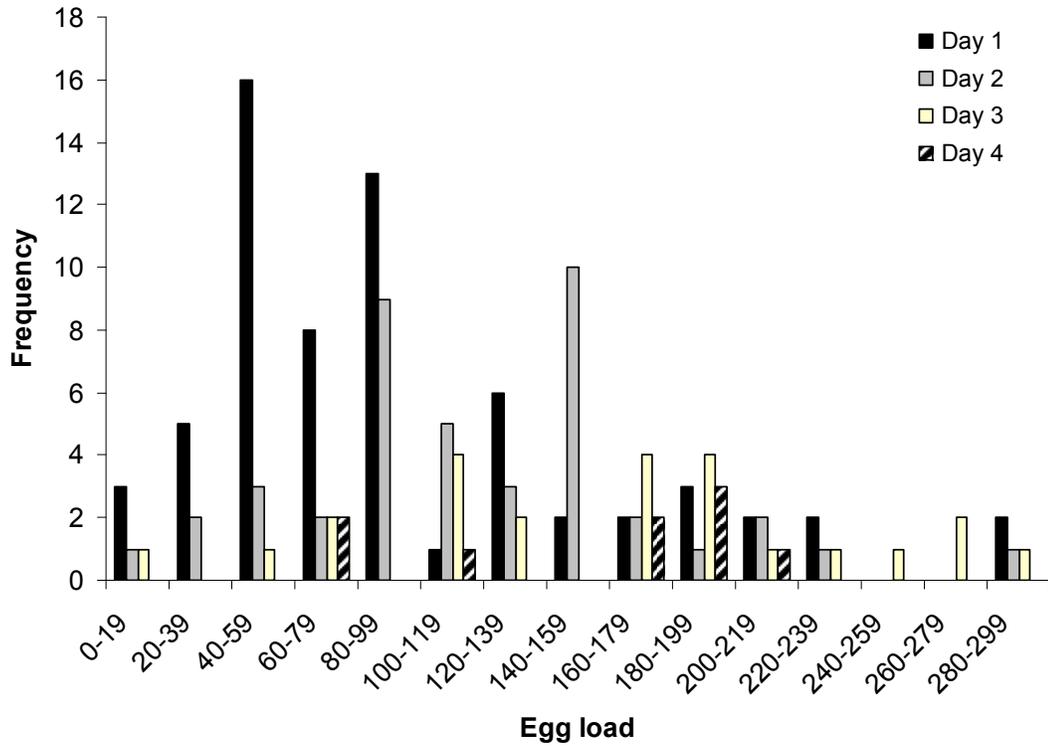
Figure 4.2: Frequency distribution of egg load in field-collected *Binodoxys communis* separated by the four consecutive days of collection.
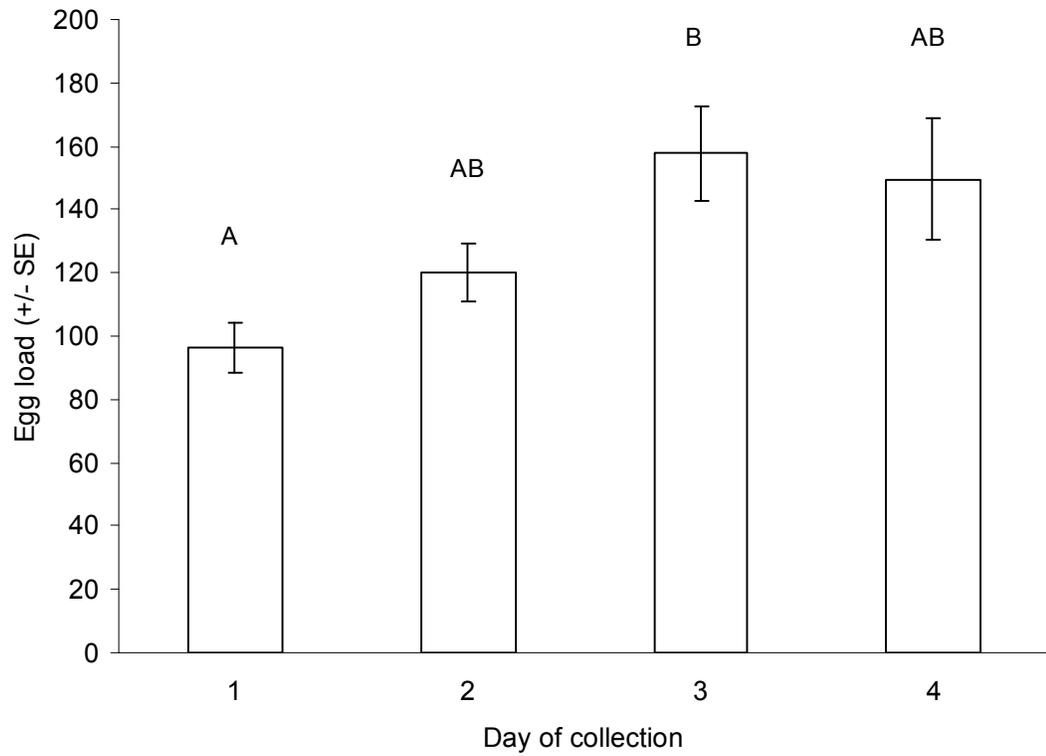
Figure 4.3: Egg load (mean ± SE) in field-collected *Binodoxys communis* separated by

the day of collection. Bars connected by the same letter are not significantly different

(Tukey HSD, $P < 0.05$).
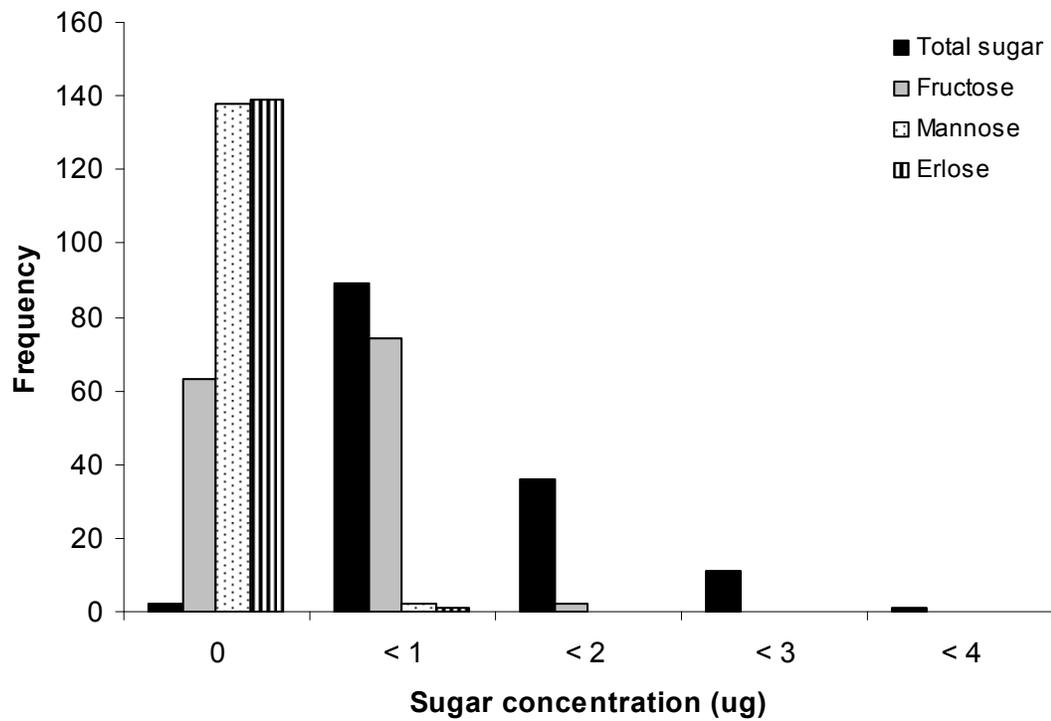
Figure 4.4: Frequency distribution of total sugar, fructose, mannose, and erlose

