PLANT NECTAR CONTRIBUTES TO THE SURVIVAL, ACTIVITY, GROWTH, AND FECUNDITY OF THE NECTAR-FEEDING WANDERING SPIDER *CHEIRACANTHIUM INCLUSUM* (HENTZ) (ARANEAE: MITURGIDAE)

DISSERTATION

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By

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ABSTRACT

Spiders are valued for their predation of insect pests, and, evaluated as an “assemblage” of species that employ different predatory strategies, constitute a natural biological control, particularly in agricultural crops. Spiders are obligate carnivores, requiring prey for normal growth, development, and reproduction. Because biologists have worked under the assumption that spiders are exclusively carnivorous, studies of the ecology of spiders and their acquisition and allocation of energy have assumed that prey is the single object of any spider’s foraging. The discovery in 1984 that orb-weaving spiderlings benefited nutritionally from pollen grains incidentally trapped by their webs, which they eat and recycle, was noteworthy.

Growing evidence indicates that a large group of spiders may routinely exploit another plant-based food source: plant nectar. Observations of nectar feeding have been reported among crab spiders (Thomisidae), jumping spiders (Salticidae), and running spiders (Anyphaenidae, Clubionidae, and Corinnidae), all non-webbuilding wanderers that occupy vegetation. Spiders have the capacity to detect and digest plant nectar, and spiders that wander in vegetation are able to encounter nectar. Lab experiments show that newly-emerged, prey-deprived spiders live longer if they are provided with sucrose, a nectar proxy. My laboratory experiments show that plant nectar contributed significantly to the survival of newly-emerged *Hibana velox* Becker (Anyphaenidae), and to the survival, growth, activity, and fecundity, of *Cheiracanthium inclusum* (Hentz) (Miturgidae, formerly in Clubionidae). Both are highly active nocturnal running (also called "sac") spiders. Experiments with *C. inclusum* measuring nighttime activity, and measuring the effects of adding plant nectar to varying levels of prey, the eggs of *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), suggest that the simple sugars of plant nectar fuel the spider's energetic activity, allowing the nutritional benefits of prey to be allocated to growth and reproduction. Spiders receiving a marginal amount of prey, for example, were able to mature and reproduce only if they also received plant nectar. During two successive growing seasons, nearly one third, and one quarter (respectively) of wandering spiders sampled from nectar-producing cotton plants tested positive for fructose, a plant-derived sugar. Pilot studies also showed increased survival and molting in *C. inclusum* provided with honeydew.

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CHAPTER 1

SPIDER NECTARIVORY: A RECENTLY RECOGNIZED NATURAL PHENOMENON

Spiders are distributed all over the world in nearly every ecological environment (Foelix 1982), even on Mt Everest at 22,000 feet (Gertsch 1949). Spiders are valued for their preying on insect pests, and, evaluated as an "assemblage" of species that employ different predatory strategies, constitute a natural biological control in agricultural crops. Spiders are such reliable predators because they are all obligate carnivores, requiring prey for normal growth, development, and reproduction (Turnbull 1973). These prey usually take the form of insects, but spiders eat other arthropods and, often, other spiders.

Biologists presume spiders to be *exclusively* carnivorous, such that any evidence to the contrary is noteworthy. Smith and Mommsen (1984), for example, discovered that some temperate-zone spiderlings gain nutrition by coincidentally eating the pollen grains that get caught in their webs, which they eat and recycle. Such a passive form of nutritional gain, note the authors, can be offset by fungal spores that are also trapped and eaten, some of which can be mildly toxic. Other exceptions to exclusive carnivory are in the anecdotal or experimental spider literature: Dondale (1965) observed a crab spider drinking fluid from its cast-off leg right after molting, Decae (1986) fed a theraphosid banana and sausage, Bonnet (1924) fed local spiders meats and fruit preserves, all of which the spiders ate. Artificial diets have been attempted for lab rearing (Peck &

Whitcomb 1968; Amalin et al. 1999) with limited success. Still, what spiders actually eat in nature remains the larger interest of biologists. Exclusive carnivory remains the presumed norm, and as generalist predators presumed to require the stimulus of live (mobile) prey, spiders continue to receive attention when they scavenge (immobile) arthropod eggs (Buschman et al. 1977; Nyffeler et al. 1990; Miliczky & Calkins 2002), scavenge dead prey (Foelix 1982; Riechert & Harp 1987; Wise 1993) or actually prefer dead prey (Sandidge 2003)—all variations of carnivory, nonetheless.

There is, however, one natural, nonproteinaceous food that has been reported more than once in the spider literature and for large families of spiders. That food is plant nectar. The consideration of plant nectar as a nutritive source for spiders is so recent that current research has gone little beyond field observations. In fact, the first suggestion of spiders benefitting from nectar came not from observation but from speculation. Vogelei and Greissl (1989) suggested nectar feeding as an important stopgap in the lives of spiders when they observed newly-emerged, unfed crab spiders *Thomisus onustus* Walckenaer (Thomisidae) surviving much longer than what their egg yolk reserves should allow. The authors surmised that the spiderlings must be filling their nutritional void with nectar and pollen, although they never observed it. In their laboratory experiments, spiderlings deprived of prey but with continuous access to a 30% sucrose solution (to imitate floral nectar) lived about four months—three, and six times as long as spiderlings maintained on pollen only or water only, respectively. The first actual observations of spider nectar feeding in the field (detailed below) were not reported until nine years later (Pollard et al. 1995). This dissertation is the first investigation of what a spider can gain from plant nectar in terms of its survival, growth and reproduction. If the gain is substantial, and spider nectar feeding more than a rare occurrence (both hypotheses of this dissertation), then some basic assumptions of spider ecology may change. For example, spiders are thought to be naturally prey dependent and often food limited, some often starving (Anderson 1974; Nentwig 1986; Nyffeler et al. 1987). Spiders that feed on nectar, however, expand their diets a trophic level and become consumers of a primary producer product, which is naturally more abundant than the insects (prey) that consume it. If nectar is a food supplement, or even a stopgap for starvation, then some spiders are far less food limited than thought. From an applied point of view, nectar feeding might help explain the different assemblages of spiders in different crops (Young & Edwards 1990). Nectar feeding may also lend valuable behavioral information to the concept of spider guilds, in the development of which some researchers have used a taxon-based approach and others have used ecological characteristics, such as where (e.g., foliage, ground) and how (e.g., running, stalking, ambushing) a spider hunts prey (Uetz et al. 1999).

My experiments to measure the nutritional contributions of nectar were precipitated by my own observations of spider nectar feeding beginning in 1984. Nighttime collections of nectar-feeding mosquitoes in Costa Rica led me to the spiders. The mosquitoes were feeding on the floral nectaries of the pantropical Indian almond, *Terminalia catappa* L., a common beachfront tree. The "flowers" of the tree are actually spiked inflorescences, each bearing hundreds of tiny shallow, white florets. The mosquitoes probed each floret with their needle-like proboscides for floral nectar. Wandering among these florets, racing up and down the entire flower spikes, were spiders. These were of the non-webbuilding type, in particular called nocturnal running spiders, or sac spiders, because of the little silken cocoon they spin and rest in during the day. The sight of nocturnal running spiders running in vegetation at night is not unusual; Uetz et al. (1999) have classified this large group of spiders as "foliage runners." These are the most active of all spiders (Riechert & Harp 1987), and running among flowers is a good way to encounter nectar-feeding insect prey. But these spiders I observed did something strange. They spent time at each floret, and seemed busy with the flowers themselves. They buried their mouthparts deep into each floret, held still for a few seconds, then systematically moved to the next. Their movements imitated what is generally termed "working" the flowers, well studied among known nectar feeders, such as bees, moths, and butterflies.

This behavior struck me immediately as nectar feeding, but for two alternative possibilities: It might be that rather than nectar, spiders sought minute prey hidden deep within the florets, or that they sought pollen, known to be nutritious. To confirm my suspicions of nectar feeding, I had to find spiders feeding at *extra*floral nectaries (EFNs). EFNs are nectar-producing structures that reside anywhere on a plant outside of a flower. They take many forms, such as slits, cups, or simply nectar-exuding undifferentiated tissue, and they often reside on leaf margins, leaf midribs, petioles, or flower bracts (Bentley & Elias 1983[eds.]; Koptur 1992). EFNs are well known to entomologists for their association with ants, presumed by many to be an evolutionary relationship, whereby plants "hire" ants with a nectar reward to protect them from herbivores (Bentley 1977). EFNs are too small and shallow to hide insect prey, and they contain no pollen. Had I known at the time, I could have looked on the underside of each leaf of the Indian almond. Only later, for my own experiments, did I learn that each of its leaves bears a pair of hugely productive petiolar EFNs (which may have explained why the tree was so attractive to running spiders). Fortunately, during this same bout of fieldwork, I did find spiders feeding at the EFNs of *Hibiscus tiliaceus* L., a seaside shrub. *Hibiscus* has three EFNs on the underside of each leaf, a longitudinal slit at the base of each leaf rib. The plants grow so tall, and the heart-shaped leaves so large, that I could stand beneath the higher branches and observe a number of EFNs at the same time. It helped also that a sooty fungus associated with the sugary exudate marks most of the nectaries. During the day, mostly ants visited the nectaries. At night, mostly mosquitoes visited, probing the slits as they did the florets. But also during the night appeared the same sorts of spiders as on the Indian almond flower spikes. The spiders splayed their legs, flattened themselves against the leaves, and pressed their mouthparts into the nectary openings.

For spiders to assume such unnatural postures at *Hibiscus* EFNs suggests that their appearance at these nectaries was not by chance. And for spiders to visit EFNs as well as flowers—which have in common nectar—weakens the argument that spiders visit flowers only for the purpose of finding hidden insects or pollen. Finally, it is unlikely that spiders sought water at the nectaries as a component of the exudate; the foliage in this tropical environment was often wet from daily downpours.

Considering spider nectar feeding a natural phenomenon worth investigating, I looked for more examples. At the time, there were only two recorded observations of spiders feeding on plant sugar in the field, both involving ant mimicking jumping spiders (Salticidae): Edmunds (1978) observed *Myrmarachne legon* Wanless taking nectar from EFNs in Ghana, and Collart (cited in Edmunds) observed *M. foenisex* Simon feeding on honeydew from coccids in Zaire. The spiders I observed nectar feeding on Indian almond dragged their palps over the surface of the leaves, stopping occasionally to pull their palps through their mouthparts, as though to clean them. This might also have been an incidence of feeding on honeydew. In 1986 and 1987, while working near a tidal marsh in Vero Beach, Florida, once again I found nocturnal running spiders apparently feeding on nectar, this time on *Eupatorium serotinum* Michaux, a common thoroughwort with tiny white florets forming flat-topped clusters. These spiders behaved like the ones in Costa Rica, wandering from floret to floret, jamming their mouthparts into each. And, also as in Costa Rica, I found the same types of spiders at extrafloral nectaries, this time at the EFNs of the common castor bean (castor oil) plant *Ricinus communis* L. The spiders pressed their mouthparts to the large, shallow, cup-shaped nectaries of castor bean, once again precluding that they sought pollen or insects. I had no permits to collect the spiders I observed in Costa Rica and therefore I have no definitive IDs. Collections of spiders in Florida, however, showed both sexes and every age of four species of spiders from three ecologically similar families, all of which are nocturnal running spiders: (1) Anyphaenidae: *Hibana velox* Becker; (2) Clubionidae: *Cheiracanthium mildei* L. Koch (now placed in the Miturgidae); and (3) Corinnidae: *Trachelas volutus* Gertsch and *Trachelas similis* F.O. Pickard Cambridge.

Among the spiders collected from the Florida castor bean nectaries, an adult female *H. velox* deposited an egg mass, from which I collected 48 spiderlings to test the effects of dietary sugar on their survival. To avoid cannibalism, individual spiderlings were isolated in small plastic containers at 27 C and 80% RH. Half received water only and the other half, water and a 25% sucrose solution. Aside from a few that died early by accidental crushing or drowning, the mean age at death of the water-only group was 18.7

days (range, 16–27); for those receiving water and sucrose mean age was 40.4 days (range, 26–56). Thus, the availability of sucrose (or its components fructose and glucose), the major component of plant nectars, more than doubled their lifetimes of newly emerged spiders. During time of low prey availability this creates a substantial window of opportunity for spiders to seek the prey that they require for normal growth, but still fulfill their energy requirements by drinking plant nectar. These spiders typically inhabit the vegetation and deposit their egg sacs there, so spiderlings emerging within proximity to floral nectaries, extrafloral nectaries, or even honeydew may gain substantially.

In another case of apparent nectar feeding by tropical spiders, in 1988 at a research station in eastern Panama, spiders on domestic cashew trees, *Anacardium occidentale* L., pressed their mouthparts to the same areas of plant tissue that every other nectar-feeding insect was probing. Although there was no specific EFN structure, mosquitoes, chironomids and other nectar feeders were all probing the angles of the pannicle branches, which Wunnachit et al. (1992) have identified as the site of nectar-bearing trichomes. All of the spiders collected were *Hibana similaris* (Banks) members of the family Anyphaenidae, one of the three nectar-feeding families collected in Florida.

In 1995, Pollard et al. were the first to publish observations of spider nectar feeding in the field. Adult male crab spiders *Misumenoides formosipes* (Thomisidae) drank floral nectar in between bouts of courtship on Queen Anne’s lace (*Daucus carota* L.) goldenrod (*Solidago* spp.), and chicory (*Cichorium intybus* L.). In this case, the nectar may have been critical to the tiny males, who are miniscule compared to females and spend most of their time on flowers courting females rather than catching prey. The males were prone to dehydration, and although the nectar replaced water lost to evaporation, in the lab, they preferred to drink a simulated nectar source (30% sucrose) even after they stopped drinking water. Males offered sucrose lived longer than those living on water only. The following year, I co-authored my observations of nectar feeding (Taylor & Foster 1996), increasing the observations of nectar feeding from two families (Salticidae and Thomisidae) to five (Anyphaenidae, Clubionidae, and Corinnidae) and from juveniles and males only, to spiders of both sexes and all stages.

Since then, two more reports have recognized the phenomenon of nectar feeding among jumping spiders. Ruhren and Handel (1999) reported that jumping spiders *Metaphidippus* sp. and *Eris* sp. fed at the EFNs of the annual legume *Chamaecrista nicitans* (L.) Moench (Caesalpineaceae). In greenhouse choice experiments, the spiders chose plants with active EFNs six times more frequently than those whose EFNs were inactive, and the presence of the spiders was correlated with enhanced seed production (Ruhren & Handel 1999). In laboratory experiments, Jackson et al. (Jackson et al. 2001) showed that 100% of 90 species of jumping spiders preferred a 30% sucrose solution to water. This was precipitated by their observations of 31 species of jumping spiders in the field with their mouthparts pressed against flower nectaries. Finally, among these "official" reports of spider nectar feeding are the observations that, because of their singularity, were not recognized as part of a common phenomenon and have been discussed only informally at conferences.

If nectar feeding is, in fact, a regular phenomenon among these large groups of spiders, why are we just now discovering it? The explanation likely lies in three aspects of a spider's activity: its level, its time of day, and its place. Our knowledge of any spider’s natural diet is tied to how closely the spider itself can be observed. It is not surprising, then, that the diets of orb weavers and other webbuilders are better understood than the diets of non-webbuilding wandering spiders. Webs are conspicuous, they tie a spider to one place for a time, and they can act as storage sites for remains of prey. But even among the wandering spiders there is a continuum of activity that allows some to be more easily observed than others. Crab spiders are nearly as sedentary as orb weavers and are therefore easy to observe once they choose their site of ambush. Running spiders, on the other hand, are in almost constant motion, lightning quick, and are impossible to follow as they dodge in and out of foliage.

Most running spiders, in addition, are nocturnal, whereas human researchers are diurnal. Jumping spiders and the majority of crab spiders are also diurnal. Unless they are looking for something specific at night, most researchers operate and make their discoveries during the day. It was looking for mosquitoes feeding at night that allowed me to discover nectar feeding among the nocturnal running spiders. Finally, to observe nectar feeding among spiders, they have to be in an environment where nectar is available. The structure of most flowers place floral nectaries too deep within the flower’s corolla to be accessible to spider mouthparts. Extrafloral nectaries, on the other hand, are exposed and accessible, and, if they are present and productive, make nectar available. For this reason, to examine the incidence of nectar feeding among spiders, I went to cotton crops to evaluate the presence of fructose, a plant-derived sugar, in the spiders’ guts. The cotton plant has EFNs on its leaf midribs and on the underside and inside of the floral bracts (Butler, Jr. et al. 1972), and studies have shown cotton crops to support populations of running spiders, including *Cheiracanthium inclusum* (Hentz), a temperate-zone nocturnal runner (Peck & Whitcomb 1970) and the subject of my laboratory experiments.

Given that discoveries and insights of biologists can be dictated by something as basic as when they sleep, other signs pointing individually to the likelihood of spider nectar feeding have been understandably overlooked. Taken together, however, they form a rationale for investigating plant nectar as contributing substantially to the life histories of some spiders. Detailed below, spiders can detect and taste sugar; they respond positively to sugar; they can digest sugar; those that wander in vegetation encounter sugar in the form of nectar; and nectar is a high energy food that fuels the adult lives of nectarivorous insects, such as bees (reviewed in Waddington 1987)and butterflies (reviewed in Boggs 1987). For spiders in particular, plant nectar is a relatively cheap resource. It saves them the energy and risk of attacking and subduing prey. Nor would spiders be the only carnivorous arachnids to use plant nectars to supplement their diets. The mite *Iphiseius degenerans* (Berlese) (Phytoseiidae), employed in Dutch greenhouses to prey on western flower thrips, populates the castor bean *R. communis* year-round in the Mediterranean and densely populates the plant in greenhouses. Castor bean is therefore known as a “banker” plant for biological control, and researchers wanted to know if the plant itself contributed to the welfare of the mite. Laboratory experiments showed that the extrafloral nectar of castor bean contributed significantly to the survival of adults and juvenile mites(van Rijn & Tanigoshi 1999). It did not contribute to reproduction directly, nor to growth or molting, but it allowed adults to survive long periods of starvation and to resume natural growth and reproduction when prey was reintroduced (van Rijn & Tanigoshi 1999).

Researchers took advantage of spiders' natural taste for sugar when they needed a vehicle to deliver mescaline and psilocybin to orb-weavers to measure the drugs' effects on the shape of their webs (Christiansen et al. 1962). They added enough glucose to each drug solution "to make it taste sweet," and the spiders drank them readily. Any drug that could be ingested, such as LSD, caffeine, and strychnine, was delivered to spiders in a "sugar water solution" (Witt 1971). Worth mentioning here is that, according to Witt (1971), the discovery of the effects of drugs on orb webs occurred because "The professor of zoology at the University of Tubingen had enlisted the help of a young pharmacologist to try to shift web-building to a convenient time," (p.98).

These researchers probably stimulated orb weavers to ingest sugar solutions by placing the drop to their mouthparts. Spiders, in fact, taste primarily with their feet. The most important chemoreceptors of spiders are contact chemoreceptors or "taste hairs," which are found mainly on the distal segments of the legs and palps and are concentrated on the tarsi of the first legs (reviewed in Foelix, 1982). Barth (2002) also found what he called "gustatory" hairs on the tarsi of the large wandering spider *Cupiennius salei* (Keyserling) (Ctenidae). Other than to some salts and hydrochloric acid, physiological responses of these hairs have not been measured. Because spiders taste with their feet, those that wander in vegetation and rely on "touch" for sensory input, are likely to touch/taste nectar at EFNs during their wandering. (My own trials with *C. inclusum* show that as soon it touches sugar with tarsus one, it immediately orients to the drop and inserts its mouthparts). Furthermore, spiders are predisposed to ingesting drops of fluid, given their extra-oral digestion (Cohen 1998) that employs a sucking stomach to take in liquified nutrients (Foelix 1982).

Were spiders not able to digest plant nectar, then their ability to detect and willingness to ingest it would be moot. Plant nectars are composed primarily of the disaccharide sucrose or its constituent monosaccharides glucose and fructose. It also contains other nutrients, such as amino acids, lipids, and vitamins (Baker & Baker 1975; 1983). Only monosaccharides can be absorbed across the invertebrate gut wall (Treherne 1967). If spiders do ingest plant nectar in the form of sucrose, then, they need the means to break it down. Researchers have known since 1877 (Plateau, cited in Pickford 1943) that spiders' digestive enzymes include the enzyme sucrase, which splits the disaccharide sucrose into its monosaccharide components. Pickford (1942) also found sucrase in the tarantula *Cyrtopholis jamaicensis* (Theraphosidae) but, like Plateau, only qualitatively. Neither questioned the presence of such an enzyme in an exclusive carnivore. In more sophisticated tests, Mommsen (1977) also found sucrase in the webbuilding spider *Tegenaria atrica* Koch (Agelenidae) and was able to quantify it and its activity. He, too, made no comment on its presence and stopped short of its complete analysis, unlike the other enzymes he tested. In addition to sucrase, Mommsen (1977) found another group of enzymes in *Tegenaria* that can also split sucrose into its components, the α-glucosidases. These enzymes can also aid in the digestion of the di-, tri- and tertrasaccharides found in honeydew, the sugary exudate of phloem-feeding insects (summarized in Wäckers 2001a). Compared to nectar-feeding, evidence that wandering spiders naturally feed on and benefit from honeydew is scant (although, see Chapter 5).

Analyses of spider energetics, competition, and distribution have always taken into consideration the numbers and types of prey available to spiders. When vegetation was considered important, it was as a support for webs, as refugia, or as a food source for the insects that spiders catch (Turnbull 1973; Uetz et al. 1999). Only since observations of spiders feeding at EFNs have researchers begun to consider vegetation as a direct source of food. Moreover, it is the proposition of this dissertation that nectar feeding is not uncommon among spiders and that 1) spiders that wander in EFN-bearing vegetation will encounter EFNs; 2) spiders encountering productive EFNs will detect their sugary nectar and ingest it; 3) spiders that ingest nectar as the disaccharide sucrose can digest it (or else the monosaccharides fructose and glucose are absorbed directly), and ultimately, 4) spiders will gain from nectar at least what other nectarivores gain, that is, energy for activity and/or added nutrition for increased survival, growth, and reproduction.

REFERENCES

Amalin, D.M., J. Reiskind, R. McSorley, & J. Peña. 1999. Survival of the hunting spider, *Hibana velox* (Araneae, Anyphaenidae), raised on different artificial diets. *Journal of Arachnology*, 27: 692-696.

Anderson, J.F. 1974. Responses to starvation on the spiders *Lycosa lenta* Hentz and *Filistata hibernalis* (Hentz). *Ecology*, 55: 576-585.

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Barth, F.G. 2002. A Spider's World: Senses and Behavior. Springer. Berlin.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Bentley, B.L. and T.S. Elias. 1983. Biology of Nectaries. Columbia University Press. New York, NY.

Boggs, C.L. 1987. Ecology of nectar and pollen feeding in lepidoptera. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Bonnet, P. 1924. Sur la nature des aliments que les araignées peuvent absorber et sur le sens du goût chez ces animaux. *C.R.Séances Soc.Biol.Fil.*, 91: 1194-1196.

Buschman, L.L., W. H. Whitcomb, R. C. Hemenway, D. L. Mays, Ru Nguyen, N. C. Leppla, & B. J. Smittle. 1977. Predators of velvetbean caterpillar eggs in Florida soybeans. *Environmental Entomology*, 6: (3) 403-407.

Butler, G.D., Jr., G. M. Loper, S. E. McGregor, J. L. Webster, & H. Margolis. 1972. Amounts and kinds of sugar in the nectars of cotton (*Gossypium* spp.) and the time of their secretion. *Agronomy Journal*, 64: 364-368.

Christiansen, A., R. Baum, & P. N. Witt. 1962. Changes in spider webs brought about by mescaline, psilocybin, and an increase in body weight. *Journal of Pharmacology and Experimental Therapeutics*, 136: 31-37.

Cohen, A.C. 1998. Solid-to-liquid feeding: The inside(s) story of extra-oral digestion in predaceous arthropoda. *American Entomologist*, Summer: 103-117.

Decae, A. 1986. Bird- or banana-spider? *Newsletter of British Arachnology Society*, 46: 3-4.

Dondale, C.D. 1965. A spider's first meal after molting. *Canadian Entomologist*, 97: 446-446.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Gertsch, W.J. 1949. American Spiders. Van Nostrand. New York, NY.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Koptur, S. 1992. Extrafloral nectar-mediated interactions between insects and plants. *In* Insect-Plant Interactions. IV. (E.A. Bernays, ed.) CRC, Boca Raton, FL.

Miliczky, E.R. & C. O. Calkins. 2002. Spiders (Araneae) as potential predators of leafroller larvae and egg masses (Lepidoptera: Tortricidae) in Central Washington apple and pear orchards. *Pan-Pacific Entomologist*, 78: (2) 140-150.

Mommsen, T.P. 1977. Digestive enzymes of a spider (*Tegenaria acrica* Koch) II. Carbohydrases. *Comp.Biochem.Physiol.*, 60A: 371-375.

Nentwig, W. 1986. Non-webbuilding spiders: prey specialists or generalists? *Oecologia (Berlin)*, 69: 571-576.

Nyffeler, M., R. G. Breene, D. A. Dean, & W. L. Sterling. 1990. Spiders as predators of arthropod eggs. *Journal of Applied Entomology*, 109: 490-501.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Peck, W.B. & W. H. Whitcomb. 1968. Feeding spiders an artificial diet. *Entomological News*, 79: 233-236.

Peck, W.B. & W.H. Whitcomb. 1970. Studies on the biology of a spider *Chiracanthium inclusum* (Hentz). 753. Agricultural Experiment Station.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 35: 33-72.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Riechert, S.E. & J.M. Harp. 1987. Nutritional ecology of spiders. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Sandidge, J.S. 2003. Scavenging by brown recluse spiders. *Nature*, 426: (6 November) 30-30.

Smith, R.B. & T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. *Science*, 226: 1330-1333.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Treherne, J.E. 1967. Gut absorption. *Annual Review of Entomology*, 12: 43-58.

Turnbull, A.L. 1973. Ecology of the true spiders (Araneomorphae). *Annual Review of Entomology*, 18: 305-348.

Uetz, G.W., J. Halaj, & A. B. Cady. 1999. Guild structure of spiders in major crops. *Journal of Arachnology*, 27: 270-280.

van Rijn, P.C.J. & L. K. Tanigoshi. 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, 23: 281-296.

Vogelei, A. & R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia (Berl.)*, 80: 513-515.

