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# Selective Utilization of Microhabitats by Web-building Spiders

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SELECTIVE UTILIZATION OF MICROHABITATS BY WEB-BUILDING SPIDERS

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DISSERTATION

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A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture
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By
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Lexington, Kentucky

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ABSTRACT OF DISSERTATION

SELECTIVE UTILIZATION OF MICROHABITATS BY WEB-BUILDING SPIDERS

Natural enemies are members of complex ecological communities, and their ability to contribute to the biological control of pest organisms is strongly influenced by a convoluted network of ecological interactions with many other organisms within these communities.  Researchers must develop an understanding of the mechanisms that shape trophic webs to predict and promote top-down effects of predators.  The behavior of predators can have a strong influence on their potential as biological control agents.

Web-building spiders are a useful example organism for the study of natural enemy behavior because of the experimentally tractable nature of their foraging behavior. Specifically, patterns in microhabitat utilization and web construction by spiders provide insights into foraging behavior and pest-suppression potential.

In field collections, spiders were found to utilize microhabitats in a species-specific manner.  Molecular gut-content analysis and a mathematical model showed that two spiders belonging to different web-building guilds differed in their dependence on microhabitat-specific prey activity-densities.  In particular, the sheet-weaving guild constructed webs in microhabitats with the highest densities of springtails (Collembola). High dependence on this non-pest prey also correlated with evidence of increased intraspecific competition, and implies a potential negative effect of springtails on the consumption of pest insects, such as aphids.

In laboratory two-choice assays, sheet-weaving spiders selected microhabitats and constructed webs in a flexible, stepwise manner, which allowed spiders to regulate their investment of silk resources to match the profitability of the microhabitat.  Spiders also exhibited prey-specific shifts in foraging behavior, constructing webs in the presence of mobile, non-pest springtails, but utilizing active foraging tactics in the presence of sedentary, pest aphids.  However, in factorial no-choice assays, pest-consumption rates were not significantly affected by the presence of non-pest springtails, indicating that prey-specific foraging-mode shifts are compatible with biological control.

From these results, it is clear that the flexible foraging behavior of web-building spiders has a strong influence on their roles in ecological communities and their position within food webs.  This dissertation highlights the importance of understanding the nuances of natural-enemy behavior for properly assessing and promoting biological control services.


KEYWORDS: Spider Web, Biological Control, Food Web, Foraging Ecology,
Alternative Prey


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SELECTIVE UTILIZATION OF MICROHABITATS BY WEB-BUILDING SPIDERS

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**CHAPTER 1: INTRODUCTION**

**1.1 Functional roles of generalist predators in biological control**

Arthropod predators can provide pest-suppression services that are economically beneficial to growers (Landis *et al.* 2000; Symondson *et al.* 2002; Cardinale *et al.* 2003). However, crop fields are complex ecosystems, and full use of the services provided by arthropod predators will require a greater understanding of how these predators interface with the agricultural environment and its many variables (Landis *et al.* 2000; Casula *et al.* 2006). Analysis of predator behavior can provide key insights into the factors that constrain or facilitate pest-suppression services. For example, patterns of space usage and diel activity can dramatically impact a predator's rate of encounters with pest prey, and thereby impact the likelihood of pest suppression (Schmitz 2007). Thus, it is crucial to understand the behavior of natural enemies when designing management programs for biological control. However, predator foraging behavior is often flexible and dynamic, and it can thus present challenges in determining how to tease apart important variables, and in assessing the effects on larger and broader scale dynamics.

The scale of investigation is a critical element of biological control research (Levin 1992). For example, many management strategies target community-scale processes by manipulating the crop habitat to promote a high diversity of natural enemies (Landis *et al.* 2000). Diversifying an agricultural landscape with strips or patches of non-crop plants, and using mulches or debris to alter the structural and biotic complexity of the habitat, are common approaches that can augment natural enemy populations (Landis *et al.* 2000; Langellotto & Denno 2004; von Berg *et al.* 2010). However, while these techniques often lead to increases in natural enemy abundance and diversity (Landis *et al.*

2000), a concomitant increase in the pest-suppression services provided is not consistently observed in all types of crop systems, indicating that the relationship between diversification and biological control is complex, and the exact mechanisms at play are often difficult to resolve (Griffiths *et al.* 2008).  In some cases, increased natural enemy diversity may improve biological control by promoting many natural enemies that attack a pest item in different ways, thereby increasing total predation pressure on pest populations  (Schmitz 2007).  In such cases, biodiversity itself (a community-scale variable) is an important component of biological control services.  However, in some cases, the apparent positive effect of biodiversity on biological control is due entirely to a single natural enemy species, whose action was enhanced by the treatment (Straub & Snyder 2006; Schneider & Brose 2013).  Additionally, increased natural enemy diversity may actually result in reduced pest suppression when natural enemies interfere with one another (Sih *et al.* 1998; Finke & Denno 2004; Casula *et al.* 2006): for example, one natural enemy may prey on another (Rosenheim *et al.* 1995), or may alter the behavior of another natural enemy in such a way as to impact its functional roles within the system (Agarwala *et al.* 2003).  Thus, it becomes important to distinguish between techniques that promote biodiversity *per se*, and techniques that promote the "right" biodiversity for maximizing pest-suppression services (Landis *et al.* 2000; Straub & Snyder 2006).  Knowledge of natural enemy behavior, and of its potential impacts on community-scale dynamics, can therefore aid in identifying and promoting this "right" biodiversity, and thereby inform and improve management decisions.

There is certainly an acute need for biological control to be guided by ecological theory, and the basic theoretical and mathematical models of predator ecology and

predator-prey interactions have much to offer for biological control research.  Here, I

provide a brief discussion of some of the relevant ecological models, and of their utility

for biological control research.

*1.1.1 Ecological theory*

One of the most influential theories in predator ecology is optimal foraging theory

(OFT), which models fundamental aspects of the decision-making processes of animals

as they forage (MacArthur & Pianka 1966).  In its basic form, this theory addresses static

decisions made by predators, although it has since been expanded to account for more

dynamic patterns of behavior.  The basic premise of OFT is that foragers balance various

environmental, physiological and biotic considerations (e.g., predation risk, prey

nutritional quality, etc) to maximize the net rate of energy gain.  OFT includes two basic

models, the prey model and the patch model, which address decisions made before and

after (respectively) attacking a prey item or entering a patch of resources.  The **prey**

**model** addresses the decision of whether to attack an encountered prey item through an

economic comparison of the availability, nutritional quality and handling time of various

prey types.  The **patch model** addresses the decision of how long to continue feeding on

a captured prey item or foraging in a patch of resources based on the rate of nutritional

gain from feeding or foraging and the potential rate of gain from searching for a new prey

item or patch (Charnov 1976).  OFT predicts that a predator will rank prey types or

patches based on their quality.  According to the prey model, prey types will be attacked

only if the gain from attacking the prey type is enough to offset the cost of missed

opportunities (i.e., the cost of foregoing the possibility of encountering a prey type of

higher quality).  This predicts a "zero-one" rule: a prey item will not be attacked unless

prey items of higher quality dip below a threshold density (Stephens & Krebs 1986).

According to the patch model, predators will leave a current resource patch when the rate

of gain from the patch equals the average rate of gain of all patches in the environment.

Although its conclusions rest on a number of unrealistic assumptions, OFT has

dominated the thinking of theoretical ecologists since its introduction.  The conceptual

premise that foraging behavior can be viewed as an economic decision-making process

has been borne out in numerous studies (e.g.  Viswanathan *et al.*, 1999; Sayers *et al.*

2010), although the strict view that all animals' diets are optimized for maximal energy

gain has long been controversial (e.g.  Pierce & Ollason 1987), and several specific

predictions, such as the zero-one rule, have not been born out in empirical studies

(Okuyama 2011; Pyke 1984).  Despite its shortcomings, OFT has provided important

insights for biological control and natural enemy research (Pyke 1984).  For example, the

effects of non-pest prey on biological control can be predicted using the prey model.  If a

non-pest prey that is high in nutritional quality is readily available in a crop system, then

predators may simply ignore any pest prey with low nutritional quality, and consequently

provide no pest-suppression services.  However, this process is not likely to be a zero-one

dynamic, in which pest prey are completely ignored at certain non-pest prey densities, but

will likely be a more gradual decline in pest consumption as non-pest prey increase.

However, nutritional quality is not a one-dimensional attribute: different prey can

fill different nutritional needs for predators, and generalist predators may therefore

employ a more dynamic strategy to meet their nutritional requirements.  For example, if

there are two potential prey species in the habitat, and each provides different essential

nutrients, then a predator may rank the two prey differently, depending on its current

nutritional state. Such dynamic decision-making hints at another important theoretical

concept in ecology: the functional response (Solomon 1949; Holling 1959). Functional

responses describe the per-capita rate of prey consumption as a function of prey density,

and can take on three basic forms, one of which (Type III) entails an increase in the

proportional effects of a predator on the population of the given prey population. And, of

course, such dynamic forces operating at the scale of individual predator behaviors can

translate into population- and community-scale effects: if prey populations are in flux,

predator populations may fluctuate in response (a numerical response) (Solomon 1949).

A numerical response may result from increased predator reproduction (due to increased

energy intake by consuming prey), or from predator immigration to a habitat with an

abundance of prey resources.

These basic theoretical principles can be used to inform biological control efforts.

For example, the aforementioned habitat management techniques make use of the

concept of numerical response: augmenting the availability of a resource (habitat

structures, alternative food resources, etc.) within an agricultural system may induce

predators to immigrate into the system in response (Alderweireldt 1994b; Jacometti *et al.*

2010). Additionally, synthesizing OFT and the functional response, a researcher might

predict that, as the relative abundance of different prey types fluctuates over time, the

economic considerations emerging from the prey model will also vary, which can alter

the decision-making processes of predators, and result in dynamic patterns of predator

impacts in biological control. This gives rise to an additional ecological concept: flexible

foraging, in which a predator adaptively modifies its foraging behavior in real time to

balance various ecological considerations (Abrams 2010). Flexible foraging behavior

may serve several different ecological functions.  Predators may modify their behavior in order to (1) regulate the efficiency of foraging (in terms of time or effort invested), (2) regulate which types of prey or other food are taken, or (3) regulate exposure to potential predation risks while foraging (Abrams 2010).

Although the principle of flexible foraging has been implicitly incorporated into biological control studies, surprisingly little experimental work has attempted to unravel the potential role of flexible foraging behaviors in driving the consumption of pests by natural enemies.  In this dissertation, I sought to characterize the behavior of a common natural enemy, web-building spiders, and to determine whether variability and flexibility in foraging behavior can influence the consumption of pests by this natural enemy.  First, I review relevant literature on behavioral ecology of predators in biological control, and on the behavior and ecology of web-building spiders.

*1.1.2 Generalists vs specialists*

Diet breadth has traditionally been considered a key attribute by which predators can be categorized into ecological groupings.  Predators that consume a wide range of prey are often referred to as *generalists*, while predators that feed on a narrow range of prey are referred to as *specialists*.  Alternative terminological systems use *polyphagous* or *euryphagous* for generalists, and *monophagous* or *stenophagous* for specialists.  The distinction between generalist and specialist is largely arbitrary, and diet breadth can vary along a continuous spectrum from nearly exclusive consumption of a single prey type to nearly indiscriminate predation on any organism that can be captured.  Thus, categorization based on diet breadth can be ambiguous.  Additionally, diet breadth does not capture all the complexity in predator feeding ecology: even highly polyphagous

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predators can show preference for certain types of prey, and can even exhibit some degree of morphological, ecological or behavioral specialization for preferred prey (Huseynov *et al.* 2005; Huseynov *et al.* 2008; Polidori *et al.* 2010). Indeed, while generalist predators are often regarded as opportunistic and indiscriminant, in reality they can display a wide range of ecological and behavioral dynamics. Therefore, treating "generalist" as a meaningful behavioral category may be inappropriate. On the other hand, nearly all predators feed on multiple types of prey, and thus few predators could truly be considered specialists in the strictest sense. Nevertheless, many predators do, indeed, display very high levels of specialization for predation on specific types of prey, and are consequently strongly associated with a narrow range of prey. Therefore, the terms "generalist" and "specialist" still have heuristic value, and still capture several relevant aspects of predator ecology and biological control potential. I will thus continue to use them throughout this dissertation for the convenience of the discussion.

Both specialist and generalist predators can play important roles in biological control. Specialist predators tend to have a few advantages that were often exploited in classical biological control: namely, more specialized predators tend to have close interrelationships with and specific adaptations to their pest prey, which enables them to efficiently target pests and suppress their populations. However, specialization also often comes with the weakness of inflexibility: specialization on a specific pest prey often entails a rigid set of adaptations in behavior, life cycle and habitat that may limit their ability to thrive in the often challenging environments of agricultural systems. In contrast, generalist predators usually lack specific adaptations to target pests, and thus tend to be less efficient at exploiting and suppressing them (Wissinger 1997). However,

generalist predators can more readily adjust to whatever conditions the environment

offers, and can take advantage of whatever prey or food resources are available

(Wissinger 1997; Symondson *et al.* 2002). These differences in ecological requirements

undoubtedly have consequences for the biological control services that predators can

provide. Agronomic practices are not always conducive to the rigid ecological

requirements of specialist predators, but are compatible with large populations of

generalist predators, whose versatility is a definite advantage in ephemeral habitats such

as annual crop fields (Wissinger 1997). This weakness of specialists has not always been

as well appreciated as it is currently. For much of the history of biological control

research, study focused strongly on importation of specialist predators, due in part to the

belief that the adaptations of generalist natural enemies to a diverse diet prevented them

from efficiently suppressing pest populations (Wardle & Buckle 1923; Symondson *et al.*

2002). Furthermore, dramatic successes in the field built a strong case for imported

specialist predators as biological control agents. As the evidence continued to

accumulate, however, it became clear that there was a characteristic profile for cases in

which specialist predators were successful (Southwood 1977; Wissinger 1997; Hawkins

*et al.* 1999). Most successes with single specialist natural enemies were accomplished in

simple systems with relatively low levels of disturbance, such as perennial crops

(Wissinger 1997), and with the natural enemy acting against an exotic pest (Hawkins *et

al.* 1999). Situations involving more complex landscapes and native pests are more suited

to pest suppression by assemblages of generalist natural enemies (Wissinger 1997).

Furthermore, because many supposed specialist natural enemies will, in fact, attack many

types of prey, importing these enemies as biological control agents often leads to negative

impacts on non-target organisms, and importation biological control is no longer considered an appropriate strategy for pest control in many cases. Therefore, generalist predators have an important niche to fill for biological control purposes.

### 1.1.3 Trophic webs and interaction webs

Inclusion of generalist predators in pest-suppression efforts entails a complex set of ecological considerations. Specialist predators only interact regularly with a few prey types, and display tightly-linked dynamical relationships with these few types. By comparison, the broad diets of generalist predators result in complex food webs, in which the predator interacts with many different types of prey on a regular basis. Consequently, each specific link in the food web may be quite weak, and these predators' dynamics will often not be strongly dependent on any one prey type (Symondson *et al.* 2002). The diffuse nature of these linkages can buffer predators against fluctuations in prey populations and allow them to persist under a wide range of conditions (Symondson *et al.* 2002). However, it also generates a complex web of trophic and non-trophic interactions that presents major challenges for researchers to disentangle or even model effectively (Krivan & Schmitz 2003; Casula *et al.* 2006; Letourneau *et al.* 2009). Many of these interactions have important implications for biological control. Here, I discuss two main types of interaction that have been considered important for biological control: alternative prey interactions, and intraguild interactions.

As generalist predators have trophic linkages with many types of prey simultaneously, they can be a vehicle by which two prey items can indirectly affect one another (Holt & Lawton 1994). For example, pest prey and alternative, non-pest prey often interact with one another indirectly, through their shared predators, whose

behaviors and feeding preferences can result in asymmetrical effects on the two prey

types. When non-pest prey have high nutritional quality, their availability in a habitat

may induce shifts in predator preferences and predator foraging behavior (Symondson *et*

*al.* 2002; Symondson *et al.* 2006), thereby releasing the pest from predation pressure and

resulting in a pest outbreak. However, for a generalist predator, feeding on a non-pest

prey and feeding on a pest prey need not be mutually exclusive. Many predators actively

diversify their diets by incorporating different types of prey. This may be a result of

partial preferences (i.e., probabilistic preferences, rather than strict, zero-one preferences)

or variation among individuals within a population (Okuyama 2011) In such cases, a

numerical response by the predator to a non-pest prey, or augmentation of predator

nutrition and fecundity by the non-pest prey, can facilitate the pest suppression activity of

the predator by sustaining underlying populations of predators in the absence of pests, so

that predators are capable of exerting control as soon as the pest arrives in the system

(Settle *et al.* 1996). Additionally, many predators are, in fact, omnivores, and non-prey

foods, such as nectar and pollen, may have similar effects to alternative prey (Lundgren

2009).

Predators may also interact with other members of their own guild, that is, they

may interact with other natural enemies. These intraguild interactions can be direct or

indirect, and can be both beneficial or detrimental to biological control (Schmitz 2007).

The most extreme case of negative interaction is intraguild predation (IGP), in which

predators actively prey on one another (Rosenheim *et al.* 1995). IGP can be

unidirectional (i.e., one predator species regularly preys on another; e.g., Dinter 2002) or

bidirectional (i.e., each predator may prey on the other under certain conditions, e.g.,

Balfour *et al.* 2003), and can therefore translate into a variety of different effects on trophic webs, depending on the structure of the predator complex. However, a variety of sub-lethal interactions can also impact the behavior of natural enemies in such a way that biological control is reduced. For example, larger predators may displace smaller predators from preferred habitats (Harwood & Obrycki 2005), and smaller predators may alter their behavior patterns to avoid larger predators (Choh *et al.* 2010). Competitive interactions such as these can be mediated by habitat structures (Finke & Denno 2002), diel activity patterns (Hill & Lodge 1994), or seasonal dynamics (Spiller 1984); and can often reduce the efficiency of the inferior competitor in biological control (Rypstra *et al.* 2007). However, occasionally, natural enemies are able to operate in a synergistic fashion. When natural enemies utilize different portions of the habitat, or utilize the habitat in a different fashion, they can exhibit complementary effects on pest populations by attacking different subsets of the pest population (Schmitz 2007; Straub & Snyder 2008). Additionally, the activity of some natural enemies may alter pest behavior in such a way that the pest's vulnerability to another natural enemy is increased (Losey & Denno 1998c). Such positive predator-predator interactions are thought to be relatively rare, but may be sufficiently common to exploit for biological control purposes in some cases.

With such complex suites of interactions with other organisms, it can be difficult to imagine that generalist predators can be sufficiently well understood and managed to provide effective biological control services. However, many of the underlying ecological and behavioral dynamics can be investigated to elucidate important mechanisms in specific cases, and thereby provide valuable insights into the functions of agroecosystems, which can be used to better inform biological control efforts in the

future.  Unfortunately, many groups of natural enemies do not receive significant attention in the literature, and ecologists therefore remain unaware of the potential services these natural enemies can provide.

## 1.2 Web-building spiders

One of the most diverse and abundant types of natural enemy in agroecosystems worldwide are the web-building spiders (Nyffeler & Sunderland 2003).  These are generalist predators that construct silk webs to capture a variety of prey items, including pest and non-pest prey (Harwood *et al.* 2004; Chapman *et al.* 2013).  Web-building spiders are a useful example organism in biological control because the construction of silk webs to trap prey renders several important behavioral variables highly discrete and quantifiable (Blackledge & Wenzel 2001).  Webs are semi-permanent structures that provide a record of an individual spider's ecological dynamics, even when the spider itself cannot be observed or recovered.  A web also confines a spider's movement and behavior to some extent, and allows researchers to examine important ecological variables on a small, non-arbitrary scale.  The architectural characteristics of a spider web often reflect its functionality and the behavioral processes of its tenant spider(s) (Blackledge *et al.* 2011); and the location of a spider web within its habitat often conveys important information about the behavior and ecology of the spider (Herberstein 1998; Harwood *et al.* 2003).  Webs are primarily regarded as foraging devices, but in fact can be multi-functional, and their construction and placement can provide information on various other ecological processes, such as abiotic stresses (Biere & Uetz 1981; Mallis & Rieske 2010) or competitive interactions (Blackledge *et al.* 2003; Blamires *et al.* 2007).

Spider webs therefore provide a unique opportunity to investigate the behavioral ecology of generalist predators.

### 1.2.1 Trophic functions of spider webs

The primary function of most spider webs is prey capture. Web-building spiders are usually generalist predators that routinely consume a wide range of prey (Nyffeler 1999). The majority of prey caught by web-building spiders come from a few arthropod orders: Hemiptera, Diptera, Lepidoptera, Coleoptera, Collembola and Araneae (other spiders) (Nyffeler 1999). However, the prevalence of a given prey item in the diet may give a false impression of its importance to the spider. Many spiders seem to be dependent on large prey that are rarely caught, but provide the bulk of prey biomass and nutritional content, and the smaller, common prey serve only to sustain the spider between rare, large catches (Venner & Casas 2005; Blackledge 2011). Additionally, prey are not the only source of nutrition: spider webs may also be used to intercept non-prey foods, such as pollen, (Peterson *et al.* 2010), which can augment spider fitness (Peterson, Lundgren & Harwood, unpublished data). Therefore, the spider web can be regarded as an important mediator of a complex trophic web, and is thus a very useful subject in the study of trophic ecology.

For web-building spiders, prey capture consists of multiple, independent components. First, the web must be placed and oriented in such a way to maximize the likelihood of intercepting a prey item (Harwood & Obrycki 2007, see section, "Microhabitat utilization," below). The location and orientation of a web may have large impacts on the types of prey that can potentially be intercepted: for example, webs that are built high above the ground and oriented vertically may intercept more flying prey,

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while webs built low to the ground and oriented horizontally may intercept more falling or epigeal prey (Eberhard 1990; Bishop & Connolly 1992). Second, the amount, density and organization of the web's silk can influence several functional characteristics, including the likelihood of intercepting prey, the size of prey that can be caught, or the visibility of the web to the prey (Rypstra 1982; Blackledge & Eliason 2007). Thus, a variety of different combinations of web size, silk density and silk patterns can be observed across species, with a variety of functional roles for spiders that may target different types of prey, or may use different behavioral tactics to capture their prey (Zschokke *et al.* 2006). Finally, web silk must have elastic and adhesive properties appropriate for the capture of targeted prey. For example, the silk must be able to absorb the kinetic energy of an intercepted prey item (Kelly *et al.* 2011), ensnare the prey to prevent it from escaping once caught (Opell 1999), and transmit vibrations to inform the spider of the prey's location within the web (Landolfa & Barth 1996). Spiders may produce several specialized types of silk to fill each of these functions, and may regulate production of each silk type to modify the functionality of the web in prey capture (Zevenbergen *et al.* 2008).

There is presumably a complex variety of tradeoffs among these web characteristics, and a concomitant diversity of strategies employed by web-building spiders. However, each of these components may be selected for independently over evolutionary time (Blackledge *et al.* 2003; Kawamoto & Japyassú 2008), and each may also show considerable plasticity of expression within a single species, or even a single individual under different conditions (Zevenbergen *et al.* 2008). Thus, web-based foraging tactics are complex, but many variables can be readily quantified and correlated

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with important ecological variables to derive robust hypotheses and conclusions about the behavior of these predators, and many of the principles gleaned from such research can be applied to other predators as well.

### 1.2.2 Non-trophic functions of spider webs

Prey-capture is not the only function of spider webs. Web-building spiders spend a substantial amount of time in their webs, and this sedentary behavior has ramifications for many other aspects of spider ecology and behavior. Web-construction can dominate the daily energy budget of a spider (Tanaka 1989), highlighting its central role in spider ecology. Therefore, a spider web can be regarded not only as a foraging device, but also as a habitat for the spider (Blackledge *et al.* 2011). Webs can provide protection for spiders, either by deterring flying parasitoids or predators that are unable to navigate a labyrinth of silk (Blackledge *et al.* 2003), by forming specialized retreats where the spider is less vulnerable to predators (Pasquet *et al.* 2007), or by altering the visibility of the web to prevent its destruction by unsuspecting birds or other large animals (Walter & Elgar 2011). Additionally, the mechanical properties of the silk can be used to transmit, not only prey vibrations, but also finely-tuned signals used during courtship (Wignall & Herberstein 2013), or as a means of communications among social spiders that live within the same web system (Burgess 1979). Webs can also serve as a water-condensation point, to retain water for drinking (Walter *et al.* 2009). These non-trophic functions introduce significant opportunity for variation among spiders, and researchers have only begun to fully understand the functional and morphological diversity of spider webs.

*1.2.3 Diversity of spider webs*

 Spider webs display a diversity of forms among the many clades that produce them. Many webs fall within three broad categories: orb webs, sheet webs and tangle webs (Fig. 1.1). Orb webs consist of a spiral of adhesive silk laid on a radial frame of supporting silk (Benjamin & Zschokke 2004). In contrast, sheet and tangle webs consist of stochastic networks of silk, arranged in two-dimensional sheets or three-dimensional masses (respectively) (Benjamin & Zschokke 2004). Each of these web types lends itself well to specific web functions, and can impact a spider's ecology in distinct ways. Orb webs, which are the most commonly studied type of spider web, are constructed using relatively small amounts of silk, which makes them less expensive (Janetos 1982) and less visible to prey (Rypstra 1982; Blackledge & Eliason 2007) than sheet or tangle webs. The behaviors associated with orb-web construction are highly stereotyped, and the web is usually highly organized and uniformly structured, making it an attractive subject for investigation (Benjamin & Zschokke 2004). Due to the adhesive qualities of the silk and the low visibility of the web, orb webs are extremely efficient at intercepting and retaining prey (Rypstra 1982). By comparison, sheet and tangle webs are constructed without stereotyped behaviors, and consequently display little specific organization and structural uniformity (Benjamin & Zschokke 2004). They also generally contain higher amounts of silk than orb webs, making them more expensive (Ford 1977; Janetos 1982) and more visible to prey (Rypstra 1982). Additionally, sheet and tangle webs have generally poorer prey-capture performance than orb webs, in terms of prey retention (Zschokke *et al.* 2006). From this comparison, it would appear that the orb web is clearly superior to the sheet and tangle webs, being both less expensive and more effective at

Orb Web                    Sheet Web                   Tangle Web

**Functional Characteristics**

Prey-Capture Efficiency

HIGH                                                              LOW

Predator Deterrence

LOW                                                              HIGH

**Construction Characteristics**

Initial Construction Cost

LOW                                                              HIGH

Daily Maintenance

HIGH                                                              LOW
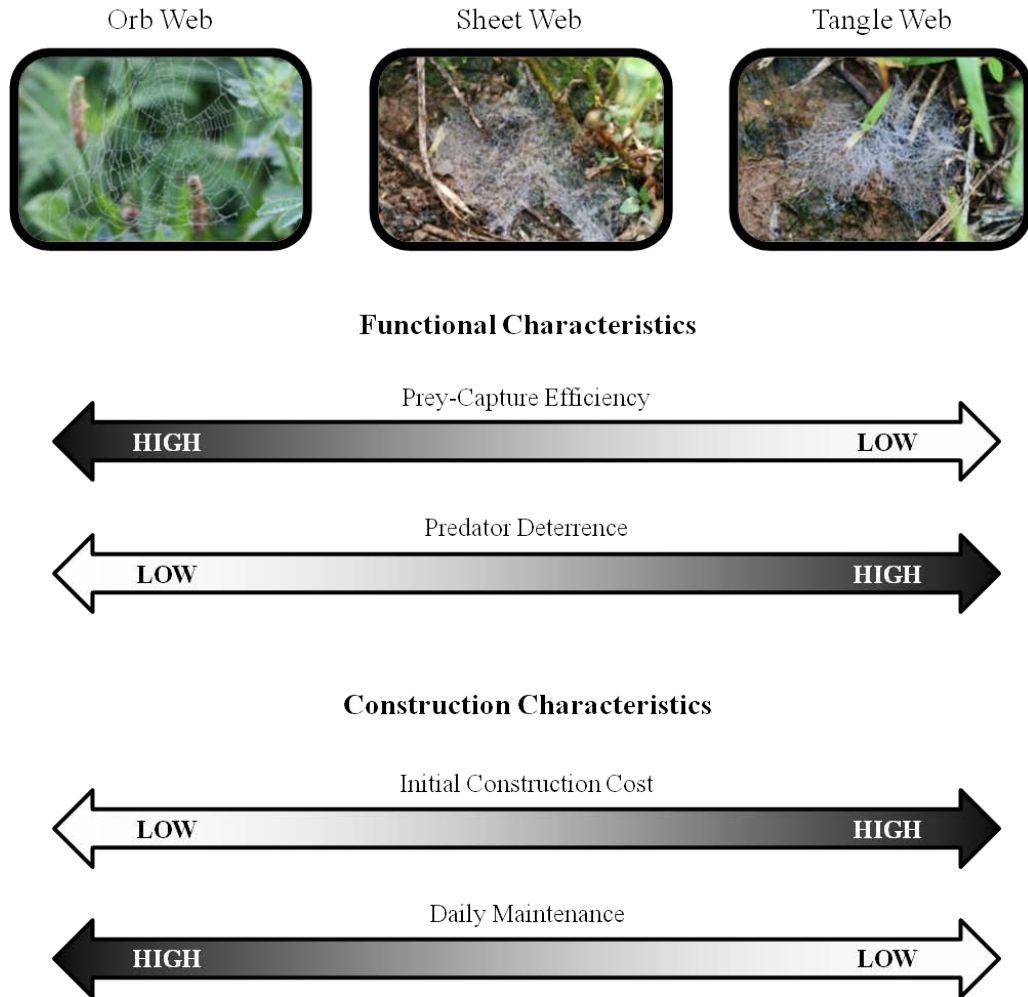
Figure 1.1  Characteristics of three primary categories of spider webs. Double-sided arrows represent gradients in each characteristic.  Based on information in Janetos (1982), Rypstra (1982), and Blackledge et al. (2003).

capturing prey. However, sheet and tangle webs have two major advantages over orb webs. First, orb webs require extensive upkeep in order to maintain optimal performance: an orb-weaving spider may even re-ingest and rebuild its web each night (Peakall & Witt 1976; Mori & Nakata 2008). By comparison, sheet and tangle webs require less maintenance over time, and can thus potentially be less expensive in the long term (Zschokke *et al.* 2006). Second, orb webs provide very little defense against flying predators, such as mud-dauber wasps (Blackledge *et al.* 2003). By comparison, sheet and tangle webs serve as a barrier of silk that denies wasps access to the spider beneath or within the web (Zevenbergen *et al.* 2008).