concentration in 140 field-collected female *Binodoxys communis*.
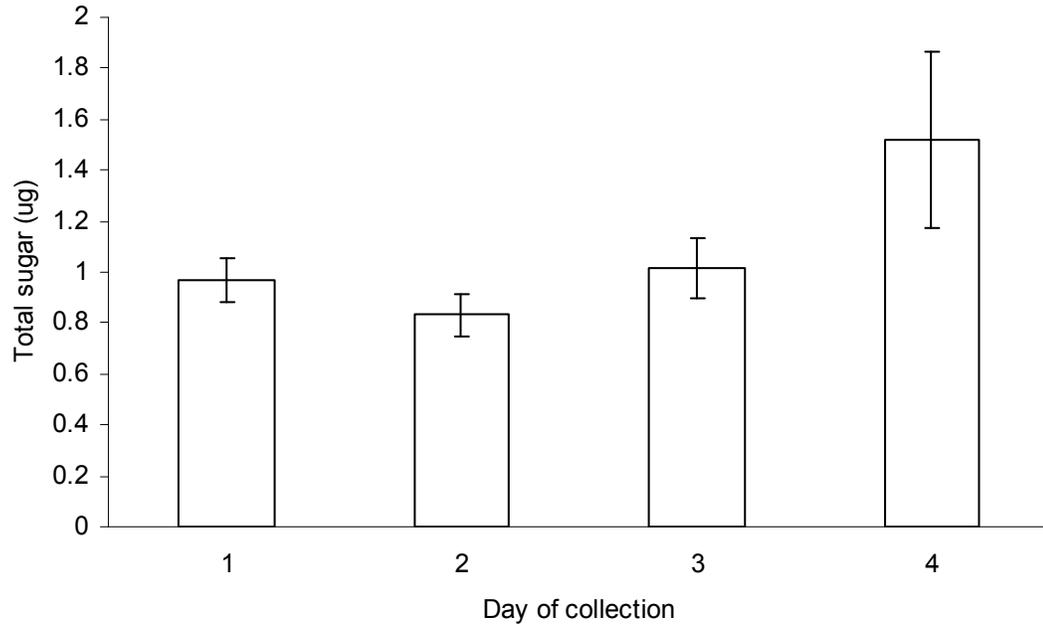
Figure 4.5: Total sugar concentrations (mean ± SE) in field-collected *Binodoxys communis* over the four days of collection.
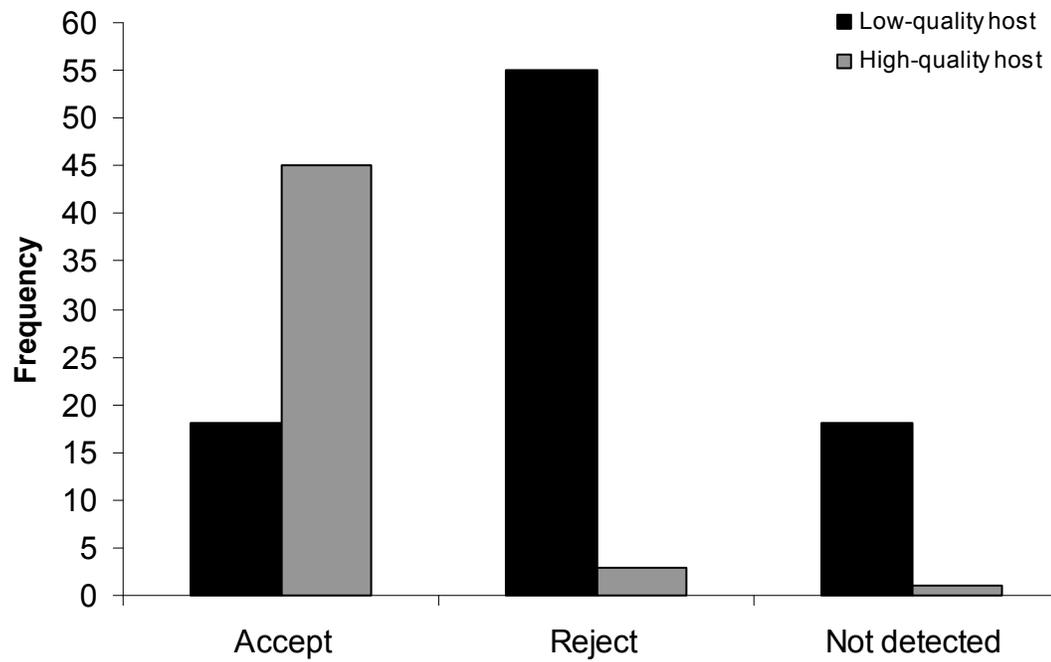
Figure 4.6: Frequency distribution of a total of 140 aphid hosts that were either accepted, rejected or not detected by female *Binodoxys communis* separated by host quality; high-quality host: n = 49, low-quality host: n = 91.
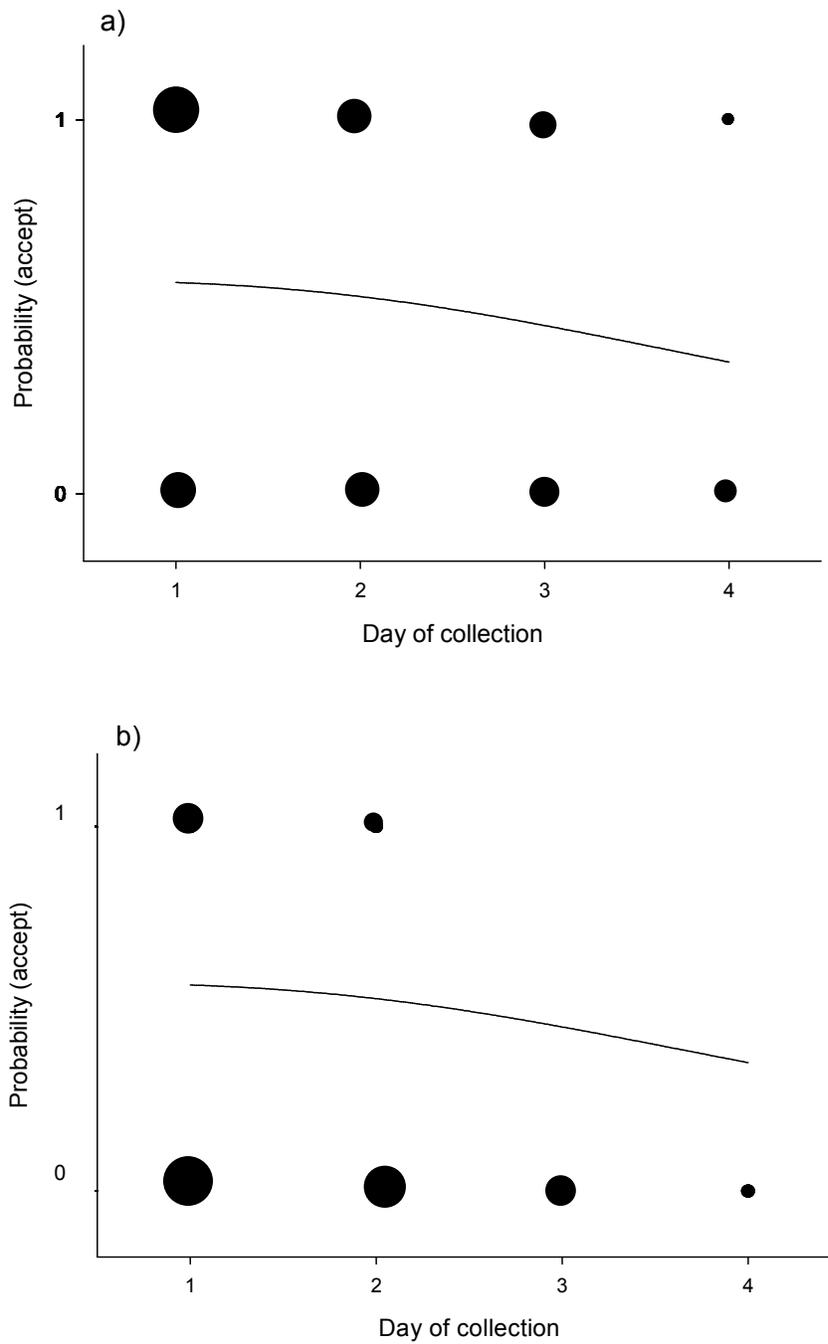
Figure 4.7: Influence of day of collection on the probability of host acceptance regardless of host quality (a) and of low-quality hosts only (b). Dot sizes represent overlapping data points.
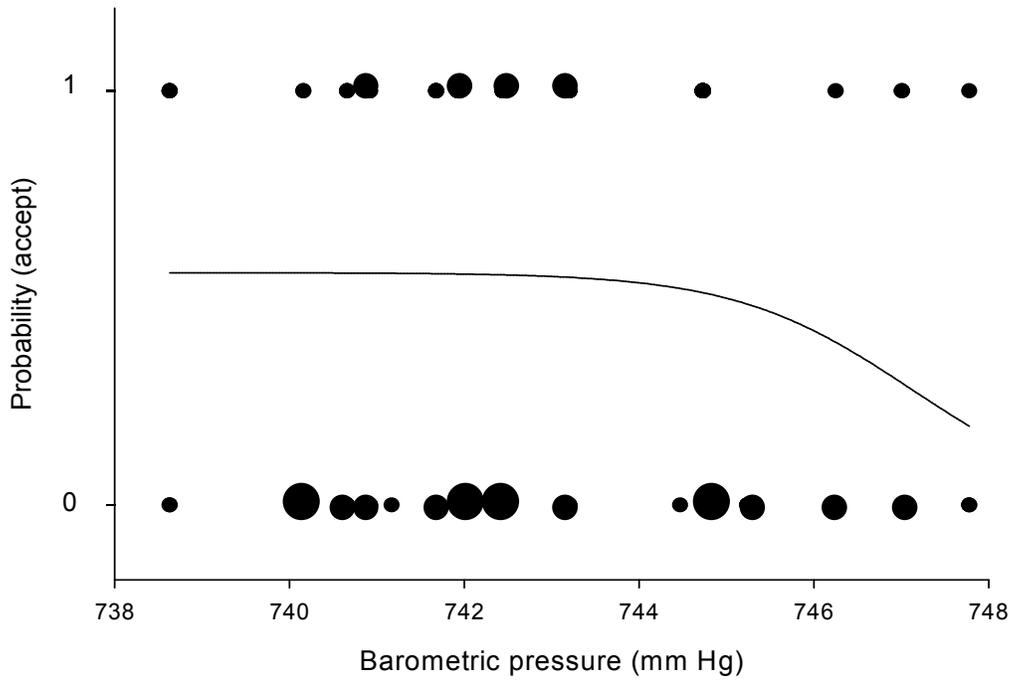
Figure 4.8: Influence of barometric pressure (mm Hg) on the probability of host