Waddington, K.D. 1987. Nutritional ecology of bees. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Wäckers, F.L. 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, 47: 1077-1084.

Wise, D.H. 1993. Spiders in Ecological Webs. Cambridge University Press. Cambridge, MA.

Witt, P. 1971. Drugs alter web-building of spiders. A review and evaluation. *Behavioral Science*, 16: 98-113.

Wunnachit, W.C., C. F. Jenner, & M. Sedgley. 1992. Floral and extrafloral nectar production in *Anacardium occidentale* L. (Anacardiaceae): an andromonoecious species. *International Journal of Plant Science*, 153: 413-420.

Young, O.P. & G. A. Edwards. 1990. Spiders in United States field crops and their potential effect on crop pests. *Journal of Arachnology*, 18: 1-27.

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CHAPTER 2
THE BENEFITS OF EXTRAFLORAL NECTAR TO THE SURVIVAL OF *HIBANA VELOX* BECKER (ARANEAE: ANYPHAENIDAE) AND THE SURVIVAL, GROWTH, AND ACTIVITY OF *CHEIRACANTHIUM INCLUSUM* (HENTZ) (ARANEAE: MITURGIDAE), TWO NOCTURNAL RUNNING SPIDERS

2.1 Abstract

As generalist predators, spiders prey on insects and other arthropods, including other spiders. Studies of spider ecology and energy allocation assume, therefore, that the single object of spider foraging is prey. Evidence indicates, however, that some large groups of spiders are exploiting an additional food source: plant nectar. Observations of nectar feeding have only recently been reported among members of large, non-webbuilding groups generally known as wandering spiders, and by family, known as crab spiders, jumping spiders, and running spiders. In laboratory experiments, I show for the first time plant nectar's significant contributions to the early survival of *Hibana velox* Becker (Anyphaenidae) and survival, growth, and activity of *Cheiracanthium inclusum* (Hentz) (Miturgidae), both nocturnal running spiders. Spiderlings lived longer, grew faster, and were more active when they received plant nectar. Both of these spiders inhabit agricultural crops; understanding nectar's role in their energy budgets, therefore, has the practical application of providing insight into their contributions as pest control agents.

2.2 Introduction

All spiders are predaceous, feeding mostly on insects and, often, other spiders (Turnbull 1973; Foelix 1982; Nentwig 1987; Wise 1993). Until recently, all spiders were presumed to be *exclusively* predaceous, relying solely on the protein of prey for their survival, growth, and reproduction. Orb weaving spiderlings, however, coincidentally eat and benefit from the pollen grains that land in their webs, which they eat to recycle (Smith & Mommsen 1984). And, involving a much larger group of spiders, a growing number of observations place some spiders at the floral and extrafloral nectaries of plants, presumably feeding on plant nectars (Edmunds 1978; Vogelei & Greissl 1989; Pollard et al. 1995; Taylor & Foster 1996; Ruhren & Handel 1999; Jackson et al. 2001). The simple sugars and amino acids of plant nectars are well known to nourish nectarivorous arthropods, many of which are also prey for spiders. As the product of a primary producer, nectar is naturally more abundant than these arthropods that drink it. As predators of many of these nectar-drinking arthropods, spiders may benefit by skipping this second trophic level and going directly to plant nectaries themselves. Field observations of nectary visiting spiders include common, familiar members of large, non-webbuilding families, crab spiders (Thomisidae) jumping spiders (Salticidae) and running spiders (Clubionidae, Anyphaenidae and Corinnidae). Considering that the hunting success rate for wandering spiders is thought to be low (Nentwig 1987) (Anderson 1974; Nyffeler et al. 1987; Nyffeler & Sterling 1994)plant nectar could serve as a substantial dietary alternate or supplement.

Plant nectars contain primarily carbohydrates and water (Percival 1961), but also amino acids, lipids, vitamins, and minerals (Baker & Baker 1975; 1983; Koptur 1992).

Nectar is exuded at floral nectaries, but is more accessible to spiders' small mouthparts by way of extrafloral nectaries (EFNs), nectar-bearing tissues or structures that reside anywhere on a plant outside of a flower. EFNs often occur on leaves or leaf petioles, and take many forms, such as slits, cups, bowls, or undifferentiated tissue. Arthropods that do not typically visit floral nectaries often visit these open, accessible EFNs, ants in particular (Bentley 1977). Although all spiders are considered "sit-and-wait" predators to various degrees (Uetz 1992; Riechert 1992), running spiders (or sac spiders, as they are also called) are deduced to occupy the "most active" end of the spider activity continuum, their wandering allowing them to prey upon immobile lepidopteran eggs (Buschman et al. 1977; Riechert & Harp 1987; Nyffeler et al. 1990). Running spiders inhabit vegetation and their degree of activity and nectar feeding may be correlated: searching for prey requires wandering, and frequent wandering means a greater likelihood of encountering EFNs and plant nectar. Plant nectar, which contains mostly sugar, could repay the energetic costs of wandering. The nocturnal running spiders *Cheiracanthium inclusum* (Hentz) (Miturgidae, [formerly in Clubionidae]) and *Hibana velox* (Becker) (Anyphaenidae) run and jump with lightening speed, making them good candidates to investigate the effects of nectar on a spider's nutrition and/or energy. Moreover, both of these spiders have been observed at plant nectaries (Taylor & Foster 1996).

Four laboratory experiments tested the effects of dietary extrafloral nectar on the survival, and activity of newly-emerged spiders. *H. velox* was the subject of initial survival tests. *C. inclusum*, which is ecologically similar, was more easily obtained and the subject of all other experiments. The experiments tested 1) the effects of nectar and two concentrations of sucrose on the survival of individually housed *H. velox*; 2) the effects of nectar on survival within crowded sibling *C. inclusum* under pressure of cannibalism; 3) the effects of adding nectar to a diet of prey (*Drosophila*) in individually housed *C. inclusum*; and 4) the effects of nectar on the nocturnal running activity of groups of prey-deprived *C. inclusum*.

2.3 Materials and Methods

Experimental subjects

Experimental *H. velox* were offspring of adults collected in Gainesville, FL, USA; experimental *C. inclusum* were offspring of adults collected in Columbus, Ohio, USA. Adults were maintained in 7-liter clear acrylic cages (15 x 21 x 27cm) with a screened opening at one end and a sleeved opening at the other. Experiments were conducted in a laboratory rearing room on a 16:8 light:dark cycle at approximately 27° C and 80% relative humidity. Spiders were checked daily for mortality. All containers in the experiments that held spiders also held fluid wells, small rectangles of plastic (1 cm X 2.5 cm) with a dimple drilled near one end (large for water, small for nectar). Each container held at least two wells; water and sugar wells were equal in number. Each experiment or trial began within 12 hours of an egg sac's emergence, which was considered day 0. "First molt" refers to a spider's first post-emergence molt.

Containers

Spiders housed individually occupied clear, lidded, plastic containers, 5.2 cm-dia. X 3.6 cm. Each container had 4 holes: 2, 12-mm, mesh-covered holes top and bottom; and 2 opposing 17-mm holes in the side wall, one mesh-covered and the other corked for introduction of prey and for changing wells. Twenty of these small containers, composing an even mix of controls and experimental individuals, filled a clear, plastic

30-cm x 25-cm lidded box (crisper). Two crispers fit on a large plastic tray, lightly dusted with sifted sulfur to repel mites. Crispers were rotated daily in place and on the shelves to avoid placement effect. Individuals were checked daily for survival and molting.

Spiders housed together occupied clear plastic containers 10 cm-dia. X 6.5 cm. Each container had 4 holes: 2, 12-mm, mesh-covered holes top and bottom; and 2 opposing 17-mm holes in the side wall, one mesh-covered and the other corked for introduction of prey and for changing wells. Each container held a total of 12 fluid wells, all filled with water, or 6 water and 6 nectar. These containers were placed in clear, plastic 30-cm x 25-cm lidded crispers. Individuals were checked daily for survival.

Spiders housed together for nighttime activity measurements occupied clear 7-cm-square plastic lidded boxes, each with a 17-mm hole on each side, 3 mesh-covered and one for introduction of spiders and changing fluid wells. Each box held 8 wells, all filled with water or, 4 water and 4 nectar. Two boxes, control and experimental, were placed side by side in a lidded clear plastic box, 3.5 by 24 cm and filmed at night with a RCA closed circuit TC7011 infrared camera with red light illumination (Peck & Whitcomb 1970). In the first replicate, each treatment held 8 spiders. In the second replicate, each treatment held 9. From tapes, I quantified nightly activity as the greatest number of spiders simultaneously running during a one-minute period, at 10-minute intervals, for 52 periods; the mean of these 52 periods is that night's activity. Individual spiders were not distinguishable.

Diets

In every experiment, every individual had water ad lib. from plastic water wells and from a No.1 (9 mm) soaked cotton dental ball. Ambient relative humidity was high, and smaller containers were kept in larger containers to keep water wells from drying out. The constant availability of free water ensured that spiders did not take nectar to obtain water. Sucrose and nectar, because of their viscosity, were delivered with a micro spatula, in the smallest transferable amount, between 1– 2µl. smeared into the smaller dimple of the 1-cm X 2.5-cm plastic feeders.

Water, sucrose, and nectar were changed daily. Prey consisted of live, vestigial-winged *Drosophila melanogaster* maintained on instant blue *Drosophila* medium (Carolina Biological Supply). For diets that combined prey and nectar, I alternated prey with nectar daily (each offered every other day) to ensure that spiders consumed nectar directly and did not ingest it by way of ingesting prey that had just eaten nectar.

All nectar was extrafloral to avoid introduction of pollen as a possible source of protein (Smith & Mommsen 1984). For the first survival experiment with *H. velox* (Fig. 2.1), extrafloral nectars came from collections of various greenhouse plants, such as orchids. For all of the following experiments, I collected nectar from the foliar EFNs of *Terminalia cattapa* L. (Indian almond), growing in the OSU greenhouse, and stored it at – 45°C. From serial dilutions on a Reichert-Jung refractometer, I determined the sugar concentration of *T. cattapa* extrafloral nectar to be 87.5%. This is nearly identical to the concentration of sugars (872mg/ml) from the EFNs of castor bean (*Ricinus communis*) (Baker et al. 1978), and is similar to the concentration of sugar (77.7%) exuded at the

EFNs of cashew (*Anacardium occidentale* L.) (Wunnachit et al. 1992). *H. velox* has been observed feeding at both of these (Taylor & Foster 1996).

Data Analysis

I analyzed data with *Statistica* for Windows (2000), StatSoft, Inc. Multiple comparisons in survival analysis employed log-rank tests and a multiple comparison procedure with an alpha level of 0.05 and a calculated comparison-wise error rate of 0.008 (based on $K = 3$ treatments) (Hardin et al. 1996). Where data could not be transformed to meet the assumptions of normality, I used nonparametric tests.

2.4 Results

Survival on sugar only

Survival experiments compared the longevity of newly emerged, sibling *H. velox* on diets of water only, sucrose, or extrafloral plant nectar, collected from various greenhouse plants (all subsequent experiments used extrafloral nectar from a single source *Terminalia cattapa*). All groups received water ad lib. In both experiments, I divided a single egg sac among the three treatments. Both experiments included a sucrose treatment to distinguish contributions of carbohydrates from possible contributions of other nectar components, such as amino acids or lipids. In one experiment, sucrose was relatively “low” (25%), in the other, “high”(69%), to more closely imitate the high sugar concentration of extrafloral nectar.

In the first experiment, spiders survived significantly longer ($\alpha = 0.05$) than water-only controls if they received 25% sucrose (log-rank test statistic = 3.71, uncorrected p =0.0002), or if they received nectar (log-rank test statistic = 4.39, uncorrected p =0.0001). The treatments 25% sucrose and nectar were not significantly different (log-rank test statistic = −1.43, uncorrected p = 0.1566) (Fig. 2.1), with some surviving over 70 days and having a higher incidence of molting (Table 2.1). The second experiment produced the same results. Spiders survived significantly longer (α = 0.05) than water-only controls if they received 69% sucrose (log-rank test statistic = 3.36, uncorrected p =0.0008), or if they received nectar (log-rank test statistic = 3.66, uncorrected p =0.0003). The treatments 69% sucrose and nectar were not significantly different (log-rank test statistic = 0.770, uncorrected p = 0.4412) (Fig. 2.1). The incidence of molting was highest in the treatment 69% sucrose (Table 2.1)

Sibling survival in prey-deprived crowds

Most spiders are solitary and disperse from their egg sac, in part, to avoid sibling cannibalism (Turnbull 1973; Wise 1993; Wagner & Wise 1996; Samu et al. 1999). The following experiment tested nectar's effects on mortality in the unusual conditions of newly emerged, sibling *C. inclusum* forced to live together in crowded conditions. The only source of prey was cannibalizing or scavenging bodies. From the day of emergence, siblings cohabited in clear plastic containers, 10 cm dia. X 6.5 cm, in two treatments, with nectar and without nectar (water available ad lib.). Each container housed 13 siblings. Three containers composed each treatment for a total of N = 39 for each. One egg sac was divided between the first 2 pairs of containers and those treatments ran concurrently. Ten days later, I set up another pair of treatments using another, single, egg sac. The comparisons ended after a 14-day period, the longest period for any spider to survive in the control treatment. After 14 days, 13 (33%) of the spiders with nectar still survived (Fig. 2.4). Also, by the fourth day, about 60% of the spiders without nectar were dead; only one had died in the treatment with nectar. Because the results in the 3 containers for each treatment were similar, I lumped the data for analysis. Spiders receiving nectar survived significantly longer than controls without nectar (log-rank test statistic = 5.81, $p < 10^{-4}$) (Fig. 2.4).

A diet of prey and nectar: survival, molting, and prey consumption

In two separate experiments, each using a different, single egg sac, newly emerged, individually housed *C. inclusum* were fed a single *Drosophila* every other day until the spider molted. The two treatments, with nectar and without nectar (= water) alternated with the *Drosophila*. Water was available ad lib. In the first experiment, the *Drosophila* diet began on day 1, in the second experiment, *Drosophila* were not introduced until day 3. Nectar-fed spiders on the delayed *Drosophila* diet received nectar for the first two days. The experiments ended when all of the spiders in one group had died or molted. In both experiments, all of the spiders without nectar died, by day 15 for the first experiment, by day 16 for the second. In both experiments, therefore, the *Drosophila* diet alone was nutritionally marginal and insufficient for normal survival (Fig. 2.4).

Nectar added to the diet of *Drosophila* significantly increased the survival and numbers of spiders that underwent their first molt (*Drosophila* on Day 1, χ^2 : without nectar, $N = 30$; with nectar, $N= 30$, d.f. = 1, $p < 0.001$; *Drosophila* on Day 3, χ^2 : without nectar, $N = 49$; with nectar, $N = 49$, d.f = 1, $p < 0.001$ Fig. 2.4). This increased survival was true whether the *Drosophila* diet began on day 1 or day 3. On the other hand, delaying the introduction of prey, and therefore protein, had a significant effect on the ability to survive molting, an energy-depleting event that can increase respiration three-fold (Stranzy & Perry 1987). The proportion of spiders that molted, receiving nectar and

Drosophila on the first day, was 29/30. Of those 29 molting events, 100% were healthy, defined by the spider having completely escaped the cast exoskeleton and surviving the molting event. The proportion of spiders that molted receiving nectar and *Drosophila* on day 3, was 38 /49 (78%) but of those 38 molting events, only 23 were healthy (61% of those that molted and 47% of the total sample). The delay of prey by two days cut the incidence of healthy molting significantly among spiders receiving nectar (χ^2: prey on Day 1, $n = 30$, prey on Day 3, $n= 49$, $p < 0.001$). Survival and molting among spiders without nectar, in both prey diets was negligible (Fig. 2.4).

In both of the above experiments, spiders without nectar consumed fewer total *Drosophila* than spiders with nectar because they did not survive nearly as long. In both experiments, however, overall, spiders without nectar consumed the same average number of *Drosophila* per day as spiders with nectar (i.e., total *Drosophila*/total spider days for entire treatment), determined by what spiders actually consumed. In the first experiment (*Drosophila* introduced on Day 1), all of the spiders in the control, without nectar, consumed a total of 74 *Drosophila* in a group total of 243 d before the spiders died (28/30) or molted (2/30). In the other treatment, all of the spiders receiving nectar consumed a total of 94 *Drosophila* in a total of 294 days before they died (1/30) or molted (29/30). In this experiment, therefore, spiders without nectar consumed an average 0.30 *Drosophila*/spider day; spiders with nectar consumed 0.32 *Drosophila*/spider day. On average, spiders were taking in nearly identical amounts of prey, but molted normally only if they were supplemented with nectar, suggesting that they were at the margins of their prey requirements. In the second experiment (*Drosophila* on Day 3) spiders without nectar consumed a total of 62 *Drosophila* in a group total 265 d before they died (49/49) or molted (0/49). Spiders with nectar consumed 122 *Drosophila* in a total 466 d before they died (11/49) or molted (38/49). Consumption of *Drosophila*/spider day for spiders without and with nectar were 0.23 and 0.26, respectively, determined from what spiders actually consumed.

Activity

Both *H. velox and C. inclusum* wander energetically in vegetation at night and are inactive during the day, typically hiding in a lightly woven sac. To investigate effects of nectar on nighttime activity, I divided newly emerged, sibling *C. inclusum* between two treatments, with nectar and without nectar. Neither of the treatments had prey; both had water ad lib. I ran two replicates, each from a separate egg sac. One replicate had 8 spiders in each treatment, the other had 9 in each treatment. With the clear, plastic containers side-by-side to fill the video frame, I filmed the spiders at night with an RCA closed circuit TC7011 infrared camera with red light illumination, to which the spiders are not sensitive (Peck & Whitcomb 1970). From tapes, I quantified nightly activity as the greatest number of spiders simultaneously moving during a one-minute period, at 10-minute intervals, for 54 periods. Individuals could not be distinguished. The mean number of spiders active during these 54 periods is that night's activity (Fig. 2.5). The experiment ended when any of the spiders died, which occurred in the treatment Without nectar, on Night 5 in the first replicate and on Night 4 in the second replicate.

Comparison of the total number of intervals of activity (216 for replicate 1, 270 for replicate 2) between the two treatments shows that nectar contributes significantly to the spider's running, in absence of prey (Mann-Whitney U: Rep 1, $n = 270$ for both treatments, $Z = -12.709$, $p < 0.001$; Rep.2, $n = 216$ for both treatments, $Z = -13.377$, $p <$

0.001). On day one, for each replicate, there was no significant difference in activity between spiders with nectar and those without. As time went on, however, spiders without nectar became increasingly quiescent until they died.

2.5 Discussion

My experimental results show that even when water was available, spiders still drank nectar when offered. Spiders with access to nectar survived significantly longer and had a higher incidence of molting than those without access. Those with access to nectar and prey survived longer, and all molted normally, as opposed to the controls, nearly all of which died without molting. How these spiders are allocating nectar, whether as fuel for activity, or to growth, or to both, can begin to be teased apart. Nectar contributes significantly to the energetic needs *of C. inclusum*, conferring not only survival but also allowing them to keep up their running all night, every night, that they were filmed. My experiments also show that nectar alone, but also sucrose alone (Fig. 2.1), contributed to a higher incidence of molting in *H. velox*, suggesting that it is the sugar component of nectar that contributes most to the spider's ability to molt. Proteins, however, comprise nearly half the components of spider cuticle (Dalingwater 1987). Because sucrose contributes to the same incidence of molting as does nectar, but molting requires protein for new cuticle, it appears that sucrose is likely allocated to fulfill a spider's activity needs, and protein, such as in a spiderling's own yolk reserves, is allocated to development and molting. This is likely why *H. velox* receiving nectar but no prey survived long, but underwent only one molt (Table 2.1), and why *C. inclusum* without nectar and receiving a marginal amount of prey (*Drosophila*) could not molt at all (Fig. 2.4): A marginal amount of prey divided among four energy demands—

maintenance, growth, activity, and molting—was not sufficient to supply all. Protein and sugar together, however, appear to work to efficient effect, and, depending on the minimum amount of protein required to reach functional maturity, a spider that nectar-feeds to support its activity may substantially reduce its prey requirements. This could be especially true for the highly active running spiders. My results show that spiders that ate a marginal number of *Drosophila* were able to molt, if they also received nectar. Furthermore, spiders that can reduce their prey intake can also reduce their energy output and risk associated with attacking and subduing prey.

Why *C. inclusum* should have reduced its overall intake of prey when *Drosophila* was delayed to day 3 is not clear. For spiders without nectar, it may have been a simple lack of energy to subdue prey. But spiders that received nectar (for days 1 and 2) would presumably have sufficient energy. The dire consequences created by a delay in prey (53% reduction of first-molt survival, Fig. 2.4), hint at some possible protein requirement within the first two days of spiderling emergence. Fulfilling such a requirement would make nectar-fueled hunting all the more valuable.

Analyses of spider energetics, competition, and distribution have routinely taken into consideration the number and types of available prey. Vegetation is considered important as a support for webs, as refugia, or as a food source for the insects that spiders catch (Turnbull 1973; Uetz et al. 1978; Hatley & Macmahon 1980; Greenstone 1984; Bradley 1993; Uetz et al. 1999). Only recently have researchers considered the possibility of vegetation as a direct food source. Vogelei and Greissl (1989) speculated that newly emerged crab spiders deprived of prey must be supplementing their diets with nectar and pollen, because they survived longer than what their yolk reserves should allow. Pollard et al. (1995) actually observed adult male crab spiders drinking nectar from a variety of flowers during bouts of courtship. Taylor and Foster (1996) observed nocturnal running spiders of all ages and both sexes at floral and extrafloral plant nectaries. Ruhren and Handel (1999) and Jackson et al. (2001) observed jumping spiders at EFNs and found that the spiders preferred plants with EFNs to plants without, and sugar solutions to water, respectively.

The likelihood that nectar can and does play a nutritional role in the lives of some spiders should not be surprising for many reasons: Nectar's value as a dietary source of energy has been well established for nectarivorous insects, such as bees (reviewed inWaddington 1987) and butterflies (reviewed in Boggs 1987). Other predaceous arthropods are known to sustain themselves on plant nectar until they can locate suitable prey (Hagen 1987; van Rijn & Tanigoshi 1999). Spiders such as crab spiders, jumping spiders, and running spiders that wander in vegetation are likely to encounter nectar, especially if the plants have EFNs, which make the nectar accessible. Spiders can detect nectar with "gustatory" hairs on their tarsi (Foelix 1982; Barth 2002); *C. inclusum*, for example, orients immediately to sugar as soon as a tarsus touches it (pers. obs.). Spiders respond positively to nectar, as shown by preference tests, or by their willingness to ingest noxious chemicals, such as LSD, caffeine, and strychnine, if they are delivered in a sucrose solution (Christiansen et al. 1962; Witt 1971). And the spiders that have been analyzed (a theraphosid and an agelenid) have the means to digest nectar (sucrose) with the digestive enzyme sucrase (Pickford 1942; Mommsen 1977).

Running spiders and other wanderers that inhabit and lay their eggs in vegetation have the potential to expose their newly emerged offspring to extrafloral nectar. Nectar could add days to the spiders' survival and to their opportunity for encountering and catching prey. Even if conditions do not allow dispersal, my experiments with *C. inclusum* siblings crowded together show that they have lower mortality if they have access to nectar, possibly because they are less subject to sibling cannibalism (Fig. 2.2). Although adults and subadults were not part of these experiments, they have been observed at nectaries and nectar does, in fact, confer the same benefits to them (Chapter 3). Ultimately, the results of these experiments suggest that whether nectar contributes to a nectar-feeding spider's growth, or solely to its energetic needs, it allows that much more nutrition from prey to be directed to growth, and to reproduction (Chapter 3).

FIGURES

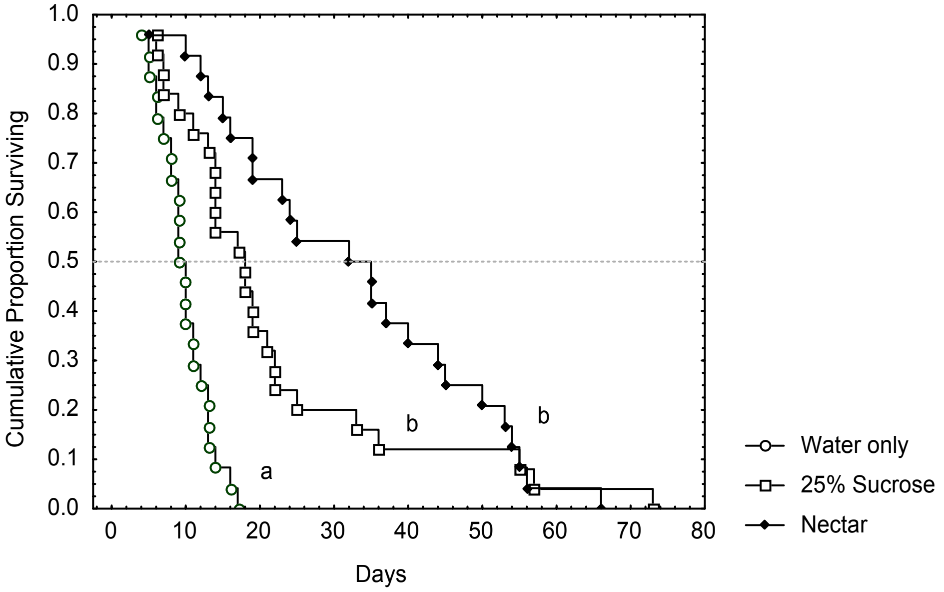

Fig. 2.1 Survival estimates (Kaplan-Meier) for newly emerged, individually housed, *H. velox*, fed water only (*N* = 24), 25% sucrose (*N* = 25), or extrafloral nectar (*N* = 24). Curves with different letters are significantly different at $\alpha < 0.05$ (adjusted pair-wise comparison rate $\gamma = 0.008$).