*1.2.4 Microhabitat utilization by web-building spiders*

For web-building spiders, every aspect of ecology is mediated through the web and through the characteristics of the specific site where the web is constructed. This site, or microhabitat, is a small unit of habitat with which the spider has a very intimate association. Thus, researchers expect patterns in microhabitat selection and utilization to be exceptionally meaningful for web-building spiders. Foraging by web-building spiders consists of the search for and utilization of specific microhabitats for the construction of webs (Harwood *et al.* 2001; Pruitt *et al.* 2011). This process can be investigated using the generalized ecological principles described above (i.e., OFT, functional responses and numerical responses). Optimally foraging predators select and utilize habitat patches that maximize their rate of energy intake (Stephens & Krebs 1986). Although this principle was originally developed to describe the behavior of active-foraging predators, it is also likely that sedentary predators, such as web-building spiders, will follow the same general pattern (Beachly *et al.* 1995). For example, microhabitat utilization by web-

building spiders has traditionally been investigated in terms of two distinct mechanisms or processes: *site selection* (Johnson *et al.* 2011; Pruitt *et al.* 2011) and *site tenacity* (Samu *et al.* 1996; Rittschof & Ruggles 2010). These processes are analogous to the processes described in the prey model and patch model (respectively) of OFT. Site selection is the initial process of locating, evaluating and choosing whether to occupy a given microhabitat (Pruitt *et al.* 2011), and is conceptually similar to the prey model's dynamic of locating and choosing whether to attack a prey item. In contrast, site tenacity (also called "site fidelity" or "site tenure") is the process of deciding when to give up or abandon the microhabitat (Kawamoto & Japyassú 2008), and is essentially identical to the patch model's dynamic of deciding how long to forage in a given patch. As such, these behavioral processes can be regarded as highly quantifiable exemplars for the study of foraging ecology.

Most web-building spiders are generalist predators that feed on a wide range of prey (Nyffeler 1999). These diverse diets raise a number of questions concerning the foraging of these predators. For example, is optimal foraging a realistic expectation for a trap-building predator (Edwards *et al.* 2009)? Web-building spiders are sit-and-wait foragers, which implies that they have little actual control over the rate at which prey are encountered (Beachly *et al.* 1995; Mori & Nakata 2008). Therefore, the availability of prey may be so low that the prey-ranking process described by the prey model of OFT simply never comes into consideration, and the cost-benefit analysis may favor an opportunistic strategy of attacking any and all prey that land in their webs (Edwards *et al.* 2009). Indeed, there is considerable evidence indicating that patterns of site selection and site tenacity do not match the expectations of the optimal-foraging paradigm (Samu *et al.*

1996; Edwards *et al.* 2009).  However, other studies have shown that foraging-related cues dominate the decision-making processes of spiders relative to their webs (Harwood *et al.* 2001, 2003; Salomon 2007; Johnson *et al.* 2011), and that, even if the strictest interpretation of OFT is inaccurate, the basic principles of that theory can still be meaningfully applied.

Microhabitat selection and utilization can also be impacted by non-trophic factors, such as structural features that provide architectural support for the spider web (McNett & Rypstra 2000), and climatic variables, such as wind direction, moisture, and temperature (Biere & Uetz 1981).  The architectural requirements of spider webs, especially orb webs, can be highly specific, and, consequently, only a narrow range of microhabitats may be capable of supporting these webs.  Also, the orientation of a web can impact its vulnerability to damage from the wind, and its exposure to sunlight, which may have implications for thermoregulation by ectothermic spiders (Biere & Uetz 1981; Mallis & Rieske 2010).

The differences in characteristics among web categories are correlated with differences in ecological strategies (Janetos 1982; Kawamoto & Japyassú 2008).  For example, the relatively low cost of an orb web (in terms of silk resources) allows orb-weavers to adopt a relatively mobile, exploratory strategy, and to take more risks (Kawamoto & Japyassú 2008).  In contrast, the high up-front costs and low ongoing maintenance costs of sheet and tangle webs favors a more sedentary strategy, in which spiders do not relocate frequently (Janetos 1982).  However, there is considerable variation across species, even within the broad categories as described above, and the strategy used by each species may differ from the generalized pattern for its web category

(Kawamoto & Japyassú 2008). Web-building spiders have also recently been found to display considerable flexibility in microhabitat-utilization and web-construction behaviors (Blamires *et al.* 2007; Zevenbergen *et al.* 2008; Pruitt *et al.* 2011; Scharf *et al.* 2011). Such behavioral plasticity may allow spiders to alter their webs to catch different types of prey (Blamires *et al.* 2011), to manage resource investments (Blackledge & Wenzel 2001), or to alter the balance between prey-capture and predator-deterrence functions (Zevenbergen *et al.* 2008). Thus, web-building spiders are expected to interact with their environment in complex ways.

All of these characteristics make web-building spiders a useful example for the study of predator ecology. In agroecosystems, web-building spiders can potentially contribute to the suppression of pest insects, such as aphids (Harwood *et al.* 2004) and leafhoppers (Sigsgaard 2007). However, their contribution to pest control is determined by a variety of factors, including the availability of suitable microhabitats (Rypstra *et al.* 1999), the presence of competitors in the environment (Sigsgaard 2007), and the availability and relative abundance of pest and non-pest prey (Harwood *et al.* 2004; Sigsgaard 2007). The most abundant web-building spiders in most agroecosystems are sheet-weavers from the family Linyphiidae, which are small-bodied spiders with diverse, yet enigmatic, behavior and ecology. This dissertation will investigate the role that microhabitat selection and utilization by these spiders plays in driving their trophic webs, and its effects on biological control.

## 1.3 Objectives

The overall goal of this dissertation was to elucidate the potential impacts of predator behavior on biological control services by characterizing patterns in foraging behavior of web-building spiders, and teasing apart mechanisms associated with prey selection and microhabitat utilization.  Because the selection and utilization of microhabitats and the construction of prey-trapping webs are of central importance to the ecology of web-building spiders, much of the behavior and ecology of these important predators can be understood and analyzed through quantification of microhabitat and web parameters.  I therefore sought to understand the potential impacts of microhabitat selection and foraging behavior on trophic linkages between spiders and their pest and non-pest prey.

Spider webs are common subjects of natural history and behavioral ecology studies, precisely because of the quantifiable nature of their behavior.  The peer-reviewed literature contains dozens of studies on the placement and construction of spider webs, dating back to the early parts of the 20th century.  However, despite this sizable record of research, many of the common spiders in agricultural systems have been largely neglected, and most of this research has focused on large, charismatic orb-weaving spiders (Eberhard 1990).  Very little research exists on the microhabitat-utilization and web-construction behaviors of linyphiid spiders, which are among the most diverse, and most abundant types of spiders found in many annual cropping systems in the northern hemisphere (Nyffeler & Sunderland 2003).  This is understandable, given the cryptic nature of these spiders, the difficulty of genus- and species-level identification, and the non-stereotyped, erratic architecture of their webs.  However, their potential usefulness in

biological control highlights the need to quantify and understand this behavior. As very little is known about microhabitat-utilization and web-construction behaviors of these spiders, my first objective was to characterize patterns in microhabitat utilization and web construction by these spiders in the field, and determine how these patterns correlate with spider diets. For this objective, it was necessary to combine field collections and measurements with molecular gut-content analysis, which allows insights into the importance of specific prey items for spiders. Additionally, it was necessary to develop a new mathematical technique to use molecular gut-content analysis to evaluate specific behavioral hypotheses.

Prior research, including preliminary work for this dissertation, indicates that several environmental variables are important drivers of spider microhabitat utilization. Among these are the structural characteristics of the habitat, and sensory cues from potential prey items. In specific, the latter cue is of interest because the primary function of a spider web is prey capture; it therefore is reasonable to predict that the presence or absence of prey from a particular microhabitat would be especially important attributes governing the decisions made by a spider to utilize a given microhabitat. However, little information exists in the literature about the how spiders use prey cues to evaluate and select microhabitats, and to construct webs. Therefore, my second objective was to evaluate spider responses to cues from prey, and assess how these cues impact behaviors associated with microhabitat selection and web construction. I used specially-designed olfactometers in controlled laboratory conditions to elucidate the behavioral process of microhabitat selection and web construction in response to cues from the principle prey item of linyphiid spiders, springtails (Collembola).

Linyphiid spiders are generalist predators, and, although springtails often make up the bulk of spider diets, they are also known to feed readily on other prey, including pest arthropods, such as aphids and leafhoppers. The behavior and ecology of these pest animals differs substantially from the behavior of non-pest springtails, and ecological theory indicates that the optimality of predator foraging tactics depends greatly on the behavior of the intended prey. Anecdotal observations from the literature indicate strongly that linyphiid spiders often utilize active foraging tactics in addition to web-based, sit-and-wait foraging tactics. It is likely that this sort of flexible behavior allows optimal foraging on prey with varying behavioral phenotypes. In order to determine how spider behavior impacts pest consumption, it is therefore necessary to quantify spider responses to both pest and non-pest prey. My third and final objective builds on the second objective, by comparing spider responses to non-pest springtails with spider responses to pest aphids. Two experiments were conducted in controlled conditions in the laboratory and greenhouse to evaluate spider behavior in response to the two types of prey, and to determine whether the presence of non-pest springtails diminishes spider consumption of aphids.

# CHAPTER 2: PATTERNS OF MICROHABITAT UTILIZATION IN THE FIELD

## 2.1 Summary

The diet of a predator can be strongly influenced by the manner in which it utilizes its habitat. For web-building spiders, the link between habitat usage and diet is particularly strong. In a field survey, I uncovered a pattern of niche partitioning among several species of web-building spider. A sheet-weaving spider, *Tennesseellum formica*, partitioned microhabitats among ontogenetic stages, suggesting competitive avoidance, while an orb-weaving spider, *Glenognatha foxi*, did not partition niches. This is explained by differences in the cost of web construction and the ecological strategies of the two guilds of spiders. However, using molecular gut-content analysis, coupled with a Bayesian inference model, I showed that *G. foxi* consumes springtail prey at a higher rate than *T. formica*, despite occupying microhabitats where springtails were encountered less frequently. This indicates that differences in prey-capture characteristics of sheet and orb webs have a strong influence on the rate at which spiders capture prey. In combination, these results also demonstrate the importance of web characteristics in driving the ecology of web-building spiders. Due to the lower prey-capture efficiency of sheet webs, *T. formica* is heavily dependent on microhabitat quality (in terms of prey availability), while *G. foxi*, with a highly-efficient orb web, is able to tolerate lower-quality microhabitats, and potentially avoid intraspecific competition.

## 2.2 Introduction

### 2.2.1 Microhabitat utilization by web-building spiders

A major consideration in determining a generalist predator's usefulness in biological control is the predator's *habitat domain*, or the way in which it utilizes its habitat (Schmitz 2007). The habitat domain of a predator will strongly influence the predator's encounter rate with prey, and thus mediate its impacts on prey populations. For example, epigeal predators will only consume foliage-dwelling prey if the predators periodically climb in the foliage to hunt the prey (Chapman *et al.* 2013), or the prey descends (or falls) to the soil surface (Losey & Denno 1998a; Kerzicnik *et al.* 2010). Construction of webs by spiders places additional constraints on habitat utilization that can influence ecology and can provide researchers with a unique opportunity to investigate the role of habitat utilization in biological control. Habitat utilization by web-building spiders has been studied extensively in the literature; however, the taxonomic resolution of this work is relatively poor, and little information currently exists on the most abundant family of agrobiont spiders, Linyphiidae. It was therefore necessary to characterize patterns in habitat utilization and web construction by these spiders in the field.

A web-building spider's foraging success is limited by the ability of its web to capture prey, which is a function of the availability of prey in the microhabitat in which the web is constructed (Harwood *et al.* 2003), and the mechanical properties of the web that determine how well it intercepts and detains prey (Blackledge & Eliason 2007). These two quantifiable aspects of spider foraging behavior therefore circumscribe two

hypotheses concerning the rate at which prey are consumed. I will refer to these as the "site-driven hypothesis," and the "web-driven hypothesis."

The first hypothesis, the site-driven hypothesis, states that prey-consumption rate is limited primarily by the availability of prey in the microhabitat. This hypothesis is in line with the reasoning of Edwards *et al.* (2009), which states that prey availability is often too limiting for web-building spiders to be selective foragers. A prediction of the site-driven hypothesis is that spiders that construct webs in microhabitats with higher prey activity-density will capture prey at a higher rate than spiders that construct webs in lower-quality microhabitats. The second hypothesis, the web-driven hypothesis, states that prey-consumption rate is limited primarily by the prey-capture properties of the web. In this view, prey availability is not equally limiting for all spiders, and selective, optimal foraging may not be an unrealistic expectation in some circumstances. A prediction of the web-driven hypothesis is that spiders with more efficient prey-trapping webs will capture prey at a higher rate than spiders with lower prey-trapping efficiency.

Spiders of the family Linyphiidae may be a special case among web-building spiders in a number of ways. This is a large family of spiders: with over 4400 described species, Linyphiidae is the most speciose family of web-building spiders, and the second most speciose of all spider families (Platnick 2013). Linyphiid spiders are usually quite small, often 1–3 mm in body length (Paquin & Dupérré 2003), and often construct webs on or very near the soil surface (Harwood *et al.* 2003; Harwood & Obrycki 2007). Springtails (Hexapoda: Collembola) are the most common prey in the diets of linyphiid spiders (Agustí *et al.* 2003; Chapman *et al.* 2013), and are likely also an important source of nutrition (Marcussen *et al.* 1999; Sigsgaard *et al.* 2001). In this way, linyphiid spiders

differ from the orb-weavers studied by Venner & Casas (2005): the diet is not dominated by rare, large prey, but by small, common prey. Therefore, the predictions of OFT may be more appropriate for linyphiid spiders than for the spiders in their study. And, indeed, data suggests that microhabitat utilization by linyphiid spiders does track the availability of common springtails (Harwood *et al.* 2003; Harwood & Obrycki 2007; Romero & Harwood 2010).

However, linyphiid spiders are not the only spiders that construct webs in or near the epigeal zone. Many orb-weavers (i.e. Araneidae and Tetragnathidae) and tangle-weavers (i.e.Theridiidae) are also of comparable body size, and construct webs similar in size to linyphiid webs, and in similar locations, low in the vegetation or on the ground. However, these spiders, particularly orb-weavers, may differ substantially from sheet-weavers in behavior and foraging ecology. Such differences in ecological strategies can potentially lead to differences in microhabitat usage and in prey consumption. Most previous research has focused on orb-weaving spiders in aerial niches high above the ground (e.g., Brown 1981), and sheet- and tangle-weavers have only recently received significant attention in the literature (e.g., Blackledge & Wenzel 2001; Harwood & Obrycki 2005; Pruitt *et al.* 2011). It is unclear how sheet- and tangle-weaving spiders might differ from orb-weaving spiders in microhabitat utilization and prey consumption. One might predict that the high costs and low prey-capture efficiency of sheet and tangle webs would require their builders to be highly discriminating in microhabitat utilization, so as to maximize prey-encounter rates and minimize potential wastage. Such selective behavior could also cause these spiders to face higher levels of competition for

microhabitats, and may be dependent on the quality of the microhabitat for foraging success.

### 2.2.2 Molecular gut-content analysis

To compare microhabitat utilization and prey consumption by web-building spiders of different web categories, it is necessary to assess and quantify interactions between spiders and their prey. Many basic techniques for the study of trophic interactions have been in use for decades, including direct field observations (e.g., Jackson 1977), laboratory observational trials (Edwards & Jackson 1993) and gut dissections (Hamilton & Pollack 1961). These techniques can often provide insights into the types of prey that are consumed by predators, but are fraught with problems, especially in the case of arthropod predators. For these predators, predation events are often cryptic, occurring in the epigeal zone, beneath the vegetation, which human observers may have difficulty accessing without disturbing the system and thereby drawing into question the genuineness of the observations. Additionally, significant amounts of time in the field are required to observe a number of predation events sufficient for analysis (e.g., Jackson (1977) observed only 33 predation events after "several thousand hours" of field observation). While laboratory trials allow researchers to artificially simulate prey encounters and a predator's willingness to attack a given prey item, these trials do not provide a good picture of events as they might unfold in the field (Huseynov *et al.* 2005). Vertebrate predators can often by dissected to identify fragments of prey from gut contents, but identification of arthropods from fragments is often impossible (Hamilton & Pollack 1961). Furthermore, many arthropod predators feed by liquid ingestion (including spiders), and thus will not have identifiable fragments of prey

in their gut contents.  For these reasons, novel approaches based on common molecular techniques are now being used widely to characterize arthropod predator-prey interactions.

Molecular gut-content analysis is a powerful technique for elucidating mechanisms and processes in trophic ecology.  Many serological techniques have been in use for decades to identify prey proteins from predator gut-content samples (reviewed in Symondson 2002; Sheppard & Harwood 2005).  However, the widespread availability of facilities and public databases for DNA identification and processing has brought PCR-based approaches to the forefront in recent years.  Molecular gut-content analysis has allowed unprecedented insights into trophic webs by providing access to information on predation events that would otherwise be undetectable, such as cryptic predation events in the field.  With PCR gut-content analysis, large sample sizes can be collected and screened for the presence of prey DNA using only small amounts of homogenate from predator specimens, providing a wealth of valuable, and otherwise inaccessible, information about trophic-web structure in the field (Symondson 2002).  However, using current analytical techniques, this information (usually reported as the percent of predator gut-content samples that test positive for the target prey DNA) can only be evaluated qualitatively (Greenstone *et al.* 2010).  One of the major goals in the field of molecular gut-content analysis is to convert these percent-positive results into an estimate of the number of prey actually consumed.  This would allow researchers to quantify trophic webs and to compare trophic ecology across species of predators.

The central challenge in quantifying predation from molecular gut-content analyses is that the probability of a positive result in a molecular assay is a function, not

only of the rate of predation on the target prey item, but also of the ability of the assay to detect prey DNA (Greenstone *et al.* 2007). Digestion of the prey DNA inside a predator's gut puts a finite limit on the interval of detection, which can vary widely with a number of environmental and physiological factors (Symondson & Liddell 1993; von Berg *et al.* 2008). Some of this variation can be accounted for using feeding trials to assess DNA decay half-lives, and thereby assess the potential impacts of DNA detectability on the results of gut-content analyses (Greenstone *et al.* 2010). In these trials, specimens are fed a standardized amount of prey, and subsets of these specimens are assayed at different time intervals after feeding. Logistic regression techniques are then used to calculate DNA decay half-lives or maximum detectability windows, which can be inserted into mathematical formulae as corrections to the percent-positive results of gut-content analyses (Naranjo & Hagler 2001; Greenstone *et al.* 2010). However, there is considerable uncertainty about the applicability of decay rates under laboratory conditions to predators collected in the field.

A previous study by Greenstone et al. (2010) ranked predator consumption rates by correlating percent-positives from field collections with species-specific DNA decay rates obtained in laboratory feeding trials, then algebraically standardizing results from all predators against a reference decay rate. This quantity was then transformed to give an adjusted proportion positive in terms of a reference species. This ranking technique has considerable heuristic value; however, it is descriptive, and makes no attempt at inference. For example, consider two species with identical half-lives in which 200 out of 400 samples of species 1 tested positive for prey DNA and 199 out of 400 samples of sample 2 tested positive. The approach of Greenstone et al. (2010) would rank species 1

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higher than species 2 without a measure of uncertainty in that ranking. Therefore, a full

probability model using Bayesian inference was developed to overcome this primary

concern. This model allows quantifiable comparisons among species, and, under realistic

assumptions, can be extended to quantify predation in the field. Hypotheses

The new model was used to evaluate the effects of prey availability and spider

web attributes on prey consumption rates by spiders, and to evaluate evidence for the two

aforementioned hypotheses related to spider microhabitat selection, which were the site-

driven hypothesis (prey-consumption rate is driven primarily by microhabitat-specific

prey activity-densities) and the web-driven hypothesis (prey-consumption rate is driven

primarily by the prey-capture attributes of spider webs). Additionally, I evaluate patterns

in microhabitat utilization among species, and among demographic categories within

species, to examine potential effects of competitive interactions on microhabitat

utilization.

## 2.3 Materials and Methods

*2.3.1 Study organisms.*

Web-building spiders are abundant in agroecosystems in eastern Kentucky (Culin

& Yeargan 1983b, a). Four species of spiders were used in this study (Fig 2.1). The

most abundant species in collections was *Tennesseellum formica* (Emerton) (Linyphiidae:

Micronetinae), a sheet-weaving spider distributed throughout most of North America, and

commonly reported from agricultural and other disturbed habitats. The spider's typical

body length is approximately 2 mm. *Glenognatha foxi* (McCook) (Tetragnathidae) was

also common in collections. This is an orb-weaving spider found in the eastern United
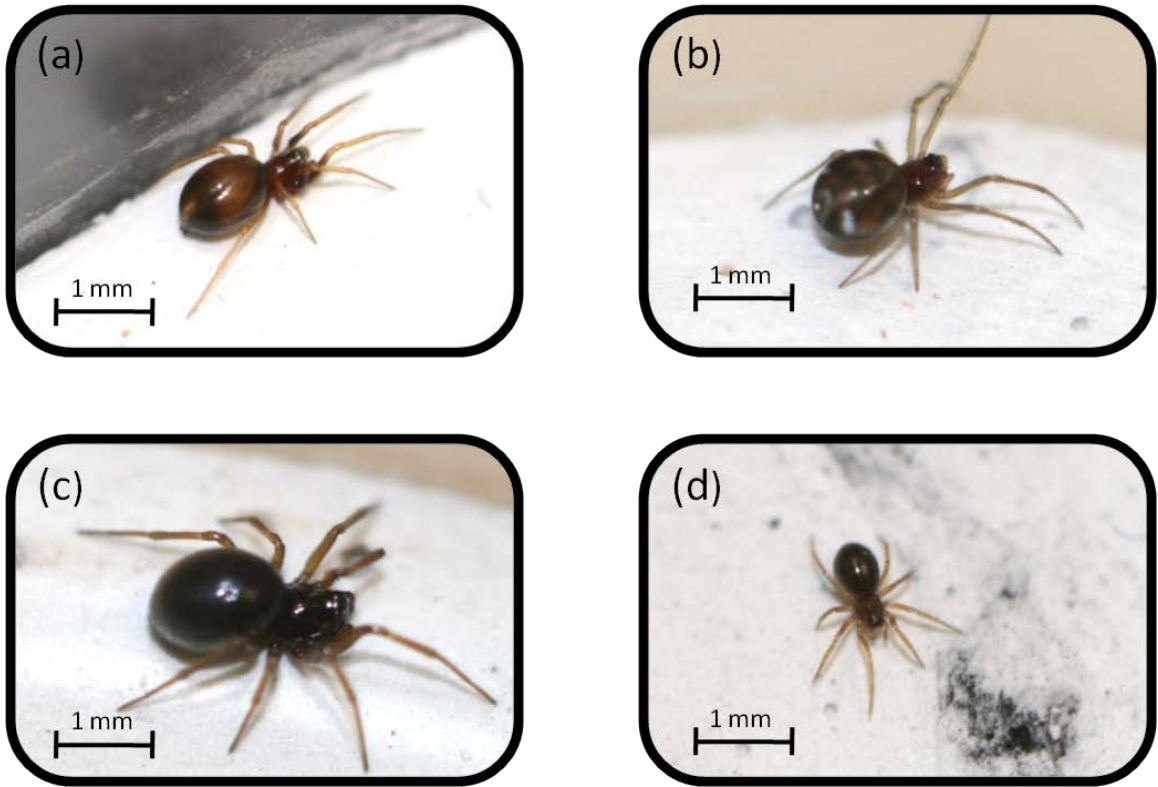
Figure 2.1 Four focal species of web-building spider in Kentucky alfalfa. Adult females are photographed here. (a) *Tennesseellum formica* (Linyphiidae: Micronetinae); (b) *Glenognatha foxi* (Tetragnathidae); (c) *Grammonota inornata* (Linyphiidae: Erigoninae); (d) *Erigone autumnalis* (Linyphiidae: Erigoninae).

States, with a body size comparable to that of *T. formica*.  For these two species, spiders

of all life stages could be reliably identified by reference to lab-reared specimens,

because of distinguishing physical characteristics.  Therefore, these two species were

used in all analyses.  Additionally, data were collected from two other spiders,

*Grammonota inornata* (Emerton), and *Erigone autumnalis* Emerton (Linyphiidae:

Erigoninae), which construct sheet and tangle webs (respectively).  Juveniles of these two

species could not be confidently distinguished from one another or from other spiders in

the system, and these spiders were thus only included in certain analyses.

For all experiments in this dissertation, laboratory colonies of springtails were

maintained as feedstock for laboratory-reared and experimental spiders.  The springtail

species raised in the laboratory was *Sinella curviseta* (Brooks) (Collembola:

Entomobryidae).  Springtails were reared in 188-mL plastic portion cups (7.5 cm

diameter, 4.5 cm depth), with a 1.5-cm layer of autoclaved, moistened potting soil.  Each

cup was provisioned with a small wedge of potato and a small amount of baker's yeast as

a food source for springtails.  All springtails were maintained in an incubator at 24 °C, on

a 16:8 h light:dark cycle.

*2.3.2 Field collections.*

Field work was carried out at the University of Kentucky Spindletop Research

Farm in Lexington, Kentucky, USA (GPS co-ordinates: 38°07'32" N, 84°30'43"W), in

two alfalfa fields (total area: 10.8 ha), between the months of April and September 2009.

The alfalfa was grown according to standard agronomic practices for Kentucky and

harvested on an approximately 40-day schedule.  Three harvests occurred during the

sampling period: 18–19 May, 26–27 June, and 7–8 August.  Alfalfa was cut early in the

morning and allowed to dry *in situ* for approximately 24 h before raking and baling, at which point it was removed from the field. No pesticides were sprayed in these fields or adjacent crop fields during the study period.

For collections, a grid system was used to ensure uniformity of collection and to prevent oversampling in any given area, which could affect spider densities and thus, competitive interactions and patterns of microhabitat utilization. The two fields were divided into a total of fifty grid squares of approximately equal area (2160 $m^2$). Spider webs were sampled from a total of 650 quadrats (0.25 $m^2$) placed in the alfalfa between May and September 2009, with no more than one quadrat placed within each grid square each week (mean: 30.9 quadrats/week). Sampling was only undertaken in the mornings between 8:00 and 12:00 to minimize diel variation in web occupancy and foraging. Each quadrat was searched exhaustively for spider webs, using a water-misting bottle during searches to aid in locating webs against the substrate. When a web was located, its surface area was recorded by measuring the longest horizontal facial dimension and the dimension perpendicular to it, and calculating an ellipse with these two measures as the radii (modified from Hesselberg 2010). In addition, web placement was classified by the presence or absence of attachments to the soil substrate and to plant stems. This resulted in three web-attachment categories: (1) **epigeal** (attached only to soil substrate), (2) **foliar** (attached only to plant stems), and (3) **basal** (attached to both soil substrate and plant stems; these were envisioned as epigeal microhabitats at the *base* of plant stems) (Fig. 2.2).

After measurements, webs were searched carefully for tenant spiders, which were collected and frozen immediately in a portable freezer and transferred to 95% ethanol for

identification. For webs with multiple tenants, only one tenant was identified as the builder of the web, using the following rules: immature spiders were selected over adult spiders, and adult females were selected over adult males. These rules are based on known behaviors of web-building spiders. Male spiders do not consistently build their own webs, but frequently enter the webs of females to mate (Suter *et al.* 1987). Thus, any web occupied by both adult male and adult female spiders was treated as the female's web. Additionally, spiders may invade and take over the webs of other spiders, with larger-bodied individuals usually having an advantage in such conflicts (Eichenberger *et al.* 2009). It is therefore unlikely that an immature spider would invade the web of an adult spider. Thus, on the very rare occasions when both adult and immature spiders were present in the same web, it was assumed that the web was constructed by the immature and recently invaded by the adult. Spider identifications were made following Ubick *et al.* 2005, with the aid of additional keys from the literature (Crosby & Bishop 1928; Hormiga & Dobel 1990; Paquin & Dupérré 2003). Where possible, sex and life stage (recorded as immature or adult) were also recorded, and immature spiders were identified to species by comparison to laboratory-reared specimens.

To analyze microhabitat distributions, web-placement data for adult females of all four major species were arranged into a 4 × 3 (species × microhabitat) contingency table, and the chi-square test of independence was performed using the open-source statistical software R, version 2.12.0 (the R Foundation for Statistical Computing), to examine how microhabitat distributions vary across species. Following this overall test, the contingency table was then subdivided into all possible 2 × 3 sub-tables for pairwise comparisons between species, using further tests of independence with Bonferroni
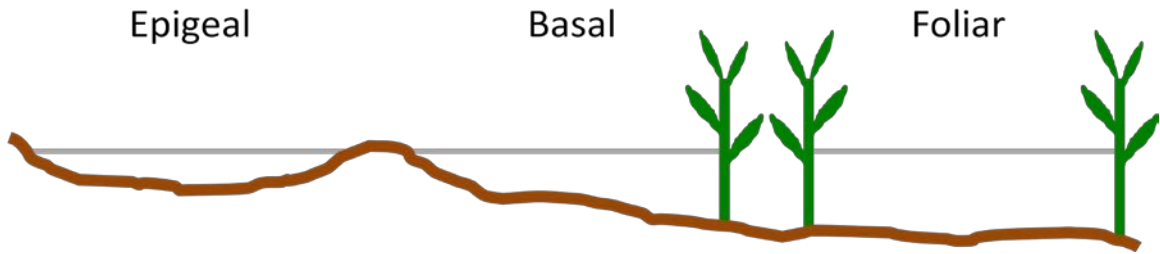
Figure 2.2  Microhabitat categorization schema used in this study.  Microhabitats are defined by the type(s) of structure to which a web is attached (soil substrate vs.  plant stem/foliage).  Light grey horizontal lines represent spider webs.

corrections applied for multiple comparisons. Fisher's exact test was substituted in cases where expected cell counts did not meet the assumptions for chi-square analysis.