acceptance. Dot sizes represent overlapping data points.
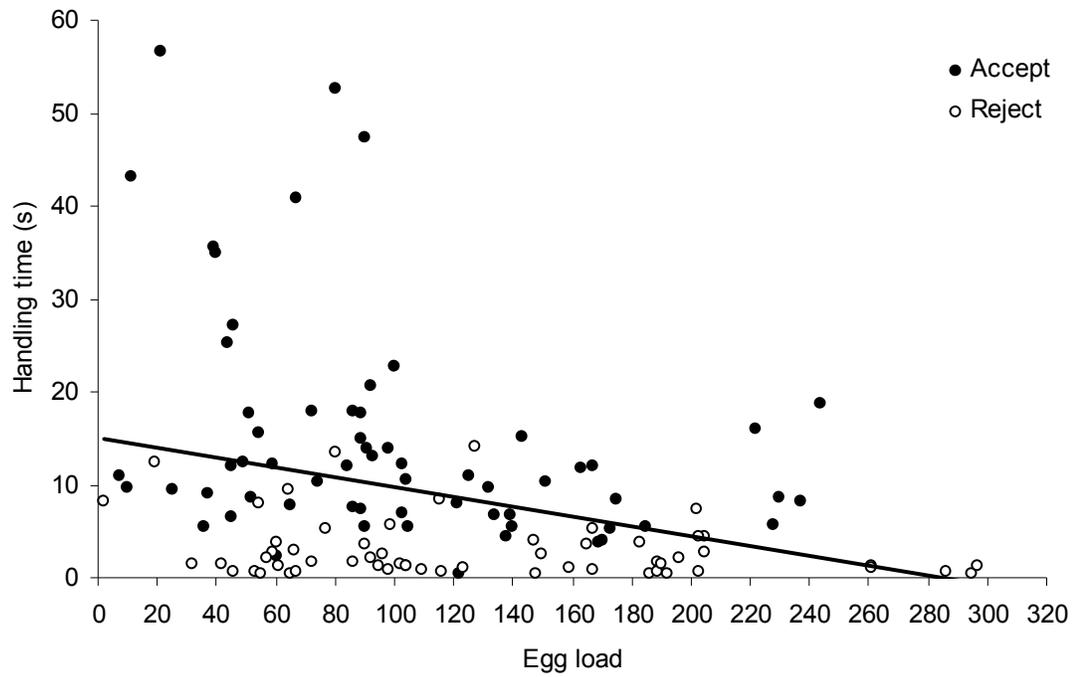
Figure 4.9: Scatterplot of handling time (s) versus egg load for *Binodoxys communis* that accepted (closed circles) or rejected (open circles) a host. Linear regression: handling time (s) = -0.0527*egg load + 15.082, r^2 = 0.1085.

Chapter 5

Nature and Nurture: Host choice behavior in free-foraging *Binodoxys communis*

Introduction

Host selection in parasitoids is a complex process involving several steps from habitat location through host location and host acceptance (Hagvar & Hofsvang, 1991; Godfray, 1994; Vinson, 1998). The outcome of this process is shaped by a multitude of internal (parasitoid nutritional state, egg load, age) and external (chemical and visual cues from the habitat and potential hosts, weather, presence of conspecifics and predators, host availability) factors acting simultaneously on a female parasitoid in the field as well as by the degree of specialization of a parasitoid species (Hagvar & Hofsvang, 1991).

Important cues in the host selection process are given by kairomones, i.e. volatile cues from the host habitat, host plant and/or the host itself. The extent to which parasitoids respond to these cues are partly dependent upon the parasitoid species and the degree of parasitoid specialization (Hagvar & Hofsvang, 1991; Geervliet et al., 1998; Kitt & Keller, 1998; Wyckhuys & Heimpel, 2007; Afsheen et al., 2008; Michereff et al., 2011). Generalist parasitoids have been shown to be more responsive to a wider range of cues compared to specialists (Geervliet et al., 1998). However, experience with a certain cue has been shown to increase parasitoid responsiveness and thus modify the outcome of host selection over time by means of priming (i.e. reinforcement of an innate response) or associative learning (Vet et al., 1995). For example, the specialist parasitoid *Binodoxys communis* showed only a weak innate response towards soybean volatile cues in an olfactometer assay (Wyckhuys & Heimpel, 2007). However, experience with the host-plant complex increased the responsiveness of female parasitoids towards that complex.

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Unfortunately, the majority of studies on parasitoid host selection have been conducted in the laboratory under controlled conditions. Rainfall, wind, and temperature can alter the reliability and availability of these cues in the field (Fink & Völkl, 1995; Weisser et al., 1997; Casas et al., 1998).

In addition, a parasitoid's physiological state is a major component influencing host choice behavior (Mangel, 1989a; Rosenheim & Rosen, 1991; Godfray, 1994; Heimpel & Collier, 1996). Egg load, in particular, has been shown to shape host selection in parasitoids, especially in the context of egg and time limitation (Mangel, 1989a; Mangel, 1989b; Rosenheim, 1996; Heimpel & Rosenheim, 1998). Parasitoids are predicted to accept a wider range of hosts when they are time limited and be more selective in their host acceptance when they are egg limited. Studies on the effect of egg load on host selection so far have strongly focused on selection of different stages or individuals of the same species by a female parasitoid (Collins & Dixon, 1986; Michaud & Mackauer, 1995; Heimpel & Collier, 1996; Mackauer et al., 1996; Minkenberg et al., 1992; Papaj, 2005). Under natural conditions though, a parasitoid is likely to encounter more than a single host species in its lifetime and we have just begun to look deeper into what shapes host species preference in parasitoids, especially under field conditions (Janssen, 1989; Brodeur et al., 1996; Geervliet et al., 2000; Heimpel et al., 2003).

The majority of our knowledge regarding parasitoid host choice behavior and host specificity comes from laboratory studies as well as theoretical models (Iwasa et al.,

1984; Collins & Dixon, 1986; Donaldson & Walter, 1988; Mangel, 1989a; Rosenheim & Rosen, 1991; Minkenberg et al., 1992; Mackauer & Völkl, 1993; Godfray, 1994; Henneman et al., 1995; Michaud & Mackauer, 1995; Heimpel & Collier, 1996; Mackauer et al., 1996; Rivero-Lynch & Godfray, 1997; Vinson, 1998; Hegazi et al., 2007; Wu & Heimpel, 2007; Desneux et al., 2009). It is in the nature of laboratory studies, though, to focus on a specific aspect of the host selection process while ignoring the rest of the process. In recent years attention has been brought to the necessity of field studies to support and validate laboratory work and to gain insight into parasitoid behavior under natural conditions (Waage, 1983; Casas, 1989; Heimpel et al., 1998; Casas et al., 2000; Geervliet et al., 2000; Heimpel & Casas, 2008). Pioneering field studies conducted so far have both validated previous laboratory studies (Geervliet et al., 2000; Casas et al., 2004) and highlighted discrepancies in parasitoid behavior (Heimpel & Casas, 2008).

The purpose of this study is to further add to our understanding of parasitoid host choice behavior under field conditions. We examined the acceptance of a low- and a high-quality host species by *B. communis* in two different crops. In addition, we manipulated the level of host experience in parasitoids prior to a release in order to test host acceptance behavior in *B. communis* under the influence of perceived egg and time limitation. This is the last experiment in a series of laboratory and field experiments to assess the influence of egg and time limitation on host choice behavior in *B. communis*, a biological control agent of the soybean aphid. We have previously shown that *B. communis* balances its risk of egg and time limitation by maintaining a constant egg load

both in the laboratory and under field conditions. Furthermore, we have shown that host availability had an indirect influence on the perceived risk of egg and time limitation in *B. communis*. In semi-field no-choice tests, field-collected parasitoids coming from a host-rich environment had a lower likelihood of accepting a low-quality host species compared to female parasitoids from a host-poor environment (see Chapter 4). Here we test the influence of perceived host availability on the acceptance of a low- and a high-host quality species under natural, non-confined conditions.

Material and Methods

Host-parasitoid system

Binodoxys communis is an oligophagous specialist parasitoid that sting aphids in the tribe Aphidini and primarily in the genus *Aphis* (Fam. Aphididae, Subf. Aphidinae) (Desneux et al. 2009). Field-collected female *B. communis* were exposed to either a high-quality host (*A. glycines* Matsumura) or a low-quality host (*Aphis craccivora* Koch) of known size. The high-quality host species of choice was the soybean aphid, *A. glycines*, which *B. communis* has been released against in North America in 2007. *Aphis craccivora* has been shown to be a low-quality host species to *B. communis* in terms of acceptability as well as parasitoid larval survival (Desneux et al., 2009). In the laboratory, female *B. communis* detected individual *A. craccivora* in over 90% of the times that they came in contact with this aphid in no-choice tests, and in about half of these encounters female parasitoids successfully stung the host individual. However, less than 5% of these ovipositions resulted in a viable adult parasitoid (Desneux et al., 2009). Therefore, we found this aphid species to be an appropriate low-quality host species.