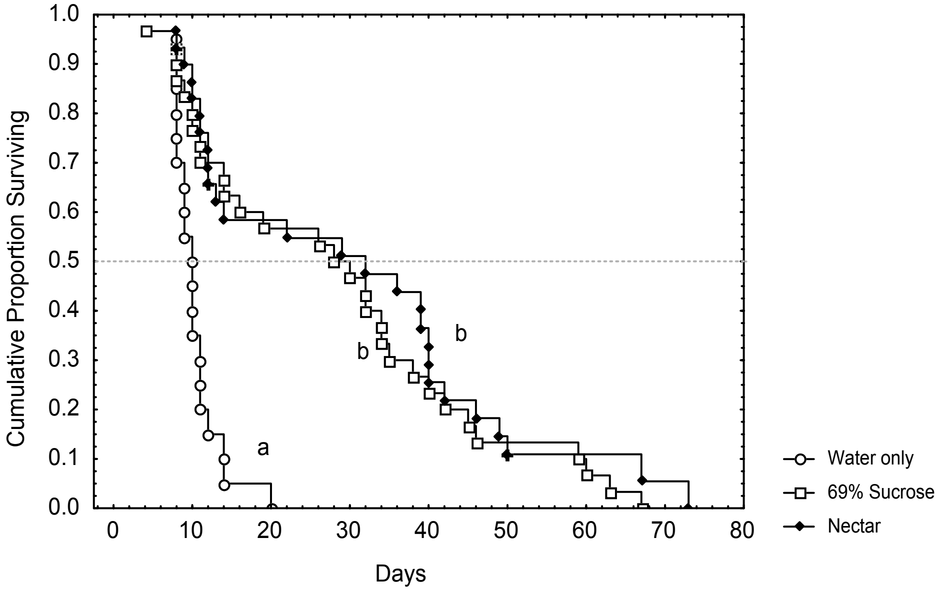

Fig. 2.2 Survival estimates (Kaplan-Meier) for newly emerged, individually housed, *H. velox*, fed water only (N = 20), 69% sucrose (N = 30), or extrafloral nectar (N = 30). Curves with different letters are significantly different at $\alpha < 0.05$ (adjusted pair-wise comparison rate $\gamma = 0.008$).

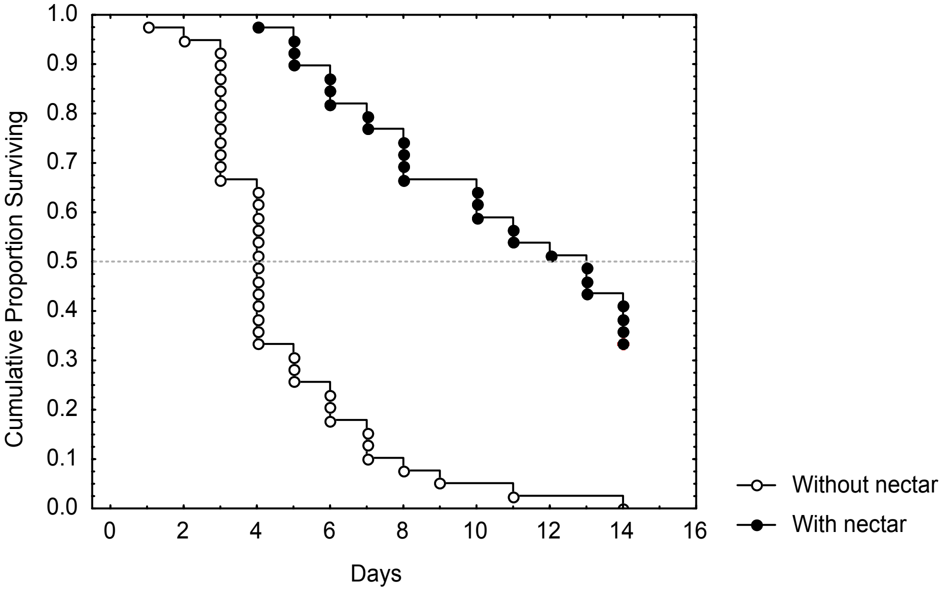

Fig. 2.3 Survival estimates (Kaplan-Meier) for newly emerged, cohabiting sibling *C. inclusum*, without (*N* = 39) or with (*N* = 39) nectar. All were able to cannibalize or scavenge bodies. Both treatment curves are the lumped results of 3 separate containers holding 13 spiders each.

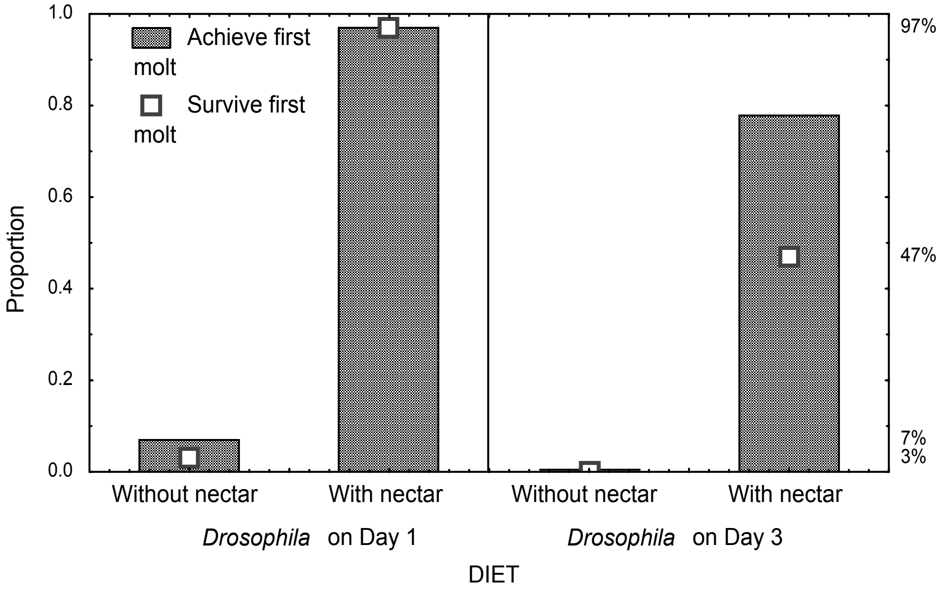

Fig. 2.4 Proportion of newly emerged, individually housed *C. inclusum* that achieved first molt, and the proportion (of total sample) that escaped the cast exoskeleton and survived first molt. Both were recorded for two separate experiments, one of which introduced *Drosophila* on Day 1, the other, *Drosophila* on Day 3. For both experiments, a single *Drosophila* was alternated daily with nectar or water.

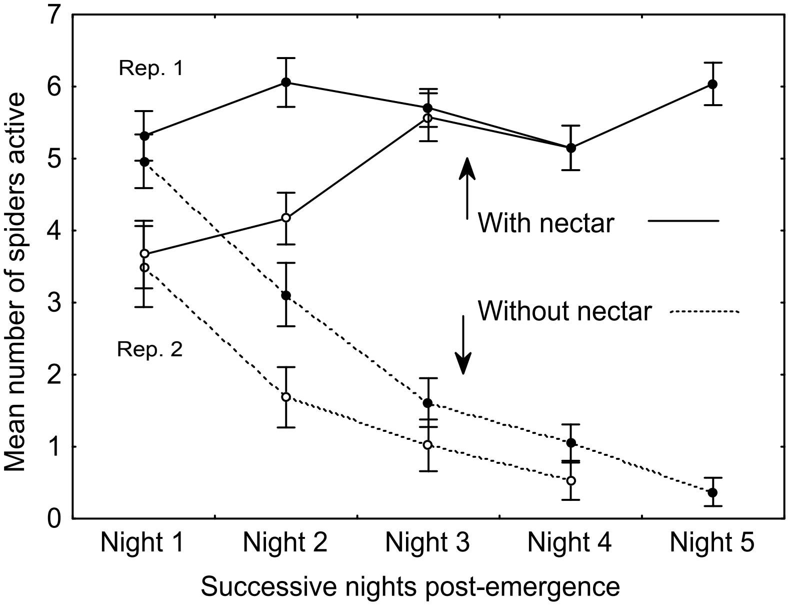

Fig. 2. 5. Replicates of nocturnal activity of newly emerged *C. inclusum* siblings, housed together, fed water only or nectar. All received water ad lib. Replicates came from separate egg sacs. Replicate 1 (solid circles), without nectar, *N* = 8; with nectar, *N* = 8. Replicate 2 (open circles), without nectar, *N* = 9, with nectar, *N* = 9. "Activity," is the average of the greatest number of spiders simultaneously running during a one-minute period at 10-minute intervals for 54 periods. Points are means, whiskers are 1.96± SE.

TABLES

Experiment	Diet	Mean ± 1 SE	Range (days))	*1st molt*	*N* =
1	Water only	9.8 ± .7	4–17	17%	24
1	25 % sucrose	22.0 ± 3.4	6–73	28%	25
1	Nectar	32.6 ± 3.6	5–66	50%	24
2	Water only	10.4 ± .7	8–20	0%	20
2	69% sucrose	28.4 ± 3.4	4–67	63%	30
2	Nectar	28.1 ± 3.5	8–73	52%	30

Table 2.1 Means (± 1 SE) and ranges of days survival and incidence of molting of newly emerged *H. velox* spiders in two experiments, on diets of Water only, 25% or 69% Sucrose, and (extrafloral) Nectar.

REFERENCES

Anderson, J.F. 1974. Responses to starvation on the spiders *Lycosa lenta* Hentz and *Filistata hibernalis* (Hentz). *Ecology*, 55: 576-585.

Baker, D.A., J. L. Hall, & J. R. Thorpe. 1978. A study of the extrafloral nectaries of *Ricinus communis*. *New Phytologist*, 81: 129-137.

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Barth, F.G. 2002. A Spider's World: Senses and Behavior. Springer. Berlin.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Boggs, C.L. 1987. Ecology of nectar and pollen feeding in lepidoptera. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Bradley, R.A. 1993. The influence of prey availability and habitat on activity patterns and abundance of *Argiope keyserlingi* (Araneae: Araneidae). *Journal of Arachnology*, 21: 91-106.

Buschman, L.L., W. H. Whitcomb, R. C. Hemenway, D. L. Mays, Ru Nguyen, N. C. Leppla, & B. J. Smittle. 1977. Predators of velvetbean caterpillar eggs in Florida soybeans. *Environmental Entomology*, 6: (3) 403-407.

Christiansen, A., R. Baum, & P. N. Witt. 1962. Changes in spider webs brought about by mescaline, psilocybin, and an increase in body weight. *Journal of Pharmacology and Experimental Therapeutics*, 136: 31-37.

Dalingwater, J.E. 1987. Chelicerate cuticle structure. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Greenstone, M.H. 1984. Determinants of web spider species diversity: vegetation structural diversity vs. prey availability. *Oecologia*, 62: 299-304.

Hagen, K.S. 1987. Nutritional ecology of terrestrial insect predators. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York.

Hardin, M.J., J. L. Willers, & T. L. Wagner. 1996. Nonparametric multiple comparisons of survivorship distributions. *Journal of Economic Entomology*, 89: (3) 715-721.

Hatley, C.L. & J. A. Macmahon. 1980. Spider community organization: Seasonal variation and the role of vegetation architecture. *Environmental Entomology*, 9: (5) 632-639.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Koptur, S. 1992. Extrafloral nectar-mediated interactions between insects and plants. *In* Insect-Plant Interactions. IV. (E.A. Bernays, ed.) CRC, Boca Raton, FL.

Mommsen, T.P. 1977. Digestive enzymes of a spider (*Tegenaria acrica* Koch) II. Carbohydrases. *Comp.Biochem.Physiol.*, 60A: 371-375.

Nentwig, W. 1987. The prey of spiders. *In* Ecophysiology of spiders. (W. Nentwig, ed.) Springer, New York.

Nyffeler, M., R. G. Breene, D. A. Dean, & W. L. Sterling. 1990. Spiders as predators of arthropod eggs. *Journal of Applied Entomology*, 109: 490-501.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Nyffeler, M. & W. L. Sterling. 1994. Comparison of the feeding niche of polyphagous insectivores (Araneae) in a Texas cotton plantation: estimates of niche breadth and overlap. *Environmental Entomology*, 23: (5) 1294-1303.

Peck, W.B. & W.H. Whitcomb. 1970. Studies on the biology of a spider *Chiracanthium inclusum* (Hentz). 753. Agricultural Experiment Station.

Percival, M.S. 1961. Types of nectar in angiosperms. *New Phytologist*, 60: 235-281.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 35: 33-72.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Riechert, S.E. 1992. Spiders as representative 'sit-and-wait' predators. *In* Natural Enemies: The Population Biology of Predators, Parasites and Diseases. (M.J. Crawley, ed.) Blackwell Scientific Publications, Oxford.

Riechert, S.E. & J.M. Harp. 1987. Nutritional ecology of spiders. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Samu, F., S. Toft, & B. Kiss. 1999. Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behavioral Ecology and Sociobiology*, 45: 349-354.

Smith, R.B. & T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. *Science*, 226: 1330-1333.

Stranzy, F. & S.F. Perry. 1987. Respiratory system: structure and function. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Turnbull, A.L. 1973. Ecology of the true spiders (Araneomorphae). *Annual Review of Entomology*, 18: 305-348.

Uetz, G.W. 1992. Foraging strategies of spiders. *Trends in Ecology and Evolution*, 7: (5) 155-158.

Uetz, G.W., J. Halaj, & A. B. Cady. 1999. Guild structure of spiders in major crops. *Journal of Arachnology*, 27: 270-280.

Uetz, G.W., A. D. Johnson, & D. W. Schemske. 1978. Web placement, web structure, and prey capture in orb-weaving spiders. *Bulletin of British Arachnological Society*, 4: (4) 141-148.

van Rijn, P.C.J. & L. K. Tanigoshi. 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, 23: 281-296.

Vogelei, A. & R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia (Berl.)*, 80: 513-515.

Waddington, K.D. 1987. Nutritional ecology of bees. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Wagner, J.D. & D. H. Wise. 1996. Cannibalism regulates densities of young wolf spiders: evidence from field and laboratory experiments. *Ecology*, 77: (2) 639-652.

Wise, D.H. 1993. Spiders in Ecological Webs. Cambridge University Press. Cambridge, MA.

Witt, P. 1971. Drugs alter web-building of spiders. A review and evaluation. *Behavioral Science*, 16: 98-113.

Wunnachit, W.C., C. F. Jenner, & M. Sedgley. 1992. Floral and extrafloral nectar production in *Anacardium occidentale* L. (Anacardiaceae): an andromonoecious species. *International Journal of Plant Science*, 153: 413-420.

CHAPTER 3
THE EFFECTS OF ADDING PLANT NECTAR TO FOUR LEVELS OF PROTEIN ON THE LIFE HISTORY OF THE RUNNING SPIDER *CHEIRACANTHIUM INCLUSUM*

3.1 Abstract

Until recently, spiders have been presumed to be exclusively carnivorous. Members of a large and diverse group of spiders known as wanderers, however, have been observed to feed on the floral and extrafloral nectaries of plants. Preference tests show that these spiders prefer a proxy nectar (sucrose solution) to water, and experiments show that sucrose contributes to their increased survival. Experiments also show that a marginal diet of prey supplemented with nectar allowed newly-emerged running spiders *Cheiracanthium inclusum* (Hentz) (Miturgidae) to survive and successfully molt, processes for the prey alone could not supply sufficient nutrition. Nectar appeared to do this by fueling the spider's activity (specifically, the high-energy, nocturnal running of *C. inclusum*) allowing it to allocate protein to maintenance and growth. The experiment herein examined the effects of dietary nectar on the life history of *C. inclusum* with special attention to fecundity. Four dietary levels of protein (estimated to be inadequate, meager, adequate and abundant) were each supplemented with extrafloral nectar. The source of nectar was extrafloral nectar from Indian almond (*Terminalia cattapa* L.). The protein source was the eggs of the lepidopteran *Helicoverpa zea*, (Boddie) (Lepidoptera: Noctuidae) the corn earworm, which *C. inclusum* encounters naturally and on which,

alone, it has been shown to develop quickly and normally. In the three protein-limited diets nectar 1) substantially increased the survival and growth of spiders on the inadequate diet; 2) provided the only means by which spiders on the meager diet reached functional maturity to mate and reproduce; and 3) increased body size and fecundity among females on the adequate protein diet, making them comparable with females receiving the abundant level of protein. Although nectar increased fecundity, it did not appear to affect offspring robustness, measured by how many days spiderlings survived without food post-emergence.

3.2 Introduction

All spiders are obligate carnivores, requiring the protein of prey for normal growth and development (Turnbull 1973; Foelix 1982). All spiders were assumed to be *exclusively* carnivorous and even to require the movement of prey to elicit a predatory response until running spiders had been observed to feed on the eggs of insects (Buschman et al. 1977; Nyffeler et al. 1990; Miliczky & Calkins 2002) and other spiders (Willey & Adler 1989). Sandidge (2003) has since demonstrated experimentally that the brown recluse *Loxoceles reclusa* Gertsch & Mulaik (Sicariidae) actually prefers dead prey to live. Outside of carnivory, juvenile orb-weavers were shown to gain nutrition by eating pollen grains trapped in their sticky webs, which they also eat and recycle (Smith & Mommsen 1984). More widespread, however, is the incidence of spider nectar feeding. Independent observations of members of a large and diverse group of spiders known as wandering spiders suggest that they are feeding at the floral and extrafloral nectaries of plants.

Vogelei and Greissl (1989) were the first to speculate that in addition to pollen, some spiders probably also feed on nectar, when they observed newly-emerged, prey-deprived crab spiders (Thomisidae) living longer than what their yolk reserves should allow. Pollard et al. (1995) actually observed adult male crab spiders drinking nectar between bouts of courtship. Taylor and Foster (1996) observed nectar feeding among all ages and both sexes of running spiders (Clubionidae, Anyphaenidae, and Corinnidae). All of these observations were accompanied by experiments showing that sucrose (to imitate nectar) contributed to the spiders' longevity. Ruhren and Handel (1999) observed jumping spiders (Salticidae) gathering nectar at the extrafloral nectaries (EFNs) of a wild legume, and showed experimentally that the spiders preferred plants with EFNs to those without. Most recently Jackson et al. (2001) demonstrated in the lab that 91 species of salticids preferred sucrose to water, after having observed 31 species in the field visiting nectaries.

Plant nectar is composed mainly of sucrose and its constituents, fructose and glucose, and is therefore high in energy (Percival 1961; Baker & Baker 1975; Baker & Baker 1983). Other predaceous arthropods, such as larval lacewings (Limburg & Rosenheim 2001), mites (van Rijn & Tanigoshi 1999), and bigeye bugs (Yokoyama 1978) have also been shown to increase their longevity on extrafloral nectar, but not their development or fecundity. On the other hand, experiments with ants, notorious nectar feeders (Bentley 1977), showed dietary sucrose to contribute to more eggs, larger larvae, and increased worker activity in *Myrmica rubra* L. (Hymenoptera: Formicidae), presumably by allowing protein to be redirected to these processes (Brian 1973). Studies of spiders have so far measured only nectar's contribution to survival, although Brian's studies with ants suggest that nectar has substantial potential as a dietary supplement. In this experiment, I followed the effect of dietary nectar on the life history of the temperate-zone, nocturnal running spider (or sac spider) *Chieracanthium inclusum* (Hentz) (now Mirturgidae, recently Clubionidae) from emergence, to functional maturity, to reproductive output. *C inclusum* and its relatives visit extrafloral nectaries (Taylor & Foster 1996) and it populates a variety of agricultural crops in the U.S., such as apple (Bajwa & Aliniazee 2001), grape (Roltsch et al. 1998; 1998), and cotton (Whitcomb et al. 1963; Agnew et al. 1982). I compared four dietary levels of protein (inadequate, meager, adequate, and abundant, each a five-fold increase), with and without plant nectar to answer these questions: 1) At various levels of protein, can the addition of nectar contribute to a spider's survival, growth, time of maturation and body size? 2) Can nectar contribute to a spider's fitness by increasing its fecundity? and, 3) Do the offspring of nectar-fed females show any gain in robustness, measured as the number of days that newly emerged spiderlings survive starvation?

3.3 Materials and Methods

3.3.1 Containers and diets for immatures

Spiders for the experiment were the offspring of lab-maintained male and female *C. inclusum* collected from USDA-BIRU cotton crops in Weslaco, Texas. I evenly distributed 160 newly emerged spiderlings from 5 egg masses of 5 different females among 8 diets: four levels of protein, with and without extrafloral nectar.

Individual spiders occupied 125 mm X 15 mm plastic petri dishes, turned upside-down so that the spider wove their sacs in the solid, uppermost part (= bottom) of the dish. Opening the dish usually did not disturb the spiders, which are nocturnal and quiescent during the day. One-half-inch cotton water wicks in each dish were resoaked three times a week when eggs and nectar were replaced. Water often condensed on that part of the dish above the wick, offering two sites of free water. Spiders were reared in incubators with a 14:10 light:dark cycle, at 24°C and relative humidity in the unhumidified incubator that cycled through daily highs (82 ± 7%) and lows (48 ± 8%). Spiders were daily rotated on the shelves and checked for mortality and molting.

For protein and lipids, spiders received the eggs of the corn earworm *Helicoverpa zea* (Boddie) (= *Heliothis zea*) (Lepidoptera: Noctuidae). *C. inclusum* encounters and eats *H. zea* eggs in crops (Nyffeler et al. 1990), and the eggs have been determined for *C. inclusum* in particular to be attractive and to meet all of the spider's nutritional requirements (R. Pfannenstiel, unpublished data). *C. inclusum* reaches normal functional maturity more quickly on a diet of *H zea* eggs alone than what had been previously determined for *C. inclusum* on a mixed diet (Peck & Whitcomb 1970). Eggs were supplied by the Weslaco, Texas, USDA-BIRU, where colonies are maintained in the Elzen lab by modified methods of Ignoffo (1965). (That is, adult moths were placed in 3.8-liter cardboard ice cream cartons lined with green florist paper for oviposition and with a feeding solution of 10% sucrose.) Four allotments of eggs—1 egg, 5 eggs, 25 eggs, and 125 eggs—delivered 3 times a week (M, W, and F) were deduced to be amounts of protein that were inadequate (i.e., near starvation), meager, adequate, and abundant.

I collected nectar from the extrafloral nectaries (EFNs) of Indian almond (*Terminalia cattapa*), a common tropical and subtropical beachfront tree growing in the OSU greenhouse, and stored it at – 45°C. From serial dilutions on a Reichert-Jung refractometer, I determined the EFN sugar concentration of *T. cattapa* to be 87.5%. This is nearly identical to the concentration of sugars (87.2%) from the EFNs of castor bean (*Ricinus communis* L.) (Baker et al. 1978), and is similar to the concentration of sugar (77.7%) from the EFNs of cashew (*Anacardium occidentale* L.) (Wunnachit et al. 1992). *Hibana velox* Becker (Anyphaenidae), a spider ecologically similar to *C. inclusum,* has been observed at the EFNs of cashew and castor bean and at the floral nectaries of Indian almond (Taylor & Foster 1996).

Nectar was delivered imprecisely with a microspatula because it was so viscous. The smallest transferable amount, about 1–2 μl, was smeared into a dimple drilled into a plastic "feeder" 1 cm X 2.5 cm. Nectar was refreshed 3 times a week, along with protein. NOTE: When 75% of the spiders on the 1-egg/with nectar diet had molted 3 times, they were switched to 69% sucrose, which has been shown to have an effect equivalent to the extrafloral nectar used here (Chapter 2). This substitution was necessary to conserve plant nectar for females that might reproduce. Spiders on the 1-egg diet were not expected to mature.

3.3.2 Containers and diet for reproductive females

Female *C. inclusum* mate only once and lay a series of egg masses (not more than 5) (Peck & Whitcomb 1970) each of which they guard until the spiderlings emerge (24 to 26 d, pers. obs.) When females reached functional maturity (usually determined by the presence of a fully sclerotized epigynum) they were mated with males, either wild-caught or from the 25-egg or 125-egg feeding groups (non-sibs). After mating, each female was housed in an upside-down 150mm X 25mm plastic petri dish to accommodate a more generous diet of eggs and a glass shell vial for egg masses. Immediately after mating females were placed on the same diet of 250 eggs, 3 times/week (M, W, F), until they deposited an egg mass. Food was then withheld while the female guarded the eggs and until the spiderlings emerged. The cycle was repeated for each female for 3 egg masses. This surfeit of eggs was to allow any female regardless of her historical nutritional status to yolk up the eggs that she had developed, presumably before she matured. Although it is generally understood that the more food a female spider gets in her lifetime, the more eggs she lays, oogenesis proper is not well understood. For the purpose of this experiment, I presumed that females were not capable of developing new eggs in response to any diet that they encountered post maturation (Miyazaki et al. 2001; Morishita et al. 2003; Choi & Moon 2003). To have maintained females on restricted diets throughout egg development and deposition risked females being too malnourished to yolk up whatever eggs they had developed (in response to the level of protein) and laying nothing at all.

When spiderlings emerged from each egg sac, 15 were selected at random (the rest, frozen and counted), and were maintained as described for immatures above. However, these spiderlings were starved and their days' survival used as a measure of offspring robustness.

Data Analysis

Analyses were performed with *Statistica* software(5.5 for Windows, StatSoft, Inc., 2000). ANOVA and ANCOVA were used if data met requirements of normality. Post hoc analysis employed Tukey's Honest Significant Difference (HSD) for unequal *N*. Offspring survival data could not be normalized and were tested pairwise with log-rank tests and corrected with Bonferroni adjustment (p / k-1).

3.4 Results

3.4.1 Effects of nectar on survival, growth, and maturation

The experiment was ended on day 266, when spiderlings from the third egg mass of the last reproductive female had emerged. This was long after females on the 25-egg (adequate) and 125-egg (abundant), with (+N) and without (–N) nectar diets had deposited and guarded 3 egg masses.

By day 266, spiders receiving 1-egg/–N had all died before their third molt (Table 3.1). No spider on the 1-egg diet had matured; however, 13/20 spiders on the 1-egg/+N diet were still alive. Of these 13, all had molted 5 or 6 times (one 7). The instar durations of these spiders were far longer than the instars of better-fed spiders (Fig. 3.1).

No spider on the 5-egg/–N diet had matured, although 11 of 20 were still alive. Of these 11, all but 1 had molted 5 or 6 times. The instar duration of these, too, were very long, compared to spiders in the higher protein levels (Fig. 3.1).

All spiders on the 5-egg (meager)/+N diet had matured and mated and, where possible, guarded 3 egg masses.

3.4.2 Effects of nectar on functional maturity and reproductive competence

No spider on the 1-egg diet matured, whether they received nectar or not.

Spiders on the 5-egg diet matured *only* if they also received nectar. All 20 spiders matured to adulthood on the 5-egg/+N diet, although reproductive competence was not guaranteed. Three of the 11 males had either malformed palps or palps that pulled away completely during the final molt. All typically dark and hard structures such as palps and chelicerae were pale, suggesting inadequate protein for proper sclerotization and/or a problem with melanisation (Dalingwater 1987). Six males contributed to 10 matings, producing seven viable egg masses (2 of these males contributed to zero egg masses). The males were mated with healthy, well-fed females reared especially for that purpose, so as not to confound potential female reproductive incompetence with male incompetence on such a low-protein diet. Likewise, females were mated with well-fed males; only 4 of 9 laid egg masses. One male, however, and one female, both from the 5-egg/+N diet were intentionally mated, and she produced 3 viable egg masses, the only female among 9 on the diet to do so. She contributed 129 of the diet's 188 total offspring (Fig. 3.4 and Table 3.2).