To examine microhabitat distributions across ontogenetic stages, I used a multinomial logistic regression model to compare microhabitat selection by different demographic categories of *T. formica* and *G. foxi*. For each species, individuals were assigned to one of three categories: immature spiders, adult male spiders and adult female spiders. Adults were separated by sex because differences in body size and behavior between the sexes likely impact habitat utilization and web characteristics (Murakami 1983). Microhabitat utilization by trap-building predators is likely to be impacted by the size of the traps that are to be constructed. Microhabitats with different attachment structural characteristics may place different constraints on the size of webs that can be constructed in them. Furthermore, body size is often correlated with web size for web-building spiders (Witt *et al.* 1972; Hesselberg 2010); therefore, any differences found in microhabitat selection among demographic categories may actually reflect variance in web sizes across microhabitats (cf. Alderweireldt 1994b), rather than ontogenetic shifts in ecology. In order to account for this contingency, both demographic category and web surface area (in mm$^2$) were incorporated into multinomial logistic regression models for microhabitat utilization by *T. formica* and *G. foxi*, using the R statistical program, with the *mlogit* package installed.

*2.3.3 Prey activity-density and prey-capture rates*

Microhabitat-specific prey activity-densities were recorded using miniature sticky-traps, after Harwood *et al.* (2001). Sticky traps were constructed from acetate sheets (5 cm × 1.5 cm) coated with Tangle-Trap Insect Trap Coating Spray (The

Tanglefoot Company, Grand Rapids, Michigan, USA). Traps were placed in pairs within the same alfalfa agroecosystem. Traps in a pair were placed approximately 20 cm apart, and pairs were separated from one another by at least 2 m. Within each pair, one trap was placed to simulate a typical *T. formica* microhabitat, and one was placed to simulate a typical *G. foxi* microhabitat. *T. formica* traps were placed on a plastic base painted to match the color of the soil substrate, and gently pressed into the soil at the base of a plant so that the trap was flush with the soil surface. *G. foxi* traps were elevated to 1 cm above the ground on a clear plastic base mounted on firm, metal wire. A second sticky trap was affixed to the underside of the clear plastic base to simulate prey capture by the underside of the sticky orb webs of *G. foxi*. Thirty pairs of traps were placed on each of four sampling days during the summer of 2009.

To translate prey activity-density measures into prey-capture rates, it was necessary to collect data on the proportion of prey intercepted by a spider web that are subsequently captured and consumed by the tenant spider. Trials were conducted using field-collected, adult female *T. formica*. Spiders were maintained in individual Petri dishes with a plaster base for five days, provided an *ad libitum* supply of water, but no prey or other food was provided during this time. Only spiders that had constructed webs were used in these assays. A single, live adult springtail (*S. curviseta*) was dropped onto the spider's web approximately 1 centimeter from the position of the spider. I recorded the number of times each spider lunged at the prey, and the number of attempts before successful capture of the prey.

*2.3.4 Molecular gut-content analysis*

After laboratory identification, spiders collected from the field were screened for predation on springtails (Hexapoda: Collembola).  Although springtails are not considered pests in alfalfa, these prey were of interest for their importance in spider diets. Springtails are the most abundant prey for epigeal, web-building spiders (Chapman *et al.* 2013), and are also a highly nutritious prey (Marcussen *et al.* 1999; Toft & Wise 1999). Therefore, predation on springtails is a central component of web-building spider trophic ecology, and may have important implications for the biological control potential of these spiders (McNabb *et al.* 2001).  Spiders were evaluated for predation on springtails using PCR gut-content analysis.  DNA was extracted from crushed, whole-body specimens using QIAGEN DNeasy Tissue Kits (QIAGEN Inc., Chatsworth, California, USA). Spider homogenates were assayed for springtail predation using Collembola-specific DNA primers Col4F2d (Chapman *et al.* 2013) and Col5R (Kuusk & Agusti 2008), which produce a 180-bp amplicon of the Collembola 18S mitochondrial gene.  These primers were previously tested for cross-reactivity against a wide selection of non-targets (Chapman *et al.* 2013).  PCR reactions (25 µL) consisted of 1X Takara buffer (Takara Bio Inc., Shiga, Japan), 2 µL of dNTP solution (2.5 mM of each nucleotide in deionized $H_2O$), 0.5 µL of each primer (10 µM in deionized $H_2O$), 0.15 µL Takara *Ex Taq*™ and 2 µL template DNA.  PCR cycling protocols were 94° C for 1 min followed by 45 cycles of 94° C for 45 s, 65° C for 45 s and 72° C for 30 s.  Reaction success was determined by electrophoresis of 10 µL of PCR product on 3% SeaKem agarose gels stained with ethidium bromide.

*2.3.5 Rate of decay of prey DNA within predator gut contents*

In order to assess the rate at which prey DNA decays within spider guts, feeding trials were performed.  Specimens of *T. formica* and *G. foxi* were collected from lawns and crop fields in Lexington, Kentucky, USA, and maintained under standard laboratory conditions in plastic portion cups (700 mm diam. $\times$ 450 mm height) with a thin layer of moistened, autoclaved potting soil.  All specimens were starved for at least five days prior to trials.  Each specimen was fed a single, adult springtail, *S. curviseta*, and ten specimens from each species were preserved in 95% ethanol for PCR assay at each of the following time intervals after feeding:  0, 1, 2, 4, 8, 18, 24, 48 and 72 hours.  For *T. formica*, it was necessary to assay additional specimens at 96 and 120 hours after feeding. Results were fit to a logit regression model, and half-lives are reported.

*2.3.6 Statistical model*

To specify the model, conditional probability models were written for both the laboratory feeding-trial data and the field collections.

For the lab data, a logistic regression model is constructed, with $t =$ time since last feeding and $p =$ probability of detecting prey DNA in the gut:

$$\text{logit}(p) = \beta_0 + \beta_1 t \qquad\qquad (\text{Eq. 1})$$

For the field data, a logistic regression model of similar form is constructed, with $\tau =$ time since last feeding, and $\pi =$ probability of detecting prey DNA:

$$\text{logit}(\pi) = \beta_0 + \beta_1 \tau \qquad\qquad (\text{Eq. 2})$$

The decay rate of prey DNA is assumed to be similar in the laboratory and in the field, therefore the parameters, $\beta_0$ and $\beta_1$, are used in both models. While potentially unrealistic, this simplifying assumption allows an effective demonstration of the model. Naranjo & Hagler (2001) proposed a complex series of greenhouse experiments that can generate more realistic models and assumptions; however, conducting such experiments is not always practical, and does not overcome the challenges of applying experimentally determined decay rates to field-collected specimens. Under more realistic assumptions, this model can be used to infer the rate of prey consumption. Under the simplifying assumptions used here, the model can still be used to compare predation rates among species.

In laboratory feeding trials, the time since last feeding, $t$, is known for each specimen. However, in field collections, the time since last feeding, $\tau$, is not known. Therefore, to complete the model, $\tau$ is assumed to fit a specified hierarchical distribution. The model was run for two different initial distributions of $\tau$ that approximate the expected relationship between time since feeding and probability of detection. These distributions were (1) a half-normal distribution (a normal distribution, truncated at 0, with a mean of 0, and unknown variance $\sigma^2$), and (2) an exponential distribution with rate parameter $\lambda$. A parameter, $\theta$, is defined to describe these distribution models. For the half-normal distribution, this parameter is equal to $\sigma$; and, for the exponential distribution, $\theta$ is equal to $1/\lambda$.

Bayesian inference was used to infer the parameters of these models. That is, weakly-infromative prior distributions were assumed for $\beta_0$, $\beta_1$, and $\theta$, and the posterior distribution for the hierarchical model was found. Prior distributions used were t-

distributions with 3 degrees of freedom, location 0 and scale 5. The scale was chosen to be 5, to reflect that it would be unlikely to have values greater 10 units for all parameters. However, the heavy tails in these prior distributions provides some robustness, allowing for values greater than 10 if the data strongly suggest such values. The prior distribution for $\theta$ was truncated to be greater than 0 since $\theta > 0$.

The posterior distribution cannot be evaluated analytically, and instead, samples were drawn from it using Markov chain Monte Carlo (MCMC), using the software package *rjags* in the statistical software R.

Of primary interest in this analysis is $\tau$, the time since last feeding for each field-collected specimen. The parameter $\theta$ describes the probability distribution of $\tau$ (that is, $\theta$ describes the distribution of "time since last feeding" within a spider population), and thus, two spider species with different values for $\theta$ differ in terms of the rate at which they consume prey. We therefore define another quantity, $\delta$, which describes the difference in $\theta$ between the two species of spider. If this quantity differs from 0, as determined by its posterior probability, then the two species differ in the rate at which they capture and consume prey.

In Bayesian inference, an important consideration is the influence of the prior distributions on the posterior distributions. To ensure that the assumed distributions were not unduly influencing the results, the posterior distributions of $\beta_0$ and $\beta_1$ obtained from the full-likelihood model were compared to the posterior distributions of $\beta_0$ and $\beta_1$ obtained when using only the data from laboratory feeding trials. No difference was

observed between the full-probability model and the laboratory-only model, indicating that the analysis was not unduly influenced by the prior distributions (data not shown).

## 2.4 Results

### 2.4.1 Web characteristics and microhabitat distributions

Characteristics of webs constructed by spiders in this study are reported in Table 2.1. Utilization of microhabitats varied with species, as determined by contingency-table analysis ($\chi^2 = 387.9$, df = 6, p < 0.001; Table 2.2; Fig. 2.3). Each of the four most common species differed significantly from each other in terms of microhabitat distribution, as determined by pairwise comparisons (Tables 2.1, 2.2). *Glenognatha foxi*, the orb-weaving spider, primarily utilized foliar microhabitats, whereas *E. autumnalis* primarily utilized epigeal microhabitats. While both *T. formica* and *Gr. inornata* favored basal microhabitats, microhabitat distribution differed significantly between these species. Examination of chi-square contributions indicated that this was primarily due to a lower utilization of epigeal microhabitats and higher utilization of foliar microhabitats by *Gr. inornata*.

Logistic regression of demographic categories revealed that microhabitat distribution varied across demographic categories for the sheet-weaving *T. formica* (Table 2.3, Fig. 2.4), but not for the orb-weaving *G. foxi* (Table 2.4; Fig. 2.4). For *T. formica*, the microhabitat distributions of immatures and females were found to differ significantly. Adult female spiders were more likely to utilize basal microhabitats, and immature spiders were more likely to utilize epigeal microhabitats. Adult male *T.*

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Table 2.1  Common species of web-building spiders collected in Kentucky alfalfa in spring-summer 2009, their proportional distributions across three microhabitat types (Ep = epigeal, Ba = basal, Fo = foliar), and characteristics of their webs.  Only the four most abundant species (above dotted line) were included in analyses.

| Species | Family: Subfamily | n | Microhabitat | | | Web Type | Web Measures (± se) | |
| | | | Ep | Ba | Fo | | Ht (mm) | Area (mm$^2$) |
|---|---|---|---|---|---|---|---|---|
| *Tennesseellum formica* (Emerton) | Linyphiidae: Micronetinae | 456 | 0.46 | 0.47 | 0.07 | Sheet | 4.1 ± 0.2 | 1230 ± 48 |
| *Erigone autumnalis* Emerton | Linyphiidae: Erigoninae | 322 | 0.73 | 0.24 | 0.02 | Tangle | 2.1 ± 0.1 | 232 ± 12 |
| *Glenognatha foxi* (McCook) | Tetragnathidae | 202 | 0.10 | 0.32 | 0.58 | Orb | 9.9 ± 0.4 | 1203 ± 48 |
| *Grammonota inornata* (Emerton) | Linyphiidae: Erigoninae | 51 | 0.14 | 0.71 | 0.16 | Sheet | 5.0 ± 0.3 | 1137 ± 128 |
| *Islandiana flaveola* (Banks) | Linyphiidae: Erigoninae | 31 | 0.81 | 0.16 | 0.03 | Tangle | 2.6 ± 0.4 | 242 ± 36 |
| *Mermessus trilobatus* (Emerton) | Linyphiidae: Erigoninae | 29 | 0.34 | 0.62 | 0.03 | Sheet | 4.1 ± 0.7 | 642 ± 128 |
| *Mermessus fradeorum* (Berland) | Linyphiidae: Erigoninae | 24 | 0.54 | 0.42 | 0.04 | Sheet | 4.3 ± 0.6 | 813 ± 217 |
| *Tetragnatha laboriosa* Hentz | Tetragnathidae | 23 | 0.04 | 0.04 | 0.91 | Orb | 75.8 ± 21 | 2031 ± 266 |
| *Meioneta micaria* (Emerton) | Linyphiidae: Micronetinae | 21 | 0.05 | 0.05 | 0.90 | Sheet | 30.2 ± 3.2 | 3180 ± 679 |
| *Mermessus tridentatus* (Emerton) | Linyphiidae: Erigoninae | 11 | 0.45 | 0.55 | - | Sheet | 4.4 ± 0.6 | 487 ± 126 |
| *Erigone atra* Blackwall | Linyphiidae: Erigoninae | 9 | 0.33 | 0.67 | - | Tangle | 3.0 ± 0.3 | 1019 ± 159 |

Table 2.2  Comparisons of microhabitat distributions of four common species of web-building spiders, using an overall chi-square test and two-species subdivided tables for pairwise chi-square tests.  Significant results are marked with an asterisk (*) (Bonferroni-adjusted p = 0.00833).  For the last comparison (*Glenognatha foxi – Grammonota inornata*), expected cell counts did not meet assumptions for the chi-square test, so Fisher's exact test was used instead.

| Test | df | $\chi^2$ | p | |
| --- | --- | --- | --- | --- |
| Overall | 6 | 297.285 | < 0.001 | * |
| *T.formica – Gr.inornata* | 2 | 16.687 | < 0.001 | * |
| *T. formica – E. autumnalis* | 2 | 71.465 | < 0.001 | * |
| *T. formica – G. foxi* | 2 | 102.767 | < 0.001 | * |
| *E. autumnalis – G. foxi* | 2 | 165.39 | < 0.001 | * |
| *E. autumnalis – Gr. inornata* | 2 | 66.822 | < 0.001 | * |
| *G. foxi – Gr. inornata* | 2 | (Fisher's exact test used) | 7.43e-5 | * |
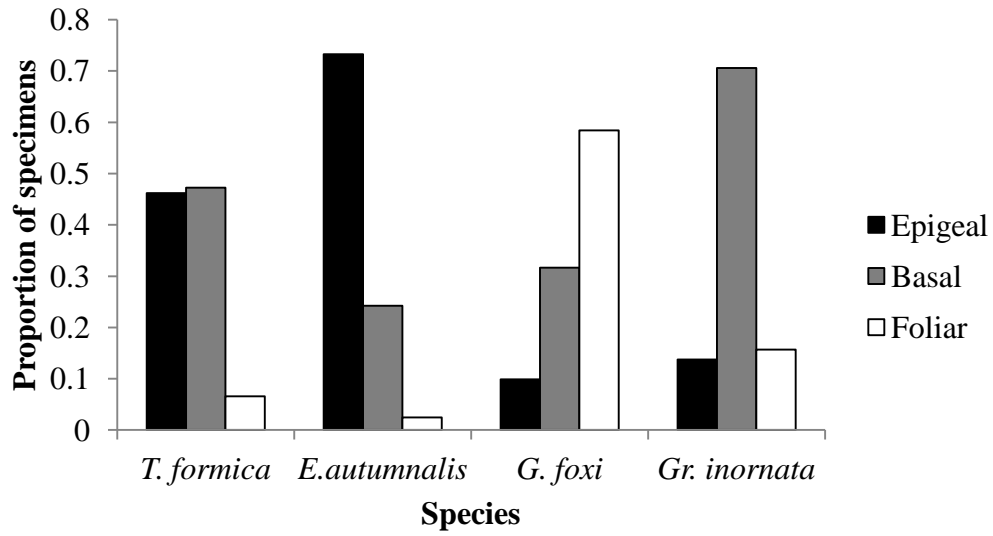
Figure 2.3  Microhabitat distribution of four species of web-building spiders in alfalfa. Only adult female spiders are included.  Chi-square analyses confirmed that the microhabitat distribution of each species differed significantly from all others.  Refer to Fig. 2.2 for microhabitat definitions, and Table 2.2 for statistics.

Table 2.3  Microhabitat utilization by *Tennesseellum formica*.  Results of a multinomial logistic regression model with web surface area and demographic category as explanatory variables.  Coefficients represent the relationship between microhabitat utilization and the factor identified.  For web surface area, for every 1 unit increase in surface area, the log of the ratio between the probabilities of the two site categories in parentheses will change by the amount of the coefficient.  For demographic category, the coefficient simply describes the change in the ratio of probabilities across the two identified demographic categories (I = immature, F = adult female, M = adult male).  Asterisks (*) denote significance at $\alpha = 0.05$.

| Comparison | Coefficient ( ± se) | T | p | |
| --- | --- | --- | --- | --- |
| Web surface area | | | | |
| (epigeal:basal) | 1.95e-5 ± 1.17e-4 | 0.1659 | 0.868 | |
| (foliar:basal) | -7.25e-4 ± 3.89e-4 | -1.8629 | 0.063 | |
| | | | | |
| Demographic category | | | | |
| I - F (epigeal:basal) | 1.051 ± 0.245 | 4.2886 | < 0.001 | * |
| I - F (foliar:basal) | -0.317 ± 0.578 | -0.5495 | 0.583 | |
| M - F (epigeal:basal) | 0.957 ± 0.566 | 1.6899 | 0.091 | |
| M - F (foliar:basal) | 1.281 ± 0.917 | 1.3971 | 0.162 | |
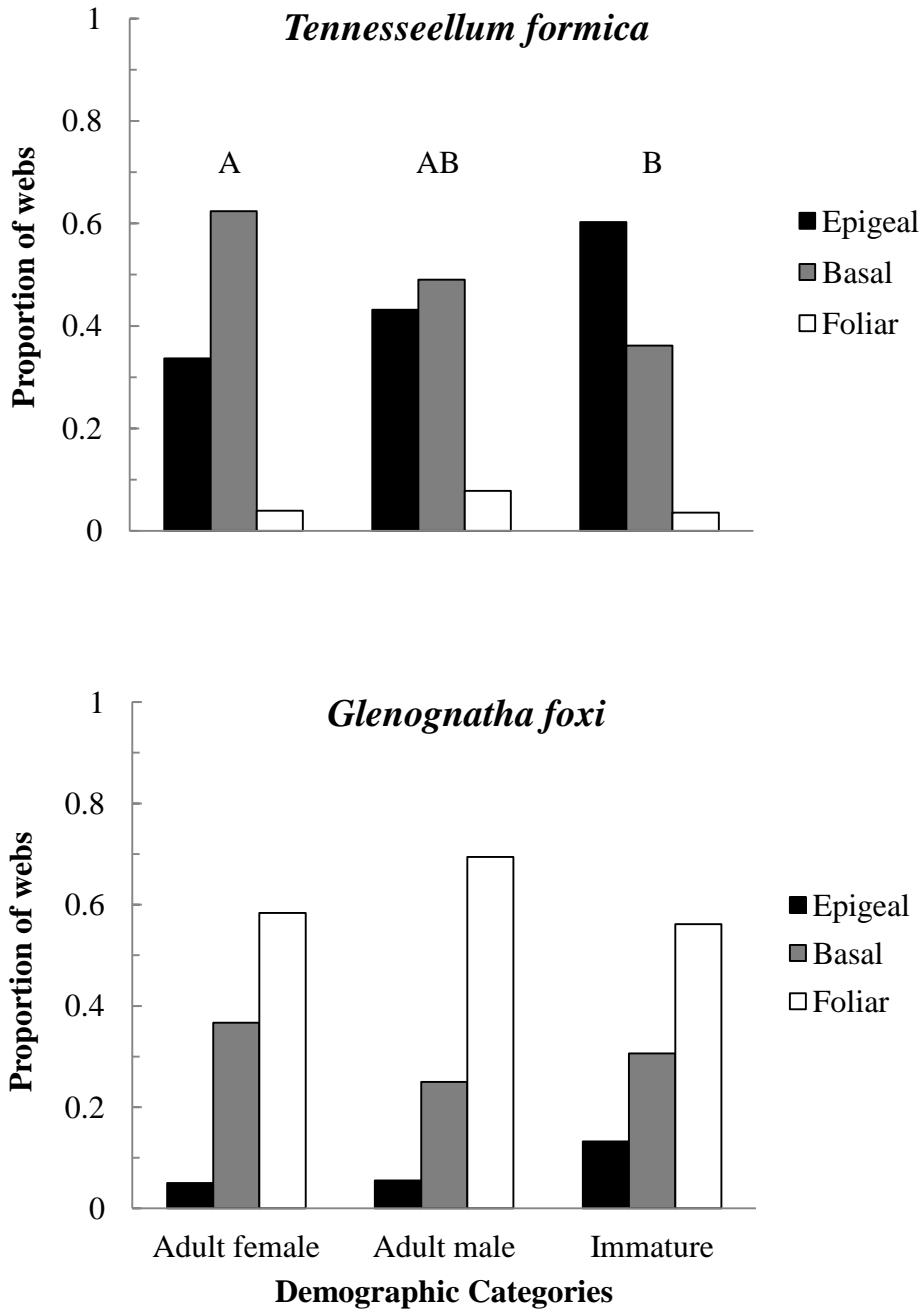
Figure 2.4  Microhabitat distribution of three demographic categories of *Tennesseellum formica* and *Glenognatha foxi*.  Letters represent significant differences between the microhabitat distributions of different demographic categories, as determined by chi-square analyses.  Refer to Fig. 2.1 for microhabitat definitions.

Table 2.4 Microhabitat utilization by *Glenognatha foxi*. Results of a multinomial logistic regression model with web surface area and demographic category as explanatory variables. Asterisks (*) denote significance at α = 0.05.

| Comparison | Coefficient ( ± se) | T | p | |
|---|---|---|---|---|
| Web surface area | | | | |
| (basal:foliar) | -0.00076 ± 2.7e-4 | -2.7622 | 0.006 | * |
| (epigeal:foliar) | -0.00025 ± 3.9e-4 | -0.6364 | 0.525 | |
| | | | | |
| Demographic category | | | | |
| I - F (basal:foliar) | -0.236 ± 0.371 | -0.6373 | 0.524 | |
| I - F (epigeal:foliar) | 0.865 ± 0.683 | 1.2664 | 0.205 | |
| M - F (basal:foliar) | -0.413 ± 0.482 | -0.8570 | 0.391 | |
| M - F (epigeal:foliar) | -0.088 ± 0.953 | -0.0926 | 0.926 | |

*formica* inhabited epigeal and basal microhabitats at similar frequencies, but showed no statistically significant differences from either adult females or immatures.

Web surface area was not significantly correlated with microhabitat utilization in *T. formica* (Table 2.3). A marginally significant positive correlation between web area and utilization of foliar microhabitats was observed; however, because foliar microhabitats are only very rarely used by this species (Fig 2.3), this correlation likely has little ecological significance. In contrast, for *G. foxi*, an increase in web surface area was correlated with an increase in use of foliar microhabitats, whereas demographic category had no significant impact on microhabitat utilization (Table 2.4).

### 2.4.2 Prey activity-density and prey-capture rates

In sticky-trap collections, prey activity-density differed significantly between simulated microhabitats of *T. formica* and *G. foxi* (Fig. 2.5). In a general linear model, the total prey activity-density varied across sampling days ($F_{3,184} = 21.7$, $p < 0.001$), and across microhabitats ($F_{1,184} = 9.05$, $p = 0.003$). Activity-densities of total prey were significantly higher in simulated *T. formica* microhabitats than in simulated *G. foxi* microhabitats (Tukey's HSD, $_{2,184} = 3.008$, $p = 0.003$). A similar trend was uncovered for springtail activity-density (Tukey's HSD, $T_{2,184} = 3.538$, $p < 0.001$). By comparison, Diptera tended to be more abundant in *G. foxi* microhabitats, but the trend was non-significant (Tukey's HSD, $T_{2,184} = 1.951$, $p = 0.053$; all tests evaluated at Bonferroni-corrected $\alpha = 0.0167$). Thus, microhabitat quality (in terms of prey resources) varies with microhabitat type, and *T. formica* microhabitats were generally higher-quality than *G. foxi* microhabitats. However, in all analyses, the interaction between
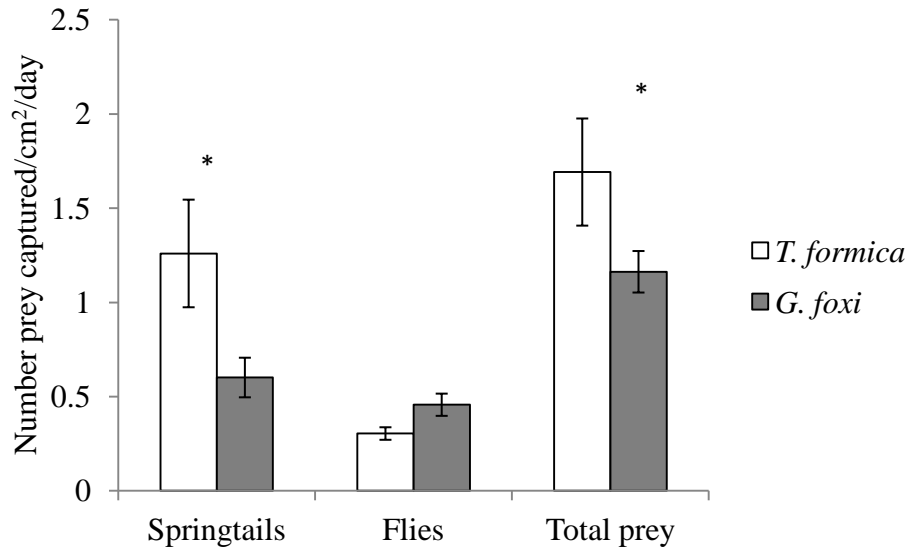
Figure 2.5  Mean activity-density of potential prey (± s.e.) collected in sticky traps placed in simulated microhabitats of *Tennesseellum  formica* and *Glenognath  foxi*.  Asterisks denote significant differences, according to pairwise Tukey's HSD tests (Bonferroni-corrected α = 0.0167).

microhabitat and sampling day was significant ($p < 0.015$ in all cases), indicating that patterns in microhabitat quality are not consistent across time.

In prey-capture trials, 18 of 70 adult female *T. formica* (25.7%) successfully captured a springtail on the first attempt (Fig. 2.6). Failures were invariably due to the springtail jumping away before the spider could envenomate it. However, in 20 out of 52 cases, escaping springtails landed back in the spider's web, and the spider made a second attempt to capture the springtail. Seven of 20 (35%) spiders that made a second attempt successfully captured the springtail; and, 3 of 7 (42.8%) spiders that made a third attempt were successful. Overall, 28 of 70 spiders successfully captured a springtail dropped into the web, for a total success rate of 40%.

### 2.4.3 Molecular gut-content analysis and decay rate of prey DNA

A total of 956 spiders from the four most abundant species were collected in alfalfa fields, and whole-body homogenates of these spiders were screened for springtail DNA using PCR gut-content analysis. All four species of spider tested positive for springtail DNA at high rates (Fig. 2.7). *T. formica* tested positive at the highest rate (~62%), while *E. autumnalis* tested positve at the lowest rate (~48%). To test the prediction of the site-driven hypothesis that prey consumption would be correlated with microhabitat, multinomial logistic regression models were constructed for *T. formica* and *G. foxi*, with percent-positive as the response variable, and microhabitat, web height and demographic category as explanatory variables (Table 2.5). For both species, percent-positives for springtails were not influenced by microhabitat attributes or by demographic category, providing no support for the site-driven hypothesis.
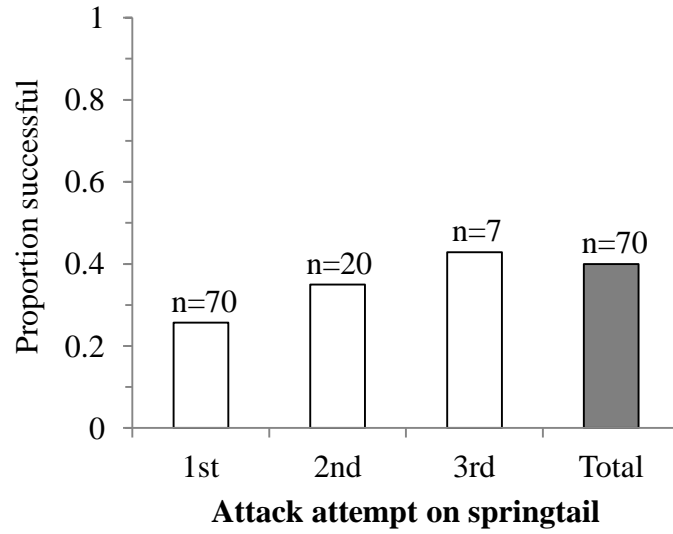
Figure 2.6  Success rate of *Tennesseellum formica* attacks on springtail prey dropped onto the web.  Failures occurred when springtails successfully escaped from the attacking spider by jumping away.  Second and third attempts occurred after failures on previous attempts, when escaping springtails landed back on the web.  The total represents the proportion of all spiders that eventually caught the springtail.
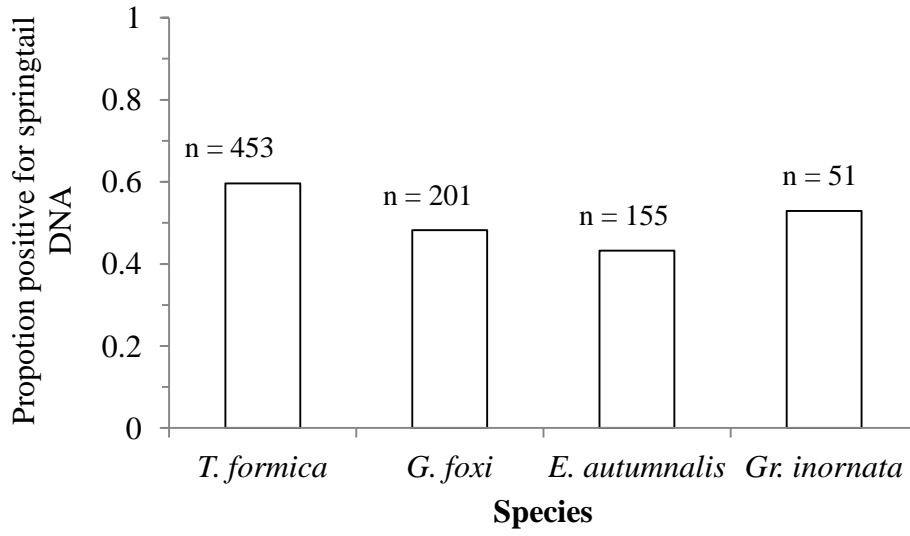
Figure 2.7 Results of PCR gut-content assays. Proportion of field-collected spiders from

the four most abundant species in alfalfa that tested positive for springtail DNA in their

gut contents.