Insects

The parasitoid colony used in this study originated from a strain collected in the Chinese province of Heilongjiang in 2002 (Wyckhuys et al., 2008). Mass-releases across the mid-western US have been conducted since 2007, but so far establishment has yet to be confirmed. All parasitoids used in the experiments were reared on *Aphis glycines*-infested soybean plants. The *Aphis glycines* colony used was originally established from

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aphids collected from a soybean field in St. Paul, MN, USA, in 2003. Since then soybean

aphids have been reared on soybean plants (Syngenta NK S19-R5) in growth chambers

(L16: D8; 60-80% r.h.; 25 ± 5 °C). The colony of *Aphis craccivora* Koch used in this

experiment was reared on *Vicia fabae* and originally kept in in growth chambers (L16:

D8; 60-80% r.h.; 25 ± 5 °C). It was originally established from individual aphids

collected on soybean crops at the Minnesota Agricultural Experiment Station, St. Paul,

MN, USA.

All insect colonies were reared in commercial insect rearing cages (BugDorm-44545,

MegaView Science Co., Ltd., Taiwan, 47.5 × 47.5 × 47.5 cm) in environmentally-

controlled greenhouses located at the Minnesota Agricultural Experiment Station at the

University of Minnesota, St. Paul, USA. The parasitoid rearing cages contained 10 9 × 9

cm plastic pots with aphid-infested soybean plants each (Syngenta NK S19-R5) and ~20

female and as many male parasitoids were added to each cage. There were a total of 4

cages per host density treatment.

Parasitoid releases

This study was conducted at the University of Minnesota Research and Outreach Centre,

Rosemount, MN, USA. The study site consisted of a single alfalfa field (9.3 ha) and a

single soybean field (12.5 ha) that were adjacent to each other. The alfalfa field was

mowed two weeks prior to the first release but not during the study. *Binodoxys*

communis was released weekly into three flagged 12 m×12 m plots on a total of 6 release

dates from 15 July – 24 August 2010. Each plot spanned across the soybean and alfalfa field and was used for a single release only with at least 30 m between individual plots (Fig. 1a). Each release lasted for a total of 36 hours. The average numbers of parasitoids released per treatment-plot combination were 1236 ± 279 parasitoids in the HHD treatment, 2730 ± 762 parasitoids in the LHD treatment, and 1667 ± 449 parasitoids in the NAÏVE treatment (please see below under "*Manipulation of pre-patch host experience/egg load*" for a detailed description of the treatments).

Manipulation of pre-patch host experience/egg load

Parasitoid pre-patch experience/egg load was manipulated by exposing parasitoids to different host densities prior to each release. There were three treatments in total: naïve, low host density (LHD), and high host density (HHD).

<u>Preparation of NAÏVE parasitoids</u>: On the evening before each release and day of parasitoid eclosion, 8 pots of soybean plants with an average of 81 ± 14.5 parasitoid "mummies' in each cage were cut down and transferred into another rearing cage containing clean soybean plants only to allow non-parasitized *A. glycines* to relocate. The following morning, the now aphid-free plant cuttings were equally distributed among two clear plastic, household storage boxes (Sterilite®, $62 \times 45 \times 18$ cm) with windows cut into the long sides and the lid for ventilation. Windows were covered with a coarse mesh (mesh size: 2×2 mm) to prevent predators from entering the boxes in the field and a layer of no-see-um mesh (mesh size: 0.6 mm, Quest Outfitters, Sarasota, FL, USA) on top of the coarse mesh during the 24hr-host exposure period and during transport.

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Preparation of LHD and HHD parasitoids: Two evenings prior to a release and the night before parasitoid eclosion, 8 pots of soybean plants covered with parasitoid "mummies' from each of the four rearing cages were cut down and transferred into another rearing cage containing clean soybean plants only to allow non-parasitized *A. glycines* to relocate. The following morning (i.e. 24 hours prior to the release), the now aphid-free plant cuttings with eclosing parasitoids were equally distributed among two clear plastic, household storage boxes per treatment. Boxes for the LHD parasitoid cohort contained 16 9 × 9 cm plastic pots each with 30 soybean aphids per pot; boxes for the HHD parasitoid cohort contained 16 9 × 9 cm plastic pots each with 150 soybean aphids per pot. To each box small plastic strips with droplets of honey were added. On the morning of the release, all plants were cut down to encourage parasitoids to leave the boxes in the field.

In order to assess parasitoid egg load in the LHD and HHD treatment an average of 20 female parasitoids from each treatment was collected from the boxes on the morning of the release. Parasitoids were stored at -80 °C until dissections and egg load counts. Parasitoid size was assessed by measuring the hind tibia length of each adult. Prior to each dissection, a single hind leg was removed from the parasitoid body and the length of the hind tibia measured using a dissecting microscope (50x magnification). Egg loads of field-collected female parasitoids were assessed by removing the ovaries from each female and counting the eggs under a dissecting microscope at 50x magnification.

Sex ratios

Sex ratios of *B. communis* prior to a release were calculated as the proportion of males. This was done to ensure that the released parasitoid cohorts did not consist of male-broods only. Two pots of soybean plants covered with parasitoid mummies were removed from each cage once mummies have formed. The mummies were collected from each plant by gently removing them from the plant with a fine paint brush and transferring them singly into gel caps (size 0). Gel caps were then kept in a growth chamber (L16: D8; 60-80% r.h.; 25 ± 5 °C) until parasitoids eclosed. Sex ratios among treatments, releases and their interaction treatment×release were analyzed using an ANOVA followed by a Tukey-Kramer HSD test for multiple comparison of means between treatments (JMP 8.0.1, SAS Inst.).

Sentinel plants

Each plot contained two transects set perpendicular to the field edges and pairs of sentinel plants were randomly placed along each transect. There were eight pairs per transect at regular distances, i.e. at 0.75 m, 2.25 m, 3.75 m, and 5.25 m from each field edge (Fig. 5.1b,c). Sentinel plant pots consisted of either a single soybean plant (V2-V3 stage) infested with 150 *A. glycines* individuals or a single *Vicia fabae* plant (similar in height to the soybean sentinel plant) with 150 *A. craccivora* individuals (Fig. 5.2). Aphids were added to each plant by gently placing a leaf cutting containing 150 aphid individuals of each species on its respective host plant 24 hours prior to each release to allow aphids to settle. Each plant pot was then covered with a 30 cm × 80 cm no-see-um mesh cover

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(mesh size: 0.6 × 0.6 mm, Quest Outfitters, Sarasota, FL, USA) until the release to avoid

parasitism of aphids. Sentinels plants were left in the field for a total of 36 hours and

afterwards returned to a greenhouse space until mummification of the host aphids.

Mummies were then collected, reared out in a growth chamber (L16: D8; 60-80% r.h.; 25

± 5 °C), and parasitoid species as well as sex (of *B. communis* only) were determined

once all mummies hatched.

Statistical analysis

Egg loads were square-root transformed to meet the assumption of normality.

Comparison of egg loads among the three host density treatments was done using an

ANOVA, followed by Tukey-Kramer HSD for multiple comparison of means (JMP

8.0.1, SAS Inst.).

The influence of distance and habitat on the mean proportion of sentinel plants

parasitized by *Binodoxys communis* was analyzed as a split-plot model with habitat as

main factor, distance as sub-plot factor, and experience as block variable using JMP 8

(SAS Institute, Inc., Cary, NC, USA). The proportions of sentinel plants with

mummified aphids among experience levels and habitat×host species combinations were

compared using an ANOVA followed by a Tukey HSD test for multiple comparison

(JMP 8, SAS Institute, Inc., Cary, NC).

Proportion of parasitism was calculated as the number of *B. communis* mummies divided

by the initial 150 aphids per sentinel plant. All proportions were averaged across the two

transects and across distances and means were arcsine(square-root)-transformed to

normalize data. Differences between parasitism rates in LHD and HHD *B. communis* were analyzed using a split-split plot on randomized nested block model, with host experience as whole plot effect, habitat as sub-plot effect, and host species as sub-sub-plot effect. Differences in naïve *B. communis* were analyzed separately with a split-plot design with habitat as whole plot effect and host species as sub-plot effect. Release date was used as a block variable. Least–square means were used to express average proportion of parasitism among host species and tested for differences in relation to parasitoid host experience and habitat using the lsmeans option of proc mixed followed by a Tukey test for multiple comparison of means. Split-split and split plot models analyzed with proc mixed in SAS 9 (SAS Institute Inc., Cary, NC, USA).