Spiders receiving 25 eggs/+N, and –N, matured and reproduced normally, although a single female in the 25 egg/–N diet spent 61 days in a sixth instar to reach maturity (Fig. 31. and Table 3.1). Spiders receiving 125 eggs/+N and –N matured quickly and normally. Females that received nectar in this group were the most fecund.

3.4.3 Effects of nectar on body size and fecundity

To measure possible contributions of dietary nectar to a female's fecundity I used analysis of covariance (ANCOVA), which factors in the size of the spider. ANCOVA models one of the strongest relationships for spiders, body size and egg production (Petersen 1950; Enders 1976; Marshall & Gittleman 1994; Simpson 1995), via regression, simultaneously providing tests of strength of any effects of the design factors (nectar supplementation and "protein," which is used herein as a shorthand reference to the *H. zea* egg component of the diets) beyond their effect on body size. If either protein or nectar come out with strong effects in the ANCOVA, it indicates effects beyond what can be explained by body size alone. However, even if protein and nectar show statistically insignificant effects on egg production directly, a simple ANOVA (with body size as the variable) will reveal their direct effects on body size. Should either nectar or protein (or both) be statistically significant in the ANOVA, then diet contributes indirectly to eggs by contributing directly to body size. I estimated body size by carapace width (measured with a microscope ocular micrometer), a sclerotized part of the spider that changes least within an instar (compared to the expandable abdomen) and is least likely to reflect a spider's current nutritional state (Dondale 1961; Hagstrum 1971). Only adult carapaces (males and females) were measured, eliminating from comparison all of the spiders on both 1-egg diets and spiders on the 5-egg/−N diet, none of which matured after 266 days. All of the spiders on the 5-egg/+N diet matured, and their average measurements are provided in graphs for comparison. They could not be included in the ANCOVA or ANOVA, however, because there were no adults from the 5-egg/−N diet for statistical comparison.

Results of ANCOVA show that carapace width was the only significant predictor of fecundity ($F_{1, 29} = 4.611$, $p = 0.040$). Protein ($p = 0.170$), nectar ($p = 0.145$), and protein*nectar ($p = 0.500$) did not show significant effects (Fig. 3.2). In an ANOVA, however, protein level was a highly significant predictor of carapace width ($F_{1,31} = 22.883$, $p < 0.0001$); nectar was not significant ($p = 0.677$). Given nectar's obvious contributions to survival, growth, and maturation in the low-protein diets, however, these data bear further analysis. Specifically, given that protein is a highly significant predictor of carapace width, which in turn predicts fecundity, and that nectar has been shown (Chapter 2) to increase a spider's productive use of protein by supplying the energy for activity, it may be that in diets with adequate and abundant protein, nectar does influence carapace width and fecundity, but to a degree that is too subtle to make nectar a predictor for ANCOVA models.

3.4.4 Post hoc analysis

Post hoc comparisons (Tukey's HSD) show that *within* each of the levels of adequate (25-egg) and abundant (125-egg) protein, nectar made no significant contribution to mean carapace width of females ($p = 0.924$, $p = 0.977$, respectively) (Fig. 3.3). Comparisons *between* levels of protein, on the other hand, suggest nectar's contribution. Although diets of 25 eggs ($N = 9$) and 125 eggs ($N = 8$), without nectar, showed a significant difference in female carapace width, as might be expected between diets with a five-fold difference in protein ($p = 0.027$), adding nectar to the 25-egg diet increased the mean carapace width enough so that there was no significant difference between diets of 25 eggs ($N = 12$) and 125 eggs (without nectar), despite the five-fold difference in protein ($p = 0.156$). Similarly, adding nectar to the 125-egg diet increased mean carapace width sufficiently to make it significantly different from those getting 25-egg/+N ($p = 0.028$) (Fig. 3.3). Spiders on the 5-egg/+N ($N = 9$) diet can be included in these comparisons, because each diet is treated separately, and although they are significantly smaller than all of the other groups ($p < 0.02$ for all), their total egg production is statistically comparable to that of females in the 25 egg/–N diet (Fig. 3.4).

Female fecundity (total number of offspring from all egg cases) follows the same trend as body size. Post-hoc comparisons (Tukey's HSD for unequal N) of total offspring from 3 sequential egg masses show that *within* levels of adequate (25-egg) and abundant (125-egg) protein, nectar makes no significant contribution to fecundity (p = 0.401 and 0.998, respectively) (Fig. 3.4). Comparisons *between* levels of protein show a significant difference between diets of 25 eggs ($N = 8$) and 125 eggs ($N = 7$) when nectar is absent ($p = 0.039$). This difference is no longer significant when nectar is present in the 25-egg treatment ($p = 0.677$) (Fig. 3.4). Furthermore, females on the 5-egg/+N diet also bore comparison with a diet five-fold higher in protein: there was no statistically significant difference in fecundity between females on the 5-egg/+N diet (although, N = 4) and females on the 25-egg/–N diet ($N = 8$, $p = 0.262$) (Fig. 3.4).

3.4.5 Offspring robustness

Offspring robustness, measured as the number of days that newly emerged spiderlings survived starvation, was apparently negatively affected by the presence of nectar in the mother's diet. Reproductive females on an adequate protein diet without nectar (25-egg/–N) produced offspring that were significantly more robust than all of the others (pairwise log-rank test with Bonferonni adjustment [p/k-1]) (Fig. 3.5). This was the trend for all 3 of the egg masses produced by females on this diet, and the reason for this difference is unclear.

3.5 Discussion

Although nectar feeding has been observed in running spiders, jumping spiders, and crab spiders, nectar's dietary contributions may be most obvious in the lightening-quick running spiders. These spiders are quiescent in a woven resting cell during the day, but are in near constant motion at night (pers. obs.). If nectar could fuel this high level of activity, then a substantial amount of the nutritional benefits of prey should be reallocated to survival, growth, and reproduction. In an inadequate diet of protein (1 egg) that allowed spiders to molt only twice then die, the addition of nectar sustained survival and allowed spiders to molt 5 to 7 times. In a meager protein diet (5 eggs), protein was sufficient for growth, but spiders matured only if they also received nectar. In diets of adequate (25 eggs) and abundant (125 eggs) protein, all of the spiders matured and reproduced, and nectar made subtle but measurable contributions. Although nectar does contain amino acids (Baker et al. 1978), studies of other nectar-feeding predators suggest that nectar makes all of these contributions indirectly, by way of supporting activity so that protein can be reallocated (van Rijn & Tanigoshi 1999; Limburg & Rosenheim 2001).

Spider nectar feeding is notable in both basic and applied biology. The presumed energy budgets of spiders, once considered to be fueled and limited exclusively by prey, must now be expanded a trophic level, recognizing some spiders as primary consumers as well as secondary. Where nectar is available, obtaining nectar costs less in risk and energy than trapping and subduing prey. Furthermore, sucrose (and its constituents, glucose and fructose), compose the bulk of plant nectar (Percival 1961; Baker & Baker 1975; Baker & Baker 1983). As simple carbohydrates high in energy, these can fuel the activity of spiders, allowing them to allocate more costly nutrients from prey to growth and reproduction. Experiments with running spiders on marginal protein diets of *Drosophila* support this scenario (Chapter 2). Although in these experiments nectar made only slight contributions to a diet that is abundant in protein, spiders in nature are thought often to be prey limited (Nentwig 1987; Nyffeler et al. 1987; Wise 1993; Nyffeler & Sterling 1994), making nectar all the more valuable as a nutritional stopgap.

From an applied aspect, in agricultural crops, all spiders are considered beneficial. Spiders are presumed to initially populate crops as the random outcome of ballooning on air currents (Bailey & Chada 1968; Riechert & Lockley 1984; Greenstone et al. 1987;

Sunderland & Samu 2000) or as a result of inhabiting nearby habitats (Uetz et al. 1999), but the assemblages of spiders that ultimately survive and reproduce is not random (Luczak 1980; Young & Edwards 1990). The North American spider fauna, for example, comprises an estimated 59% web spinners and 41% wanderers; in North American field crops, however, this representation is reversed—44% webspinners and 56% wanderers. Moreover, some spiders occupy some crops more readily than they occupy others. This preponderance of some spiders over others has been attributed to a particular crop's characteristics such as its various microclimates, insect prey that inhabit it, or plant architecture that can determine favorable web sites (Turnbull 1973; Uetz et al. 1978; Luczak 1979; Young & Edwards 1990; Riechert 1998; Uetz et al. 1999). It may be that a favorable resting cell site explains why *C inclusum* prefers to occupy the underside of cotton plant leaves early in the season and then move to the underside of floral bracts when blossoms form (Whitcomb et al. 1963). It may also be that the underside of leaves is the site of cotton's foliar EFNs (Yokoyama 1978; Wäckers et al. 2001), and the underside of bracts, the site of cotton's subbracteal EFNs (Butler, Jr. et al. 1972), which produce an even richer nectar (Limburg & Rosenheim 2001). For this reason, plants, which are usually thought to serve as web attachment sites or shelter for spiders, should be reconsidered as a direct source of food for some. Spider nectar feeding and the presence of accessible nectar on plants, such as cotton, should be examined as one of the possible "sieves" (Luczak 1980) that help determine what types of spiders survive and reproduce in different crops.

FIGURES

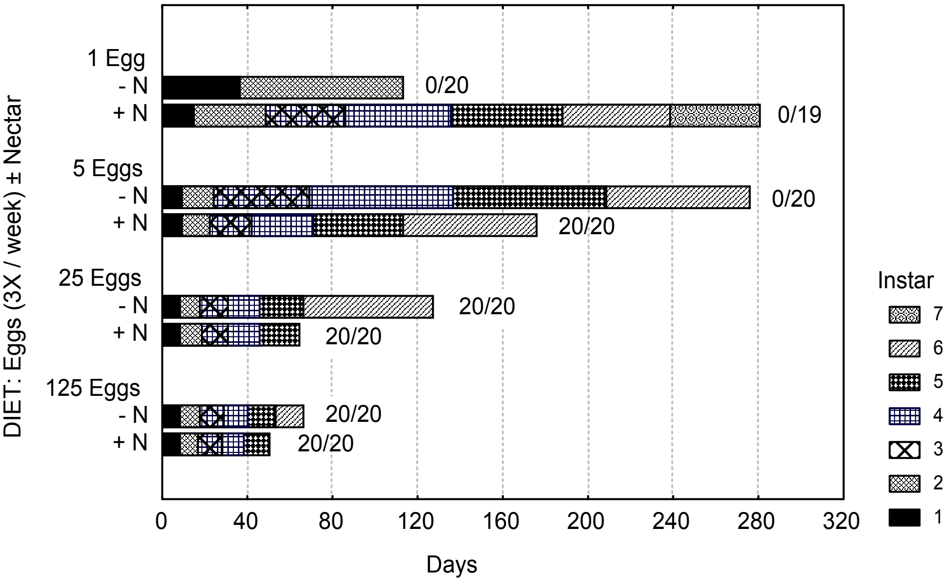

Fig. 3.1. Average instar duration (days) on 8 diets. All groups started with 20 spiders. Proportions at the ends of bars are numbers of total sample that had reached maturity by the end of the experiment (day 266) (See Table 3.1).

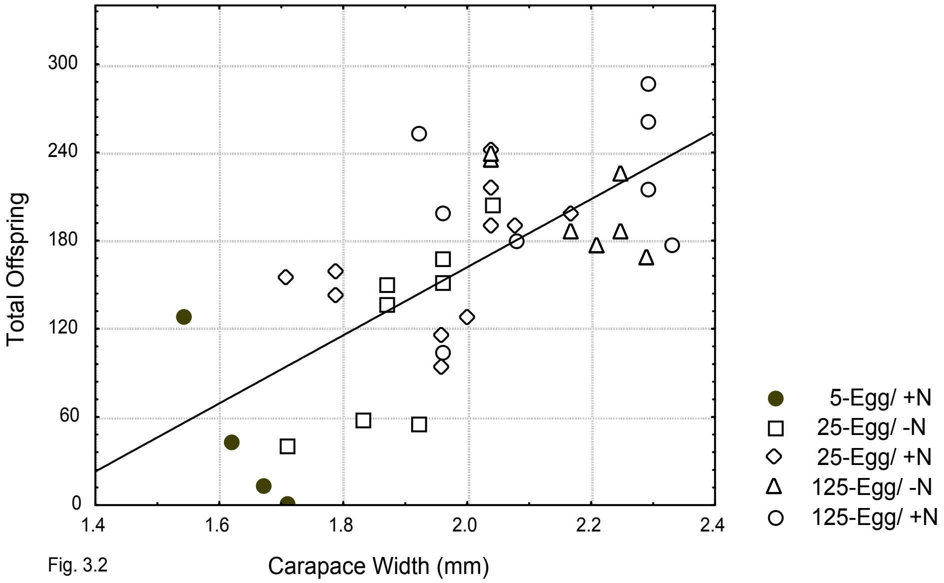

Fig. 3.2 Regression of body size (carapace width) on total number of off spring (fecundity).

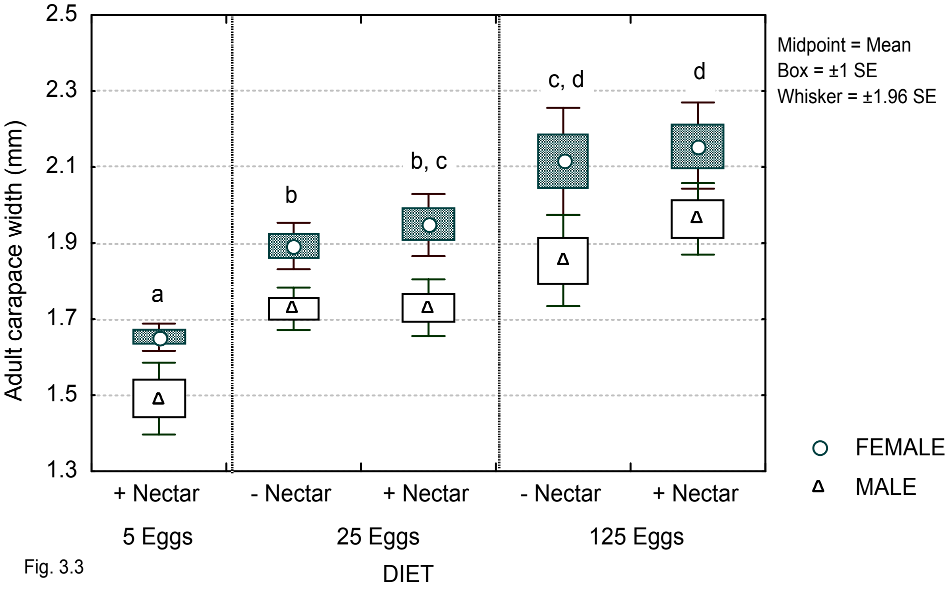

Fig. 3.3 Adult carapace widths (mm), of spiders on a fixed protein diet, with and without nectar. None of the spiders on the 5-egg/–N diet matured. Boxes ("female") labeled with different letters are significantly different (post hoc analysis, $p < 0.05$).

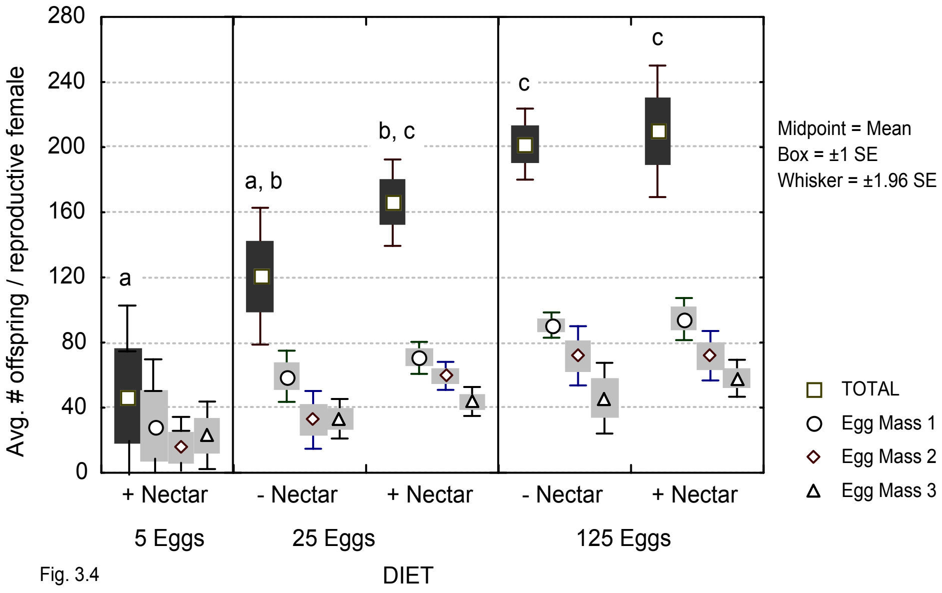

Fig 3.4 Average offspring production per reproductive female in five diets, per egg mass and overall. A single female produced 65% of the eggs in the 5-egg/+Nectar diet. Boxes ("Total") labeled with different letters are significantly different (post hoc analysis, $p < 0.05$).

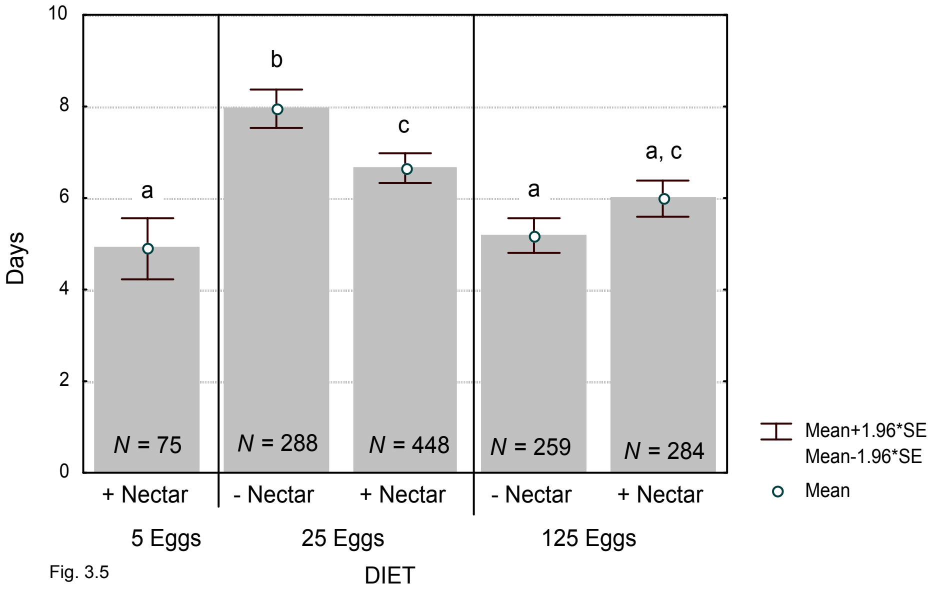

Fig. 3.5 Average survival in days of newly emerged spiderlings that were starved. Fifteen spiderlings were randomly collected from each egg sac (Table 3.2). Columns with the same letter are not significantly different (log-rank pairwise comparison with Bonferonni correction, $p < 0.05$).

TABLES

Diet	Instar / Avg. Duration (d)							Day 266	
Egg/Nectar	1	2	3	4	5	6	7	Survive	Adult
1 –Nectar	36.	77							
(*n* / *N*)	17/20	2/20	0					0/20	0/20
1 +Nectar	15	34	37	49	52	51	42		
n / *N*	20/20	19/19	18/19	18/19	15/19	10/19	1/19	15/19	0/19
5 –Nectar	9	16	44	68	72	68			
n / *N*	20/20	20/20	19/20	16/20	13/20	2/20		13/20	0/20
5 + Nectar	9	14	19	29	41	63			
n / *N*	20/20	20/20	20/20	20/20	19/20	1/20		20/20	20/20
25 –Nectar	8	10	13	15	20	61			
n / *N*	20/20	20/20	20/20	20/20	15/20	1/20		20/20	20/20
25 +Nectar	9	10	12	14	19				
n / *N*	20/20	20/20	20/20	20/20	9/20			20/20	20/20
125–Nectar	9	9	11	12	13	13			
n / *N*	20/20	20/20	20/20	20/20	14/20	1/20		20/20	20/20
125+Nectar	8	9	11	11	12				
n / *N*	20/20	20/20	20/20	20/20	15/20			20/20	20/20

Table 3.1 Average duration of instars in days; individuals alive at day 266; and individuals that reached adulthood. Change in *N* (from 20 to 19) indicates an escape. *n*/*N* = subsample of sample for the particular state (e.g. all of 125 eggs/+N reached instar 4. Five became adult during that instar and the other 15 reached adulthood in instar 5).

Diet: Eggs/Nectar	Adult Carapace Width		Reproductive Females, *N*=	Total Egg Masses	Total Offspring
	Males, *N*=	Females, *N*=			
5 / +N	11	9	4	9	188
25 / –N	11	9	8	23	966
25 / +N	8	12	11	31*	1825
125 / –N	10	9	7	20*	1413
125 / +N	11	9	8	22*	1678

Table 3.2 Numbers of adults measured for carapace width, and female fecundity by diet. * Survival data from five initial egg masses (from 3 diets) were lost to high humidity: 2 each from 25/+N and 125/–N, and 1 from 125/+N.

REFERENCES

Agnew, C.W., W. L. Sterling, & D. A. Dean. 1982. Influence of cotton nectar on red imported fire ants and other predators. *Environmental Entomology*, 11: 629-634.

Bailey, C.L. & H. L. Chada. 1968. Spider populations in grain sorghums. *Annals of the Entomological Society of America*, 61: (3) 567-571.

Bajwa, W.I. & M. T. Aliniazee. 2001. Spider fauna in apple ecosystem of western Oregon and its field susceptibility to chemical and microbial insecticides. *Journal of Economic Entomology*, 94: (1) 68-75.

Baker, D.A., J. L. Hall, & J. R. Thorpe. 1978. A study of the extrafloral nectaries of *Ricinus communis*. *New Phytologist*, 81: 129-137.

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Baker, H.G., P. A. Opler, & I. Baker. 1978. A comparison of the amino acid complements of floral and extrafloral nectars. *Botanical Gazette*, 139: (3) 322-332.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Brian, M.V. 1973. Feeding and growth in the ant *Myrmica*. *Journal of Animal Ecology*, 42: 37-53.

Buschman, L.L., W. H. Whitcomb, R. C. Hemenway, D. L. Mays, Ru Nguyen, N. C. Leppla, & B. J. Smittle. 1977. Predators of velvetbean caterpillar eggs in Florida soybeans. *Environmental Entomology*, 6: (3) 403-407.

Butler, G.D., Jr., G. M. Loper, S. E. McGregor, J. L. Webster, & H. Margolis. 1972. Amounts and kinds of sugar in the nectars of cotton (*Gossypium* spp.) and the time of their secretion. *Agronomy Journal*, 64: 364-368.

Choi, Y.-S. & M.-J. Moon. 2003. Fine structure of the ovarian development in the orb-web spider *Nephila clavata*. *Korean Journal of Entomology*, 33: (1) 25-32.

Costello, M.J. & K. M. Daane. 1998. Influence of ground cover on spider populations in a table grape vineyard. *Ecological Entomology*, 23: 33-40.

Dalingwater, J.E. 1987. Chelicerate cuticle structure. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Dondale, C.D. 1961. Life histories of some common spiders from trees and shrubs in Nova Scotia. *Canadian Journal of Zoology*, 39: 777-787.

Enders, F. 1976. Clutch size related to hunting manner of spider species. *Annals of the Entomological Society of America*, 69: (6) 991-998.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Greenstone, M.H., C. E. Morgan, A.-L. Hultsch, R. A. Farrow, & J. E. Dowse. 1987. Ballooning spiders in Missouri, USA, and New South Wales, Australia: Family and mass distributions. *Journal of Arachnology* , 15: 163-170.

Hagstrum, D.W. 1971. Carapace width as a tool for evaluating the rate of development of spiders in the laboratory and the field. *Annals of the Entomological Society of America*, 64: (4) 757-760.

Ignoffo, C.M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). II. Biology and propagation of diet-reared *Heliothis*. *Journal of Invertebrate Pathology*, 7: 217-226.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Limburg, D.D. & J. A. Rosenheim. 2001. Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology*, 30: (3) 595-604.

Luczak, J. 1979. Spiders in agrocoenoses. *Polish Ecological Studies*, 5: (1) 151-200.

Luczak, J. 1980. Spider communities in crop fields and forests of different landscapes of Poland. *Polish Ecological Studies*, 6: (4) 735-762.

Marshall, S.D. & J. L. Gittleman. 1994. Clutch size in spiders: is more better? *Functional Ecology*, 8: 118-124.

Miliczky, E.R. & C. O. Calkins. 2002. Spiders (Araneae) as potential predators of leafroller larvae and egg masses (Lepidoptera: Tortricidae) in Central Washington apple and pear orchards. *Pan-Pacific Entomologist*, 78: (2) 140-150.

Miyazaki, K., R. Ueshima, & T. Makioka. 2001. Structure of the female reproductive system and oogenetic mode in a schizomid, *Schizomus sawadai* (Arachnida, Schizomida). *Invertebrate Reproduction and Development*, 40: (1) 1-7.

Morishita, R., S. A. Ferreira, A. S. Filha, & C. D. Faraco. 2003. Studies on oogenesis and oviposition in the brown spider *Loxosceles intermedia* (Araneae: Sicariidae). *The Anantomical Record Part A*, 273A: 575-582.

Nentwig, W. 1987. The prey of spiders. *In* Ecophysiology of spiders. (W. Nentwig, ed.) Springer, New York.

Nyffeler, M., R. G. Breene, D. A. Dean, & W. L. Sterling. 1990. Spiders as predators of arthropod eggs. *Journal of Applied Entomology*, 109: 490-501.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Nyffeler, M. & W. L. Sterling. 1994. Comparison of the feeding niche of polyphagous insectivores (Araneae) in a Texas cotton plantation: estimates of niche breadth and overlap. *Environmental Entomology*, 23: (5) 1294-1303.

Peck, W.B. & W.H. Whitcomb. 1970. Studies on the biology of a spider *Chiracanthium inclusum* (Hentz). 753. Agricultural Experiment Station.

Percival, M.S. 1961. Types of nectar in angiosperms. *New Phytologist*, 60: 235-281.