Table 1.5  Springtail consumption by *Tennesseellum  formica* and *Glenognatha foxi*.
Results of multinomial logistic regression models with web characteristics and
demographic categories as explanatory variables.  Asterisks (*) denote significance at α =
0.05.

*Tennesseellum formica*

| Comparison | Coefficient | p |
| --- | --- | --- |
| Web Height | -0.032 | 0.348 |
| Microhabitat | | |
| Epigeal - Basal | -0.153 | 0.487 |
| Foliar - Basal | 0.166 | 0.743 |
| Demographic Category | | |
| Immature - Ad.  Female | 0.288 | 0.184 |
| Ad.  Male - Ad.  Female | -0.954 | 0.101 |

*Glenognatha foxi*

| Comparison | Coefficient | p |
| --- | --- | --- |
| Web Height | -0.019 | 0.546 |
| Microhabitat | | |
| Epigeal - Foliar | -0.907 | 0.108 |
| Basal - Foliar | -0.125 | 0.713 |
| Demographic Category | | |
| Immature - Ad.  Female | 0.294 | 0.426 |
| Ad.  Male - Ad.  Female | -0.115 | 0.797 |

In laboratory feeding trials, springtail DNA was found to decay at a faster rate inside the gut of the orb-weaving *G. foxi* (half-life $\approx$ 9.5 hrs) than inside the gut of the sheet-weaving *T. formica* (half-life $\approx$ 34 hrs). Given that these decay-rates differ substantially, while the percent-positives from field-collected specimens differ much less dramatically, it is likely that a direct comparison of percent-positives between *T. formica* and *G. foxi* would reflect DNA detectability, rather than springtail-predation rate. In order to compare springtail-predation rates, statistical inference is necessary. Using the new full-probability model, the rates of predation by these species could be determined, which allows a comparison of predatory impact between species.

Two models were analyzed with different prior probability distributions assumed for $\tau$ (time since feeding). These distributions were a normal distribution with mean 0 and unknown variance, truncated at 0 (referred to as the *half-normal model*), and an exponential distribution with an unknown rate parameter (referred to as the *exponential model*). Within each model, the parameter $\theta$ describes the relationship between time since last feeding and probability of feeding on a springtail. The difference ($\delta$) between the $\theta$ parameters of the two species is shown in Fig 2.8. Estimates of the posterior probability distributions derived from the half-normal and exponential models did not differ substantially, so model selection did not seem to significantly alter the results. Monte Carlo estimates of the posterior probabilities of $\delta$ provide supporting evidence that the mean time since last feeding differs between *T. formica* and *G. foxi* ($\Pr(\delta > 0) =$ 0.004 for the half-normal model, and $\Pr(\delta > 0) = 0.013$ for the exponential model). From this data, I conclude that *G. foxi* consumes springtails at a faster rate than *T. formica*.
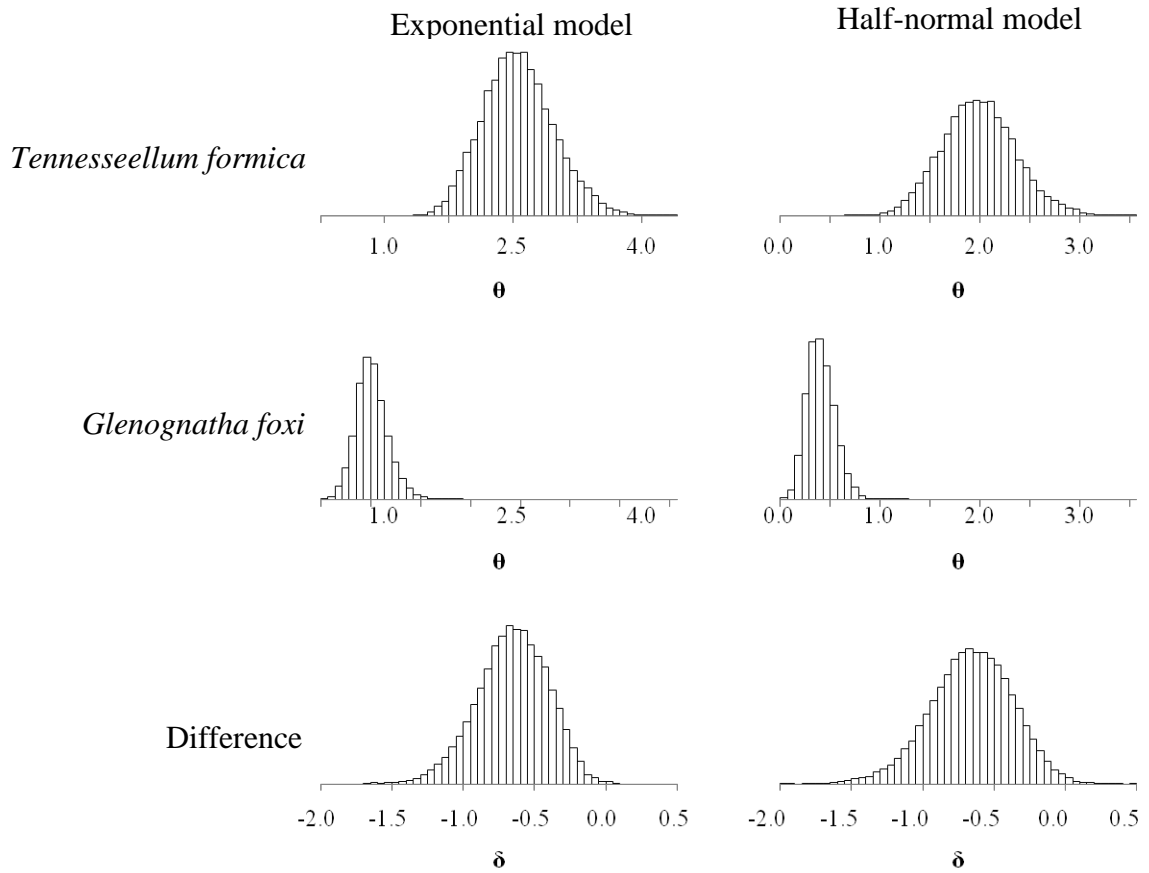
Figure 2.8 Monte Carlo estimates of variables relevant to predation rate. $\theta$ describes the posterior distribution of time since last feeding for each species. $\delta$ describes the difference in $\theta$ between species. The height of the bars represents the probability that the parameter is equal to the value on the horizontal axis.

This conclusion is further supported by examination of the posterior distributions of springtail consumption over time (Figs. 2.9 and 2.10). The probability distribution function (Fig. 2.9) shows that the detectability of springtail DNA in gut contents declines sharply over time for *G. foxi*, and less sharply for *T. formica*. The cumulative distribution function (Fig. 2.10) shows that a larger proportion of *G. foxi* are expected to feed on springtails within a given time interval. All of these results demonstrate that *G. foxi* does, indeed, capture springtails at a faster rate than does *T. formica*, despite utilizing microhabitats with lower activity-densities of springtails. This data supports the web-driven hypothesis, and indicates that prey availability is more limiting for *T. formica* than it is for *G. foxi*.

Model validation consisted of investigating the influence of the prior distributions for each quantity on the corresponding posterior distributions. The half-normal and exponential models were compared to a third model, in which only the data from laboratory feeding trials was included (Fig. 2.9). The model showed little sensitivity to prior-distribution assumptions, and I am therefore confident that these assumptions did not overly influence the results.

### 2.5 Discussion

In this study, I have uncovered evidence for several ecological factors influencing microhabitat utilization and diet by web-building spiders, and have also demonstrated that differences in web characteristics among spiders can impact dependence on microhabitat quality. I observed an overarching pattern of niche partitioning among and within species of spiders in this alfalfa agroecosystem. Similar patterns have been observed many times in a variety of web-building spiders in many different ecosystems
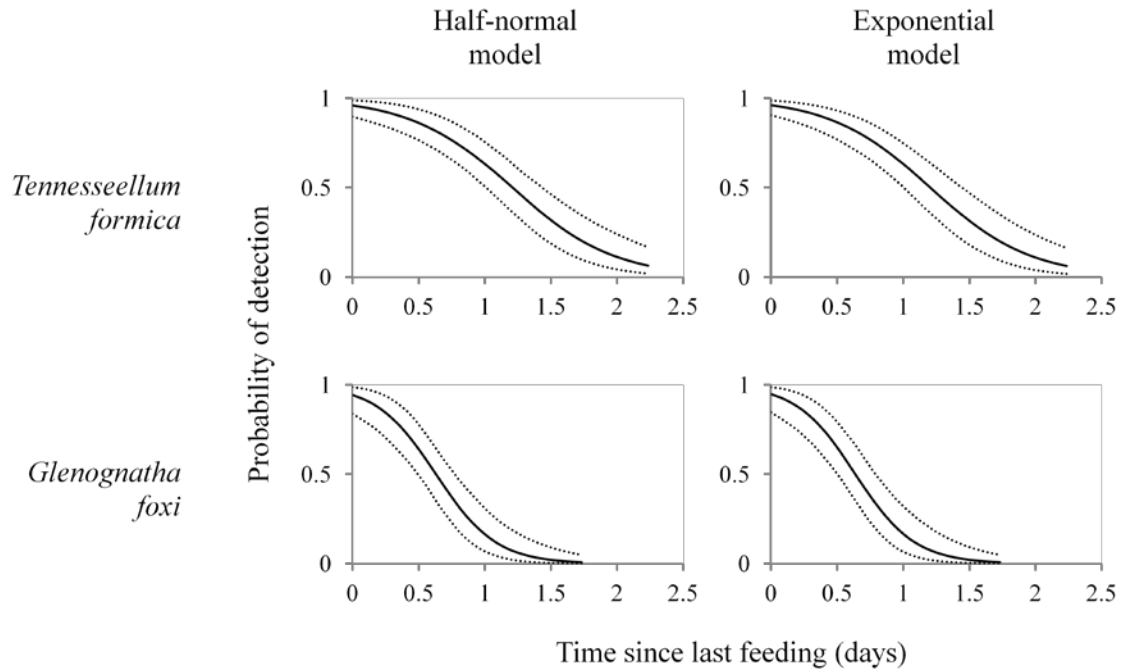
Figure 2.9  The decay rate of prey in the guts of two species of web-building spiders,

under two models for the distribution of time since last feeding (half-normal and

exponential).  In each plot, the solid line is the median of the posterior distribution, and

the two dashed lines are the 2.5% and 97.5% posterior quantiles.
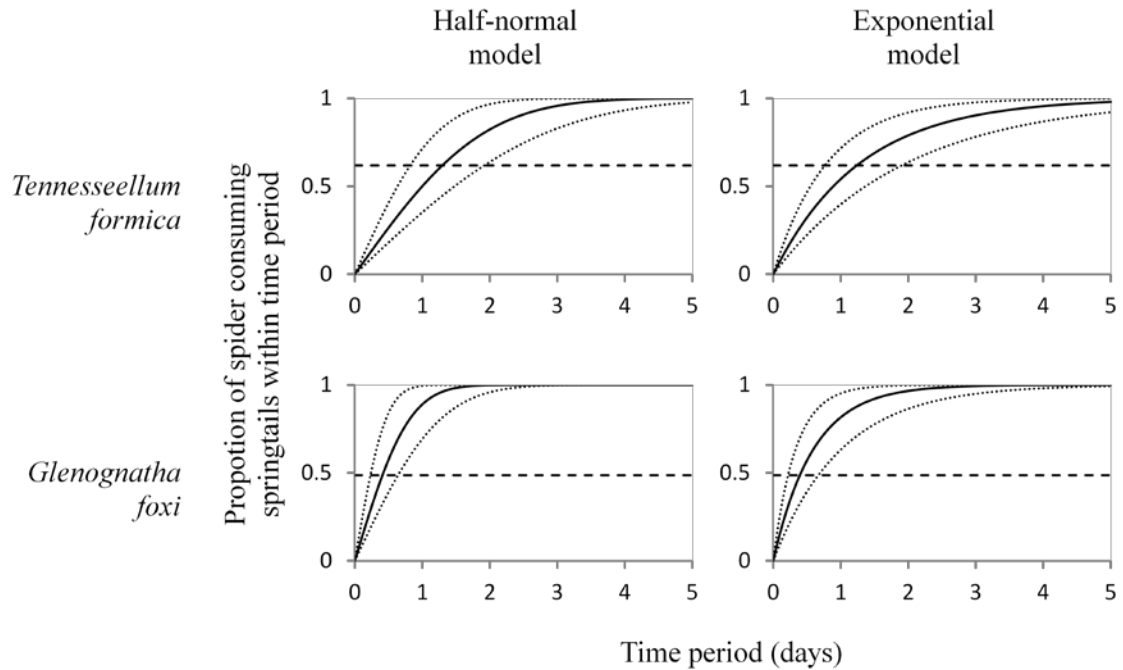
Figure 2.10 Cumulative distribution function for the time since last feeding, estimated from a new Bayesian inference model. In each plot, the solid line is the median estimate for the posterior distribution, and the two dashed lines are the 2.5% and 97.5% posterior quantiles. The horizontal, dashed line represents the percent-positive obtained from PCR gut-content assays on field-collected specimens. The intersection of this line with the posterior median indicates the time frame during which detected predation events are estimated to have occurred.

(e.g., Enders 1974; Brown 1981; Herberstein 1998; Harwood & Obrycki 2007), and have

been explained by several different ecological mechanisms.  These patterns have  often

been interpreted as evidence of interspecific competition (e.g., Herberstein 1998).  In

these arguments, it is suggested that different species inhabit different habitats to avoid

competitive interference or intraguild predation.  However, clear evidence of competitive

mechanisms is generally lacking in most cases (Wise 1993).  More recent work has

invoked mechanisms involving dietary divergence, with microhabitat-specific differences

in prey availability driving different species to utilize different microhabitats (Harwood *et

al.* 2001, 2003; Harwood & Obrycki 2007).

However, some recent work with linyphiid spiders suggests that competition may,

in fact, play an important role in the ecology of these spiders (Harwood & Obrycki 2005,

Eichenberger *et al.* 2009).  This conclusion is supported, in part, by my research, because

of the patterns observed in linyphiid and orb-weaver microhabitat utilization (Figure 2.4).

In *T.  formica*, niche partitioning was observed among demographic categories: small-

bodied juveniles utilized different microhabitats from large-bodied adult females.  This is

not easily explained by differences in architectural support requirements among webs of

different sizes, because web size was not significantly correlated with microhabitat in this

species (Table 2.3)  A better explanation is ontogenetic niche partitioning, in which

juveniles avoid niche overlap with adults to minimize the risks of cannibalism or

competitive displacement (which entails significant wastage of silk resources).  In

contrast to *T.  formica*, the orb-weaving *G.  foxi* did not partition niches among

ontogenetic stages (Figure 2.4).  This may indicate that the less expensive orb webs of *G.*

*foxi* constitute a lower cost to the spider than the sheet webs of *T. formica*, and that web loss due to competitive displacement is of minimal concern.

Competitive mechanisms likely do not explain microhabitat utilization on their own: after all, the existence of competition for microhabitats implies either an insufficient supply of microhabitats for all occupants (which is unlikely in this system), or some metric of microhabitat quality that makes a given microhabitat worth the effort and risk of competing for. It is therefore more accurate to say that microhabitat quality, rather than competition, drives patterns in microhabitat utilization, and that competition is a side effect of the search for high-quality microhabitats. For spiders, the rate of prey encounter is a primary metric of microhabitat quality (Harwood & Obrycki 2007; Kawamoto & Japyassú 2008; Rittschof & Ruggles 2010). Linyphiid spiders in this system consistently utilized microhabitats with relatively high activity-densities of the principle prey item, springtails (i.e. high-quality microhabitats); whereas orb-weaving *G. foxi* did not (Figure 2.5). This indicates that microhabitat quality has a stronger influence on microhabitat utilization for sheet-weaving linyphiid spiders than for orb-weaving spiders. This higher dependence on microhabitat quality would undoubtedly mean that linyphiids experience higher levels of competition for microhabitats than orb-weavers. This is in line with previous hypotheses of spider-web diversity. For example, Kawamoto and Japyassú (2008) hypothesized that the low construction costs of orb webs allow flexible, exploratory strategies of microhabitat utilization, because the relatively low silk investments mitigate the risk of resource wastage. My work therefore provides suggestive evidence for the corollary that high silk investments decrease tolerance for low-quality microhabitats, decrease the total number of microhabitats that could be

profitably exploited, and thereby increase the likelihood of competitive interactions with other individuals. However, stronger evidence can be found in the prey-consumption data.

The prey-capture efficiency of a spider web can also have important impacts on the tolerance of the spider to different microhabitats: spider webs with low prey-capture efficiencies would require high rates of prey encounter in order to capture the same amount of prey as a web with higher prey-capture efficiency. In PCR gut-content analyses, the sheet-weaving *T. formica* tested positive for springtail DNA at a higher rate than the orb-weaving *G. foxi* (Figure 2.7). However, using a full-probability model to account for the rate of prey DNA digestion, it was demonstrated that *G. foxi* actually captured springtails at a higher rate than *T. formica* (Figure 2.9 and 2.10), and that *T. formica*'s higher percent-positive is primarily due to a longer retention time of prey DNA within the gut. Thus, despite utilizing microhabitats with higher rates of encounter with springtail prey, *T. formica* was less successful at capturing springtails. In fact, in the laboratory, only 40% of springtail prey that were intercepted by *T. formica* webs were captured by the tenant spider, and this high rate was only attained because many individuals were given multiple chances to attack the prey (Figure 2.6). It is, in fact, reasonable to suggest that *T. formica* constructs relatively large webs for its body size precisely to increase the chances that intercepted springtails will contact the web multiple times after successful escapes. Thus, the web-driven hypothesis is supported: the prey-capture efficiency of a spider's web has a strong influence on its rate of prey capture, and also determines whether or not the rate at which prey are encountered in the microhabitat is limiting on predation rates.

*Glenognatha foxi* was able to capture prey at high rates, even in relatively low-quality microhabitats, which indicates that the characteristics of the web allow this spider to tolerate a wider range of microhabitats, and thereby avoid significant competitive interactions; while *T. formica*, with its less efficient prey trap, was apparently more dependent on the quality of the microhabitat for successful prey capture, and was thereby subject to higher levels of competitive interactions with other individual spiders.

This example highlights the importance of understanding the rate of prey-DNA decay within predator guts. Without correcting field results by DNA decay rates, it would appear that *T. formica* consumes springtails at a higher rate than *G. foxi*, a conclusion that is consistent with the observation that *T. formica* utilizes microhabitats that are relatively rich in springtails. The intuitiveness of this conclusion is attractive, but decay-rate correction of percent-positive results reveals that *G. foxi* actually consumes prey at a higher rate than *T. formica*. However, caution must be exercised in interpreting these results, because the unrealistic assumptions associated with estimating field data remain in this model. Prey activity-density was high enough in this system that spiders likely had ample opportunity to take more than a single prey item within the measured detectability intervals; consequently, many spiders testing positive likely consumed more than a single prey item. However, the large disparity in decay rates and low difference in percent-positives between the two species suggests that the comparison is still valid: *G. foxi* does indeed consume springtails at a higher rate than *T. formica*. This model represents an important step in molecular gut-content analyses, as it provides a basic mathematical framework for drawing quantitative inferences from field data. Future work must focus on improving the reliability of the assumptions, using either model

simulations or controlled experiments to gain insights into plausible rates of prey encounter and prey consumption in the field.

From these results, perhaps there is an answer to the question posed by Edwards *et al.* (2009): is optimal foraging a realistic expectation for orb weavers? For some spiders, such as these epigeal predators, the answer may very well be, "yes." Unlike many of the aerial orb-weavers, the epigeal spiders in this study inhabit microhabitats where high-quality prey can be intercepted in time intervals measured in hours. Therefore, the rate at which prey are encountered is likely high enough that spiders can assess the quality of the microhabitat through direct foraging experience. Additionally, the epigeal environment in which these spiders forage can retain olfactory cues from prey, which can also provide useful information for spiders to evaluate, and allow them the ability to identify and selectively utilize high-quality microhabitats.

Finally, this research also highlights the importance of understanding the behavior of generalist predators for biological control. Not only the habitat preferences of a predator, but also the way in which a predator utilizes the habitat, can impact its rate of prey consumption. In this chapter, I have found evidence that the foraging ecology of web-building spiders is impacted by microhabitat utilization and competitive interactions, and have also demonstrated that a non-pest prey item plays a central role in spider ecological webs.

# CHAPTER 3: PHENOLOGICAL DYNAMICS OF WEB-BUILDING SPIDER POPULATIONS

## 3.1 Summary

Web-building spiders form a major component of the generalist predator fauna in arable fields.  They have been purported to contribute to the biological control of pests such as aphids and leafhoppers.  However, their successful contribution to pest suppression is contingent upon their ability to adapt to highly-disturbed agroecosystems. We examined the population dynamics of these important natural enemies to compare phenological patterns in relation to crop cycles among species in an alfalfa agroecosystem using quadrat-based sampling and time-series analysis.  Three common species of web-building spiders had generation times similar to the duration of a crop cycle (31 to 44 days), with peak abundances of adult spiders occurring at 15-18 days after harvest.  The timing of these peaks corresponds with the critical early phase of the pest population cycle, during which natural enemies can have the maximum impact on pest populations, suggesting that these spiders are capable of contributing to pest suppression as part of an assemblage of natural enemies.

## 3.2 Introduction

Spiders are an important component of the community of arthropods frequenting agroecosystems, often outnumbering most or all other groups of natural enemies (Nyffeler & Sunderland 2003), and feeding on many important pest species, such as aphids and leafhoppers (Nyffeler 1999; Harwood et al. 2004). The potential for generalist predators, such as spiders, to contribute to pest regulation has been an important focus of the biological control literature (Riechert & Lockley 1984; Nyffeler & Sunderland 2003). Generalist predators usually experience loose, diffuse dynamical links with any specific prey item, and thus, are thought to be less likely to respond heavily to changes in the populations of a specific pest (Hagen & Vandenbosch 1968; Symondson et al. 2002). This contrasts with the tightly-coupled relationships of specialist natural enemies with their pest prey, which provide them an advantage in terms of responding to specific prey items. However, such highly synchronized dynamics can place them at a disadvantage in shifting environments (e.g., frequently harvested crops), in which pest population cycles can deviate unexpectedly from the conditions to which the specialist is adapted. In such instances, species with polyphagous habits and multivoltine life cycles have the flexibility to persist even as pest populations fluctuate, or before pest populations have been established (Settle *et al.* 1996; Scheu 2001), thereby exerting predatory pressure when pest populations are establishing and growing (Landis & Van der Werf 1997; Harwood *et al.* 2004; Harwood *et al.* 2007). This is important because the early, establishment phase of pest population growth is critical in the context of biological control, and is the time during which the greatest impact by natural enemies can be realized (Ekbom *et al.* 1992).

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Despite these favorable traits, the ability of generalist predators to contribute to pest regulation through early-season predation is challenged by the disturbances caused by agronomic practices, which impact predators as much as, if not more than, pests. Thus predators are required to adapt to cyclical agronomic disturbances (such as crop harvests), which can potentially neutralize their advantage as natural enemies. Such adaptations become increasingly important as the level of disturbance in crops increases. For example, forage crops, such as alfalfa, *Medicago sativa* L. (Fabales: Fabaceae), are harvested several times during any growing season, presenting spiders with only ephemeral habitats in which to forage for prey and complete development. These conditions are highly disruptive to the synchronized dynamics of specialist predators and their prey, and may be conducive to ruderal, generalist predators with rapid life cycles, such as web-building spiders. Indeed, the cyclical disturbances in these systems may favor spiders with very specific life-cycle characteristics.

Web-building spiders constitute a large portion of the spider fauna in North American alfalfa fields (Birkhofer *et al.* 2007). The family Linyphiidae, for example, comprises a large number of agrobiont species with varying life cycle characteristics, including univoltine, bivoltine and multivoltine species (Wise 1984; Thorbek *et al.* 2003; Bolduc *et al.* 2005). In alfalfa systems, a polyphagous diet and a multivoltine life cycle could allow persistence in spite of intensive disturbances.

Herein, I present population data on web-building spiders collected by absolute population sampling in quadrats in a North American alfalfa agroecosystem throughout an entire growing season. The objective of this research is to examine the dynamics of spider populations in a highly disturbed agroecosystem, with the intent of determining

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life-cycle and phenological patterns in relation to agronomic disturbances. My hypothesis is that the disturbances of crop management will select for specific life-cycle phenologies. I thus predict that the most common species will display life cycles that closely match the crop cycles in alfalfa.

### 3.3 Materials and Methods

Phenological data were recorded and analyzed for spiders collected in chapter 2. Collection protocols were reported in chapter 2. All spiders collected between the first alfalfa cut in May 2009, and the last alfalfa cut, in August, are included in these analyses. The three most abundant species, *T. formica*, *G. foxi* and *E. autumnalis* were included in these analyses. Population density data for adult spiders of all three species were analyzed using sample autocorrelation. Density (spiders/m$^2$) of each species was calculated for each sampling day. Sampling data was not collected uniformly, and since autocorrelation analysis requires uniformly distributed time series, the seven-day moving average of each date was calculated. A seven-day moving average was chosen because seven days corresponds to the largest gap between sampling dates (see Rasmussen *et al.* (2001) for discussion of time series analysis). Each uniform time series was analyzed using the autocorrelation function in MATLAB version 7.10.0.499 R2010a (function *autocorr*, The MathWorks, Inc., Natick, MA, USA). This technique can qualitatively determine the stationarity and periodicity in time-series data (Turchin & Taylor 1992), and can identify regions in a time-series that are significantly correlated with one another. In effect, it estimates the lag time between successive peaks (a positive correlation) or between an adjacent trough and peak (a negative correlation) over a time series. In the

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present study, positive correlations were interpreted as generation times in alfalfa, while negative correlations were interpreted as establishment times, i.e. the time between a density trough (which occurred at each harvest) and a subsequent density peak.

In addition, a similar analysis was run on two life stages (immatures versus adults) of *T. formica* and of *G. foxi*. *E. autumnalis* was omitted because immatures could not be reliably identified. Protocols were identical to those used to analyze the adult data sets, except that the crosscorrelation function in MATLAB (function *crosscorr*) was used. This function works similarly to the autocorrelation function, except it compares a pair of time series to one another instead of comparing different regions of the same time series In this case, a positive correlation corresponds to the lag between the peak of one time series and the peak of the other, while a negative correlation corresponds to the lag between the peak of one time series and the trough of the other. For species with discrete generations, positive correlations estimate the duration of the final instar. Negative correlations, while producing significant signals in analyses, can be regarded as an artifact of the analysis, and do not carry any biological meaning for this data.

### 3.4 Results

Collection data show two peaks in density of adult females for each species (Fig. 3.1), indicating that two generations were completed within the study period. Each of the three spider species had unique time lags (generation times) between successive peaks in adult densities (Fig. 3.2): *T. formica* had a significant positive autocorrelation at a 44-day lag, *E. autumnalis* had a significant positive autocorrelation at a 40-day lag, and *G. foxi*

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had a significant positive autocorrelation at a 31-day lag.  Significant negative
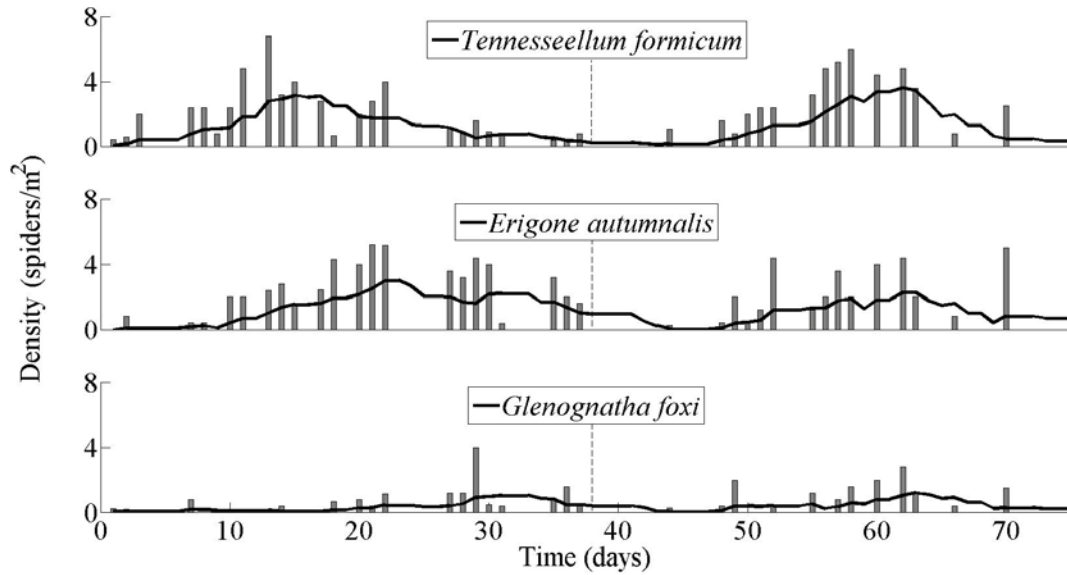


Figure 3.1  Density of three spider species during two crop cycles in Kentucky alfalfa, calculated with a seven-day moving-average window.  Raw density data are shown as bars on the day of collection.  Harvests occurred at day 0 (19 May), day 37 (26 June; vertical dotted line), and day 75 (7 Aug; end of sampling period).
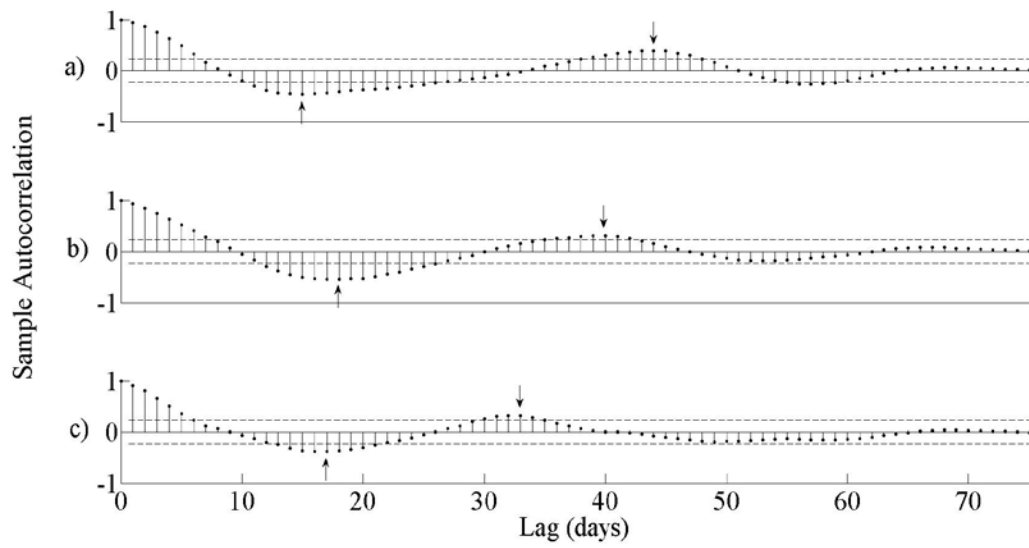
Figure 3.2 Autocorrelation functions for three species of spider in Kentucky alfalfa. In each panel the dashed lines represent 95% confidence intervals and arrows mark the strongest negative and positive autocorrelation. a) *Tennesseellum formica*; b) *Erigone autumnalis*; c) *Glenognatha foxi*.

autocorrelations occurred at a lag (establishment time) of 15 days for *T. formica*, 18 days for *E. autumnalis*, and 17 days for *G. foxi*.