Results

Distribution of egg load and sex ratios

Egg loads in *Binodoxys communis* between the three host density treatments were not

significantly different ($F_{2,280} = 0.09$, P = 0.9114; Fig. 5.3). The average parasitoid egg

load ($\pm$ SE) in the HHD cohort was 88.38 $\pm$ 11.23 eggs, in the LHD cohort 97.59 $\pm$ 12.27

eggs, and in the naïve cohort 97.92 $\pm$ 12.26 eggs. Thus, parasitoids differed only in the

level of host experience upon their release. Sex ratios of *B. communis* were slightly

male-biased with an average of 0.61 $\pm$ 0.03 in the HHD cohorts, 0.64 $\pm$ 0.03 in the LHD

cohorts, and 0.58 $\pm$ 0.03 in the NAÏVE cohorts. There was no significant difference in

sex ratios between treatments ($F_{2,12} = 1.04$, P = 0.3840), releases ($F_{1,12} = 2.28$, P =

0.1575), or treatment×release ($F_{2,12} = 0.87$, P = 0.4419).

Host acceptance behavior

Binodoxys communis located sentinel plants at all four distances from the release point

into the soybean and alfalfa field (Fig. 5.4). The proportion of sentinel plants that were

located by *B. communis* was significantly higher in the soybean field than the alfalfa field

($F_{1,20} = 33.06$, P = 0.0289) and decreased with distance from the release point ($F_{1,20} =$

20.88, P = 0.0447).

A comparison of the proportion of sentinel plants with mummified aphids showed that

significantly more sentinel plants with the high-quality host species placed in the soybean

field contained *B. communis* mummies compared to any of the other habitat×host species

sentinel plant combinations ($F_{3,6} = 13.14$, P = 0.0048) (Fig. 5.5). There was no

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significant difference between the proportions of sentinel plants with mummified aphids among the three levels of host experience ($F_{2,6} = 0.44$, $P = 0.6607$). Comparison of the average proportion of parasitism on sentinel plants showed that both LHD and HHD *B. communis* parasitized more aphids on sentinel plants in the soybean field as opposed to plants set in the alfalfa field (Table 5.1a, Fig. 5.6). This effect was significant in *B. communis* with low host density pre-patch experience while it was only marginally significant in females with high host density pre-patch experience. There was a significant interaction between HHD parasitoids and the high-quality host species (*A. glycines*) (Table 5.1a, Fig. 5.7). That is, HHD parasitoids parasitized significantly more *A. glycines* on sentinel plants compared to *A. craccivora* on sentinels as well as compared to parasitism by LHD females on either sentinel plant. Furthermore, there was a significant three-way interaction between experience×habitat×host species stating that HHD parasitoids parasitized significantly more soybean aphids on sentinel plants compared to any other sentinel-habitat combination (Table 5.1a). This suggests that female *B. communis* with high host density pre-patch experienced showed a stronger preference for the target host (i.e. soybean aphid) compared to the non-target host as well as a preference for the target habitat (i.e. soybean field). There was no significant difference in mean parasitism between the low- and the high-host quality aphid species by LHD female *B. communis*. But the three-way interaction between experience×habitat×host species showed a marginally significant effect for increased parasitism in LHD parasitoids on low-quality host aphid species in the soybean field. On average, sentinel plants with the high-quality host species placed in soybean fields

suffered the highest attack by HHD and LHD *B.communis* (Table 5.1a,b, Figs. 5.8a,b).

Naïve *B. communis* also attacked high-quality host species sentinels placed in the

soybean field the most (Table 5.1b, Figs. 5.8b). However, there was also a significant

amount of parasitism of high-quality host species sentinels as well as low-quality host

species sentinels in the alfalfa field.

Discussion

Binodoxys communis was able to locate sentinel plants in both habitats across the four

distances. Egg load did not differ among the two host density treatments suggesting that

host availability rather than egg load influenced parasitoid host choice behavior in *B.*

communis. Pre-patch host experience had an influence on host choice behavior in *B.*

communis that was in accordance with the predictions of egg and time limitation. Female

parasitoids with prior host experience showed a stronger preference for the soybean field

and the high-quality host species, i.e. soybean aphid, compared to naïve parasitoids.

Moreover, this preference was more pronounced in females with high host density

experience compared to females with low-host density experience. This suggests that

female parasitoids that previously experienced a host-rich environment were more

selective in their host acceptance compared to LHD and naïve females.

Binodoxys communis attacked aphids on sentinel plants in both the soybean and the

alfalfa field. This confirms previous laboratory findings on this species indicating that *B.*

communis does not have a strong innate response towards soybean (Wyckhuys &

Heimpel, 2007). Wyckhuys & Heimpel (2007) showed that *B. communis* was attracted to

odors from both soybean and non-soybean host-plant complexes in an olfactometer assay.

B. communis did not distinguish between aphid-infested and aphid-free soybean plants,

indicating that it responded to plant-emitted volatiles rather than aphid-induced volatiles

(e.g. honeydew) in the laboratory (Wyckhuys & Heimpel, 2007). Our field study

supports this notion based on the lack of significant preference in *B. communis* for the

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soybean environment. Parasitoid drift into the alfalfa field due to wind might have contributed to parasitism in that field if parasitoids were passively blown into the alfalfa field. In the laboratory study by Wyckhuys & Heimpel (2007), experience on a given host-plant complex increased *B. communis'* response toward that complex compared to naïve females. This would to some extent explain the pronounced preference of HHD parasitoids for soybean aphid sentinel plants that we saw in our study here. Essentially, HHD females were more or less equally likely to enter either crop but parasitized more of the high-quality host in either habitat. Proportions of parasitism by naïve female parasitoids suggested the same trend towards a preference for the high-quality host species but results were not conclusive. Interestingly, LHD parasitoids also showed a preference for the soybean habitat but parasitized equal proportions of high- and low-quality hosts. Thus, contact with a given host-plant complex alone does not appear to be sufficient in B. communis to reinforce a potentially weak innate response towards the high-quality host species complex or the host-plant complex it developed on (Vet et al., 1995; Grasswitz & Paine, 1993). It appears also to be a matter of intensity (high vs. low host density) or time. We have previously shown that field-collected *B. communis* were more likely to accept a low-quality host species within the first 48 hours following a release and rejected all low-quality hosts offered on the third and fourth day in the field (Chapter 4). That is, *B. communis* became more selective in their host choice with age and therefore prolonged exposure to the high-quality host environment. In our study here, parasitoid age was confounded by the extent of host exposure prior and during a

release might explain some of the parasitism we observed on the low-quality host species in the field.

The results from this study suggest that perceived host availability in *B. communis* influences host choice behavior in the field. Despite the low parasitism rates, the trends in parasitism of the two different host species by *B. communis* in this study meet the overall predictions of egg- and time limitation (Collins & Dixon, 1986; Mangel, 1989b; Rosenheim, 1996; 1999). We have previously shown that *B. communis* balances its risk of egg and time limitation in the field by maintaining a constant egg load over time (Dieckhoff & Heimpel, 2010). We have also previously shown that perceived host availability influenced the acceptance of low-quality host species in *B. communis* in no-choice tests (see Chapter 4). Field-collected female parasitoids coming from a host-rich environment were more likely to reject the low-quality host species compared to females from a host-poor environment. Host acceptance behavior in that case, though, was tested under no-choice conditions in a Petri dish environment and not in a complex host environment with multiple visual and chemical stimuli acting on a parasitoid (Heimpel & Casas, 2008). Here, we show that *B. communis* also appears to vary its host choice behavior in the field in response to perceived host avaiability. Female parasitoids that had been exposed to a high host density (HHD) early in their life showed the strongest response to the high-quality host habitat and the high-quality host itself. HHD parasitoids may have perceived an increased risk of becoming egg limited due to their pre-exposure to a host-rich environment. LHD and naïve parasitoids, on the other hand, were less

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pronounced in their host choice showing no significant differences in their acceptance of the high- and low-quality host species. In this study, even though pre-patch host experience, i.e. the perceived host availability, had been manipulated in the laboratory, parasitoids foraged freely in the two field crops. Thus, host choice on either sentinel plant-host species combination therefore represents the outcome of various internal (egg load, perceived egg or time limitation, nutrition) and external (host availability, abiotic factors, accessibility of sentinel plants) variables. Egg load and host experience inevitably confound each other in the field (Rosenheim & Rosen, 1991; Heimpel et al., 1998; Heimpel & Casas, 2008). Thus, even though there was no significant difference in egg load as a result of pre-patch host experience egg load may still have had an influence on the behavior at the individual level (Rosenheim & Rosen, 1991; Heimpel & Casas, 2008). However, in the present study we consider the effect of host experience to be the main factor influencing observed parasitism in the field. The more so as *B. communis* has previously been shown to maintain a constant egg load in the field and only a small proportion of field-collected parasitoids had what could be considered a low egg load in this species (< 40 eggs) (Dieckhoff & Heimpel, 2010).