Petersen, B. 1950. The relation between size of mother and number of eggs and young in some spiders and its significance for the evolution of size. *Experientia*, 6: (3) 96-98.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Riechert, S.E. 1998. The role of spiders and their conservation in the agroecosystem. *In* Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. (C.H. Pickett & R.T. Bugg, eds.) Univ. of Calif. Press, Berkeley, CA.

Riechert, S.E. & T. Lockley. 1984. Spiders as biological control agents. *Annual Review of Entomology*, 29: 299-320.

Roltsch, W., R. Hanna, F. Zalom, H. Shorey, & M. Mayse. 1998. Spiders and vineyard habitat relationships in central California. *In* Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. (C.H. Pickett & R.T. Bugg, eds.) Univ. of Calif. Press, Berkeley.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Sandidge, J.S. 2003. Scavenging by brown recluse spiders. *Nature*, 426: (6 November) 30-30.

Simpson, M.R. 1995. Convariation of spider eggs and clutch size: The influence of foraging and parental care. *Ecology*, 76: (3) 795-800.

Smith, R.B. & T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. *Science*, 226: 1330-1333.

Sunderland, K. & F. Samu. 2000. Effects of agricultural diversification on the abundance, distribution, and pest control potential of spiders: a review. *Entomologia Experimentalis et Applicata*, 95: 1-13.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Turnbull, A.L. 1973. Ecology of the true spiders (Araneomorphae). *Annual Review of Entomology*, 18: 305-348.

Uetz, G.W., J. Halaj, & A. B. Cady. 1999. Guild structure of spiders in major crops. *Journal of Arachnology*, 27: 270-280.

Uetz, G.W., A. D. Johnson, & D. W. Schemske. 1978. Web placement, web structure, and prey capture in orb-weaving spiders. *Bulletin of British Arachnological Society*, 4: (4) 141-148.

van Rijn, P.C.J. & L. K. Tanigoshi. 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, 23: 281-296.

Vogelei, A. & R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia (Berl.)*, 80: 513-515.

Wäckers, F.L., D. Zuber, R. Wunderlin, & F. Keller. 2001. The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, 87: 365-370.

Whitcomb, W.H., H. Exline, & R. C. Hunter. 1963. Spiders of the Arkansas cotton field. *Annals of the Entomological Society of America*, 56: 652-660.

Willey, M.B. & P. H. Adler. 1989. Biology of *Pucetia viridans* (Araneae, Oxyopidae) in South Carolina, with special reference to predation and maternal care. *Journal of Arachnology*, 17: 275-284.

Wise, D.H. 1993. Spiders in Ecological Webs. Cambridge University Press. Cambridge, MA.

Wunnachit, W.C., C. F. Jenner, & M. Sedgley. 1992. Floral and extrafloral nectar production in *Anacardium occidentale* L. (Anacardiaceae): an andromonoecious species. *International Journal of Plant Science*, 153: 413-420.

Yokoyama, V.Y. 1978. Relation of seasonal changes in extrafloral nectar and foliar protein and arthropod populations in cotton. *Environmental Entomology*, 7: 799-802.

Young, O.P. & G. A. Edwards. 1990. Spiders in United States field crops and their potential effect on crop pests. *Journal of Arachnology*, 18: 1-27.

CHAPTER 4
NECTAR FEEDING AS A COMMON OCCURRENCE AMONG WANDERING SPIDERS: EVIDENCE FROM A TEXAS COTTON CROP

4.1 Abstract

The temperate-zone, nocturnal running spider *Cheiracanthium inclusum* has been shown experimentally to utilize the sugars of plant nectar to sustain its high level of activity. This allocation of sugar to activity allowed the spider to reallocate more precious protein to growth and reproduction. In nature, protein costs more to obtain in terms of energy and risk. Running spiders and other wanderers that occupy vegetation have been observed at floral and extrafloral nectaries, and sucrose (a proxy nectar) has been shown to increase their longevity. The cursorial nature of these spiders gives them the opportunity to encounter nectar, but they also have the means to detect nectar, respond positively to nectar, ingest nectar, and to digest and utilize nectar. Wandering spiders that occupy vegetation with productive extrafloral nectaries, therefore, should be ingesting nectar. Likewise, a test that can detect the presence of ingested fructose, a plant-derived sugar, should show some spiders to be positive. These two predictions were borne out by samples of spiders collected from cotton plants (*Gossypium hirsutum*) with productive extrafloral nectaries. During two consecutive growing seasons, 32% and 23% (respectively) of wandering spiders tested positive for fructose. Four families of

spiders were represented among the positives Anyphaenidae, Miturgidae, Thomisidae, and Oxyopidae. Oxyopidae is new to the growing list of nectar-feeding spiders.

4.2 Introduction

All spiders are predaceous; most are generalists that feed on insects and often other spiders. Until recently, all spiders were presumed to be *exclusively* predaceous, relying solely on the protein of prey for their maintenance, growth, and reproduction (Foelix 1982). Orb-weaving spiderlings, however, benefit nutritionally from pollen grains trapped in their webs (Smith & Mommsen 1984), and a small but growing number of anecdotal observations place some spiders at the floral and extrafloral nectaries of plants, presumably feeding on plant nectars. Sucrose, a constituent of plant nectar, has been shown experimentally to increase the longevity of these spiders (Edmunds 1978; Vogelei & Greissl 1989; Pollard et al. 1995; Taylor & Foster 1996; Ruhren & Handel 1999; Jackson et al. 2001). Plant nectars contain primarily simple sugars and water. Nectars also contain amino acids, lipids, vitamins, and minerals (Baker & Baker 1975; 1983; Koptur 1992). They are well known to fuel the adult lives of nectarivorous insects, many of which are also prey for spiders. It may be, then, that some spiders have expanded their diet an additional trophic level to engage in nectarivory themselves. By drinking nectar directly, spiders tap into a source of fuel that can be more abundant than prey, that poses less risk to obtain than prey, and that is more fuel efficient taken directly (primary consumption) rather than by way of nectarivorous prey (secondary consumption).

Laboratory experiments with two spiders previously observed at nectaries, *Cheiracanthium inclusum* (Hentz) (Miturgidae, formerly Clubionidae) and *Hibana velox*

(Becker) (Anyphaenidae), (Taylor & Foster 1996) showed that plant nectar contributed significantly to survival, growth, and fecundity, apparently by allowing a spider to allocate more rare and expensive protein to these processes, while allocating nectar to activity (Chapters 2 and 3). Other predaceous arthropods have also been shown to survive periods of prey deprivation by feeding on plant nectars (Yokoyama 1978; van Rijn & Tanigoshi 1999; Limburg & Rosenheim 2001), and laboratory experiments with ants showed a nectar proxy (sucrose) also to increase egg number, larval size, and worker activity (Brian 1973).

Nectar is exuded at floral nectaries and also at extrafloral nectaries (EFNs), which, as their name implies, reside outside of floral structures, often on leaves or petioles, in the form of slits or cups or slightly differentiated tissue. EFNs make plant nectar available to arthropods, such as ants (Bentley 1977) that do not ordinarily visit flowers but that do occupy vegetation. Among the spiders reported to have visited plant nectaries, all live in vegetation foliage, and all are members of a large group of spiders called wanderers. Unlike spiders that use webs, wandering spiders catch their prey by active hunting. They exhibit activity levels at both extremes, from the globular, almost sedentary crab spiders to the frenetic, elusive, running spiders. *C. inclusum* and *H. velox* are nocturnal running spiders that are particularly elusive during their periods of activity. They spend so much time in motion, presumably in search of prey, that, where EFNs are present and active, these spiders in particular are likely to encounter extrafloral nectar. Upon encountering nectar, some spiders can undergo a sequence of events, that, together, support the investigation of nectar-feeding as a common occurrence: 1) gustatory hairs on their tarsi allow spiders to taste the nectar (Barth 2002); 2) they respond positively to the sweet taste of nectar and readily drink it (Christiansen et al. 1962; Witt 1971); 3) if the nectar is in the form of the disaccharide sucrose, they can enzymatically break it into its monosaccharide components fructose and glucose (Pickford 1942; Mommsen 1977); 4) the monosaccharides can be absorbed (Treherne 1967) and utilized to fuel activity, as nectar fuels the adult lives of other nectarivorous arthropods, such as bees (reviewed in Waddington 1987) and butterflies (reviewed in Boggs 1987).

To test this hypothesis that wandering spiders should ingest plant nectar whenever they encounter it, I collected spiders from cotton crops and analyzed their gut contents for fructose—a plant-derived sugar. Cotton crops are known to support populations of running spiders and other wanderers (Whitcomb et al. 1963; Agnew et al. 1982; Adjei-Maafo & Wilson 1983; Nyffeler et al. 1987; Nyffeler & Sterling 1994), and the cotton plant itself has productive foliar, and subbracteal and circumbracteal EFNs, (Butler, Jr. et al. 1972; Yokoyama 1978; Wäckers et al. 2001).

4.3 Materials and Methods

For the summers of 2002 and 2003, I collected spiders from experimental crops of unsprayed USDA cotton, *Gossypium hirsutum* L. (Deltapine 54-14 RR) covering 2 acres at the USDA-BIRU research center in Weslaco, Texas. Although the collection focused on three species of running spiders, *Cheiracanthium inclusum, Hibana futilis* and *H. arunda*, other wandering spiders were also collected but identified only to family. The first summer's collection comprised 14 nights between 19 July and 6 August, between 2100 and 0400 hours. The second season's collection comprised 36 nights between 30 May and 21 August, between 2100 and 0100 hours. I collected spiders haphazardly (although as the plant canopy became more dense, spider were easier to spot on the edge rows), individually by hand in, clear plastic 3.5-cm snap-cap vials. Because the sampling effort was not systematic, the sample abundances do not necessarily reflect field abundances. The sample of thomisids in particular is almost all female, because female crab spiders are bigger and easier to see.

To test spiders for nectar feeding, I employed Van Handel's cold anthrone test for determining nectar feeding in mosquitoes. The test detects the presence of fructose, a plant derived sugar, as a monosaccharide by itself or as half of the disaccharide sucrose, both of which occur in flower nectar (Van Handel 1972). Fructose detection requires that the organism's activity be halted as soon as possible to slow any further digestion and utilization of sugars. Following this, heat treatment permanently halts all enzymatic action that could convert fructose to products that would not react with anthrone. To halt spider activity in the field, in the first season for the first six collecting nights, spiders were knocked down with ethyl acetate, then frozen at the lab during the same collecting night to −86°C to completely arrest enzymatic activity. Ethyl acetate proved to be too slow-acting and unsatisfactory, however, and for the following 8 nights and for the entire next season, spiders were chilled to immobility in a portable ice chest in the field, then, during the same night, stored in a freezer at −86°C. To prepare the spiders for storage and transport, each spider was heat-treated in its own screw-top test tube. For the first season, I heated spiders in a water bath, for the second season, in a heat block (Van Handel 1972). Anthrone testing, which uses an arthropod's entire body, took place in Columbus, Ohio.

Quantifying fructose followed the colorimetric methods of Haramis and Foster (1983). The authors employed Van Handel's method of detecting fructose and developed color standards for quantifying fructose in the field. Specimens were measured by visually comparing each to a series of standard sucrose solutions corresponding to 0, 1, 2, 4, 8, 16, 32, 64, 128, and 256 μg/μl (0.1 to 25.6% solutions) (Haramis & Foster 1983). The anthrone reagent in the presence of fructose turns from yellow to green, and the green fructose in the standard sucrose solutions represents one half the total concentration of sugar. In the specimens, however, green indicates presence of fructose, but whether it is fructose alone or fructose combined with glucose to form sucrose cannot be determined within the limits of the test. Specimens can be described as positive for fructose as low as 1 μg/μl, at which a distinct green distinguishes the solution from a fructose-free yellow.

4.4 Results

In the first season, (19 July–6 August, 14 nights), of 324 spiders tested, 102 (31.5%) were positive for fructose (Table 4.1), ranging from 1 to 16 μg/μl. (Table 4.2). Among the 201 spiders that could be sexed, 85 were male, 116 female. Seventeen of the males (20%) were positive for fructose; 64 (55%) of the females were positive, a significant difference ($\chi^2 = 24.3$, d.f. = 1, $p < 0.005$). This bias in females held true for each of the 3 species of running spiders, although positive cases between *H. arunda* males and females were more evenly divided (Table 4.3). My analyses did not distinguish between spiders that were knocked down with ethyl acetate the first six nights and spiders that were knocked down by chilling the following eight nights.

In the second season (30 May–21 August, 36 nights), a lower percentage of spiders were positive for nectar, but the range of measured fructose was broader. Of 821 spiders tested, 190 (23%) were positive for fructose (Table 4.4), ranging from 1 to 128

μg/μl (Table 4.5). Among the 574 spiders that could be sexed, 244 were male, 330 were female. Thirty-eight of the males (15.6%) were positive for fructose; 112 (33.9%) of the females were positive, a signifcant difference ($\chi^2 = 24.2$, d.f. = 1, $p < 0.005$), and a bias that once again held for each the 3 species of running spiders (Table 4.6). A subset of Season 2 (14 nights from 5 July–19August) covers the same calendar period as Season 1 (Tables 4.7–4.10). This subset has a higher representation of adult thomisids, which appear more likely to test positive for fructose than juveniles.

Broad comparisons of both seasons and the season 2 subset appear in Table 4.10. Generally, for both seasons (and the subset), more adults were positive for fructose than juveniles, and more females were positive than males (Table 4.10).

The likelihood of spiders that tested positive for fructose having derived fructose from preying on nectar-feeding insects rather than by directly nectar-feeding themselves is small. Nectar-feeding insects quickly convert ingested sucrose, fructose, and glucose into the disaccharide trehalose after the monosaccharides fructose and glucose cross the gut wall into the haemolymph. This maintains a steep concentration gradient across the gut wall, which allows for continued absorption of ingested sugars. The release of ingested sugar from the insect's crop controls the rate of this process (Treherne 1967).

4.5 Discussion

Cold-anthrone testing has been used to determine nectar feeding in mosquitoes by measuring fructose (a plant-derived sugar) in their crops. This same method applied to spiders has indicated for the first time that some spiders also feed at plant nectaries. Although nectaries are not always productive, and spiders will encounter competition for nectar from other nectarivorous arthropods, spiders should glean what nectar they can whenever they can. Nectar has been shown to fuel a spider's activity (Chapter 2)—a boon to running spiders—allowing the nutritional benefits of prey, such as protein and lipids, to be directed to growth and reproduction.

In these samplings of wandering spiders from cotton crops (predominated by running spiders), fewer juveniles were positives for fructose than adults. Juveniles may feed on nectar less often than adults, or, they may feed as often, but because they are smaller, their fructose content may register below the cold anthrone test's sensitivity. Juveniles might also burn fuel at a different rate than adults, possibly spending more energy in pursuit of prey for growth and egg development. Juveniles may also be more abundant when nectar is less abundant. For example, although 8-week-old cotton plants with only 5 or 6 leaves produce nectar at foliar EFNs early in the season (Wäckers et al. 2001) when juvenile running spiders appeared to be most abundant, extrafloral nectar production is highest later in the season with anthesis (Butler, Jr. et al. 1972; Yokoyama 1978), when adult spiders appear to be more abundant.

A higher proportion of adult females tested positive for fructose than males, for both seasons and within each of the three species of running spiders. Male running spiders are smaller than females, but not dramatically enough, it would seem, to account for fewer positive readings. The sexes are apparently feeding differentially, therefore, or are burning fuel differentially. It is the nature of these runners to spend much of the night in motion, but perhaps males are even more active than females as they search for mates. That thomisids should show so many positive for fructose might be expected, given their slow-moving, near sedentary lifestyle, but with also a preference for branch terminals, where bracteal EFNs occur. By the same token, I never observed lycosids wandering in the vegetation, but, rather, they were stock-still on a leaf on the lower half of the plant, most often detected by eyeshine.

From a broad, biological perspective, the relevance of spider nectar feeding is obvious: if nectar can fuel a spider's activity, then its protein requirements decrease. A spider can grow and reproduce on fewer prey than without nectar. From an applied perspective, what this capability suggests is unclear. In the aggregate, do these spiders consume fewer prey because they can supplement their diet with nectar, or do they consume more prey because greater numbers are able to survive starvation and are able to fulfill their reduced protein requirements? Such a conundrum is new to arachnologists who until now knew only one nutritional currency for spiders: live arthropod prey. Without yet knowing the effects of spider nectar feeding, arachnologists can nonetheless use this behavior as one more means by which to help elucidate spider guilds and possibly predict spider guild structure in major agricultural crops, as suggested by Uetz et al. (Uetz et al. 1999). Although such prediction is unrealistic now, according to the authors, such an effort is not impossible, provided enough information of spider ecology. To that end, my experiments suggest that in addition to the architecture of plants having an influence on the spider community, arachnologists should also consider whether the plants offer accessible nectar.

TABLES

Species	**Total collected (*N*)**	**% Sample (*N*/324)**	**Fructose Positive (*n*)**	**% Positive (*n*/*N*)**
Cheiracanthium inclusum	47	14.5	26	55.3
Hibana futilis	61	18.8	18	29.5
Hibana arunda	58	17.9	23	39.7
Juvenile runners	105	32.4	13	12.4
Thomisids	35	10.8	17	48.6
Oxyopids	1	0.3	1	--
Unknown	17	5.2	4	23.5

Table 4.1 Season 1**:** Indication of nectar feeding (% positive) in wandering spiders by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance. Juvenile runners comprise the 3 species of running spiders.

Fructose μg/μl	***C. inclusum***	***H. futilis***	***H. arunda***	**Juvenile runners**	**Thomisid**	**Oxyopid**	**Unknown**
0	21	43	35	92	18	0	13
1	9	8	13	10	9	0	2
2	7	7	1	3	1	0	0
4	7	2	8	0	2	1	2
8	3	1	1	0	4	0	0
16	0	0	0	0	1	0	0
(Total)	47	61	58	105	35	1	17

Table. 4.2 Season 1: Frequency of nectar feeding among wandering spiders, indicated by cold-anthrone detection of ingested fructose. Measurements relied on visual colorimetric comparisons to standard solutions. Total sample size was 324.

Species/sex	Total collected (N)	# Fructose positive (n)	% Positive (n/N)
C. inclusum **/male**	20	5	25.0
/female	24	20	83.3
H. futilis **/male**	30	3	10.0
/female	23	11	47.8
H. arunda **/male**	25	9	36.0
/female	31	14	41.2

Table 4.3. Season 1: Nectar feeding in running spiders, by species and by sex, indicated by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance.

Species	Sample collected (N)	% Total (N/822)	Fructose Positive (n)	% Positive (n/N)
Cheiracanthium inclusum	134	16.3	30	22.4
Hibana futilis	293	35.6	70	23.9
Hibana arunda	56	6.8	17	30.4
Hibana juveniles	196	23.8	33	16.8
Thomisids	115	14.0	39	33.8
Oxyopids	8	1.0	1	12.5
Lycosids	20	2.4	0	0.0

Table 4.4. Season 2**:** Indication of nectar feeding (% positive) in wandering spiders by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance. Only one of the thomisids was a male (31, unknown), and all ages of *C. inclusum* are lumped.

Fructose $\mu g/\mu l$	***C. inclusum***	***H. futilis***	***H. arunda***	***Hibana* juveniles**	**Thomisid**	**Oxyopid**	**Lycosid**
0	104	223	39	163	70	7	20
1	4	13	5	8	8	0	0
2	19	36	9	21	13	0	0
4	5	10	2	2	7	0	0
6	0	1	0	0	1	0	0
8	1	5	1	0	6	0	0
12	0	2	0	1	0	0	0
16	1	1	0	1	3	0	0
24	0	1	0	0	0	0	0
32	0	0	0	0	1	0	0
64	0	0	0	0	0	1	0
128	0	1	0	0	0	0	0
(Total)	134	293	56	196	109	8	20

Table. 4.5 Season 2: Frequency of nectar feeding among wandering spiders, indicated by cold-anthrone detection of ingested fructose. Measurements relied on visual colorimetric comparisons to standard solutions. Only one of the thomisids was male (31 were unknown). All ages of *C. inclusum* are lumped. Total sample size was 822.

Species/sex	**Total collected (*N*)**	**# Fructose positive (*n*)**	**% Positive (*n*/*N*)**
***C. inclusum* /male**	41	7	17.0
/female	63	21	33.3
***H. futilis* /male**	139	18	12.1
/female	140	48	34.3
***H. arunda* /male**	34	9	26.5
/female	15	6	40.0

Table 4.6. Season 2: Nectar feeding in running spiders, by species and by sex, indicated by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance.

Species	**Total collected (*N*)**	**% Sample (*N*/365)**	**Fructose Positive (*n*)**	**% Positive (*n*/*N*)**
Cheiracanthium inclusum	59	16.2	13	22.0
Hibana futilis	179	49.0	45	25.1
Hibana arunda	38	10.4	12	31.6
Hibana juveniles	19	5.2	3	15.8
Thomisids	57	15.6	26	45.6
Oxyopids	3	1.0	1	--
Lycosids	9	2.5	0	0

Table 4.7 Season 2 Subset (a 14-night subset of the same calendar days as Season 1): Indication of nectar feeding (% positive) in wandering spiders by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance. All ages of *C. inclusum* are lumped.

Fructose $\mu g/\mu l$	***C. inclusum***	***H. futilis***	***H. arunda***	***Hibana* juveniles**	**Thomisid**	**Oxyopid**	**Lycosid**
0	46	134	26	16	31	2	9
1	4	9	4	1	7	0	0
2	9	21	5	2	8	0	0
4	0	8	2	0	4	0	0
6	0	1	0	0	0	0	0
8	0	3	1	0	3	0	0
12	0	1	0	0	0	0	0
16	0	0	0	0	3	0	0
24	0	1	0	0	0	0	0
32	0	0	0	0	1	0	0
64	0	0	0	0	0	1	0
128	0	1	0	0	0	0	0
(Total)	59	179	38	18	57	3	9

Table. 4.8 Season 2 Subset (a 14-night subset of the same calendar days as Season 1): Frequency of nectar feeding among wandering spiders, indicated by cold-anthrone detection of ingested fructose. Measurements relied on visual colorimetric comparisons to standard solutions. Total sub-sample size was 365. The four highest readings (24 through 128 $\mu g/\mu l$) occurred during this period.

Species/sex	Total collected (*N*)	# Fructose positive (*n*)	% Positive (*n*/*N*)
***C. inclusum* /male**	24	2	8.3
/female	32	11	34.4
***H. futilis* /male**	78	11	14.1
/female	94	31	33.0
***H. arunda* /male**	24	7	29.2
/female	9	3	33.3

Table 4.9. Season 2 Subset (a 14-night subset of the same calendar period as Season 1): Nectar feeding in running spiders, by species and by sex, indicated by cold-anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance.

Age/ Sex	# Fructose Positive Season 1	% Pos.	# Fructose Positive Season 2	% Pos.	Subset Season 2	% Pos.
Juvenile	25/141	**17.7**	50/319	**15.7**	10/61	**16.3**
Subadult	39/80	**48.8**	41/148	**27.7**	15/60	**25.0**
Adult	31/79	**39.2**	99/353	**28.0**	75/244	**30.7**
Males	17/85	**20.0**	38/244	**15.6**	21/127	**21.3**
Females	64/116	**55.1.**	112/330	**33.9**	72/203	**35.5**

Table 4.10 Indication of nectar feeding among wandering spiders for two seasons by age and sex, by cold anthrone detection of ingested fructose. Sampling effort was not systematic and does not necessarily reflect field abundance. Subset of Season 2 are 14 (of 36) nights covering the same calendar period as Season 1.

REFERENCES

Adjei-Maafo, I.K. & L. T. Wilson. 1983. Factors affecting the relative abundance of arthropods on nectaried and nectariless cotton. *Environmental Entomology*, 12: (2) 349-352.

Agnew, C.W., W. L. Sterling, & D. A. Dean. 1982. Influence of cotton nectar on red imported fire ants and other predators. *Environmental Entomology*, 11: 629-634.

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Barth, F.G. 2002. A Spider's World: Senses and Behavior. Springer. Berlin.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Boggs, C.L. 1987. Ecology of nectar and pollen feeding in lepidoptera. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Brian, M.V. 1973. Feeding and growth in the ant *Myrmica*. *Journal of Animal Ecology*, 42: 37-53.

Butler, G.D., Jr., G. M. Loper, S. E. McGregor, J. L. Webster, & H. Margolis. 1972. Amounts and kinds of sugar in the nectars of cotton (*Gossypium* spp.) and the time of their secretion. *Agronomy Journal*, 64: 364-368.

Christiansen, A., R. Baum, & P. N. Witt. 1962. Changes in spider webs brought about by mescaline, psilocybin, and an increase in body weight. *Journal of Pharmacology and Experimental Therapeutics*, 136: 31-37.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Haramis, L.D. & W. A. Foster. 1983. Visual quantification of sugar in mosquitoes using anthrone reagent. *Mosquito News*, 43: (3) 362-364.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Koptur, S. 1992. Extrafloral nectar-mediated interactions between insects and plants. *In* Insect-Plant Interactions. IV. (E.A. Bernays, ed.) CRC, Boca Raton, FL.

Limburg, D.D. & J. A. Rosenheim. 2001. Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology*, 30: (3) 595-604.

Mommsen, T.P. 1977. Digestive enzymes of a spider (*Tegenaria acrica* Koch) II. Carbohydrases. *Comp.Biochem.Physiol.*, 60A: 371-375.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Nyffeler, M. & W. L. Sterling. 1994. Comparison of the feeding niche of polyphagous insectivores (Araneae) in a Texas cotton plantation: estimates of niche breadth and overlap. *Environmental Entomology*, 23: (5) 1294-1303.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 35: 33-72.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Smith, R.B. & T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. *Science*, 226: 1330-1333.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Treherne, J.E. 1967. Gut absorption. *Annual Review of Entomology*, 12: 43-58.

Uetz, G.W., J. Halaj, & A. B. Cady. 1999. Guild structure of spiders in major crops. *Journal of Arachnology*, 27: 270-280.

Van Handel, E. 1972. The detection of nectar in mosquitoes. *Mosquito News*, 32: (3) 458-458.

van Rijn, P.C.J. & L. K. Tanigoshi. 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, 23: 281-296.

Vogelei, A. & R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia (Berl.)*, 80: 513-515.

Waddington, K.D. 1987. Nutritional ecology of bees. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Wäckers, F.L., D. Zuber, R. Wunderlin, & F. Keller. 2001. The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, 87: 365-370.