In the life-stage analysis for *T. formica* (Fig. 3.3), the positive crosscorrelation at -4 days suggests that the final instar for *T. formica* lasts only 4 days, and indicates that this species undergoes a cyclical pattern of discrete generations. For *G. foxi* (Fig. 3.4), a pattern of discrete generations was also uncovered, and the final instar lasts 9 days; however, strong crosscorrelation values were also present at other lag times, suggesting *G. foxi* populations experience additional periodic fluctuations that *T. formica* populations do not experience. These results suggest other periodic factors, such as seasonal effects (as opposed to crop cycle), influence the life-stage distribution of this species.

### 3.5 Discussion

This research has provided evidence that web-building spider population dynamics closely match the cyclical disturbance patterns in an alfalfa agroecosystem. Harvests occurred approximately 40 days apart, and spider generation times closely matched this. In addition, the lowest densities of active life stages for these spiders occurred very close to harvest, while peak densities occurred within 2.5 weeks of harvest for all three species. This has important implications for the pest suppression activity of these spiders. Emmen *et al.* (2004) showed that potato leafhopper immigration into Pennsylvania alfalfa reaches its highest rates 2–3 weeks after the alfalfa is harvested, indicating that adult spiders are at their highest densities during the critical establishment phase in the leafhopper population cycle, thereby suggesting that these spiders can play an important role in leafhopper suppression early in the growth cycle. However, Birkhofer *et al.* (2007) showed that pea aphid populations in Kentucky alfalfa can return
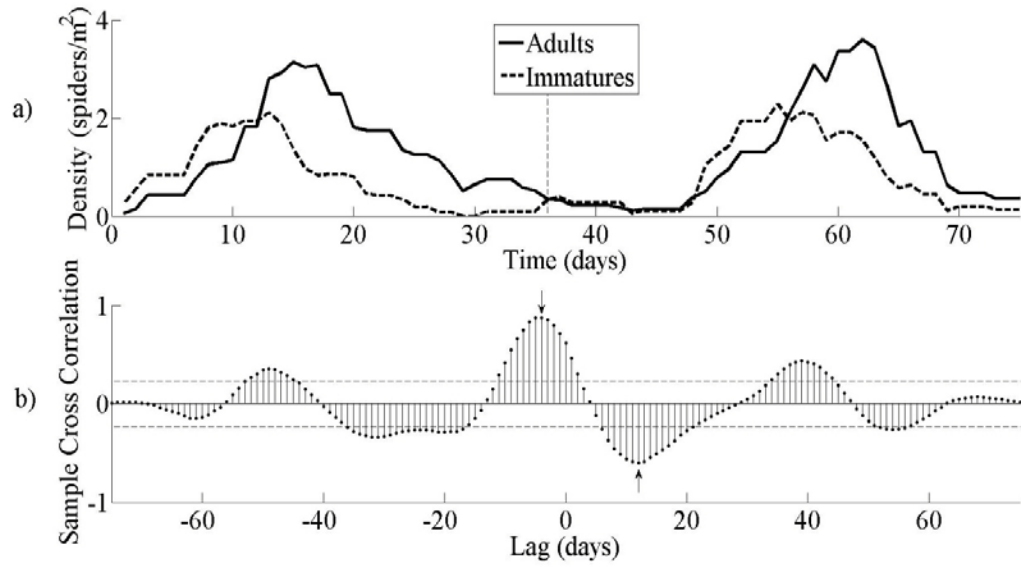
Figure 3.3 Phenology of *Tennesseellum formica* in Kentucky alfalfa. a) Seven-day moving average density of *T. formica* adults and immatures during an alfalfa growing season. At day 0 and 37, the alfalfa was harvested. b) Crosscorrelation analysis between adult and immature *T. formica*. Crosscorrelation analysis finds temporal patterns in correlated time series.

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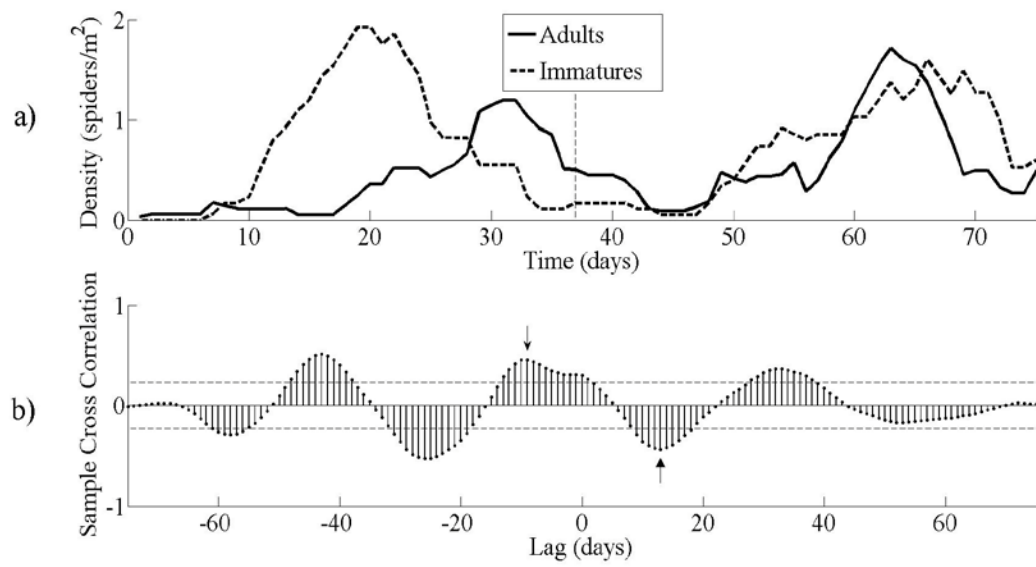
Figure 3.4  Phenology of *Glenognatha foxi* in Kentucky alfalfa.  a) Seven-day moving average density of *G. foxi* adults and immatures during an alfalfa growing season.  b) Crosscorrelation analysis between adult and immature *G. foxi*.

to pre-harvest densities within two weeks after harvest, and similar results were found in

Canadian alfalfa (Harper *et al.* 1990). This indicates that web-building spider dynamics

are not optimized for suppression of aphids by adult spiders; however, immature spiders

are present and accumulating in the habitat during the critical establishment phase of the

aphid population cycle, and thus may still contribute to aphid suppression to some extent.

Particularly noteworthy is the timing of population peaks and troughs. Densities

of adults of each species reached their lowest densities very near the time of harvest (Fig.

3.1). Other authors have previously shown strong negative effects of management

disturbances on predator populations (Thorbek & Bilde 2004; Öberg & Ekbom 2006). It

is tempting to apply this explanation to the dynamics of *G. foxi*, which appear to have not

completed a full population cycle before the end of the first crop cycle, and to have

declined in density after the harvest. However, the density of active stages of *T. formica*

clearly declined steadily throughout the latter half of the crop cycle until reaching its

lowest level at harvest. The decline of this species thus does not appear to be related to

the disturbance from harvest, but to a pattern of discrete generational phenology in which

the egg stage—which was not sampled in this study—coincides with harvest. Thus, the

phenology of *T. formica* may be a pre-adaptation to the crop cycles of alfalfa, and allow

this spider to persist in high numbers in this agroecosystem. Interestingly, Bolduc *et al.*

(2005) uncovered a bivoltine/trivoltine life cycle for this species in Quebec vineyards,

with a time lag between successive generational peaks that closely approximated the time

lag we uncovered, indicating that the findings of the present study are not a specific

adaptation to the alfalfa system, but a general characteristic of the species. However, the

egg stage for *T. formica* is completed in approximately 10 days under laboratory

conditions (Welch, unpublished data).  Thus, we should expect the peak density of immatures to occur within 10 days of the prior adult peak.  However, in field collections, the gap between the peak density of adults and the subsequent peak density of immatures is approximately 40 days, four times the duration of the laboratory egg stage.  This suggests that either (1) the egg stage lasts considerably longer in the field than in the lab (although, Thorbek *et al.* (2003) found that linyphiid eggsac development times of 40 days occurred only at temperatures of $\leq 12°$ C); (2) the subsequent peak of immatures does not derive from eggs laid at peak adult densities, but from eggs laid later in the population cycle; or (3) spider hatchlings undergo a prolonged stage in which they do not construct webs, and thus were not collected by our web-centric sampling protocols.  Additionally, a qualitative assessment of the collection data suggests that, during the first crop cycle, adult density peaks were staggered across species, producing a succession-like dynamic in which each species is associated with a specific phase in the crop cycle (Fig. 3.1).  However, in the second crop cycle, this staggered pattern in density peaks disappeared, and, consequently, the time lags calculated when both cycles are incorporated did not differ substantially.  The staggered dynamic may have been simply a coincidence, or it may have been an effect of phenological shifts in the community across crop cycles due to seasonality and the timing of population cycles for different species.  For *T. formica*, peak densities of each life stage were comparable across crop cycles, both in timing (relative to one another and relative to harvest) and in magnitude, which may indicate a stable generational dynamic in which the observed densities represent a maximum enforced by resource limitations or predation/competition pressures, and suggest that the 4-day estimate of the final instar duration is accurate.  However, in the

life-stage analysis of *G. foxi*, strong periodic signals were uncovered at time lags longer than the generation time (Fig. 3.4).  This is likely related to a seasonal phenological dynamic: immature *G. foxi* peaked at higher densities than adults during the first crop cycle, while adults peaked at higher densities than immatures during the second crop cycle.  It is possible that the adult peaks for this species do not represent two discrete generations at all, but a single generation (or pattern of overlapping or indiscrete generations) interrupted by a harvest, with migration modulating the dynamics following harvest (cf.  Thorbek & Bilde 2004; Gavish-Regev *et al.* 2008).  This is supported by the observation that adult *G. foxi* reached peak densities much sooner after the second harvest than they had after the first, and the observation that, during the second crop cycle, the adult density peak occurs *before* the immature density peak.  This may explain why the "generation time" obtained for *G. foxi* was so much shorter than for *T. formica*: both generation time and final instar duration were likely underestimated for *G. foxi* by assuming that the two peaks in adult density represented two discrete generations.  It also suggests that the timing of harvest will impact whether or not these spiders' pattern of occurrence in the latter part of the season will repeat the staggered pattern of the earlier part of the season, and that agronomic disturbances in crop systems have the potential to alter patterns of natural enemy community structure across the growing season.

# CHAPTER 4: MICROHABITAT EVALUATION AND UTILIZATION BY A SHEET-WEAVING SPIDER

## 4.1 Summary

Sit-and-wait foraging is a distinct strategy that involves significant investments prior to prey encounters. However, the complexity of decisions involved in selecting, maintaining and abandoning ambush sites provides considerable opportunity for learning and flexibility, particularly for trap-building predators, such as web-building spiders. Most research into the behavior of trap-building predators has focused on the initiation and completion of foraging bouts (site selection and site abandonment, respectively), and less consideration has been given to the ecological significance of behaviors that occur between these end points. In this study, I sought to determine whether an interim behavior, flexible trap construction, is a means of real-time decision making during foraging, and test a new hypothesis that site selection is not a single decision, but a cumulative series of several, distinct decisions based on evaluation of relevant site characteristics. In a laboratory assay, web-building spiders, *Mermesses fradeorum* (Araneae: Linyphiidae) were allowed to choose between microhabitats with and without prey, and site occupancy and web construction behaviors were evaluated over a foraging period, to determine how prey cues impact the evaluation and selection of foraging sites by predators. Spiders responded to prey availability across successive phases of site searching and web construction (i.e., site selection, web initiation, and web expansion), demonstrating that multiple, distinct decision-making steps are involved in foraging-site selection and utilization. These results indicate that site maintenance behaviors, such as

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multiple phases of web-construction by spiders, are an integral part of the decision-making framework of foraging predators.

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## 4.2 Introduction

In the previous chapter, I showed that microhabitat utilization by web-building spiders correlates with the activity of prey. This is consistent with the conclusions of previous work (Harwood *et al.* 2003; Harwood & Obrycki 2007; Pruitt *et al.* 2011). However, the behavioral mechanisms behind this correlation remain incompletely understood. The foraging behavior of sit-and-wait predators, such as web-building spiders, has been and continues to be studied through the paradigm of Optimal Foraging Theory, with behaviors typically being reconstructed as discrete, often dichotomous, decisions contingent on evaluation of external stimuli related to food quality and availability (Stephens & Krebs 1986). OFT has provided useful insights into ecological dynamics in many systems (e.g. Crowley & Linton 1999; Mori & Nakata 2008; González-Bernal *et al.* 2011). However, recent work has repeatedly demonstrated that sit-and-wait foraging in the field is significantly more complex than is represented in the classical model, and newer optimality paradigms that incorporate behavioral flexibility are being more commonly applied (Venner & Casas 2005; Tso *et al.* 2007; Blackledge *et al.* 2011; Blamires *et al.* 2011; Scharf *et al.* 2011).

The optimal foraging models most commonly used were developed primarily for active foragers and may not be appropriate for sit-and-wait foragers (Beachly *et al.* 1995). One of the basic models, the marginal value theorem, states that an optimal forager maximizes its rate of energy gain by preferentially foraging in highly productive patches (Charnov 1976). For active foragers, which deplete the food in a patch as they forage, there is a threshold (in terms of time or food density) beyond which the patch would optimally be abandoned (Charnov 1976). These patch departure thresholds have been

termed the 'giving-up time' (Krebs *et al.* 1974) and 'giving-up density' (Brown 1988). However, for sit-and-wait predators, foraging sites are characterized by rates of food arrival from outside the site; these rates are not influenced by the foraging of the predator, and thus, the rate of gain within a site is linear, rather than decreasing (Beachly *et al.* 1995). Models based on patch departure criteria for active foragers may thus be unsuitable for the study of sit-and-wait foragers (Beachly *et al.* 1995).

However, some sit-and-wait predators must invest considerable amounts of resources into patch use, and the rules governing these investments may be qualitatively similar to the rules governing patch departure criteria for active foragers. Web-building spiders (Blackledge *et al.* 2011), and other predators that construct traps, such as pit-building antlions (Scharf *et al.* 2011), may optimize their rate of gain by regulating the allotment of resources into trap construction (Blackledge & Wenzel 2001; Nakata & Ushimaru 2004; Mori & Nakata 2008; Scharf *et al.* 2009). In contrast to active foragers, trap-building predators make the bulk of investments prior to contact with the intended prey (Tanaka 1989). The disproportionately large up-front costs demand a significantly elevated level of accuracy in foraging site selection to prevent wastage of resources (Nakata & Ushimaru 2004), and also place constraints on the ability of the predator to relocate (Blackledge *et al.* 2011; Ruch *et al.* 2012), forcing the predator into a prolonged, intimate association with a very small unit of habitat. Therefore, trap-building predators are expected to be highly discriminating in their selection of foraging sites (Harwood & Obrycki 2007; Pruitt *et al.* 2011) and to optimize their investments into trap construction.

Among trap-building predators, web-building spiders have attracted a great deal of interest because of the elaborate nature of their prey-trapping webs and associated

behaviors (Blackledge *et al.* 2011). In particular, orb-weaving spiders construct elaborate webs that can record the details of the spider's web-construction behavior, and much of their web-construction behavior has been investigated. Orb-weaving spiders frequently recycle and reconstruct prey-trapping webs between foraging bouts to maximize the prey-capture efficiency of the trap (Opell 1998; Nakata & Ushimaru 2004). They therefore incur a high cost at each foraging bout, regardless of whether they remain in the same foraging site or relocate to a new site. When relocating to a new site, orb-weavers must make investment decisions with very limited information about the quality of the microhabitat (Mori & Nakata 2008). Therefore, spiders often rely on previous foraging experience to dictate future web-construction decisions (Mayntz *et al.* 2009). A recent model (Mori & Nakata 2008) indicates that the optimal strategy for an orb-weaving spider entering a novel, unfamiliar habitat is to invest in a full-size web for the first foraging bout to maximize information gathering capacity and minimize lost opportunity costs. In contrast, the less-studied sheet- and tangle-weaving spiders construct multifunctional webs with higher silk densities and thus, greater initial costs (Janetos 1982; Tanaka 1989). Sheet and tangle webs are not frequently recycled or rebuilt (Janetos 1982), but are often built small and gradually expanded to full size over a protracted period, encompassing several foraging bouts (Benjamin & Zschokke 2004). This suggests that web construction decisions can be spread out temporally, allowing investments into foraging to accumulate in response to ongoing assessment of the current patch, in a manner qualitatively similar to the predictions of the marginal value theorem.

In this chapter, I evaluate the web-construction behavior of sheet-weaving spiders (Linyphiidae), which are abundant predators in many ecosystems, including agricultural

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fields. These spiders have received attention for their potentially beneficial roles in pest consumption (Harwood *et al.* 2004; Chapman *et al.*, in press), which may be contingent on an understanding of their habitat-use decisions (Harwood & Obrycki 2007). I hypothesized that linyphiid sheet-weaving spiders continuously assess the quality of the current foraging site and incorporate this information into ongoing web construction decisions. I predicted that spiders would (1) prefer to construct webs in microhabitats with prey over microhabitats without prey, and (2) invest more resources into webs constructed in microhabitats with prey than in microhabitats without prey. We further predicted that spiders might increase resource investments by: (a) expanding webs beyond their initial size in prey-rich microhabitats and (b) spending more time lying-in-wait within webs in prey-rich microhabitats.

### 4.3 Materials and Methods

*4.3.1 Study Species and Laboratory Rearing Protocols*

Spiders used in this trial were reared in laboratory colonies at the University of Kentucky. Founding stock for the colony was collected from alfalfa fields at the University of Kentucky Spindletop Research Farm, Lexington, KY, U.S.A. (GPS coordinates: 38°07'32"N, 84°30'43"W). The species selected for this study was *Mermessus fradeorum* (Berland) (Linyphiidae: Erigoninae), which is a common web-building spider in North American grasslands and agricultural fields. This species is a small spider (average body length ≈ 2.5 mm) that constructs two-dimensional sheet webs or three-dimensional tangle webs within 1 cm of the ground, with an average surface area of approximately 8 cm$^2$ (Table 2.1). Spiders were reared on a diet of springtails (*Sinella curviseta* Brooks, Collembola: Entomobryidae) from laboratory colonies (springtail-

rearing methodologies are described in Chapter 2). Spiderlings were placed in cups in groups of four to six with established springtail populations and allowed to grow to maturity. Upon reaching adulthood (which typically took 3–4 weeks), spiders were placed individually or in male–female pairs in springtail colonies. All springtails and spiders were maintained in an incubator at 24 °C, on a 16:8 h light:dark cycle.

Only adult female spiders were used in trials because I observed that males of this species do not consistently build webs. Spiders used in trials had been housed individually, without male partners, for at least 1 week. Five days prior to behavioral experiments, trial spiders were removed from springtail colonies and placed in individual Petri dishes (6 cm diameter × 1.5 cm depth) with a plaster base to maintain humidity. Spiders were allowed to establish webs overnight and were then fed one adult springtail, after which food was withheld for 4 days to standardize hunger level. During starvation, spiders were monitored daily and provided an *ad libitum* supply of water.

### 4.3.2 Experimental Setup

Controlled two-way behavioral choice assays were conducted to allow spiders to choose between two simulated microhabitats that were identical in physical structure but differed in prey availability. Assays were run in 12 specially constructed olfactometer apparatuses (Fig. 4.1), which consisted of three chambers connected by tubes. The central chamber was constructed from a clear plastic container (8.4 cm diameter × 3.3 cm depth) and connected to two side chambers made from larger, clear plastic containers (10.3 × 12 cm). Connecting tubes (2.5 cm diameter × 7 cm length) were constructed

**Figure 4.1**
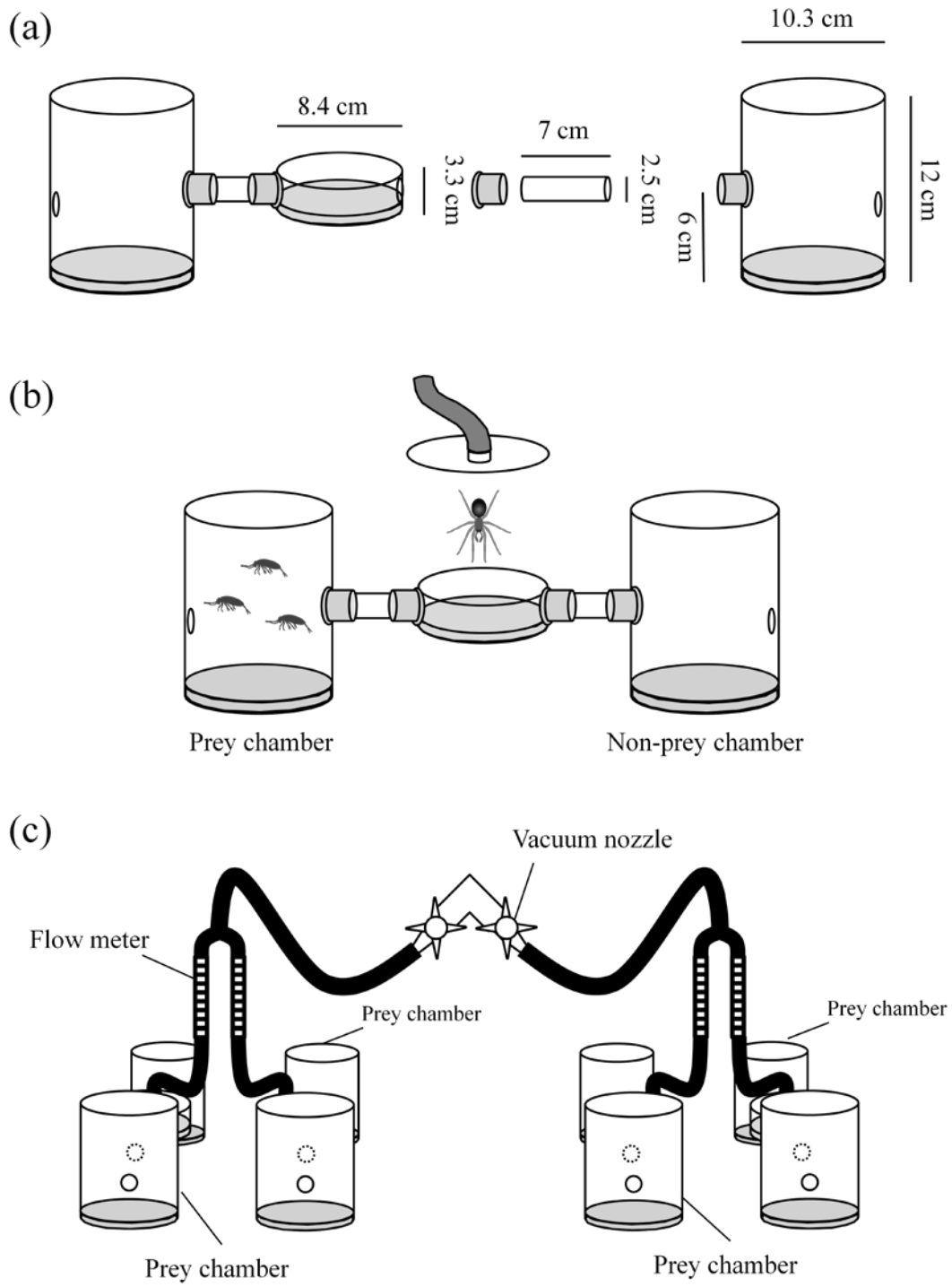
Figure 4.1 Experimental setup for olfactometer trials. (a) Olfactometer parts explosion, showing dimensions (lids of chambers not shown). (b) Assembled olfactometer showing placement of study organisms (not shown to scale) during trials; side chambers were lined with vellum paper (not shown). (c) Schematic of experiment station layout during trials, showing use of vacuum heads and flow meters, and the orientation of each olfactometer. On each trial day, three such stations were set, for a total of 12 simultaneous replicates.

from plastic vials and affixed to the experimental chambers by means of a socket constructed from craft foam (see Fig. 4.1a for design and dimensions of the apparatus). Sockets consisted of a ring of craft foam surrounding a circular opening cut into the chamber and a collar that fit tightly around the connecting tube, which was slid into the socket without adhesives. This modular design allowed the apparatus to be disassembled easily for cleaning and also allowed for manipulation of the apparatus during the trial (described below). The openings and connecting sockets on side chambers were raised 6 cm from the bottom to prevent prey from moving between chambers. A thin layer of plaster of Paris was poured into each of the three chambers as a substrate, which was moistened prior to trials. The walls of the side chambers were also lined with a sheet of vellum paper to facilitate climbing by spiders. Plaster bases and paper linings were replaced after each trial, and chambers were washed thoroughly in distilled water and 95% ethanol.

To promote the transmission of prey olfactory cues, vents were created in the lid of the central chamber and on the walls opposite the connecting tubes in the side chambers. All vents were fitted with fine-mesh screen to prevent escape of test animals, and the central chamber vent was connected by means of a plastic adaptor to the wall-mounted nozzle of the building's central vacuum system. Airflow was monitored with the use of Thermo Scientific Gilmont flow meters (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) and maintained at $1.23 \pm 0.22$ litres/min in all apparatuses.

This design has two major advantages over alternative olfactometer designs. First, it allowed not only an assessment of instantaneous spider responses to prey cues, but also a documentation of subsequent spider behavior within the selected foraging site after the

initial response.  Second, the apparatus can accommodate live prey, providing a more accurate simulation of natural foraging than could be attained using only chemical cues.

I used a total of 59 adult female spiders, which had been reared in the laboratory for three to six generations, in these trials.

### 4.3.3 Behavioral Choice Experiments

Twelve assays were run simultaneously in a dark room.  Two apparatuses were attached to each of six available vacuum nozzles, and an individual flow meter monitored flow to each apparatus separately (Fig. 4.1c).  In each apparatus, live springtail prey were introduced into one of the two side chambers (designated the 'prey chamber'), while the other side chamber (the 'non-prey chamber') was left empty (Fig. 4.1b).  Between 40 and 45 adult springtails (total mass 4–6 mg) were placed in the prey chamber of each apparatus prior to spider introduction.  The orientation of the apparatuses was alternated to avoid the confounding effects of non-experimental variables (Fig. 4.1c).  Although springtails were able to climb the vellum and the chamber walls, preliminary trials revealed that they would aggregate at the base of the chamber within 90 min after introduction and not climb again thereafter.  Springtails were therefore allowed a 90 min acclimation period in the prey chamber prior to trials, during which the prey chamber was disconnected from the remainder of the apparatus and sealed to avoid springtail escape.

Following the springtail acclimation period, the apparatus was reassembled and airflow was initiated.  This was timed to coincide with the beginning of the dark phase in the incubator's light cycle, because preliminary observations indicated that night-time was the period of greatest web-building and foraging activity by *M. fradeorum*.  An

experimental trial was initiated by gently coaxing a spider to drop from an aspirator by a silk thread and land in the centre of the central chamber (Fig. 4.1b). The lid of the central chamber, connected to the activated vacuum head, was quickly replaced. Spiders were allowed to forage and construct webs for the entire, 8 h nocturnal period. Observations were recorded immediately after all 12 spiders were placed in the chamber and at 30 min intervals thereafter throughout the trial period. At each observation, I recorded the following data for each spider: (1) location of the spider within the apparatus, with five locations recognized (prey chamber, non-prey chamber, central chamber and the two connecting tubes); (2) web status (whether or not the spider was occupying a web); and (3) airflow.

### 4.3.4 Statistical Analysis

To evaluate web construction investments, I constructed a series of nested contingency tables. I assessed initial microhabitat selection using a binomial test to compare the first chamber entered by each spider, and I analyzed two phases of web construction (initiation of web construction and subsequent expansion of the web) as a series of contingent behaviors using Fisher's exact test. Lighting conditions during trials did not permit evaluation of web structure without disturbing the spiders, so it was not possible to document the expansion of webs during trials. However, in preliminary trials, *M. fradeorum* constructed webs in repeated bouts of silk laying, interspersed with short periods of inactivity (cf. Benjamin & Zschokke 2004). Periods of inactivity during web construction did not exceed 30 min and were followed either by resumption of silk laying (web expansion) or abandonment of the site. Therefore, all webs that were occupied for at least two consecutive experimental observations were assumed to have been expanded,

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and this was verified by documenting the presence or absence of a completed sheet web at the end of the trials.

I used time-budget analysis to assess how spiders allotted time to foraging behaviors (lying-in-wait within a web) and exploratory behaviors (actively moving about within the site). The number of experimental observations was taken as a surrogate measure for the amount of time spent in a given location or within a web. The apparatus was divided into three roughly equal-sized zones: (1) prey chamber, (2) non-prey chamber and (3) central chamber + connecting tubes. The time spent within each zone was determined for each specimen, and the time spent in a web was calculated as a proportion of the time spent in a given zone, yielding three measures (one proportion for each zone) for each specimen, and a repeated measures ANOVA was performed on the arcsine square-root-transformed data (after Bakeman *et al.* 1992). Only spiders that were observed at least once in each zone were included in this analysis (n = 35).

### 4.4 Results

In binary choice assays, spiders preferred microhabitats that contained prey over microhabitats that lacked prey. All 59 specimens entered at least one of the two side chambers during the trial period. More spiders entered the prey chamber first (binomial test: $P = 0.02$; Fig. 4.2), meaning that the spiders were responding directly to the presence of prey while initially searching for foraging sites. This confirms the hypothesis that the initial selection of foraging sites is driven by prey-related cues.