Overall, parasitism rates on sentinel plants on a per-plant basis in this field study were low and did not exceed 3% of the total number of aphids present per plant. This may be the result of restricted searching efficiency on part of the parasitoid. Plant architecture in the field, for example, may have restricted *B. communis* from efficiently accessing or locating the sentinel plants in either habitat. Such an effect of plant complexity on

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searching efficiency has been shown in two *Trichogramma spp.* parasitizing eggs of the European corn borer (Wang et al., 1997). However, given that sentinel plants at all four distances in either habitat were successfully attacked indicates that any effect of plant architecture is probably small. Predation by resident predators, on the other hand, may have contributed to overall low observed parasitism rates. That is, these sentinel plants probably constituted high-quality patches in either crop, and thus may have attracted its fair share of predators throughout the 36 hours that the plants were left in the field. Coccinellids as well as Anthocoridae, for example, have been shown to be able to exert strong predation pressure on aphids in soybean as well as other crops (Obrycki & Kring, 1998; Costamagna & Landis, 2007). Moreover, the sentinel plants in this study were open to the environment inadvertently allowing migration of aphids to and from the sentinel plants. Thus, it is possible that parasitized aphids were either eaten by predators or moved off the plant and/or that healthy field aphids moved onto the plants throughout the 36 hours of this study. Either scenario would result in us underestimating the true rate of parasitism on sentinel plants by *B. communis*, and thus explain at least partly the overall low observed parasitism rates.

In conclusion, *B. communis* showed no habitat preference under field conditions but host experience had an influence on the parasitism rate of high- and low-quality host species. Furthermore, some of our observations on *B. communis* host choice behavior in the field suggested that host specificity in the field is under the influence of the perceived risk of egg and time limitation. We were able to validate some conclusions regarding *B.*

communis behavior from laboratory observations and experiments including olfactometer assays. And while our results do not allow us to draw clear-cut conclusions – mainly due to the overall low parasitism – we were able to shed some more light on parasitoid behavior in the field and on the importance of bringing research into the field.

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Table 5.1: Proportion of parasitism by a) LHD and HHD (n = 48) and b) naïve (n = 24) *Binodoxys communis* on two different host species. Shown are least square means effects with estimates of means are back-tranformed.

a) HHD – LHD

Experience×Habitat

	Estimate	t	P
HHD×Alfalfa	0.009	1.74	0.101
HHD×Soybean	0.013	2.06	0.056
LHD ×Alfalfa	0.005	1.22	0.240
LHD×Soybean	0.016	2.26	0.039

Experience×Host species

	Estimate	t	P
HHD×*A. craccivora*	0.005	1.50	0.160
HHD×*A. glycines*	0.019	2.88	0.014
LHD ×*A. craccivora*	0.010	1.97	0.074
LHD ×*A. glycines*	0.010	2.04	0.065

b) Naïve

Habitat×Host species

	Estimate	t	P	Estimate	t	P
				(Habitat×Host species)		
Alfalfa×*A. craccivora*	0.004	1.22	0.242	0.008	2.23	0.045
Alfalfa×*A. glycines*	0.010	2.00	0.065	0.012	2.78	0.016
Soybean×*A. craccivora*	0.011	2.06	0.059	0.003	1.45	0.172
Soybean×*A. glycines*	0.018	2.65	0.019	0.017	3.21	0.007

Experience×Habitat×Host species

	Estimate	t	P
HHD×Alfalfa×*A. craccivora*	0.005	1.09	0.286
HHD×Alfalfa×*A. glycines*	0.016	1.99	0.058
HHD× Soybean×*A. craccivora*	0.006	1.22	0.236
HHD×Soybean×*A. glycines*	0.023	2.43	0.023
LHD×Alfalfa×*A. craccivora*	0.003	0.09	0.379
LHD×Alfalfa×*A. glycines*	0.006	1.27	0.218
LHD×Soybean×*A. craccivora*	0.018	2.13	0.044
LHD×Soybean×*A. glycines*	0.014	1.87	0.074

a) Schematic of the field setup of 1 replicate containing 2 plots. Replicate sets were aligned accordingly.

b) Detailed diagram of a single plot used for one of the two egg load-manipulated parasitoid cohorts

Alfalfa   Soybean

~ 30-50m

12 m

12 m

5.25   3.75   2.25   0.75   0.75   2.25   3.75   5.25

Distance of subplots from the field edges (m)

X

0

X = *Aphis glycines* on soybean
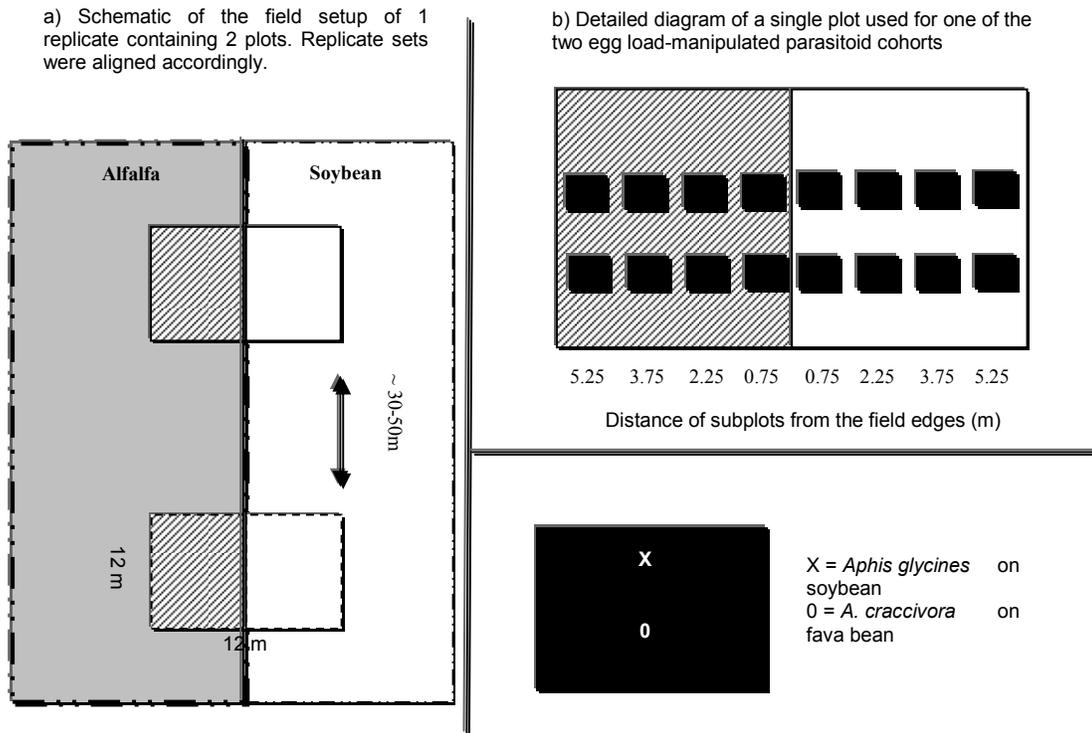0 = *A. craccivora* on fava bean

Figure 5.1: Schematic of the split-split-split plot design illustrated on a single release replicate. a) Placement of plots within the soybean and alfalfa habitat. Grey = alfalfa field, white = soybean field. b) Placement of subplots within each plot along two transects. Black boxes designate position of paired outplants along each transects. c) Schematic of a single subplot each containing a pair of outplants. Outplants were arranged randomly within a subplot along each transect.
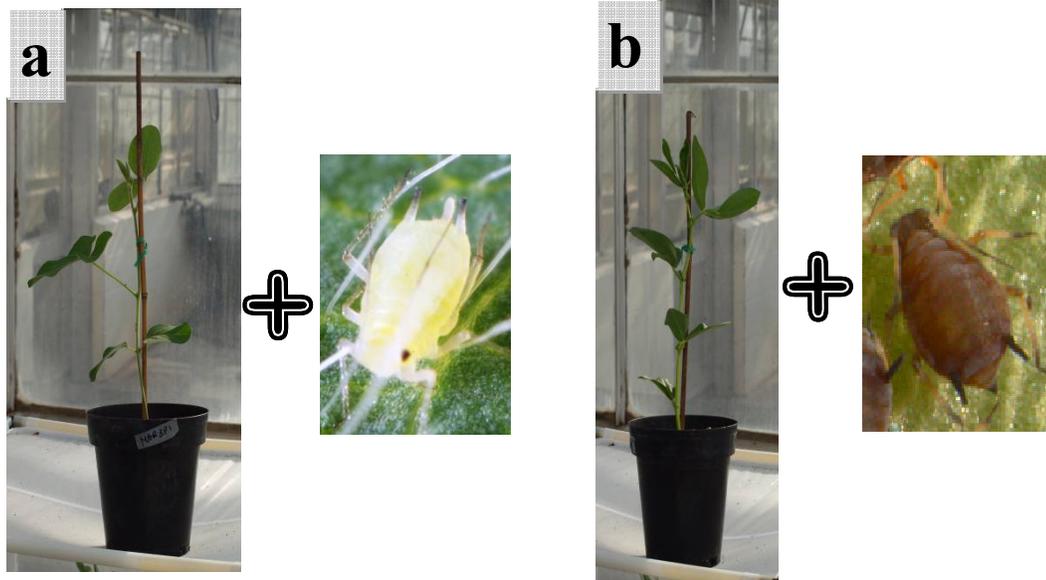
Figure 5.2: Outplants consisting of single potted plants with 150 aphids each of either quality. (a) High-quality host (*A. glycines*) on soybean (*Glycine max*), (b) Low-quality host (*A. craccivora*) on fava bean (*Vicia fabae*). Photo credits for Potted plants: Christine Dieckhoff; *A. glycines*: Dave Hansen; *A. craccivora*:

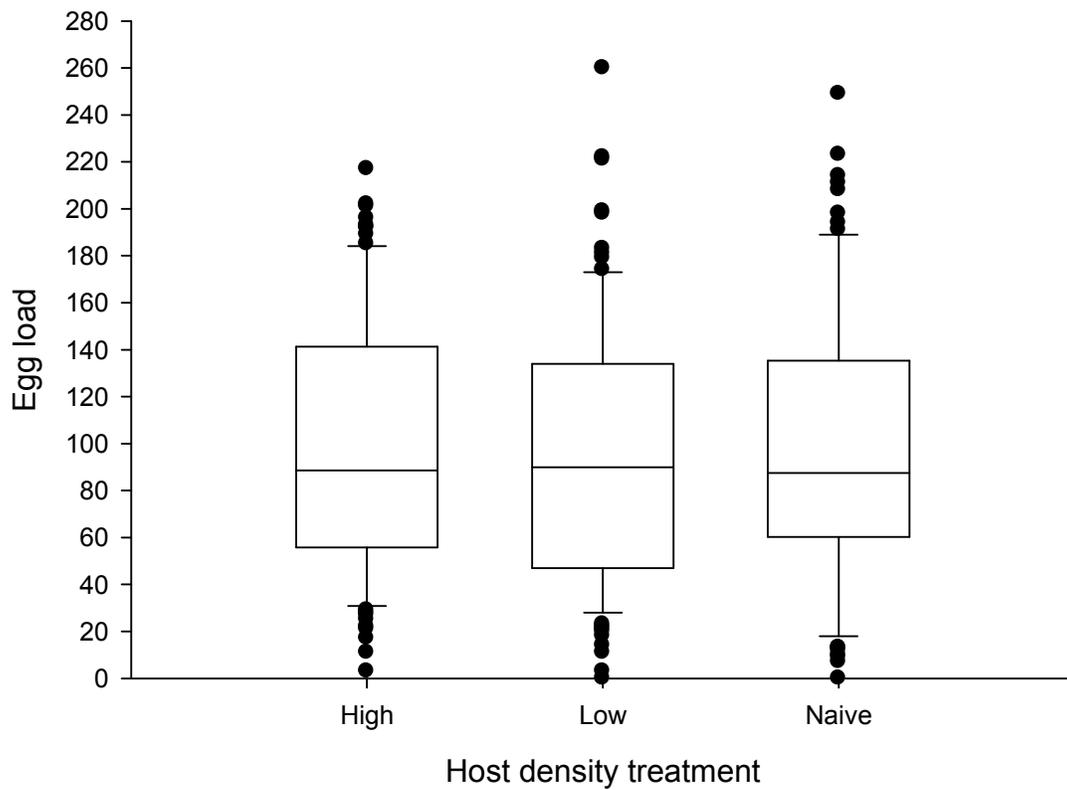http://www.aphidweb.com/Aphids%20of%20Karnataka/Aphiscraccivora.htm

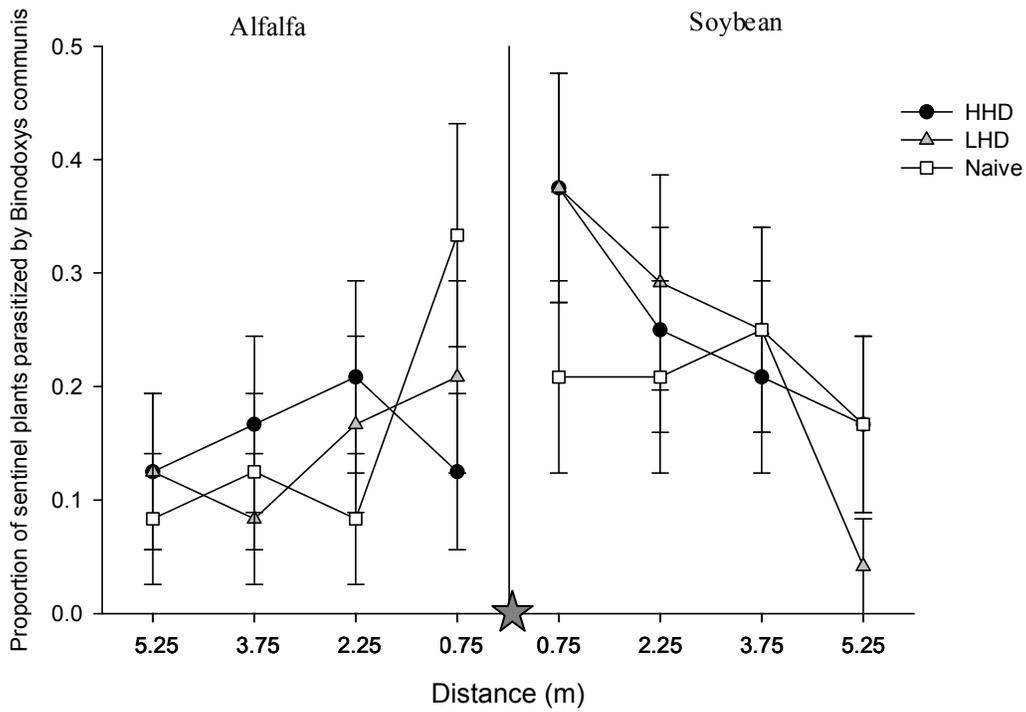Figure 5.3: Box plot of egg loads of female *Binodoxys communis* in the three host density treatments.

Figure 5.4: Average proportion of sentinel plants (± SE) parasitized by *Binodoxys communis* across the four distances in the soybean and alfalfa field. The grey star indicates the release point.
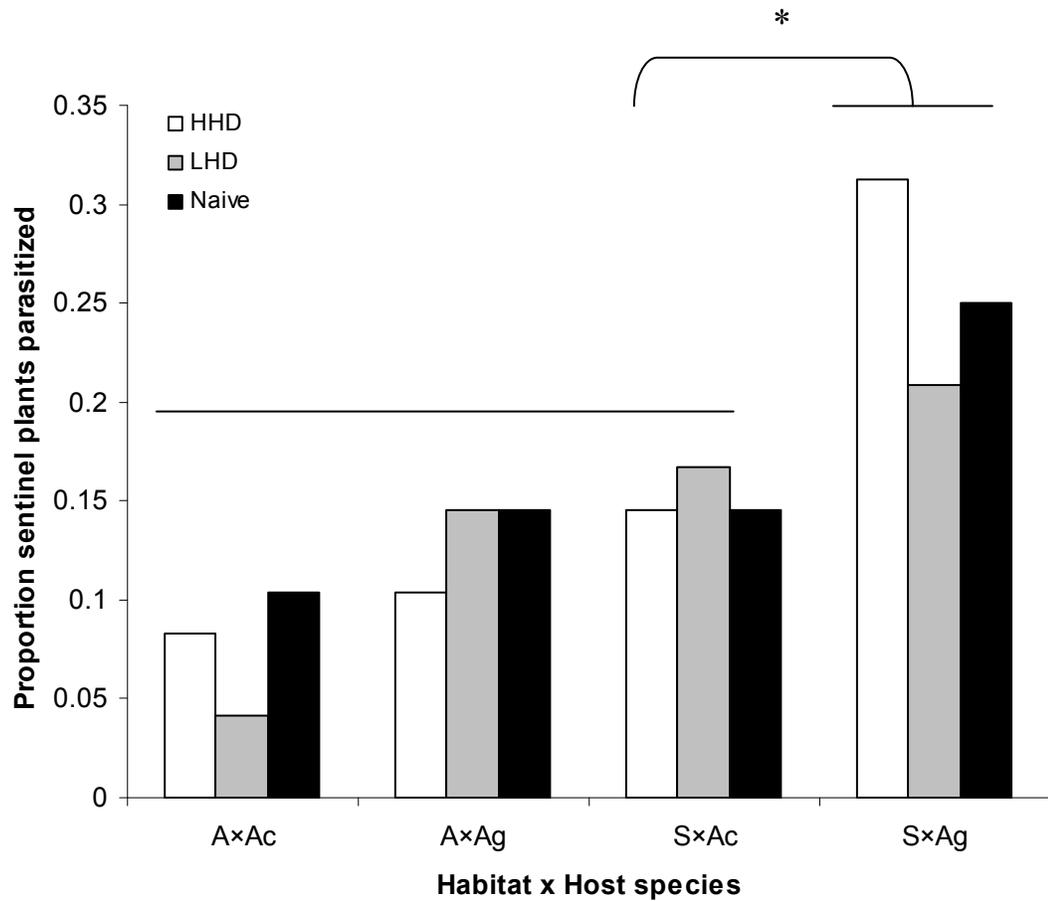
Figure 5.5: Average proportion of parasitized sentinel plants separated by level of host experience and habitat×host species. Asterisk indicates significant difference (Tukey HSD, P < 0.05). Abbreviations: HHD = high host density pre-patch experience, LHD = low host density pre-patch experience, A = alfalfa, S = soybean, Ac = *Aphis craccivora*, Ag = *Aphis glycines.*