Whitcomb, W.H., H. Exline, & R. C. Hunter. 1963. Spiders of the Arkansas cotton field. *Annals of the Entomological Society of America*, 56: 652-660.

Witt, P. 1971. Drugs alter web-building of spiders. A review and evaluation. *Behavioral Science*, 16: 98-113.

Yokoyama, V.Y. 1978. Relation of seasonal changes in extrafloral nectar and foliar protein and arthropod populations in cotton. *Environmental Entomology*, 7: 799-802.

CHAPTER 5

THE CONTRIBUTION OF HONEYDEW TO THE SURVIVAL AND GROWTH OF NEWLY EMERGED *CHEIRACANTHIUM INCLUSUM*: (A PILOT STUDY)

5. 1 Abstract

Although as generalist predators spiders have been considered to be exclusively carnivorous, wandering spiders, such as crab spiders, jumping spiders, and running (also called “sac”) spiders, have been observed to feed at the floral and extrafloral nectaries of plants. Plant nectar has been shown experimentally to contribute to the survival, activity, growth, and fecundity of the running spider *Cheiracanthium inclusum* (Hentz) (Miturgidae). Extrafloral nectaries make nectar available to these spiders and other arthropods, such as ants, that do not typically visit floral nectaries. Another source of sugar that can be even more accessible is honeydew, which is excreted by phloem-feeding insects. These insects can coat leaves in a sugary exudate, which wandering spiders can and detect with contact chemoreceptors on their tarsi and palps. The pilot studies herein suggest that honeydew can make some of the same contributions as plant nectar to the livelihood of *C. inclusum*. Newly emerged spiders provided the honeydew of long-tailed mealybugs, *Pseudococcus longispinus* (Targioni-Tozzetti), survived significantly longer than spiders without honeydew. Likewise, spiders provided with prey (*Drosophila melanogaster*) underwent their first molt significantly sooner if they also received honeydew.

5.2 Introduction

Although studies of spider ecology and nutrition treat all spiders as exclusive carnivores, a large group of spiders, all members of what are generally called wanderers, have been observed to feed at the floral and extrafloral nectaries of plants (Edmunds 1978; Pollard et al. 1995; Taylor & Foster 1996; Ruhren & Handel 1999; Jackson et al. 2001). As generalist predators, all spiders require prey for normal development, but in the same way that webbuilding spiders utilize protein for silk to entrap prey, these non-webbuilding wanderers may be utilizing the simple carbohydrates of plant nectar (fructose, sucrose and glucose) (Percival 1961; Baker & Baker 1975; 1983) to sustain their active hunting for prey. Indeed, my laboratory experiments with the running spider *Cheiracanthium inclusum* showed that plant nectar sustained that spider's nocturnal running activity, allowing protein, which costs more to obtain, to be allocated to growth and reproduction (Chapters 2 and 3). Brian (1973) showed this same use of sugar for activity and allocation of protein to growth and fecundity in laboratory experiments with the ant *Myrmica rubra* L. (Formicidae).

Ants, known sugar feeders, obtain their sugar from the extrafloral nectaries of plants (Bentley 1977) and from honeydew, the sugary exudate of phloem-feeding insects (reviewed in Stradling 1987). Because experiments with ants and spiders have shown them to gain the same positive effects of adding sugar to a diet of protein, and because some spiders share the cursorial lifestyle of ants and also feed at extrafloral nectaries, it seemed reasonable to investigate whether spiders also could benefit from honeydew. More particularly, spiders feeding on honeydew seemed a distinct possibility when I observed running spiders dragging their palps across the surfaces of leaves then stopping to clean them with their mouthparts. Populations of phloem feeders can makes leave surfaces sticky with honeydew, and the exudate made available this way has been found in the guts of black flies (Burgin & Hunter 1997) and mosquitoes (Burkett et al. 1999), and has been shown, incidentally, to support populations of cotton leafworm (Lukefahr & Rhyne 1960).

An infestation of long-tailed mealybugs on greenhouse mulberry trees provided the opportunity to measure the response of newly emerged *C. inclusum* to the availability of honeydew. I offered spiders honeydew on two substrates, as it naturally was deposited on leaves, and transferred to pieces of heavy-gauge plastic. In separate experiments I measured the effects of honeydew on 1) the survival of newly emerged spiders housed together, and 2) the number of days to a spider's first post-emergent molt.

5.3 Materials and Methods

During spring 2002, an unexpected and unwelcome (by some) infestation of long-tailed mealybugs (*Pseudococcus longispinus* (Targioni-Tozzetti)) (Pseudococcidae) in the university greenhouse provided a short-term opportunity to gather honeydew and test its attractiveness to, and effects on, newly emerged spiderlings. Of the red mulberry trees (*Morus rubra* L.) infested, only a few could be spared to maintain the infestation. Those were soon defoliated. The experiments that follow, therefore, necessarily involved small sample sizes and protocols that were developed during the course of the experiments.

The spiders for all of the experiments were the offspring of gravid *C. inclusum* collected from Columbus OH during the spring of 2002. Each experiment and replicates (four total) used a single egg mass divided among the treatments, except the first survival experiment (Fig. 5.1), which used a separate (unrelated) egg mass for the treatment

"Honeydew on plastic." This treatment was added two days after the experiment began as an assurance that spiders were responding only to honeydew and not to prey that might be hidden along the leaf ribs. Experiments were conducted in a laboratory rearing room on a 16:8 light:dark cycle at approximately 27°C and 80% relative humidity. Spiders were checked daily for mortality in the survival experiment, for molting in the growth experiment.

Containers

For survival experiments, spiders in each treatment were housed together in 7-liter clear acrylic cages with a screened opening at one end and a sleeved opening at the other. Water was available from soaked cotton wicks inserted into water-filled vials. Spiders in the molting experiment were housed individually in lidded, clear plastic containers, 5.2 cm-dia. X 3.6 cm, each of which had four holes, two, 12-mm, mesh-covered holes top and bottom; and two opposing 17-mm holes in the side wall, one mesh-covered and the other corked. Water was available from a soaked cotton dental ball resting on the top of the container.

Diet

Spiders occupying the larger cages living in groups were allowed to cannibalize or scavenge sibling bodies. In the smaller containers in which spiders lived individually, they received frozen *Drosophila melanogaster*, two the first day and one daily thereafter. In all honeydew treatments, the amount of honeydew was undetermined and was refreshed every 24–48 hours. For the group treatment "Honeydew leaves," two or three mulberry leaves coated in honeydew were cut from an infested tree and each petiole inserted into a water-filled glass test tube covered with parafilm (care was required to avoid even a drop of water spilling on a leaf, which resulted in a fungal bloom). Leaves were chilled while each was removed from the test tube, cleaned of debris and potential prey under a dissecting scope, and reinserted into a new water-filled (parafilm covered) test tube and placed in the cage. To control for potential prey that might have been hiding deep within the angle of a leaf rib, I created a third treatment, "Honeydew on plastic," by pressing an undetermined amount of honeydew onto pieces of clear, heavy-gauge plastic (freezer bags). These were also checked under a dissecting scope to eliminate debris and potential prey. The control treatment "Without honeydew" contained artificial leaves, in case the physical presence of leaves offered any benefit.

Honeydew in the molting experiment was offered on pieces of mulberry leaves cut from the margins of honeydew-coated leaves. Leaf pieces were also cleaned using the dissecting scope and mounted onto a small piece of heavy-gauge plastic by moistening the back of the leaf with a damp cotton swab. Using dead *Drosophila* allowed prey and honeydew to be offered at the same time without the risk of prey eating the honeydew themselves. The flies were frozen and are known to be acceptable to the spiders. Two were offered on the first day and one each day thereafter until the spider's first post-emergence molt.

Data analysis

Analyses were performed with *Statistica* software (5.5 for Windows, StatSoft, Inc., 2000). Survival analysis employed log-rank tests and a multiple comparison correction procedure with an alpha level of 0.05 and a calculated comparison-wise error rate (γ)of 0.008 (based on $K = 3$ treatments) , a more conservative correction than Bonferroni (Hardin et al. 1996).

5.4 Results

In the pilot survival experiment, spiders that received honeydew, on leaves or on plastic, lived significantly longer than controls without honeydew (Fig. 5.1). There are two caveats to this interpretation: 1) the sample size was small, and 2) the sample for the treatment Honeydew on plastic came from a separate egg sac and began on day 1 post emergence, whereas the other two treatments started on day 2. A log-rank pairwise comparison of survival in the two treatments Without honeydew and Honeydew leaves (both from the same egg sac) produces a significant ($\alpha = 0.05$) difference, uncorrected $p = 0.0046$ (corrected value = 0.008). Likewise, survival in the treatment Without honeydew is significantly less than survival in the treatment Honeydew on plastic (uncorrected $p = 0.0008$). Pair-wise comparison of the two honeydew treatments (leaf and plastic) results in no significant difference (uncorrected $p = 0.0173$) (Fig 5.1).

The results of the first replicate of the survival experiment (Fig. 5.2) suggest the same trends as the pilot. Again, the sample size is small, and in addition, more than half of the data are censored, the result of necessarily ending the experiment on day 42 when 6/10 and 5/10 spiders were still alive in the honeydew treatments (Honeydew leaf and Honeydew on plastic, respectively). Because of the combination of small sample size and censored data, I did not analyze these data, but present here them for their relevance (Fig. 5.2).

The results of the second replicate of the survival experiment somewhat resemble the results of the pilot (Fig. 5.3). Mean survival in the treatment Without honeydew is 5.0 days vs. 6.6 days for the pilot; mean survival in the treatment Honeydew leaf is 15.3 days vs. 16.9 days. On the other hand, mean survival in the treatment Honeydew on plastic is 14.9 days vs. 27. 9 days for the pilot experiment, which may have something to do with the unmeasured amounts of honeydew that were offered. Furthermore, in this replicate, there was no significant difference ($\alpha = 0.05$, $\gamma = 0.008$) in mean survival between the treatments Without honeydew and Honeydew leaf (uncorrected $p = 0.0107$). There was, however, a significant difference between the treatments Without honeydew and Honeydew on plastic (uncorrected $p = 0.0037$), even though mean survival in the treatment Honeydew on plastic is less than mean survival in the treatment Honeydew leaf (14.9 days vs. 16.9 days). This incongruity with other data that support the positive effects of honeydew is likely the result of a small sample size. Specifically, it occurs as a result of a few spiders dying early ("a few" making up a large portion of a small sample) and a statistical test that compares medians rather than means. There was no significant difference in survival between the honeydew treatments (uncorrected $p = 0.8370$).

In the single experiment to measure honeydew's contribution to a newly emerged spider reaching first molt, spiders given prey only (*Drosophila*) reached first (post-emergent) molt in a mean 9.2 days (range 7–12 days). Spiders given honeydew in addition to prey molted in a mean 7.2 days (range 6–8 days). The difference is significant (Mann-Whitney U, $U = 22.5$, $p = 0.0043$).

Discussion

Despite small sample sizes, these pilot studies strongly suggest that *C. inclusum* can benefit from the sugars of honeydew in some of the same ways that they benefit from the sugars of plant nectar. These experiments also illustrate that, as with nectar feeding, the occurrence of some spiders feeding on honeydew lies more with opportunity than with the spider. That is, to nectar feed, spiders must rely on production at extrafloral nectaries and then compete with other nectar feeders. To feed on honeydew, spiders must rely on the presence of phloem feeders (the efficacy of eating the phloem feeder is a possibility but may not be an option for first instar spiderlings). Given the opportunity, however, to feed on nectar or honeydew, some spiders are fully equipped to take advantage of dietary sugar, from detection, to attraction and ingestion, to utilization, described below.

Spiders possess contact chemoreceptors, or "taste hairs," on the distal parts of their legs and palps (Foelix 1982). Barth (2002) refers to these as "gustatory hairs" in his study of the wandering spider *Cupiennius salei* (Keyserling) (Ctenidae). Spiders that wander in vegetation and over the surface of leaves are able to detect the sugars of honeydew wherever they encounter it. That spiders find sugar attractive and readily ingest it, enabled researchers to study the famed effects of LSD on the webs of orb-weavers, as well as the effects of caffeine and strychnine (Witt 1971), mescaline, and psilocybin, (Christiansen et al. 1962), all delivered to spiders by way of sugar water.

Evidence to support the hypothesis of honeydew feeding in spiders comes from two avenues: 1) direct evidence from more experiments of the sort here showing that spiders *do* use honeydew , and 2) indirect evidence from digestive enzyme profiles showing that spiders *can* use honeydew. To that end, Pickford (1942) had identified the enzyme sucrase in the tarantula *Cyrtopholis jamaicensis* but only qualitatively. In more sophisticated tests, Mommsen (1977) also found sucrase in the webbuilding spider *Tegenaria atrica* Koch and was able to quantify it and its activity. Sucrase splits the disaccharide sucrose into its monosaccharide components, glucose and fructose, which can then be absorbed across the invertebrate gut wall (Treherne 1967). Sucrose is one of the predominant sugars of honeydew (Wäckers 2001). Unlike plant nectar, however, which consists of various fractions of sucrose, fructose, and glucose (Percival 1961; Baker & Baker 1975;1983) honeydew is composed of many sugars, which include, in addition to sucrose, another disaccharide, maltose; the trisaccharides, melezitose, and erlose; and the tertrasaccharide, stachyose. These sugars contain an α-glucosidic bond (Wäckers, 2001) requiring the enzyme α-glucosidase to digest them. As it turns out, in addition to the enzyme sucrase, Mommsen (1977) also found α-glucosidase among the digestive enzymes of *Tegenaria atrica*. Despite these findings, *Tegenaria*, builds a sheet funnel web and may not be the best candidate to illustrate the digestive profile of a sugar-feeding spider. This predicament, and tantalizing evidence, makes obvious the need for new evaluations of the digestive enzymes of spiders, particularly among those that are likely to feed on sugar in the form of plant nectar or honeydew.

FIGURES

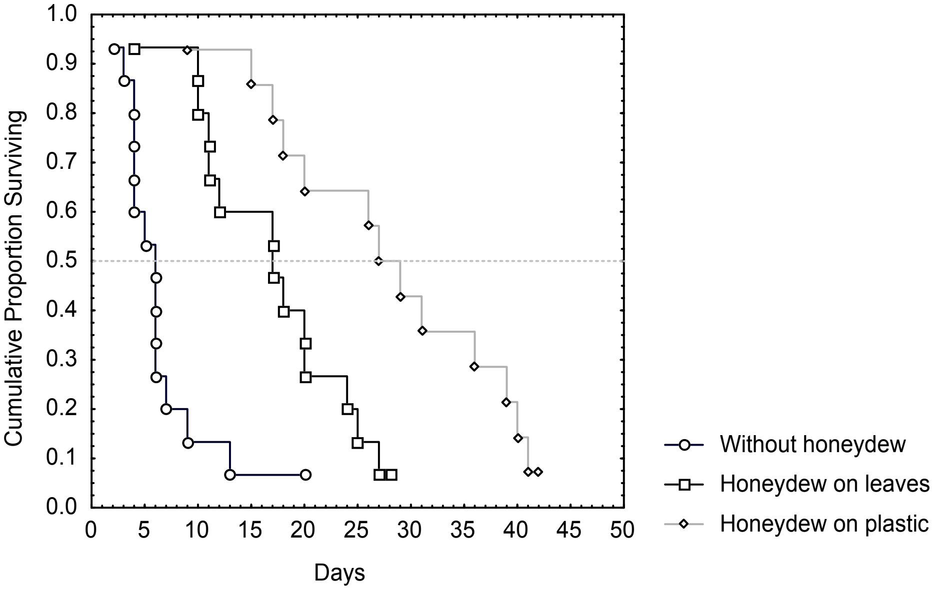

Fig. 5.1 Plot of survival estimates (Kaplan-Meier) (pilot experiment), for newly emerged, *C. inclusum* housed together, without honeydew ($N = 15$) or receiving honeydew, on leaves ($N = 15$) or pressed onto plastic ($N = 14$). All received water ad lib. and were able to cannibalize or scavenge bodies.

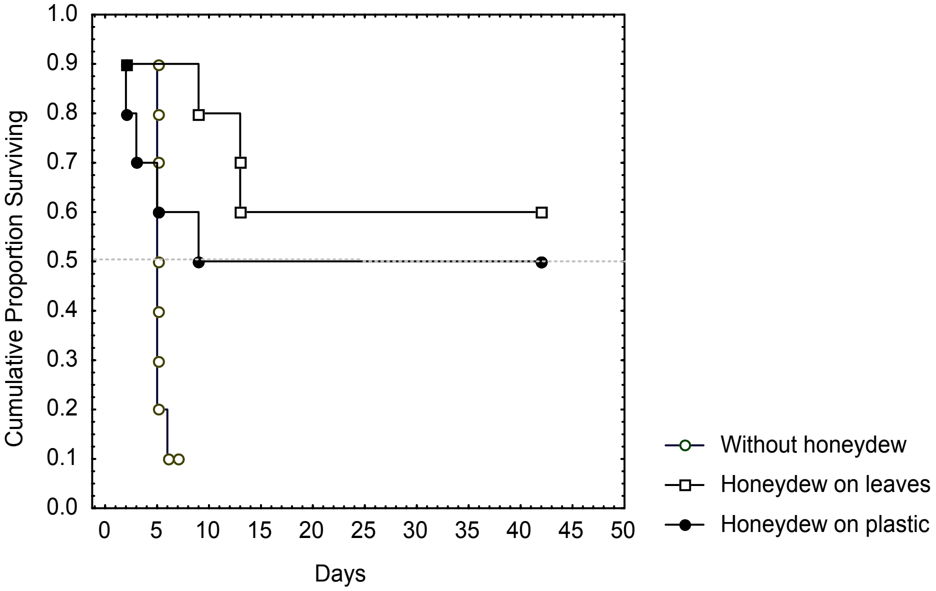

Fig. 5.2 Survival estimates (Kaplan-Meier) (first replicate), for newly emerged, *C. inclusum* housed together, without honeydew (*N* = 10) or receiving honeydew, on leaves (*N* = 10) or pressed onto plastic (*N* = 10). All received water ad lib. and were able to cannibalize or scavenge bodies. By day 42, half of the spiders in Honeydew on plastic and 60% in Honeydew on leaves still survived (censored data).

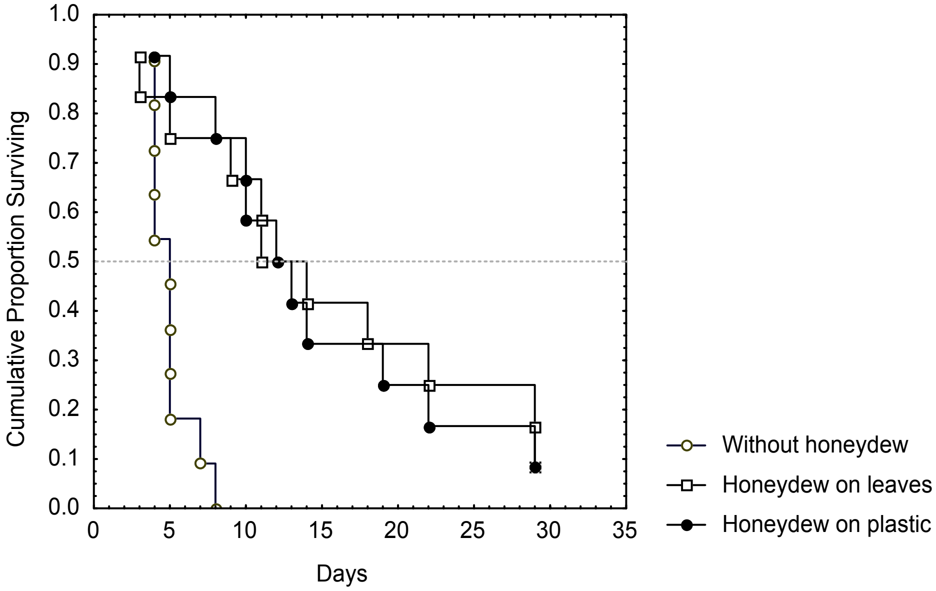

Fig. 5.3 Survival estimates (Kaplan-Meier) (second replicate), for newly emerged *C. inclusum* housed together, without honeydew ($N = 11$) or receiving honeydew, on leaves ($N = 12$) or pressed onto plastic ($N = 12$). All spiders received water ad lib. and were able to cannibalize or scavenge bodies.

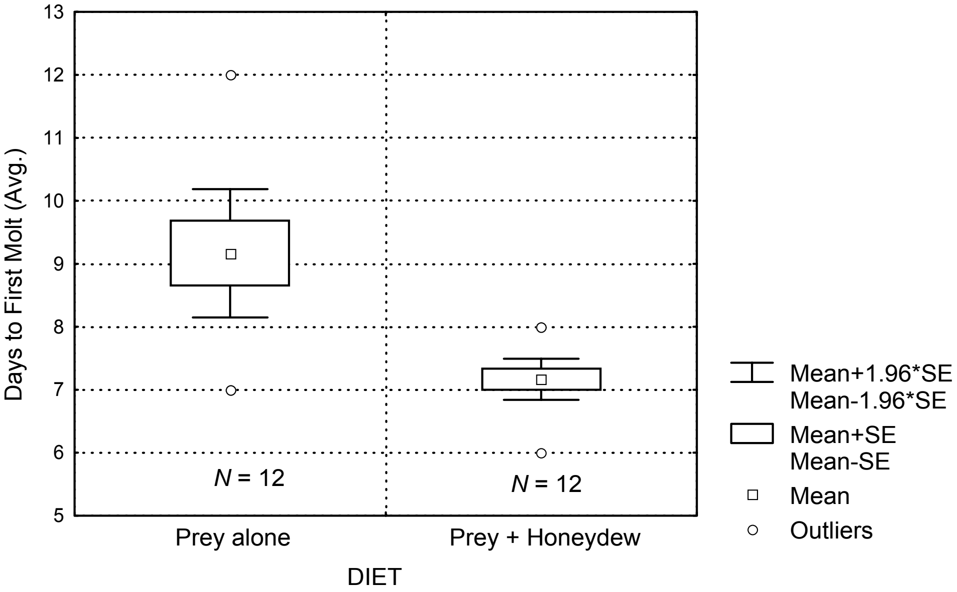

Fig. 5.4 Mean number of days to first molt on a diet of *Drosophila*(frozen, two the first night, one daily thereafter), without and with honeydew.

REFERENCES

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Barth, F.G. 2002. A Spider's World: Senses and Behavior. Springer. Berlin.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Brian, M.V. 1973. Feeding and growth in the ant *Myrmica*. *Journal of Animal Ecology*, 42: 37-53.

Burgin, S.G. & F. F. Hunter. 1997. Nectar versus honeydew as sources of sugar for male and female black flies (Diptera: Simuliidae). *Journal of Medical Entomology*, 34: (6) 605-608.

Burkett, D.A., D. L. Kline, & D. A. Carlson. 1999. Sugar meal composition of five North Central Florida mosquito species (Diptera: Culicidae) as determined by gas chromatography. *Journal of Medical Entomology*, 36: (4) 462-467.

Christiansen, A., R. Baum, & P. N. Witt. 1962. Changes in spider webs brought about by mescaline, psilocybin, and an increase in body weight. *Journal of Pharmacology and Experimental Therapeutics*, 136: 31-37.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Hardin, M.J., J. L. Willers, & T. L. Wagner. 1996. Nonparametric multiple comparisons of survivorship distributions. *Journal of Economic Entomology*, 89: (3) 715-721.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Lukefahr, M.J. & C. Rhyne. 1960. Effects of nectariless cottons on populations of three lepidopterous insects. *Journal of Economic Entomology*, 53: (2) 242-244.

Mommsen, T.P. 1977. Digestive enzymes of a spider (*Tegenaria acrica* Koch) II. Carbohydrases. *Comp. Biochem. Physiol.*, 60A: 371-375.

Percival, M.S. 1961. Types of nectar in angiosperms. *New Phytologist*, 60: 235-281.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 35: 33-72.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Stradling, D.J. 1987. Nutritional ecology of ants. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Treherne, J.E. 1967. Gut absorption. *Annual Review of Entomology*, 12: 43-58.

Wäckers, F.L. 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, 47: 1077-1084.

Witt, P. 1971. Drugs alter web-building of spiders. A review and evaluation. *Behavioral Science*, 16: 98-113.

CHAPTER 6
MICROMANIPULATING A SPIDER'S NUTRITION WITH A COMPLETE AND NATURAL DIET OF INSECT EGGS AND SUCROSE

6.1 Abstract

As obligate carnivores and generalist predators, most spiders reared for experimental purposes require a variety of insect prey to develop and mature normally. Experiments, intended and unintended, have shown that fruit flies alone (*Drosophila melanogaster*) fail to fulfill most spiders' nutritional requirements, and yet *Drosophila* is one of the few insects that is relatively easy to rear and maintain in any lab. This leaves researchers the alternative of collecting insects in the field or maintaining multiple insect colonies that can be even more difficult to rear than the spiders themselves. Furthermore, a diet of "a variety of prey" precludes investigating a spider's minimum nutritional requirements, which would necessitate a food source that is not only nutritionally complete and homogeneous, but that can be parceled out in small, measurable quantities. For this reason, studies of spider nutrition have examined what makes a spider well-fed, not what meets its minimum needs. The nocturnal wandering spider *Cheiracanthium inclusum* (Hentz) (Miturgidae), however, is an excellent model for micromanaging a spider's nutrition. Laboratory-reared *C. inclusum* grew quickly and normally solely on the eggs of lab-maintained *Helicoverpa zea* (Boddie) (Noctuidae) the corn earworm.

Days to functional maturity were fewer than half the number previously recorded for a mixed-species diet. *H. zea* eggs can be frozen, stored, and dispensed to spiders singly, providing a minute, homogeneous, quantifiable source of protein, lipids, and other nutrients. *C. inclusum* also feeds on plant nectar, the simple sugars of which the spider appears to allocate to activity, such as its energetic nocturnal foraging. In an experiment to measure plant nectar's effect on survival and fecundity, spiders reared at what were thought to be starvation-level quantities of *H. zea* eggs unexpectedly survived and continued to grow and reproduce if they were also fed plant nectar (for which sucrose can serve as a proxy). These low-nutrient diets are investigated in detail here to illustrate the useful combination of sucrose and insect eggs. Together, they allow for the micromanipulation of nutrients and the possibility to determine of what is nutritionally necessary and sufficient for a spider's activity, growth, and reproduction.