**p = 0.02 \***
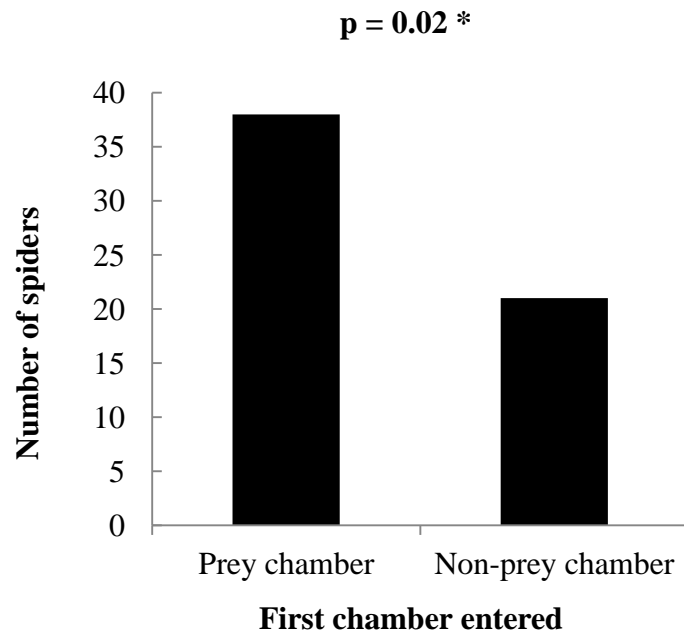


Figure 4.2  Effect of prey cues on site-searching behavior of spiders within a two-way

olfactometer.  P-value determined by binomial test.

After selecting a foraging site, spiders initiated foraging by constructing a web. Of spiders that entered the prey chamber first, 68% (26 of 38) initiated web construction, versus 52% (11 of 21) for spiders entering the non-prey chamber. The difference was non-significant (Fisher's exact test: $P = 0.27$; Fig. 4.3a), providing no evidence for the hypothesis that prey availability drives the decision to initiate web construction. However, spiders that constructed a web within the prey chamber were more likely to complete the web than spiders that constructed a web within the non-prey chamber (85% versus 45%; $P = 0.038$), supporting the hypothesis that prey availability drives the decision to expand a web after it is constructed (Fig. 4.3b).

Additionally, spiders that entered the prey chamber allotted more time to lying-in-wait within a web than spiders that entered the non-prey chamber. In a repeated measures ANOVA, a model incorporating both specimen and zone of the apparatus did not significantly explain the trends in the proportion of within-zone time spent in a web ($F_{36,68} = 0.69$, $P = 0.89$). However, because the effect of specimen was non-significant (type III sum of squares: $F_{34,68} = 0.5$, $P = 0.99$), I pooled data across specimens and performed an analysis using only zone of apparatus as an explanatory variable. In this analysis, zone of apparatus had a significant effect on the proportion of time spent in webs ($F_{2,102} = 4.69$, $P = 0.011$), indicating that the availability of prey strongly affected the spiders' relative allocation of time to site searching and lying-in-wait. Spiders spent a significantly higher proportion of time in webs while in the prey chamber than they did while in the non-prey chamber (67% versus 19% of the time; Fig. 4.4)
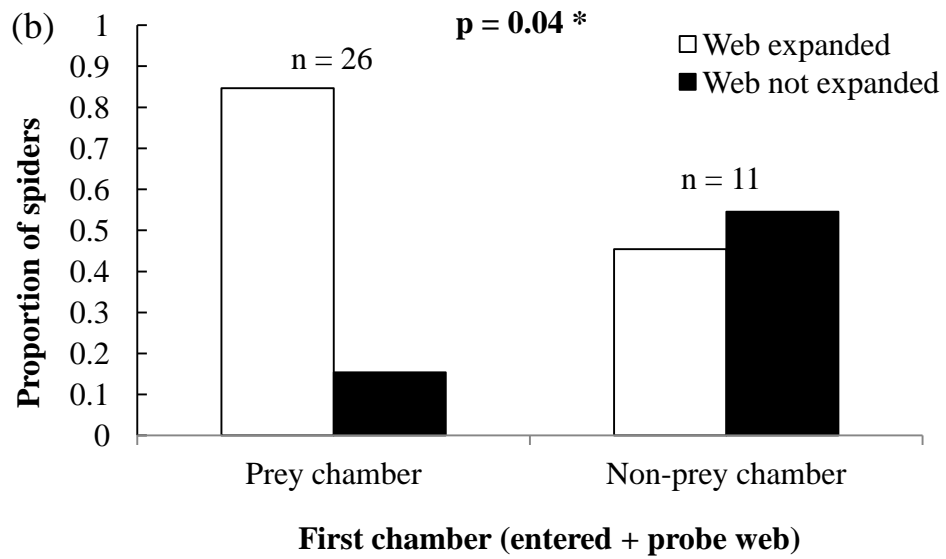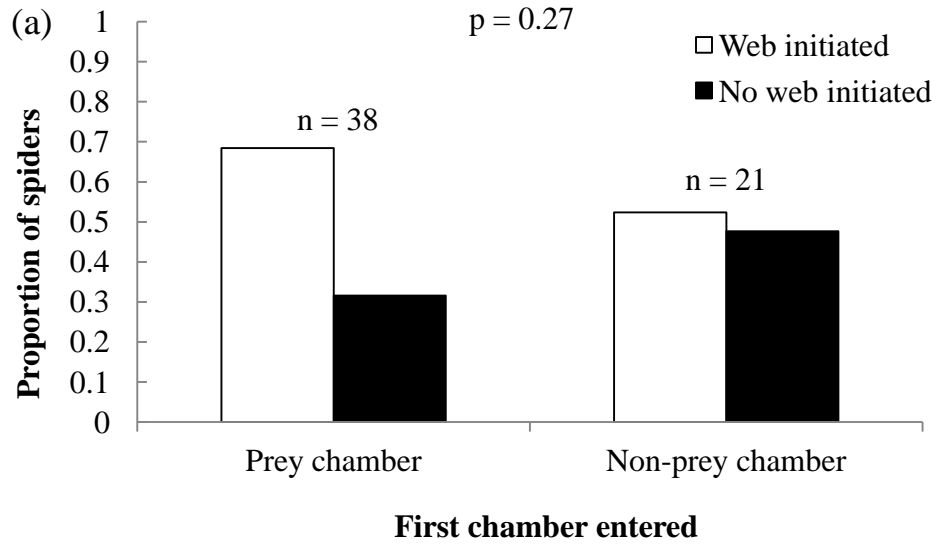
Figure 4.3  Effect of initial chamber selection on subsequent web construction. (a) Effect

of initial chamber selection on the decision to initiate web construction.  (b) Effect of

chamber selection on the decision to expand the initial web.  P-values determined by

Fisher's exact test.
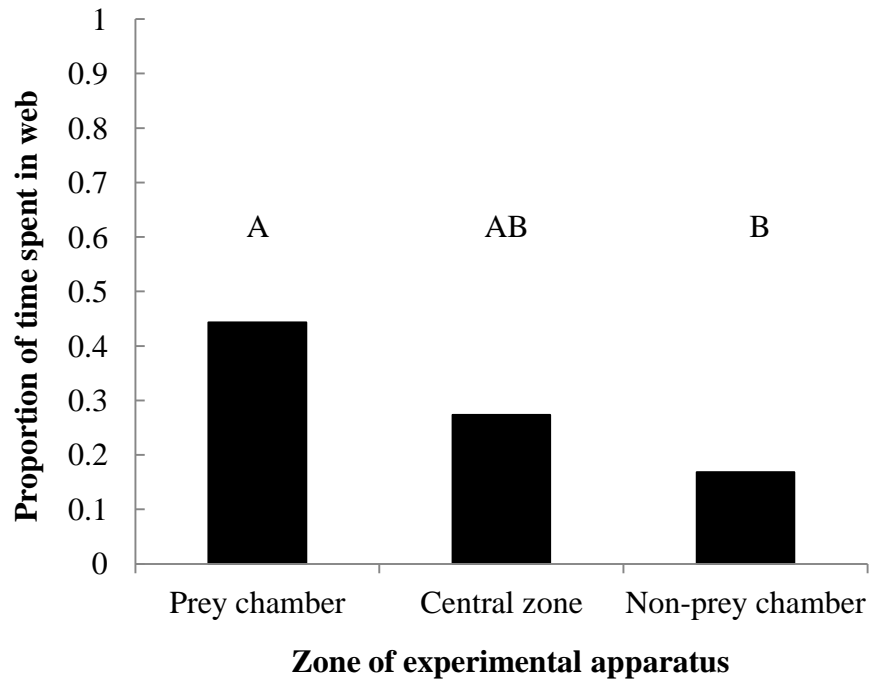
Figure 4.4 Average time spent in a web (i.e. foraging) as a proportion of total time within each zone of an experimental apparatus. Letters reflect significance groupings based on Tukey's HSD test ($N = 35$ spiders).

## 4.5 Discussion

The results of this study indicate that web-building spiders evaluate foraging sites based on the availability of prey prior to and during the construction of a web, and adjust their behaviors in response. Prey availability influenced spiders' allotment of time to different foraging activities (Fig. 4.4) as well as spiders' investment of silk resources into the construction of webs (Fig. 4.3). Spiders spent more time in webs in the prey chamber, indicating that they responded directly to prey availability while making decisions related to site tenacity, and that the absence of prey cues during a critical period of early web establishment may result in abandonment of the web or diminishment of investments into its construction. Thus, the behavior of a sheet-weaving spider is qualitatively similar to the predictions of the marginal value theorem: spiders spend more time foraging in sites where the likelihood of capturing prey is high and spend more time exploring sites where the likelihood of capturing prey is low.

However, these results also indicate that foraging time was not the only investment that accumulated over time. Disparity was observed between occupation of prey and non-prey chambers across several, sequential behavioral steps (chamber selection, web initiation and web expansion), indicating that spiders evaluate foraging sites in a surprisingly sophisticated manner, with quality assessment continuing throughout a multiphase behavioral process culminating in the successful establishment of a full foraging web in a site with suitable abundance of prey. In sites with no access to prey, web construction was initiated but frequently aborted before a full sheet structure could be observed (Fig. 4.3b), indicating that investment of silk resources was also regulated in a manner qualitatively similar to the predictions of the marginal value

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theorem. One previous study (Blackledge & Wenzel 2001) showed a similar behavioral process of web expansion over time in a tangle-weaving spider, *Dictyna volucripes*. However, this study focused on a longer time interval and restricted spiders' site selection. Our study demonstrates that the same processes observed at these larger timescales occurs during the initial phases of site selection and site evaluation. Therefore, the initial site selection process is more complex than a single, dichotomous appraisal of site quality: clearly, site evaluation and investment regulation continue throughout the tenure of a predator in its microhabitat. Many sedentary predators, including sheet-weaving spiders, are known to use active foraging tactics under certain conditions (Inoue & Matsura 1983; Chapman et al., in press); therefore, the decision to build a trap is distinct from the decision to forage within a particular microhabitat. Interestingly, the effect of prey availability in this study was not consistent across all decision-making steps (Figs. 3.2, 3.3), indicating that different features of the site may be evaluated at each phase, and that each phase has a distinct and crucial role in determining microhabitat utilization patterns. An analogous decision-making process was reported by Roces (1993), in which successive behaviors of successful foraging worker ants had a cumulative effect on recruitment of new workers to a food patch, indicating that complex site evaluation and site selection processes are not unique to trap-building predators. Future studies into habitat use by foraging predators should therefore account for evaluation behaviors that occur at all phases of site occupancy.

Our experimental set-up did not allow us to isolate the specific sensory cues that mediated the behavior of the spiders, but previous studies have shown that web-building spiders respond to olfactory cues while selecting foraging sites (Johnson *et al.* 2011;

Pruitt *et al.* 2011).  Therefore, olfactory cues were likely important for spiders in the present study as well.  However, in my preliminary trials that incorporated only olfactory cues from prey, and not live prey (data not shown), spider responses to prey were less clear, suggesting that other factors are also important.  Spiders are known to make extensive use of seismic and airborne vibratory cues, often using web silk to enhance transmission of these vibrations to aid in prey capture (Landolfa & Barth 1996; Blamires *et al.* 2011).  Additionally, orb-weaving spiders are known to adjust web architecture in response to prey vibrations (Blamires *et al.* 2011).  Thus, I conclude that seismic cues were also used by the spiders in the present study to evaluate prey availability, especially after a web was constructed to enhance transmission of these cues.

I propose a prey-centric model of site utilization by trap-building predators, which consists of a series of dichotomous decisions (Fig. 4.5).  I hypothesize that the active search through the habitat for suitable foraging sites is guided by prey-oriented chemotaxis (Pruitt *et al.* 2011), while the decision to initiate trap construction is based on evaluation of the physical features of an encountered site, such as support structures and microclimate (cf.  Biere & Uetz 1981; Rypstra *et al.* 1999).  Web construction behavior proceeds in bouts, with pauses between silk-laying bouts used to further evaluate the potential suitability of the foraging site based on prey activity in the vicinity, assessed through olfactory and seismic cues.  A web begins as a proto-web, or a loose network of just a few silk threads (described for orb-weavers in Zschokke 1996).  I propose that this proto-web serves as a platform from which the spider continues to evaluate site quality, and therefore refer to such a proto-web as a 'probe web'.  This probe web can later be
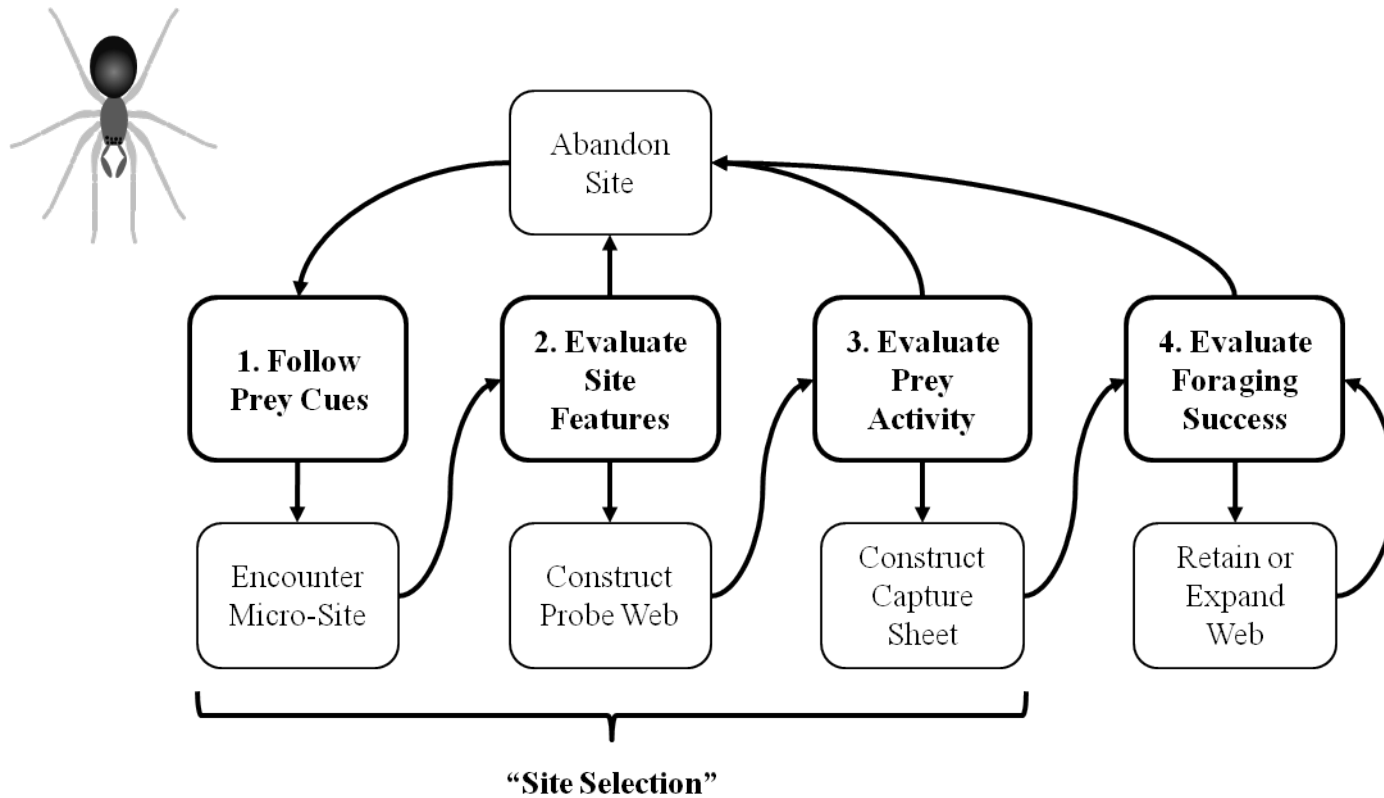
Figure 4.5 The probe web hypothesis. Hypothesized series of behaviors during site selection and web construction, based on the

present study and information from the literature. Steps in which prey cues or other external stimuli are evaluated by the spider are

numbered and written in bold face.

expanded into a full-sized sheet web in suitable foraging sites, or may be abandoned with minimal wastage in unsuitable foraging sites. Over succeeding nights, webs in suitable sites will be retained, expanded and modified (cf. Benjamin *et al.* 2002; Zevenbergen *et al.* 2008).

The present study provides an important empirical test for current theory on sit-and-wait foraging. While our results suggest a general agreement with basic optimality models, it conflicts with the model of Mori & Nakata (2008), which suggests that animals with high site tenacity should invest maximally into site evaluation to maximize information gathering for assessing the potential for future exploitation. However, in our study, sheet-weaving spiders, which have relatively high site tenacity (Janetos 1982), invested minimally at first, and gradually expanded their investments in accordance with site evaluation. However, there are two problems with applying this model here. First, Mori & Nakata (2008) assumed a trade-off between the cost of investment and the quality of information collected. While this assumption is reasonable in some cases (e.g. Nakata & Ushimaru 2004; Bollazzi & Roces 2011), the present study demonstrates that foraging site quality can be assessed based on sensory information, such as olfactory and seismic cues, that can be collected with minimal investment of resources into trap construction. It is therefore reasonable to assume that epigeal trap-building predators do not experience a trade-off between the quality of information and the cost of gathering it (in terms of trap construction costs), and that trap size thus does not restrict the ability of a predator to assess the quality of a foraging site.

The second assumption of Mori & Nakata (2008) is that the forager would be making separate investments into each foraging bout within the same site. For sheet-

weaving spiders, which do not repeatedly construct new webs in the same site, this assumption does not hold. Sheet- and tangle-weaving spiders are more accurately viewed as deciding between making a large investment up-front, or making many small investments over a longer period. Spiders using a probe web strategy will still undoubtedly incur a missed opportunity cost because foraging capacity during the information gathering phase will be diminished. For sheet-weaving spiders, whose webs represent a substantial investment of non-recoverable resources, the benefits of gathering accurate information before committing, and potentially wasting, large amounts of silk resources may significantly exceed the missed opportunity costs (cf. foraging benefit of information, Olsson & Brown 2006). Thus, the model of Mori and Nakata (2008) does not apply here, and more information will be needed on the energetic trade-offs experienced by trap-building predators before existing theory can be extended to cover these and other trap-building predators.

In summary, my results indicate that current optimality models can be applied to some aspects of sit-and-wait foraging (time spent foraging within a patch), but that resource investment, rather than foraging time, is a better indicator of foraging effort for trap-building predators. Current optimality models are not adequate for generally addressing the dynamics of trap-building foraging tactics, and newer models are needed to describe microhabitat utilization behavior by trap-building predators.

# CHAPTER 5: PREY-SPECIFIC FORAGING BEHAVIOR

## 5.1 Summary

Predator foraging behavior can have important impacts on trophic-web structure, and on biological control. In agroecosystems, foraging-tactic selection may determine whether the predator feeds on pest or non-pest prey. I examined the foraging behavior of the flexibly-foraging web-building spider, *Grammonota inornata* (Araneae: Linyphiidae) in response to pest and non-pest prey that differ in behavior, to assess the potential role of predator foraging behavior in biological control. Spiders were allowed to choose between simulated microhabitats with and without a given prey item, to determine what tactics spiders would use to forage for the given prey. Spiders preferred microhabitats with aphid prey over paired microhabitats with no prey, but showed no preference for non-pest springtail prey. Spiders rarely constructed webs in response to aphid prey, but frequently utsed web-construction tactics in the presence of springtail prey. This demonstrates that predators can regulate their foraging activity to maximize consumption of specific prey items. In the many cases where pest and non-pest prey differ in ecology, prey-specific foraging tactics such as these may limit the usefulness of natural enemies in pest suppression: high-quality, non-pest prey may cause shifts in predator behavior that reduce consumption of pest prey. Therefore, non-pest prey can have behavior-mediated, indirect effects on biological control.

## 5.2 Introduction

In previous chapters, I showed that web-building spiders respond to prey cues while choosing which microhabitats to utilize as foraging sites. I also showed that microhabitat-utilization and web-construction behavior is flexible: spiders can choose to abort or modify web-construction in response to ongoing evaluations of relevant cues. This raises the question of how flexible spider foraging behavior may be.

Foraging tactics utilized by a predator impact the types of prey it encounters and the rates at which it can successfully capture the prey. Predators that use a sit-and-wait mode of foraging are most likely to encounter active, mobile prey, but are less likely to encounter sedentary prey (Huey & Pianka 1981). Therefore, the rate at which a predator captures a prey item can be treated as a function of the activity level of the prey and the foraging tactic utilized by the predator. However, much research has demonstrated that many predators forage flexibly: that is, predators are capable of adaptively modifying their foraging behavior to optimize prey-capture success under a variety of conditions (reviewed in Abrams 2010). Flexibly foraging predators may alternate between sedentary and active foraging tactics, often as a result of tradeoffs between prey-encounter rates and the risk of attack from other predators (Tsao & Okuyama 2012), or in response to changes in prey densities (Formanowicz & Bradley 1987). Additionally, many predators have been found to use specialized behavioral sequences to attack specific types of prey (Huseynov *et al.* 2008). Many predators are trophic generalists that feed on a wide variety of prey, and their selection of foraging tactics may thus be influenced by the relative abundances of sedentary and mobile prey in their environment. Such flexible foraging behavior has the potential to greatly affect food-web structure by

altering the rates at which predators encounter and consume different types of prey (Abrams 2010).  In the context of agroecology, behavioral differences between pest and non-pest prey may mediate trophic interactions by altering the behavior of flexibly-foraging predators.  Therefore, in order to assess potential biological control services provided by predators, it is important to quantify how pest and non-pest prey influence the foraging behavior of a predator.

As established in previous chapters, springtails are a common, detritivorous prey that are high in nutritional quality (Toft & Wise 1999; Bilde *et al.* 2000; Hvam & Toft 2005), and are among the most important prey for many types of epigeal generalist predators, including web-building spiders (Kuusk & Ekbom 2010; Romero & Harwood 2010; Chapman *et al.* 2013).  Given the high nutritional quality of springtails as prey, predators may prefer springtails over lower-quality pest prey, such as aphids, and thereby reduce the pest-suppression services provided (Harwood *et al.* 2004; Kuusk & Ekbom 2010).  In addition, springtails are very mobile, and use their furcula to jump away from an attacking predator (as observed in Chapter 2).   The high mobility of springtails suggests that their predators, such as web-building spiders, would optimally utilize sit-and-wait tactics (Kuusk & Ekbom 2010).  These same tactics would be sub-optimal for the capture of sedentary aphids, suggesting that predators may experience a tradeoff between aphid and springtail consumption.

Web-building spiders are among the major predators of springtails (Chapman *et al.* 2013).  Spider webs are ideal devices for capturing springtails, because they include elastic silk and often sticky adhesives, which can effectively absorb kinetic energy and may render springtail escape mechanisms ineffective.  Additionally, as observed in

Chapter 2, a springtail jumping out of a horizontal spider web may simply land in the web again, allowing the spider a second opportunity to capture the prey. However, horizontal spider webs can also potentially be used to capture prey that fall from the vegetation, such as aphids, although these prey would be more accessible through active-foraging tactics (Chapman *et al.* 2013). Although linyphiid spiders typically forage in webs, previous research has indicated that they will also forage actively, away from their webs, and that such active-foraging tactics may increase consumption of foliar prey, such as aphids (Alderweireldt 1994a; Harwood *et al.* 2004; Gavish-Regev *et al.* 2009; Chapman *et al.* 2013). However, these dual foraging tactics have not yet been investigated in manipulative, behavioral experiments. Similar research on foraging modes has been conducted extensively in lizards, where the dichotomy between active and sit-and-wait foraging modes has been attributed to divergent evolutionary adaptations (e.g., Cooper 1995; Perry 1999), or intra-species phenotypic variation (Williams & McBrayer 2011). However, the behavioral dichotomy indicated in linyphiid spiders is distinct from the sit-and-wait/active-foraging dichotomy that has been extensively studied in lizards: while trap-construction tactics can be considered an extreme form of the sit-and-wait mode, non-trapping tactics utilized by linyphiid spiders are not necessarily active foraging, but may also include ambush tactics (Welch, personal observation). In order to avoid confusion, I therefore use the term *free-foraging* to refer to foraging tactics that do not involve trap construction. Also, in contrast to vertebrate model systems, such as lizards (Perry 1999), the foraging-tactic dichotomy for linyphiid spiders is discrete (i.e., there is little possibility of an intermediate foraging mode between trap-building and free-foraging), and is thus a highly useful model system for quantifying foraging behavior.

Linyphiid spiders feed extensively on both pest aphids and non-pest springtails (Harwood *et al.* 2004; Gavish-Regev *et al.* 2009), and have often been investigated in the context of biological control (e.g., Harwood *et al.* 2004; Öberg & Ekbom 2006; Opatovsky *et al.* 2012; Chapman *et al.* 2013). Consequently, for linyphiid spiders, the dichotomy in foraging tactics may overlap with two other, important dichotomies. First, the two separate foraging tactics may function as optimal strategies for capturing the two different types of prey (Harwood *et al.* 2004; Chapman *et al.* 2013). This means that, while other flexibly-foraging predators have been demonstrated to utilize different foraging modes under different physiological states (Inoue & Matsura 1983; Tsao & Okuyama 2012) or predation risks (Gillespie & Caraco 1987), linyphiid spiders may switch from trap-building to free-foraging tactics as a mechanism of prey switching. Second, these two important prey types represent a pest and a non-pest prey item. The non-pest prey, springtails, are high-quality prey for spiders (Marcussen *et al.* 1999; Toft & Wise 1999; Sigsgaard *et al.* 2001), while the pest prey, aphids, are lower-quality prey (Hvam & Toft 2005). Therefore, foraging-tactic selection in linyphiid spiders may correlate with prey behavior, and may have important ramifications for biological control by these predators. Specifically, a strict interpretation of Optimal Foraging Theory predicts that a zero-one rule ought to be employed by web-building spiders. That is, if springtail prey are highly available, optimal behavior by spiders would be to eliminate aphids from their diet.

In this chapter, three controlled experiments are presented to evaluate the foraging behavior of linyphiid spiders in response to sedentary aphids and mobile springtails. I hypothesize that prey type drives foraging-tactic selection, and predict that spiders would

respond to both prey in terms of foraging site selection, but utilize trap-building tactics in response to springtails, and free-foraging tactics in response to aphids. I also hypothesize that prey-specific foraging-tactic utilization would have negative impacts on biological control, because spiders would preferentially forage for springtails, and the tactics utilized to capture springtails would be incompatible with extensive consumption of pest aphids.

## 5.3 Materials and Methods

Three experiments were designed to investigate spider foraging behavior in the context of alternative prey. The first experiment is a laboratory, two-choice assay derived from the experiment reported in the previous chapter, designed to characterize the foraging behavior of spiders in response to two different types of prey: an epigeal, detritivorous, non-pest springtail (*S. curviseta*) and a foliar, herbivorous pest aphid (*Rhopalosiphum padi* Linnaeus, Hemiptera: Aphididae). The second and third experiments are greenhouse, no-choice assays in which spider foraging behavior and prey consumption were investigated across mixed-prey and single-prey treatments. PCR gut-content analysis was incorporated into experiment 3 to examine spider trophic linkages under different prey treatments.

### 5.3.1 Experimental organisms

In all three experiments, the spiders species used was *Grammonota inornata*. This spider is similar in body size and web architecture to *Mermessus fradeorum*, and is in the same subfamily (Linyphiidae: Erigoninae). *G. inornata* is a common web-building spider in grasslands and agricultural fields in the eastern United States, including central

Kentucky (Culin & Yeargan 1983a; Birkhofer *et al.* 2007; Welch *et al.* 2011).

Experimental specimens were adult females collected by hand from residential lawns in

Lexington, Kentucky, USA.  Colony-reared spiders were not used, in order to avoid the

confounding effects of a controlled diet.  After collection, spiders were kept individually

in laboratory incubators in Petri dishes (6 cm diam.  $\times$ 1.5 cm depth) with a plaster base

for at least 4 days, during which water was provided *ad libitum*, but no food was provided.

Light cycle was maintained at 16 L: 8 D, and temperature was maintained at 24° C.

For these experiments, a colony of *R.  padi* was established from founding stock

collected in Princeton, Kentucky, in addition to springtail colonies.  This aphid is a

common vector of Barley Yellow Dwarf Virus (BYDV) in winter wheat (D'Arcy &

Burnett 1995).  Field-collected aphids were quarantined and monitored for the presence

of virus for at least three generations before being introduced into colonies, where they

were maintained on virus-free winter wheat (*Triticum aestivum* Linnaeus, Pembroke red

winter wheat, Kentucky Small Grain Growers Association, Eastwood, Kentucky, USA)

in greenhouse enclosures (BugDorm 60 × 60 × 60 cm insect rearing tent, MegaView

Science, Taichung, Taiwan), with a light cycle of 16:8 (L:D) hrs.  Temperature was 25° C

during the light period, and 21° C during the dark period.  Within each greenhouse

enclosure, wheat was grown in sixteen flower pots (11 cm diamater × 11 cm ht), and each

enclosure was maintained for three weeks, after which it was discarded (this reduced

parasitoid infestations).  Wheat was planted in one new enclosure weekly, and new

enclosures were populated after one week of wheat growth with aphids from older

enclosures.

### 5.3.2 Experiment 1: Prey-Specific Foraging Behavior

Behavioral responses of spiders to prey were assessed in two-choice assays utilising an experimental arena developed and described in the previous chapter. Arenas were designed to confine prey in one of the two side chambers, while simultaneously facilitating spider movement throughout the arena (Fig. 5.1). In these assays, spiders were introduced into the central chamber of a three-chamber arena, and allowed to choose between two side chambers simulating microhabitats with and without available prey. Half of the assays were provisioned with aphid prey, and the other half were provisioned with springtail prey. This approach allows comparisons of the tactics utilized by the spiders when the target prey item is the only prey stimulus. Specifically, I documented, not only whether spiders prefer a microhabitat with available prey over a microhabitat without prey, but also assessed whether spiders constructed webs in order to capture the prey available to them in the particular microhabitat.