Figure 5.6: Average proportion of parasitism by the interaction of experience×habitat in *Binodoxys communis*. Abbreviations: HHD = high host density pre-patch experience, LHD = low host density pre-patch experience, A = alfalfa, S = soybean.
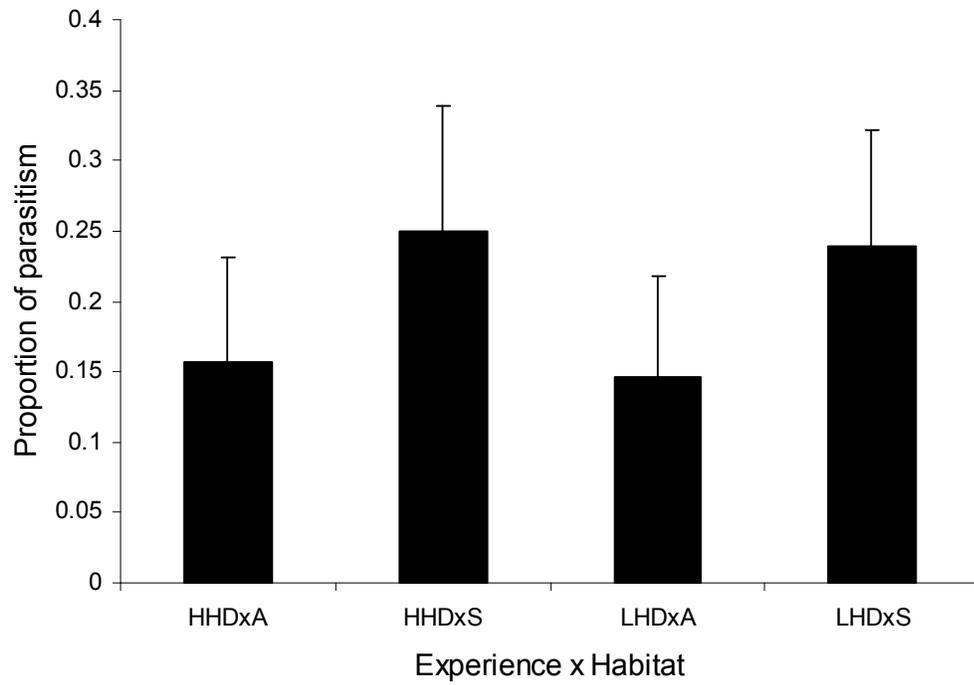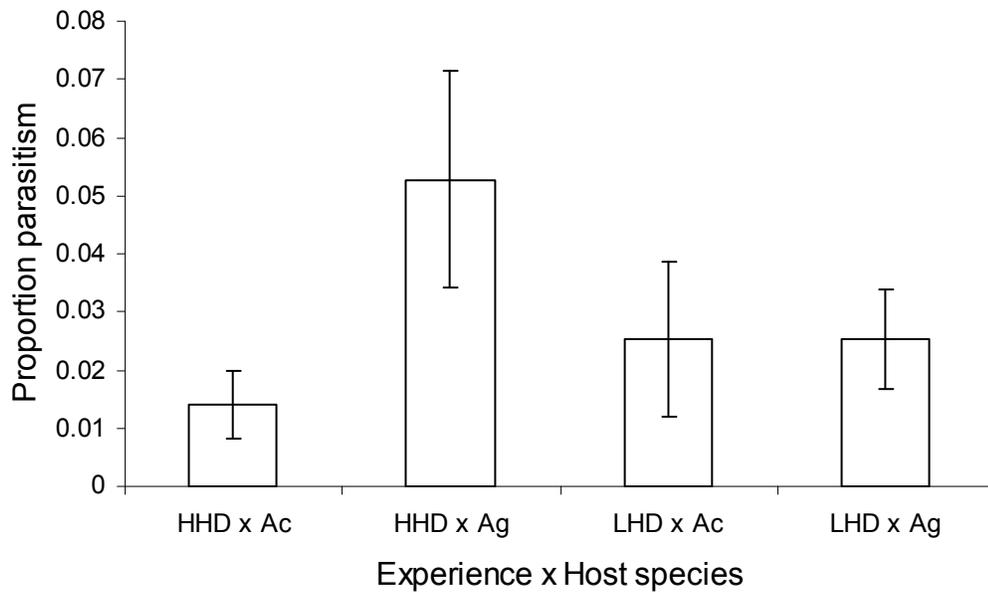
Figure 5.7: Average proportion of parasitism by the interaction of experience×host species in *Binodoxys communis*. Abbreviations: HHD = high host density pre-patch experience, LHD = low host density pre-patch experience, Ac = *Aphis craccivora*, Ag = *Aphis glycines*.

a) HHD - LHD
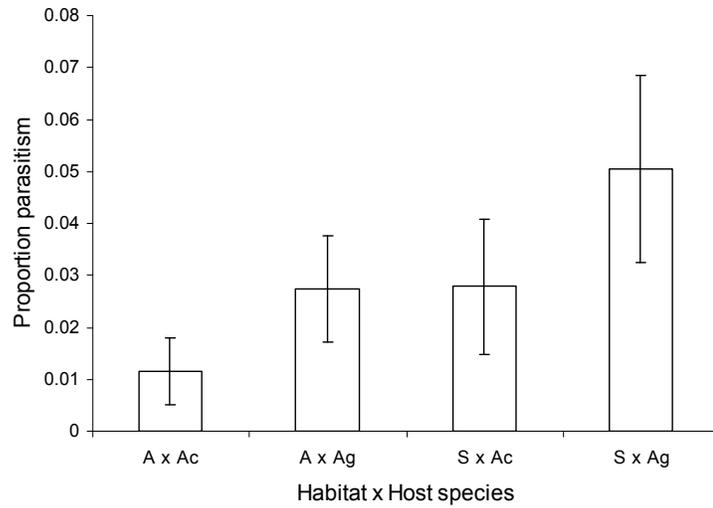
b) Naïve

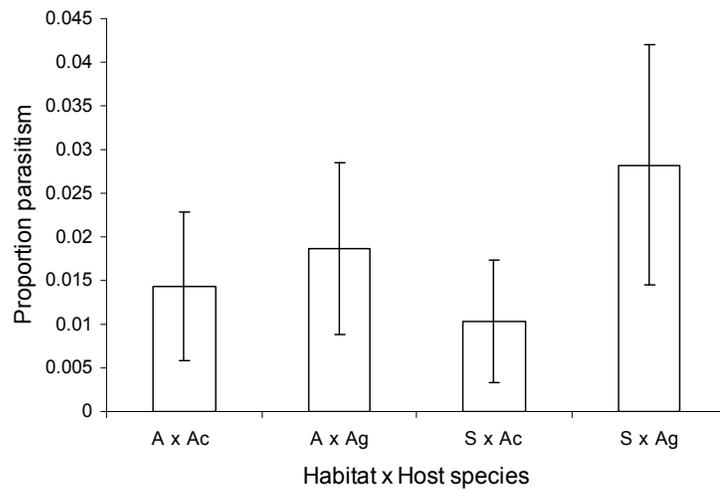

Figure 5.8: Average proportion of parasitism by the interaction of habitat×host species in

a) HHD and LHD *Binodoxys communis* and b) naïve *Binodoxys communis*.

Abbreviations: A = alfalfa, S = soybean, Ac = *Aphis craccivora*, Ag = *Aphis glycines*.

Chapter 6

Conclusion

Binodoxys communis showed a low risk of egg limitation under both laboratory and field conditions through a strategy of maintaining a constant egg load in the wake of oviposition (Chapter 2 & 3). Furthermore, there was a strong indication that honeydew feeding in free-foraging *B. communis* is a common occurrence and thus helps minimize a female parasitoid's risk of becoming time limited due to starvation (Chapter 3). Host specificity in *B. communis* was maintained even under conditions that have been shown to maximize the risk of time limitation and the acceptance of low-quality hosts in other parasitoid species. Host availability appeared to be the dominant factor influencing host choice behavior in *B. communis*. In accordance with the predictions stemming from optimality theory, low host availability and changes in the environment which indicate a drastic decline of favorable conditions increased the probability to accept low-quality hosts in female *B. communis* presumably as a result of an increase in the perceived risk of time limitation by the parasitoids (Chapter 4). Finally, our observations on *B. communis* host choice behavior in the field suggested that host specificity in free-foraging female parasitoids is under the influence of the perceived risk of egg and time limitation as was predicted based on the no-choice tests in field-collected parasitoids (Chapter 4 & 5).

The research outlined above is a contribution to the ongoing study of the role of parasitoid physiological state in dynamic host choice behavior and the first of its kind (to my knowledge) that explicitly looked at the influence of physiological state on interspecific host choice in parasitoids. It was conducted in the context of host risk assessment of classical biological control and provided some insight into the potential of

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host range expansion in a biological control agent in response to physiological state variables. Furthermore, the combination of laboratory and field experiments allowed us to build a bridge between the control environment of a laboratory and the complex world of a free-foraging parasitoid. That is, it brought "… a dose of realism into an otherwise deterministic world found only in the laboratory" (quote Heimpel & Casas, 2008; page 65).

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