6.2 Introduction

Because all spiders are obligate carnivores requiring prey for normal maintenance, growth, and reproduction, mass rearing healthy spiders for experiments has always posed a daunting challenge. As generalist predators, most spiders not only prefer live prey (however, see Sandidge 2003), they require a variety of prey (Greenstone 1979; Lowrie 1987; Uetz et al. 1992; Li 2002; Oelbermann & Scheu 2002). The fruit fly *Drosophila melanogaster*, relatively easy to obtain and rear in numbers, has been shown to supply a complete diet to only a few spiders, such as linyphiids (Turnbull 1962) and crab spiders (Thomisidae). Linyphiids, however, pose the added complication of needing web sites, and survival in thomisids is low (Li 2002). Otherwise, *Drosophila* is notorious for supporting various genera of spiders through four or five molts followed by near

100% mortality before maturation (Miyashita 1968; Jakob & Dingle 1990; Uetz et al. 1992; Toft & Wise 1999; Oelbermann & Scheu 2002; pers. obs.), although *Drosophila* raised on nutritious media, such as dogfood, were more nutritious to spiders (Mayntz & Toft 2001). When a spider can successfully be raised on a monotypic diet, such as mosquitoes (Turnbull 1965) or collembola (Marcussen et al. 1999), colonies of these can be difficult to maintain. Researchers have tried to make artificial diets (Peck & Whitcomb 1968; Amalin et al. 1999), but perhaps because these diets have been composed of liquids rather than the solid prey that extra-oral digestors typically catch, they have met with limited success (Cohen 1998).

These complicated dietary requirements of spiders make it difficult to measure precisely the upper and lower limits of a spider's nutritional needs. Even a single-species diet is difficult to mete out in quantities that do not exceed necessary and sufficient (Turnbull 1965). Field studies suggest that some wandering spiders have a low incidence of prey capture based on observations of spiders carrying prey (Nentwig 1986; Nyffeler et al. 1987). Researchers have also described spiders as "starving," (Nakamura 1987), some by comparing the body size of field-caught spiders to the body-size of laboratory-reared spiders that were well-fed or starved (Miyashita 1968; Anderson 1974). The description of starving, however, would seem more clearly defined by knowing the minimum nutrition a spider needs to mature and reproduce.

Considering these challenges with spider rearing, *Cheiracanthium inclusum* stands out as a fast-growing, non-webbuilding wandering spider that reaches functional maturity on a diet composed exclusively of the eggs of the corn earworm *Helicoverpa zea* (Boddie) (= *Heliothis zea*) (Lepidoptera: Noctuidae) (R. Pfannenstiel, unpublished data).

The eggs of *H. zea* can be meted out one-by-one as minute packets of complete and homogenous protein, lipids, and other nutrients. On a diet of eggs, *C. inclusum* matured in less than half the time—51 days vs.127days—that had been reported for it on a mixed-species diet of adult insects (sexes averaged; 143 days for females, 112 days for males) (Peck & Whitcomb 1970). *C. inclusum* is a nocturnal running spider that is often one of the most numerous spiders collected in crops such as vineyards (Costello & Daane 1997), cotton (Whitcomb et al. 1963), citrus (Amalin et al. 2001), and apple (Bajwa & Aliniazee 2001). It is known to prey on the eggs of *H. zea* (Nyffeler et al. 1990; Neussley & Sterling 1994), the eggs of other crop pests (Buschman et al. 1977; Miliczky & Calkins 2002), and on the eggs of other spiders (Willey & Adler 1989). Peck and Whitcomb (1965) tried rearing *C. inclusum* on *Drosophila* alone and failed. They succeeded by adding other insect prey to the diet and discovered that spiderlings grew best and fastest on a mixed-species diet rich in the larvae of *H. zea*. It now appears that the eggs alone of *H. zea* can be a nutritionally complete diet for this particular spider (R. Pfannenstiel, unpublished data). The ability of *C. inclusum* to mature quickly on a single-species diet has been documented elsewhere (Amalin et al. 2001).

In addition to its ability to thrive on insect eggs, *C. inclusum* is among the wandering spiders that have been observed to feed at plant nectaries (Edmunds 1978; Pollard et al. 1995; Taylor & Foster 1996; Ruhren & Handel 1999; Jackson et al. 2001). My experiment to measure the contributions of plant nectar to the spider's fecundity (Chapter 3) produced unexpected results regarding survival and development, the details of which are investigated here. More than half (12/20, Table 6.1) of the spiders that were expected to die of starvation on what was considered an inadequate supply of nutrients (3

H. zea eggs /week) survived and continued to grow if they also received extrafloral plant nectar (or a nectar proxy, 69% sucrose). In a separate treatment, spiders receiving what was thought to be a meager nutrition, matured and reproduced, but, also, only if they received nectar. These data suggest that it is possible to quantify not only *C. inclusum*'s optimal dietary requirements but also its minimum requirements. Furthermore, it appears that feeding a spider sucrose allows the energy requirements of activity to be distinguished from the energy/nutritional requirements for maintenance growth, and reproduction.

6.3 Materials and Methods

In addition to its simple dietary demands and the fact that it does not require a web site, *C. inclusum* is quiescent during the day in a woven resting cell. This simplifies the chores of feeding, watering, and cleaning. In 2003, 160 offspring of 5 lab-reared females (reared from spiders collected from a USDA cotton crop in Weslaco, Texas) were reared individually in 125 X 15 mm petri dishes, turned upside-down to encourage the spiders to weave their cells in the solid, uppermost corner of the dish. Opening the dish from the bottom usually didn't disturb the spiders, which were fed and had their half-inch cotton water wicks resoaked three days a week. Water from the wicks often condensed on the plastic surface above it, offering another source of free water. Spiders were reared in incubators with a 14:10 light:dark cycle, at 24°C. The humidity inside the incubator was not controlled, but was monitored, and cycled through daily highs (82 ± 7%) and lows (48 ± 8%). Spiders were daily rotated on the shelves and checked for mortality and molting. References to instars refer to post-emergent stadia.

Spiders were reared from emergence, to functional maturity, to multiple egg sac production on eight different diets, 4 levels of nutrition (*H zea* eggs), with and without plant nectar. The USDA-BIRU supplied the moth eggs, maintaining the colonies according to modified methods of Ignoffo (1965). (That is, adult moths were placed in 3.8-liter cardboard ice cream cartons lined with green florist paper for oviposition and with a feeding solution of 10% sucrose.) Eggs of *H. zea* can also be purchased from on-line biological supply houses such as Benzon Research or Agripest. Eggs were frozen right after collection to prevent hatching. For experimental purposes, I used only eggs that had been frozen no more than a month. For colony maintenance, however, frozen eggs of any age seemed sufficient. Parcels of 1, 5, 25 and 125 eggs (cut from the oviposition paper) offered three times per week (M, W, F), were deduced to be nutritionally inadequate (i.e., near-starvation), meager, adequate, and surplus, respectively.

The plant nectar for this experiment I had collected (and stored at –45°C) from the extrafloral nectaries of Indian almond (*Terminalia cattapa*, L. growing in the OSU greenhouse), a common tropical and subtropical beachfront tree. I had observed running spiders feeding from the floral nectaries of this plant in Costa Rica and presumed that they feed from the extrafloral nectaries (Taylor & Foster 1996). A previous experiment showed that 69% sucrose worked as a nectar proxy (Chapter 2). In the present experiment, spiders received the limited supply of available nectar exclusively only if they had been determined likely to reproduce, because the effect of nectar on female fecundity was the focus of the experiment. These reproductive spiders were in 5 diets: both diets with 125-eggs, both diets with 25 eggs, and the one diet with 5 eggs receiving nectar. Spiders in three diets, therefore (1-egg/–N, 1-egg/+N, 5-egg/–N), received nectar initially and were later switched to 69% sucrose to conserve nectar. Because nectar and 69% sucrose are very viscous, measurements for feeding were inexact, and the smallest transferable amount, about 1–2 µl, was smeared into a dimple drilled into a plastic "feeder," 1 cm X 2.5 cm. Nectar and sucrose were refreshed three times a week (M,W, F), along with the *H. zea* eggs and water.

In an earlier experiment, the growth of 22 *C. inclusum* was monitored from hatching to adult on a diet solely of *H. zea* eggs offered ad lib. This diet is referred to as "ad lib." in Tables 6.1 and 6.2. The exact numbers of eggs eaten by each spider for each instar were counted using a dissecting microscope (R. Pfannenstiel, unpublished data), and the means are presented here for comparison to means of the 1-, 5-, 25-, and 125-egg diets. For these diets, time was too limited to count precisely how many eggs each spider ate. Rather, the mean number of eggs that spiders ate per instar is based on the number of eggs *provided* (Table 6.2), which results from the following calculation: for each spider, for each instar, I divided the exact number of days duration for each instar by 7 to produce the number of weeks duration, and that number I multiplied by 3, 15, 75 or 375 eggs, that is 1, 5 , 25 or 125 eggs, 3 times per week (M, W, F). I summed the totals for each instar and divided by the sample size.

6.4 Results

Mean numbers of days duration of each instar, based on exact counts, and mean numbers of eggs eaten per instar, calculated from instar duration and number of eggs provided per diet, appear in Tables 6.1 and 6.2, respectively, to which all of the results refer. For comparison, included in the tables are results of a previous experiment (R.

Pfannenstiel, unpublished data), referred to as "ad lib.," which provided *C. inclusum* the eggs of *H. zea* ad lib. and for which the *exact* numbers of eggs eaten per spider per instar were counted. The 21 subadults from the 1- and 5-egg diets that still survived but had not yet reached adulthood were 46 weeks (319 days) old.

Spiders nearing adulthood consumed hundreds of *H. zea* eggs offered ad lib. The number of eggs offered in the 125-egg diets with (+N) and without (−N) nectar were intended to be a surplus and did surpass what spiders ate when eggs were offered ad lib. (Table 6.2). Their nearly identical developmental times suggest a reliable, necessary developmental duration for each instar (Table 6.1). Compared to the instar durations of these three diets (ad lib., 125 eggs/−N and +N), the instar durations of spiders receiving 25 eggs with and without nectar were both comparable until the 4th and 5th instars (Table 6.2). By the 5th instar, mean duration in the 25-egg diets increased from 12 days to 20 days. These longer instar durations suggest a suboptimal amount of nutrients for this point of development at this feeding level. Suboptimal nutrition here suggests that at this point and at lower dietary levels (1-egg and 5-eggs), the number of eggs provided reflects the number of eggs consumed, making comparisons worthwhile. Within this level of nutrition (25-egg) the spiders were robust and survival and maturation, 100%.

After 46 weeks (319 days), half (10/20) of the spiders receiving 5 eggs/−N still survived. This diet was presumed at the beginning of the experiment to be nutritionally meager, one level up from starvation. One of the spiders in this group, however, had matured to adulthood in the 6th instar; 8 others had reached the 6th instar, and 1, the 7th instar. Given enough time, all of these spiders might reach adulthood. On the other hand, all 20 spiders at this protein level supplemented with nectar (5 eggs/+N) had all long since matured and (where possible) reproduced. [The substantial contribution of nectar to the life history of *C. inclusum* is discussed in Chapter 3]. These spiders were significantly smaller than spiders receiving surplus eggs (ANOVA, *p* =0.000), and the typically dark sclerotized parts of their body, such as their chelicerae and palps, were pale, suggesting improper tanning, which involves protein(Dalingwater 1987). At this feeding level some males also experienced problems with abnormal or underdeveloped palps or palps that separated and remained in the cast skin during molting. Nonetheless, some of the individuals on this diet were able to reproduce, and a mated pair from this diet produced three viable egg masses.

The lowest level of nutrition, 1-egg, (three times per week), had been presumed at the beginning of the experiment to lead to death by starvation. As anticipated, the spiders died, during their second (= 18) or third (= 2) instars, although they survived a mean 80 days. This was the case only for the spiders without nectar (1 egg/–N), however. Although the addition of plant nectar to the 1-egg diet was hypothesized to forestall starvation, not prevent it, after 46 weeks, 12 spiders on this protein diet were still alive. Three were in their 7th instar, 9, in their 8th instar.

The number of eggs provided to spiders in the surplus diet give no indication of what the spiders actually ate (lack of time precluded counting remaining eggs). On the other hand, eggs consumed on the ad lib. diet were exact counts, and eggs provided on the limited 1- and 5-egg diets (and for later instars, on the 25-egg diet) were likely all consumed (except during periods of molting) making these numbers worth comparing.

6.5 Discussion

Two aspects of the natural feeding behavior of the wandering spider *Cheiracanthium inclusum* make it an excellent candidate for laboratory experiments: it develops quickly and normally on a diet solely of *H. zea* eggs (R. Pfannenstiel, unpublished data), and it can utilize plant nectar, apparently to fuel its activity (Chapter 2). As a quantifiable, homogeneous, complete nutritional source, the minute eggs of *H. zea* can be meted out precisely, allowing the micromanipulation of nutrition that researchers have previously lacked. Coupled with sucrose, the diet allows researchers 1) to determine minimum and optimal nutritional requirements, and 2) to distinguish the energetic requirements for activity from the energetic/nutritional requirements for maturation and reproduction.

The results of these experiments broadly suggest the optimal and minimum nutritional (egg) requirements of *C. inclusum*. Spiders fed ad lib. consumed a mean 780 eggs (range, 474–1275) to reach adulthood in 53 days. Spiders reached adulthood in the same amount of time (54 days) if they were fed 25% fewer eggs (581, on the 25-egg/+N diet) and nectar. This total of 581 eggs is an overestimation, however, because far more eggs were provided on this diet during the first three instars than could actually have been consumed. Results for the first three instars from spiders fed ad lib. suggest that , for future experiments, *C inclusum* might reach adulthood on a total of 563 eggs with sucrose (28 + 55 + 118 from instars 1, 2, and 3 on the ad lib. diet + 156 +206 from instars 4 and 5 on the 25-egg/+N diet) (Table 6.2). Without sucrose, spiders are calculated to require 578 eggs (28 + 55 + 118 + 159 +218), and an additional week to mature (54–61days)

(Table 6.1). Thus, about 600 eggs appear, roughly, to meet the protein requirements of *C. inclusum* to mature as quickly as possible.

A rough estimate of minimum nutritional (egg) requirements appears to lie somewhere between the 5- and 25-egg diets. Spiders receiving 25 eggs/+N lengthened their fifth instar by 7 days (compared to diets with 125 eggs or eggs ad. lib.) (Table 6.1), as though the diet might be limiting. Spiders on this diet consumed a mean 206 eggs (range, 183–229) to complete their fifth instar, after which all became adults (Table 6.1). Spiders receiving one fifth the amount of protein, 5 eggs/+N, on the other hand, consumed a mean 89 eggs (range, 70 to 109), significantly fewer than those on the higher level diet (Mann- Whitney U, $p = 0.000$) and yet they also completed their fifth instar, after which 18 became adults. These adults were noticeably and significantly smaller than adults on the higher level diet (ANOVA, $p = 0.000$). Body size may explain the fewer eggs required for maturation, but that begs the question, when and what was the factor that allowed these spiders, molt by molt, to achieve adulthood at such a small size? These spiders may indicate having fulfilled some minimum protein requirement, which cued continued development. This consideration is strengthened by the fact that among the spiders receiving the same amount of protein, but without nectar (5 egg/−N), only one matured to adulthood. In this diet without nectar, the nutrition is marginal enough that the demands of activity and growth leave an insufficient amount for maturation.

It is also at the 5-egg feeding level that energy for activity can begin to be distinguished from energy/nutrition for development. The one adult that matured without nectar did so on a mean total of 643 eggs (subadults had so far consumed 551 eggs). Spiders with nectar, all of which became adults, required only a mean total of 245 eggs.

This two-and-a-half-fold difference in eggs is the first indication that, for an extremely active spider at least, sustaining activity demands more prey than sustaining growth. *C. inclusum* and other spiders that engage in nectar feeding, therefore, may be reducing their requirement for prey substantially.

The spiders in the 1-egg/+N diet that continue to molt on so little nutrition remain a puzzle. Although after 46 weeks, more than half (12/20) survive and are in their 6th or 7th instars, reaching adulthood seems impossible. Spiders in the 5-egg/+N diet, consuming more than twice the protein (245 vs. 123 eggs) indicated difficulty with the tanning process (see Results), leaving hardened parts of the body, such as palps and chelicerae soft and vulnerable.

TABLES

Instar	Ad lib.	1 egg		5 eggs		25 eggs		125 eggs	
		-N	+N	-N	+N	-N	+N	-N	+N
1	9	30	15	9	9	8	9	9	8
2	9	80*	34	15	13	10	10	9	9
3	11		37	44	19	13	12	11	10
4	13		49	68	29	15	15	12	11
5	12		56	72	42	20	19	13	12
6	12		54	54	63	61		13	
7			55	71					
Adult	53 (*N*=21)	(*N*=0)	(*N*=0)	300 (*N*=1)	115 (*N*=20)	61 (*N*=20)	54 (*N*=20)	50 (*N*=20)	47 (*N*=20)
Sub-Adult	NA	(*N*=0)	287 (*N*=12)	261 (*N*=9)	NA	NA	NA	NA	NA

Table 6.1 Mean duration in days of each instar and to reach adulthood (or subadulthood) for nine different diets. Ad. lib. means are from R. Pfannenstiel, unpublished data. Initial sample size: *N* = 22 for ad lib. diet, for all other diets, *N* = 20.

*Only two spiders survived their entire second instar.

Instar	Ad lib.	1 egg		5 eggs		25 eggs		125 eggs	
		–N	+N	–N	+N	–N	+N	–N	+N
1	28	13	7	19	20	90	93	466	439
2	55	33*	14	33	28	102	108	492	488
3	118		16	95	42	142	131	594	538
4	216		21	144	62	159	156	624	576
5	327		24	162	89	218	206	689	664
6	482**		22	156	135**	653**		604**	
7			23	152**					
Adult	780 (*N*=22)	(*N*=0)	(*N*=0)	643 (*N*=1)	245 (*N*=20)	657 (*N*=20)	581 (*N*=20)	2695 (*N*=20)	2539 (*N*=20)
Sub-adult	NA	(*N*=0)	123 (*N*=12)	559 (*N*=9)	NA	NA	NA	NA	NA

Table 6.2 Average number of *H. zea* eggs consumed ad lib. and parceled out, with (+N) and without (–N) nectar, per instar and to reach adulthood (or subadulthood) for nine different diets. Ad. lib. means are calculated from exact counts of eggs eaten by 22 spiders for each instar (R. Pfannenstiel, unpublished data). Means for the other diets are based on the number of eggs *provided* to each individual during each instar, calculated by dividing each individual's instar duration in days (or days to adulthood) by 7 and multiplying by the cumulative number of eggs in the weekly diet (3, 15, 75 or 375). Sample sizes: *N* = 22 for eggs ad lib.; for all other diets, *N*=20. Adult means are calculated from all individuals in the sample, not from the column means.

*Only two spiders survived the second instar, but mean survival was 80 days.

**Represents only one spiderling (see Table 3.1).

REFERENCES

Amalin, D.M., J. Reiskind, R. McSorley, & J. Peña. 1999. Survival of the hunting spider, *Hibana velox* (Araneae, Anyphaenidae), raised on different artificial diets. *Journal of Arachnology*, 27: 692-696.

Amalin, D.M., J. Reiskind, J. E. Peña, & R. E. McSorley. 2001. Predatory behavior of three species of sac spiders attacking citrus leafminer. *Journal of Arachnology*, 29: 72-81.

Anderson, J.F. 1974. Responses to starvation on the spiders *Lycosa lenta* Hentz and *Filistata hibernalis* (Hentz). *Ecology*, 55: 576-585.

Bajwa, W.I. & M. T. Aliniazee. 2001. Spider fauna in apple ecosystem of western Oregon and its field susceptibility to chemical and microbial insecticides. *Journal of Economic Entomology*, 94: (1) 68-75.

Buschman, L.L., W. H. Whitcomb, R. C. Hemenway, D. L. Mays, Ru Nguyen, N. C. Leppla, & B. J. Smittle. 1977. Predators of velvetbean caterpillar eggs in Florida soybeans. *Environmental Entomology*, 6: (3) 403-407.

Cohen, A.C. 1998. Solid-to-liquid feeding: The inside(s) story of extra-oral digestion in predaceous arthropoda. *American Entomologist*, Summer: 103-117.

Costello, M.J. & K. M. Daane. 1997. Comparison of sampling methods used to estimate spider (Araneae) species abundance and composition in grape vineyards. *Environmental Entomology*, 26: (2) 142-149.

Dalingwater, J.E. 1987. Chelicerate cuticle structure. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Greenstone, M.H. 1979. Spider feeding behavior optimizes dietary essential amino acid composition. *Nature*, 282: 501-503.

Ignoffo, C.M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). II. Biology and propagation of diet-reared *Heliothis*. *Journal of Invertebrate Pathology*, 7: 217-226.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Jakob, E.M. & H. Dingle. 1990. Food level and life history characteristics in a pholcid spider (*Holocnemus pluchei)*. *Psyche*, 97: 95-110.

Li, D. 2002. The combined effects of temperature and diet on development and survival of a crab spider, *Misumenops tricuspidatus* (Fabricius) (Araneae: Thomisidae). *Thermal Biology*, 27: 83-93.

Lowrie, D.C. 1987. Effects of diet on the development of *Loxosceles laeta* (Nicolet) (Araneae, Loxoscelidae). *Journal of Arachnology*, 15: 303-308.

Marcussen, B.M., J. A. Axelsen, & S. Toft. 1999. The value of Collembola species as food for a linyphiid spider. *Entomologia Experimentalis et Applicata*, 92: 29-36.

Mayntz, D. & S. Toft. 2001. Nutrient composition of the prey's diet affects growth and survivorship of a generalist predator. *Oecologia*, 127: 207-213.

Miliczky, E.R. & C. O. Calkins. 2002. Spiders (Araneae) as potential predators of leafroller larvae and egg masses (Lepidoptera: Tortricidae) in Central Washington apple and pear orchards. *Pan-Pacific Entomologist*, 78: (2) 140-150.

Miyashita, K. 1968. Growth and development of *Lycosa T-insignata* Boes. et Str. (Araneae: Lycosidae) under different feeding conditions. *Appl.Ent.Zool.*, 3: (2) 81-88.

Nakamura, K. 1987. Hunger and starvation. *In* Ecophysiolgy of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Nentwig, W. 1986. Non-webbuilding spiders: prey specialists or generalists? *Oecologia (Berlin)*, 69: 571-576.

Neussley, G.S. & W. L. Sterling. 1994. Mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in cotton as a function of oviposition sites, predator species, and dessication. *Environmental Entomology*, 23: (5) 1189-1202.

Nyffeler, M., R. G. Breene, D. A. Dean, & W. L. Sterling. 1990. Spiders as predators of arthropod eggs. *Journal of Applied Entomology*, 109: 490-501.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Oelbermann, K. & S. Scheu. 2002. Effects of prey type and mixed diets on survival, growth and development of a generalist predator, *Pardosa lugubris* (Araneae: Lycosidae). *Basic and Applied Ecology*, 3: 285-291.

Peck, W.B. & W. H. Whitcomb. 1968. Feeding spiders an artificial diet. *Entomological News*, 79: 233-236.

Peck, W.B. & W.H. Whitcomb. 1970. Studies on the biology of a spider *Chiracanthium inclusum* (Hentz). 753. Agricultural Experiment Station.

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Sandidge, J.S. 2003. Scavenging by brown recluse spiders. *Nature*, 426: (6 November) 30-30.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Toft, S. & D. H. Wise. 1999. Growth, development, and survival of a generalist predator fed single- and mixed-species diets of different quality. *Oecologia*, 119: 191-197.

Turnbull, A.L. 1962. Quantitative studies of the food of *Linyphia triangularis* Clerck (Araneae: Linyphiidae). *Canadian Entomologist*, 94: (12) 1233-1249.

Turnbull, A.L. 1965. Effects of prey abundance on the development of the spider *Agelenopsis potteri* (Blackwall) (Araneae: Agelendidae). *Canadian Entomologist*, 97: 141-147.

Uetz, G.W., J. Bischoff, & J. Raver. 1992. Survivorship of wolf spiders (Lycosidae) reared on different diets. *Journal of Arachnology*, 20: 207-211.

Whitcomb, W.H., H. Exline, & R. C. Hunter. 1963. Spiders of the Arkansas cotton field. *Annals of the Entomological Society of America*, 56: 652-660.

Willey, M.B. & P. H. Adler. 1989. Biology of *Pucetia viridans* (Araneae, Oxyopidae) in South Carolina, with special reference to predation and maternal care. *Journal of Arachnology*, 17: 275-284.

.

BIBLIOGRAPHY

Adjei-Maafo, I.K. & L. T. Wilson. 1983. Factors affecting the relative abundance of arthropods on nectaried and nectariless cotton. *Environmental Entomology*, 12: (2) 349-352.

Agnew, C.W., W. L. Sterling, & D. A. Dean. 1982. Influence of cotton nectar on red imported fire ants and other predators. *Environmental Entomology*, 11: 629-634.

Amalin, D.M., J. Reiskind, R. McSorley, & J. Peña. 1999. Survival of the hunting spider, *Hibana velox* (Araneae, Anyphaenidae), raised on different artificial diets. *Journal of Arachnology*, 27: 692-696.

Amalin, D.M., J. Reiskind, J. E. Peña, & R. E. McSorley. 2001. Predatory behavior of three species of sac spiders attacking citrus leafminer. *Journal of Arachnology*, 29: 72-81.

Anderson, J.F. 1974. Responses to starvation on the spiders *Lycosa lenta* Hentz and *Filistata hibernalis* (Hentz). *Ecology*, 55: 576-585.

Anderson, J.F. 1990. The size of spider eggs and estimates of their energy content. *Journal of Arachnology*, 18: 73-78.

Austin, A.D. 1984. Life history of *Clubionia robusta* L. Koch and related species (Araneae, Clubionidae) in South Australia. *Journal of Arachnology*, 12: 87-104.

Bailey, C.L. & H. L. Chada. 1968. Spider populations in grain sorghums. *Annals of the Entomological Society of America*, 61: (3) 567-571.

Bajwa, W.I. & M. T. Aliniazee. 2001. Spider fauna in apple ecosystem of western Oregon and its field susceptibility to chemical and microbial insecticides. *Journal of Economic Entomology*, 94: (1) 68-75.

Baker, D.A., J. L. Hall, & J. R. Thorpe. 1978. A study of the extrafloral nectaries of *Ricinus communis*. *New Phytologist*, 81: 129-137.

Baker, H.G. & I. Baker. 1975. Studies of nectar constitution and pollinator-plant coevolution. *In* Coevolution of Animals and Plants. (L.E. Gilbert & P.H. Raven, eds.) University of Texas Press, Austin, TX.