### 5.3.3 Experiment 1: Protocols

The construction of the experimental arenas used in this study is described in the previous chapter, and a brief summary is provided here. Arenas were constructed from transparent plastic containers, with plastic tubes connecting a central chamber to two side chambers (called the "prey chamber" and the "non-prey chamber") via sockets made of craft foam (Fig. 5.1). A 0.5-cm layer of plaster was poured into the base of each of the three chambers and allowed to harden, and the walls of the two side chambers were lined with vellum paper to facilitate climbing by spiders. A vacuum system connected to the lid of the central chamber draws air through screen-covered vents on the opposite sides of the side chambers, and airflow is monitored with Thermo Scientific Gilmont flow meters

(Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA). Experimental

methodologies followed Chapter 3, with two modifications (Fig. 5.1). Six-day-old wheat

seedlings (Pembroke cultivar) were placed in each side chamber of each arena, in order to

provide a substrate for aphid settlement. Seedlings were planted individually in small,

plastic tubes filled with water and pressed into the plaster base of the chamber.

Parafilm® was used to seal the top of the tube and prevent experimental animals from

falling into the water. Two seedlings were placed in each chamber because, in

preliminary testing, aphids regularly dropped from seedlings. The second seedling

therefore provided an alternative host for dropping aphids within the designated prey

chamber, and stopped these aphids from leaving the prey chamber in search of new host

plants. Second, the prey chamber was isolated from the rest of the arena for a 24-hour

acclimation period prior to the introduction of spiders, during which time prey were

allowed to move freely within the prey chamber and establish on the seedlings (aphids) or

on the ground (springtails), but were denied access to the remainder of the arena.

Throughout the experiment, no prey were observed outside of the prey chamber in any

trials, and I am therefore confident that control (non-prey) treatments remained

uncontaminated.

Figure 5.1



Legend:
- *G. inornata*
- *S. curviseta* (springtails)
- *R. padi* (aphids)

(a)

5 cm

10.3 cm

8.4 cm

7 cm

3.3 cm

2.5 cm

6 cm

12 cm

(b)

or

prey chamber          non-prey chamber

(c)

vacuum nozzle

flowmeter

no prey

no prey
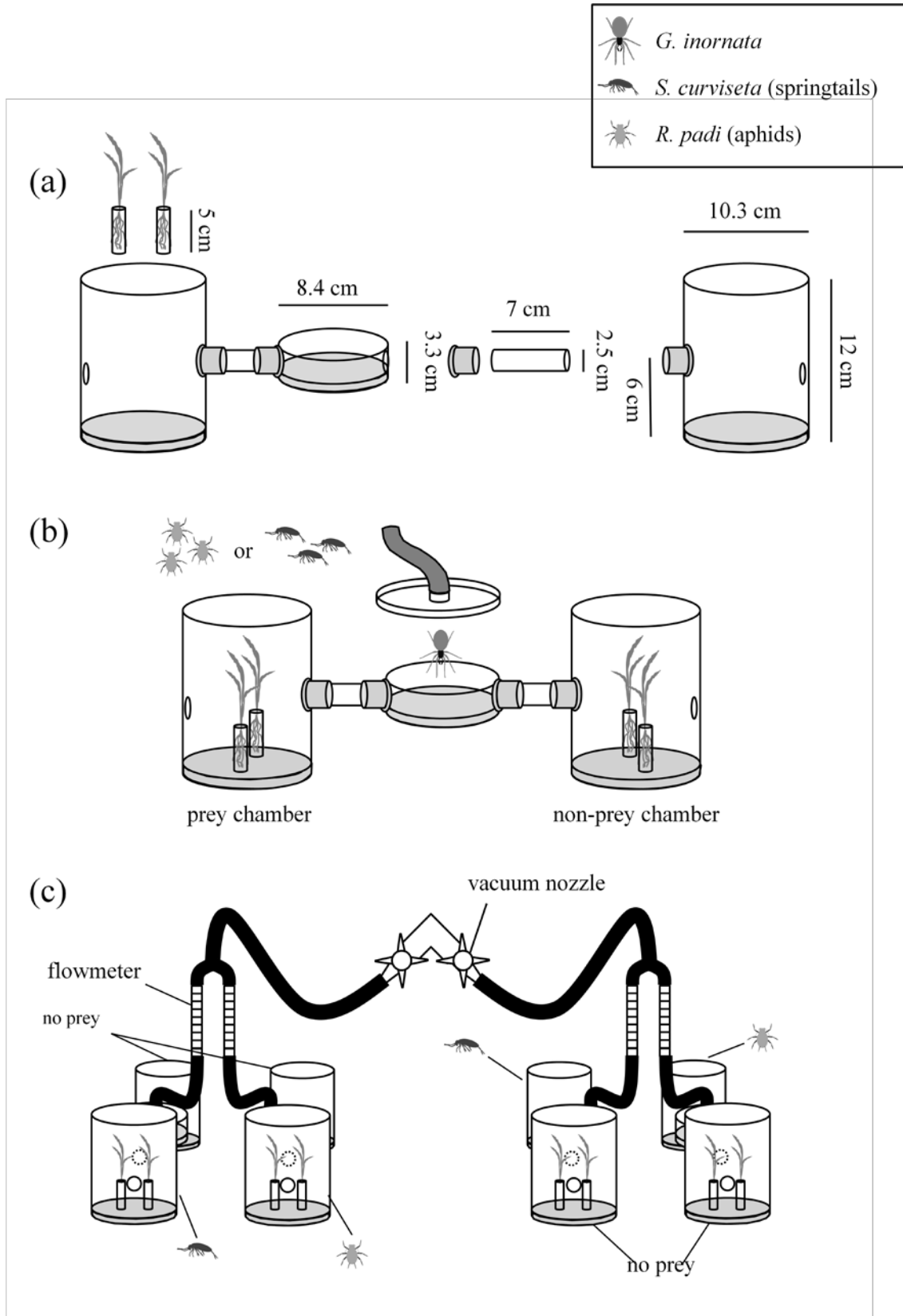
Figure 5.1  Setup of experiment 1 (Prey-specific foraging behavior assay).  (a) Arena

parts explosion, showing dimensions (lids of chambers not shown).  (b) Assembled

olfactometer showing placement of study organisms (not shown to scale) during trials;

side chambers were lined with vellum paper (not shown).  (c) Schematic of experiment-

station layout during trials, showing use of vacuum heads and flowmeters, and the

orientation of each olfactometer.  On each trial day, three such stations were set, for a

total of 12 simultaneous replicates.

On a given trial date, ten or twelve assays were run simultaneously in a dark room, and the start of the trial was timed to coincide with the beginning of the dark period of the greenhouse and laboratory incubator. In half of these assays, the prey chamber was provisioned with aphid prey (these spiders will be referred to hereafter as "aphid-prey spiders"), while the other half of assays were provisioned with springtail prey (referred to hereafter as "springtail-prey spiders") (Fig. 5.1b). Body mass for adults of the two prey is similar, so number of prey was standardised at forty adult individuals across both prey treatments; these numbers resulted in activity-densities comparable to measured activity-densities in the field. Following the 24-hour prey acclimation period, spiders were introduced into the central chamber of the arena by allowing them to lower from an aspirator via a silk line. Experimental observations were recorded immediately after all spiders had been placed, and again at 30-minute intervals throughout the nocturnal period (8 hours). At each observation, the following data were recorded for each spider: (1) location of the spider within the arena, with five locations recognized (prey chamber, non-prey chamber, central chamber and the two connecting tubes); (2) web status (whether or not the spider was occupying a web); and (3) presence or absence of prey in the spider's jaws. Airflow was also monitored during each observation, and maintained at $1.4 \pm 0.01$ L/min in all arenas.

A total of 94 spiders were used in these trials (47 spiders in each treatment). One aphid-prey spider was eliminated from analysis due to technical problems during the assay.

*5.3.4 Experiment 1: Statistical Analysis*

Experimental results were analyzed using the statistical software R (ver. 2.12.0). To test for effects of prey availability on foraging behavior, a generalized linear model was constructed using the *mlogit* package of R, with two indicator variables, (1) prey treatment and (2) the first chamber entered by the spider. Foraging-tactic utilization (trap-building vs. free-foraging) was the lone response variable. Only spiders that entered at least one side chamber during the trial period were included in analysis. Additionally, prey-consumption behavior was analyzed using multivariate analysis of variance (MANOVA), with total number of prey taken and average handling time as dependent variables, and prey treatment as the predictor variable.

*5.3.5 Experiment 2: Density-Dependent Effects of Alternative Prey*

Spider foraging-tactic utilization was investigated in the presence of multiple prey cues in greenhouse microcosm experiments. Microcosms for this experiment were constructed from plastic flower pots (11 cm diameter × 10 cm height) covered with 3.78-L paint strainers (Fig. 5.2). Each microcosm was filled with autoclaved, moistened potting soil to approximately 7.5 cm deep, and seven Pembroke wheat seedlings were planted in a row across the center of the microcosm. Microcosms were placed in BugDorm enclosures, and seedlings were allowed to grow for seven days prior to trials. I assigned microcosms to four prey treatments. Each treatment was provided 100 aphids, and either 0, 100, 300 or 500 springtails (designed "no-springtail," "low-springtail," "medium-springtail," and "high-springtail"). A fifth treatment with no aphids or springtails provided was used as a control.

To test the effects of prey treatments on prey activity-density, a calibration trial was conducted. In this trial, microcosms were placed in a greenhouse, and prey were introduced after seven days of wheat growth. After allowing prey a 24-hr acclimation period to settle on plants and burrow in the soil, a single sticky trap (surface area = 7.5 cm$^2$; as in Chapter 2) was placed at the base of the wheat seedlings in each microcosm, and left *in situ* for 24 hrs. Sticky traps were recollected after 24 hrs, and the number of prey was counted and averaged for each treatment. I expected the low-springtail treatment to yield an activity-density comparable to activity-densities collected in the field in Chapter 2. To my knowledge, such a density/activity-density calibration trial has never been conducted for the experimental study of spider foraging behavior.

### 5.3.6 Experiment 2: Protocols

For this assay, 3 replicates for each treatment were run simultaneously in the greenhouse. Wheat was allowed to grow for seven days, and prey were introduced on the eighth day. Prey were allowed a 24-hr acclimation period before spiders were introduced. One adult female *G. inornata* was introduced into each microcosm by aspirator, and allowed 48 hrs to construct webs and forage on prey. I documented web status at 24 hours and 48 hours. Web status, in this case, consists of three components: (1) whether or not a web was constructed; (2) whether the web was a probe web or sheet web; and (3) what structures the web was attached to (soil, wheat stems or walls of the pot). At 48 hrs, I also determined the surface area of the spider's web, by measuring the longest facial dimension and its perpendicular, and assuming an ellipse (as in Chapter 2). Finally,
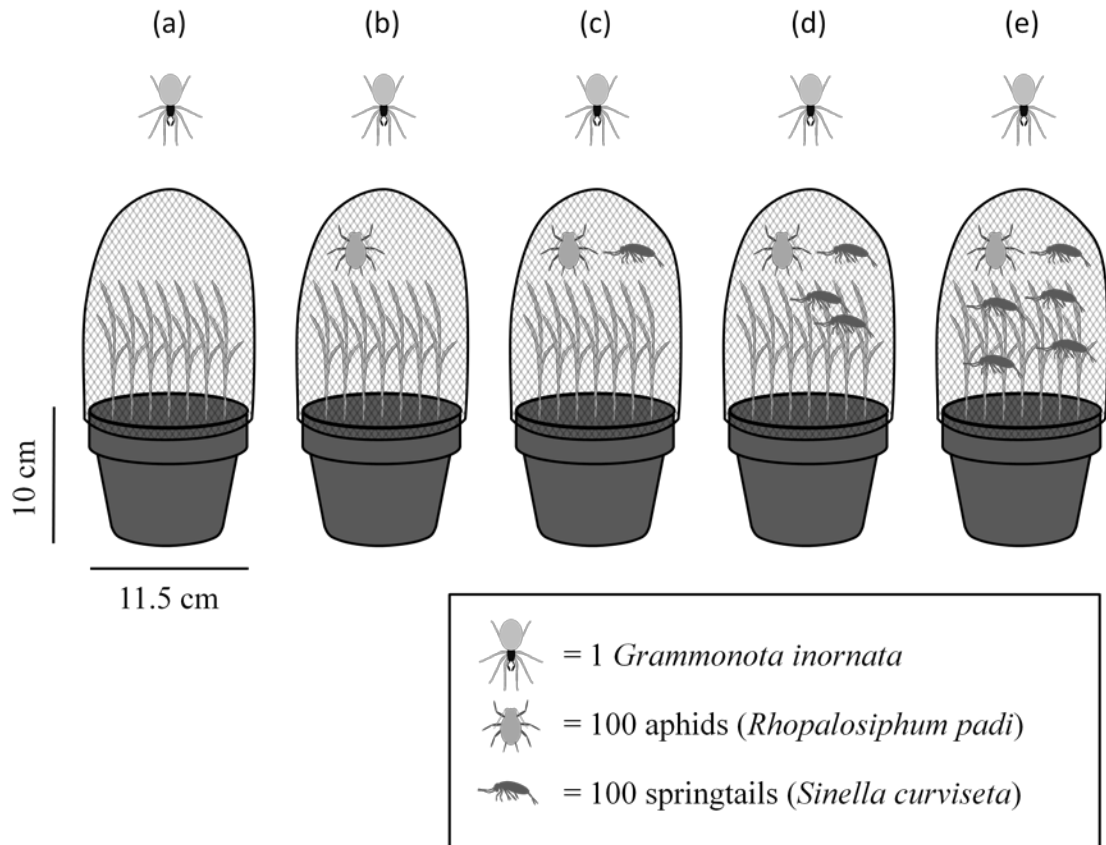
Figure 5.2 Setup of experiment 2 (Density-dependent effects of alternative prey), showing microcosm layout and treatment design. Treatments are (a) no-prey control, (b) no-springtail, (c) low-springtail, (d) medium-springtail, and (e) high-springtail.

I took the mass of all spiders immediately before and immediately after trials to evaluate spider weight gain in response to different prey treatments.

### 5.3.7 Experiment 2: Statistical Analysis

The primary quantities of interest in this experiment were foraging-tactic utilization (trap-building vs. free-foraging), change in spider body mass, and expected springtail catch. I calculated the expected springtail catch for each specimen by multiplying the surface area of the spider's web by the mean activity-density of springtails from the corresponding treatment in the calibration trial. I used general linear models and Fisher's exact test to examine key differences in behavioral variables.

### 5.3.8 Experiment 3: Alternative Prey and Pest Consumption

Aphid consumption by spiders was investigated under single-prey and mixed-prey treatments in a second greenhouse microcosm experiment. Microcosms were constructed from cylindrical, clear plastic containers (10.3 cm diameter $\times$ 12 cm height), with two, screen-covered vents drilled in the lid to allow air circulation and prevent overheating in the greenhouse environment (Fig. 5.3). Smaller holes were also drilled in the underside of the pots, to allow water drainage. A 3-cm layer of potting soil was placed in each microcosm, and a single, seven-day seedling of Pembroke winter wheat was transplanted into each microcosm 1 day before trials began. I used four prey treatments: springtails (*S. curviseta*), aphids (*R. padi*), mixed (aphids + springtails) and control (no prey). Treatments were laid out in an additive, factorial design: mixed-prey treatments contained twice as many total prey as the single-prey treatments (40 prey/species). This additive design allowed me to evaluate the effects of non-pest springtail prey without confounding effects from differences in prey density.

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On each trial date, twelve microcosms (three replicates of each prey treatment) were placed simultaneously in the greenhouse.  Prey were introduced 24 hrs after wheat seedlings were transplanted, and allowed a 24-hr acclimation period before spiders were introduced.  One adult female *G. inornata* was introduced into each microcosm, and recollected after 24 hrs.  Web status was recorded after 24 hrs, as in Experiment 2, and spiders were preserved in 95% ethanol for molecular analysis.

Protocols for molecular gut-content analysis followed Chapter 2, with the following exceptions.  Each spider was screened for the DNA of both springtails and aphids.  Primers used to detect springtail DNA were described in Chapter 2.  For detection of aphid DNA, *R. padi*-specific primers developed by Chen *et al.* (2000) were used.  This primer pair, BcoaCOIIF4 and BcoaCOIIR2, amplify a 148-bp amplicon of the *R. padi* COII mitochondrial gene.  Cross-reactivity testing revealed that this primer responds to some specimens of other aphid species as well (*Myzus persicae* and *Brevicoryne brassicae*); however, these species were not used in this experiment, and were not present in the greenhouse environment, so there was no danger of contamination. In this experiment, I ran 12.5-µL reactions, so reagent quantities were reduced (relative to Chapter 2) by one-half.  PCR cycling protocols for the springtail-DNA assays were identical to Chapter 2.  For the aphid-DNA assays, cycling protocols were 94° C for 1 min followed by 45 cycles of 94° C for 30 s, 55° C for 30 s and 72° C for 45 s.  Reaction success, in all cases, was determined by electrophoresis of 10 µL of PCR product on 3% SeaKem agarose gels stained with ethidium bromide.
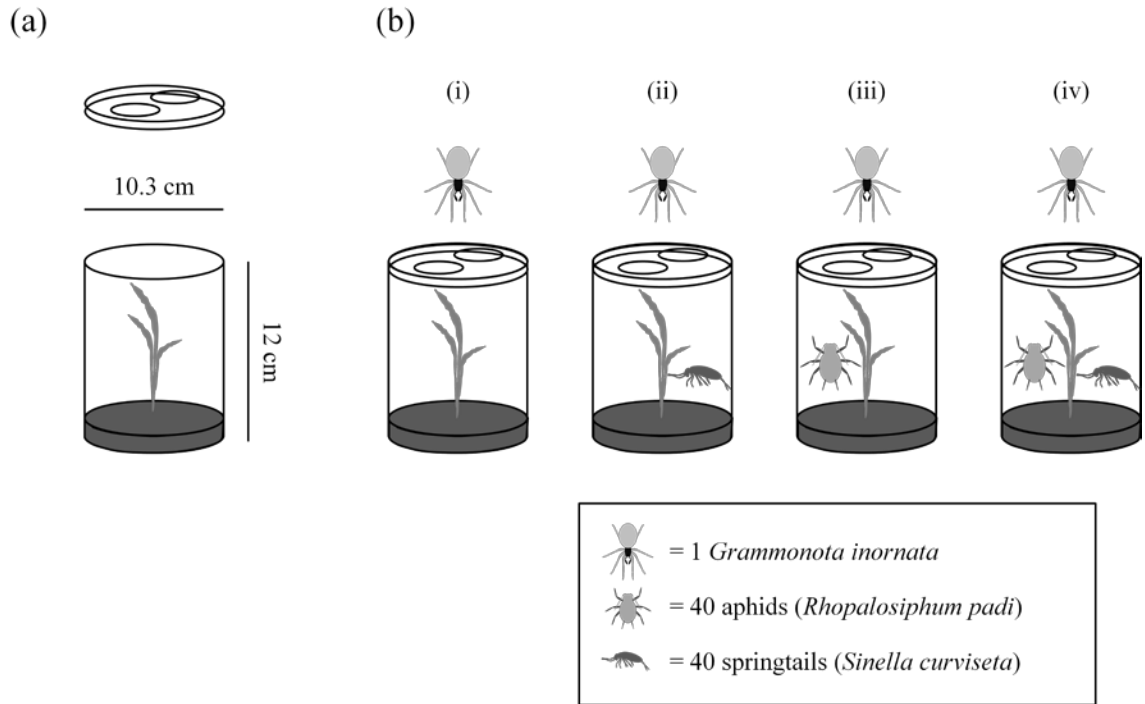
Figure 5.3 Setup of experiment 3 (Alternative prey and pest consumption). (a)

Microcosm parts explosion; (b) prey treatments: (i) control, (ii) springtail, (iii) aphid, (iv)

mixed.

## 5.4 Results

*5.4.1 Experiment 1: Prey-Specific Foraging Behavior*

In two-choice assays, spider foraging behavior was found to differ across prey treatments in a manner that is generally consistent with the results of Chapter 2. In a general linear model, foraging-tactic selection was not found to be significantly influenced by either prey treatment (coefficient = -1.79, $t_1$ = -1.67, p = 0.09) nor first chamber selected (coefficient = -0.63, $t_1$ = -0.69, p = 0.48) alone, but a significant interaction between prey treatment and first chamber selected was uncovered (coefficient = 3.87, $t_1$ = 3.02, p = 0.0025). Springtail-prey spiders were more likely to construct webs in the prey chamber than in the non-prey chamber (coefficient = 3.24, $t_1$ = 3.57, p = 0.0003), whereas aphid-prey spiders were not (coefficient = -0.63, $t_1$ = -0.69, p = 0.49) (Fig. 5.4). This supports the hypothesis that spiders use trap-building tactics to catch mobile springtails.

However, the model provides no indication that spiders behaved differently in response to aphids than in the absence of prey stimuli. Thus, from the model alone, it is uncertain that spiders responded to aphid prey at all. I therefore conducted binomial tests on each treatment group separately to test for effects of prey treatment on initial site selection, to determine whether spiders responded to aphid prey. Aphid-prey spiders were found to prefer the prey chamber in terms of initial site selection (p = 0.002), while springtail-prey spiders were not (p = 0.26) (Fig. 5.4a). Thus, while spiders did not utilize trap-building tactics to forage for aphids, they were responding to aphid prey while searching for foraging sites; so I can be confident that observed behaviors in response to

aphids represent a foraging effort. This confirms the hypothesis that spiders utilize different foraging tactics to capture aphid and springtail prey.

In addition, 65 predation events were recorded during trials (39 springtails and 26 aphids, by 18 and 17 individual spiders, respectively). Thirty-five of 39 captured springtails were captured in webs (90%), whereas 11 of 26 captured aphids were captured in webs (42%). Springtail-prey spiders that utilized trap-building tactics had a higher prey-capture success rate than spiders that used free-foraging tactics, or both springtail prey (17/21 vs 1/4). Aphid-prey spiders showed a similar trend (8/8 web-builders caught aphids vs 9/15 free-foragers). In total, 18 of 25 springtail-prey spiders (72%), and 17 of 22 aphid-prey spiders (77%) that entered the prey chamber successfully caught prey. The number of consecutive trial observations during which spiders were observed with a single prey item in the jaws was taken as a surrogate measure for the handling time for each predation event. In a MANOVA model, prey treatment was found to significantly impact prey-consumption behavior (Pillai's trace = 0.272, df = 1,33, p = 0.006): specifically, handling time was longer for aphids than for springtails ($F_{1,33}$ = 10.79, p = 0.0025), but average number of prey taken did not differ significantly between prey treatments ($F_{1,33}$ = 3.025, p = 0.091) (Fig. 5.5). This confirms that spiders foraged for and fed on both types of prey, and that prey-consumption behavior differs with prey type.
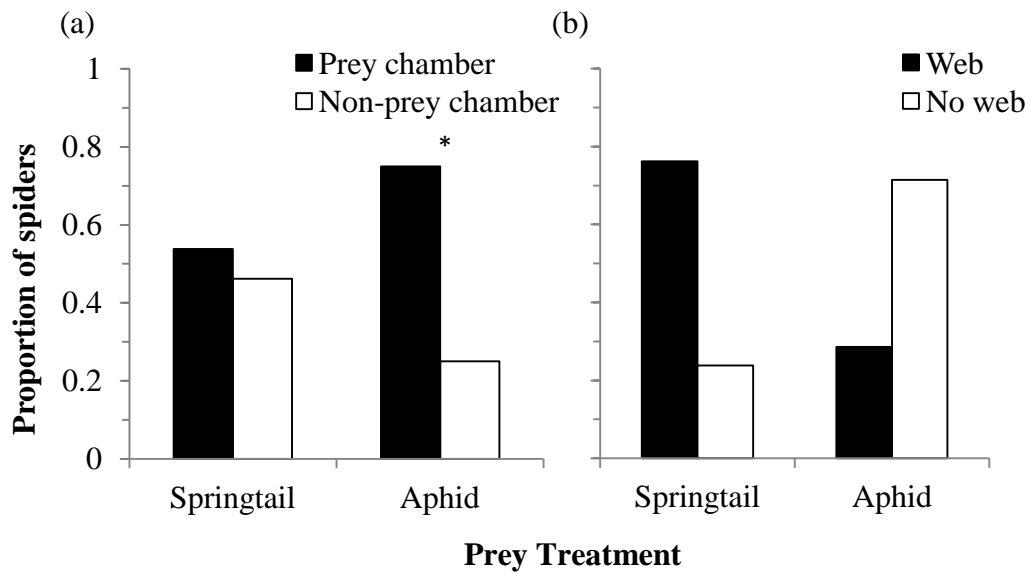
Figure 5.4 Experiment 1: Microhabitat utilization by spiders within two-way choice arenas. (a) first chamber (microhabitat) selected —asterisks represent significant differences as determined by binomial test; (b) web construction by spiders that had entered the prey chamber first; P-value based on comparison of log-odds ratios between prey.

*5.4.2 Experiment 2: Density-Dependent Effects of Alternative Prey*

In the second experiment, the effects of increasing springtail density on spider behavior were investigated. In calibration trials, the activity-density of springtails showed the expected positive relationship with springtail-density treatment ($F_{3,8} = 17.17$, $p = 0.001$, $R^2 = 0.866$) (Fig. 5.6). Mean activity-density of the low-springtail treatment was $0.8 \pm 0.08$ springtails/cm$^2$. This is comparable to activity-densities collected in alfalfa in Chapter 2 ($1.26 \pm 0.29$ springtails/cm$^2$ in simulated *T. formica* microhabitats on the soil surface, and $0.60 \pm 0.11$ springtails/cm$^2$ in simulated *G. foxi* microhabitats 1 cm above the soil surface; Fig. 2.5). It is also comparable to springtail activity-densities collected in a neighboring wheat field ($0.65$ springtails/cm$^2$; Chapman *et al.* 2013) in the same year. Aphid activity-density was not found to vary with springtail density ($F_{3,8} = 1.46$, $p = 0.298$, $R^2 = 0.353$), so I am confident that any confounding effects of behavioral interactions between the two prey types were minimal.

In microcosm assays, prey treatment had a significant effect on foraging-tactic utilization (Fisher's exact test: $p = 0.0009$; Note: all tests in this experiment evaluated at a Bonferroni-corrected $\alpha = 0.006$) (Fig. 5.7). Spiders in the medium-springtail treatment used trap-building tactics most frequently (i.e., 100% of spiders constructed webs), while the treatments without springtails were least likely to construct webs (33% and 67%, for the no-prey control and no-springtail treatment). However, there was no effect of prey treatment on proportional change in spider body mass ($F_{3,30} = 1.30$, $p = 0.294$, $R^2 = 0.115$). In a general linear model, I observed that the expected springtail catch increased with springtail density ($F_{4,48} = 11.9$, $p = 0.001$). In pairwise comparisons, the expected springtail catch did not differ significantly between medium-springtail and
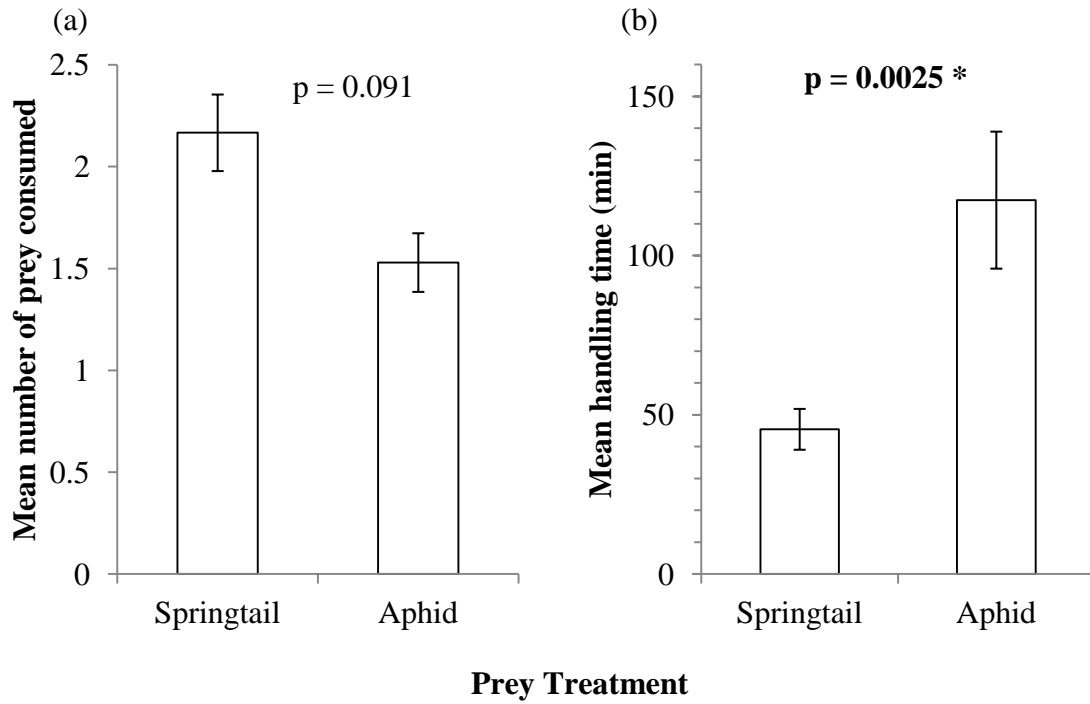
Figure 5.5  Experiment 1: Mean number of prey consumed and mean handling time of observed predation events during two-way choice trials.  P-values determined from univariate F-tests in a MANOVA model.
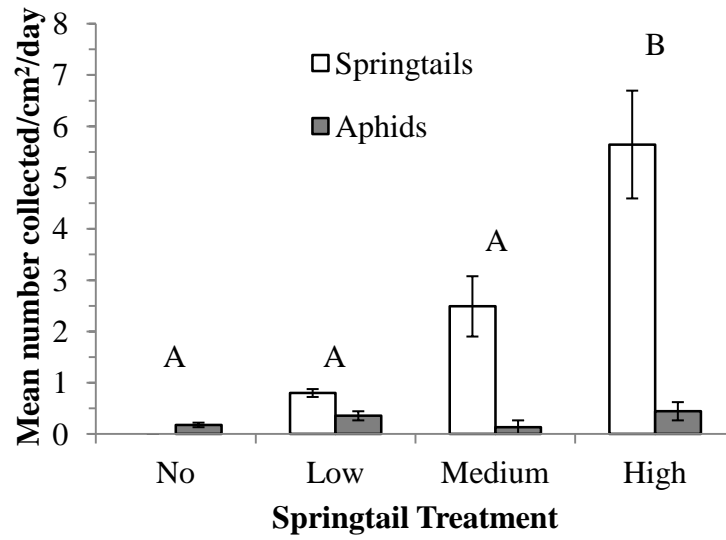
Figure 5.6  Experiment 2: Density/activity-density calibration trials.  Prey activity-densities collected by sticky trap, across prey-addition treatments.  Letters represent significant differences among springtail activity-densities, as determined by Tukey's HSD test.  Aphid activity-densities did not differ significantly between treatments.
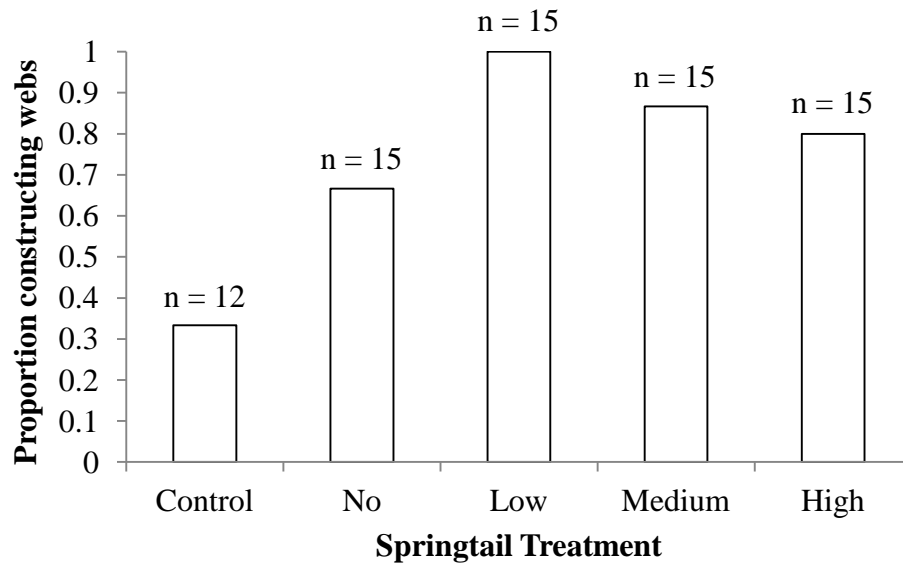
Figure 5.7 Experiment 2: Proportion of spiders constructing webs across springtail-density treatments in behavioral microcosm trials. All treatments except the control treatment contained low aphid densities. Total sample size appears above each bar.

high-springtail treatments (Tukey's HSD test: $t_{2,48} = 1.38$, $p = 0.64$), but did differ

significantly between other treatments (Fig. 5.8a). This is potentially explainable by an

observed trend in web surface area: mean web surface area was consistently between 11-

13 $cm^2$ across no-springtail, low-springtail and medium-springtail treatments, but

dropped to approximately 7 $cm^2$ in the high-springtail treatment (Fig. 5.8b).

*5.4.3 Experiment 3: Alternative Prey and Pest Consumption*

In the third experiment, I investigated the effects of non-pest springtails on spider

consumption of pest aphids. In a logistic regression model, the proportion of spiders

testing positive for aphid DNA within their gut contents did not differ significantly

between the aphid-only and mixed-prey treatments ($t = 0.883$, $p = 0.38$) (Fig. 5.9). This

indicates that spiders will still consume aphids at a high rate, even when higher-quality,

non-pest springtail prey are available, and provides no support for the zero-one rule of

OFT.

However, spiders tested positive for springtail DNA at low rates in treatments in

which no springtails were added (Fig. 5.9). This is attributable to contamination from the

greenhouse environment. I observed a population of entomobryid springtails (not *S.*

*curviseta*) in the greenhouse during these trials, and these springtails routinely took up

residence beneath the experimental microcosms. These springtails were undoubtedly

able to infiltrate the microcosms through the drainage holes on the underside of the

container. Despite the contamination, however, springtail consumption by spiders

differed significantly between treatments with and without *S. curviseta* added (Fig. 5.9),

so I am confident that this contamination did not have any undue influence on my results.
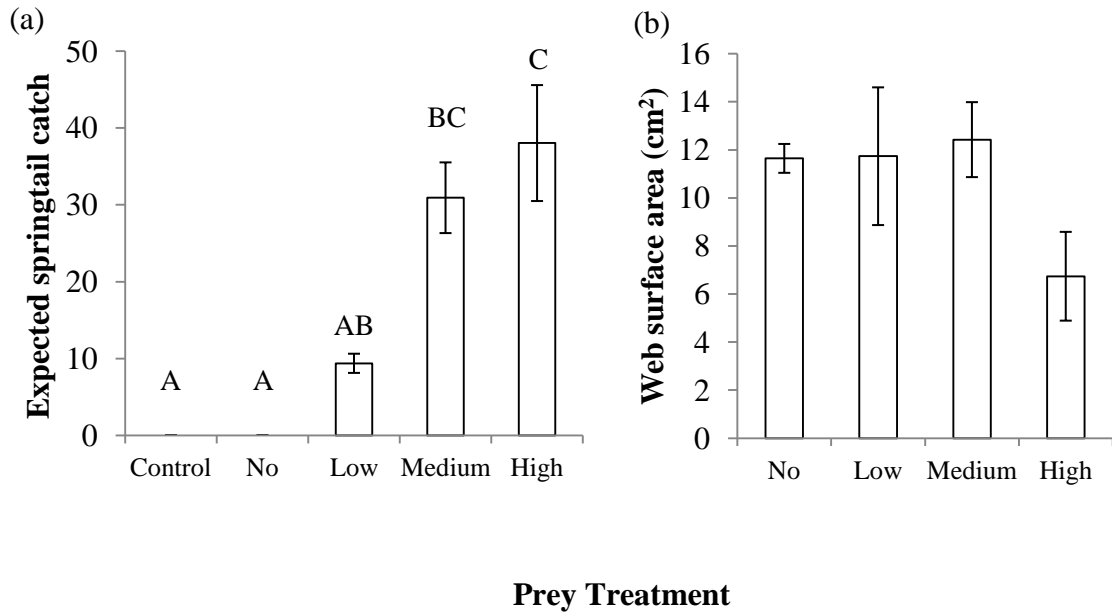
**Prey Treatment**

Figure 5.8  Experiment 2: Web construction and foraging.  (a) Mean expected number of springtails caught by spiders in 24 hrs, calculated from the web surface area of each specimen, and the mean activity-density of springtails in each treatment from calibration trials.  (b) Mean surface area by treatment (the control treatment is excluded because all webs in the control were probe webs).  Letters represent significant differences according to Tukey's HSD post hoc tests.
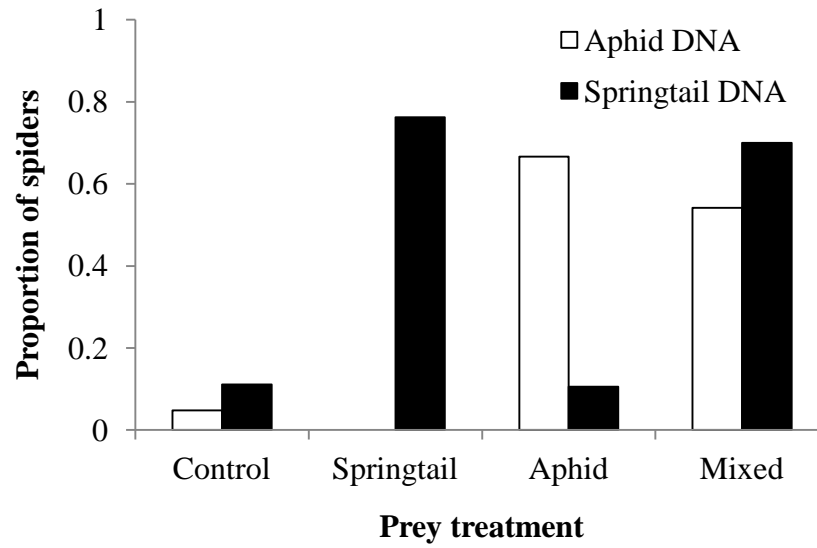
Figure 5.9  Experiment 3:  PCR gut-content assays.  Proportion of spiders in microcosm

trials that tested positive for springtail and aphid DNA in gut-content samples, across four

prey treatments.

Foraging-tactic utilization by spiders in experiment 3 was inconsistent with observations in the previous two experiments. Spiders readily constructed webs in all treatments (Fig. 5.10). It is possible that contamination of the treatments by springtails from the greenhouse environment contributed to the lack of difference in foraging-tactic utilization between treatments, as spiders in aphid-only treatments still had access to sensory cues from springtails. In treatments with no aphids present, spiders attached webs to the soil surface and to the microcosm walls; however, when aphids were present, a minority of spiders (18% in the aphid-only treatment, and 22% in the mixed-prey treatment) also attached webs to the wheat seedling (Fig. 5.10), indicating that the selection of attachment structures may be influenced by the availability of prey.

### 5.5 Discussion

In experimental assays, I observed prey-specific foraging behaviors in web-building spiders. In two-way choice assays, there is a two-phase process in spider responses to prey: first, spiders select microhabitats in which to forage; and second, spiders select a foraging tactic to utilize within the microhabitat. This confirms my findings in Chapter 4, that spider foraging behavior consists of a series of decision-making steps, which allows spiders to forage flexibly in response to importants cues in the environment. In this chapter, I uncovered evidence of flexibility in response to different types of prey. In two-way choice assays, spiders responded to aphid prey while searching for a microhabitat, but, upon arrival in an aphid-rich microhabitat, did not utilize trap-building tactics to capture the aphids.
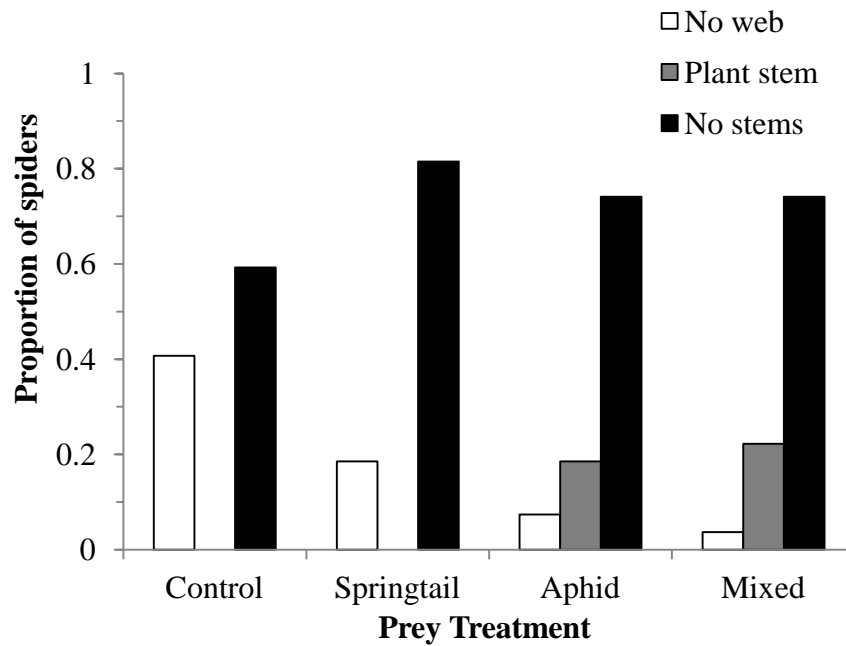
Figure 5.10  Experiment 3: Web-construction behavior by spiders in single-prey and mixed-prey treatments.  "Plant stem" refers to spiders that constructed webs, using plant stems as attachment points, while "no stems" refers to spiders that constructed webs without attaching to plant stems.

In contrast, spiders showed no response to springtails during the microhabitat-selection phase, but constructed webs upon locating a springtail-rich microhabitat. I am confident that the lack of web construction by aphid-prey spiders does not indicate a lack of foraging response to aphid prey, because these spiders still preferred the prey chamber over the non-prey chamber (Fig. 5.4). The difference in web-construction behavior therefore indicates a difference in foraging tactics, rather than a difference in prey preference. Thus, it is clear that web-building spiders forage flexibly, opting to switch foraging tactics from trap-building to free-foraging when different prey stimuli are detected within the environment.

Additionally, prey-consumption behavior differed with prey type: springtails were handled and consumed more rapidly than aphids (Fig. 5.5). Given the documented importance and nutritional value of non-pest springtails for spiders (Bilde *et al.* 2000; Harwood *et al.* 2004; Opatovsky *et al.* 2012), it is possible that spiders have specialized behaviors for efficiently handling and consuming this type of prey, but use less efficient, generalized behaviors for consuming aphids (cf. Huseynov *et al.* 2008). Indeed, I have observed that colony spider responses to springtail prey are more rapid and more uniform than responses to aphid prey (Welch, personal observation), suggesting that spiders do have a more specialized, efficient attack behavior for springtails. However, despite the importance of springtails and potential presence of specialized attack and handling behaviors, preference for the prey chamber was not clearly observed in springtail-prey spiders, but was observed in aphid-prey spiders (Fig. 5.4). There are three possible explanations for this. First, the vertical position of the prey within the prey chamber (epigeal versus foliar) may have influenced the transmission of olfactory signals and led

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to greater detectability of aphids in our assays. However, in a similar experiment in Chapter 4, M*ermessus fradeorum* did respond positively to springtail prey; so the arena is capable of effectively transmitting springtail cues. Second, herbivore-induced plant volatiles may have added a supplemental signal and elicited a greater response from aphid-prey spiders than from springtail-prey spiders (cf. Kessler & Baldwin 2001; Verheggen *et al.* 2008). However, this still does not explain the lack of response to springtail cues. Finally, it is possible that spiders sought out aphid prey specifically. Springtails are relatively more available to epigeal web-building spiders (Harwood *et al.* 2001; Romero & Harwood 2010; Chapman *et al.* 2013) than are aphids. Greater behavioral response to the signals of rare aphid prey may thus suggest a strategy of dietary diversification, which is suggested by previous studies (Toft 1995; Chapman *et al.* 2013). Feeding trials conducted by other researchers suggest that web-building spiders that incorporate a pest prey (aphids or planthoppers) to a diet of non-pest prey (springtails or fruit flies) experienced increases in fitness (Toft 1995; Sigsgaard *et al.* 2001), leading to further support of the dietary-diversification hypothesis. This explanation is also in line with the results of experiment 3, in which aphid consumption remained high, despite the availability of high-quality, springtail prey (Fig. 5.9). Therefore, I conclude that spiders actively diversify their diets, incorporating both pest and non-pest prey, to improve fitness.

The lack of a searching response to springtail cues found in this chapter contrasts sharply with the results of the previous chapter, in which *M. fradeorum* responding to springtial cues showed clear preference for the prey chamber over the non-prey chamber (Fig. 4.2). It is therefore possible that these two species utilize different foraging tactics.

Throughout laboratory work with these two species, *M. fradeorum* was a much more active and fast-moving spider, while *G. inornata* was very sedentary and slow-moving (personal observation). A comparison of behavior in these two olfactometer experiments shows that *M. fradeorum* moved around the apparatus constantly throughout the trial period, and consequently frequently spent only part of the total trial period in one chamber; while *G. inornata* tended to remain in the first chamber entered for the rest of the trial period, resulting in a bimodal distribution of time spent in one chamber (Fig. 5.11). Thus, even within this single subfamily (Linyphiidae: Erigoninae), spiders can display a diversity of foraging behaviors and tactics.

Even though *G. inornata* showed much more static, sedentary behavior in two-way choice assays than *M. fradeorum*, the species' behavior still demonstrated considerable flexibility in no-choice assays. Spiders tailored web-construction decisions to match the availability of prey in the microhabitat they were forced to occupy. In Experiment 2, spiders constructed webs more frequently when springtails were at high densities than when they were not present in the microhabitat (Fig. 5.7). In Experiment 3, spider webs were only attached to plant stems when aphids were present, possibly to increase the likelihood of capturing aphids that fell from the foliage (Fig. 5.10).

It is possible that the differential responses to pest and non-pest prey uncovered in Experiment 1 were due to differences in the types of sensory signals transmitted by each prey type. Spiders are known to respond to prey cues using both olfactory and seismic/vibrational sensory modalities (Pruitt *et al.* 2011; Blamires *et al.* 2011; Johnson *et al.* 2011). Both types of signals were available to spiders in my experimental arenas. However, the generation of seismic cues is undoubtedly correlated with the activity level
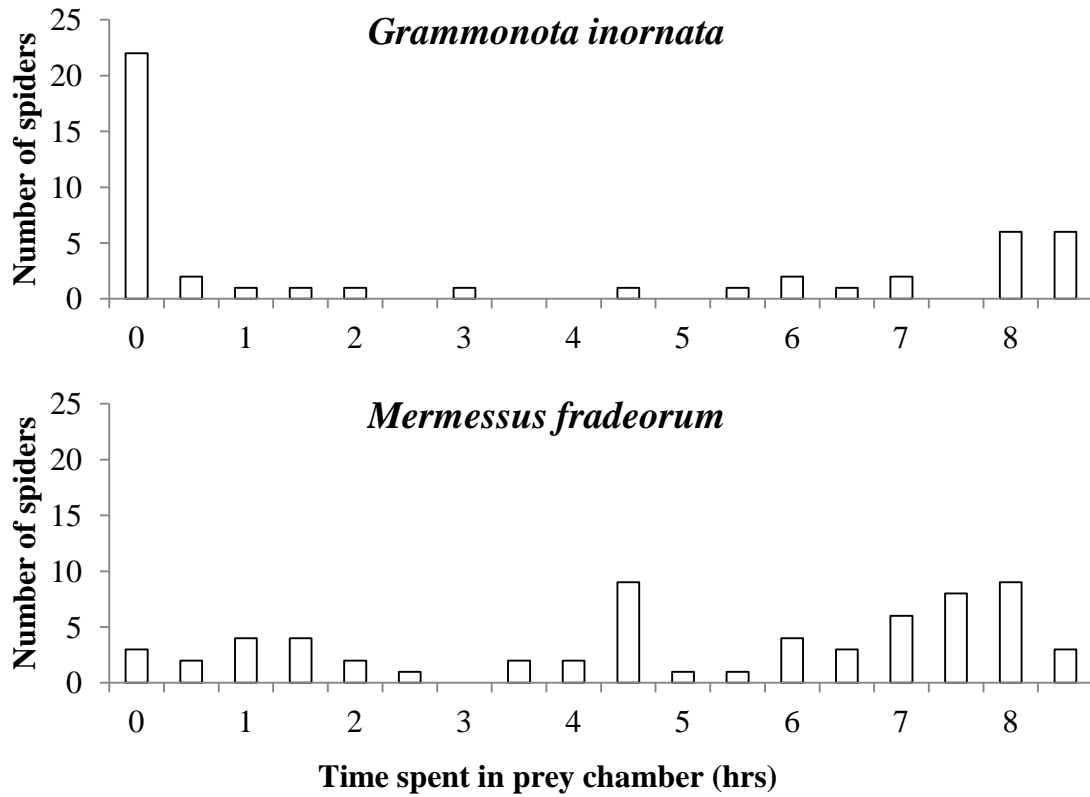
Figure 5.11  Comparison of *Mermessus fradeorum* and *Grammonota inornata* foraging

behavior in two-way choice assays, from Chapters 4 and 5 (respectively).  Only *G.*

*inornata* with springtail prey are included here.

of the prey, and this correlation may thus serve as an important cue in spider foraging-mode selection. Mobile, epigeal prey, such as springtails, may transmit more seismic signals, which serve as a stimulus for web-building behaviors (cf. Blamires *et al.* 2011), whereas sedentary aphids produce fewer seismic signals, and thus, do not illicit the same behavioral response. Thus, a predator's behavioral responses to sensory cues can potentially influence its foraging ecology.

The utilization of distinct foraging tactics to catch pest and non-pest prey has significant implications for food webs in agroecosystems, and for biological control. Optimally foraging predators can be expected to utilize tactics that maximize capture rates of the highest-quality prey item, and to ignore prey of lesser quality. As the nutritional quality of aphids for many generalist predators is known to be quite low (Toft 1995), OFT predicts that generalist predators will not consume aphids in the presence of high-quality, non-pest prey, such as springtails. For web-building spiders, who feature stark differences in foraging tactics when foraging for aphids and for springtails, a zero-one rule is expected, because the tactics used to capture non-pest prey may not be compatible with pest consumption. In this scenario, high availability of alternative prey would create a behaviorally-mediated disruption of biological control. However, the results of Experiment 3 suggest that trap-building tactics are compatible with aphid consumption, and show no support for a zero-one rule: spiders clearly continue to consume aphids, even when springtails are readily available (Fig. 5.9). Aphids are known to drop from their host plants in response to predation risks (Losey & Denno 1998b; Losey & Denno 1998a), and this is often the manner in which they are likely to become available to epigeal predators, such as linyphiid spiders (Losey & Denno 1998c;

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Kerzicnik *et al.* 2010).  In my two-choice assays, aphid-prey spiders that constructed webs suffered no apparent reduction in aphid-capture success.  Indeed, one experimental spider web was found to have collected six aphids within half an hour of its initial construction.  Thus, sheet webs can, in fact, intercept falling aphids at high rates.  Furthermore, regardless of whether the spider goes on to consume the aphid in its web, undoubtedly many of these aphids will fail to escape the web.  Therefore, spiders can be expected to contribute to the suppression of aphids.

However, caution should be exercised when interpreting these results: as stated in Chapter 2, molecular gut-content assays are only capable of determining the presence of prey DNA, and cannot determine how many prey were eaten.  Although there was no significant difference in percent-positive for aphid DNA in experiment 3, it is possible that spiders in the aphid-only treatment consumed more aphids than spiders in mixed-prey treatments.  I also do not know at what point during the trial period the spiders constructed their webs, and I cannot determine what tactics they utilized to capture the aphids they consumed.  I therefore cannot be certain that the presence of springtails had no effect on aphid consumption rates.  However, given the results of these experiments, I have no reason to suspect that aphid suppression would be diminished in the presence of springtails.  And, at the very least, I can be confident that there is no zero-one rule in operation here: spiders do not immediately drop aphids from their diet when springtail prey are present.

For web-building spiders, foraging-tactic selection is closely tied to habitat usage, and thus, can dramatically impact, not only prey consumption, but also spider interactions with their environment.  Therefore, due to the flexible nature of their foraging behavior,

the functional position of these spiders within food webs and interactions webs can potentially be variable, because they use two very distinct types of foraging tactics. Flexibly-foraging predators with only subtle differences between foraging tactics, such as distinct attack sequences for specific types of prey (e.g., Huseynov *et al.* 2008), but homogeneous prey-searching behaviors, may have less variable functional positions within their food webs than these spiders.  However, for many predators, prey-specific shifts in foraging tactics would undoubtedly alter habitat use and the relative magnitudes of various trophic links (Krivan & Schmitz 2003).  Additionally, while foraging tactics may alter a predator's interaction with its habitat, the reverse is also true: interaction with the habitat can also alter the success rates of predator foraging (Denno *et al.* 2005), and thereby modify the behavior of flexibly-foraging predators.  Many habitat-management techniques, such as weed strips and mulch additions, aim to increase natural enemy density by altering the structural complexity of the habitat (Landis *et al.* 2000).  While studies that use these techniques are often very successful at increasing natural enemy densities, they rarely uncover a concomitant increase in biological control services (Griffiths *et al.* 2008).  It is possible that behavioral dynamics, such as the prey-specific foraging tactics uncovered in the present study,  account for many of these negative observations.  However, few studies have attempted to examine the effects of habitat structure on the behavior of flexibly-foraging predators.  As structural complexity is known to be an important factor in habitat use by many natural enemies, including spiders, beetles and hymenopterans (Sunderland & Samu 2000; Langellotto & Denno 2004), further research into these systems is also needed to understand the interactions between habitat structure and predator behavior.

In conclusion, I observed differential foraging responses of a natural enemy to a pest prey and a non-pest prey with different activity levels and life histories, which indicates that flexible foraging behaviors may play a major role in natural enemy food webs.  I have found no evidence that prey-specific foraging tactics have a negative impact on biological control, but this work is only the first step in unraveling the roles of flexible foraging in pest suppression by generalist predators.  The potential implications of this type of prey-specific, flexible foraging behavior for biological control are quite large, and further studies into natural enemy foraging behavior are needed to determine the impacts of flexible foraging on pest consumption.

# CHAPTER 6: SYNTHESIS

In this dissertation, I have examined web-building spider behavior as a model system for biological control by generalist predators. I have uncovered several key variables that influence microhabitat-utilization and web-construction decisions by web-building spiders. The ecology of a spider revolves around its web, so variables that influence web construction and placement drive the ecology of the spider, and strongly constrain its ability to provide beneficial biological control services in agroecosystems. The spider web therefore serves as an excellent metric of spider ecology and behavior, and spiders can provide valuable insights into the importance of predator behavior in biological control.

I observed that web construction by epigeal, web-building spiders is correlated with a variety of ecological factors. Most importantly, I observed a clear effect of sensory cues from prey on spider behavior. Both pest and non-pest prey influenced spider foraging behavior, and they had different influences. Spider foraging behavior proved to be highly flexible, using different foraging modes and ongoing evaluation of relevant cues to regulate resource investments to match the quality of the microhabitat. However, I also observed that spiders consumed pest prey at high rates, even when the availability of high-quality alternative prey influenced foraging behavior. This indicates that spiders either forage indiscriminately on any prey that land in their webs, or actively diversify their diets by consuming a range of prey. In light of the evidence I have collected, I suggest that dietary diversification is the more likely explanation: spiders in my experiments responded positively to both types of prey and readily consumed both in treatments where prey activity was high enough to allow discriminate foraging.

Spiders with different types of webs showed differential dependence on the availability of prey, which not only indicates variation in foraging behavior, but also, variation in the ecological implications of those behaviors. Sheet-weaving spiders captured springtail prey at a lower rate than orb-weavers, despite utilizing microhabitats with higher springtail prey availability. This is probably due to the lower prey-capture efficiency of the webs they construct. Because of their relatively inefficient exploitation of microhabitats, sheet-weavers are highly constrained by microhabitat quality, and showed indication of elevated intraspecific competitive interactions as a consequence. These effects can have important implications for the biological-control capacity of each type of spider, and also showcase the ecological diversity of generalist predators.

These two types of web-building spiders are limited by different factors, and thus, likely cannot be managed by a simple management scheme. For example, sheet-weavers, who will be more limited by springtail density, may benefit more from management practices that seek to augment springtail populations through detrital subsidies. Such tactics may improve biological control of aphids by these spiders, because springtails may improve nutrition for spiders, but do not apparently diminish aphid consumption. In contrast, orb-weavers, who are able to secure a larger food supply at current springtail densities, will be unlikely to respond significantly to detrital subsidies, but may respond to manipulation of some other habitat feature, such as structural complexity.

Many of the basic principles of Optimal Foraging Theory can be applied to web-building spiders. However, these principles may be best applied to variables associated with the search for microhabitats and the construction of webs, rather than to the search for and capture of prey directly. Additionally, flexible and state-dependent foraging have

shown that the zero-one prediction of OFT is unrealistic: spiders forage on prey of varying quality, and do not adhere strictly to a prey-quality ranking to determine which prey will and will not be consumed.

From this example predator, web-building spiders, it is clear that the behavior of generalist predators can impact their potential roles in biological control. The very high diversity among these predators suggests that no one habitat management strategy could simultaneously facilitate the biological control activity of the entire community. It is thus critical for researchers to identify the "right" diversity of predators for biological control, in order to determine which management strategies will be most productive. Unfortunately, this is not an easy task: it requires considerable investment of research effort toward understanding the behavior of a range of natural enemies.

As I have shown in this dissertation, there can be considerable ecological variation among and within predator taxa, and a manager is therefore required to learn a great deal of information about his or her specific community of predators before an effective decision can be made about which management strategy to pursue. My research also suggests that knowledge about the behavior and ecology of one predator is not necessarily transferrable to another. Consequently, the insights I have gained into microhabitat utilization by web-building spiders may be specific to this group of predators, and may be less meaningful for predators that do not show the same, intimate association with their habitats. Therefore, care must be taken in extrapolating any conclusions derived from an example organism. Nevertheless, many recent works (including this dissertation) have shown that the generalized principles developed for active-foraging predators also apply to sedentary predators, such as web-building spiders.

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For example, spiders do regulate their foraging efforts to maximize nutritional intake, in partial accordance with the principles of OFT. I therefore fully expect that other common types of agrobiont predators, such as carabid beetles, various predatory Hemiptera, and wolf spiders, will follow similar patterns of behavior: they will regulate their foraging behavior in ways that will maximize the nutritional rewards, and care will be needed in determining how this behavior will impact the biological control services they provide. Many of the salient factors for web-building spiders will be comparable for other predators, as well: prey cues, structural features, competition avoidance, etc. I thus argue that my results can be meaningfully applied to a wide range of taxa. From this, I can justifiably conclude that web-building spiders are suitable as behavioral models for generalist predators in biological control, because the principles governing trap-building predator behavior are comparable to the principles governing active-foraging predator behavior, and because trap-building predators offer considerably clearer metrics for quantifying important variables. I was able to identify many important attributes of linyphiid spider ecology with high precision due to the highly quantifiable nature of web-building spider behavior. The principles behind these behavioral and ecological dynamics are transferrable among predators, but it will still be necessary to determine, for each predator, which characteristics and which metrics are most appropriate for evaluating the operation of these basic principles. Therefore, further research into the behavior of natural enemies, and its potential influences on larger-scale ecological dynamics, and on biological control, is needed.

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- Chapman EG, Schmidt JS, **Welch KD**, Harwood JD (2013, in press) Molecular evidence for dietary selectivity and pest suppression potential in an epigeal spider community in winter wheat. *Biological Control*.
- **Welch KD**, Pfannenstiel RS and Harwood JD (2012) The role of generalist predators in terrestrial food webs: lessons for agricultural pest management. In

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## Awards and Honors:

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- 36th American Arachnological Society Annual Meeting, Student Paper Competition, Runner-up (2012). $50
- University of Kentucky Graduate School, Student Travel Grant (2010). $800
- International Society of Arachnology, Congress Support Program (2010). Title: "The role of web placement in structuring linyphiid spider communities and food webs." $250
- Tracy Farmer Center for the Environment, Karri Casner Environmental Sciences Fellowship (2010). Title: "The impact of microhabitat-selection on dietary intake by web-building spiders." $1000
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## Society Memberships:

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