Baker, H.G., P. A. Opler, & I. Baker. 1978. A comparison of the amino acid complements of floral and extrafloral nectars. *Botanical Gazette*, 139: (3) 322-332.

Baker, H.G. & I. Baker. 1983. A brief historical review of the chemistry of floral nectar. *In* The Biology of Nectaries. (B. Bentley & T. Elias, eds.) Columbia University Press, New York.

Barth, F.G. 2002. A Spider's World: Senses and Behavior. Springer. Berlin.

Bentley, B.L. 1977. Extrafloral nectaries and protection by pugnacious bodyguards. *Annual Review of Ecology and Systematics*, 8: 407-427.

Bentley, B.L. and T.S. Elias. 1983. Biology of Nectaries. Columbia University Press. New York, NY.

Boggs, C.L. 1987. Ecology of nectar and pollen feeding in lepidoptera. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Bonnet, P. 1924. Sur la nature des aliments que les araignées peuvent absorber et sur le sens du goût chez ces animaux. *C.R.Séances Soc.Biol.Fil.*, 91: 1194-1196.

Bradley, R.A. 1993. The influence of prey availability and habitat on activity patterns and abundance of *Argiope keyserlingi* (Araneae: Araneidae). *Journal of Arachnology*, 21: 91-106.

Brian, M.V. 1973. Feeding and growth in the ant *Myrmica*. *Journal of Animal Ecology*, 42: 37-53.

Brown, C.A., B. M. Sanford, & R. R. Swerdon. 2003. Clutch size and offspring size in the wolf spider *Pirata sedentarius* (Araneae, Lycosidae). *Journal of Arachnology*, 31: 285-296.

Buckley, R. 1990. Ants protect tropical Homoptera against nocturnal spider predation. *Biotropica*, 22: (2) 207-209.

Bultman, T.L., G. W. Uetz, & A. R. Brady. 1982. A comparison of cursorial spider communities along a successional gradient. *Journal of Arachnology*, 10: 23-35.

Burgin, S.G. & F. F. Hunter. 1997. Nectar versus honeydew as sources of sugar for male and female black flies (Diptera: Simuliidae). *Journal of Medical Entomology*, 34: (6) 605-608.

Burkett, D.A., D. L. Kline, & D. A. Carlson. 1999. Sugar meal composition of five North Central Florida mosquito species (Diptera: Culicidae) as determined by gas chromatography. *Journal of Medical Entomology*, 36: (4) 462-467.

Buschman, L.L., W. H. Whitcomb, R. C. Hemenway, D. L. Mays, Ru Nguyen, N. C. Leppla, & B. J. Smittle. 1977. Predators of velvetbean caterpillar eggs in Florida soybeans. *Environmental Entomology*, 6: (3) 403-407.

Butler, G.D., Jr., G. M. Loper, S. E. McGregor, J. L. Webster, & H. Margolis. 1972. Amounts and kinds of sugar in the nectars of cotton (*Gossypium* spp.) and the time of their secretion. *Agronomy Journal*, 64: 364-368.

Choi, Y.-S. & M.-J. Moon. 2003. Fine structure of the ovarian development in the orb-web spider *Nephila clavata*. *Korean Journal of Entomology*, 33: (1) 25-32.

Christiansen, A., R. Baum, & P. N. Witt. 1962. Changes in spider webs brought about by mescaline, psilocybin, and an increase in body weight. *Journal of Pharmacology and Experimental Therapeutics*, 136: 31-37.

Cohen, A.C. 1998. Solid-to-liquid feeding: The inside(s) story of extra-oral digestion in predaceous arthropoda. *American Entomologist*, Summer: 103-117.

Collart, A. 1929. Quelques observations sur une araignée mimétique. *Rev.Zool.Bot.Afr.*, 18: 147-161.

Collart, A. 1929. Quelques notes sur les *Myrmarachne*, araignées oecophylliformes. *Bull.Cerc.Zool.Congol.*, 5: 117-118.

Costello, M.J. & K. M. Daane. 1995. Spider (Araneae) species composition and seasonal abundance in San Joaquin Valley grape vineyards. *Environmental Entomology*, 24: (4) 823-831.

Costello, M.J. & K. M. Daane. 1997. Comparison of sampling methods used to estimate spider (Araneae) species abundance and composition in grape vineyards. *Environmental Entomology*, 26: (2) 142-149.

Costello, M.J. & K. M. Daane. 1998. Influence of ground cover on spider populations in a table grape vineyard. *Ecological Entomology*, 23: 33-40.

Dalingwater, J.E. 1987. Chelicerate cuticle structure. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Decae, A. 1986. Bird- or banana-spider? *Newsletter of British Arachnology Society*, 46: 3-4.

Dondale, C.D. 1961. Life histories of some common spiders from trees and shrubs in Nova Scotia. *Canadian Journal of Zoology*, 39: 777-787.

Dondale, C.D. 1965. A spider's first meal after molting. *Canadian Entomologist*, 97: 446-446.

Downes, M.F. 1986. The production and use of inviable eggs by *Theridion rufipes* Lucas (Araneae: Theridiidae). *Australian Arachnology, The Australian Entomological Society Miscellaneous Publication No.5*, 127-135.

Edmunds, M. 1978. On the association between *Myrmarachne* spp. (Salticidae) and ants. *Bulletin of British Arachnological Society*, 4: (4) 149-160.

Enders, F. 1976. Clutch size related to hunting manner of spider species. *Annals of the Entomological Society of America*, 69: (6) 991-998.

Foelix, R.F. 1970. Chemosensitive hairs in spiders. *Journal of Morphology*, 132: 313-334.

Foelix, R.F. 1982. Biology of Spiders. Harvard University Press. Cambridge, MA.

Foelix, R.F. 1996. Biology of Spiders. Oxford University Press. New York.

Fritz, R.S. & D. H. Morse. 1985. Reproductive success and foraging of the crab spider *Misumena vatia*. *Oecologia*, 65: 194-200.

Gertsch, W.J. 1949. American Spiders. Van Nostrand. New York, NY.

Gonzaga, M.O. & J. Vasconcellos-Neto. 2001. Female body size, fecundity parameters and foundation of new colonies in *Anelosimus jabaquara* (Araneae, Theridiidae). *Insectes Sociaux* , 48: 94-100.

Greenstone, M.H. 1979. Spider feeding behavior optimizes dietary essential amino acid composition. *Nature*, 282: 501-503.

Greenstone, M.H. 1984. Determinants of web spider species diversity: vegetation structural diversity vs. prey availability. *Oecologia*, 62: 299-304.

Greenstone, M.H., C. E. Morgan, A.-L. Hultsch, R. A. Farrow, & J. E. Dowse. 1987. Ballooning spiders in Missouri, USA, and New South Wales, Australia: Family and mass distributions. *Journal of Arachnology* , 15: 163-170.

Hagen, K.S. 1987. Nutritional ecology of terrestrial insect predators. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York.

Hagstrum, D.W. 1971. Carapace width as a tool for evaluating the rate of development of spiders in the laboratory and the field. *Annals of the Entomological Society of America*, 64: (4) 757-760.

Hanna, R., F.G. Salom, & A. Corbett. 1995. Use of rubidium marking to determine the impact of vineyard ground cover on spiders. *In* Poster Session: Ecological Society of America (ESA). Las Vegas, Nevada.

Haramis, L.D. & W. A. Foster. 1983. Visual quantification of sugar in mosquitoes using anthrone reagent. *Mosquito News*, 43: (3) 362-364.

Hardin, M.J., J. L. Willers, & T. L. Wagner. 1996. Nonparametric multiple comparisons of survivorship distributions. *Journal of Economic Entomology*, 89: (3) 715-721.

Harrington, C.L. 1978. Field studies on reproduction in the funnel-web spider *Agelenopsis potteri* (Araneae: Agelenidae). *Oikos*, 31: 368-375.

Hatley, C.L. & J. A. Macmahon. 1980. Spider community organization: Seasonal variation and the role of vegetation architecture. *Environmental Entomology*, 9: (5) 632-639.

Helbert, J.R. & K. D. Brown. 1955. Factors influencing quantitative determination of methylpentoses and ketohexoses with anthrone. *Analytical Chemistry*, 27: (11) 1791-1796.

Hespenheide, H.A. 1985. Insect visitors to extrafloral nectaries of *Byttneria aculeata* (Sterculiaceae): relative importance of roles. *Ecological Entomology*, 10: 191-204.

Ignoffo, C.M. 1965. The nuclear-polyhedrosis virus of *Heliothis zea* (Boddie) and *Heliothis virescens* (Fabricius). II. Biology and propagation of diet-reared *Heliothis*. *Journal of Invertebrate Pathology*, 7: 217-226.

Jackson, R., S. D. Pollard, X. J. Nelson, G. B. Edwards, & A. T. Barrion. 2001. Jumping spiders (Araneae: Salticidae) that feed on nectar. *Journal of Zoology, London*, 255: 25-29.

Jakob, E.M. & H. Dingle. 1990. Food level and life history characteristics in a pholcid spider (*Holocnemus pluchei)*. *Psyche*, 97: 95-110.

Janetos, A.C. 1982. Active foragers vs. sit and wait predators: a simple model. *Journal of Theoretical Biology*, 95: 381-385.

Jennings, D.T. & M. W. Houseweart. 1978. Spider preys on spruce budworm egg mass. *Entomological News*, 89: (78) .

Keeler, K.H. 1978. Insects feeding at extrafloral nectaries of *Ipomoea carnea*. *Entomological News*, 89: (78) 163-168.

Keeler, K.H. 1985. Extrafloral nectaries on plants in communities without ants: Hawaii. *Oikos*, 44: 407-414.

Kessler, A. 1971. Relation between egg production and food consumption in species of the genus *Pardosa* (Lycosidae, Araneae) under experimental conditions of food-abundance and food-shortage. *Oecologia (Berl.)*, 8: 93-109.

Killebrew, D.W. & N. B. Ford. 1985. Reproductive tactics and female body size in the green lynx spider, *Peucetia viridans* (Araneae, Oxyopidae). *Journal of Arachnology*, 13: 357-382.

Koptur, S. 1992. Extrafloral nectar-mediated interactions between insects and plants. *In* Insect-Plant Interactions. IV. (E.A. Bernays, ed.) CRC, Boca Raton, FL.

Laster, M.L. & J. R. Brazzel. 1968. A comparison of predator populations in cotton under different control programs in Mississippi. *Journal of Economic Entomology*, 61: (3) 714-719.

Li, D. & R. R. Jackson. 1997. Influence of diet on survivorship and growth in *Portia fimbriata*, an araneophagic jumping spider (Araneae: Salticidae). *Canadian Journal of Zoology*, 75: 1652-1658.

Li, D. 2002. The combined effects of temperature and diet on development and survival of a crab spider, *Misumenops tricuspidatus* (Fabricius) (Araneae: Thomisidae). *Thermal Biology*, 27: 83-93.

Limburg, D.D. & J. A. Rosenheim. 2001. Extrafloral nectar consumption and its influence on survival and development of an omnivorous predator, larval *Chrysoperla plorabunda* (Neuroptera: Chrysopidae). *Environmental Entomology*, 30: (3) 595-604.

Linzen, B. & P. Gallowitz. 1975. Enzyme activity patterns in muscles of the lycosid spider, *Cupiennius salei*. *Journal of Comparative Physiology*, 96: 101-109.

Lowrie, D.C. 1987. Effects of diet on the development of *Loxosceles laeta* (Nicolet) (Araneae, Loxoscelidae). *Journal of Arachnology*, 15: 303-308.

Luczak, J. 1979. Spiders in agrocoenoses. *Polish Ecological Studies*, 5: (1) 151-200.

Luczak, J. 1980. Spider communities in crop fields and forests of different landscapes of Poland. *Polish Ecological Studies*, 6: (4) 735-762.

Lukefahr, M.J. & C. Rhyne. 1960. Effects of nectariless cottons on populations of three lepidopterous insects. *Journal of Economic Entomology*, 53: (2) 242-244.

Mansour, F., D. Rosen, & A. Shulov. 1980. Biology of the spider *Cheiracanthium mildei* (Arachnida: Clubionidae). *Entomophaga*, 25: (3) 237-248.

Mansour, F., D. Rosen, & A. Shulov. 1980. Functional response of the spider *Cheiracanthium mildei* (Arachnida: Clubionidae) to prey density. *Entomophaga*, 25: (3) 313-316.

Marcussen, B.M., J. A. Axelsen, & S. Toft. 1999. The value of Collembola species as food for a linyphiid spider. *Entomologia Experimentalis et Applicata*, 92: 29-36.

Marshall, S.D. & J. L. Gittleman. 1994. Clutch size in spiders: is more better? *Functional Ecology*, 8: 118-124.

Miliczky, E.R. & C. O. Calkins. 2002. Spiders (Araneae) as potential predators of leafroller larvae and egg masses (Lepidoptera: Tortricidae) in Central Washington apple and pear orchards. *Pan-Pacific Entomologist*, 78: (2) 140-150.

Miyashita, K. 1968. Quantitative feeding biology of *Lycosa T-insignata* Boes. et Str. (Araneae: Lycosidae). *Bull.Nat.Inst.Agric.Sci.(Japan)(C)*, 22: 329-334.

Miyashita, K. 1968. Growth and development of *Lycosa T-insignata* Boes. et Str. (Araneae: Lycosidae) under different feeding conditions. *Appl.Ent.Zool.*, 3: (2) 81-88.

Miyazaki, K., R. Ueshima, & T. Makioka. 2001. Structure of the female reproductive system and oogenetic mode in a schizomid, *Schizomus sawadai* (Arachnida, Schizomida). *Invertebrate Reproduction and Development*, 40: (1) 1-7.

Mommsen, T.P. 1978. Comparison of digestive α -amylases from two species of spiders (*Tegenaria atrica* and *Cupiennius salei*). *Journal of Comparative Physiology B*, 127: 355-361.

Mommsen, T.P. 1977. Digestive enzymes of a spider (*Tegenaria acrica* Koch) II. Carbohydrases. *Comp.Biochem.Physiol.*, 60A: 371-375.

Morishita, R., S. A. Ferreira, A. S. Filha, & C. D. Faraco. 2003. Studies on oogenesis and oviposition in the brown spider *Loxosceles intermedia* (Araneae: Sicariidae). *The Anantomical Record Part A*, 273A: 575-582.

Nakamura, K. 1987. Hunger and starvation. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Nentwig, W. 1986. Non-webbuilding spiders: prey specialists or generalists? *Oecologia (Berlin)*, 69: 571-576.

Nentwig, W. 1987. The prey of spiders. *In* Ecophysiology of spiders. (W. Nentwig, ed.) Springer, New York, NY.

Neussley, G.S. & W. L. Sterling. 1994. Mortality of *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in cotton as a function of oviposition sites, predator species, and dessication. *Environmental Entomology*, 23: (5) 1189-1202.

Nyffeler, M., D. A. Dean, & W. L. Sterling. 1987. Predation by green lynx spider, *Pucetia viridans* (Araneae: Oxyopidae), inhabiting cotton and woolly croton plants in East Texas. *Environmental Entomology*, 16: (2) 356-359.

Nyffeler, M., R. G. Breene, D. A. Dean, & W. L. Sterling. 1990. Spiders as predators of arthropod eggs. *Journal of Applied Entomology*, 109: 490-501.

Nyffeler, M. & W. L. Sterling. 1994. Comparison of the feeding niche of polyphagous insectivores (Araneae) in a Texas cotton plantation: estimates of niche breadth and overlap. *Environmental Entomology*, 23: (5) 1294-1303.

Nyffeler, M. & W. O. C. Symondson. 2001. Spiders and harvestmen as gastropod predators. *Ecological Entomology*, 26: 617-628.

Oelbermann, K. & S. Scheu. 2002. Effects of prey type and mixed diets on survival, growth and development of a generalist predator, *Pardosa lugubris* (Araneae: Lycosidae). *Basic and Applied Ecology*, 3: 285-291.

Peck, W.B. & W. H. Whitcomb. 1968. Feeding spiders an artificial diet. *Entomological News*, 79: 233-236.

Peck, W.B. & W.H. Whitcomb. 1970. Studies on the biology of a spider *Chiracanthium inclusum* (Hentz). 753. Agricultural Experiment Station.

Percival, M.S. 1961. Types of nectar in angiosperms. *New Phytologist*, 60: 235-281.

Petersen, B. 1950. The relation between size of mother and number of eggs and young in some spiders and its significance for the evolution of size. *Experientia*, 6: (3) 96-98.

Pickford, G.E. 1942. Studies on the digestive enzymes of spiders. *Transactions of the Connecticut Academy of Arts and Sciences*, 35: 33-72.

Platnick, N.I. 2004. The world spider catalog, version 4.5. American Museum of Natural History. Online at http://research.amnh.org/entomology/spiders/catalog81-87/INTRO2.html

Pollard, S.D., M. W. Beck, & G. N. Dodson. 1995. Why do male crab spiders drink nectar? *Animal Behaviour*, 49: (6) 1443-1448.

Riechert, S.E. & J.M. Harp. 1987. Nutritional ecology of spiders. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Riechert, S.E. 1992. Spiders as representative 'sit-and-wait' predators. *In* Natural Enemies: The Population Biology of Predators, Parasites and Diseases. (M.J. Crawley, ed.) Blackwell Scientific Publications, Oxford.

Riechert, S.E. 1998. The role of spiders and their conservation in the agroecosystem. *In* Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. (C.H. Pickett & R.T. Bugg, eds.) Univ. of Calif. Press, Berkeley, CA.

Riechert, S.E. & R. C. Tracy. 1975. Thermal balance and prey availability: bases for a model relating web-site characteristics to spider reproductive success. *Ecology*, 56: 265-284.

Riechert, S.E. & J. Luczak. 1982. Spider foraging: behavioral responses to prey. *In* Spider Communication: Mechanisms and Ecological Significance. (P.N. Witt & J.S. Rovner, eds.) Princeton University Press, Princeton, New Jersey.

Riechert, S.E. & T. Lockley. 1984. Spiders as biological control agents. *Annual Review of Entomology*, 29: 299-320.

Riechert, S.E., L. Provencher, & K. Lawrence. 1999. The potential of spiders to exhibit stable equilibrium point control of prey: tests of two criteria. *Ecological Applications*, 9: (2) 365-377.

Roltsch, W., R. Hanna, F. Zalom, H. Shorey, & M. Mayse. 1998. Spiders and vineyard habitat relationships in central California. *In* Enhancing Biological Control: Habitat Management to Promote Natural Enemies of Agricultural Pests. (C.H. Pickett & R.T. Bugg, eds.) Univ. of Calif. Press, Berkeley.

Ruhren, S. & S. N. Handel. 1999. Jumping spiders (Salticidae) enhance the seed production of a plant with extrafloral nectaries. *Oecologia*, 119: 227-230.

Russel, C.B. & F. F. Hunter. 2002. Analysis of nectar and honeydew feeding in *Aedes* and *Ochlerotatus* mosquitoes. *Journal of the American Mosquito Control Association*, 18: (2) 86-90.

Samu, F., S. Toft, & B. Kiss. 1999. Factors influencing cannibalism in the wolf spider *Pardosa agrestis* (Araneae, Lycosidae). *Behavioral Ecology and Sociobiology*, 45: 349-354.

Sandidge, J.S. 2003. Scavenging by brown recluse spiders. *Nature*, 426: (6 November) 30-30.

Scheer, B.T. & V.R. Meenakshi. 1961. The metabolism of carbohydrates in arthropods. *In* Physiology of carbohydrate metabolism in heterothermic animals. (A.W. Martin, ed.) University of Washington, Seattle.

Simpson, M.R. 1995. Convariation of spider eggs and clutch size: The influence of foraging and parental care. *Ecology*, 76: (3) 795-800.

Skow, C.D. & E. M. Jakob. 2003. Effects of maternal body size on clutch size and egg weight in a pholcid spider (*Holocnemus pluchei)*. *Journal of Arachnology*, 31: 305-308.

Slansky, F.J. & J.G. Rodriguez. 1987. Nutritional ecology of insects, mites, spiders and related invertebrates: an overview. *In* Nutritional Ecology of Insects, Mites, Spiders and Related Invertebrates. (F.J. Slansky & J.G. Rodriguez, eds.) John Wiley & Sons, New York.

Smith, R.B. & T. P. Mommsen. 1984. Pollen feeding in an orb-weaving spider. *Science*, 226: 1330-1333.

Stradling, D.J. 1987. Nutritional ecology of ants. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Stranzy, F. & S.F. Perry. 1987. Respiratory system: structure and function. *In* Ecophysiology of Spiders. (W. Nentwig, ed.) Springer-Verlag, Berlin.

Sunderland, K. & F. Samu. 2000. Effects of agricultural diversification on the abundance, distribution, and pest control potential of spiders: a review. *Entomologia Experimentalis et Applicata*, 95: 1-13.

Sunderland, K.D. & G. P. Vickerman. 1980. Aphid feeding by some polyphagous predators in relation to aphid density in cereal fields. *Journal of Applied Ecology*, 17: 389-396.

Suter, R.B., C. M. Shane, & A. J. Hirscheimer. 1987. Communication by cuticular pheromones in a linyphiid spider. *Journal of Arachnology*, 15: (2) 157-162.

Symondson, W.O.C., K. D. Sunderland, & M. H. Greenstone. 2002. Can generalist predators be effective biocontrol agents? *Annual Review of Entomology*, 47: 561-594.

Tanaka, K. 1995. Variation in offspring size within a population of the web-building spider *Agelena limbata*. *Researches on Population Ecology*, 37: (2) 197-202.

Taylor, R.M. & W. A. Foster. 1996. Spider nectarivory. *American Entomologist*, (Summer) 82-86.

Toft, S. & D. H. Wise. 1999. Growth, development, and survival of a generalist predator fed single- and mixed-species diets of different quality. *Oecologia*, 119: 191-197.

Toyama, M. 2003. Relationship between reproductive resource allocation and resource capacity in the matriphagous spider, *Chiracanthium japonicum* (Araneae: Clubionidae). *Journal of Ethology*, 21: 1-7.

Treherne, J.E. 1967. Gut absorption. *Annual Review of Entomology*, 12: 43-58.

Turnbull, A.L. 1962. Quantitative studies of the food of *Linyphia triangularis* Clerck (Araneae: Linyphiidae). *Canadian Entomologist*, 94: (12) 1233-1249.

Turnbull, A.L. 1965. Effects of prey abundance on the development of the spider *Agelenopsis potteri* (Blackwall) (Araneae: Agelendidae). *Canadian Entomologist*, 97: 141-147.

Turnbull, A.L. 1973. Ecology of the true spiders (Araneomorphae). *Annual Review of Entomology*, 18: 305-348.

Uetz, G.W., J. Bischoff, & J. Raver. 1992. Survivorship of wolf spiders (Lycosidae) reared on different diets. *Journal of Arachnology*, 20: 207-211.

Uetz, G.W., A. D. Johnson, & D. W. Schemske. 1978. Web placement, web structure, and prey capture in orb-weaving spiders. *Bulletin of British Arachnological Society*, 4: (4) 141-148.

Uetz, G.W. 1992. Foraging strategies of spiders. *Trends in Ecology and Evolution*, 7: (5) 155-158.

Uetz, G.W., J. Halaj, & A. B. Cady. 1999. Guild structure of spiders in major crops. *Journal of Arachnology*, 27: 270-280.

Van Handel, E. 1972. The detection of nectar in mosquitoes. *Mosquito News*, 32: (3) 458-458.

van Rijn, P.C.J. & L. K. Tanigoshi. 1999. The contribution of extrafloral nectar to survival and reproduction of the predatory mite *Iphiseius degenerans* on *Ricinus communis*. *Experimental & Applied Acarology*, 23: 281-296.

Vogelei, A. & R. Greissl. 1989. Survival strategies of the crab spider *Thomisus onustus* Walckenaer 1806 (Chelicerata, Arachnida, Thomisidae). *Oecologia (Berl.)*, 80: 513-515.

Waddington, K.D. 1987. Nutritional ecology of bees. *In* Nutritional Ecology of Insects, Mites, Spiders, and Related Invertebrates. (F. Slansky, Jr. & J.G. Rodriguez, eds.) John Wiley & Sons, New York, NY.

Wagner, J.D. & D. H. Wise. 1996. Cannibalism regulates densities of young wolf spiders: evidence from field and laboratory experiments. *Ecology*, 77: (2) 639-652.

Walker, S.E., A. L. Rypstra, & S. D. Marshall. 2003. The relationship between offspring size and performance in the wolf spider *Hogna helluo* (Araneae: Lycosidae). *Evolutionary Ecology Research*, 5: 19-28.

Walker, S.E., S. D. Marshall, A. L. Rypstra, & D. H. Taylor. 1999. The effects of hunger on locomotory behaviour in two species of wolf spider (Araneae, Lycosidae). *Animal Behaviour*, 58: 515-520.

Wäckers, F.L., D. Zuber, R. Wunderlin, & F. Keller. 2001. The effect of herbivory on temporal and spatial dynamics of foliar nectar production in cotton and castor. *Annals of Botany*, 87: 365-370.

Wäckers, F.L. 2001. A comparison of nectar- and honeydew sugars with respect to their utilization by the hymenopteran parasitoid *Cotesia glomerata*. *Journal of Insect Physiology*, 47: 1077-1084.

Whitcomb, W.H., H. Exline, & R. C. Hunter. 1963. Spiders of the Arkansas cotton field. *Annals of the Entomological Society of America*, 56: 652-660.

Willey, M.B. & P. H. Adler. 1989. Biology of *Pucetia viridans* (Araneae, Oxyopidae) in South Carolina, with special reference to predation and maternal care. *Journal of Arachnology*, 17: 275-284.

Wise, D.H. 1993. Spiders in Ecological Webs. Cambridge University Press. Cambridge, MA.

Wise, D.H. 1979. Effects of an experimental increase in prey abundance upon the reproductive rates of two orb-weaving spider species (Araneae: Araneidae). *Oecologia*, 41: 289-300.

Witt, P. 1971. Drugs alter web-building of spiders. A review and evaluation. *Behavioral Science*, 16: 98-113.

Wunnachit, W.C., C. F. Jenner, & M. Sedgley. 1992. Floral and extrafloral nectar production in *Anacardium occidentale* L. (Anacardiaceae): an andromonoecious species. *International Journal of Plant Science*, 153: 413-420.

Yokoyama, V.Y. 1978. Relation of seasonal changes in extrafloral nectar and foliar protein and arthropod populations in cotton. *Environmental Entomology*, 7: 799-802.

Young, O.P. & G. A. Edwards. 1990. Spiders in United States field crops and their potential effect on crop pests. *Journal of Arachnology*, 18: 1-27.